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Process optimisation for fractionating Jerusalem artichoke fructans with ethanol using response surface methodology

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Abstract

Optimal conditions for fructan precipitation from Jerusalem artichoke concentrate by adding ethanol were established by response surface methodology. The combination of the starting syrup concentration (9.9–40 °B), ethanol-to-syrup (E/S) ratio (2.3:1–15.7:1) and precipitation temperature (3.1–62 °C) was varied at design points of a central composite rotatable design. Significant regression models describing the changes of average chain length, fructan precipitation yield and purity value of fructans were developed with the coefficient of determination greater than 0.8. The results suggested that higher syrup concentration resulted in an increased fructan yield, but further increase in concentration had a reverse effect on average chain length and purity values. The optimum conditions for fractionating fructan composition by ethanol were estimated to be 32 °B initial syrup concentration, E/S ratio of 13 and temperature of 42 °C.

Keywords: Fructans; Jerusalem artichoke; Ethanol precipitation; Response surface methodology

1. Introduction

Jerusalem artichoke (JA-*Helianthus tuberosus* L.) is native to North America and is presently cultivated in Europe, Asia and Australia. The tubers accumulate high levels of polysaccharides (fructans) during their growth. On a dry weight basis, the tubers contain 68–83% fructans; 15–16% proteins; 13% insoluble fibre and 5% ash (Fleming & GrootWassink, 1979). Remarkably, the JA tubers do not contain starch. It has traditionally been used as food and animal feed and, more recently, as a raw material for the industrial production of fructose and fructans (Kosaric, Cosentino, Wieczorek, & Duvnjak, 1984). The fructans are composed of linear β-D-(2 → 1)-linked fructose units and generally have a terminal unit (Waterhouse, 1993). They are characterised by the degree of polymerisation (DP_n) defined as the number of fructosyl units linked to the terminal glucose. By applying this criterion, fructans are classified being either as inulin ($DP_n > 10$) or oligofructose ($DP_n < 10$) (Cabezas, Rabert, Bravo, & Shene, 2002). The distribution of chain lengths in JA tubers is dependent on the cultivar and time of harvest (Baldini, Danuso, Turi, & Vannozzi, 2004).

Fructans are not digestible and are not absorbed in the human small intestine, but do promote the proliferation of beneficial bacteria particularly bifidobacteria in the large intestine. They induce fibre effects on gut function, including relief of constipation, reduction in stool pH and increase in stool weight and frequency (Franck, 2000). Human and animal studies have also shown that fructan-containing foods enhance mineral absorption, reduce acylglycerol and cholesterol levels, stimulate the immune system and suppress intestinal infection (Kaur & Gupta, 2002). Fructans play an important role in functional foods. The functional attributes of fructans including prebiotic activity, indigestibility, energy value and health promoting potential are dependent on the DP_n (Moerman, Van Leeuwen, & Delcour, 2004). Fructans with high DP_n have the ability to form food gels with high water-binding capacity and bestow similar rheological characteristics to those of fat. Therefore, they may be used as texture modifiers and

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fat replacers in food systems. On the other hand, low DP_n fructans are used as bulking agents, having a sweetness of 35% compared with sucrose, and thus can be utilised in foods for diabetic patients (Franck, 2000).

A number of studies have been conducted on the production of fructans with high DP_n. For this purpose, at least five fractionation techniques have been investigated, namely ultrafiltration (Berghofer, Cramer, Schmidt, & Veigl, 1993; Laurenzo, Navia, & Neiditch, 1999), crystallisation/precipitation from aqueous solution (Silver, 2003), treatment with solvent (Smits, Daenekindt, & Booten, 2001), treatment with enzymes (Kunz, Munir, & Vogel, 1995) and chromatographic separation (Van Leeuwen, Slaghek, De Wit, Kuzee, & Raaymakers, 1997). Many researchers have studied the effects of different types of solvents, and generally have used commercial standard grade inulin (Moerman et al., 2004). The technical processes for crude inulin extract concentration are neither well documented nor clearly understood.

To date, the conventional method, the so-called change-one-factor-at-a-time approach has been used for optimisation. As a result, a large number of experiments are needed to describe the effect of individual factors. This is not only laborious and time-consuming, but also suffers from major drawbacks of giving unreliable results and less accurate conclusions since it is unable to determine interactive effects among the factors. On the other hand, applying response surface methodology (RSM) for optimisation studies has been shown to overcome those weaknesses (Montgomery, 1996). RSM is a useful group of mathematical and statistical techniques, used for analysing the influence of several independent factors on one or more characteristics of the processes or product. These techniques have been successfully applied for optimising microbiological media composition, enzyme hydrolysis conditions and food processes (Faveri, Torre, Perego, & Converti, 2004; Pinelo, Rubilar, Sineiro, & Nunez, 2004).

In spite of more specific studies giving protocols for inulin production from several inulin-containing plants (Berghofer et al., 1993; Lopez-Molina et al., 2005; Mitchell & Mitchell, 1995), little is known about the mutual effects of different process conditions during inulin precipitation by ethanol. In addition, there have been no reports on the use of RSM to optimise the precipitation conditions. Accordingly, the present work has been undertaken to find optimum conditions for inulin precipitation by starting from a concentrated JA extract. The conditions were established on the basis of obtaining a high yield of fructans and having high purity as well as high average chain length.

2. Materials and methods

2.1. Jerusalem artichoke

Jerusalem artichoke tubers were obtained from local markets in Melbourne, Victoria, Australia during the harvest season from June to August, 2005. The tubers were cleaned in cold water to remove soil and soaked in 100 ppm sodium hypochlorite solution at 15 °C for 30 min to reduce microbial load before storing at 4 °C.

2.2. Preparation of fructan concentrate

To extract fructans, 2 kg lots of peeled tubers were chopped into fine pulp in 10 l of hot water containing 100 ppm sodium metabisulphite to minimise browning, in a Stephan Kettle (Model UMM/SK 24E, Germany) at 95–98 °C for 10 min. The resulting extract was filtered through muslin cloth and then concentrated to 50% of the original volume using a single-stage climbing film evaporator (James A Jobling & Co Ltd., England), operating under a vacuum of 68 kPa with steam supply at 138 kPa. The resulting concentrate was turbid due to the presence of particulate and colloidal matter, i.e., pectin, protein, and cell wall materials (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 ca. 8–9, causing the precipitation of excess calcium and coagulated organic material. The mixture was allowed to stand at 60 °C for 2–3 h before re-filtering (Whatman No. 4). The clarification process was repeated twice. Under these conditions, the dry matter of the clarified juice was ca. 7% (w/w). Activated carbon powder was added to the filtrates at 60 °C and mixed for 15–30 min in order to remove coloured materials. The treated syrup was filtered (Whatman No. 1) and the clear syrup obtained was further concentrated by rotary evaporation at $\leqslant$70 °C, to obtain syrups with five different soluble solids levels (9.9, 16, 25, 34 and 40.1 °B) which were then stored at −20 °C.

2.3. Precipitation in ethanol

Aliquots (2 g) of JA concentrate (9.9–40.1 °B) were weighed into preweighed test tubes and mixed with 2.3–15.7 parts by weight of ethanol (abs. 99%). The test tubes were vortexed and hermetically sealed before storage at temperatures 3.1, 15, 32.5, 50 and 61.9 °C for 3 days. After storage, the supernatents were removed by aspirator and the precipitates washed with 5 ml of ethanol, that was subsequently discarded and the tubes containing precipitates were placed in a hot-air oven at 102 °C for 30 min to remove excess solvent and reweighed. The precipitate, formed as a pasty substance, was then analysed for total carbohydrate, total fructans, reducing sugar and dry matter content. The following equations were used to calculate the fructan yield, average chain length, and purity value of fructans.

The fructan yield was calculated according to

$$Y_1 = \frac{F_P}{F_M} \times 100 \quad (1)$$

where Y_1 is fructan yield (%), F_P is the amount of fructans contained in the precipitate (g) and F_M is the amount of fructans in JA concentrate utilised for precipitation (g).

The average chain length was calculated according to

$$Y_2 = \frac{TC}{RS} \quad (2)$$

where Y_2 is average chain length, TC is total amount of carbohydrate (g) and RS is total amount of reducing sugar (g).

The purity value was calculated according to

$$Y_3 = \frac{F_P}{DM} \times 100 \quad (3)$$

where Y_3 is purity value and DM is the dry matter content of the precipitate (g).

2.4. Experimental design

A central composite rotatable design (CCRD) was adopted to evaluate the combined effects of the initial syrup concentration, E/S ratio and precipitation temperature (Arteaga, Li-Chan, Vazquez-Arteaga, & Nakai, 1994). Based on preliminary trials, five levels of each variable (Table 1) were selected to cover the process conditions (Kunz et al., 1995; Moerman et al., 2004; Smits et al., 2001). The axial distance was 1.68 which made this design orthogonal. The complete designs consisted of 20 combinations i.e., eight factorial points, six axial points and six centre points (Montgomery, 1996; Moyo, Gashe, Collison, & Mpuchane, 2003; Myers, 1976). Two replications were carried out for all design points except the centre points.

2.5. Analytical methods

2.5.1. Determination of total carbohydrate

Total carbohydrate was assayed colorimetrically using the Phenol–sulphuric acid method (Southgate, 1991). Sample weights were adjusted to obtain a reading of 10–70 μg. Sample solution (1 ml) was mixed with 1 ml of 5% phenol and 5 ml of sulphuric acid. The mixture was incubated in a water bath at 30 °C for 20 min. The solution appeared as yellow–orange colour and its absorbance was measured at 490 nm using UV–VIS spectrophotometer (model UV-1601, Shimadzu). A series of glucose solutions of known concentration were used to establish a standard curve.

2.5.2. Determination of reducing sugar

The amount of reducing sugar was determined spectrophotometrically at 440 nm using *para*-hydroxy benzoic acid hydrazide (PHBAH) reagent. Using the method of Southgate (1991), 1 ml of sample solution was added to 5 ml of reagent and subsequently boiled for 6 min. Calibration curves were prepared using glucose as standard.

2.5.3. Determination of fructan content

The enzymatic, spectrophotometric method was used for quantitative fructan determination according to the method of McCleary and Blakeney (1999). The samples were analysed using a commercial kit supplied by Megazyme International (Deltagen Australia Pty. Ltd., Australia).

2.5.4. Determination of dry matter

Dry matter content of precipitate was determined gravimetrically (AOAC, 1990). To facilitate transfer of the precipitate from the test tube to drying dish, this was first dissolved with 10 ml of distilled water. The sample was then dried on a steam bath, before further drying in a hot-air oven at 102 °C until the weight remained constant. Each determination was performed in duplicate.

2.6. Statistical analysis

The least square regression methodology by SPSS 10.0 for Windows (SPSS, USA) was used to fit the data to the second-order equations:

$$Y = b_0 + \sum_{i=1}^{3} b_i X_i + \sum_{i=1}^{3} b_{ii} X_i^2 + \sum_{i<j=1}^{3} b_{ij} X_i X_j \quad (4)$$

where Y is dependent or response variable; b_0, b_i, b_{ii} and b_{ij} are intercept, linear, quadratic and interaction coefficients, respectively; and X_i and X_j are independent variables (Myers, 1976). The Student's t-test was employed to evaluate the statistical significance of regression coefficients. Non-significant terms ($p > 0.05$) were deleted from the second-order polynomial and a new polynomial was recalculated to obtain a predictive model for each dependent

Table 1
Independent variables and their levels used for this study

Variables	Symbols		Levels[a]				
	Coded	Uncoded	−1.68 (−α)[b]	−1	0	1	−1.68 (−α)
Syrup concentration (°B)	X_1	A	9.9	16.0	25.0	34.0	40.1
E/S ratio	X_2	B	2.3	5.0	9.0	13.0	15.7
Temperature (°C)	X_3	C	3.1	15.0	32.5	50.0	61.9

[a] Transformation of variable levels from coded to uncoded could be calculated using: $X_1 = (A - 25)/9$, $X_2 = (B - 9)/4$ and $X_3 = (C - 32.5)/17.5$.
[b] Levels based on the central composite rotatable design.

variable (Faveri et al., 2004). Once the fitted regression equations were determined, the response surface plots were drawn using STATISCA software (version 5.0, Stasoft Inc., USA). One independent factor was kept constant at a centre point and the other two factors were varied within the experimental range. The optimum set of conditions was searched using Design Expert software (trial version 7.0.2, Stat-Ease Inc., USA).

3. Results and discussion

3.1. Model fitting

The combined effects of syrup concentration, E/S ratio and temperature on fructan precipitation yield, average chain length and purity are presented in Table 2.

Table 3 shows the coefficients of variables in the models calculated by the least square technique and their statistical significances were judged by Student's *t*-test at a probability of 0.001, 0.01 or 0.05. The table also summarises the statistical parameters, namely the determination coefficient (R^2) and *F*-test probability, both of which are used for measuring the correlation and significance of the models. Results of the R^2 values showed a good agreement between experimental data and predicted data for all regressions (0.865, 0.888 and 0.815 for Y_1, Y_2 and Y_3, respectively). The results of the *F*-test showed a statistically significant relationship between the variables within a 95% confidence interval.

3.2. Effects of precipitation conditions on inulin precipitation yield

The results in Table 3 indicate that the initial syrup concentration had a strong linear effect on fructan precipitation yield, followed by linear effect of E/S ratio. Analysis of regression coefficients also indicated that temperature

Table 3
Regression coefficients, R^2 and *F*-test probability for three dependent variables

Regression coefficients	Y_1	Y_2	Y_3
b_0 (constant)	56.925*	22.147*	70.676*
b_1	10.513*	3.501*	2.750**
b_2	4.703***	0.750	0.054
b_3	−1.187	0.397	1.000
b_1^2	−2.221	−2.158**	−2.485***
b_2^2	−1.735	−1.638***	2.050***
b_3^2	−0.273	−1.479***	−1.955***
b_{12}	−1.137	−1.179	0.395
b_{13}	0.670	−0.206	0.350
b_{23}	1.687	1.136	1.475
R^2	0.865	0.888	0.815
F-test probability	0.003**	0.001*	0.010***

Subscripts: 1 = syrup concentration; 2 = E/S ratio; 3 = precipitation temperature.
R^2 = coefficient of determination.
* Significant at 0.001 level.
** Significant at 0.01 level.
*** Significant at 0.05 level.

Table 2
Scheme of central composite rotatable design with the results of independent variables on three dependent variables

Run[a]	Independent variables						Dependent variables		
	Coded level			Uncoded level					
	X_1[b]	X_2[c]	X_3[d]	A[b]	B[c]	C[d]	Y_1	Y_2	Y_3
1	−1	−1	−1	16.0	5.0	15.0	44.41	11.87	67.78
2	−1	−1	+1	16.0	5.0	50.0	34.86	10.50	63.63
3	−1	+1	−1	16.0	13.0	15.0	45.60	11.80	60.50
4	−1	+1	+1	16.0	13.0	50.0	46.48	16.42	68.28
5	+1	−1	−1	34.0	5.0	15.0	67.61	22.65	71.64
6	+1	−1	+1	34.0	5.0	50.0	64.42	21.90	74.92
7	+1	+1	−1	34.0	13.0	15.0	67.93	19.31	71.97
8	+1	+1	+1	34.0	13.0	50.0	67.81	21.66	75.12
9	−1.68	0	0	9.9	9.0	32.5	33.52	12.01	61.09
10	1.68	0	0	40.1	9.0	32.5	61.56	19.67	63.53
11	0	−1.68	0	25.0	2.3	32.5	34.73	14.94	74.30
12	0	1.68	0	25.0	15.7	32.5	63.10	19.68	75.99
13	0	0	−1.68	25.0	9.0	3.1	54.31	17.59	62.74
14	0	0	1.68	25.0	9.0	61.9	51.79	17.93	64.88
15	0	0	0	25.0	9.0	32.5	59.41	25.10	70.39
16	0	0	0	25.0	9.0	32.5	59.35	23.93	71.44
17	0	0	0	25.0	9.0	32.5	55.54	20.13	71.60
18	0	0	0	25.0	9.0	32.5	55.04	21.85	70.19
19	0	0	0	25.0	9.0	32.5	58.12	20.89	70.86
20	0	0	0	25.0	9.0	32.5	55.15	21.05	70.03

Y_1, Y_2 and Y_3 represent fructan yield (%), average chain length and purity value (%), respectively.
[a] Does not corresponded to order of processing.
[b] X_1 and A, initial syrup concentration (°B).
[c] X_2 and B, ethanol-to-syrup ratio.
[d] X_3 and C, precipitation temperature (°C).

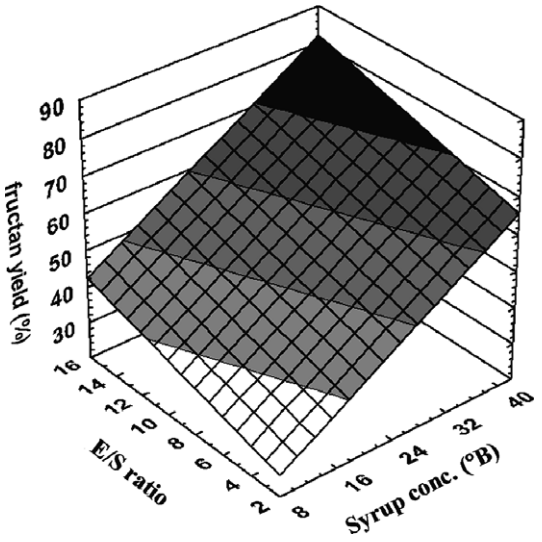

Fig. 1. Response surface graph of fructan precipitation yield (%) as a function of initial syrup concentration (°B) and E/S ratio.

had no significant effect on the yield, and that neither quadratic terms nor all interaction terms were significant ($p > 0.05$). After rejecting the statistically insignificant terms, the predictive model for the yield was found to be a first-order equation. As a result, the response surface plot generated for the fructan yield showed flat areas and no maximum or minimum responses were present (Fig. 1). It was observed that the precipitation yield increased linearly as the initial syrup concentration increased. However, in order to achieve more than 50% fructan yield from the precipitation, syrup with a minimum concentration of 16 °B should be utilised. It is also observed that for the same level of syrup concentration, it was necessary to raise the E/S ratio for those to be an increase in the yield of fructan.

3.3. Effects of precipitation conditions on average chain length

The average chain length was calculated by dividing the total number carbohydrate present in the precipitate by the

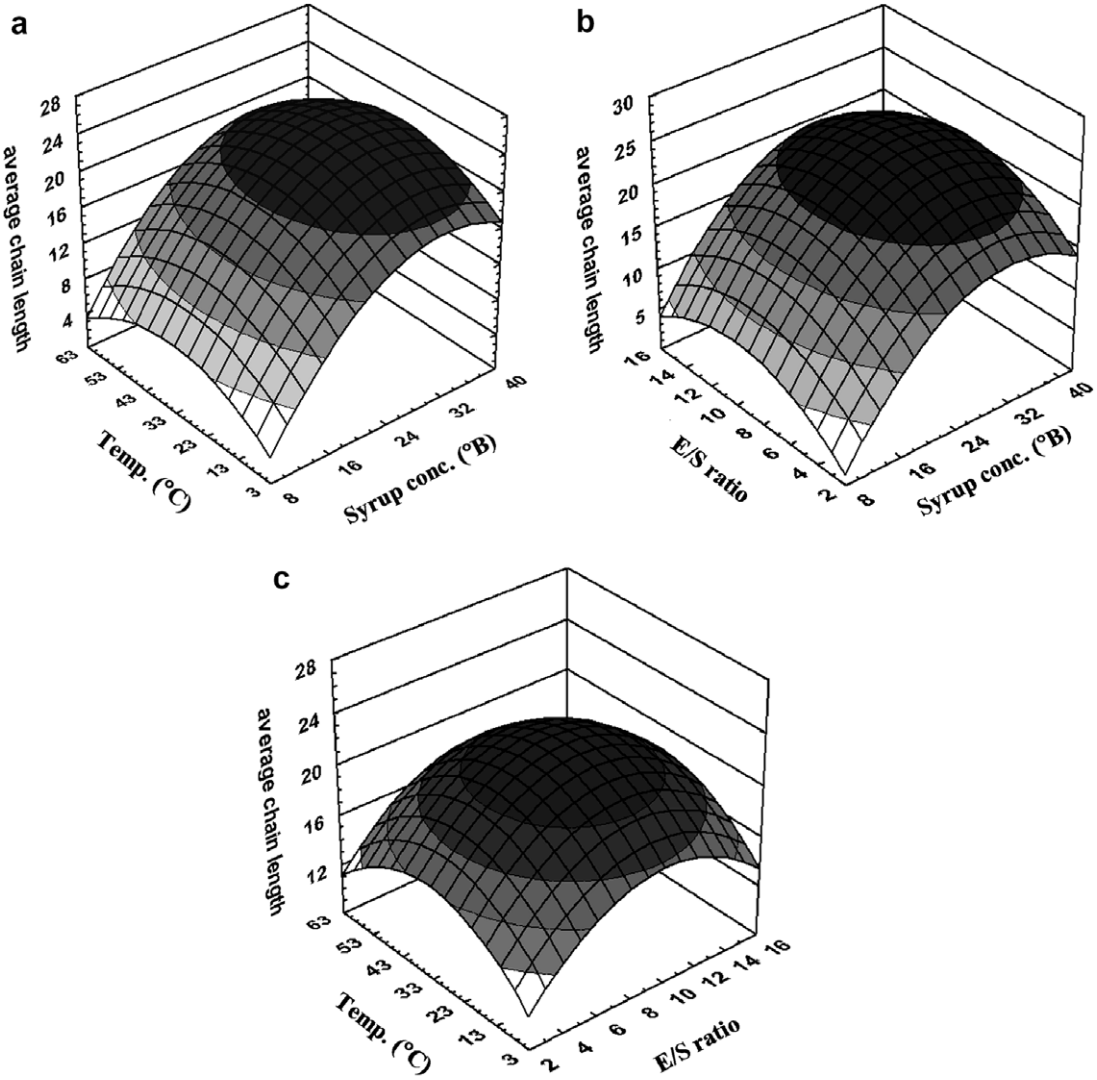

Fig. 2. Response surface graphs of average chain length at (a) E/S ratio of 9:1, (b) temperature of 32.5 °C and (c) syrup concentration of 25 °B.

amount of reducing end groups. It is considered as a qualitative index of the precipitate since it is closely related to the functional properties when used in food applications. The average chain length also indicates the ability of the production process to avoid excess hydrolysis of polysaccharides or lower molecular weight carbohydrates. Higher average chain length values indicate the presence of a higher proportion of long fructan chains.

The concentration of syrup was the most significance determinant of the average chain length in precipitate since its linear and quadratic effects mainly contributed to the total variation (Table 3). The relationship between three independent variables on average chain length is demonstrated in Fig. 2. The behaviour of response surface graphs (Fig. 2a and b) indicated that increasing syrup concentration up to 32 °B had a positive effect on average chain length in the precipitate. However, there seemed to be less effect of increased syrup concentration on the chain length. As can be seen in Fig. 2a and b, a similar average chain length was obtained from syrup with concentrations of 24 and 40 °B. The effects of E/S ratio and temperature on the average chain length were similar to the effect of syrup concentration (Fig. 2c). It was found that the optimum precipitation should be carried out at 33 °C with E/S ratio of 10:1 to yield the highest average chain length and any further increase of both resulted in lower average chain length. The results of this study were in agreement with those of Moerman et al. (2004) who found that the average chain length of commercial grade chicory inulin and dahlia inulin decreased with more added solvent (ethanol, acetone or methanol). It is believed that the initial increase in solvent amount leads to the increase of average chain length due to the induced precipitation of longer fructan chains. Nonetheless, excess solvents accelerate the precipitation of the shorter chain carbohydrates resulting in a decrease in average chain length of the precipitate (Ku, Jansen, Oles, Lazar, & Rader, 2003; Moerman et al., 2004).

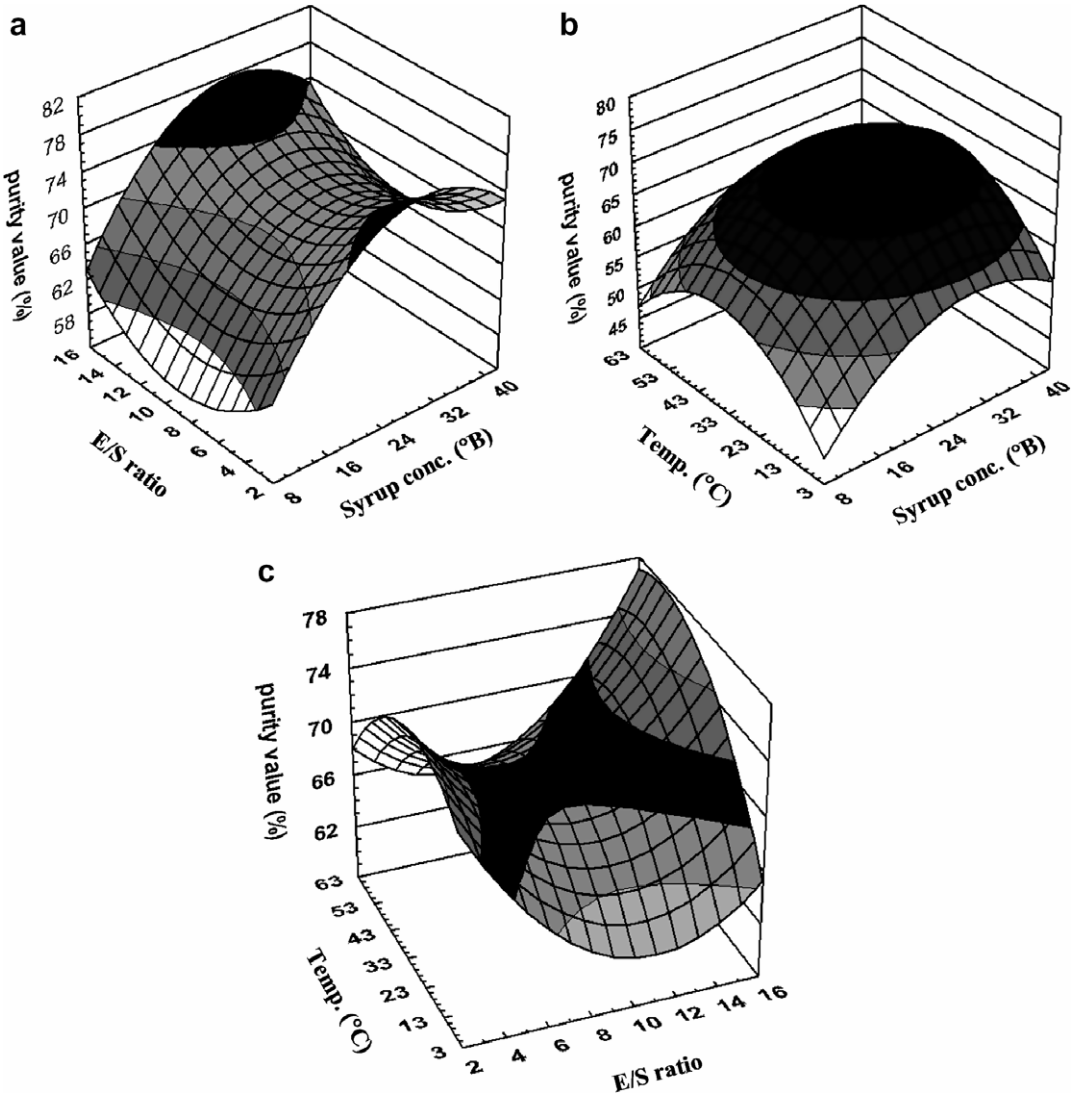

Fig. 3. Response surface graphs of purity value (%) at (a) temperature of 32.5 °C, (b) E/S ratio of 9:1 and (c) syrup concentration of 25 °B.

3.4. Effects of precipitation conditions on purity of fructans

Purity represents an index of the quantity of fructans in the precipitate obtained. High values mean that fructans represent as the main components of the precipitate. Low purity value, on the other hand, indicates the high contents of oligosaccharides, and/or non-sugar substances including ash in the precipitate. Fig. 3 describes the dependence of purity value on syrup concentration, E/S ratio and temperature. With the increase in syrup concentration, the purity of precipitate increased gradually, but decreased after the concentration reached a maximum at 30 °B (Fig. 3a and b). The effects of E/S ratio and temperature were not profound in comparison to syrup concentration. At constant temperature of 32.5 °C, the precipitates with syrup concentration between 8 and 24 °B showed a high purity if using intermediate E/S ratios (8:1–10:1). It was also found that either low E/S ratio (less than 7:1) or high E/S ratio (more than 11:1) was required when syrup concentration was between 24 and 36 °B (Fig. 3a). Fig. 3c presents the combined effects of temperature and E/S ratio on the purity of fructan at syrup concentration of 25 °B. It was found that using moderate temperature between 23 and 43 °C resulted in high purity values, particularly when using either extremely low or high ratios of E/S. The maximum response was obtained at 37 °C.

3.5. Optimisation of precipitation conditions

The optimum precipitation conditions for JA fructan concentrate were achieved by means of graphical techniques using design expert software (Faveri et al., 2004). Three contour plots generated from the predicted equations were superimposed to achieve the experimental region giving desired values of the responses (Arteaga et al., 1994). In this study, the independent variables would be considered optimal if all dependent variables were as high as possible. Therefore, the criteria applied for the graphical optimisation were $68\% > Y_1 > 50\%$, $Y_2 > 20\%$ and $76\% > Y_3 > 70\%$. Fig. 4 shows the overlaid plot of three dependent variables. The white area represents the region produced by the criteria outlined above and the flag depicted within the same area indicates the optimised point. The best combination of process variables was found to be the initial syrup concentration of 32 °B, E/S of 13:1 and temperature of 42 °C. Under these conditions, the model gave predicted values of Y_1, Y_2 and Y_3 being 66%, 22% and 75%, respectively.

3.6. Verification of the results

Verification experiments were carried out to confirm the adequacy of the models for predicting the values of dependent variables. The experimental results obtained under the selected conditions from RSM optimisation and the predicted values are listed in Table 4. An experimental fructan yield of 65% with an average chain length of 21 was close to the predicted values since the R^2 values of the models were reasonably high (Table 3). Meanwhile, the fructan purity of 75% (Table 4) did not closely match the experimental value (68%), possibly reflecting the lower R^2 value of the model (0.815), indicating that some of the variability was not predicted by the model.

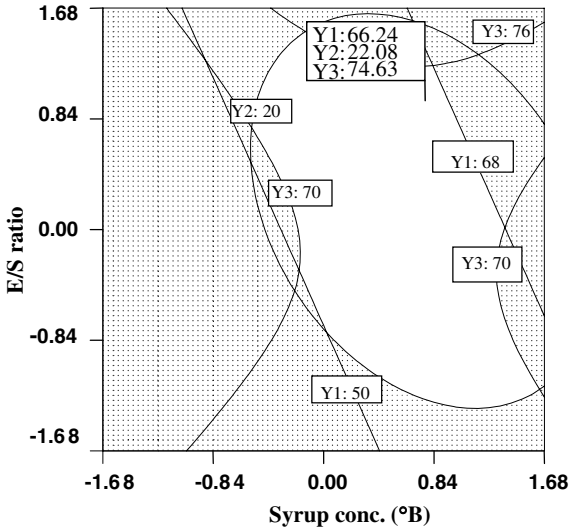

Fig. 4. Optimum region identified by the overlaid plots of the three responses: fructan yield (Y_1), average chain length (Y_2), purity value (Y_3).

Table 4
Experimental and predicted values of verification experiment under optimum conditions[a]

Dependent variables[b]	Experimental values[c]	Predicted values
Y_1	64.85 ± 0.76	66.23
Y_2	21.40 ± 0.35	22.08
Y_3	68.30 ± 1.04	74.63

[a] Optimum reaction conditions: JA syrup concentration 32 °B, E/S 13:1 and temperature 42 °C.
[b] Y_1, Y_2 and Y_3 represent fructan yield (%), average chain length and purity value (%), respectively.
[c] Results shown are mean ± standard deviation for three experiments each with two replications.

4. Conclusions

Response surface methodology which combines factorial designs and regression analysis is a useful tool for optimising process conditions for fructan precipitation from JA concentrate. The models developed allowed not only identification of the optimum reaction conditions (32 °B syrup concentration with 13:1 ethanol-to-syrup ratio at 42 °C) but also the prediction of the quality of fructan precipitate. Good agreement was found between the values predicted and those determined experimentally, particularly for the inulin precipitation yield and the average chain length.

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