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Steam-explosion-modified optimization of soluble dietary fiber extraction from apple pomace using response surface methodology

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**ABSTRACT**

To make full use of apple pomace, pretreatment with steam explosion (SE) was investigated to determine its effects on the yield and functionality of soluble dietary fiber (SDF). Response surface methodology (RSM) was used to optimize the effects of SE parameters. According to the quadratic polynomial model, the optimum SE parameters were as follows: steam pressure, 0.51 MPa; residence time, 168 s; and sieving mesh size, 60. Under the optimized conditions, the SDF yield from apple pomace after SE was 29.85%, which is 4.76 times the yield of SDF (6.27%) in untreated apple pomace. The SDF prepared with SE also exhibited higher functionality values. Scanning electron micrographs (SEM) indicated that the SDF surface from apple pomace was poriferous, rough and lax after SE treatment; therefore, it can be concluded that the SDF morphology was altered by the steam explosion.

**OPTIMIZACIÓN DE LA EXTRACCIÓN DE FIBRA DIETÉTICA soluble de la pulpa de manzana modificada por explosión de vapor y usando la metodología de superficies de respuesta**

**RESUMEN**

Con el fin de aprovechar plenamente la pulpa de manzana, se investigaron los efectos que su tratamiento previo con explosión de vapor (EV) tiene sobre el rendimiento y la funcionalidad de la fibra dietética soluble (FDS). Asimismo, a fin de optimizar los efectos de los parámetros de EV, se usó la metodología de superficies de respuesta (MSR). El modelo del polinomio cuadrático permitió establecer los parámetros óptimos de EV: presión del vapor, 0.51 MPa; tiempo de residencia, 168 s; y medida de la malla de criba, 60. Bajo estas condiciones óptimas, el rendimiento de la FDS extraída de la pulpa de manzana tras ser sometida a EV fue de 29.85%, es decir, 4.76 veces superior al rendimiento de la FDS extraída de la pulpa de manzana sin tratar (6.27%). Por otra parte, la FDS tratada con EV también presentó valores de funcionalidad más elevados. La micrografía por barrido electrónico (MBE) indicó que, tras el tratamiento con EV, la superficie de la FDS extraída de la pulpa de manzana era porosa, áspera y laxa, lo que permite concluir que la morfología de la FDS fue alterada por el tratamiento con explosión de vapor.

**1. Introduction**

Dietary fiber, the indigestible portion of plants, are non-starch polysaccharides that can be divided into soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) (Zhang & Wang, 2013). The consumption of dietary fiber can prevent many human diseases, such as coronary heart disease, colon cancer, obesity and diabetes when added to food products (Esposito et al., 2005; Kosmala et al., 2013). Dietary fiber has also been an important raw material for health foods to provide lower levels of calories and cholesterol (Macagnan et al., 2015). China is the world’s largest producer of apples, a large proportion of which are used to produce apple juice. Apple pomace is the primary by-product of this process, but with microbial decomposition, it can easily pollute the environment (Ramful, Tarnus, Aruoma, Bourdon, & Bahorun, 2011). Previous research has found that apple pomace contains more than 70% dietary fiber, so its extraction not only makes full use of this apple by-product but can also add value to other food products and thereby increase economic benefits (Fabek, Messerschmidt, Brulport, & Goff, 2014). Of these advantages, the SDF content in dietary fiber is an important indicator of effective physiological function (Wang, Xu, Yuan, Fan, & Gao, 2015). It is also important for improving the SDF content of dietary fiber, especially for developing fiber-containing food products (Feng, Dou, Alaxi, Niu, & Yu, 2017).

Steam explosion (SE) is a high-efficiency processing pretreatment method that has developed rapidly in recent years. The main function of SE is to destroy the cell walls of biomass as a pretreatment for lignocellulosic materials. In this method, the sample is re-exposed under high-temperature and high-pressure steam for a given time. These conditions force steam through the cells and tissues within the samples, after which an explosive decompression occurs in milliseconds (Shafiei, Kabir, Zilouei, Sarvari, & Karimi, 2013). During this short time, high-temperature steam and liquid water contained within the samples rapidly enlarge and break from the original structure. SE has many advantages...
over other pretreatment methods, e.g. low energy use, no chemical additives and little environmental pollution (Alvira, Tomas, Ballesteros, & Negro, 2010). Other results have shown that SE can substantially enhance the amount of extract as well as the physicochemical features of SDF from orange peels (Wang, Xu, Yuan, Fan, & Gao, 2015). Romero-Garcia et al. (2016) also obtained mannitol, flavonoids, hydroxytyrosol and oleuropein from olive leaves using SE treatment.

Response surface methodology (RSM) is an important method used to appraise the influence of several indicators and their interactive effects on different response variables. Through RSM, it is possible to establish a mathematical model, find the optimum response and provide statistically meaningful results (Liu, Mu, Sun, Zhang, & Chen, 2013). RSM has been widely applied for optimizing conditions in extraction processes involving vegetables, fruit peels and pomace (Ma, Wang, Tang, & Yang, 2016; Oliveira et al., 2016). However, there have been no studies on the optimal SE conditions to increase the SDF yield from apple pomace.

Therefore, the purpose of the present study is to use RSM to determine the appropriate SE treatment conditions to optimize SDF extraction from apple pomace. This study will provide a reference for obtaining a high yield of SDF in subsequent industrial production.

2. Materials and methods

2.1. Materials

Apple pomace, provided by the Beijing Zhonglu Juice Co., Ltd. (Beijing, China), was washed in water repeatedly until clean and then dried in a drying oven. To ensure a consistent starting material and prevent apple pomace deterioration in the test, the apple pomace was stored at −20°C until use. All reagents and chemicals used were of analytical grade.

2.2. SE treatment

Apple pomace was turned into powder using a grinder (FW-400A, Beijing Zhongxingweiye Co., Ltd., Beijing, China) at a moderate rate for 30 s. A sieve analyzer consisting of multiple stainless steel sieves (mesh no. 20, 40, 60, 80 and 100) was used. Then, the sieved apple pomace was weighed (300 g) and placed in the SE system cylinder. The piston was immediately placed on the cylinder, the pressure (0.2, 0.4, 0.6, 0.8, 1.0 Mpa) and time (60, 120, 180, 240, 300 s) were set, and the hot steam was quickly injected into the cylinder from the steam inlet. After the pressure was maintained for a certain period of time, the inlet for steam was switched off while setting a trigger on the piston device. The explosion was completed in 0.0875 s (Yu, Zhang, Yu, Xu, & Song, 2012). The treated apple pomace was then collected.

2.3. Experimental design to optimize SE parameters

RSM was used to analyse the influence of the three variables affecting SE conditions (steam pressure, residence time and sieving mesh size) on the SDF yield from apple pomace. The factorial levels were chosen by considering the result of single-factor experiments varied as follows: steam pressure, 0.4, 0.6 and 0.8 Mpa; residence time, 120, 180 and 240 s; and sieving mesh size, 40, 60 and 80.

According to the single-factor tests, RSM was adopted to enhance the SDF yield from apple pomace. A central composite design (CCD) was performed with the same three independent variables (A, steam pressure; B, residence time; C, sieving mesh size) at three levels (Kaushik, Rao, & Mishra, 2016). According to the range of the independent variables, each variable was turned into a three-level code, leading to the following second-order polynomial Equation (1):

\[ Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{12} A^2 + \beta_{22} B^2 + \beta_{33} C^2 + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC \]  

where \( Y \) is the predicted response; \( \beta_0 \) is the intercept; \( \beta_1, \beta_2 \) and \( \beta_3 \) indicate linear coefficients; \( \beta_{12}, \beta_{22} \) and \( \beta_{33} \) are the squared coefficients; and \( \beta_{13}, \beta_{23} \) are the interactive coefficients. Analysis of variance (ANOVA) was used to appraise the appropriateness of the model as well as to define the regression coefficients and statistical significance.

2.4. SDF extraction from apple pomace

The apple pomace pretreated by SE was dispersed in petroleum ether 4 times and then shaken at 37°C with constant stirring at 100 rpm for 2.5 h. The apple pomace was collected by suction filtration, dispersed 4 times in 70% (w/w) ethanol and then placed in a water bath at 80°C for 1 h. The apple pomace was again collected by suction filtration. Cellulase (0.1%, w/w) was added, followed by further hydrolysis for 3 h at 25°C with constant stirring at 100 rpm. The sample was quenched at 90°C for 10 min to initiate enzymatic hydrolysis, and the supernatant and sediment were then collected. Next, 95% (v/v) ethanol was added into the concentrated supernatant. The mixture was retained for 12 h, followed by centrifugation at 10,000 g for 15 min. Finally, the precipitated flocculate was collected and placed in an electrically heated drum wind drying oven (101-3ABS, Guangming, Beijing, China) at 70°C for 24 h. The dried flocculate was ground, forming the SDF powder, and then stored at −20°C.

2.5. Chemical composition of untreated and SE-treated apple pomace

The moisture (method 925.09), protein (method 955.04), ash (method 942.05), fat (method 920.39), total dietary fiber and soluble and insoluble fractions (method 991.43) in the SE treated and untreated apple pomace were measured according to standard AOAC (2000) methods.

2.6. Physicochemical properties of SDF

2.6.1. Water-holding capacity (WHC)

The WHC was determined in triplicate in accordance with a published method (Gao, Yan, Xu, Ye, & Chen, 2015). A total of 0.5 g (\( w_1 \)) of SDF from treated and untreated apple pomace was dissolved in distilled water; then, the two samples were left at room temperature for 24 h. After centrifugation at 10,000 g for 10 min, the two samples were collected and weighed (\( w_2 \)). The WHC value was calculated using the following equation:

\[ \text{WHC}(g/g) = \frac{w_2 - w_1}{w_1} \]  

(2)
2.6.2. Swelling capacity (SC)
The SC was determined using the method described by Chen, Ye, Yin & Zhang (2014). A total of 0.5 g (W) of SDF from untreated and treated apple pomace was placed in test tubes. Then, 10 mL of water was added to the test tubes, and the samples were allowed to hydrate for 24 h at room temperature. Records of the samples’ volume (mL) and volume fraction were kept. The final volume of the SDF samples was \( v \). The SC value was calculated with the following equation:

\[
SC\text{(mL/g)} = \frac{v}{m}
\]

(3)

2.6.3. Oil-holding capacity (OHC)
The OHC was determined as reported by Du, Zhu, and Xu (2014). A total of 0.5 g (W) of SDF extracted from treated and untreated apple pomace was mixed with corn oil in centrifuge tubes then left at room temperature for 2 h. The mixture was centrifuged under 10,000 g for 15 min, and the sediment was weighed (\( W_1 \)). The OHC value was calculated using the following equation:

\[
OHC\text{(g/g)} = \frac{W_1 - W}{W}
\]

(4)

2.6.4. Scanning electron microscopy (SEM)
The SDF microstructure from the untreated and treated apple pomace was obtained using a scanning electron microscope (S-3400 scanning electron microscope, Hitachi Ltd., Tokyo, Japan). SDF samples were prepared according to a previously described method (Chen, Gao, Yang, & Gao, 2013). Using double-sided adhesive tape, the gold-coated samples were placed on specimen holders (10 min, 2 mbar). The samples were then placed into the scanning electron microscope, with an accelerating voltage of 15.0 kV.

2.7. Statistical analysis
All tests were conducted in at least triplicate, and statistical significance was determined using the mean values ± standard deviation. Tukey’s test was used to determine any significant differences (\( p < 0.05 \)) between means using SPSS Statistics 17.0 (SPSS Inc., Chicago, IL, USA). Outcomes analysis of the response surface design was performed using Design-Expert 8.0.6 software (trial version, Stat-Ease Inc., Minneapolis, MN, USA).

3. Results and discussion
3.1. Response surface optimization of the SE pretreatment conditions
3.1.1. Statistical analysis and model fitting
RSM is an efficient statistical tool to optimize processing, as it requires fewer experimental tests and can provide statistically acceptable results (Neta, Peres, Teixeira, & Rodrigues, 2011). The 17 response values (SDF yield) under different experimental combinations for the coded variables are shown in Table 1. Using multiple regression analysis on the experimental data, the SDF yield and the test variables could be modeled using the following second-order polynomial Equation (5):

\[
Y = 28.78 - 3.15A - 1.01B + 0.75C + 1.2AB - 0.94AC + 0.42BC - 3.36A^2 - 3.03B^2 - 2.52C^2
\]

The regression equation from the variance analysis of the results for the CCD is shown in Table 2. This data indicates that the polynomial model of the second order was statistically significant (\( p < 0.0001 \)). The \( p \)-value for lack of fit was 0.4523, which suggests that it was not significant relative to the pure error. Therefore, these two statistics indicate that the model was suitable for describing the SDF yield. The coefficient of determination (\( R^2 \)) of the model was 0.993, which explains 99.3% of the total variation in the response. The coefficient of variation was 1.88%, a value less than the 5% that indicates that the CCD model is reproducible (Ma, Sun, Tian, Luo, Zheng & Zhan 2016). These last two statistics also indicate the good fitness of the regression model. The determination of each coefficient’s significance was undertaken using the \( p \)-value. Table 2 shows that the steam pressure (A), residence time (B) and sieving mesh size (C) all had a substantial influence over the SDF yield (Y). One interactive term (AB) and the quadratic terms (\( A^2, B^2, C^2 \)) were also significant. In contrast, two interaction terms (AC, BC) were not significant. The adjusted coefficient of determination (Adj \( R^2 \)) was 0.9839, which shows that the model was fit to properly demonstrate the real relationship between the variables and the responses (Zhang, Zhang, Che, & Wang, 2016).

3.1.2. SE pretreatment conditions optimization
The surface plots and contour plots were obtained using Equation (2). These plots represent the interactions between two variables and the results for the SDF yield by steam pressure, residence time and sieving mesh size (Figure 1). Figure 1(a, b) show that the SDF yield was affected by different steam pressures and residence times when the sieving mesh size was 60. In addition, the SDF yield increased as the retention time and steam pressure increased, and the maximum SDF yield was obtained at 0.51 MPa and 168.24 s. Figure 1(c, d) show that the SDF yield was affected by different steam pressures and sieving mesh sizes for a residence time of 180 s. The SDF yield increased as the sieving mesh size increased, with the
| Source | Sum of squares | df | Mean square | Coefficient (β) | F-value | p-Value |
|--------|---------------|----|-------------|----------------|---------|---------|
| Model  | 215.59        | 9  | 23.95       | 29.18          | 109.98  | <0.0001 |
| A      | 53.51         | 1  | 53.51       | −2.59          | 245.67  | <0.0001 |
| B      | 9.03          | 1  | 9.03        | −1.06          | 41.46   | 0.0004  |
| C      | 12.18         | 1  | 12.18       | 1.23           | 55.91   | 0.0001  |
| AB     | 2.48          | 1  | 2.48        | 0.79           | 11.39   | 0.0118  |
| AC     | 0.55          | 1  | 0.55        | −0.37          | 2.51    | 0.1568  |
| BC     | 0.71          | 1  | 0.71        | 0.42           | 3.28    | 0.1131  |
| A²     | 41.5          | 1  | 41.5        | −3.14          | 190.54  | <0.0001 |
| B²     | 48.3          | 1  | 48.3        | −3.39          | 221.77  | <0.0001 |
| C²     | 33            | 1  | 33          | −2.80          | 151.5   | <0.0001 |
| Residual | 1.52      | 7  | 0.22        |              | 29.18   |         |
| Lack of fit | 0.68   | 3  | 0.23        | −2.59         | 1.08    | 0.4523  |
| Pure error | 0.84   | 4  | 0.21        |              |         |         |
| Cor total | 217.11 | 16 |              |              |         |         |
| R²     | 0.993         |    |             |                |         |         |
| Adj. R² | 0.9839     |    |             |                |         |         |
| C.V. % | 1.88          |    |             |                |         |         |

* p < 0.05 indicates statistical significance.

* p < 0.05 indica el significado estadístico.

Figure 1. Response surface plot and contour plot showing the effects of the variables on the yield of SDF. Three independent variables set were steam pressure, residence time and sieving mesh size.

Figura 1. Gráfico de superficie de respuesta y gráfico de contorno que indican los efectos de las variables en el rendimiento de la FDS. Las tres variables independientes seleccionadas son presión del vapor, tiempo de residencia y medida de malla de cribado.
maximum obtained at a sieving mesh size of 64.72. The SDF yield was affected by different residence times and sieving mesh sizes at a steam pressure of 0.6 MPa as shown in Figure 1(e, f). The SDF yield increased rapidly as the sieving mesh size increased above 20, reaching the maximum at the sieving mesh size 64.72 and then dropping up to the sieving mesh size 100.

Based on these results and the significance of the regression coefficients of the quadratic polynomial model, the optimal pretreatment conditions for SE were as follows: steam pressure, 0.51 MPa; residence time, 168.24 s; and sieving mesh size, 64.72. The predicted SDF yield under these conditions was 30.02%.

3.1.3. Model verification
To provide the actual operating conditions for production, the modified optimal conditions for SE were as follows: steam pressure, 0.51 MPa; residence time, 168 s; and sieving mesh size, 60. Under these conditions, the actual SDF yield should be 29.85 ± 0.47%, close to the predicted value (30.02%). These data demonstrate the validity of the RSM model and that the model was adequate for SE pretreatment conditions.

3.2. Chemical composition of untreated and treated apple pomace
The apple pomace composition under the modified optimal conditions is shown in Table 3. The moisture, fat, protein and ash content in the apple pomace did not change significantly after SE. The DF content decreased from 77.35% to 73.62%, the IDF content decreased from 70.82% to 45.25% and the SDF content increased from 6.27% to 29.85%.

3.3. SDF properties
The effects of SE on the WHC, OHC and SC values under the modified optimal conditions are shown in Table 4. The WHC value increased from 7.24 to 11.51 g/g, the OHC value increased from 2.55 to 4.25 g/g and the SC value increased from 3.57 to 5.66 g/g.

3.4. Scanning electron microscopy (SEM)
The surface morphology for the SDF extracted out of SE-treated apple pomace under the modified optimal conditions (0.51 MPa, 168 s and sieving mesh size 60) and from untreated apple pomace is shown in Figure 2. The electron micrograph in Figure 2a shows intact and smooth SDF surface extracted from the untreated apple pomace. However, after SE treatment, the electron micrograph (Figure 2b) shows that the cell wall had disintegrated and the texture had obviously begun to loosen with some honeycomb-like holes appearing on the surface of the cell.

In general, using RSM to optimize the pretreatment conditions of SE resulted in a 476% increase in increased SDF yield compared to the untreated apple pomace. This finding demonstrated that RSM is a powerful tool for optimization of the target values (Xu et al., 2016). The SDF extraction yield after SE increased much more than after the extrusion process (increased by 37.3%) (Zhang, Bai, & Zhang, 2011). The changes in chemical composition observed after SE show that the increase in SDF was mainly from the degradation of IDF. Raghavendra and Esposito also found that the SDF content

| Table 3. Chemical composition of the untreated and treated apple pomace. |
|---------------------------------|---------------------------------|
|                                | Untreated apple pomace (%)      | Treated apple pomace (%)       |
| Moisture                        | 3.50 ± 0.27a                    | 3.59 ± 0.31a                   |
| Fat                             | 7.82 ± 0.18a                    | 7.42 ± 0.22a                   |
| Protein                         | 6.22 ± 0.47a                    | 6.57 ± 0.29a                   |
| Ash                             | 2.49 ± 0.08a                    | 2.62 ± 0.11a                   |
| DF                              | 77.35 ± 0.89a                   | 73.62 ± 0.95b                  |
| IDF                             | 70.82 ± 0.66a                   | 45.25 ± 0.71b                  |
| SDF                             | 6.27 ± 0.55a                    | 29.85 ± 0.47b                  |

Values followed by the different letters indicate significant differences (p < 0.05).

| Table 4. The effect of SE on physicochemical properties of SDF in apple pomace. |
|---------------------------------|---------------------------------|---------------------------------|------|
|                                | WHC (g/g)                       | OHC (g/g)                       | SC (ml/g) |
| Untreated                       | 7.24 ± 0.17a                    | 2.55 ± 0.22a                    | 3.57 ± 0.20a |
| Treated                         | 11.51 ± 0.16b                   | 4.25 ± 0.29b                    | 5.66 ± 0.24b |

Values followed by the different letters indicate significant differences (p < 0.05).

IDF content decreased from 70.82% to 45.25% and the SDF content increased from 6.27% to 29.85%.

Figure 2. Scanning electron microscopy (SEM) images of SDF from apple pomace ((a) untreated, (b) treated by SE at 0.51 Mpa, 168 s and sieving mesh size 60).
increased after different treatments (Esposito et al., 2005; Raghavendra, Rastogi, Raghavarao, & Tharanathan, 2004). The increases in WHC and OHC were probably caused by the increase in short-chain dietary fiber after SE treatment, which improved the water and oil uptake (Esposito et al., 2005), while the increase in SC may be attributed to the increase in the surface area of SDF after SE, the outcomes of which agreed with those of Zhang et al. (2011). The micrographs from SEM showed that SDF’s spatial structure was altered due to the SE modification. Because of changes in chemical properties caused by changes in porosity, looseness and dilatability, SDF can hold more water or oil molecules after SE treatment, leading to increased WHC, OHC and SC values (Ubandomheri, 2005).

4. Conclusions
RSM was successfully used to optimize the pretreatment conditions for SE. Under the optimal conditions (steam pressure, 0.51 MPa; residence time, 168 s; and sieving mesh size, 60), the SDF yield reached 29.85%. This was significantly higher than the SDF yield from untreated apple pomace (6.27%), an increase of 4.76 times. This result indicates that SE can substantially improve the amount of SDF extracted from apple pomace and increase the WHC, OHC and SC values. The changes in surface morphology showed that SDF’s spatial structure was altered due to SE modification.

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Disclosure statement
No potential conflict of interest was reported by the authors.

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