Fractionation of *Eremurus spectabilis* fructans by ethanol: Box–Behnken design and principal component analysis

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ARTICLE INFO

ABSTRACT

The fructans, inulin and oligofructose, are known to exert many food and pharmaceutical applications and are widely used in functional foods throughout the world for their nutritional and techno-functional properties. In the present study, the Box–Behnken design was used to determine the optimal conditions for fructan precipitation from *Eremurus spectabilis* root powder (Serish) by adding ethanol that gave the maximum yield. Ethanol-to-syrup (*E/S*) ratio (2:1–15:1), precipitation temperature (30–60 °C) and syrup concentration (10–40 °B) were considered variables of fructan precipitation. The most compatible model among mean, linear and quadratic expressions was fitted to each response and the regression coefficients were determined using least square method. There was a good agreement between the experimental data and their predicted counterparts. The optimum conditions for fractionating fructan composition of Serish by ethanol were estimated to be *E/S* ratio of 8.56, temperature of 23.51 °C and initial syrup concentration of 40 °B. Precipitation under these optimized conditions achieved the best yield (85.81%), average chain length (12.92) and purity (80.18%). In addition, principal component analysis (PCA) allowed discriminating among precipitated fructan specialties.

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1. Introduction

Serish (*Eremurus spectabilis*) belongs to the family of Liliaceae and geographically distributed in the region of South Asia and Central Asia, including Iran, West Pakistan, Afghanistan, Iraq, Turkey, Palestine, Lebanon, Syria and Caucasus. Its roots accumulate high levels of fructans during their growth (Dashti, Tavakoli, Zarif Ketabi, & Paryab, 2005; Hajebi, 1974; Khorasani, Yusefi, & Ershad Langroudi, 2006; Rubin, 2002). This root has traditionally been used to cure of jaundice, liver disorders, stomach irritation, pimples and bone fractures. Also, it has been applied as a glue for industrial application (Brayan, 1989; Brickell, 1996; Crockett, 1972; Dashti et al., 2005).

A prebiotic is defined as a nondigestible food ingredient that beneficially influences the consumer by selectively exciting the growth and/or activity of one or a limited number of bacteria in the colon. It is a substance that modifies the composition of the intestinal microflora in such a way that a few of the potentially health-promoting bacteria (especially lactobacilli and bifidobacteria) become predominant in numbers (Gibson & Roberfroid, 1995).

Fructans are an important product of the industry of prebiotics. In addition to its interesting nutritional and health benefit properties, fructans are also used in food formulations for its techno-functional properties such as fat substitute, bulk agent and water retention (Blecker et al., 2001; O'Brien, Mueller, Scannell, & Arendt, 2003). They are oligo- and polysaccharides consisting of short chains of fructose units with a single D-glucosyl unit at the nonreducing end. While the terminology in this area can be confusing, fructans with a short chain length (i.e. average chain length, CL) of 2–9 units are generally referred to as fructooligosaccharides (FOS) or oligofructose, and the longer chain (CL ≥ 10) are called inulins (Muir et al., 2007; Prosky & Hoebregs, 1999).

Due to growing interest in the food industries, oligofructoses have to be separated from inulins, because their properties (digestibility, prebiotic activity and health promoting potential, caloric value, sweetening power and water binding capacity) differ substantially (de Gennaro, Birch, Parke, & Stancher, 2000). Numerous fractionation procedures are examined for their potential to enrich fructans with high CL. A series of fractionation techniques known to increase CL of polysaccharides are studied as follows: ultrafiltration, specific precipitation/crystallization from aqueous

solutions, and specific precipitation through addition of a solvent, which decreases inulin solubility (Defloor, Vandenreyken, Grobet, & Delcour, 1998; Laurenzo, Navia, & Neiditch, 1999; Paseephol, Small, & Sherkat, 2007; Silver, 2003; Smits et al., 2001).

To the best of our knowledge, there are no reports on the precipitation of Serish fructans. Thus, the present study is considered the first attempt aiming to: (a) examine the effect of conditions of fructan precipitation, i.e. syrup concentration, ethanol-to-syrup (E/S) ratio and temperature on yield, purity and average chain length of fructans; (b) determine the optimum situations for fructan precipitation by ethanol; (c) check the validity of Box–Behnken design to analyze the synergistic and/or antagonistic effects of precipitation conditions on fructans properties; and (d) obtain the relationship between precipitated fructan parameters by using principal component analysis (PCA).

2. Materials and methods

2.1. Preparation of Serish roots powder

The Serish root powders were obtained from the local medical plant market, Mashhad, Iran. Then, the prepared samples were passed through a 50 μm sieve and stored in dry container for further use.

2.2. Preparation of fructan concentrate

The Scheme for preparation steps of fructan concentrate from Serish root powder is shown in Fig. 1. Fructan extraction was carried out in a water bath (model WB/0B7-45, Memmert Company, Schwabach, Germany). Serish root powder was suspended with distilled water at a ratio of 1:50 (w/v) and was allowed to stand at 85–90 °C for 30 min (In preliminary study, extraction conditions, i.e. water to solid ratio (30–50, v/w), temperature (40–90 °C) and time (5–40 min) were optimized for achieving the best yield, average chain length and purity). The suspension was then filtered through muslin cloth to remove the insoluble residues. The resulting solution was turbid due to the presence of particulate and colloidal matter, i.e. pectin, protein, and cell wall substances (Hansen & Madsen, 1992). To remove these impurities, the concentrate was mixed with a 5% slurry of calcium hydroxide at 50–60 °C for 30 min, resulting in the formation of a flocculent precipitate and a brighter yellow supernatant. By this technique, the pH of juice rose from 5–6 to 10–12. After filtration under vacuum using paper filter (Whatman No. 4), 10% phosphoric acid was added to the filtrate with vigorous stirring to adjust its pH to 8–9, causing the precipitation of surplus calcium and coagulated organic material. The mixture was permitted to stand at 60 °C for 2–3 h before re-filtration (Whatman No. 4). The clarification process was repeated twice. Activated carbon powder was added to the filtrates at 60 °C and mixed for 15–30 min to facilitate elimination of colored materials. The treated syrup was filtered (Whatman No. 1) and the clear syrup achieved was further concentrated by rotary evaporation at 70 °C, to obtain syrups with three different soluble solids levels (10, 25 and 40 °B) which were then stored at −20 °C (Paseephol et al., 2007).

2.3. Precipitation in ethanol

Aliquots (2 g) of Serish concentrate (10–40 °B) were weighted into preweighted test tubes and mixed with 2–15 parts by weight of ethanol (abs. 99%). The test tubes were vortexed and hermetically sealed before storage at 3–60 °C for 3 days. After storage, the supernatants were removed and the precipitates washed with ethanol. Then the tubes containing precipitates
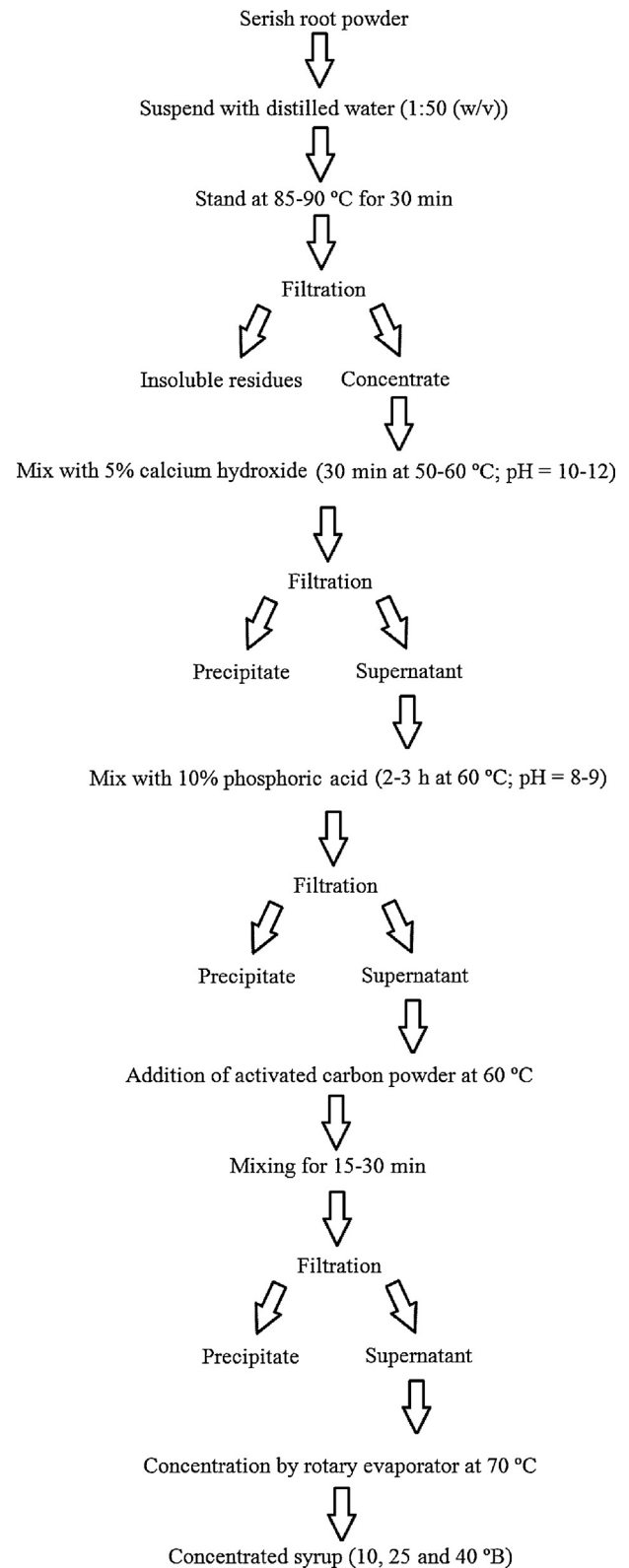

Fig. 1. Scheme for preparation steps of fructan concentrate from Serish root powder.

were placed in a hot-air oven at 102 °C for 30 min to remove surplus solvent and reweighted. The precipitate was then analyzed for total carbohydrate, reducing sugar, fructan content, dry matter content, and average chain length (Paseephol et al., 2007).

2.4. Experimental design

A Box–Behnken design (BBD) was constructed using the software Design Expert Version 6.0.10 (Stat-Ease Corporation, Minneapolis, MN, USA) and was used for estimating the effect of independent variables on the yield, purity and average chain length. Three precipitation variables considered for this research were ethanol: syrup, temperature and syrup concentration (Table 1). The design consists of 17 sets of test conditions for each precipitation method where three levels were attributed to each factor at high, central, and low levels, with additional four replicated center points. Maximum and minimum treatment levels were chosen by carrying out preliminary screening tests and according to the literature reports and instrumental aspects.

2.5. Analytical methods

2.5.1. Determination of total carbohydrate

Total carbohydrate was assayed colorimetrically using the phenol–sulphuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Sample weights were changed to obtain a reading of 10–70 μg. Sample solution (1 ml) was mixed with 1 mL of 5% phenol solution and 5 mL of 96% sulphuric acid. The mixture was incubated in a water bath at 30 °C for 20 min. The solution appeared as yellow-orange color and its absorbance was measured at 490 nm using UV–vis spectrophotometer (model S2000, WPA Lightwave, England). A series of D(−)-fructose (M_w = 180.16, Fluka) solutions of known concentration were used to establish a standard curve.

2.5.2. Determination of reducing sugar

The concentrations of soluble reducing sugars were measured using a 3,5-dinitrosalicylic acid (DNS) method (Miller, 1959). Three mL of the sample solution was transferred to a test tube where it was mixed with 3 mL of the DNS reagent. After mixing it with a vortex, samples were heated in water bath at 90 °C for 10 min followed by rapid cooling to ambient temperature. The absorbance of all samples was measured at 575 nm using a UV–vis spectrophotometer. Calibration curve was prepared using D(−)-fructose as standard.

2.5.3. Determination of fructan content (precipitation yield)

The fructan content was measured with the difference between total carbohydrate and reducing sugars. The percentage fructan yield (%) was evaluated based on the following equation (Paseephol et al., 2007; Wei et al., 2007):

$$\text{Fructan yield}(\%) = \left(\frac{\text{Fructan content of precipitate}}{\text{Fructan content of Serish concentrate utilised for precipitation}} \right) \times 100 \quad (1)$$

2.5.4. Purity determination

The purity value was calculated as follow (Paseephol et al., 2007):

$$\text{Purity value}(\%) = \left(\frac{\text{Fructan content}}{\text{Dry matter content of the precipitate}} \right) \times 100 \quad (2)$$

Dry matter content of precipitate was determined gravimetrically by drying in a hot-air oven at 102 °C until the weight remained constant (AOAC, 2005).

2.5.5. Average chain length

The average chain length, as an index of degree of polymerization, was calculated according to (Paseephol et al., 2007):

$$\text{Average chain length} = \frac{\text{Total amount of carbohydrate}}{\text{Total amount of reducing sugar}} \quad (3)$$

2.6. Statistical analyses

2.6.1. Modeling of variables

For each of the response variables, a second-degree polynomial model was used to data fitting as below equation:

$$Y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{11}x_1^2 + b_{22}x_1^2 + b_{33}x_3^2 + b_{12}x_1x_2$$
$$+ b_{13}x_1x_3 + b_{23}x_2x_3 + \varepsilon \quad (4)$$

where Y is the predicted response; x_1, x_2 and x_3 the variables; b_0 a constant; b_1, b_2 and b_3 the linear effects; b_{11}, b_{22} and b_{33} the quadratic coefficients; and b_{12}, b_{13} and b_{23} the interaction coefficients.

In this study, predictor variables were permitted to be at any level within the range of the design. All experiments were carried out in triplicate. Statistical significance of the terms in the regression equations was examined. The significant terms in the model were found by analysis of variance (ANOVA) for each response. In addition, Lack of fit, coefficients of determination (R^2), adj-R^2, coefficient of variation (CV) and significant probabilities were calculated to check the model adequacy. The above quadratic equation was used to build surfaces for the variables. The software Design Expert Version 6.0.10 was used to analyze the results.

2.6.2. Optimization and verification procedures

Besides explaining the behavior of variables by the contour curves, the models fitted in this study could also be utilized for optimization purposes using the desirability function. This approach consists in first converting each response variable into a desirability function d_i, that varies from 0 to 1 (Wu & Hamada, 2000). That is, if we want to find the factor levels that take to a maximum response variable value, we need to set d_i = 1 for high values and d_i = 0 for low values of this response variable. In case, we want a minimum response variable value, we need to set d_i = 0 for high values and d_i = 1 for low values of this response variable. The idea is that this desirability function acts as a penalty function that leads the algorithm to regions where we can find the desired response variable values. The factor levels that take to a maximum or a minimum of the response variable are called "optimum points". Eq. (5) expresses the global desirability function, D, defined as the geometric mean of the individual desirability functions. The algorithm should search for response variable values where D tends to 1.

$$D = (d_1 d_2 ... d_m)^{1/m} \quad (5)$$

where m is the number of response variables (Derringer & Suich, 1980).

Optimization was based on generation of the best results for fructan precipitation yield, purity and CL. The precipitation yield and purity was determined under optimal conditions. Design Expert Version 6.0.10 was used to optimization. In order to determine the validity of the model, the experimental and predicted values were compared by paired t-test using Minitab 15 (Minitab Inc., State College, PA, USA) software.

In order to verification of fructan determination by colorimetric methods, fructan content in precipitate and optimized concentrate was measured by HPLC (Knauer, Germany). The method was carried out under the following condition; column: Aminex HPX-87C column (Bio-rad Laboratories, Milan, Italy); detector: refractive index detector (Knauer, Germany); eluent: water, flow rate: 0.3 mL/min; injected volume: 20.0 μL; column temperature: 80 °C; detector

Table 1
Variables and levels used in Box–Behnken design.

Trial	Sample codes	Variable codes			Actual values		
		Ethanol: syrup	Precipitation temperature	Syrup concentration	Ethanol: syrup	Precipitation temperature (°C)	Syrup concentration (°B)
1	$A_{-1}B_{-1}C_0$	−1	−1	0	2	3	25
2	$A_1B_{-1}C_0$	1	−1	0	15	3	25
3	$A_{-1}B_1C_0$	−1	1	0	2	60	25
4	$A_1B_1C_0$	1	1	0	15	60	25
5	$A_{-1}B_0C_{-1}$	−1	0	−1	2	31.5	10
6	$A_1B_0C_{-1}$	1	0	−1	15	31.5	10
7	$A_{-1}B_0C_1$	−1	0	1	2	31.5	40
8	$A_1B_0C_1$	1	0	1	15	31.5	40
9	$A_0B_{-1}C_{-1}$	0	−1	−1	8.5	3	10
10	$A_0B_1C_{-1}$	0	1	−1	8.5	60	10
11	$A_0B_{-1}C_1$	0	−1	1	8.5	3	40
12	$A_0B_1C_1$	0	1	1	8.5	60	40
13	$A_0B_0C_0$	0	0	0	8.5	31.5	25
14	$A_0B_0C_0$	0	0	0	8.5	31.5	25
15	$A_0B_0C_0$	0	0	0	8.5	31.5	25
16	$A_0B_0C_0$	0	0	0	8.5	31.5	25
17	$A_0B_0C_0$	0	0	0	8.5	31.5	25

A: ethanol:syrup ratio; B: precipitation temperature; C: syrup concentration.

temperature: 40 °C (Figueira, Park, Brod, & Luis Honório, 2004). The commercially available inulin from Sigma used as a standard in this work is from Dahlia tubers.

Average chain length of precipitated optimized sample was calculated by dividing the molecular weight of it to the molar mass of glucose or fructose (180.16 g/mol). The molecular weight was determined by viscometry and according to the famous Mark–Houwink equation:

$$[\eta] = KM_w^\alpha \tag{6}$$

where $[\eta]$ and M_w are intrinsic viscosity of fructan solutions and molecular weight of precipitated fructan, respectively. K and α are constants for a given polymer, solvent and temperature (Kwaambwa, Goodwin, Hughes, & Reynolds, 2007). Intrinsic viscosity of prepared solutions were determined in triplicate using a No. 75 Cannon-Ubellohde semimicro dilution type capillary viscometer (Cannon Instruments Co., USA) immersed in a thermostated water bath under precise temperature control (±0.1 °C). The flow time of sample through capillary was measured with an accuracy of 0.1 s using a digital chronometer. The equilibration time for each solution at the bath temperature was 15 min, after loading (2 mL) and each time diluting the sample. The viscosity measurements were carried out at 25 °C. The relative viscosities, η_{rel}, were corrected for solvent and solution densities. The intrinsic viscosity was calculated using the well-known Huggins and Kraemer relations (Mohammad Amini & Razavi, 2012):

Huggins equation:

$$\frac{\eta_{sp}}{c} = [\eta] + k_H[\eta]^2 c \tag{7}$$

Kraemer equation:

$$\frac{\ln \eta_r}{c} = [\eta] - k_K[\eta]^2 c \tag{8}$$

where is the η_r is the relative viscosity, $\eta_{sp} = \eta_r - 1$ is the specific viscosity, c is the polymer concentration, k_H and k_K are the respective constants. Sigma Dahlia inulin was used as a standard for validation of calculations.

2.6.3. Principal component analysis

A principal component analysis (PCA) using Minitab 15 Software was done on the quantities of precipitated fructan properties to differentiate them.

3. Results and discussion

3.1. Model fitting

Precipitation variables were ethanol: syrup (x_1), temperature (x_2) and syrup concentration (x_3). The design for combined effects consisted of 17 experiments according to Table 1. The results of ANOVA for the effects of variables on fructan precipitation yield, average chain length and purity with the corresponding coefficients of multiple determinations (R^2) are shown in Table 2. The regression models were highly significant with satisfactory coefficients of determination ($R^2 = 0.917 - 0.922$). Therefore, it is more appropriate to use an adj-R^2 to evaluate the model adequacy. The values of the adjusted determination coefficients (adj-$R^2 = 0.868 - 0.904$) also confirmed that the models were highly significant. Moreover, coefficient of variation (CV) describes the extent to which the data were dispersed. The CV for conventional extraction yield was within the acceptable range (1.20–2.24). Since CV is a measure of expressing standard deviation as a percentage of the mean, the small value of CV give better reproducibility. In general, a high CV shows that variation in the mean value is high and does not satisfactorily develop a suitable response model (Daniel, 1991). The lack-of-fit test, which measures the fitness of the model, did not result in a significant F-value, indicating that the models are sufficiently accurate for predicting the yield, purity and average chain length.

3.2. Effects of precipitation conditions on fructan precipitation yield

The results in Table 2 indicated that the linear coefficient of E/S ratio was significant. In addition, quadratic terms of E/S ratio, precipitation temperature and syrup concentration were significant. The other term coefficients were not significant ($P \leq 0.05$). Based on the sum of squares, the importance of the independent variables on yield could be ranked in the following order: quadratic term of E/S ratio (x_1x_1), quadratic term of precipitation temperature (x_2x_2), quadratic term of syrup concentration (x_3x_3) followed by the linear term of E/S ratio (x_1).

Three-dimensional (3D) plots for fructan yield as a function of precipitation conditions are given in Fig. 2. The data were generated through keeping one variable at center value of the testing ranges and varying the other two within the experimental range.
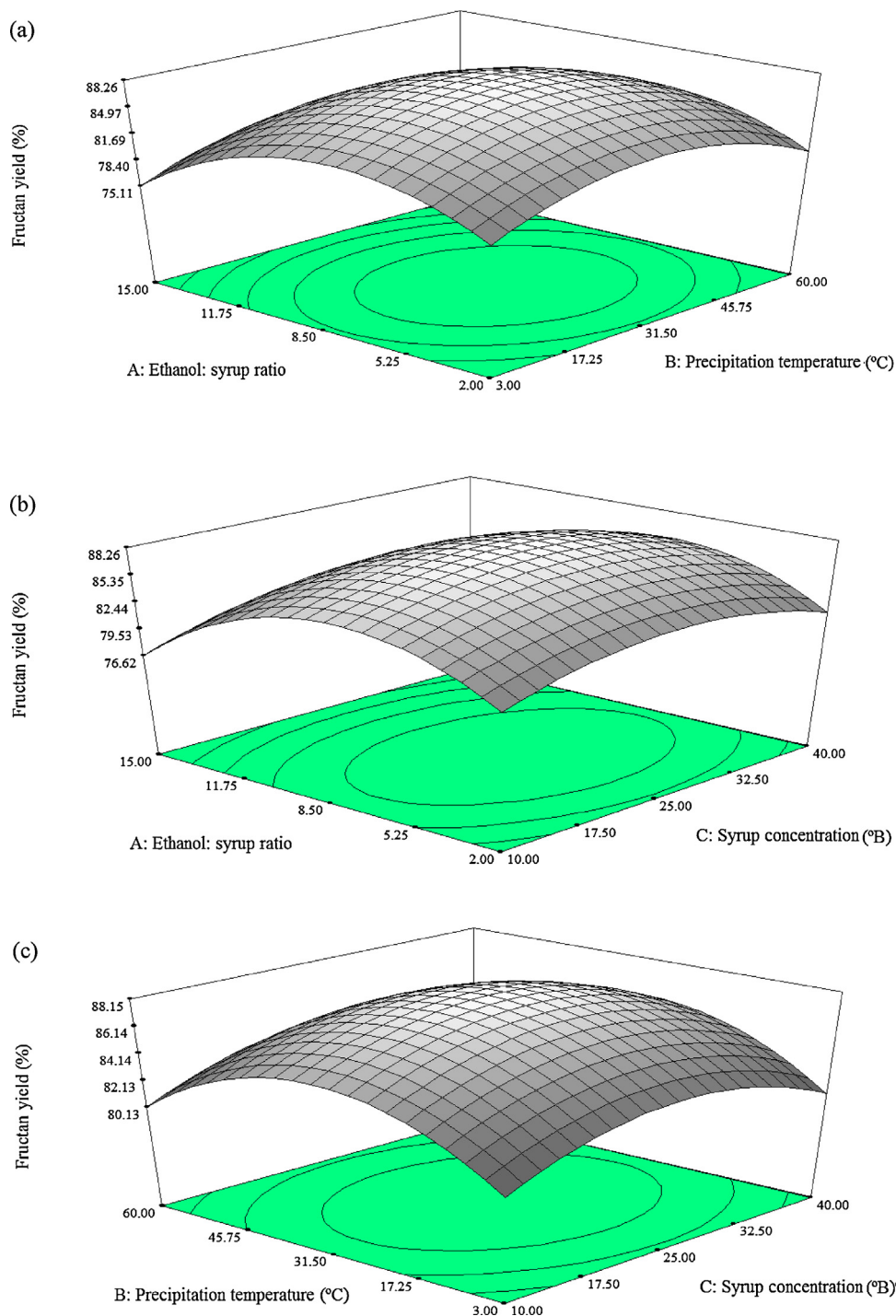
(a)

(b)

(c)

Fig. 2. Response surface plots for fructan yield; other variables are held at medium level.

The response surfaces showed that the E/S ratio had a quadratic significant effect on the yield. This effect is related to the decrease of polysaccharides solubility as the ethanol-to-syrup ratio increased (Bouchard, Hofland, & Witkamp, 2007; Gong, Wang, Li, & Qu, 2013). The dielectric constant of solvent implies its polarity. Generally, the higher the dielectric constant, the more polar the solvent is. The dielectric constant of ethanol is about one third of water. In addition, by adding ethanol, the polarities of solution decrease. This means that adding ethanol to solution disrupts the screening of charges by water. If enough ethanol is added, the electrical attraction between present groups in solution becomes strong enough to

form stable bonds and fructan precipitation (Kim, Faqih, & Wang, 2001).

The yield showed a quadratic behavior when the precipitation temperature was increased. This is maybe due to diminish of the diffusion rate for promoting precipitation formation at lower temperatures (Humphreys & Hatherly, 2004). Conversely, yield decrease after peak value might be attributed to the solubility increase of carbohydrate polymers at higher temperatures (Moerman, Van Leeuwen, & Delcour, 2004).

Results showed that, the yield rose as the syrup concentration increased from 10 to 25 °B and approached its maximum value at

Table 2
Analysis of variance for the predicted quadratic polynomial models for properties of precipitated fructan.

Source	df	Fructan yield		Average chain length		Purity	
		Coefficient	Sum of squares	Coefficient	Sum of squares	Coefficient	Sum of squares
Model	6	64.76***	369.69	11.63***	3.05	61.53***	244.69
Linear							
b_1	1	2.34*	25.18	0.18**	0.33	2.79*	8.00
b_2	1	0.37ns	0	−0.012***	0.92	ns	ns
b_3	1	0.69ns	0.45	0.026***	1.22	0.17***	55.36
Quadratic							
b_{11}	1	−0.15***	177.76	−0.0088***	0.58	−0.15***	181.33
b_{22}	1	−0.0059***	95.55	ns	ns	ns	ns
b_{33}	1	−0.013**	38.35	ns	ns	ns	ns
Interaction							
b_{12}	1	ns	ns	ns	ns	ns	ns
b_{13}	1	ns	ns	ns	ns	ns	ns
b_{23}	1	ns	ns	ns	ns	ns	ns
Lack of fit	3	ns	24.97	ns	0.026	ns	0.11
Pure error	4		8.25		0.25		20.55
Total	16		402.91		3.32		265.36
CV		2.24		1.20		1.67	
R^2		0.918		0.917		0.922	
adj-R^2		0.868		0.890		0.904	

Subscripts: (1) E/S ratio; (2) precipitation temperature; (3) syrup concentration.
ns: no significant effect at level <0.05.
CV, coefficient of variation.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

this concentration (Fig. 2). This might be due to the acceleration of aggregation and nucleation by initial increase that leads to increase of fructan precipitation.

3.3. Effects of precipitation conditions on average chain length

The values of the regression coefficients give an idea as to what extent the control variables affect the responses quantitatively. The results in Table 2 indicated that linear terms of E/S ratio, precipitation temperature, syrup concentration and quadratic term of E/S ratio were significant. The interaction terms exhibited no obvious significant effect on the CL. Based on the sum of squares, the importance of the independent variables on CL could be ranked in the following order: syrup concentration (x_3), precipitation temperature (x_2), quadratic term of E/S ratio (x_1x_1) and linear coefficient of it (x_1).

As it can be seen in Fig. 3, the response surfaces showed that the E/S ratio had a quadratic significant effect on the CL. This effect is probably due to the induced precipitation of longer fructan chains that increased the CL because of initial increase in solvent amount. Conversely, CL decrease might be due to precipitation acceleration of the shorter chain carbohydrates with more added solvent (Ku, Jansen, Oles, Lazar, & Rader, 2003; Moerman et al., 2004). On the other hand, CL decreased with the elevation of the temperature. This is probably because the disruption of fructan branch to reducing sugars with increasing temperature that leads to CL decrease.

According to Fig. 3, increase of syrup concentration resulted in increase of the CL. Similar trend has also been reported by Moerman et al. (2004) and Paseephol et al. (2007) who also concluded that the average chain length of precipitated inulin increased at higher syrup concentration. It is probably due to the fact that the increase in syrup concentration leads to the increase of average chain length due to the induced precipitation of total carbohydrates, i.e. longer fructan chains.

3.4. Effects of precipitation conditions on purity

Purity signifies an index of the amount of fructans in the precipitate achieved. The results in Table 2 indicated that the linear effect of E/S ratio, syrup concentration and quadratic term of E/S ratio were significant on the purity. The other term coefficients exhibited no obvious significant effect on the purity ($P \leq 0.05$). Based on the sum of squares, the importance of the independent variables on purity could be ranked in the order of the quadratic term of E/S ratio (x_1x_1), linear term of syrup concentration (x_3) followed by the linear term of E/S ratio (x_1).

As it can be seen in Fig. 4, the response surfaces showed that the E/S ratio had a quadratic significant effect on the purity. This behavior was in agreement with the results of Paseephol et al. (2007) about the purity of precipitated Jerusalem artichoke fructans. It is believed that the initial increase in solvent amount leads to the increase of purity due to the induced precipitation of longer chain fructans. Nonetheless, excess solvents accelerate the precipitation of the shorter chain carbohydrates, oligosaccharides, and/or non-sugar substances including ash in the precipitate that lead to purity reduction.

According to Fig. 4, purity increased with the elevation of the syrup concentration. This is probably as a result of the improvement of fructan precipitation with increasing syrup concentration due to the aggregation and nucleation increase.

(Fig. 5)

3.5. Optimization procedure and verification of results

Multiple response optimizations were performed to measure the optimum levels of independent variables to achieve the desired response goals. Fructan yield, purity and CL were desired maximal. Then, the optimal conditions were extracted by Design Expert software.

The final result for this optimization suggested that a precipitation process containing E/S ratio of 8.56, temperature of 23.51 °C
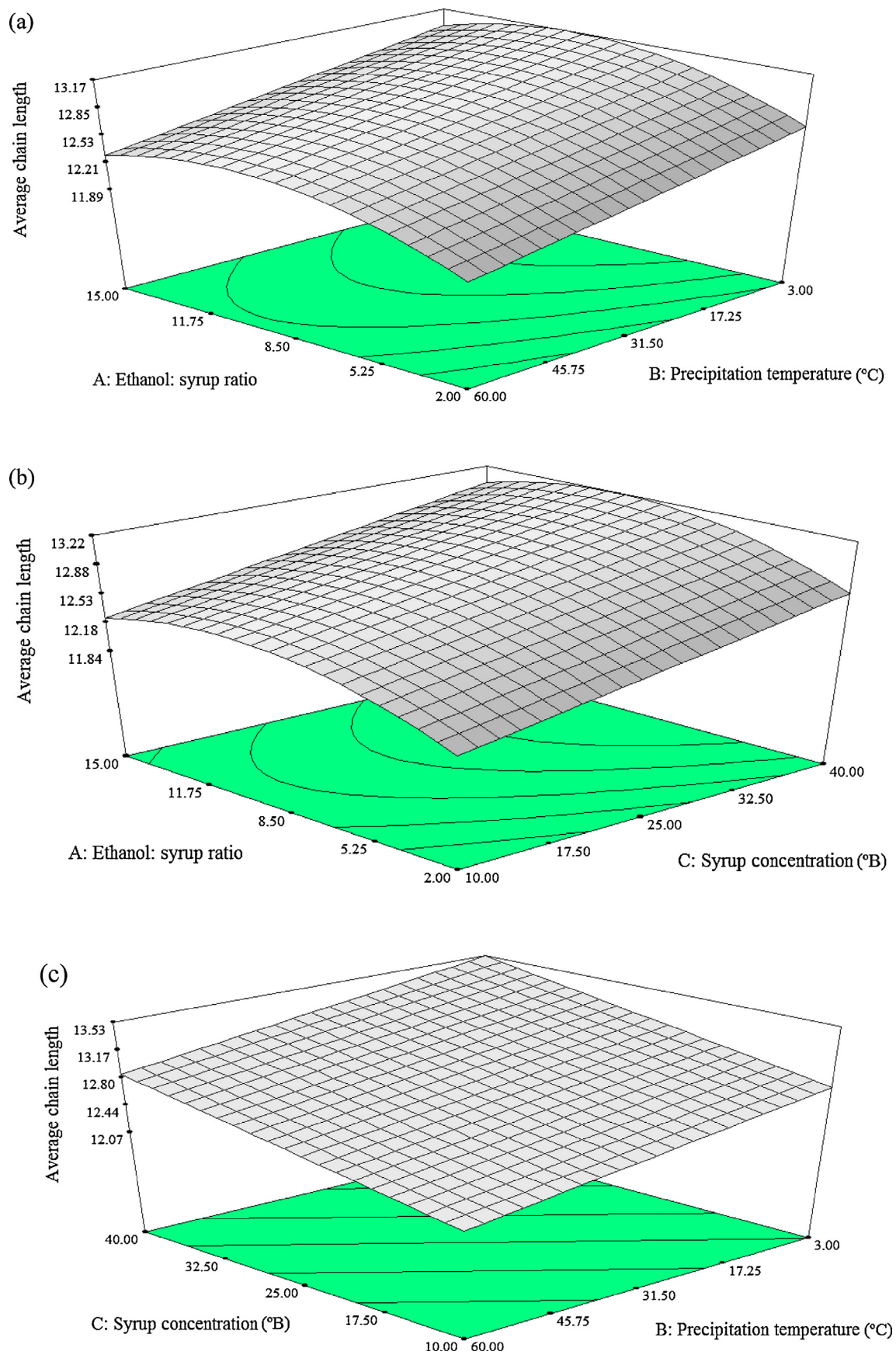
Fig. 3. Response surface plots for average chain length of fructan; other variables are held at medium level.
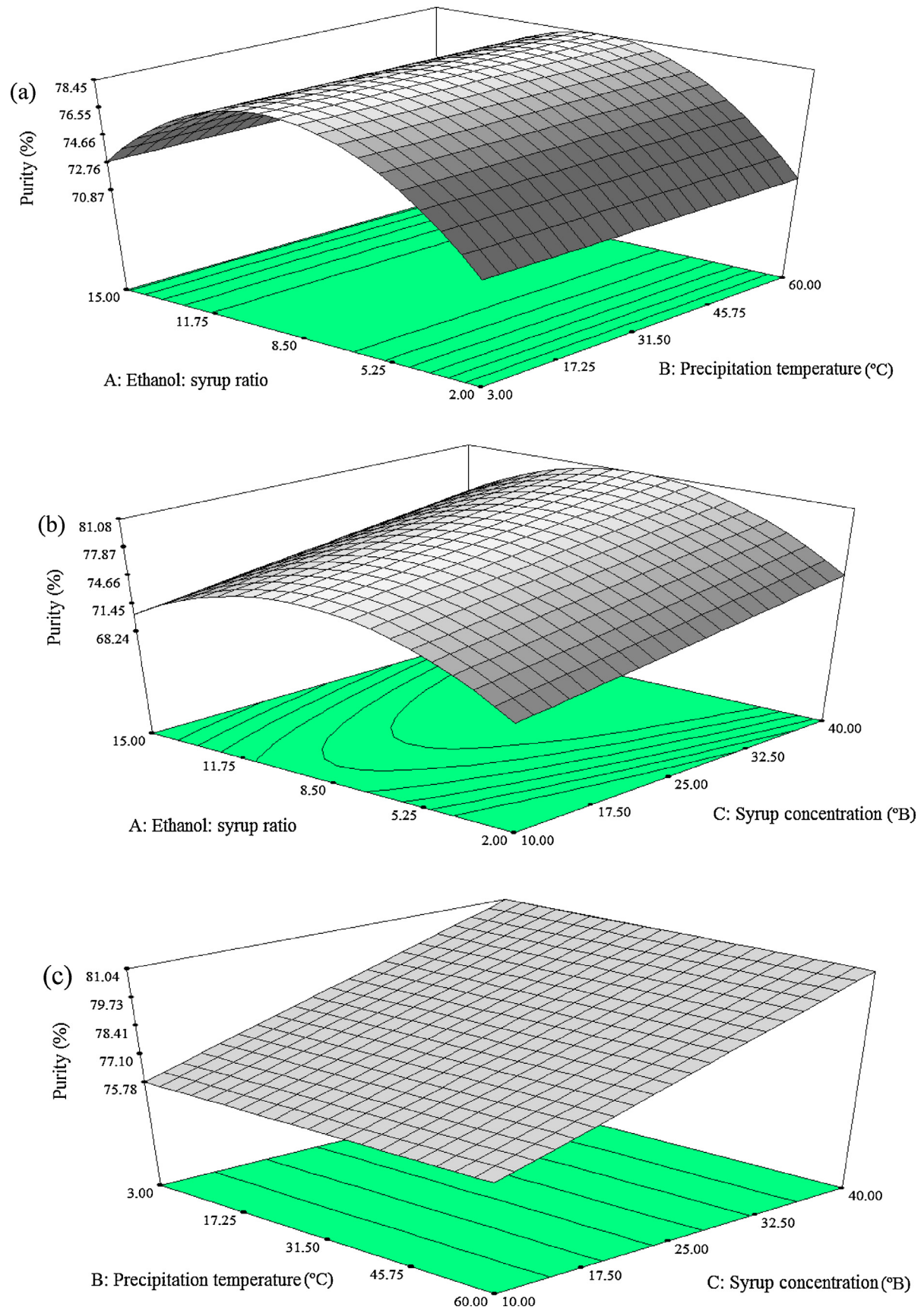
Fig. 4. Response surface plots for fructan purity; other variables are held at medium level.
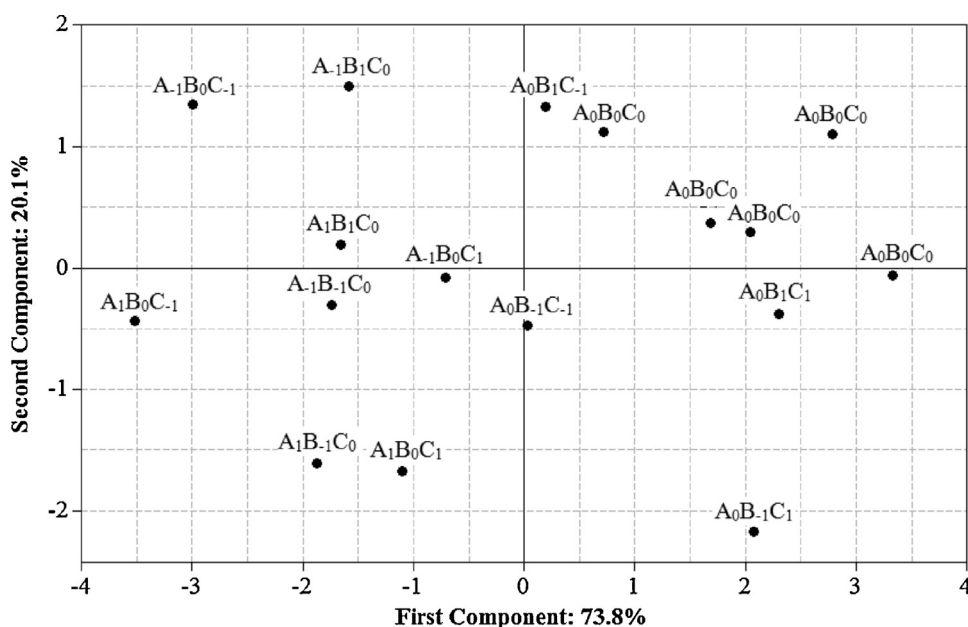
Fig. 5. Principal component analysis of the precipitated fructan properties (for the codes of the samples, see Table 1).

and initial syrup concentration of 40 °B could be a good mixture of these three components in order to achieve the best yield, CL and purity. These new precipitation conditions were submitted to the same experimental procedures applied as those from the beginning of this study. There was no significant difference between the estimated and observed values ($P < 0.05$), suggesting a good fit between the models to the experimental data (Table S1).

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2014.01.048.

To establish the reliability of the applied method for the analysis of fructan in the Serish samples, the results obtained using spectrophotometry were compared to that using HPLC (Table S2), where both fructan content of concentrate and precipitate were quantified. It is clearly seen (from HPLC) that fructan content of concentrate (93.21%) and precipitate (76.84%) is relatively high. In addition, the paired t test at 95% confidence limit ($P < 0.05$) showed insignificant differences between the results obtained from spectrophotometry and from HPLC. On the other hand,

viscometry method was used to verification of average chain length of precipitated sample which determined by colorimetric method. Results verified that there was no significant difference ($P < 0.05$) between viscometric and colorimetric data. Thus, colorimetric method, which is relatively inexpensive compared to the chromatographic, could be used as a routine method for analysis of Serish.

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2014.01.048.

3.6. Principal component analysis

Data on fructan properties were subjected to PCA . The multivariate treatment of the data obtained for the fructan samples permitted the reduction of the variables to two principal components, which together explained 93.9% of the total variability. The first axis accounted for 73.8% and the second axis for 20.1%. The factor loadings plot is presented in Fig. 6. According to Fig. 6,
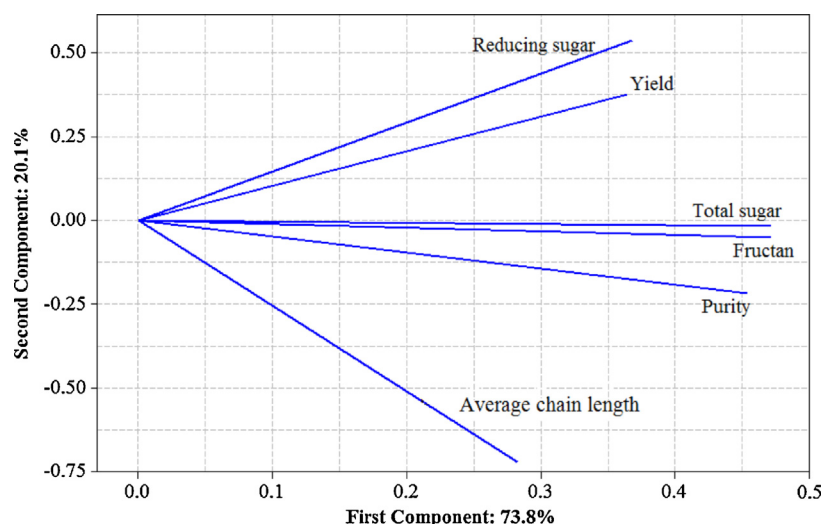

Fig. 6. Factor loadings for principal component analysis.

all of precipitated fructan properties, especially total sugar and fructan, were positively correlated to PC1 axis. Reducing sugar and yield were positively correlated to the PC1 axis whereas total sugar, fructan, purity, and CL were negatively correlated to it.

As it is clearly revealed in PCA plot (Fig. 5) chemical attributes were found high for those samples precipitated by moderate level of E/S ratio ($A_0B_0C_0$, $A_0B_1C_{-1}$, $A_0B_1C_1$, $A_0B_{-1}C_{-1}$, $A_0B_{-1}C_1$). Fructan samples precipitated at high temperatures, especially those that produced by moderate E/S ratio ($A_0B_1C_{-1}$, $A_0B_1C_1$), were also rated high in fructan, yield, reducing and total sugar, but they exhibited lower CL. While, fructans created at low temperatures, especially those that produced by moderate E/S ratio ($A_0B_{-1}C_{-1}$, $A_0B_{-1}C_1$), were characterized by low amount of fructan, yield, reducing and total sugar while they were rated long in CL. The results depicted by PCA seemed to be in accordance with the response surface methodology results discussed above.

4. Conclusion

Box–Behnken design was an efficient statistical tool to examine the influence of precipitation conditions of fructan from Serish root powder on the yield, CL and purity. These results also suggested that by modifying the proportion of syrup concentration, ethanol-to-syrup ratio and temperature, a large range of variations might be obtained. Based on these models, the optimal conditions of precipitation process were obtained (E/S ratio of 8.56, temperature of 23.51 °C and initial syrup concentration of 40 °B). There was a good agreement between the experimental data and their predicted counterparts, showing the effectiveness of the proposed conditions and reliability of Box–Behnken analysis on fructan precipitation. It was proved that PCA is able to extract relevant information and offer an easy and promising approach for the interpretation of chemical properties of produced fructan.

Acknowledgement

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