**Research Article**

**Optimization of technological parameters for osmotic dehydration of black chokeberry**

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**Abstract**

This Response surface methodology (RSM) was used to investigate the effect of the temperature of osmotic treatment (43, 50, 60, 70, 77°C), the concentration of osmotic solution (47, 50, 55, 60, 63°Brix) and fruit to solution ratio (1:2, 1:3, 1:4, 1:5, 1:6 w/w) on mass transfer (water loss, solid gain) and antioxidant activity during osmotic dehydration of black chokeberry. It was found that the temperature of osmotic treatment and the concentration of osmotic solution were the most significant factors affecting water loss (WL), followed by fruit to solution ratio. For solid gain (SG), the effect of temperature and concentration of osmotic solution were found to be more significant factors. The temperature of the osmotic treatment and fruit: solution ratio (linear and quadratic effect) had the highest impact on total antioxidant capacity (TAC). The increasing temperature resulted in decreased antioxidant activity. As a result of the applied RSM methodology, the osmotic dehydration process was optimized to achieve high values of the parameters water loss, solid gain, and total antioxidant capacity.

**Keywords:** black chokeberry, osmotic dehydration, mass transfer, anthocyanin content, optimization

**Abbreviations:**

WL - water loss
SG - solid gain
TAC - total antioxidant capacity
RSM - response surface methodology

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Introduction

Aronia, also known under the name chokeberry is a shrub that belongs to the Rosaceae family. This genus includes three species: Aronia melanocarpa [Michx.] (black-fruit), A. arbutifolia [L. Elliot] (red-fruit) and A. prunifolia [Marsh. Rehd] (purple-fruit) which are commercially cultivated in Eastern Europe, Russia, North America and East Canada. In Europe, black chokeberry grows to a height of 2-3 m and gives fruits of purplish black berries with a diameter of 6-13 mm and a weight of 0.5-2.0 g (Ara 2002; Seidemann 1993). Chokeberry fruits are characterized with high nutritional and biological value. They contain (in 100 g) carbohydrates (glucose and fructose, total up to 17.6 mg), organic acids (1.3 mg malic acid, 2.0 mg citric acid, 0.08 mg succinic acid), proteins (0.7 mg), pectins (0.34–0.58 mg), fibers (5.6 mg), ash (0.44–0.58 mg), vitamin C (up to 2.7 mg), etc. (Jeppsson and Johansson 2000; Lehmann 1990; Tanaka and Tanaka 2001). Fresh fruits are a good source of polyphenols including proanthocyanidins, anthocyanins and flavonols, vitamins B (B1, B2, B6, niacin, pantothenic acid, β-carotene (Benvenuti et al. 2004; Koponen et al. 2007; Kulling et al. 2008; Walther and Schnell 2009). In the last few decades Aronia fruits have gained popularity for the production of health-promoting juices, syrups, jams, dried and dietary foods rich in antioxidants. One method to produce dried fruits with good quality is to use a pre-drying treatment such as osmotic dehydration. The method is used for the partial removal of water from the plant tissues by immersion of the fruit in hypertonic water solution (Sereno et al. 2001). Osmotic dehydration involves three mass transfer processes: (1) water transfer from the plant cells to the osmotic solution, (2) migration of solutes from the solution into the fruit tissue and (3) excretion of soluble solids from the plant tissues to the osmotic solution. The third transfer is quantitatively negligible compared to the first two types of transfer but it is essential for the composition of the product (Akbarian et al. 2014, Spiazz and Mascalceroni 1997).

The characteristics of the osmotic dehydrated fruits are varied by controlling the temperature of the osmotic treatment, concentration of osmosis solution, product:solution ratio, time of osmosis, etc. to speed up osmotic concentration process (Phisut 2012; Tortoe 2010).

Temperature of the osmotic solution. The most important variable affecting the kinetics of mass transfer during osmotic dehydration is temperature (Tortoe 2010). Increasing the temperature of the osmotic solution results in increased rate of water removal and sugar uptake (Phisut 2012). Diffusion of flavour and odour compounds from the fruits to the solution is also increased at high temperature. According to the literature on osmotic treatment, temperatures around 50°C have been used for fruits and vegetables for the following reasons: 1) deterioration of flavour, texture, and thermo-sensitive compounds of the plant materials is limited at this temperature, 2) enzymatic browning and flavour deterioration of fruits start at 49°C, and 3) this temperature has proven efficient to maintain the viscosity of the solution and to ensure adequate infusion time without deterioration of fruit quality (Khan 2012; Shi and Xue 2009).

Type and concentration of the osmotic agent. Phisut (2012) reported that osmotic agents with low molecular weight penetrate easier into the fruit cells compared to high molecular weight osmotic agents. The most commonly used osmotic agents are sucrose, glucose, fructose, sorbitol, glycerol, corn syrup and fructo-oligosaccharides. During osmotic dehydration, the increase of solute concentration results in higher water loss and solid gain rates (Phisut 2012). The increased osmotic solution concentration results in higher water loss and weight reduction and increased drying rate (Conway et al. 1983; Marcotte et al. 1991; Tortoe 2010).

Fruit: osmotic solution ratio. The increase of fruit sample: osmotic solution ratio results in higher osmosis rate up to a certain level. However, it is essential to use an optimum ratio...
since large ratios offer practical difficulties in handling the processing of osmotic solution and fruit mixture. A ratio of 1:2 or 1:4 sample:osmotic solution is reported as optimal for practical purposes (Tiwari and Jalali 2004). The major advantages of osmotic dehydration process in the food industry include quality aspects (improvement of colour, flavour and texture), product stability and retention of nutrients during storage, energy efficiency, packaging and distribution cost reduction, chemical treatment not required, product stability during storage (Raoult-Wack 1994). The aim of the present study was to explore the influence of process parameters on mass transfer processes during osmotic dehydration of black chokeberry by using a non-traditional osmotic solution from fruit concentrates and inulin. The effects of process parameters on the antioxidant capacity of the fruits was also studied.

Materials and Methods

Raw materials. Black chokeberry (Aronia melanocarpa) fruits with dry matter of 24-27% were supplied by the Agricultural and stockbreeding experimental station, Bulgaria. The fresh fruits were sorted and stored in a refrigerator at 4-6°C until used.

Osmotic agents: concentrated sour cherry juice, concentrated apple juice, and inulin (oligofructose - 87%, sum of sucrose, glucose, and fructose - 12% and average degree of polymerization - 8) were purchased from Krichimfrukt Ltd. (Bulgaria), Agrobiotech Ltd. (Bulgaria), and Food consulting Ltd. (Bulgaria), respectively. The containers of concentrated sour cherry juice and concentrated apple juice were stored in at 4-6°C until used.

Chemicals: the following reagents were used: DPPH [2, 2-diphenyl-1-picrylhydrazyl] (Sigma-Aldrich, Steinheim, Germany); Trolox [(+/-)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid] (Sigma-Aldrich, Steinheim, Germany); methanol (Sigma-Aldrich, Steinheim, Germany)

Sample preparation and osmotic process. Black chokeberry was washed with tap water and cuts were made in the surface tissue. Osmotic solutions were prepared in five concentrations (47, 50, 55, 60 and 63°Brix) (Table 1) using concentrated sour cherry juice with 63°Brix (60% w: w), concentrated apple juice with 72°Brix (20% w:w), and inulin (20% w:w). Concentration of the osmotic solutions was monitored by Abbe refractometer (VEB Carl Zeiss JENA, Germany). Osmotic dehydration of fruits was performed in a water bath (VEB MLW Prüfgeräte Werk, Medingen, Sitz Freital, Germany) to achieve the necessary solution temperature according to the experimental design (Table 1). The choice of the preferred process conditions was based on literature on osmotic dehydration (Marcotte et al. 1991; Ferradji et al. 2015). The berries were dipped in osmotic solution of a specified concentration for 3 hours. The fruit:solution ratio was 1:2, 1:3, 1:4, 1:5, and 1:6 (w:w) according to the experimental design (Table 1). Further, the fruits were removed from the solutions, quickly rinsed with hot water and gently dried with paper towel to remove surface solution.

Calculation of basic mass transfer indicators (WL, SG) for the osmotic process. Moisture content, total dry matter and drained weight (final sample weight) of the fresh and osmotic dehydrated fruits were determined according to BDS EN 12143:2000 and BDS EN 12145:2000. Water loss (WL) and solid gain (SG) were calculated according to the method of Panagiotou et al. (1999):

\[
WL = \frac{x_0^{ST} M_f^0 - x_f^{ST} M_f^0}{M_0^0} \times 100, \% \tag{1}
\]

\[
SG = \frac{x_0^{ST} M_f^0 - M_0}{M_0^0} \times 100, \% \tag{2}
\]

Where: \(M_0^0\) - initial sample weight (g), \(M_f^0\) - final sample weight (g), \(x_0^{ST}\) - initial dry matter (%), \(x_f^{ST}\) - final dry matter.
Further, the fresh and osmotically dehydrated berries were analyzed for total antioxidant capacity (TAC).

**Total antioxidant capacity.** Extract preparation: Osmotic dehydrated black chokeberries (5.00 g) were mixed with acidified methanol HCl (1000 ml MeOH with 2.3 ml c. HCl) in 50 ml volumetric flask. After 12 hours at 4-6°C, the extracts were filtered through filter paper and transferred into flasks.

DPPH (TAC) assay: The ability of the fruit extract to react with free radicals (scavenger against DPPH•) was estimated by the colorimetric method of Brand-Williams et al. (1995), with some modifications - Dinkova et al. (2013). An amount of 2250 μl of DPPH - ethanol solution (2.4 mg DPPH in 100 cm³ ethanol) was mixed with 250 μL of extract. The samples were incubated at room temperature without light exposure. The change in absorbance after 15 minutes was measured at 515 nm by spectrophotometer (UV-Vis Thermo Fisher Scientific, Madison, WI, USA). The standard curve for the method was obtained with ethanol solution of Trolox in concentration range between 100 and 1000 μmol per 100 ml. Total antioxidant capacity (TAC) was expressed as μmol Trolox equivalent per 100 g of samples on dry weight basis (dw). All analyses were performed in triplicate (n = 3).

**Experimental design and data analysis.** Central composite rotatable design (CCRD) was used to optimize the osmotic dehydration of black chokeberry by response surface methodology with three variables at five levels. The actual factor values were selected based on the literature review. Independent variable values and the corresponding levels are presented in Table 1. The complete design consisted of 17 experimental runs with three replications of the center point. Experimental runs were carried out in random order. The generalized second-order polynomial model used in the response surface analysis was the following:

\[ Y = b_0 + \sum_{i=1}^{n} b_i x_i + \sum_{i=1}^{n} b_{ii} x_i^2 + \sum_{i=1}^{n} \sum_{j=1}^{n} b_{ij} x_i x_j \]  

Where: \( y \) is the dependent variable (response), \( \beta_0, \beta_i, \beta_{ii}, \) and \( \beta_{ij} \) are the regression coefficients for intercept, linear, quadratic, and interaction terms. SYSTAT statistical software (SPSS Inc., Chicago, USA, version 7.1) and Excel were used to analyse the data.

**Results and Discussion**

**Effect of osmotic dehydration parameters on the dependent variables (WL, SG, TAC).** Results of the experimental design for WL, SG and TAC of black chokeberries subjected to different osmotic dehydration conditions are presented in Table 1. The average values for WL varied from 11.93 to 41.58% and SG values varied from 5.31 to 11.49%, respectively. Total antioxidant capacities varied from 20 972 to 58 256 μmol/TE 100g (dw). Statistical analysis of variance (ANOVA) for WL and SG showed that four (temperature of the osmotic treatment, linear and quadratic effect of the concentration of the osmotic solution and fruit to solution ratio) and three effects (temperature of the osmotic treatment, concentration of the osmotic solution and quadratic effect of the fruit to solution ratio), respectively, have p-values less than 0.05.
Table 1. Central composite rotatable design in coded form and natural units of independent variables and experimental data for WL, SG, and TAC

| Variant of osmotic dehydration № | Osmotic treatment temperature X₁ (°C) | Solution concentration X₂ (°Brix) | Fruit / solution ratio X₃ (w/w) | Water loss (WL), % | Solids gain (SG), % | Total antioxidant capacity (TAC), µmol/TE 100 g dw |
|---------------------------------|----------------------------------------|-----------------------------------|-------------------------------|------------------|------------------|----------------------------------|
| 1                               | 50 (-)                                  | 50 (-)                            | 1:3 (-)                       | 11.93            | 7.73             | 58256.5                          |
| 2                               | 70 (+)                                  | 50 (-)                            | 1:3 (-)                       | 20.55            | 8.93             | 46307.8                          |
| 3                               | 50 (-)                                  | 60 (+)                            | 1:3 (-)                       | 17.27            | 9.03             | 40582.3                          |
| 4                               | 70 (+)                                  | 60 (+)                            | 1:3 (-)                       | 28.88            | 10.13            | 36837.3                          |
| 5                               | 50 (-)                                  | 50 (-)                            | 1:5 (+)                       | 20.63            | 5.31             | 47786.2                          |
| 6                               | 70 (+)                                  | 50 (-)                            | 1:5 (+)                       | 28.25            | 8.93             | 35045.9                          |
| 7                               | 50 (-)                                  | 60 (+)                            | 1:5 (+)                       | 34.97            | 6.86             | 35991.6                          |
| 8                               | 70 (+)                                  | 60 (+)                            | 1:5 (+)                       | 41.58            | 10.35            | 29675.3                          |
| 9                               | 43 (-1.68)                              | 55 (0)                            | 1:4 (0)                       | 15.30            | 6.96             | 55226.2                          |
| 10                              | 77 (+1.68)                              | 55 (0)                            | 1:4 (0)                       | 28.22            | 10.03            | 20972.7                          |
| 11                              | 60 (0)                                  | 47 (-1.68)                       | 1:4 (0)                       | 15.35            | 6.78             | 35060.8                          |
| 12                              | 60 (0)                                  | 63 (+1.68)                       | 1:4 (0)                       | 38.17            | 9.78             | 32639.0                          |
| 13                              | 60 (0)                                  | 55 (0)                            | 1:2 (-1.68)                   | 16.64            | 11.04            | 52367.6                          |
| 14                              | 60 (0)                                  | 55 (0)                            | 1:6 (+1.68)                   | 18.87            | 11.49            | 24928.8                          |
| 15                              | 60 (0)                                  | 55 (0)                            | 1:4 (0)                       | 17.47            | 8.63             | 26877.0                          |
| 16                              | 60 (0)                                  | 55 (0)                            | 1:4 (0)                       | 16.87            | 8.87             | 27666.9                          |
| 17                              | 60 (0)                                  | 55 (0)                            | 1:4 (0)                       | 17.11            | 8.67             | 26725.1                          |

Table 2. Regression coefficients and analysis of variance for water loss (WL) of black chokeberry fruits

| Regression coefficient | Sum of Squares | F – value | P – value |
|------------------------|----------------|-----------|-----------|
| Constant               | 460.836        |           |           |
| A:X₁                   | -2.357         | 241.266   | 35.15     | 0.0006*   |
| B:X₂                   | -14.137        | 157.515   | 22.95     | 0.0020*   |
| C:X₃                   | -5.661         | 67.167    | 9.78      | 0.0167*   |
| AA                     | 0.017          | 31.494    | 4.59      | 0.0694    |
| AB                     | 0.016          | 4.977     | 0.73      | 0.4227    |
| AC                     | -0.024         | 0.466     | 0.07      | 0.8020    |
| BB                     | 0.127          | 114.333   | 16.66     | 0.0047*   |
| BC                     | -0.036         | 0.256     | 0.04      | 0.8525    |
| CC                     | 1.412          | 22.462    | 3.27      | 0.1134    |

*Significant at P < 0.05
indicating that they are significantly different from zero at the 95.0% confidence level - Table 2 and 3. Results from statistical analysis (ANOVA) for TAC show that four effects (linear and quadratic effects of the temperature of the osmotic treatment and linear and quadratic effects of fruit to solution ratio) have p-values less than 0.05, indicating that they are significantly different from zero at the 95.0% confidence level (Table 4). For the indicators WL, SG and TAC, the R-squared are 0.93, 0.89 and 0.86, respectively.

Based on the results from ANOVA, the following regression equations were obtained after removing the insignificant effects:

WL = 460.84 – 2.36X₁ – 14.14X₂ – 5.66X₃ + 0.13X₂², %

SG = 34.88 + 0.25X₁ + 1.78X₂ + 0.68X₃², %

TAC = 937828 – 8216.6X₁ – 56563.5X₃ + 47.5X₁² + 4941.6X₃², µmol/TE 100g

Each of the estimated effects of regression model coefficient on dependent variables (WL, SG and TAC) and interactions are shown in the standardized Pareto charts (Fig. 1).

Our research shows that linear effect of temperature of the osmotic treatment and concentration of the osmotic solution had the highest impact on WL and SG. Fruit to solution ratio also had a significant effect on the two analyzed indicators (Fig. 1a and 1c). The increase in temperature, solution concentration and fruit to solution ratio resulted in higher water loss (Fig. 1a) and solids gain (Fig. 1b) values throughout the osmosis period. Increasing WL and SG by increasing temperature could be attributed to the effect of
Table 3. Regression coefficients and analysis of variance for solids gains (SG) of black chokeberry fruits

|          | Regression coefficient | Sum of Squares | F – value | P – value |
|----------|------------------------|----------------|-----------|-----------|
| Constant | -34.879                |                |           |           |
| A:X1     | 0.255                  | 15.551         | 21.89     | 0.0023*   |
| B:X2     | 1.784                  | 8.097          | 11.39     | 0.0118*   |
| C:X3     | -9.974                 | 0.956          | 1.35      | 0.2841    |
| AA       | -0.003                 | 0.997          | 1.40      | 0.2749    |
| AB       | -0.001                 | 0.007          | 0.01      | 0.9259    |
| AC       | 0.060                  | 2.892          | 4.07      | 0.0834    |
| BB       | -0.015                 | 1.571          | 2.21      | 0.1806    |
| BC       | 0.012                  | 0.028          | 0.04      | 0.8493    |
| CC       | 0.682                  | 5.243          | 7.38      | 0.0299*   |

*Significant at P < 0.05

Table 4. Regression coefficients and analysis of variance for total antioxidant capacity (TAC) of black chokeberry fruits

|          | Regression coefficient | Sum of Squares | F – value | P – value |
|----------|------------------------|----------------|-----------|-----------|
| Constant | 937828.0               |                |           |           |
| A:X1     | -8216.57               | 6.25E8         | 15.05     | 0.0061*   |
| B:X2     | -18179.2               | 1.71E8         | 4.13      | 0.0816    |
| C:X3     | -56563.5               | 4.64E8         | 11.19     | 0.0123*   |
| AA       | 47.4761                | 2.54E8         | 6.12      | 0.0425*   |
| AB       | 36.5692                | 2.67E7         | 0.64      | 0.4484    |
| AC       | -42.0362               | 1.41E6         | 0.03      | 0.8588    |
| BB       | 129.805                | 1.19E8         | 2.86      | 0.1346    |
| BC       | 249.488                | 1.24E7         | 0.30      | 0.6009    |
| CC       | 4941.63                | 2.75E8         | 6.64      | 0.0367*   |

*Significant at P < 0.05

Temperature on the membrane permeability by making it more permeable to water and sugar exchanges. On the other hand, by increasing the concentration of the osmotic solution and the fruit to solution ratio, osmotic pressure on the fruit tissue was increased, which led to higher water loss and solid gain. Similar results were reported by Khatir et al. (2013), Mizkahi et al. (2001), Telis et al. (2004) and others.

Fig. 1c shows that temperature of the osmotic treatment had the highest impact on TAC, followed by the fruit:solution ratio. The increasing temperature resulted in decreased antioxidant activity in the osmotic dehydrated chokeberries. This is explained by the thermal effects.
instability of the major antioxidant components in the chokeberry fruits – anthocyanins such as cyanidin 3-galactose and cyanidin 3-arabinoside. Similar conclusions were reported by Biglari (2008), Saxena et al. (2012) and Kucner et al. (2013) for the osmotic dehydration of blackberries in sugar syrup. The increasing fruit to solution ratio had a negative effect on the total antioxidant capacity of the osmotically dehydrated chokeberries. This is explained by the excretion of antioxidant components from the plant tissues into the osmotic solution.

Determination of the optimal conditions for osmotic dehydration of black chokeberry fruits

The criterion established to determine the optimal osmotic dehydration conditions for black chokeberry was to find the conditions to achieve high values of water loss (WL>25%), solid gain (>8%), and total antioxidant capacity (TAC > 27000 µmol/TE 100g). Optimization was carried out by the superposition of several contour surfaces of competing responses. The optimized parameters for osmotic dehydration of *aronia* with an osmotic solution from concentrated sour cherry juice, concentrated apple juice and inulin are as follows: concentration of osmotic solution – 55°Brix to or greater, temperature within the range of 50-55°C, and fruit to solution ratio 1:4 to or greater. The applied osmotic solution from fruit concentrates and inulin could be further used as an ingredient of other foods.

**Conclusions**

Results from the present study showed that the temperature of the osmotic treatment and concentration of osmotic solution, followed by fruit to solution ratio, had significant effect on the mass transfer (water loss, solid gain) during osmotic treatment. The increase of the temperature of osmotic treatment and fruit: solution ratio resulted in degradation of the biologically active components and, respectively, in decreased total antioxidant capacity. The criterion established to determine the optimal conditions for osmotic dehydration of black chokeberry was to find the conditions leading to high values of water loss (WL>25%), solid gain (>8%), and total antioxidant capacity (TAC>27000 µmol/TE 100g). Optimization was carried out by the superposition of several contour surfaces of competing responses. The optimized parameters for osmotic dehydration of *aronia* with an osmotic solution from concentrated sour cherry juice, concentrated apple juice and inulin are as follows: concentration of osmotic solution – 55°Brix to or greater, temperature within the range of 50-55°C, and fruit to solution ratio 1:4 to or greater. The applied osmotic solution from fruit concentrates and inulin could be further used as an ingredient of other foods.
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