Effects of culinary strategies on the concentration and bioaccessibility of cadmium and bioactivity of *Lentinula edodes*

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ABSTRACT: Objective To investigate the effect of the common treatment methods in family kitchen on the cadmium (Cd) content, bioaccessibility and the biological activity of the *Lentinula edodes*. **Methods** The content of heavy metal from different parts of *Lentinula edodes* by household acid, alkali soaking and boiling were detected by atomic absorption, bioaccessibility of exceeded Cd were studied, the change of biological activity by different treatments were detected by electron spin resonance (ESR). **Results** The results showed that the content of Cd in caps was higher than that in stipes, both were also above the national maximum limiting values. Soaking or boiling with weak acid solution decreased the content of cadmium markedly in both caps and stipes. Based on ultrasound biomicroscopy (UBM) *in vitro* extraction, Cd bioaccessibility was measured to show that Cd available in gastric stage was much more than that in intestinal stage, indicating both adult and children had 4~5 times health risk compared to provisional tolerable daily intake value. Within certain concentration, antioxidant activity of both superoxide radicals and hydroxyl radicals in treated caps or stipes decreased greatly compared to untreated ones. At the same concentration (5 mg/mL), scavenging superoxide radicals in caps treated with 1% $NaHCO_3$ and 1% CH_3COOH were enhanced markedly while to hydroxyl radical scavenging activity, only in 1% CH_3COOH treated caps increased significantly. **Conclusion** Soaking and boiling with 1% CH_3COOH decreased the content of Cd markedly in *Lentinula edodes*, the bioaccessibility of Cd in gastric was higher than that in intestinal simulation, and health risk was 4~5 times of Cd maximum tolerance value for adult and children respectively. The free radical scavenging activity of *Lentinula edodes* decreased after treatment with 1% $NaHCO_3$ or 1% CH_3COOH below the concentration at 5 mg/mL.

KEY WORDS: heavy metals; cadmium; bioaccessibility; antioxidant; *Lentinula edodes*

厨房措施对香菇中镉离子的浓度和生物可给性及生物活性的影响

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摘 要: 目的 探讨家庭厨房普通的处理方式对香菇中的镉(Cd)含量、镉的生物可给性以及其生物活性的影响。**方法** 用原子吸收检测家用酸、碱浸煮香菇不同部分的重金属含量变化, 对严重超标的 Cd 的生物可给性进行研究, 并采用电子自旋共振仪(ESR)对不同处理方式导致的生物活性变化进行检测。**结果** 香菇菇伞中的 Cd 含量高于菇梗中的 Cd 含量, 且都高于国家规定的最高允许值。家庭弱酸浸煮可以显著降低菇伞和菇梗中的 Cd 含量。用 UBM 法对体外模拟人体胃肠消化过程中 Cd 在胃和小肠的生物可给性研究表明, 胃液中的生物可给性显著比小肠阶段高, 并由此计算出人体健康风险因子, 其呈现出成人和儿童的每日摄入量均超过可耐受的每日摄入量。处理过的菇伞和菇梗的自由基清除能力都较未处理样品有显著降低。在相同浓度时(5 mg/mL), 1% $NaHCO_3$ 和 1% CH_3COOH 处理的菇伞对超氧阴离子自由基的清除能力均有显著增强, 但只有 1% CH_3COOH 处理的菇伞对羟基自由基的清除能力有显著增强。**结论** 家庭弱酸浸煮可以显著降低香菇中的 Cd 含量; Cd 在胃液中的生物可给性显著比小肠阶段高, 由此得出成人和儿童的每日摄入量均超过可耐受的每日摄入量各为 4~5 倍。酸、碱浸煮处理过的菇伞和菇梗的自由基清除能力都显著降低。

关键词: 重金属; 镉; 生物可给性; 抗氧化; 香菇

1 Introduction

Edible mushrooms are used worldwide as being valuable health foods, not only for their unique and subtle flavor, but also for their nutritional properties—as proved by its richness in carbohydrates, proteins, fibers, vitamins, and minerals, and the presence of unsaturated fatty acids and the poorness in fat as well. These characteristics render mushrooms as excellent food in low caloric content with certain health care effect [1-8]. *Lentinula edodes (L edodes)*, is the second most popular edible mushroom on the global market due to its nutritional value and the possibility of its medical application such as antioxidant, antimicrobial, antiallergenic and antitumor properties [9-14]. However, *L. edodes,* as other species of edible mushrooms, accumulates heavy metals from their living environment at considerable levels due to the serious circumstance of soil pollution all over the world, which can undoubtedly lead to impairment of human health [15-22]. Therefore, monitoring the heavy metal content of *L. edodes* has a tremendous significance. A number of ways have been employed to reduce or inhibit the accumulation and transformation of heavy metals from contaminated soils to agricultural products. Nevertheless, data are limited, esp. about common culinary strategies such as washing, soaking or cooking with different media such as acid or alkaline solution for a period of time to decrease the content of heavy metals in agricultural products. All these household treatments should be taken into consideration to reduce the content of heavy metals such as cadmium (Cd), lead (Pb), arsenic (As) and mercury (Hg) in mushrooms, with the purpose to instruct consumers to safeguard their personal health and to reduce their health risk. This is undoubtedly of great benefit.

Among the heavy metals with high bioaccumulation levels of *L. edodes,* Cd is usually reported as the main element with the content above the maximum permission values. So this study was based on concentrations of Cd, its bioaccessibility would be assessed to achieve a sound evaluation of potential health risks. Bioaccessibility of a substance is the fraction that is soluble in the gastro-intestinal environment and is available for absorption, which can be assessed by *in vitro* methods, and is also generally regarded as reproducible and cost-affordable *in vitro* digestive methods which can make further hazard and risk assessment to metals and minerals in soils, consumer products, and evaluation of metal substances [23-26] since the traditional human health risk assessment was mostly based on the total content of heavy metal which would lead to over-estimation of risk in the past decades.

So far, bioaccessibility test has several methods such as PBET (physiologically based extraction test), SBET (simplified bioaccessibility extraction test), IVG (*in vitro* gastrointestinal method), USP(pharmacopoeia method), MB & SR (mass balance & soil recapture), SHIME (simulator of human intestinal microbial ecosystem of infants), RIVM (rijksinstituut voor volksgezondheid en milieu), TIM (TNO gastrointestinal model) and UBM(unified bioaccessibility model) as well[1, 25-28]. In comparison, PBET, SBET, and UBM was wildly applied in many investigations, esp. UBM was undertaken by an international inter-laboratory

exercise, which involved in 7 laboratories (five in European countries such as United Kingdom, Netherlands, France, Denmark, and Belgium, and two in North American: Canada and USA) in the Western countries[29-31]. So Cd bioaccessibility will be used following UBM protocol in this investigation to assess the possible health risk of mushrooms. In addition, since *L. edodes* is so attractive food owing to its excellent bioactivity, we want to know whether the household ways would have impact on its bioactivity, such as antioxidant activity and antitumor effects. This investigation would focus on its scavenging activity (SA) to free radicals which was measured to identify whether the activity changed or not after taking common culinary strategies.

2 Materials and methods

2.1 Reagents

Some reagents were purchased from Sigma Chemical (St. Louis, MO, USA), including Uric acid (≥99%, catalogue No. U2625), Pancreatin (catalogue No. P-7545), Mucin (catalogue No. M-2378). Other enzymes and bile salts were bought from Solabio Chemical (Beijing, China), including α-Amylase (catalogue No. G-8290), Lipase (catalogue No. L-8070), Pepsin (catalogue No. P-8160), *D*-Glucosamine hydrochloride ((catalogue No. G-8240), *D*-Glucose anhydrous (≥99.8catalogue No. G-8150), Bile salt ((catalogue No. G-8210), Urea (≥99.5, catalogue No. G-8020), Albumin bovine (catalogue No. G-8010). The following reagents were ultrapure or analytical grade: sodium chloride, citric acid, malic acid, lactic acid, glacial acetic acid, hydrochloric acid, sodium bicarbonate, iron trichloride, calcium chloride, acetic acid, and alcohol. Deionized water (18.2 MΩ cm) was made using a super-pure water machine (Pine-tree, Desheng Science and Technology Park, Beijing, China) and was used to prepare the reagents and standards. Before being used, all glassware was treated with 10% (*V*/*V*) HNO_3 for at least 24 h and then rinsed 3 times with deionized water.

2.2 Sample collection, preparation and analysis of the total content of heavy metals

Sample collection: *L. edodes* samples were bought from the Yuege Zhuang, whole sale market in Beijing, China.

Sample preparation and analysis: all the procedures followed the GB/T 5009-14 to prepare for Cd analysis, GB/T 5009-17 for Pb analysis, GB/T 5009-11 for As analysis and GB/T 5009-12 for Hg analysis. Microwave digester MARS5 (CEM, USA) and Atomic Absorption Spectrometer (PinAAcle 900T, PerkinElmer, USA) were used to dissolute samples and then to detect samples respectively.

2.3 Bioaccessibility analysis

Bioaccessibility were done following ultrasound biomicroscopy (UBM) Method as previously described [30] with a little modification about the final total volume. To ensure complete dissolution of all reagents, all simulated fluids including saliva, gastric, duodenal fluid and bile were prepared 1d prior to the bioaccessibility extraction test, which was in accordance to Wragg *et al*[30-32]. In brief, 0.6 g dry mushroom powder was added to polypropylene conical centrifuge tubes (50 mL). Also 4.5 mL saliva was added. This mixture was vigorously mixed for 5 min. Subsequently, gastric juice (13.5 mL) was added to each tube. The pH was adjusted to 1.2±0.05 using Hall and the suspension was shaken (100 r/min) at 37 ℃ for 1 h. Check the pH to ensure the variance between 1.2~1.5. Subsequently, duodenal juice (13.5 mL) and bile (4.5 mL) were added and the pH was adjusted to 6.3 ±0.05 with NaOH (1.0 mol/L). The tubes were returned to the shaker for an additional 4 h. The experiment was conducted in duplicates.

All samples taken from mimic stomach, intestine and colon stages were centrifuged at 5,000 *g* for 10 min. The supernatants were filtered over a 0.45-μm filter and subsequently mixed with 1 mol/L HNO_3 to store at 4 ℃ until analysis. The samples were analyzed for total Cd contents by Atomic Absorption Spectrometer (PinAAcle 900T, PerkinElmer, USA). All samples were randomized prior to analysis. Cd bioaccessible fraction and bioaccessibility (%) were calculated according to the following equation:

$$\text{Cd Bioaccessibility (\%)} = \{[Cd]_{Extracted} / [Cd]_{food}\} \times 100\%$$

Where $[Cd]_{extracted}$ is the Cd concentration in the total volume of the gastric, intestinal and colon fluid (L), $[Cd]_{food}$ is the Cd concentration in the mushroom used in the extraction.

2.4 Health risk assessment

The health risk assessment (HRA, μg kg^{-1} BW d^{-1}) of Cd in the intestinal phase (HRA) were calculated as follows:

$$HRA=BAC\times(Wv/Wb)$$

BAC is the bioaccessibility of Cd in the small intestinal phase. Wv (kg/d) is the total consumption of food per adult per day and expected to be 400 g. Wb (kg/p) is the average Chinese adult body weight, 63.9 kg. The average Chinese children body weight is 32.7 kg, and consumption of food per day for children is 257 g [33-34].

2.5 Free radical Scavenging activity by Self-Spinning ESR assay

Scavenging activity (SA) of superoxide- and hydroxyl-radical was measured using an X-Band 300 ESR spectroscopy (Bruker Co, Germany). Superoxide radical was generated by using hypoxanthine/xanthine oxidase system (DMPO-OOH), and hydroxyl radical by the Fenton reaction system (DMPO-OH) as well. Then, different concentration of samples was added to the system to detect the spectra changes to find out the scavenging activity. All the experimental conditions followed the reference [35-36] and have been set up by finding the best signal-to-noise ratio at the same time. Every sample was performed at least 3 times. SA to superoxide or hydroxyl-radical estimated by ESR spectra was calculated by the following equation.

The SA value of the samples coped with varied medium was defined as:

$$SA=\{(h0-hx)/h0\}\times100\%$$

Where h0 and hx are the height of the second peak in the ESR spectrum of DMPO-O/DMPO -OH spin adduct of the blank and the probes, respectively.

2.6 Statistical analysis

The data were analyzed using analysis of variance (ANOVA). All the statistical analyses were carried out using Excel software for Windows with alpha levels for critical ranges at $P<0.05$.

3 Results and discussion

3.1 Concentration of heavy metals in stipes and caps of *L. edodes* with different coping solution

L. edodes not only acted as a delicacy, but also accumulator of heavy metals. Table 1 indicated that two parts, caps and stipes were burdened with toxic Cd content above the national maximum permission values, which was consistent with previous reports about general higher Cd concentration no matter whether it was treated or not due to its sensitivity to Cd[36]. In comparison, caps accumulated nearly double Cd content to stipes.

As for the unavoidable higher content of Cd in *L. edodes*, some easy and convenient ways need to be found to guide consumers how to enjoy this edible mushroom and safeguard health at the same time. After soaking and boiling with different media, it seems that tap water works better than any other solution. The second is weak acid media that can decrease the Cd content greatly both in caps or stipes. Other 3 media did not change the content in caps or stipes markedly. Maybe that's the reason Cd is easy to be dissolved under acid conditions. In a contrary, weak alkaline shows no stronger activity to decrease the Cd content. Anyway, soaking and boiling treatments may destroy tissues and cells of *L. edodes* to the greatest extent, thus enabling release of metal-binding compounds [37,38].

As to other 3 heavy metals, Pb, As and Hg were far lower than the permission value. So no more further studies will carried out about these 3 elements in the investigations. There was a little bit difference from the investigation that was carried out in another institute of China [16], which resulted from the different place of origin. Though it reported that boiling was proved to be a more efficient operation for decreasing the metal levels in mushroom than soaking [38], it seemed out of work for lead content in this investigation with the combined ways, which need to do more work to identify it.

3.2 Cd bioaccessibility in stipes and caps at mimic gastric and intestinal stages

Heavy metal contamination of food leads to the health risk which depends on its release from the food matrix, one of the important values is bioaccessibility, which is simulating gastro- intestinal extraction in comparison with the total content that may result in overestimation of health risk [36,39].

Cd bioaccessibility was generally higher in gastric fluid extractions than in intestinal fluid extractions, which was also identified in our studies (Fig. 1). At gastric stage Cd available varied narrowly between caps and stipes, or between treated and untreated groups, except in acid treated stipes which showed the highest bioaccessibility with pronounced difference.

Enhanced Cd bioaccessibility occurred *in vitro* gastric fluid extraction of acid treating caps and stipes as well, which was consistent with previous studies showing an increased Cd mobility at lower pH [25]. The

Cd bioaccessibility of the stipes in mushrooms increased sharply after soaking with 1% CH_3COOH and then boiling for 30 min. These results might be due to the chemical form changes after household coping ways. Similarly, in intestinal fluid extractions it showed the same changes in stipes, but bioaccessibility decreased in caps.

It has been increasingly recognized that the response of an at-risk population is not controlled by the total metal concentration, but instead by only the biologically available portion [22-24]. Even when a cooking process led to a decrease in Cd content, the bioaccessibility of Cd remained high. So the bioaccessibility could be commonly used to make more accurate evaluation about health risk.

3.3 Health risk assessment of Cd by *L. edodes* consumption

Based on the bioaccessibility data at intestinal phase, both the adult and children daily intake values were all higher than that of the provisional tolerable daily intake (PTDI) of the FAO/WHO (0.83 μg/kg body weight for Cd) (FAO/WHO, 2004) [33,40-41], esp. the untreated caps owed the highest health risk to adult and children as well, which was 4.4 and 5.6 times of the PTDI values respectively. The daily intake of Cd in the caps treated by NaCl was the lowest while that in stipes was the highest treated by CH_3COOH among five groups as it showed in table 2. The results revealed that soaking with 1%NaCl should be preferred if only caps were chose to be eaten. But if someone liked to eat stipes of the *L. edodes*, no more treated ways were better to reduce health risk. The HRA difference lies in not only the variance of the bioaccessibility, but the main compound differs in caps and stipes to a great extent.

3.4 Effects on the scavenging activity to superoxide radicals

The antioxidant activity was usually assessed by free radicals scavenging activity. Among most of the technology about free radical study, ESR coupled with spin-trapping is one of the most traditional methods to evaluate free radicals[42,43]

Table 1　Metal content in caps and stipes of *L. edodes* with different culinary treatments.

Samples	Cd(mg/kg)	Pb (mg/kg)	AS(mg/kg)	Hg(mg/kg)
Caps				
Untreated	1.170±0.205	0.053±0.002	0.058±0.003	0.0135±0.0007
Tap water	0.769±0.015 ^{a}	0.104±0.011 ^{a}	0.011±0.001	0.0118±0.0003
NaCl	1.078±0.031 ^{b}	0.161±0.062 ^{ab}	-	0.0321±0.0016
$NaHCO_3$	1.116±0.097 ^{b}	0.163±0.079 ^{ab}	-	0.0139±0.002
CH_3COOH	0.858±0.009 ^{abcd}	0.193±0.016 ^{abcd}	-	0.0330±0.002
Stipes				
Untreated	0.750±0.018	0.102±0.012	0.035±0.005	0.005±0.0004
Tap water	0.687±0.009	0.149±0.004^{a}	-	0.009±0.0005^{a}
NaCl	0.579±0.102	0.104±0.008 ^{b}	-	0.016±0.0005^{a}
$NaHCO_3$	0.612±0.013	0.025±0.003^{abc}	-	0.005±0.0002
CH_3COOH	0.644±0.046	0.107±0.009 ^{bd}	-	0.015±0.0003^{a}
GB[2]	0.5	1.0	0.5	0.100

Note: 1. In each row, different letters mean significant differences compared with the four groups in turn respectively (P<0.05). 2. GB is the national maximum permission value of China to the whole mushroom (GB 2762-2012)

The antioxidative and free radical scavenging properties of the phenolic and polysaccharide content of mushroom extracts have been reported [44,45] though no more work related to effects of heavy metals. This investigation showed that the scavenging activity to superoxide radicals, both treated parts, that is, caps or stipes dramatically decreased in comparison with untreated cap or stipe. It may due to the possibility that antioxidant ingredients decreased after soaking and boiling for a period of time. Between untreated two parts, caps showed a little bit stronger scavenging activity to superoxide radicals than stipes, esp. marked difference occurred at the concentration 1 mg/kg. Between treated two parts, stipes show a significantly stronger scavenging activity to superoxide radicals than caps at lower concentration (1 mg/kg), while at higher concentration (5 mg/kg) it shows reversal results, that is, caps show a significantly stronger scavenging activity to superoxide radicals than stipes. Within a certain concentration, the activity increased with a dose-dependent manner. Though it was reported that boiling was proved to be a more efficient operation for decreasing the metal levels in mushroom than soaking [38], it was clear to lead to the loss of bioactivity.

3.5 Effects of soaking and boiling on the scavenging activity to hydroxyl radicals

Not only untreated caps but also untreated stipes showed a stronger activity to get rid of hydroxyl radicals than treated caps or stipes at concentration varied from 0.5 mg/mL to 5 mg/mL. The activity of scavenging hydroxyl radicals reveals an increasing in a concentration-dependent manner. With concentration reaching higher to 5 mg/mL, the significant difference among groups generally disappeared, while the activity of treated caps increased markedly than stipes with concentration going higher. It's very clear that the separated parts of *L. edodes*, untreated caps owes higher activity of scavenging hydroxyl radicals than treated caps within the detected concentration, while to the stipes , marked difference occurred only at lower concentration, that is, 0.5 mg/mL and 1 mg/mL between the untreated samples and treated samples, as Fig. 3 shows. Though the use of the entire mushroom is recommended for the sake of economics, the mushroom cap proved to be better than the stipe; particularly for scavenging effects that provide a good protection against the oxidative damage, which occurs both in the body and our daily foods (Ferreira et al.2007).

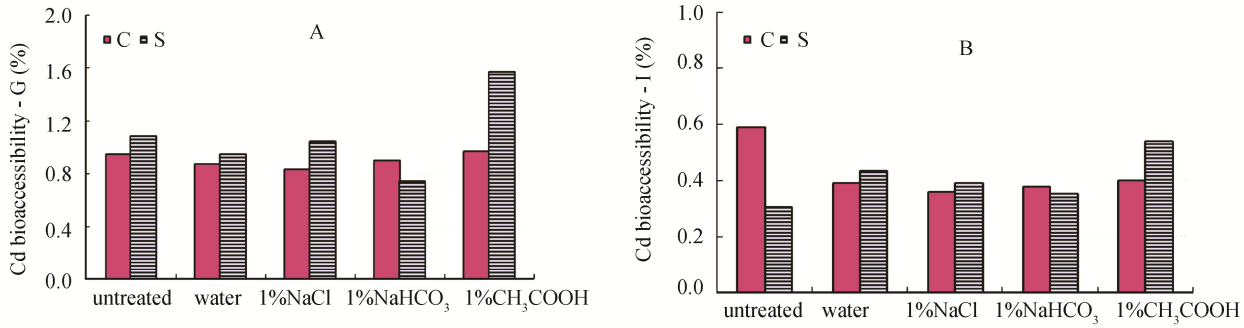

Fig. 1 Cadmium bioaccessibility of *L. edodes* coping with different solutions at gastric (A) and intestinal (B) stage.

Table 2 Estimation of adult and children dietary intake of Cd through *L. edodes* consumption. (μg·kg^{-1}BW·days^{-1}).

	Cd bioaccessibility (100%)		adult		Children	
	Caps	stipes	caps	stipes	caps	stipes
untreated	58.9	30.5	3.69	1.91	4.63	2.40
water	39.1	43.4	2.45	2.72	3.08	3.41
NaCl	36.0	39.1	2.25	2.45	2.83	3.07
$NaHCO_3$	37.7	35.2	2.36	2.20	2.97	2.76
CH_3COOH	40.2	53.9	2.52	3.38	3.16	4.24

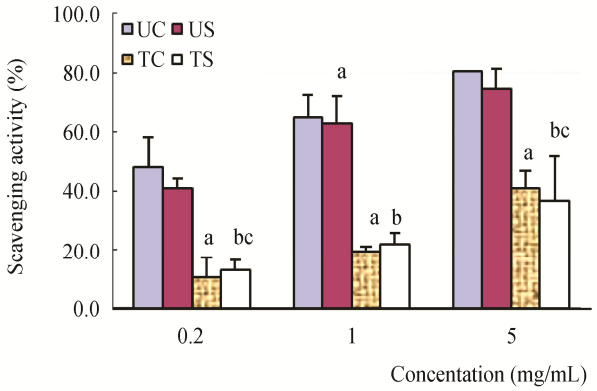

Fig. 2　Effects of soaking and boiling on the scavenging activity to superoxide radicals .

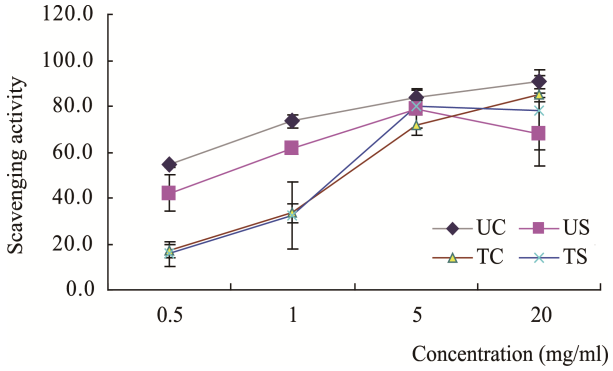

Fig. 3　Effects of soaking and boiling on the scavenging activity to hydroxyl radicals.

3.6　Effects of soaking and boiling with different solution on the scavenging activity to superoxide radicals

As Fig.4 showed, soaking caps with 1%$NaHCO_3$ and then boiling would markedly increase scavenging activity of superoxide radicals (P<0.05) at the same concentration (5 mg/mL). In comparison with caps, 1% $NaHCO_3$ could decrease the activity of stipes. As to the coping media effects on hydroxyl radicals, no difference occurred between different groups in stipes. However, in caps, comparing with soaking and boiling with tap water, 1% NaCl reduced the scavenging activity markedly, while 1% CH_3COOH enhanced the scavenging activity markedly in comparison with 1% NaCl or 1%$NaHCO_3$ coped groups.

As to scavenging activity to hydroxyl radical, *L. edodes* showed higher antioxidant properties. Samples treated with 1% NaCl performed reducing scavenging activity markedly (P<0.05) while 1% CH_3COOH enhanced the scavenging activity significantly in comparison with tap water treated caps. No more difference existed among coped stipe groups. The variance of scavenging activity may relate to its presence changes of antioxidant ingredients, such as monosaccharide, polysaccharide, phenolic and flavonoid compounds and so on. In fact, these ingredients are known as strong free radical scavengers and metal chelators [11,45-50]. These compounds also result in various bioactivity such as antioxidant, anti-virus, anti-tumor and anti-inflammation, which also attracted more exploration for health care or medical use.

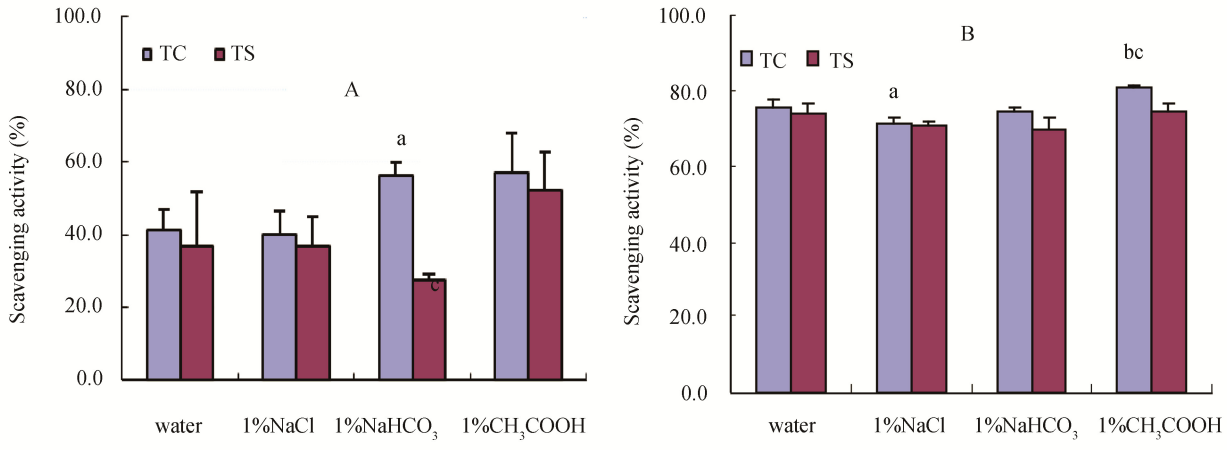

Fig. 4　Effects of soaking and boiling on the scavenging activity to superoxide (A) and hydroxyl radicals(B).

4 Conclusion

Compared to green plants, mushrooms built up large concentrations of some heavy metals, such as Cd, Pb, As, and Hg, esp. Cd is one of the elements easy to be accumulated to higher levels. Weak acid soaking and then boiling for a period of time helped reduce the content of Cd, which is higher than maximum national permission value. Cd bioaccessibility, which mimic the availability in gastric or intestinal stage, indicates a gastric simulating extraction which could pose more effect on the availability than in intestinal stage. Furthermore, bioactivity of total *L. edodes* was taken into consideration after coping with different solution, which showed a reduced antioxidant activity within a certain concentration. Even so, to decrease health risks of Cd by certain coping ways is preferred with loss of some bioactivity inevitably.

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