Effect of xylose on the biological activity, physical property and antioxidant capacity of dried *Aronia melanocarpa*

**Wanting Sun**¹,², **Sai Wang**¹,², **Jun Zhang**³, **Guihua Sheng**¹,², **Meng Wang**¹,², **Xuanhong Chen**¹,², **Leichao Dong**¹,², **Quancheng Zhou**¹,²,*

¹Department of Food Science, School of Agricultural Engineering and Food Science, Shandong University of Technology, Zibo, 255049, China
²Key Laboratory of Shandong Provincial Universities for Technologies Agricultural Products, Zibo, 255049, China
³Zibo Tieshan Forest Farm Centre, Zibo, 255000, China

*Corresponding author e-mail: zhouquancheng@sdut.edu.cn

**Abstract.** This research investigated effect of xylose on drying *Aronia melanocarpa* using response surface methodology (RSM) to produce the optimal drying conditions for high bioactive compounds. The conditions were drying temperature 58.70°C, drying time 8 h 48 min, and xylose solution concentration 50.70%. Subsequently, effects of drying conditions on physical properties of dried *Aronia melanocarpa* (DAM) were evaluated in Scanning electron microscope (SEM), Fourier transform infrared spectrum (FTIR), X-ray diffraction (XRD) and Different scanning calorimetry (DSC). The physical properties showed that DAM with xylose changed from amorphous to crystalline during drying process, which would extend the shelf life of DAM. Besides that, the physical properties results also showed that a Maillard reaction occurred during drying, which explained the improved antioxidant capacity of DAM with xylose. The results showed that xylose could reduce spoilage, prolong shelf-life of DAM, and indicated its use as a potential functional ingredients in the food industry.

**1. Introduction**

Nowadays, extensive studies are conducted to berries due to the growing interest in health foods. *Aronia melanocarpa* (AM), common chokeberries, is one of the most abundant sources of polyphenols in fruits, which contains flavonoids, anthocyanins, caffeic acid derivatives and other compounds [1]. In view of these, AM benefits are mainly attributed to strong antioxidant activity and anti-inflammations [2], cardiovascular disease [3] and antiviral [4] as well as effective anti-cancer drug [5]. Due to it can adjust to the cold weather and humid soils, it grows not only in Russia and Baltic countries, but also in Poland and Germany [6]. Most products are in the form of fresh, dried fruits, or juice [7].

Xylose is a five-carbon sugar, which is the second most abundant sugar after glucose [8-9]. Compared with other functional sugar, xylose can’t provide heat for human body or increase blood sugar value, and has special function of increasing intestinal bifid bacteria, which can adjust intestinal tract and improve human microbial environment. However, xylose, which is pre-active biological components, has not been effectively exploited in the drying industry. In the process of food drying, adding a small
amount of xylose not only increases the flavor and aroma of the food, but also occurs a Maillard reaction between xylose and amino acids under heating conditions, which can inhibit lipid oxidation. Therefore, xylose has excellent functions and high added value, which can be used as an important food additive in the field of food and medicine with broad prospects.

Due to the high moisture content of fresh fruits, the seasonality is strong, and it is not resistant to storage. Hence, if it is not treated in time, it will be perishable. Adding substance in drying process has proven to be a good alternative to prolong the shelf life and reduce transportation costs. Up to now, hot air drying is the most widely used drying method, with the advantages of low production cost, high flexibility and high output [10, 11] However, compared with microwave drying and freeze drying, this drying method has a great damage to product quality, such as dried blueberry with the lowest polyphenol content and the least antioxidant capacity [12]. So pre-treatment was often used to reduce the loss of berry during hot air drying [13, 14]. The preparation of xylose-added DAM (Add-X) by hot air drying, and research on the bioactive components, physical properties and antioxidant capacity of DAM were rarely reported.

In this paper, the research investigated the optimization of crucial hot air drying process parameters using Response Surface Methodology (RSM) with the expectation to maintain the bioactive components. Later on, in order to study whether the DAM with xylose had improved biological and storage capacity. Physical properties, antioxidant capacity of DAM were characterized using Scanning electron microscope (SEM), Fourier transform infrared spectrum (FTIR), X-ray diffraction (XRD), Different scanning calorimeter (DSC), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, O2· radical scavenging capacity, respectively. The findings provide a new thought for reducing the biological activity loss of AM during hot air drying, extending DAM shelf life, and increasing the value of xylose utilization.

2. Materials and methods

2.1. Sample preparation
AM berries were supplied by the ZiBo Tieshan Forestry Centre in August 2018 and stored at -20℃ until used.

2.2. Chemicals and reagents
Gallic acid (98%), rutin (98%), 2, 2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu phenol reagent were supplied by Shanghai Yuanye Biological Technology. Co (Shanghai, China). Aluminium nitrate, sodium carbonate, sodium nitrite and the other reagents were all purchased from Yantai Shuangshuang Chemical Co., Ltd. (Yantai, China). Xylose, calcium carbonate, sodium sulfite and potassium chloride were all food-grade and purchased from Shanghai Aichun Biological Technology Co (Shanghai, China).

2.3. Sample preparation and processing
AM berries with the same color and size were carefully selected for fresh, no mechanical damage. Before each test, the berries were well washed and blanched for 2 min in order to conserve anthocyanins and polyphenols [15], and then soaked in the solution of 0.45% calcium carbonate for 12 h and 0.06% sodium sulfite for 2 h. Lastly, the berries were immersed in the xylose solution for 24 h (1 g sample/1 mL xylose solution) and dried in the electric thermostat drying oven.

2.4. Physical characteristics and biological activity of AM
The moisture content and ash content were determined according to the methods described in the literature [16, 17], respectively. Total polyphenol (TP) content was determined according to the Sharif’s method [18]. Total anthocyanins (TA) were determined using the pH-differential method [19]. Total flavonoids (TF) content was measured by Liu’ method [20]. All the experiments were repeated three measurements.
2.5. Response surface methodology
The optimization scheme was determined by RSM according to the Box-behnken design principle in order to optimize the drying conditions for retaining the nutrients of the AM. After preliminary tests, drying temperature A (50, 60, 70°C), drying time B (8, 9, 10 h), and xylose solution concentration C (40, 50, 60 %) were selected as important factors, and the total scores of TP, TA and TF in DAM were calculated according to a certain proportion. Additionally, validation tests were performed under optimal drying conditions to verify that the optimization test was feasible, repeatable and reliable. Actual and coded levels of response surface are shown in Table 1.

Table 1. Box-behnken design and experimental results of the total score of dried samples.

| Run | Independent Variables | Uncoded values | Dependent variables |
|-----|-----------------------|----------------|-------------------|
|     | Code values | A | B | C | A | B | C | Y   |
| 1   | -1 | -1 | 0 | 50 | 8 | 50 | 63.036 |
| 2   | -1 | 0 | 1 | 50 | 9 | 60 | 68.282 |
| 3   | -1 | 1 | 0 | 50 | 10 | 50 | 57.087 |
| 4   | -1 | 0 | -1 | 50 | 9 | 40 | 66.783 |
| 5   | 0 | 0 | 0 | 60 | 9 | 50 | 87.885 |
| 6   | 0 | 0 | 0 | 60 | 9 | 50 | 83.390 |
| 7   | 0 | 0 | -1 | 60 | 8 | 60 | 59.866 |
| 8   | 1 | 0 | 1 | 70 | 9 | 60 | 68.202 |
| 9   | 1 | 0 | -1 | 70 | 9 | 40 | 55.604 |
| 10  | 0 | 1 | 1 | 60 | 10 | 60 | 54.368 |
| 11  | 0 | 1 | -1 | 60 | 8 | 40 | 42.430 |
| 12  | 0 | -1 | -1 | 60 | 8 | 40 | 66.076 |
| 13  | 0 | 0 | 0 | 60 | 9 | 50 | 82.068 |
| 14  | 0 | 0 | 0 | 60 | 9 | 50 | 81.252 |
| 15  | 1 | 1 | 0 | 70 | 9 | 50 | 41.375 |
| 16  | 1 | 1 | -1 | 70 | 9 | 50 | 62.655 |
| 17  | 0 | 0 | 0 | 60 | 9 | 50 | 87.225 |

A Temperature (°C), B Time (H), C Xylose solution concentration (%), Y Total score (%)

2.6. Physical properties

2.6.1. Scanning electron microscope (SEM). The sample was stored in a dry box at 25°C for 48 h, and cut into pieces of 1 mm ×1 mm × 2 mm, and photographed at 100 μm, 50 μm scale.

2.6.2. Fourier transform infrared spectrum (FT-IR). Attenuated total reflectance FT-IR spectrum of dried sample (400-4000 cm⁻¹) was characterized by Nicolet 5700 spectrometer (Thermo Nicolet, USA).

2.6.3. X-ray diffraction (XRD). XRD patterns of the dried sample was obtained by a Bruker AXS D8 Advance X-ray diffract meter (Bruker Inc., Germany), at an X-ray tube voltage of 70 kV, current of 40 μA.

2.6.4. Different scanning calorimetry (DSC). DSC was performed to characterize the structure and interactions between polymers, using the DSC Q100 (TA Instruments, USA) for determination. 7 mg dry powder was put in a sealed aluminum pan. In this programme, the sample was cooled to -65°C at 20°C/min and kept constant for 2 min, then heated to 200°C with the rate of 10°C/min, in inter atmosphere of N₂[10].
2.7. Antioxidant capacity

2.7.1. \( \cdot \text{O}^2 \)-radical scavenging capacity. \( \cdot \text{O}^2 \) free radical scavenging activity of the Add-X and DAM without xylose (N-X) were evaluated by a modified method of Xuan’s [21].

2.7.2. DPPH radical scavenging capacity. The ability of dried samples to scavenge DPPH radical were determined by the method of Teng’s [22].

2.8. Statistical analysis
All measurements were performed in triplicate and the results were presented with at least three replicates. Taking the total score of bioactive components of dried samples as an indicator, set the total score as 100%, each bioactive ingredient contributes one-third of each: Total score (Y) = (TP/TPmax + TA/TAmax + TF/TFmax)×1/3.

3. Results and discussion

3.1. Moisture content and ash content
The moisture content and ash content of AM were 79.60±0.28% and 3.10±0.42%, respectively. The fresh fruit moisture content of AM was up to 79.11%, which was consistent with the literature [6].

3.2. Response surface test analysis

3.2.1. Establishment of quadratic regression models. Using the Box-behnken design of Design-expert 8.0 software, the drying temperature, drying time and xylose solution concentration were used as the response variable, and the total score was the response value. The response surface experiment results were shown in Table 1. The quadratic regression models in correlation of total score with three variables are shown as follows:

\[
Y=84.36-3.42*A-7.05*B+2.48*C-3.83*A*B+2.77*A*C+4.54*B*C-9.65*A^2-18.68*B^2-10.00*C
\]

\( (R^2=0.9851, \text{RAdj}^2=0.9661) \)

It showed that the regression equation could explain 98.51% of the response surface change, and the regression simulation results were good.

3.2.2. Analysis of total score regression model. As shown in Fig. 1 (A), the graph showed the interaction of temperature and time on the total score. It had been found that heat treatment affected the level of phytochemical levels. Therefore, heat treatment should reduce the loss of active ingredients in the sample and increase bioavailability. The active ingredient in the DAM decreased with increasing drying time and drying temperature, which was consistent with results in literature [23]: the content of polyphenol, flavonoids and anthocyanins decreased with the prolongation of heating time at 100°C. The decrease in TA content might be due to the fact that the degradation rate of anthocyanins was directly related to the heat exposure time. Because a cyclic adduct was formed during heating, resulting in loss of hydroxyl [24]. While the drying temperature had less effect on the total score, compared with the drying time, and the elliptical shape of the contour map was obvious, indicating that the interaction between drying temperature and drying time was definite.
Figure 1. Response surface plot showing effects of temperature and time A; temperature and xylose solution B; time and xylose solution C on the total score of dried samples.

As shown in Fig. 1 (B), the concentration of xylose solution had less effect on the total score of DAM. The concentration of xylose solution increased at first and then decreased, and the interaction between xylose solution concentration and drying temperature was poor (p<0.05). As shown in Fig. 1 (C), the interaction between drying time and xylose solution concentration was obvious. Because flavonoids were degraded by heat during drying processes, so the longer the drying time, the lower the total score, and the longer the heat, the faster the degradation. The slope of the response surface was steep, indicating that the response value was sensitive to the change of operating conditions and had a great influence on the total score.

As shown in Table 2, the F value of the regression model was 51.60, P<0.0001, indicating that the model was extremely significant; the F-value of the missing term was 0.44, P=0.7350>0.05, indicating that the model was valid. Therefore, the above regression model could be used to analyze and predict the results of the effects of drying time, drying temperature, xylose solution concentration on the comprehensive bioactivity of dry samples. In the regression analysis of the regression equation, the F-value could judge the influence of the independent variable on the dependent variable. Therefore, the order of influence of each factor on the total score was B>A>C, that is to say, drying time > drying temperature > xylose solution concentration. The significant results of the total score regression model showed that the drying temperature primary term A and the xylose solution concentration C had
significant effects on the total score regression model (p<0.05), and the drying time primary term B and quadratic term B² were combined to the composite score regression. Model impact was extremely significant (p<0.01), the interaction terms AB and BC of drying time, drying temperature and xylose solution concentration all had significant effects, which was in agreement with Fig. 1 (A) and Fig. 1 (C).

Table 2. ANOVA analysis of the response surface quadratic for optimizing the total score of dried samples. A is drying Temperature, B is drying time, C is xylose solution concentration.

| Source  | Sum of Squares | df | Mean Square | F-Value | P-Value |
|---------|----------------|----|-------------|---------|---------|
| Model   | 3225.29        | 9  |             |         |         |
| A       | 93.51          | 1  | 358.37      | 51.80   | <0.001  |
| B       | 397.23         | 1  | 397.23      | 57.19   | 0.0001  |
| C       | 49.13          | 1  | 49.13       | 7.07    | 0.0325  |
| AB      | 58.75          | 1  | 58.75       | 8.46    | 0.0227  |
| AC      | 30.80          | 1  | 30.80       | 4.43    | 0.0732  |
| BC      | 82.34          | 1  | 82.34       | 11.85   | 0.0108  |
| A²      | 1469.15        | 1  | 1469.15     | 211.53  | <0.0001 |
| B²      | 391.81         | 1  | 391.81      | 56.41   | 0.001   |
| C²      | 421.05         | 1  | 421.05      | 60.62   | 0.0001  |
| Lack of Fit | 12.13 | 3 | 4.04 | 0.44 | 0.7350 |
| R²      | 0.985          |    |             |         |         |
| Total   | 3273.91        | 16 |             |         |         |

3.2.3. Model optimization and validation. According to the analysis of Design-Expert 8.0 in this system, the total score Y value was set to the maximum, so the optimal test parameters were obtained: drying temperature 58.70°C, drying time 8 h 48 min, xylose solution concentration 50.70%. The predicted value of the regression equation under this condition was 85.26%. The test was carried out on the predicted value, each experiment was carried out in triplicate, and the total score was 85.51%, which was more than the predicted score, suggesting that the models was suitable for optimizing and predicting the hot air drying process.

3.3. Physical properties

3.3.1. SEM analysis. The effects of Add-X and N-X on microstructure were observed by SEM images. Fig. 2. Showed SEM images of the surface of Add-X and N-X at 100 μm, 50 μm scale. After the hot air drying of the N-X, the shell had a rough surface and showed honeycomb-shaped. The pores of the inner pulp were connected to each other, deep and excessive, and the structure was destroyed, hence the texture of the AM were more damaged. Fig. 2 displayed that the xylose particles in Add-X adhered to the surface of the outer and inner pulp, and cross-linked with AD. Compared with the N-X, the Add-X volume became larger, and the pores were reduced.

3.3.2. FTIR analysis. FTIR spectroscopy was used for the analysis of intermolecular interactions in Add-X and N-X, which could be reflected in infrared spectra by location and intensity of characteristic absorption peaks as shown in Fig. 3 (A). The peaks noticed at 3420 cm⁻¹ was attributed to N-H stretching vibration of amide. Peaks observed in the region of 2924 cm⁻¹ were related to the stretching vibration of –CH₂ symmetrical stretching vibrations. The intense bands at 1736 cm⁻¹, 1622 cm⁻¹ were assigned to carbonyl functional group [25]. The band at ν = 1244.24 cm⁻¹ was attributed to ester group. Notably, some differences were observed between N-X and Add-X. Compared with N-X, Add-X had an absorption peak of the hydroxyl stretching vibration occurred at 3263.7 cm⁻¹, and the carbonyl functional group also appeared at 1622 cm⁻¹. The absorption bands at 1395.06, 1305.3, 1236.3, 1191.3, 1149.9, 1128.29 cm⁻¹ were the characteristic absorption peak of C=O and hydroxyl stretching vibration interaction, and the bands between 1175 and 1000 cm⁻¹ were typical of xylose [26]. The band at 672.7
cm$^{-1}$ and 634 cm$^{-1}$ indicating the hydroxyl out-of-plane bending vibration, which was attributed to phenol association. The emergence of new functional groups might contribute to a good compatibility between xylose and AM.

3.3.3. XRD analysis. XRD was an important tool to give useful information about the sample crystallinity. In general, since the amorphous material was disordered, the dispersion peak and the broad peak appearing in the X-ray diffraction spectrum represent an amorphous structure, thereby producing a dispersion band. However, crystalline materials produce sharp and defined peaks due to their well-ordered state [27]. The XRD patterns of N-X and Add-X were measured in Fig.3 (B). N-X showed amorphous materials. However, XRD patterns of Add-X exhibited strong diffraction peaks at 12.06, 17.12, 18.54, 19.92, 21.28, 23.26, 26 and 28.3 2θ angles, which confirmed the crystalline nature of Add-X under these conditions. A new diffraction peak appeared in the map, indicating that the chemical combination between the AM and the xylose during hot air drying produced a new substance with a crystal structure.

Figure 2. SEM images of surface and inside of N-X and Add-X ((A and B) N-X shell; (C and D) N-X inside; (E and F) Add-X shell; (G and H) Add-X inside).
The crystalline and amorphous state were also related to the storage stability. Generally, amorphous samples had more strong hygroscopicity than crystalline materials, and were easy to absorb water during storage [28]. So, the property affects sample storage, as water absorption could lead to weight gain, nutrient degradation, microstructural collapse, and potential microbial instability [29]. The sample after adding xylose changed state from amorphous to crystal, which was hard to absorb water for low water content, and improved the storage stability.

3.3.4. DSC analysis. Glass transition temperature (Tg) could be defined as the temperature, which was an amorphous system changes from the glassy to the rubber state [30]. Tg was related to stickiness, collapse, and crystallization during food processing and storage [31]. It was generally believed that high-sugar foods should be dried as much as possible below Tg to reduce stickiness and improve product quality [32]. Different parameters characteristic of glass transition such as Tg onset, Tg midpoint and Tg end were shown in Fig.3 (C). In this study, Add-X showed a high Tg than the N-X, which could lead to a better stability at room temperature. The possible cause was the addition of xylose produce a non-enzymatic browning reaction with AM during hot air drying.

The DSC thermo grams of N-X and Add-X were shown in Fig. 5. N-X and Add-X had a melting endothermic peak between 100-160°C, but the Add-X melting peak position was postponed, which the peak temperature was increased from 109.71°C to 152.36°C. This was due to the crystal structure of xylose was destroyed at this temperature [33]. It was indicated that Add-X had good thermal stability. The possible reason was that a Maillard reaction occurred between xylose and the amino in AM, forming a more stable complex, which lead to the extension of the Add-X melting endothermic peak. The thermal stability of macromolecules was related to the crystal structure. The substances with higher crystallinity had higher thermal stability and required more energy to destroy the crystal structure. Add-X might form a crystal structure during hot air drying, which results were in accordance with the XRD results.

3.4. Antioxidant capacity analysis

Figure 3. FTIR spectra (A), XRD (B), DSC (C) and Antioxidant capacity (D) of N-X and Add-X
Up to now, there were a few reports on adding xylose into AM. As shown in Fig. 3 (D), Add-X had greater O₂⁻ and DPPH radical scavenging activity than N-X. The Maillard reaction occurred between amino acids in the AM and xylose under high temperature conditions, resulting in browning pigments, which could remove most of O₂⁻, such as the browning pigment produced by xylose reacted with lysine [34]. DPPH radical scavenging ability of Add-X was significantly improved to 84.41%, which might be due to the formation of carbonyl species in the Maillard reaction. Furthermore, the physical property test also proved that the Maillard reaction occurred.

4. Conclusion
This article creatively added the functional food additive xylose to DAM, and explored the best bioactive preservation through RSM. The results demonstrated to be an effective optimization technique for modeling the parametric effects. A quadratic models was developed and validated to optimize the hot air drying conditions. Under optimal drying conditions, the highest bioactive components total score was 85.51%. This score indicated that the optimal conditions maintained the nutrients of the DAM. Based on physical properties, the addition of xylose changed structure from amorphous to crystal of the DAM, which showed better quality retention and shelf life than the N-X. Additionally, physical properties also indicated that the Add-X had a Maillard reaction during the hot air drying, which also explained that Add-X had higher antioxidant activity than N-X. These finding could be helpful for the development and utilization of xylose resources, and increased the nutritional value of the DAM, and could be applied to food preservation and extended the shelf life.

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