Bioactive compounds and antioxidant activity of beetroots prepared by different drying methods

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Abstract

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Introduction. The aim of the research is to investigate the influence of different drying methods, including freeze drying, vacuum drying, microwave drying and microwave vacuum drying, on the bioactive compounds and antioxidant activity of beetroots.

Materials and methods. Bioactive compounds and antioxidant activity of dried beetroots prepared by freeze drying (FD), vacuum drying (VD), microwave drying (MD), and microwave vacuum drying (MVD) were investigated. Moisture analyzer was used to determine moisture content of beetroots. Beetroot powder was extracted three times with 50% ethanol (v/v). Colorimetric methods were used to determinate contents of betalain, ascorbic acid and total phenolic, and 1,1-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, ferric reducing antioxidant power (FRAP) and 2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical scavenging activity of dried beetroots.

Results and discussion. Drying methods significantly affected the drying time, bioactive compounds and antioxidant activity of beetroots. The shortest drying time (0.56 ± 0.01 h) was occurred at MVD, while FD required the longest drying time of 29.67 ± 0.58 h. There was no significant difference in the final moisture content of dried beetroots prepared by different drying methods. It was found that the dried beetroots prepared by VD showed the highest ascorbic acid content of 8.73 ± 0.23 mg/g, and the beetroots dried by FD displayed the highest betaxanthin content of 2.57 ± 0.01 mg/g, while the beetroots obtained by MD showed the lowest betacyanin content of 2.98 ± 0.02 mg/g, and FD led to significantly lower total phenolic content of beetroots in comparison with other drying methods. In terms of antioxidant activity, the beetroots prepared by MD and MVD showed significantly higher DPPH radical scavenging activity than the beetroots prepared by VD and FD. Meanwhile, the beetroots obtained by VD exhibited the largest ABTS radical scavenging activity of 17.22 ± 0.35 mg trolox equivalent (TE)/g. Besides, the beetroots prepared by MVD displayed the highest FRAP value of 13.36 ± 0.17 mg TE/g. Compared to beetroots prepared by other drying methods, the beetroots prepared by FD showed the lowest FRAP, DPPH and ABTS values, indicating the weakest antioxidant activity.

Conclusions. Considering the drying time, bioactive compounds and antioxidant activity, MVD is recommended to prepare dehydrated beetroots, which can reduce drying time and better preserve bioactive compounds and antioxidant activity of beetroots.

Introduction

Beetroot (*Beta vulgaris* L.) is an important root vegetable and widely grown in Russia, Germany, Ukraine, Turkey, Poland, France, Egypt, America, United Kingdom and China. According to the macronutrient data reported by the US Department of Agriculture, 100 g of raw beetroot contains an average energy of 43 kcal, total sugar 6.76 g, total dietary fiber 2.8 g, proteins 1.61 g, and total lipids 0.17 g (United States Department of Agriculture, 2013). Beetroot can not only be consumed as fresh vegetable, but also can be processed into canned, pickled, frozen and dehydrated products (Kanner et al., 2001; Paciulli et al., 2016). Beetroot contains highly bioactive compounds, such as betalains (betacyanins and betaxanthins), polyphenols, flavonoids, carotenoids, ascorbic acid, nitrate and saponins. Antioxidant activity of bioactive compounds and successful utilization of beetroot in disease prevention and health promotion is increased in last few decades (Chhikara et al., 2019). Beetroot spoils rapidly due to high moisture content, continuous metabolism and microbial attack.

Drying is one of most widely used methods for preservation foods by inhibiting enzyme activity, avoiding microbiological spoilage, and delaying deterioration (Arikan et al., 2012). In order to obtain dried vegetables, a great number of drying methods have been applied such as hot air drying, vacuum drying, microwave drying, microwave vacuum drying, and freeze drying. During drying process, it causes nutrient losses and changes the raw material's chemical and physical properties. Thus, choosing the most suitable drying method mainly depends on the quality of the final products (Wei et al., 2019). Vacuum drying (VD) is a very good drying technology because of its sealed environment, low content of oxygen and cost superiority (Dai et al., 2021). A number of studies have demonstrated that VD can well preserve the nutritional components, physical and chemical properties, organizational structure and sensory quality of products (Mitra et al., 2011; Liu et al., 2021). Microwave drying (MD) is also widely used in the drying of agricultural products, it is generally recognized as rapid drying rate, short drying time, rapid process control, and fast-switching (Jin et al., 2017). At the same time, MD causes non-uniform heating, textural damage, nutrient degradation, and limited penetration depth (Vadivambal et al., 2010). Microwave vacuum drying (MVD) is an alternative drying method for a variety of foodstuffs. The intensive heating of microwave and low boiling point generated by the vacuum make the food to be dried at relatively low temperature and in a short time, helping to maintain a high level of healthy nutrition and sensory quality (Scaman et al., 2005). As the best dehydration method for heat sensible foods, freeze drying (FD) involves high energy consumption, high equipment costs, and long drying time, which limits its use in the production of high-value products (Köprüalan et al., 2021).

The drying method significantly influences the bioactive compounds and antioxidant activity of the final product. Therefore, it is important to choose a suitable drying method to obtain high quality of dried products. In literature, there are rarely studies on the effects of different drying methods on the quality attributes of dried beetroots. So we conducted different types of drying methods to dry beetroots, exploring the effect of different drying methods on the quality of dried beetroots, and looking forward to obtaining high-quality dried beetroots in this study. Therefore, the purpose of the research is to investigate the influence of four different drying methods, including freeze drying, vacuum drying, microwave drying and microwave vacuum drying, on the bioactive compounds and antioxidant activity of dried beetroots.

Objectives of research:

- To explore the drying time and final moisture content of dried beetroots under four different drying methods.

- To investigate the effect of four different drying methods on the bioactive compounds of beetroots.
- To evaluate determine the antioxidant activity of dried beetroots obtained by four different drying methods.

Materials and methods

Materials

Fresh beetroots were purchased from a local market in Xuzhou, Jiangsu province, China. The fresh beetroots were washed, peeled, and cut into slices 75 mm in diameter and 4 mm in thickness. Ascorbic acid assay kit was obtained from Nanjing Jiancheng Institute of Bioengineering, Nanjing, China. Folin-Ciocalteu's phenol reagent (1.0 mol/L), gallic acid (purity≥98%), tri-2-pyridyl-s-triazine (TPTZ, purity≥98.0%), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, purity≥98.0%), 1,1-diphenyl-2-picrylhydrazyl radical (DPPH, purity≥98.0%) and 2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS, purity≥98.0%) were obtained from Shanghai Yuanye Bio-Technology Co., Ltd, Shanghai, China.

Drying procedures

The experimental procedures of four different drying methods are listed as follows:

Microwave vacuum drying (MVD): The 300-g fresh beetroot slices were placed uniformly on a tray (61 cm × 43 cm × 5 cm), and the tray was put into a microwave vacuum dryer (WBZ-10, Guiyang Xinqi Microwave Industry Co., Ltd, Guiyang, China). The vacuum degree was –90 KPa. Microwave power of 1000W was investigated in microwave vacuum drying for beetroots at constant vacuum degree.

Microwave drying (MD): The 300-g fresh beetroot slices were spread evenly as a thin layer on the circular fiberglass tray (diameter-30 cm) of microwave drying system (SAM-255, CEM Corporation, USA), and dried at 390W.

Vacuum drying (VD): The 300-g fresh beetroot slices were spread a thin layer on a tray (31 cm × 29 cm × 2 cm) of vacuum drying oven (BPZ-6033B, Shanghai Yiheng Scientific Instrument Co., Ltd, Shanghai, China). The vacuum degree was –95 KPa. The temperature of vacuum drying was set at 60 °C.

Freeze drying (FD): The 300-g fresh beetroots were frozen at –20 °C for 12 h and were placed on the cavity in the freeze dryer (FDU-2110, Tokyo Rikakikai Co., Ltd, Tokyo, Japan) at 4 Pa. The condenser temperature was set at –80 °C.

All the drying process stopped when the final moisture content of beetroots was less than 6.0% on a wet basis. All the drying experiments were conducted in three replications.

Determination of moisture content

The moisture content (wet basis) of beetroots was measured by a moisture analyzer (HX204, Mettler Toledo Co. Ltd., Switzerland) at 105 °C until it reached to a constant weight. The average initial moisture content of fresh beetroots was 90.07±0.72% (wet basis).

Extraction process

Dried beetroots obtained from triplicate were mixed and then ground into powders (pass through a 60-mesh sieve) to obtain samples with representative chemical components for particular drying methods. 2.0 g of beetroot powder was placed in a 50-mL centrifuge tube, 20 mL of 50% ethanol (v/v) was added, and then fully mixed by a vortex mixer (VORTEX-5 Kylin-Bell Instrument Manufacturing Co., Ltd, Jiangsu, China) for 2 min. After centrifugation using a centrifuge (H1850, Xiangyi Centrifuge Instrument Co., Ltd, Hunan, China) at 5000 rpm for 10 min, the supernatant was collected, and the precipitate was extracted twice with 20 mL of 50% ethanol (v/v). The supernatants obtained from three extractions were combined and diluted to 100 mL with 50% ethanol (v/v). The extracts were stored at 4 °C until further analysis.

Determination of bioactive compounds

Betalains are natural water-soluble pigments containing nitrogen, which are subdivided into betacyanins (red-violet pigments) and betaxanthins (yellow-orange pigments) (Coy-Barrera, 2020). The betalain content was determined spectrophotometrically (722N, Shanghai Youke Instrument Co., Ltd, Shanghai, China) as described by Stintzing et al., 2005. The extract was diluted with 0.05 mol/L phosphate buffer solutions (pH 6.5) to obtain absorption values of $0.8 \leq A \leq 1.0$ at 538 nm, and then the absorbance of diluted extract was measured at 480 nm, 538 nm, and 600 nm, respectively. The betalain content was expressed as micrograms per gram (mg/g).

Ascorbic acid content of beetroots was determined by colorimetric method using a detection kit (Nanjing Jiancheng Institute of Bioengineering, Nanjing, China). Results were calculated on a dry weight basis and expressed as mg/g.

Total phenolic determination was adopted from Folin-Ciocalteu method (Alvarez-Parrilla et al., 2011). Diluted extract (0.5 mL) was mixed with 2.5 mL of 10% Folin-Ciocalteu's reagent (v/v), and then 2 mL of 7.5% sodium carbonate (w/v) was added. The mixture was incubated for 15 min at 50 °C and then cooled to room temperature, and the absorbance was measured at 760 nm by a visible spectrophotometer (722, Shanghai Youke Instrument Co., Ltd, Shanghai, China). A stand curve was obtained using different concentrations (0–0.1 mg/mL) of gallic acid. The total phenolic content of dried beetroots was expressed as milligrams of gallic acid equivalent (GAE) per gram.

Determination of antioxidant activity

Antioxidant activity of dried beetroots was evaluated by three methods.

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay was analyzed according to the colorimetric method with slight alterations (Brand-Williams et al., 1995). The DPPH assay was performed by adding 2 mL of diluted extract to 4 mL of 0.2 mM DPPH solution and reacting for 30 min in the dark at room temperature. The absorbance of the mixture was read at 517 nm. A calibration curve was obtained using different concentrations (0–200 µmol/L) of trolox. Results were expressed as milligrams trolox equivalent (TE) per gram.

The 2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) assay was evaluated according to the method reported by Re et al., 1999, with minor modifications. Equal quantities of 7 mM ABTS and 2.45 mM K₂S₂O₈ solution were mixed to obtain ABTS^{•+} solution. The ABTS^{•+} solution was allowed to stand for 16 h in the dark at room temperature. The ABTS^{•+} solution was diluted with 80% ethanol (v/v) to obtain an absorbance of

0.70±0.02 at 734 nm before the measurement. Diluted extract (0.4 mL) was reacted with 3.6 mL diluted ABTS⁺⁺ solution for 6 min at room temperature. Absorbance was recorded at 734 nm. Trolox with a concentration of 0–150 µmol/L was used as the standard curve. The results were expressed as trolox equivalent (TE) in mg/g.

The ferric reducing antioxidant power (FRAP) assay was conducted by the colorimetric method described by Benzie et al., 1996. First of all, FRAP reagent was prepared by mixing 0.01 M Tri-2-pyridyl-s-triazine (TPTZ) solution (prepared in 0.04 M HCl), 0.02 M FeCl₃ solution and 0.3 M acetate buffer (pH 3.6) at the volumetric ratio of 1:1:10. The diluted extract (0.2 mL) was fully reacted with 6 mL of FRAP reagent. After incubating at 37 °C for 10 min, the absorbance at 593 nm was read. A calibration curve was obtained using different concentrations (0–600 µmol/L) of trolox. All solutions were prepared on the day of use. Results were calculated as mg TE/g.

Statistical analysis

All experiments were conducted at least in triplicate and results were expressed as mean±standard deviation (SD). The differences among samples were determined by one way analysis of variance (ANOVA) followed by the Tukey's multiple range test using the SPSS Statistics Version 20 (IBM Corporation, Chicago, IL, USA). The mean values with different letters are significantly different at 95% confidence level ($p<0.05$). Origin 2017 (Origin Lab, MA, USA) was used to draw figures.

Results and discussion

Drying time and moisture content

The drying time and final moisture content of beetroots are depicted in Table 1. Different drying methods resulted in significant differences in drying time. FD had the longest drying time (29.67±0.58 h). Although FD has less thermal damage to products, it takes longer time. It is an expensive process and is only applicable to high-value products (Hu et al., 2006). MVD was much faster drying method than VD and FD. It required the shortest drying time (0.56±0.01 h) for MVD to reach the final moisture content. The drying time of MVD was reduced by 98.1% compared to FD, and was only 7.2% of the vacuum drying time. While the drying time for MD was 0.77±0.03 h and very close to MVD. The drying time required from MD reduced up to 90.2% in comparison with VD, and was about 2.6% of FD time. This indicated that the drying time was significantly reduced as microwave was used.

Table 1
Drying time and final moisture content of beetroots

Drying methods	MVD	MD	VD	FD
Drying time, h	0.56±0.01 ^c	0.77±0.03 ^c	7.83±0.29 ^b	29.67±0.58 ^a
Final moisture content, %	5.47±0.71 ^a	4.82±0.60 ^a	4.41±0.81 ^a	4.36±0.70 ^a

Note: Different superscript letters in the same row indicate a significant difference ($p<0.05$). MVD, microwave vacuum drying; MD, microwave drying; VD, vacuum drying; FD, freeze drying.

Moisture content is essential for the quality control and stability of dried foods. Lower moisture content usually represents longer shelf life. As presented in Table 1, the final moisture content of beetroots obtained by four different drying methods ranged from $4.36 \pm 0.70\%$ to $5.47 \pm 0.71\%$. VD and FD beetroots had slightly lower final moisture content comparing to MVD and MD, and there was no significant difference in final moisture content among all the beetroots prepared by different drying methods.

Effect of different drying methods on the bioactive compounds of beetroots

The influence of different drying methods on betalain content of dried beetroots is displayed in Figure 1. As can be seen, the betacyanin content of dried beetroots obtained by four different drying methods ranged from 2.98 ± 0.02 to 4.04 ± 0.05 mg/g. It was found that the beetroots obtained by MD showed the lowest betacyanin content (2.98 ± 0.02 mg/g). The greatest betacyanin content (4.04 ± 0.05 mg/g) of dried beetroots was observed in VD, and the beetroots prepared by VD showed no significant difference from that prepared by FD in betacyanin content ($p > 0.05$). As it can be seen from Figure 1, the betaxanthin content of dried beetroots prepared by four different drying methods ranged from 2.40 ± 0.02 to 2.57 ± 0.01 mg/g, and the greatest betaxanthin content (2.57 ± 0.01 mg/g) of dried beetroots was obtained from FD. No significant difference was observed in MVD and MD ($p > 0.05$).

Figure 1. Betalain content of dried beetroots as affected by different drying methods. MVD, microwave vacuum drying; MD, microwave drying; VD, vacuum drying; FD, freeze drying. Means with different letters in each column indicate significantly different ($p < 0.05$).

Ascorbic acid is relatively unstable to heat, oxygen, and light, and easily is degraded during drying process, so it can be used as an indicator for the quality of dried products (Hu et al., 2006). The higher the temperature, the longer the drying time, and the more degraded ascorbic acid in dried fruits and vegetables (Cui et al., 2004). The effect of different drying methods on the ascorbic acid content is provided in Figure 2. The results showed that the beetroots prepared by VD displayed the highest ascorbic acid content of 8.73 ± 0.23 mg/g, followed by MD, FD, and MVD beetroots of 8.32 ± 0.10 , 7.44 ± 0.06 , and 7.17 ± 0.09 mg/g, respectively. MVD and FD beetroots had relatively lower ascorbic acid content than that of MD and VD beetroots, and there was no significant difference between MVD and FD ($p > 0.05$). This can be explained that microwave vacuum drying caused some higher temperature areas on the surface of the beetroots, resulting in loss of the heat-sensitive ascorbic acid (Wei et al., 2019).

Figure 2. Ascorbic acid content of dried beetroots as affected by different drying

Figure 3 shows the total phenolic content of dried beetroots obtained by different drying methods. The highest total phenolic content was observed in VD (10.23 ± 0.08 mg GAE/g) beetroots, followed by MVD (10.20 ± 0.08 mg GAE/g), MD (9.31 ± 0.14 mg GAE/g), whereas FD (8.01 ± 0.11 mg GAE/g) beetroots showed the lowest content. Similar results have been reported by other researchers. It was reported that freeze drying of willow led to a lower total phenolic content than thermal drying methods such as hot air drying, oven drying and tray drying (Harbourne et al., 2009). It was found that freeze drying of raspberry fruits much lower polyphenol retention compared with other drying methods such as hot air drying, infrared radiation drying, and hot air combined explosion puffing drying (Si et al., 2016). The use of low-temperature dehydration led to more serious degradation of polyphenols, which was understandable from the long drying time of 29.67 ± 0.58 h. VD and MVD led to more higher total phenolic content, which can be explained that higher temperature drying caused more damage to the tissue, so that more phenolic compounds to be extracted or caused changes in other compounds, thereby producing more total phenols (Si et al., 2016). There was no significant difference between MVD and VD ($p > 0.05$).

Figure 3. Total phenolic content of dried beetroots as affected by different drying methods

Effect of different drying methods on the antioxidant activities of beetroots

Beetroot contains a variety of phytochemicals with high antioxidant activity. At least two methods should be employed to evaluate the total antioxidant capacity due to different mechanisms of antioxidants, such as reducing capacity, decomposition of peroxides, free radical scavenging, and binding of transition-metal ion catalysts (Mao et al., 2006; Inchuen et al., 2010). In this study, the antioxidant activity of dried beetroots was evaluated by three methods: DPPH radical scavenging activity, ferric reducing antioxidant power (FRAP), and ABTS radical scavenging activity.

The DPPH radical scavenging activity of dried beetroots is presented in Figure 4. The beetroots prepared by FD were found to show the weakest DPPH radical scavenging activity (5.16 ± 0.02 mg TE/g). The beetroots prepared by MD and MVD showed significantly ($p < 0.05$) higher DPPH radical scavenging activity than the beetroots prepared by VD and FD.

The ABTS radical scavenging activity of dried beetroots is illustrated in Figure 5. Among the dried beetroots, MD beetroots showed the highest ABTS radical scavenging activity (17.22 ± 0.35 mg TE/g), followed by MVD, VD and FD. The result was in agreement with the results reported by Nistor et al., 2017 showed that betacyanin degradation leads to other phenolic compounds, thereby increasing antioxidant activity. The beetroots prepared by MD showed the lowest betacyanin content (Figure 1), while its ABTS radical scavenging activity was the highest. Compared to other drying methods, FD led to the lowest ABTS radical scavenging activity. It has been reported that the ABTS radical scavenging ability of freeze-dried sour cherries was significantly lower than that of vacuum microwave-dried sour cherries under several drying conditions (Wojdyło et al., 2014).

Figure 4. Effect of drying methods on the DPPH radical scavenging activity of dried beetroots

Figure 5. Effect of drying methods on the ABTS radical scavenging activity of dried beetroots

The FRAP values of dried beetroots can be seen in Figure 6. Drying methods caused the degradation of bioactive compounds that were responsible for FRAP in beetroots to different degrees. Similar to DPPH radical scavenging activity and ABTS radical scavenging activity, the beetroots obtained by FD showed the lowest FRAP value (9.89 ± 0.13 mg TE/g).

Figure 6. Effect of drying methods on the FRAP of dried beetroots

Through the antioxidant activity data, it was found that FD did not exhibit any advantages in the antioxidant activity of beetroots in comparison with the other drying methods. This result was in agreement with results published by Si et al., 2016, but contradicted that of Chan et al., 2009. Chan et al. reported that sun drying, oven drying, and microwave drying, caused more serious losses of antioxidant capacity than freeze drying. The change of antioxidant activity can be attributed to a variety of reasons. Firstly, during the thermal process, intense and/or long-term heat treatment may lead to the loss of natural antioxidants, most of which are relatively unstable (Lim et al., 2007). Moreover, degradation caused by enzymes or heating leads to the loss of antioxidant ability. In addition, the intermediate products of degradation and Maillard reaction during heating process can enhance the antioxidant capacity (Cheigh et al., 1995). The enzymatic and non-enzymatic browning reactions during drying process may also result in antioxidative characteristics (Samoticha et al., 2016).

Conclusions

The drying time, bioactive compounds and antioxidant activity of beetroots were greatly affected by drying methods. Among the four different drying methods, FD led to the longest drying time, while MVD required the shortest drying time of 0.56 ± 0.01 h. There was no

significant difference in final moisture content of beetroots among all drying methods. In terms of bioactive compounds, the beetroots dried by FD displayed the highest betaxanthin content, while the beetroots obtained by MD showed the lowest betacyanin content. Besides, the beetroots prepared by VD showed the highest ascorbic acid content of 8.73 ± 0.23 mg/g. Meanwhile, FD led to significantly lower total phenolic content of beetroots in comparison with other drying methods. For antioxidant activity, the beetroots prepared by MD and MVD showed significantly higher DPPH radical scavenging activity than the beetroots prepared by VD and FD. Besides, the beetroots prepared by VD exhibited the largest ABTS radical scavenging activity. Moreover, the beetroots obtained by MVD exhibited the highest FRAP value. It is noteworthy that the antioxidant activity of beetroots prepared by FD was the weakest, with the lowest values of FRAP, DPPH and ABTS. MVD led to higher antioxidant activity of beetroots in comparison with other drying methods. Comprehensive consideration of the drying time, bioactive compounds and antioxidant activity of beetroots, MVD is a suitable method for preparing dried beetroots. Dried beetroots with a large number of bioactive compounds and antioxidant activity can be utilized as food additives, functional foods and high value-added food products.

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