Black goji berry (Lycium ruthenicum) tea has higher phytochemical contents and in vitro antioxidant properties than red goji berry (Lycium barbarum) tea

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Abstract

Goji berry tea, a traditional herbal tea, is the main ate mode of goji berry in Asia, yet few studies in comparison with red goji berry tea and black goji berry tea are carried out. This study investigated the effects of water temperature and soak time on the colour, phytochemicals, and the antioxidant capacity [2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), and the ferric-reducing antioxidant power (FRAP)] of two goji berry tea. A comparison of the bioactive compounds and antioxidant activities between black and red goji berry tea was conducted. Results showed that both red and black goji berry tea were rich in phytochemicals, giving high antioxidant ability. The levels of bioactive compounds and the antioxidant activity of the two goji berry tea increased as the increases in soak temperature and time. Black goji berry tea had higher phytochemicals and antioxidant property than those of red goji berry tea. Infused at 100° water for the same time, the levels of total polysaccharides (150 mg/100 ml), total polyphenols (238 mg/ml), and antioxidant capacity (550 μmol/100 ml) of black goji berry tea were 3.5, 2, and 5 times higher, respectively, in comparison with red goji berry tea. The results of this study demonstrate that hot drink of goji berry in China is a good habit and black goji berry tea may be a better choice.

Key words: red goji berry (L. barbarum); black goji berry (L. ruthenicum); goji tea; phytochemicals; antioxidant activity.

Introduction

Lycium plants (goji berries or wolfberries) are prevailing in China and other Asian areas (Seeram and Navindra, 2008). There are around 80 species of Lycium L. (Solanaceae) in the world (Hitchcock, 1932; Miller, 2002; Levin and Miller, 2005), with only seven species have been reported in China (Yisilam et al., 2018). However, only three species (Lycium barbarum, Lycium chinense, and Lycium ruthenicum) are consumed as medicine and food in China. Recently, goji berry has been marketed as food and dietary supplement in various retail outlets in the world. Of particular, consumers from Europe and America show increasing interests in wolfberries due to their potential health benefits (Amagase and Farnsworth, 2011; Pedro et al., 2019; Sa et al., 2019).

Red goji berry occupied approximately 90% of all commercially available goji berries (Wang et al., 2018), and it has been planted in Northwest China for about 600 years (Chen et al., 2013). Red goji berry has high economic significance as Traditional Chinese Medicine and nutritional purposes (Chang and So, 2008; Ruiz-Salinas, et al., 2020).

Recently, wild black goji berry (L. ruthenicum) inhabiting in the Qinghai-Tibet Plateau is attracting attentions of consumers (Han et al., 2014). Health care products containing Lycium ruthenicum Murray (LR) have also been sold in Chinese markets. Currently, the price of black goji berry is expensive, which is 10 times of red goji berry. The high price of black goji mainly is due to the scarcity of wild black goji...
berry and higher functional value (Ni et al., 2013; Islam et al., 2017; Abduaibifu and Tamer, 2019). Islam et al. (2017) found that black goji berry has higher polyphenols and antioxidant than red goji berry. Ni et al. (2013) found that black goji berry has higher polysaccharide content than red goji berry. However, given that goji berry tea is the main ate form of goji berry (Sun et al., 2017), the phytochemicals and bioactive activity of black goji berry were evaluated using the extracts of organic solvents (e.g. acetone and ethanol) in the existing studies which may not reflect the real values in daily consume form—goji berry tea.

Therefore, the aim of this study was to analyse and compare the phytochemicals and antioxidant abilities of red goji berry and black goji berry tea at different soak conditions, to provide scientific knowledge on traditional red and black goji berry tea for consumers’ daily life.

Materials and Methods

Chemicals

Folin–Ciocalteu phenol reagent, 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH), 2,4,6-Tri-2-pyridyl-s-triazin (TPTZ), 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and other reagents were analytical grade acquired from Aladdin Reagent Database Inc. (Shanghai, China). The following chemicals and compounds were purchased from Sigma-Aldrich (St. Louis, MO, USA): chlorogenic acid, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), gallic acid, and rutin.

Materials

Dried red goji berries (L. barbarum L.) and black goji berries (L. ruthenicum) were purchased from Golmud Yilin Goji berry Technology Development Co., Ltd, Golmud City, Qinghai Province (Figure 1). The moisture content was 6.20% and 10.45% (w/w), respectively.

Preparation of black goji berry tea and red goji berry tea

Dried black or red goji berries were added to a flask, then purified water (100 ml) was added to the flask, and the samples were infused a different number of times for different times at different temperatures. The tea was filtered by passage through a 45 µm pore size polytetrafluoroethylene (PTFE) disposable syringe filter and kept at a constant volume of 100 ml.

Determination of total phenolics content (TPC)

Black or red goji berry tea (0.2 ml) was added to a 10 ml colourimetric cylinder which contained Folin–Ciocalteu reagent (0.5 ml), shaken thoroughly, and kept for 1 min. Then Na₂CO₃ [1.5 ml, 20% (w/v)] was added, and the solution was vortex mixed and made to 10 ml with distilled water. After 10 min, A761 was measured by a UV-752N ultraviolet spectrophotometer (Yidian Analytical Instrument Co, Ltd, Shanghai, China) using distilled water as a blank. A calibration curve was prepared using a standard solution of gallic acid and the results for TPC were expressed as mg gallic acid equivalents per 100 ml of tea (Sun et al., 2017).

Colourimetric analysis of goji berry tea

The colour of the goji berry tea was monitored with colourimeter (Sun et al., 2017). The infusion of red goji berry and black goji berry was added to cuvettes, and then subjected to a colourimeter (ColorQuest XE, Hunter Associates Laboratory Inc., Reston, VA, USA) for assessing L*, a*, b* with a D65 light source and an aperture size of 9.5 mm.

Total polysaccharides content (TPOC) analysis

Total polysaccharides were determined by the phenol-sulfuric acid method using glucose as the standard reported by Sun et al. (2017) with slight modifications.

Filtered black or red goji berry tea (1 ml) was placed into a 10 ml volumetric flask, made to 10 ml with distilled water, and mixed well. The diluted solution (1 ml) was used and A490 was determined according to the method of the standard curve using distilled water as the blank. The concentration and content of glucose in the goji berry polysaccharide tea were calculated according to the regression equation. The conversion factor was calculated as follows:

\[
\text{Polysaccharide} = \frac{C}{D} f M \times 100\%,
\]

where C is the glucose concentration in the sample, D is the dilution, f is the conversion factor, and M is the mass of dried goji berry.

Evaluation of antioxidant activity

Antioxidant activity (AA) of goji berry tea was determined by 2, 29-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) 2,
2-diphenyl-1-picrylhydrazyl radical (DPPH), and the ferric-reducing antioxidant power (FRAP) assays.

**ABTS free radical-scavenging assay**
Potassium persulfate was dissolved in distilled water (140 mM, 440 μl), ABTS was dissolved by H₂O₂ (7 mM); then potassium persulfate solution was added to 25 ml ABTS solution and was kept in darkness at 25 °C for 12–16 h, and then diluted with ethanol to A₇₃₄ of 0.70 ± 0.02 (Xu et al., 2007). ABTS cation solution (7 mM, 3.9 ml) was added to black or red goji berry tea (0.1 ml), mixed thoroughly, and then kept at 25 °C for 10 min. A₇₃₄ was determined by a UV-752N ultraviolet spectrophotometer. A control (0.1 ml of methanol, 3.9 ml of ABTS solution) was prepared, and a calibration curve was constructed for the absorbance reduction and concentration of the Trolox standard. The ABTS radical-scavenging ability is expressed as TEAC mg/g DW.

**DPPH free radical-scavenging assay**
DPPH was dissolved in ethanol (0.1 mM), and then a black or red goji berry tea (0.2 ml) was added to 3.9 ml DPPH solution and then kept in darkness for 30 min at 25 °C (Xu et al., 2008). A₅₁₇ was determined by a UV-752N ultraviolet spectrophotometer. The trolox solution was used to make the calibration curves and the results are expressed as TEAC mg/g DW.

**FRAP assay**
Fresh FRAP reagent was prepared by mixing 10 mM TPTZ dissolved in 40M HCL and 20 mM ferric chloride dissolved in distilled water and 0.1M acetate buffer (pH 3.6) (ratio 1:1:10, by vol.) (Xu et al., 2008). Black or red goji berry tea (0.2 ml) was added to FRAP reagent (5.0 ml). After 10 min, A₅₉₃ was measured by a UV-752N ultraviolet spectrophotometer and Trolox solution was used to make the calibration curves. The results are expressed as Trolox equivalent antioxidant capacity (TEAC) mg/g sample dry weight (DW).

**Statistical analysis**
Experiments were repeated three times. Data were expressed as the mean ± standard deviation. Statistical analyses were carried out using SPSS software, version 17.0 (SPSS Inc.). Data were compared with ANOVA. Statistically significant differences were set at P < 0.05.

**Results and Discussion**
**Variation of colour of red goji berry tea and black goji berry tea with soak temperature and time**
The colour of black and red goji berry tea is visually shown in Figure 2 and precisely quantified by L*, a*, and b* (Figure 3). It was found that red goji berry tea and black goji berry tea presented light yellow and purple, respectively, and the colour of both teas gradually changed to darker with the increase of time and temperature (Figure 2).

The L* value (representing the lightness) of black and red goji berry tea significantly decreased with the increasing soak temperature and time (P < 0.05) (Figure 3a and 3b). Generally, the L* value of black goji berry tea was lower than that of red goji berry tea.

**Figure 2.** Red (a and b) and black (c and d) goji berry tea prepared at 25 and 100 °C for 5, 10, 15, 30, 60, and 100 min.
The $a^*$ value (presenting its position between red and green) of black goji berry tea significantly increased with the increase of soak temperature time when soak time was 5 min, and the $a^*$ value of black goji berry firstly increased with the increase of temperature and then decreased with the increase of temperature when soak time varied from 10 to 60 min ($P < 0.05$), whereas the $a^*$ value of red goji berry tea was not significantly different at different soak conditions. The $a^*$ value of black goji berry tea was higher than red goji berry tea ($P < 0.05$) (Figure 3c and 3d).

The $b^*$ value (meaning its yellow value) of red goji berry tea significantly increased with soak temperature varied from 0 to 100 °C and time ranging from 5 to 100 min ($P < 0.05$) (Figure 3f). However, the $b^*$ value of black goji tea significantly decreased with the increasing soak temperature and time a ($P < 0.05$) (Figure 3e).

Figure 3. The L*, a*, b* value of black and red goji berry tea prepared at different temperatures for different lengths of time (a–f) (significant differences were set at $P < 0.05$).
The $b^*$ value of red goji berry tea was higher than that of black goji berry tea.

The variations of $L^*$, $a^*$, $b^*$ of red goji berry tea were connected with the contents of soluble solid, polyphenols, and carotenoids at different tea conditions, and the hue of red goji berry tea was determined by the soluble carotenoids. The variations of $L^*$, $a^*$, $b^*$ of black goji berry tea were connected with the contents of soluble solid and anthocyanins at different soak conditions, and the hue of black goji berry tea was determined by the soluble anthocyanins.

Effects of soak temperature, time, and times on the total polysaccharides content (TPOC) of black and red goji berry tea

Polysaccharides are one of the water-soluble phytochemicals in goji berry, which are focused on by many researchers. Total polysaccharides contents of black and red goji berry tea both significantly varied with soak temperature, time, and times ($P < 0.05$). Total polysaccharides contents significantly increased with the increase of soak temperature and time ($P < 0.05$) (Figure 4a and 4b). TPOC of black

![Black goji berry tea](image1)

![Red goji berry tea](image2)

Figure 4. Polysaccharides content of black and red goji berry tea at different temperatures for different time (a and b), and for different soak times (c and d) (significant differences were set at $P < 0.05$).
and red goji berry tea at 100 °C, 60 min was significantly higher compared with the tea in other conditions \((P < 0.05)\), indicating that the polysaccharides are stable and high temperature and long time is good for its diffusion. The TPOC value in black goji berry at 100 °C, 60 min was about 30 times of TPOC at 40 °C, 60 min, and similarly the contents of TPOC at 100 °C, 60 min was about 14 times of TPOC at 100 °C, 5 min. The TPOC significantly decreased with increased times \((P < 0.05)\) (Figure 4c and 4d). From Figure 4a and 4b, it was found that TPOC of black goji berry tea was far higher than red goji berry. Take 100 °C as an example, the TPOC of black goji berry tea was 3.5 times of red goji berry. Figure 4c and 4d suggested that the polysaccharides in black goji berry could be released into water easier than red goji berry, and most of the polysaccharides were released in the water during the first time, but red goji berry needed two times tea. The difference between two goji berry tea may be the following reasons: firstly, because of the differences in the surface-to-volume ratios, black goji berry are much smaller than red goji berry, which means much faster rates in heat and mass transfer; secondly, maybe due to the higher in the thickness and compactness of the red goji fruits’ skin \((Lx, 2012)\); and thirdly, maybe due to the higher contents in raw black goji dried fruits \((Ni et al., 2013)\) and lower molecular weight of polysaccharide in black goji berry \((Lx, 2012)\).

Effects of soak temperature, time, and times on the total phenolics content (TPC) of black and red goji berry tea
Flavonoids and phenolic acids are the main polyphenols in red goji berry, and anthocyanins are the main polyphenols in black goji berry. Figure 5 shows the variation of TPC of black and red goji berry tea with soak temperature, time, and times. TPC in both goji berry tea significantly increased with temperature ranging from 0 to 100 °C and time ranging from 5 to 60 min, but the difference of TPC in goji berry tea between 80 and 100 °C was not significant \((P < 0.05)\); it could contribute to the unstable condition of polyphenols at higher temperature, and the results are in agreement with the in tea colour of Figure 2. The contents of TPC in black goji berry at 100 °C,
60 min were about 2 times of TPC at 40 °C, 60 min, and similarly, the contents of TPC at 100 °C, 60 min were about 5 times of TPC at 100 °C, 5 min. Comparing Figure 5a and 5b, TPC of black goji berry tea (243.4 mg/100 ml) was far higher than red goji berry tea (108.90 mg/100 ml) at 100 °C, which is about 2 folds, and the results can be explained by that black goji berry has abundant anthocyanins while red goji berry does not (Wang et al., 2018). Different from polysaccharides, the difference of the TPC of goji berry between the first tea time and the second tea time was not significantly different, so the second tea was important for more polyphenols can be dissolved (P < 0.05).

Effects of soak temperature, time, and times on the antioxidant abilities of black and red goji berry tea

The antioxidant abilities of goji berry tea were determined using FRAP, ABTS, and DPPH assays (Figure 6). The antioxidant abilities of black and red goji berry tea significantly increased with soak temperature and time and significantly decreased with soak times (P < 0.05), which was similar to variation trend of the polysaccharides and polyphenols in goji berry tea. The antioxidant ability in black goji berry at 100 °C, 60 min was about 3 times of those at 40 °C, 60 min and the contents of antioxidant ability at 100 °C, 60 min were about 4 times of those at 100 °C, 5 min. The antioxidant
ability of black goji berry tea was far higher than red goji berry, and the AA of black goji berry tea was about two to five folds of red goji berry tea. The antioxidant ability of goji berry tea was related to the soluble polyphenols in tea.

Conclusions

In the present study, the effects of making tea with water on the colour, and the content of phytochemicals and antioxidant ability of black and red goji berry tea were investigated and compared, which will directly provide a scientific basis for daily diet. It is found that both red goji berry and black goji berry tea are rich in phytochemicals and show high antioxidant capacities. The contents of phytochemicals and the antioxidant capacity of a goji berry tea rised with soak temperature and time. Soaking using the cold or warm water, even at long time, will waste most of the phytochemicals and antioxidant activity. Black goji berry tea has far higher phytochemicals, antioxidant property, and more desirable colours than red goji berry tea. The results of this study suggest that high temperature and long soak time can be used to make a healthy goji berry tea, hot drink of goji berry is a good habit, and black goji berry may be a better choice, but we need to change the habit of short tea time.
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Conflict of Interest
The authors declare no conflicts of interest.

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