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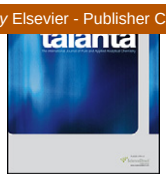

Chemometrics optimization of carbohydrate separations in six food matrices by micellar electrokinetic chromatography with anionic surfactant

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ABSTRACT

Multivariate statistical design modeling and the Derringer–Suich desirability function analysis were applied to micellar electrokinetic chromatography (MEKC) results with anionic surfactant to separate carbohydrates (CHOs) in different food matrices. This strategy has been studied with success to analyze compounds of difficult separation, but has not been explored for carbohydrates. Six procedures for the analysis of different sets of CHOs present in six food matrices were developed. The effects of pH, electrolyte and surfactant concentrations on the separation of the compounds were investigated using a central composite design requiring 17 experiments. The simultaneous optimization of the responses for separation of six sets of CHOs was performed employing empirical models for prediction of optimal resolution conditions in six matrices, condensed milk, orange juices, rice bran, red wine, roasted and ground coffee and breakfast cereal samples. The results indicate good separation for the samples, with appropriate detectability and selectivity, short analysis time, low reagent cost and little waste generation, demonstrating that the proposed technique is a viable alternative for carbohydrate analysis in foods.

1. Introduction

The analysis of carbohydrates in foods has extreme nutritional importance since they contribute with 40–50% of the caloric intake of human beings [1]. The constant increase in obesity indices, diabetes and some CHO intolerances intensify the necessity of their rigorous control. In the food industry, they are used as technological coadjuvants in order to obtain physicochemical and sensorial characteristics of foods [2,3], but they also serve as quality markers during processing [4]. Milk, when subjected to excessive heating, suffers a lactose isomerization reaction forming lactulose and epilactose. Both have ssmaller biological values than lactose and may cause negative effects like flatulence [4–7]. The presence of an excessive amount of glucose in ground coffee after hydrolysis indicates product adulteration from the addition of other grains [8]. In rice bran, a food industry by-product, xylose is the predominant carbohydrate, which has been used as a source for obtaining xylitol (a compound with high sweetening power) [9]. In wines, the quantification of CHOs is correlated with final product conservation. Studies indicate that the majority of microorganisms used for the fermentation process consume more glucose than fructose. So, high contents of fructose in wine can compromise its preservation [10]. Also, the control of CHO compositions in wine is fundamental for the standardization of its alcoholic content [11].

The determination of different CHOs is a significant challenge, since they are analytes of difficult separation and present similar physicochemical characteristics [12,13]. Several analytical techniques have been studied for this purpose, among them high performance liquid chromatography (HPLC) and capillary electrophoresis (CE). CE is an ascending technique for this type of analysis, since the fused-silica capillary withstands highly alkaline pHs, above 11.5, allowing the ionization of CHOs, which facilitates separation [14–21]. Besides this, the technique has been largely employed owing to its low reagent consumption and waste generation [14,22–26]. Various kinds of separations have already been studied with CE, emphasizing capillary zone electrophoresis (CZE), with or without derivatization reactions, and micellar electrokinetic chromatography (MEKC) with cationic surfactant [14–21]. Despite several investigations developed to separate CHOs in food matrices, one can still verify some separations difficulties still exist, mainly between maltose and glucose [14], glucose and galactose [22], galactose, maltose and maltotriose [23], fructose and xylose [24] and fructose and mannose [8], separations that are very important for the food industry. The MEKC with anionic surfactant is a type of separation employed in several food analyses and is based on analyte partition into two phases, the micellar phase and the run electrolyte. The formation of micelles occurs by the addition of surfactant agents to the electrolyte, in concentrations above the critical micellar concentration [27]. Bao et al. [28] observed good results

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for the separation of oligosaccharides in human milk using sodium dodecyl sulfate surfactant. However, there are still few studies on CHOs separation using this method.

In general, method optimization depends on several variables, which directly effect compound separation such as pH, the type and concentration of electrolytes and surfactants, voltage, capillary length and temperature [8,14–18]. Multivariate optimization strategies have been increasingly and efficiently used for developing and optimizing separation methods, since they allow the evaluation of interaction effects among the variables, reduce the number of experiments and make possible the construction of empirical models to predict the most adequate experimental conditions [20,29]. On the other hand, optimization of the simultaneous separation of several compounds involves a large number of responses, each of which may have different optimal regions within the experimental domain. The Derringer and Suich method [30], which is suitable for optimizing several responses, is based on the construction of a global desirability function involving all the responses of the system. Recently the central composite design [31] and the Derringer and Suich method have been successfully utilized in several works like the studies of the separation of resveratrol in nutraceuticals [32], rizatriptan in medical drugs [33], separation of chiral compounds [34], optimization of biochemical experiments [35] and separation of phenolic compounds from extra-virgin olive oil [36].

In this study the MEKC technique with anionic surfactant was investigated exploring its potential to separate carbohydrates which are difficult to analyze owing to their structural similarities. To reduce the number of experiments to a minimum, this study was made using a central composite design to evaluate the effect of three important variables, pH, electrolyte and surfactant concentrations on separation. With the Derringer and Suich method, empirical predictions for the separation of the most important analytes in each sample were determined. Six procedures applied to the separation of CHOs in coffee, rice bran, red wine, breakfast cereal, orange juice and condensed milk were optimized and developed.

2. Materials and methods

2.1. Chemicals

Saccharose (SAC), D-lactose (LAC), lactulose (LTU), epilactose (EPI), maltotriose (MTO), D-(+)-maltose (MAL), D-(+)-glucose (GLU), D-(−)-arabinose (ARA), D-(+)-mannose (MAN), D-(−)-fructose (FRU) and D-(−)-ribose (RIB) standards were purchased from Sigma–Aldrich (USA). D-(+)-galactose (GAL) and D-xylose (XYL) were from Chem. Service (USA). Sodium hydroxide was purchased from Nuclear (Brazil), sorbic acid (SOR) from Sigma–Aldrich (USA) and sodium dodecyl sulfate (SDS) from Riedel-de-Haën (Germany). All reagents used were of high analytical purity. Ultra-pure water was obtained from a Direct-Q 3 UV ultra-pure water system (Millipore Corporation, France).

The stock solutions of CHO standards (10 g L^{-1}) were maintained at −18 °C until use for preparing the solutions for the experiments. All the standard solutions were degassed by ultrasonication (Microsonic SX-20, Arruda Ultra-sons LTDA, Brazil) before the injection. Condensed milk, orange juice, roasted and ground coffee, breakfast cereal, red wine and rice bran samples were purchased at supermarkets in the region of Campinas, SP, Brazil.

2.2. Equipment

The experiments were performed using a capillary electrophoresis Agilent G1600AX instument (Agilent Technologies, Germany), assembled with a diode array detector. All the multivari-

Table 1
Factor levels and their experimental peak-pair resolutions.

Laboratory variables			Resolutions														
pH	SDS	SOR[a]	SAC-GLU	GLU-FRU	LAC-LTU	LTU-EPI	EPI-GAL	GAL-GLU	GLU-ARA	ARA-FRU	FRU-XIL	SAC-GAL	ARA-MAN	MAN-FRU	XIL-RIB	EPI-MTO	MTO-MAL	MAL-GAL	SAC-LAC
12.5	15.0	10.0	22.8	7.3	2.4	0.0	0.0	1.7	1.8	5.5	1.3	10.7	3.0	0.9	3.2	0.0	0.0	0.0	11.1
12.5	15.0	20.0	29.5	8.6	2.3	0.0	0.0	2.1	1.9	6.3	1.4	12.5	3.3	0.9	4.0	0.0	0.0	0.0	14.0
12.5	60.0	10.0	27.6	7.5	3.3	0.0	0.0	1.5	2.5	5.4	1.6	14.3	2.9	1.0	4.0	0.0	0.0	0.0	16.2
12.5	60.0	20.0	33.1	8.8	2.6	0.0	0.0	1.8	2.6	6.2	1.7	11.8	3.2	1.4	4.6	0.0	0.0	0.0	17.8
12.7	15.0	10.0	19.6	7.0	1.5	0.0	1.6	1.5	3.5	2.4	3.2	11.1	0.7	2.6	3.3	1.6	0.0	0.0	9.7
12.7	15.0	20.0	28.4	7.9	1.5	0.0	2.4	2.6	3.7	4.0	2.9	21.2	2.0	2.2	3.8	1.2	1.3	0.0	13.4
12.7	60.0	10.0	28.5	7.2	2.4	1.2	1.0	2.9	3.3	3.6	2.8	22.7	1.9	2.3	3.4	0.0	1.0	0.0	16.3
12.7	60.0	20.0	33.5	9.3	3.1	2.0	5.4	3.6	5.3	2.6	4.7	30.8	0.0	2.6	4.8	2.6	1.5	1.3	20.1
12.6	37.5	15.0	29.9	8.2	2.1	1.4	0.9	3.1	3.8	4.7	2.8	25.2	2.3	2.1	3.7	0.0	0.9	0.0	13.2
12.6	37.5	15.0	32.8	7.4	2.7	0.7	1.7	2.2	3.7	3.4	3.2	22.4	1.3	2.4	3.8	0.0	1.7	0.0	18.3
12.6	37.5	15.0	29.3	7.7	2.2	1.2	1.3	2.5	3.9	4.1	3.1	22.4	1.9	2.4	4.0	0.0	1.3	0.0	16.3
12.43	37.5	15.0	20.1	7.7	2.8	0.0	1.5	0.0	1.8	8.2	0.9	20.1	5.2	1.1	5.1	0.0	0.0	1.5	16.0
12.77	37.5	15.0	31.5	7.6	2.5	1.7	6.7	2.7	4.3	2.3	4.4	31.0	0.6	2.6	4.1	3.7	0.8	1.5	18.6
12.6	0.0	15.0	24.8	7.0	3.2	0.0	0.0	1.5	2.6	5.0	1.6	12.7	3.1	1.0	3.2	0.0	0.0	0.0	12.1
12.6	75.3	15.0	38.8	9.5	2.6	1.6	1.8	2.9	4.5	3.9	3.6	24.0	1.4	2.7	4.4	0.0	1.8	0.0	22.1
12.6	37.5	6.6	24.5	6.8	2.5	0.0	0.0	1.1	2.1	5.1	1.5	11.0	2.8	0.9	3.4	0.0	0.0	0.0	11.5
12.6	37.5	23.4	42.2	8.7	2.3	2.1	1.5	2.7	4.6	4.6	3.7	25.3	2.4	2.5	4.8	0.0	1.5	0.0	23.5

SDS: sodium dodecyl sulfate; SOR: sorbic acid; RIB: Ribose; XYL: Xylose; FRU: Fructose; MAN: Mannose; ARA: Arabinose; GLU: Glucose; GAL: Galactose; MAL: Maltose; MTO: Maltotriose; EPI: Epilactose; LTU: Lactulose; LAC: Lactose; and SAC: Saccharose.
[a] Concentrations in mmol L^{-1}.

ate optimization experiments were performed using a fused-silica capillary with 50 μm internal diameter and 80 cm total length (72 cm of effective length), with extended light path, purchased from Agilent (Germany). The temperature, voltage and injection conditions remained fixed at 25 °C, 20 kV, and hydrodynamic injections of 50 mbar during 5 s. The detection was performed in an indirect way at 350 nm, with reference to 275 nm.

2.3. Multivariate optimization

The analytical methods were optimized using a central composite design, with central and axial points [37,38]. The influences of pH, electrolyte (SOR) and surfactant (SDS) concentrations were investigated, as well as the interaction effects among the variables. All the experiments were carried out by injection of a standard solution containing a mixture of all 13 carbohydrates to be separated in all matrices, so that optimization in six analytical methods can be achieved by performing just one central composite design. Since the standards were present in just one aqueous solution for determining results for response surface modeling, six subsequent studies were performed, each one conducted for the specific separation of the compounds of interest in each food matrix.

The levels studied for the factors of the central composite design were: pH values from 12.43 to 12.77, SDS concentrations from 0.00 (absent) to 75.3 mmol L^{-1} and SOR concentrations from 6.6 to 23.4 mmol L^{-1}. The central point was performed in triplicate to measure experimental error. The experiments were randomly performed.

The responses studied were the resolution between adjacent peaks, which co-eluted in at least one of the experimental design conditions and based on the carbohydrate composition already found in the literature mentioned above and the order of compound migration. For condensed milk the resolutions were observed between LAC-LTU, LTU-EPI, EPI-GAL and GAL-GLU; for orange juice the resolutions were observed between SAC-GLU and GLU-FRU; for rice bran the resolutions were observed between GLU-ARA, ARA-FRU and FRU-XYL; for red wine between SAC-GAL, GAL-GLU, GLU-ARA, ARA-FRU, FRU-XYL and XYL-RIB; for coffee GAL-GLU, GLU-ARA, ARA-MAN, MAN-FRU, FRU-XYL and XYL-RIB; and for breakfast cereals resolutions were observed between SAC-LAC, LAC-LTU, LTU-EPI, EPI-MTO, MTO-MAL, MAL-GAL, GAL-GLU and GLU-FRU. In cases where more than one set of satisfactory conditions were found, additional responses, like run time and the analytical signal to noise ratio, were used as selection criteria. The resolutions were calculated using:

$$R_s = \frac{2\,(t_2 - t_1)}{w_2 + w_1} \quad (1)$$

where R_s is the resolution, t, the migration time and w, the peak width.

The data treatment was made using the Design Expert 6.0.10 software (Minneapolis, USA). Model quality was evaluated by the Analysis of Variance (ANOVA, 95% confidence level). The Derringer and Suich technique was used, stipulating desirability criteria for each resolution. The conditions predicted by the model were confirmed experimentally within the sets of analytical standards. The optimal conditions were applied to real samples to evaluate the effect of the matrices.

2.4. Analytical procedures using CE

Before determination of the analytical results for each set of conditions of the factorial design, the capillary was conditioned by washing under 1000 mbar pressure with 1 mol L^{-1} sodium hydroxide, followed by 5 min of washing with purified water and 10 min with the background electrolyte. To avoid adsorption of the solutes on the capillary walls, the capillary was washed with the electrolyte for 2 min between injection replicates of identical factor levels. All the electrolytes were filtered through cellulosic membranes with 0.45 μm porosity and centrifuged for 10 min at 5000 rpm (Excelsa II, mod. 206 BL, Fanem, Brazil) to remove dissolved air.

To analyze the food samples, the capillary was conditioned at the beginning of the day by washing under 1000 mbar pressure with 1 mol L^{-1} sodium hydroxide, followed by 5 min of washing with purified water and 10 min with electrolyte. Between injections, the pre-conditioning treatment was made by running the electrolyte for 2 min. At the end of the day, the capillary was washed for 5 min with a 1 mol L^{-1} sodium hydroxide solution, followed by 5 min of washing with purified water, followed by storage in water.

2.5. Sample preparation

Orange juice and red wine samples were diluted with deionized water (1:20 and 1:6, respectively) [16,39]. For condensed milk, 0.5 g of sample was solubilized in water and transferred to a volumetric flask of 25 mL, the volume being completed with purified water. Subsequently, 100 μL of acetic acid was added to 10 mL of the solution and centrifuged at 5000 rpm for 10 min, collecting the supernatant for analysis [16]. The samples of breakfast cereals were triturated and 5 g were taken to the ultrasound with 80 mL of water for 5 min. The extract was transferred to a 100 mL volumetric flask and the volume was completed with water [20]. For the roasted ground coffee samples, 50 mL of 1 mol L^{-1} HCl was added to 5 g of sample and heated (under agitation) in a water bath at 90 °C for 150 min. The sample was neutralized with NaOH, transferred to a 100 mL volumetric flask and the volume was completed with water. Rice bran was hydrolyzed to extract xylose with the same procedure used for coffee [8]. All the sample extracts were filtered through membranes with 0.45 μm porosity and subjected to 5 min of ultrasound before injection into the capillary electrophoresis instrument.

3. Results and discussion

3.1. Optimization of the analytical procedures

Experimental resolution results for the factors and levels studied are shown in Table 1. All three investigated factors significantly affected peak separations. In all the experiments the analytes presenteda, SAC, LAC, LTU, EPI, MTO, MAL, GAL, GLU, ARA, MAN, FRU, XYL, XYL and RIB elution order. The effects of pH increase were positive for the resolutions of the majority of peak pairs, except for the ARA-FRU, ARA-MAN and XYL-RIB pairs, where negative effects were observed, indicating that the increase in the pH values, for levels higher than those of the experimental domain studied, leads to an inversion in their migration order.

The addition of SDS to the electrolyte led to a positive effect for the majority of the resolutions studied, except for the one between ARA-MAN where this addition decreased separation. The significant effect of SDS on the separation may be related to the association of the solutes with the micelle surface, through dipole–dipole or ion–dipole (or ionic) interactions at the surfactant head. Strong coulomb interactions (attractive and repulsive) can also occur when the solute is charged, as described by Tavares [40]. The increase of sorbic acid concentration also resulted in positive effects on resolution.

It is possible to observe that the simultaneous increase of the three factors contributed positively to an increase in the resolutions for the majority of the compounds. However, it was also shown that the increase in each of the three factors, individually or simultaneously, resulted in a reduction of analytical signal, accom-

Table 2
Statistical model coefficients, their standard errors and F-distribution parameters for model validation.

Resolutions Studied	Indicated Model	Coefficients (standard error)[a]										Regression Significance ($p < 0.05$)	Model Fit ($p > 0.05$)
		Intercept	A-pH	B-SDS	C-SOR	A^2	B^2	C^2	AB	AC	BC		
SAC-GLU	Linear	29.23 (0.89)	1.18 (0.99)	3.36 (0.99)	4.09 (0.99)							0.0011	0.1993
GLU-FRU	Linear	7.89 (0.10)	−0.08 (0.11)	0.46 (0.11)	0.65 (0.11)							<0.0001	0.6231
LAC-LTU	Constant	2.47 (0.12)										–	0.3339
LTU-EPI	Linear	0.71 (0.14)	0.45 (0.16)	0.44 (0.16)	0.31 (0.16)							0.0056	0.2434
EPI-GAL	Quadratic	1.36 (0.42)	1.40 (0.19)	0.39 (0.19)	0.56 (0.19)	0.85 (0.22)	−0.30 (0.22)	−0,35 (0.22)	0.30 (0.25)	0.65 (0.25)	0.44 (0.25)	0.0022	0.1932
GAL-GLU	Linear	2.14 (0.14)	0.58 (0.16)	0.31 (0.16)	0.37 (0.16)							0.0035	0.4436
GLU-ARA	Linear	3.28 (0.13)	0.82 (0.14)	0.46 (0.14)	0.49 (0.14)							<0.0001	0.0528
ARA-FRU	Linear	4.55 (0.16)	−1.52 (0.18)	−0.15 (0.18)	0.09 (0.18)							<0.0001	0.5628
FRU-XIL	Linear	2.61 (0.12)	0.99 (0.14)	0.40 (0.14)	0.40 (0.14)							<0.0001	0.0943
SAC-GAL	Quadratic	23.59 (1.80)	4.01 (0.85)	3.16 (0.85)	3.05 (0.85)	−0.03 (0.93)	−2.58 (0.93)	−2.66 (0.93)	2.30 (1.10)	2.35 (1.10)	−0.78 (1.10)	0.0058	0.1765
ARA-MAN	Linear	2.24 (0.16)	−1.16 (0.18)	−0.28 (0.18)	−0.06 (0.18)							0.0002	0.3402
MAN-FRU	Linear	1.85 (0.11)	0.59 (0.12)	0.27 (0.12)	0.21 (0.12)							0.0007	0.1304
XIL-RIB	Linear	3.98 (0.08)	−0.15 (0.09)	0.32 (0.09)	0.41 (0.09)							0.0005	0.1254
EPI-MTO	Quadratic	0.00 (0.31)	0.85 (0.14)	−0.01 (0.14)	0.16 (0.14)	0.66 (0.16)	0.00 (0.16)	0.00 (0.16)	−0.02 (0.19)	0.28 (0.19)	0.37 (0.19)	0.0093	[b]
MTO-MAL	Linear	0.69 (0.13)	0.37 (0.14)	0.31 (0.14)	0.30 (0.14)							0.0133	0.3866
MAL-GAL	Quadratic	0.02 (0.21)	0.09 (0.10)	0.10 (0.10)	0.10 (0.10)	0.45 (0.11)	−0.08 (0.11)	−0.08 (0.11)	0.16 (0.13)	0.16 (0.13)	0.16 (0.13)	0.0569	[b]
SAC-LAC	Linear	15.90 (0.50)	0.34 (0.55)	2.86 (0.55)	2.35 (0.55)							0.0002	0.7765

SDS: sodium dodecyl sufate; SOR: sorbic acid; A: pH; B: SDS; C: SOR; RIB: Ribose; XYL: Xylose; FRU: Fructose; MAN: Mannose; ARA: Arabinose; GLU: Glucose; GAL: Galactose; MAL: Maltose.

[a] Coefficients obtained using design (−1.68, −1, 0, 1, 1.68) variables (p 0.95).

[b] Lack of fit was not calculated since experimental error could not be estimated at the central point (complete coelution occurred in the three center point experiments).

panied by an increase in noise and run time, which are undesirable characteristics for routine analysis. To avoid these side effects the Derringer–Suich optimization criteria were modified to obtain adequate separation for the matrix, combined with the shortest run time and the best signal-to-noise ratio. Thus, experimental conditions were chosen so that minimum NaOH (pH), surfactant and electrolyte concentrations were used while maintaining an adequate resolution.

Initially linear and quadratic models were determined for each central composite design response. In those cases where none of the coefficients of the quadratic and cross terms were significant, a linear model was assumed and a new fit to the data was carried out. This is automatically done by the Design Expert program.

Table 2 presents the final models and their significant coefficients, as well as test results for lack of fit and regression significance from the analysis of variance (ANOVA). Most of the mathematical models obtained showed adequate fits ($p > 0.05$) according to the response data. It was not possible to evaluate the fit of the models for the resolutions between the compounds EPI-MTO and MAL-GAL, since a complete coelution occurred for all three replicates at the central point. Thus, given the difficulty of making accurate estimates, resolution was considered to be zero for all three tests, so that experimental error could not be estimated. As for regression significance, the p values were all below 0.05, indicating that factor changes significantly affect resolution. For the MAL-GAL resolution, the p value was 0.0569, slightly higher than 0.05. This difficulty could be explained by the small response variation for this pair of compounds since, of the 17 experiments performed, values greater than zero were observed only for three sets of conditions. For all others complete coelution occurred.

The only resolution for which no significant effects occurred was between LAC-LTU. The constantmodel indicates resolutions with random fluctuations around 2.47. Using the Derringer and Suich desirability method, the models were used to calculate predictions of the optimized separation conditions for the pairs of carbohydrates present in each of the six matrices. Table 3 shows the criteria established for the models, their coded optimal experimental variables, the corresponding predicted resolution responses and the responses observed from the experimental tests under these conditions.

It is worth mentioning that for factor levels inside the experimental domain optimum CHO separations were found for condensed milk, wine, coffee, cereal and rice bran matrices. As for the method applied to the separation of CHOs in fruit juice, whose compounds of interest showed excellent separation in all tests, an extrapolation of all factor levels was possible, in an attempt to further reduce run time. Considering that increases in factor levels for this matrix cause increases in run time as well as reductions in analytical signal for all the desirable conditions, Derringer–Suich criteria for minimizing factor concentrations, while maintaining resolutions higher than 2 were imposed.

Table 3
Desirable conditions, predicted responses of the models and experimentally observed responses for the mixture of standards.

Matrix	Variables and resolutions	Desirability criteria for factors and resolutions			Predicted conditions (Codified)	Predicted resolutions	Observed resolutions
		Aim	Minimum Limit	Maximum Limit			
Breakfast cereals	pH	Minimize	−1.68	1.68	1.59		
	SDS	Minimize	−1.68	1.68	1.42		
	SOR	Minimize	−1.68	1.68	1.44		
	SAC-LAC	Maximize	2.00	30.00		23.90	22.43
	LAC-LTU	Maximize	2.00	10.00		2.47	2.76
	LTU-EPI	Maximize	2.00	10.00		2.49	1.66
	EPI-MTO	Maximize	2.00	10.00		4.59	4.54
	MTO-MAL	Maximize	2.00	10.00		2.15	2.16
	MAL-GAL	Maximize	2.00	10.00		2.31	2.43
	GAL-GLU	Maximize	2.00	10.00		4.04	4.84
	GLU-FRU	Maximize	2.00	10.00		9.35	9.25
Coffee	pH	Minimize	−1.68	1.68	−0.14		
	SDS	Minimize	−1.68	1.68	1.46		
	SOR	Minimize	−1.68	1.68	−0.74		
	GAL-GLU	Maximize	2.00	10.00		2.24	3.94
	GLU-ARA	Maximize	2.00	10.00		3.48	3.20
	ARA-MAN	Maximize	2.00	10.00		2.04	3.74
	MAN-FRU	Maximize	2.00	10.00		2.01	1.72
	FRU-XIL	Maximize	2.00	10.00		2.77	2.51
	XIL-RIB	Maximize	2.00	10.00		4.16	4.20
Condensed milk	pH	Minimize	−1.68	1.68	0.82		
	SDS	Minimize	−1.68	1.68	1.48		
	SOR	Minimize	−1.68	1.68	0.89		
	LAC-LTU	Maximize	2.00	10.00		2.46	2.88
	LTU-EPI	Maximize	2.00	10.00		2.07	2.19
	EPI-GAL	Maximize	2.00	10.00		3.50	2.33
	GAL-GLU	Maximize	2.00	10.00		3.74	3.92
Orange Juice	pH	Between	−3.00	0.00	−1.97		
	SDS	Between	−1.68	3.00	−1.68		
	SOR	Between	−2.00	3.00	−2.00		
	SAC-GLU	Minimize	3.00	42.2		13.07	22.42
	GLU-FRU	Minimize	3.00	9.50		5.98	7.53
Rice bran	pH	Minimize	−1.68	1.68	−0.10		
	SDS	Minimize	−1.68	1.68	−0.60		
	SOR	Minimize	−1.68	1.68	0.46		
	GLU-ARA	Maximize	2.00	30.00		3.14	2.76
	ARA-FRU	Maximize	2.00	10.00		2.51	3.66
	FRU-XIL	Maximize	2.00	10.00		2.45	2.26
Red wine	pH	Minimize	−1.68	1.68	0.02		
	SDS	Minimize	−1.68	1.68	0.18		
	SOR	Minimize	−1.68	1.68	−0.56		
	SAC-GAL	Maximize	2.00	40.00		23.97	26.2
	GAL-GLU	Maximize	2.00	10.00		2.00	3.54
	GLU-ARA	Maximize	2.00	10.00		3.10	2.69
	ARA-FRU	Maximize	2.00	10.00		4.44	4.98
	FRU-XIL	Maximize	2.00	10.00		2.48	2.93
	XIL-RIB	Maximize	2.00	10.00		3.80	4.90

The algorithm predicted experimental conditions that satisfied the resolution criteria established for all six matrices. In some cases more than one satisfactory set of conditions were found. In these cases, those conditions with lower factor concentrations were chosen since they are expected to result in the lowest possible analysis times. These conditions were confirmed experimentally. The simultaneous optimization method allowed the development of six different procedures, which, when tested experimentally, resulted in the separation of the sets of CHO standards and were effective for the use in real samples after slight changes to adjust for

Table 4
Analytical conditions for the procedures developed for the six matrices.

Matrix	Analytical conditions					
	pH	SDS (mmol L^{-1})	SOR (mmol L^{-1})	Capillary length (cm)[a]	Voltage (kV)	Injection (pressure/time)
Coffee	12.59	70.35	11.35	60	20	50 mbar/5 s
Breakfast cereal	12.76	69.50	22.20	80	20	50 mbar/5 s + 50 mbar/1 s de NaCl[b]
Orange Juice	12.40	0.00	5.00	60	25	50 mbar/5 s
Rice Bran	12.59	24.00	17.30	60	25	50 mbar/5 s
Condensed Milk	12.68	70.80	20.00	80	25	50 mbar/5 s + 25 mbar/1 s NaCl[b]
Red wine	12.60	41.50	12.20	80	20	50 mbar/5 s

[a] Total capillary length, 50 μm diameter with extended light path.
[b] 1.0 mol L^{-1} NaCl solution. DAD system at 350 nm (reference to 275 nm).

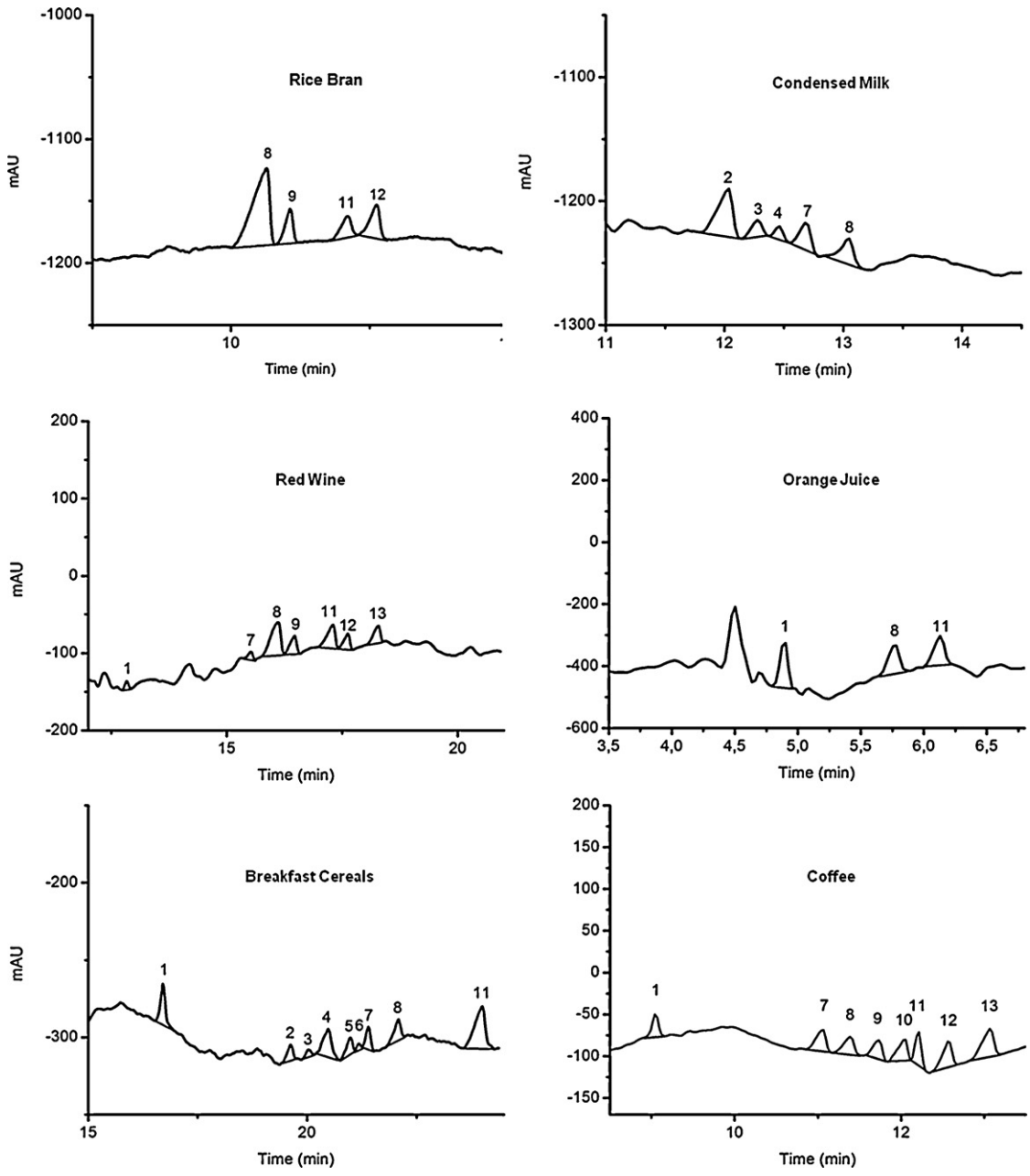

Fig. 1. Electropherograms for samples of orange juice, roasted and ground coffee, condensed milk, breakfast cereals, red wine and rice bran. Peaks identification: (1) saccharose, (2) lactose, (3) lactulose, (4) epilactose, (5) maltotriose, (6) maltose, (7) galactose, (8) glucose, (9) arabinose, (10) mannose, (11) fructose, (12) xylose, and (13) ribose. Analytical conditions are described in Table 4.

matrix effects. The optimum conditions found for the six matrices are presented in Table 4.

In most cases, the optimum conditions were very distinct for the different sets of CHOs. The exceptions were the electrolyte compositions for condensed milk and breakfast cereal matrices, which were very similar, with only a slight difference in the pH level. This difference occurred because the observed pairs in the separation method for the CHOs of the condensed milk samples were LAC-LTU LTU-EPI, EPI-GAL and GAL-GLU. In order to separate them, experimental conditions that presented the best resolutions for thses peak pairs were used.

In the CHO cereals procedure, in addition to the analytes studied for condensed milk, two other compounds eluted, MTO and MAL, between the EPI-GAL peaks, which needed to be separated. Thus, the pairs of target compounds increased to six, since it was necessary to separate: LAC-LTU LTU-EPI, EPI-MTO, MTO-MAL, MAL-GAL and GAL-GLU. The pH condition for this was a little lower than that of the condensed milk sample, and was the only pH that allowed adequate resolutions for the six pairs of interest. It was possible to observe that the most critical pair was MAL-GAL, which coeluted in 14 of the 17 experiments of the factorial design and only presented separations in a few experiments: the experiment with the coded −1.68, 0 and 0 levels, where the resolution was 1.52; the +1.68, 0, 0 levels with 1.45 resolution and the +1, +1 and +1 levels where resolution was 1.31, confirming that a reduction in pH is favorable for the separation of these compounds in this matrix.

Within the experimental region studied the models did not predict analytical conditions for separating all 13 compounds simultaneously for later application to untested matrices. In this case, the peak pairs that showed the greater separation difficul-

ties were LTU-EPI, MAL-GAL, ARA-MAN and MAN-FRU. From the predictions of the empirical models, when it was possible to separate the first two pairs, the other two coeluted completely and vice versa. A Derringer–Suich desirability analysis permitting extrapolation out of the experimental domain predicted separation of the 13 compounds if levels close to +3.00 were used. However, in replicate experimental confirmatory tests under these conditions severe reductions of analytical signal, increases in noise and run time as well as a lack of reproducibility in migration times and peak areas were observed.

It was possible to verify that the investigated procedure allowed the separation of the strategic sets of carbohydrates present in each sample, separating the critical CHOs. The central composite design and desirability optimization of Derringer and Suich resulted ingood resolutions among the CHOs in these matrices, and also yielded valuable information about the system in only 17 experiments.

3.2. Application in food and beverage samples

The six optimized procedures for the CHO standards were applied to real samples. However, some adjustments were necessary to solve matrix effect problems, and further reduce run time.

For the procedure applied to the orange juice sample, good separation was obtained while also reducing the capillary length to 60 cm and using 25 kV of voltage. The analysis was performed in 6.5 min.

For the condensed milk sample good separation was not achieved even with a 80 cm capillary, since the lactose peak overlapped the lactulose peak due to its high sample levels. Since the empirical model for LAC-LTU resolution showed that, unlike the other pairs of carbohydrates studied, none of the factors showed significant effects, some adjustments were necessary to provide better separation between these two carbohydrates. Initially the effect of increasing the total capillary length to 112 cm was tested, which did not improve separation. In this situation, investigations were conducted towards reduction of the bandwidth of the lactose peak. In the investigations carried out by Li et al. [41], NaCl was added to the sample as a method of online pre-concentration, resulting in the reduction of peak broadening caused by an excess of injected sample. Here, it was decided to inject the sample and then inject a 1 mol L^{-1} NaCl solution at 25 and 50 mbar pressures for 1, 2, 3, 4 and 5 s. For the sample used in this study, complete peak separation was obtained by injecting NaCl for 1 s at 25 mbar pressure. This simple procedure, realized by programming the equipment, showed that the addition of NaCl reduced the width of the lactose and lactulose peaks, which allowed separation in an 80 cm capillary within 13.5 min for the condensed milk sample.

The method developed for the group of carbohydrates present in cereal, when applied to real samples, showed a slight coelution between maltose and galactose. Using the same strategy applied in the condensed milk adaptation, peak separation was obtained by NaCl injection at 50 mbar for 1 s. The method had a 23 min run time.

In the procedure for roasted and ground coffee, a capillary reduction to 60 cm in total length and the use of 20 kV voltage was found to maintain adequate separation in the sample while run time was reduced to 14 min. In the procedure for samples of red wine, good separation in an 80 cm capillary during 19 min was found, but it was impossible to reduce the length to 60 cm. In the rice bran procedure, a reduction of the capillary to 60 cm maintained peak separation, with a 20 kV voltage, presenting excellent separation within 11.5 min. Fig. 1 shows the electropherograms for the procedures developed, optimized and with the necessary adaptations, applied to the individual food matrices.

4. Conclusions

The using of the MEKC technique with anionic surfactant, combined with multivariate modeling to study the effects of pH, electrolyte and surfactant concentrations on peak pair resolutions, followed by Derringer and Suich desirability optimization was efficiently used for the development of six different analytical procedures applicable to the quantification of CHOs in foods. The multivariate optimization methods have providedmuch information about factor effects permitting empirical predictions of peak resolutions, performing only 17 experiments. After slight adjustments it was possible to obtain excellent results in real samples. The methods were successfully applied to the separation of CHOs in condensed milk (lactose, lactulose, epilactose, galactose and glucose), rice bran (glucose, arabinose, xylose and fructose), red wine (saccharose, galactose, glucose, arabinose, fructose, xylose and ribose), roasted and ground coffee (galactose, glucose, arabinose, mannose, fructose, xylose and ribose), orange juice (sucrose, glucose and fructose) and even breakfast cereals (saccharose, lactose, lactulose, epilactose, maltotriose, maltose, galactose, glucose and fructose). The methods presented good compound separations and were cost-effective due to their fast analytical runs, use of simple and accessible electrolytes and reduced volumes of generated waste, demonstrating that micellar electrokinetic chromatography using anionic surfactant is a viable alternative for carbohydrate analysis in foods.

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