JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Selective Recovery of Tagatose from Mixtures with Galactose by Direct Extraction with Supercritical CO_2 and Different Cosolvents

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A selective fractionation method of carbohydrate mixtures of galactose/tagatose, using supercritical CO_2 and isopropanol as cosolvent, has been evaluated. Optimization was carried out using a central composite face design and considering as factors the extraction pressure (from 100 to 300 bar), the extraction temperature (from 60 to 100 °C), and the modifier flow rate (from 0.2 to 0.4 mL/min, which corresponded to a total cosolvent percentage ranging from 4 to 18% vol). The responses evaluated were the amount (milligrams) of tagatose and galactose extracted and their recoveries (percent). The statistical analysis of the results provided mathematical models for each response variable. The corresponding parameters were estimated by multiple linear regression, and high determination coefficients (>0.96) were obtained. The optimum conditions of the extraction process to get the maximum recovery of tagatose (37%) were 300 bar, 60 °C, and 0.4 mL/min of cosolvent. The predicted value was 24.37 mg of tagatose, whereas the experimental value was 26.34 mg, which is a 7% error from the predicted value. Cosolvent polarity effects on tagatose extraction from mixtures of galactose/tagatose were also studied using different alcohols and their mixtures with water. Although a remarkable increase of the amount of total carbohydrate extracted with polarity was found, selective extraction of tagatose decreased with increase of polarity of assayed cosolvents. To improve the recovery of extracted tagatose, additional experiments outside the experimental domain were carried out (300 bar, 80 °C, and 0.6 mL/min of isopropanol); recoveries >75% of tagatose with purity >90% were obtained.

KEYWORDS: Tagatose; galactose; monosaccharides; supercritical fluid extraction; fractionation; cosolvents

INTRODUCTION

Tagatose, an isomer of galactose, occurs naturally in some lichens (*1*), and it is also formed in small amounts from lactose during heat treatment of milk (*2*, *3*). Although it has approximately the same sweetness as sucrose and has the bulk of sucrose, tagatose provides only 1.5 cal/g in contrast to 4 cal/g for sucrose; it has been affirmed as generally recognized as safe (GRAS) in the United States, and the Joint FAO/WHO Expert Committee on Food Additives has recommended tagatose for use as a food additive (*4*, *5*). Ingested tagatose is incompletely absorbed from the small intestine but selectively stimulates the growth and activity of beneficial bacteria in the colon (*6*).

Tagatose may be obtained from galactose using chemical methods involving isomerization of galactose in the presence of basic catalysts such as calcium hydroxide (*7*) and aluminates (*8*). The best source of D-galactose is lactose from cheese whey. Upon acid- or enzyme-catalyzed hydrolysis, lactose yields an equimolar mixture of D-glucose and D-galactose, and the two monosaccharides can be separated before isomerization or used directly in the isomerization step and the glucose and its isomerization products separated afterward. The chemical methods have disadvantages, including complex purification steps, byproduct formation due to the alkaline reaction conditions, and chemical waste formation (*9*). Biological tagatose production from galactose using L-arabinose isomerase has received much research attention during the past few years, and commercially viable processes have been reported (*9*−*12*). Although considerable bioconversion yield can be achieved, removal of the remaining galactose is required.

Previous studies on solubility enhancement of carbohydrates in supercritical carbon dioxide have shown that, using polar

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10.1021/jf0618123 CCC: $33.50
Published on Web 09/26/2006

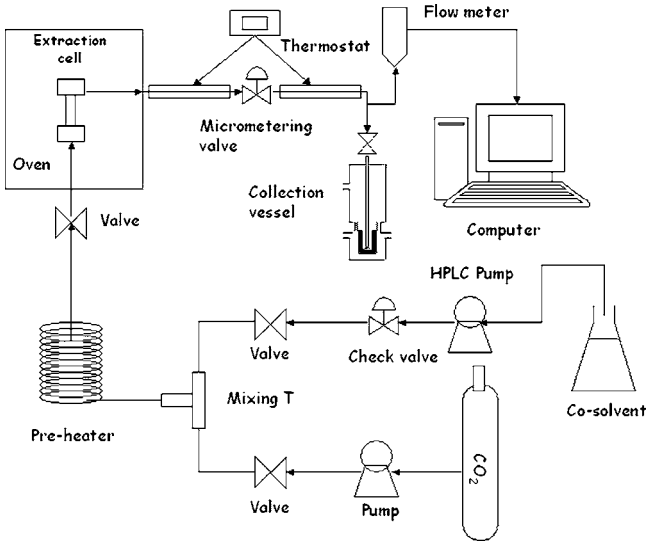

Figure 1. Scheme of the supercritical fluid extractor used in this work.

cosolvents, solubility may considerably increase, and separation of carbohydrates from mixtures was possible (*13*).

The aim of the present work was to develop an extraction process based on the use of supercritical carbon dioxide with isopropanol as cosolvent for recovery of tagatose from mixtures with galactose. Further experiments have been done to test the efficiency of different cosolvents in the recovery and purity of the tagatose extracted.

MATERIALS AND METHODS

Samples and Reagents. Reagents employed for GC analysis including sugar standards [D-(+)-galactose and D-(−)-tagatose], internal standard (phenyl-β-glucoside), methanol, and two derivatizing reagents (*N*-trimethylsilylimidazole and chlorotrimethylsilane redistilled) were obtained from Sigma (St. Louis, MO). The other derivatizing agent, pyridine dried, was supplied by Merck (Darmstadt, Germany). 1-Propanol extra pure and 2-propanol extra pure were purchased from Scharlau Chemie S.A. (Barcelona, Spain). Sea sand, glass wool washed chemically pure, and 1-butanol were acquired from Panreac Química S.A. (Barcelona, Spain). Ethanol absolute was from Prolabo (Fontenay sous Bois, France). Ultrapure water quality (18.2 MΩcm) with 1−5 ppb of TOC and <0.001 EU/mL pyrogen levels (Milli-Q) was produced in-house using a laboratory water purification Milli-Q Synthesis A10 system (Millipore, Bellerica, MA) and was used throughout. The carbon dioxide liquefied at high pressure used in supercritical extraction was supplied by Praxair Inc. (Danbury, CT).

Supercritical Fluid Extraction (SFE) System. Figure 1 shows a diagram of the supercritical fluid extractor used to perform the experiments. The system is based on a Suprex Prep Master (Suprex Corp., Pittsburgh, PA) with several modifications. It has a thermostatic oven heated by air convection where the extraction cell containing the sample is placed. A Waters 510 HPLC pump (Waters Corp., Milford, MA) was used to introduce the modifier in the extraction system. A preheater system was employed by placing a heating coil inside a glycerine bath (JP Selecta Agimatic N, JP Selecta S.A., Abrera, Spain) to guarantee that the fluid employed in all of the experiments reaches the extraction cell at the target temperature. After the modifier pump, a check valve (Swagelok SS-CHS2-BU-10, Swagelok Corp., Solon, OH) was used. A micrometering valve (Hoke SS-SS4-BU-VH, Hoke Inc., Spartanburg, SC) was placed after the extraction cell to manually control the flow, and a computer-controlled mass flow meter (EL-FLOW Mass Flow Meter/Controller F-111C, Bronkhorst High-Tech BV, AK Ruurlo, The Netherlands) was used to adjust the carbon dioxide flow rate to the values selected for each experiment.

Extraction processes were performed on samples consisting of 1 part of either 70:30 or 30:70 tagatose/galactose mixed with either 4 or 9 parts of sea sand in the mill (Janke and Kunkel IKA A-10, Labortechnik, Staufen, Germany).

The sea sand was selected as inert material to hold the sample inside the extraction cell and to improve efficiency while avoiding formation of preferential flow paths. One gram of the carbohydrate−sea sand mixture was introduced into the extraction cell and packed with glass wool. Once the experimental conditions were reached, the extraction solvent (consisting of a mixture of CO_2 and cosolvent) passed through the extraction cell for 2 h.

After depressurization, extracts were collected in a collection vessel described previously (*14*). Inside the collection vessel, a 30 mL volume glass vial was placed to recover the extracts. The collected extracts were weighed with an analytical balance.

Experimental Design. The effects of three factors, pressure (*P*), extraction temperature (*T*), and modifier flow rate (*M*), on the amount of tagatose (Ta) and galactose (Ga) extracted [also Ta and Ga recovery (%)] were studied using a central composite face (CCF) design. Isopropanol was selected as cosolvent for the selective extraction of monosaccharides. A total of 17 experiments (2^3 points of a factorial design, 6 star points placed on the faced of the sides, and 3 center points to verify the experimental errors) were carried out in randomized run order. By using this design, the three variables were tested at three different experimental levels: extraction pressure at 100, 200, and 300 bar; extraction temperature at 60, 80, and 100 °C; and modifier at 0.2, 0.3, and 0.4 mL/min in correspondence with the coded levels −1, 0, and +1, respectively. The response variables selected were the amounts of tagatose (Ta) and galactose (Ga) extracted and the corresponding recoveries (percent dry weight). **Table 1** shows the experimental matrix design, with the experimental levels of the independent variables (factors), along with the results obtained for the response analyzed variables. The quadratic model proposed for each one of the variables (Y_i) was

$$Y_i = \beta_0 + \beta_1 P + \beta_2 T + \beta_3 M + \beta_{1,1} P^2 + \beta_{2,2} T^2 + \beta_{3,3} M^2 + \beta_{1,2} PT + \beta_{1,3} PM + \beta_{2,3} TM + \epsilon \quad (1)$$

where β_0 is the intercept, β_i the first-order model coefficient, $\beta_{i,i}$ the quadratic coefficient for the *i*th variable, $\beta_{i,j}$ the interaction coefficients for the interaction of variables *i* and *j*, and ϵ the error variable. The parameters of the model were estimated by multiple linear regression (MLR) using the Statgraphics Plus v. 5.1 program (Statistical Graphics Corp., Manugistics Inc., Rockville, MD, 2000). This program permits the creation and analysis of experimental designs. The effect of each term in the model, and their statistical significance, for each of the response variables, was analyzed from the standardized Pareto chart. The terms not significantly different from zero ($P > 0.10$) were excluded from the model, and the mathematical model was refitted by MLR. The goodness of fit of the model was evaluated by the coefficient of determination (R^2) and the residual standard deviation (RSD). From the new fitted models, the surface plot of response variables, and the optimum conditions that maximized each of the response variables were obtained.

For mean comparison a two-sample *t* test was used (Statgraphics Plus v. 5.1.).

GC Analysis of Galactose and Tagatose. *Sample Preparation.* Half a milliliter of the extract collected in the collection vessel was added to 0.5 mL of a solution of 0.01% (w/v) phenyl-β-D-glucoside in methanol/water (70:30, v/v) as internal standard. Prior to derivatization, this sample was dried at 38−40 °C in a Rotavapor R-200 (from Büchi Labortechnik AG, Flawil, Switzerland).

Derivatization and GC Analysis. The dried mixtures were added to 100 μL of pyridine, 100 μL of *N*-trimethylsilylimidazole, and 100 μL of chlorotrimethylsilane for silylation; the reaction was carried out instantly at room temperature. Silylated carbohydrates were extracted with 0.1 mL of hexane and 0.2 mL of water. Volumes in the range of 2−3 μL of the organic phase containing silyl derivatives were injected onto the column.

The trimethylsilyl ethers were analyzed as previously described (*14*) using a 30 m × 0.32 mm internal diameter and 0.5 μm film fused silica capillary column SPB-17, bonded, cross-linked phase poly (50% diphenyl/50% dimethylsiloxane) (Supelco, Bellefonte, PA). Separation

Table 1. Experimental Matrix Design and Results Obtained for Each of the Response Variables Studied Using a 70:30 wt % Mixture of Tagatose/Galactose

	factors			response variables					
expt no.	*P* (bar)	*T* (°C)	*M* (mL/min)	extracted Ta (mg)	extracted Ga (mg)	Ta purity (%)	Ga purity (%)	recovery of Ta (%)	recovery of Ga (%)
1	100	60	0.2	0.676	0.067	90.99	9.01	0.97	0.22
5[a]	100	60	0.4						
9	100	80	0.3	6.859	0.863	88.82	11.18	9.80	2.88
3	100	100	0.2	0.913	0.124	88.06	11.94	1.30	0.41
7	100	100	0.4	18.965	3.151	88.72	11.28	27.09	10.50
11	200	60	0.3	4.616	0.397	92.09	7.91	6.59	1.32
13	200	80	0.2	3.900	0.387	90.98	9.02	5.57	1.29
15	200	80	0.3	5.804	0.797	87.92	12.08	8.29	2.66
16	200	80	0.3	4.811	0.492	90.72	9.28	6.87	1.64
17	200	80	0.3	5.553	0.717	88.56	11.44	7.93	2.39
14	200	80	0.4	20.853	2.217	90.39	9.61	29.79	7.39
12	200	100	0.3	6.898	0.685	90.95	9.05	9.85	2.28
2	300	60	0.2	1.932	0.203	90.51	9.49	2.76	0.68
6	300	60	0.4	26.341	2.504	91.32	8.68	37.63	8.35
10	300	80	0.3	7.992	0.746	91.47	8.53	11.42	2.49
4	300	100	0.2	4.416	0.394	91.80	8.20	6.31	1.31
8	300	100	0.4	24.034	3.108	88.55	11.45	34.33	10.36

[a] This experiment was done in a two-phase (liquid–vapor) region and was not considered in the statistical analysis.

was performed at 165 °C for 13 min, followed by an increase to 270 °C at rate of 50 °C/min and keeping this temperature for 5 min. The temperatures of the injector and detector were 280 and 300 °C, respectively. Injections were carried out in split mode 1:50. Data were acquired by means of HP ChemStations (Agilent Technologies Inc., Wilmington, DE).

RESULTS AND DISCUSSION

As has been mentioned, previous studies have demonstrated the importance of using polar cosolvents, mainly alcohols, to enhance the solubility of carbohydrates in supercritical CO_2 (*13*). On the basis of previous experience, isopropanol was selected as polar cosolvent because it was expected to provide reasonable extraction yields for monosaccharide extraction and enough selectivity between tagatose and galactose.

As mentioned under Materials and Methods, **Table 1** lists the values of the responses obtained for all of the experiments corresponding to the matrix design. Tagatose with a high degree of purity was obtained in all assayed conditions, and no significant differences were found in the tagatose purity achieved among experiments.

MLR was applied to estimate the parameters of the proposed model (eq 1) for each of the two response variables considered, which were related to the amount of monosaccharide extracted. Experiment 5 ($P = 100$ bar, $T = 60$ °C, $M = 0.4$ mL/min) was excluded from the statistical analysis because two phases (liquid−vapor) were formed at these conditions, the extraction solvent therefore not being a homogeneous state. The rest of the combinations of *P*, *T*, and *M* lead to supercritical conditions, so it seems reasonable to fit a mathematical model to represent the fractionation of the monosaccharides only under supercritical extraction conditions. **Figure 2** shows the standardized Pareto charts for the amount of tagatose (Ta) and galactose (Ga) extracted, showing the importance and statistical significance of the different terms in the model. Pareto charts are presented only for the amounts of Ta and Ga because recoveries are correlated, and therefore the significance of the different terms has to be similar. The effects, computed as twice the MLR coefficients for centered and scaled factors, are plot-sorted (in absolute value) in descending order. As can be seen, the terms that have the strongest influence on the response variables are, for tagatose extraction, the modifier (*M*) and its quadratic term

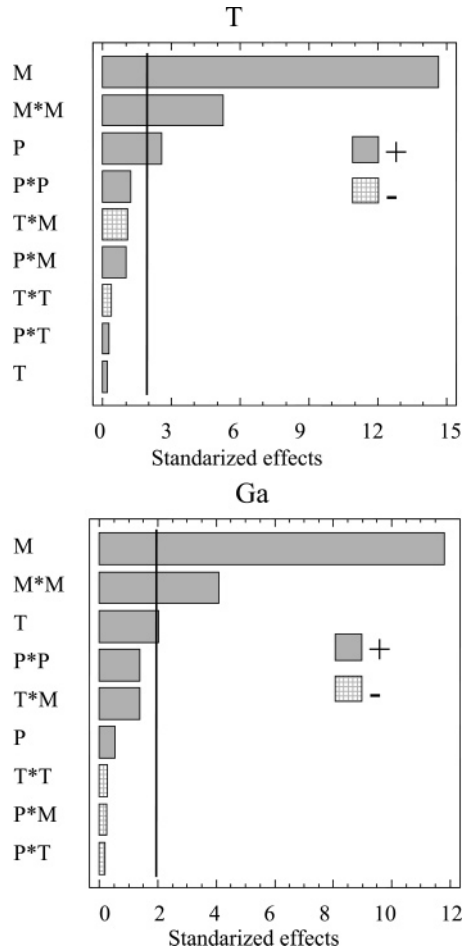

Figure 2. Standardized Pareto chart plot with the effect of each term in the model divided by its standard error for two response variables (milligrams of tagatose extracted and milligrams of galactose extracted). The vertical line in the chart tests the significance of the effects at the 95% confidence level. *P* = extraction pressure; *T* = extraction temperature; *M* = modifier flow rate.

($M \times M$) and the extraction pressure (*P*) or, for galactose extraction, extraction temperature (*T*), all of them having a positive effect (**Figure 2**). This means that, in the experimental

Table 2. Regression Coefficients for Unscaled Factors and Statistics for the Fit Obtained from MLR[a]

	Response Variables	
terms of the model	amount of Ta (mg)	amount of Ga (mg)
constant	32.3957	4.9681
P	–0.01138*	
T		–0.01339*
M	–276.007*	–39.719*
P × *P*		
T × *T*		
M × *M*	586.107*	75.675*
P × *T*		
P × *M*	0.10015**	
T × *M*		0.07837**
	Statistics for Goodness of Fit of the Model	
R^2	0.973	0.965
RSD	1.502	0.230

[a] R^2, determination coefficient; RSD, residual standard deviation; *, regression coefficient significantly different from zero, $p < 0.05$; **, regression coefficient significantly different from zero, $p < 0.10$.

region tested, the amount of monosaccharides extracted will be maximum by increasing the amount of modifier while keeping the extraction solvent at supercritical conditions. An increase in the extraction pressure or the extraction temperature is also expected to increase the amount of sugars extracted.

Table 2 shows the results for the mathematical model refitted by MLR after removal of the terms not significantly different from zero; the following information is included: the regression coefficients obtained, for unscaled factors, the determination coefficient (R^2), and the residual standard deviation (RSD). From these results it can be seen that the fraction of variation of response explained by the model (R^2) was >96%. **Figure 3** shows the surface plot for the two selected response variables (amounts of Ta and Ga). The amount of Ta is represented as a function of pressure and modifier and considering a medium value of temperature, that is, 80 °C. On the other hand, the amount of Ga is represented as a function of temperature and modifier, which were the factors that mostly contribute to the recovery of this compound, considering a medium pressure equal to 200 bar. By analyzing the plots and considering a maximization of the response for the amount of Ta extracted, it can be seen that an increase in modifier yields a huge increase in the amount of Ta recovered from the mixture, whereas an increase in pressure slightly improves the response. The optimum conditions of the extraction process to get the maximum recovery of Ta, provided by the statistical program using the fitted model in **Table 2**, are the following: extraction pressure equal to 300 bar, extraction temperature equal to 60 °C, and modifier equal to 0.4 mL/min. By using these conditions, the predicted value was 24.37 mg of tagatose (35% recovery). What is also true is that, at these conditions, also a maximum amount of galactose is recovered but, considering that the selectivity has been previously tuned by selecting the appropriate modifier of the polarity of the CO_2, this means only 8% of galactose in the final product. As can be seen, the optimum conditions are coincident with experiment 6 of the experimental design. The amount of Ta recovered in the experiment was indeed the maximum and was equal to 26.34 mg of Ta (37% recovery), which indicates a 7% error from the predicted value.

Because the modifier flow rate is the main factor influencing sugars recovery, **Figure 4** shows tagatose recovery (percent), at some of the experimental conditions tested, as a function of the amount of isopropanol present in the supercritical CO_2 solvent. Different symbols correspond to different modifier flow rate (*M*), and lines represent extractions carried out at the same temperature and pressure corresponding to a constant CO_2 density of 189, 662, and 830 kg/m^3, respectively. As can be

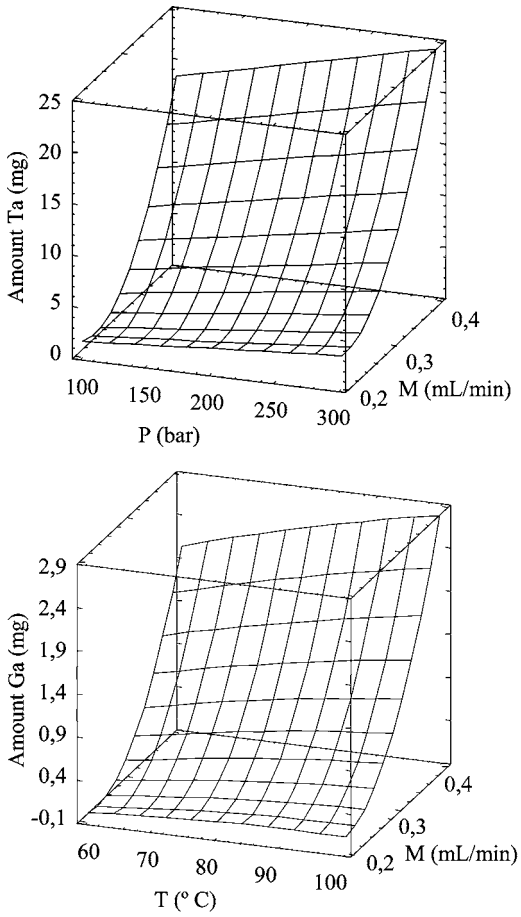

Figure 3. Surface plot of the response variables: milligrams of tagatose extracted [Ta (mg)] and milligrams of galactose extracted [Ga(mg)]. For Ta, temperature was fixed at medium level ($T = 80$ °C), and for Ga, pressure was fixed at medium level ($P = 200$ bar).

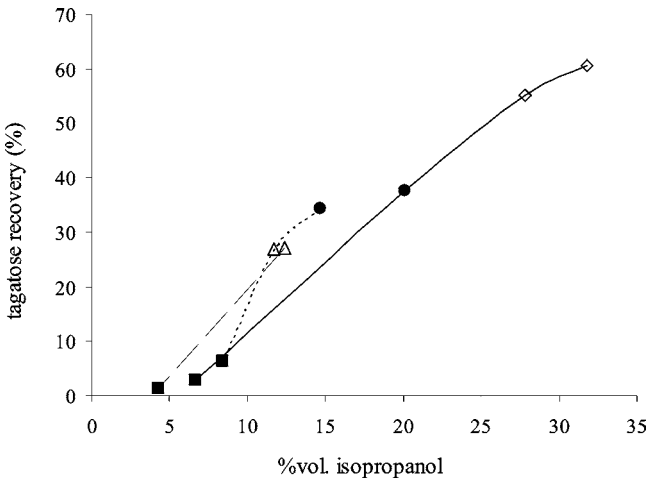

Figure 4. Tagatose recovery obtained in the experiments as a function of the amount (% vol) of isopropanol present in the supercritical phase. Modifier flow: (■) 0.2 mL/min; (△) 0.3 mL/min; (●) 0.4 mL/min; (◇) 0.6 and 0.7 mL/min. Constant CO_2 density lines: (—) 300 bar, 60 °C (ρ_{CO_2} = 830 kg/m^3); (· · ·) 300 bar, 100 °C (ρ_{CO_2} = 662 kg/m^3); (– –) 100 bar, 100 °C (ρ_{CO_2} = 189 kg/m^3).

Table 3. Additional Extraction Assays at Modifier Flow Rates Higher than in the Initial Experimental Design, Using a 70:30 wt % Mixture of Tagatose/Galactose

P (bar)	*T* (°C)	*M* (mL/min)	extracted Ta (mg)	extracted Ga (mg)	Ta purity (%)	Ga purity (%)	recovery of Ta (%)	recovery of Ga (%)
300	60	0.6	38.628	3.723	91.21	8.79	55.18	12.41
300	60	0.7	42.438	4.870	89.70	10.30	60.62	16.23
300	80	0.6	52.338	6.980	88.23	11.77	74.77	23.27
300	80	0.7	50.507	5.243	90.55	9.45	72.15	17.48

Table 4. Effect of the Type of Cosolvent and Initial Composition of the Extracted Samples on the Selective Extraction of Tagatose

cosolvent	extracted Ta (mg)	extracted Ga (mg)	Ta purity (%)	Ga purity (%)	recovery of Ta (%)	recovery of Ga (%)
n-butanol[a]	36.441	4.019	90.06	9.94	52.06	13.40
n-propanol[a]	37.057	4.247	89.72	10.28	52.94	14.16
isopropanol[a]	52.338	6.980	88.23	11.77	74.77	23.27
ethanol[a]	56.550	8.230	87.30	12.70	80.78	27.43
ethanol/water[a] (95:5 v/v)	65.930	11.721	84.91	15.09	94.18	39.07
ethanol/water[b] (90:10 v/v)	119.641	34.036	77.85	22.15	85.46	56.73
methanol[b]	124.803	46.280	72.96	27.04	89.14	77.13
isopropanol[c]	49.271	7.106	87.41	12.59	82.12	5.07
ethanol[c]	46.494	12.283	79.31	20.69	77.49	8.77

[a] Initial sample composition: 1 part of Ta:Ga 70:30 and 9 parts of sea sand. [b] Initial sample composition: 1 part of Ta:Ga 70:30 and 4 parts of sea sand. [c] Initial sample composition: 1 part of Ta:Ga 30:70 and 4 parts of sea sand.

deduced from the figure, the amount of isopropanol present in the supercritical phase has a significant effect on tagatose recovery, showing almost a lineal increase of tagatose yield with increasing modifier content. On the other hand, the supercritical phase density has a rather lower effect on the amount of tagatose extracted; that is, the tagatose recovery could be highly increased even at low CO_2 density if enough isopropanol modifier is dissolved in the CO_2 solvent. Thus, at low CO_2 density tagatose extraction is limited by the small amount of isopropanol that can be dissolved in CO_2 while maintaining a homogeneous supercritical phase. When density is increased, higher amounts of modifier can be dissolved in the supercritical phase, and thus tagatose recovery is considerably increased.

To have a really viable process, additional experiments outside the experimental domain were performed to improve the recovery (37%) of extracted tagatose. **Table 3** shows the experimental conditions tested and the amounts of Ta and Ga extracted along with the percent of each monosaccharide in the final mixture. As can be seen, maximum pressure and medium temperatures were selected for the experiments; because the amount of modifier tested was around 30% vol, temperatures $\geq$60 °C were needed to work in a homogeneous supercritical extraction phase. Therefore, 300 bar and 60 and 80 °C extraction temperatures were tested with modifier flow rates equal to 0.6 and 0.7 mL/min. As can be clearly seen, although selectivity is almost constant in all of the experiments, the amount of tagatose extracted increases with the amount of modifier added to the carbon dioxide. Maximum extraction of Ta is obtained in a homogeneous phase (300 bar, 80 °C) and with a modifier of 0.6 or 0.7 mL/min (**Figure 3**, experiment ◇). An increase from 0.4 mL/min (which was the optimum in the experimental region provided by the model) to 0.6 mL/min (in a one-region phase) implies an increase in recovery of Ta from 37 to 75%. A higher percentage of modifier was not added in the mixture due to experimental constraints.

Taking into account that cosolvent polarity variations may have effects on the relative solubility of tagatose and galactose, the behavior of several alcohols and mixtures of ethanol/water have been also evaluated, and the results are shown in **Table 4**. As expected, a remarkable increase of the amount of total carbohydrate extracted with polarity was found; however, selective extraction of tagatose decreased with increasing polarity of the assayed solvent. In the case of very polar solvents (methanol and ethanol/water 90:10) the compositions of the extracts were similar to that of the original sample. No significant differences were observed in the compositions of extracts obtained using ethanol, *n*-propanol, isopropanol, or *n*-butanol as cosolvents.

Extractions of samples of Ta/Ga 30:70 using ethanol and isopropanol as cosolvents were also assayed, and the results are shown in **Table 4**. As can be observed, the increase of galactose content in initial sample did not affect the amount of total carbohydrate extracted or the selective extraction of tagatose.

In conclusion, in the present study, SFE conditions and the composition of the supercritical phase have been evaluated to selectively extract and fractionate a carbohydrate mixture containing a solid tagatose/galactose in different proportions. After experimental design optimization, the conditions to obtain the maximum tagatose extraction were 300 bar, 60 °C, and 0.4 mL/min of isopropanol, which accounted for the maximum amount of modifier added. Under these conditions, recoveries of around 37% were achieved. To improve Ta recovery, modifier addition was increased to higher values (30% vol) because it was the factor that mostly influenced the amount of Ta extracted. The final conditions selected in the study allowed extraction of tagatose with purity of >90% and with extraction recoveries of >75%.

LITERATURE CITED

(1) Cheetham, P. S. J.; Wootton, A. N. Bioconversion of D-galactose into D-tagatose. *Enzyme Microb. Technol.* **1993**, *15*, 105−108.

(2) Troyano, E.; Olano, A.; Martinez-Castro, I. Changes in free monosaccharides of dried milk. *J. Agric. Food Chem.* **1994**, *42*, 1543−1545.

(3) Troyano, E.; Martinez-Castro, I.; Olano, A. Kinetics of galactose and tagatose formation during heat treatment of milk. *Food Chem.* **1992**, *45* (1), 41−43.

(4) Rollini, M.; Manzoni, M. Bioconversion of D-galactitol to tagatose and dehydrogenase activity induction in *Gluconobacter oxydans*. *Process Biochem.* **2005**, *40*, 437−444.
(5) FAO/WHO recommends tagatose. *Int. Sugar J.* **2001**, *103* (1232), 321.
(6) Bertelsen, H.; Jensen, B. B.; Buemann, B. D-Tagatose—a novel low-calorie bulk sweetener with prebiotic properties. *World Rev. Nutr. Diet.* **1999**, *85*, 98−109.
(7) Beadle, J. R.; Saunders, J. P.; Wadja, T. J. Process for manufacturing tagatose. U.S. Patent 5,078,796, 1992.
(8) Ekeberg, D.; Morgenlie, S.; Stenstron, Y. Base catalysed isomerization of aldose of the arabino and lyxo series in the presence of aluminate. *Carbohydr. Res.* **2002**, *337*, 779−786.
(9) Jung, E. S.; Kim, H. J.; Oh, D. K. Tagatose production by immobilized Escherichia coli cells containing *Geobacillus stearothermophillus* L-arabinose isomerase mutant in a packed-bed bioreactor. *Biotechnol. Prog.* **2005**, *21*, 1335−1340.
(10) Kim, P. Current studies on biological tagatose production using L-arabinose isomerase: a review and future perspective. *Appl. Microbiol. Biotechnol.* **2004**, *65*, 243−249.
(11) Jorgensen, F.; Hansen, O. C.; Stougaard, P. Enzymatic conversión of D-galactose to D-tagatose: heterologous expression and characterization of a thermostable L-arabinose isomerase from *Thermoanaerobacter mathranii*. *Appl. Microbiol. Biotechnol.* **2004**, *64*, 816−822.
(12) Oh, H. J.; Kim, H. J.; Oh, D. K. Increase in D-tagatose production rate by site-directed mutagenesis of L-arabinose isomerase from *Geobacillus thermodenitrificans*. *Biotechnol. Lett.* **2006**, *28*, 145−149.
(13) Dohrn, R.; Buenz, A. P. Solubility enhancement of carbohydrates in carbon dioxide. *ZFL, Intern. Z. Lebensm.-Technol. Marketing, Verpack. Anal.* **1995**, *46* (7/8) 10−12, (9) 30−31.
(14) Ibañez, E.; Oca, A.; de Murga, G.; López-Sebastián, S.; Tabera, J.; Reglero, G. Supercritical fluid extraction and fractionation of different preprocessed rosemary plants. *J. Agric. Food Chem.* **1999**, *47*, 1400−1404.

Received for review June 28, 2006. Revised manuscript received August 29, 2006. Accepted August 31, 2006. F.M. thanks MEC for a FPI grant. T.F. acknowledges the financial support of the Ramon y Cajal Program from the Ministry of Education and Science. This work has been financed under a R+D program of the Spanish Ministry of Education and Science, Project AGL-2004-07227-C02-02, and Project ALIBIRD S-0505/AGR/000153 from the Comunidad Autónoma de Madrid.

JF0618123