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Inulin Determination by an Improved HPLC-ELSD Method

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Abstract

Since inulin utilization as a sugar or fat replacer, texture modifier, and emulsifier is increasing in the food industry, there is a need for its determination for food control and labeling. This study reports the development and validation of an advanced high-performance liquid chromatographic method with evaporative light-scattering detection (HPLC-ELSD) method for inulin quantification, with optimized extraction conditions (1:10 w/v solid-to-solvent ratio, 1 h/80 °C in a water bath continued with 30 min ultrasonication for the extraction step, and precipitation during the night with four volumes of acetone). The rapid HPLC-ELSD analysis (16 min) has been performed by isocratic elution with water as a mobile phase and optimized detector parameters (temperature of evaporator 80 °C, temperature of nebuliser 80 °C, gas flow rate 1.3 standard liters per minute, detector gain 1). The method was validated in terms of limits of detection and quantification, intra- and inter-day precision and accuracy, and recovery. The proposed method was carried out using a cation exchange (Ag +) column for the first time for the inulin determination, avoiding the hydrolysis step in order to simplify and accelerate the quantification.

Keywords Inulin · HPLC-ELSD · Cation exchange column · Method development · Validation

Introduction

Inulin is a non-digestible fructan-type polysaccharide made of 2 to 60 fructose monomers linked with β (2→1) glycosidic bonds (Mensink et al. 2015). Chicory root is one of the richest inulin source (15–20%), as well as dahlia, kuth, and salsify (15–20%), followed by Jerusalem artichoke (12–19%), dandelion (12–15%), garlic (9–16%), and agave (3–7%) (Singh et al. 2019). Inulin is used as an ingredient in different food products in the role of fat or sugar replacer, a texture modifier, a stabilizer of foams and emulsions, or to increase dietary fiber content (Nair et al. 2010). Inulin is also classified as *generally recognized as safe* compound from the American Food and Drug Administration (Mensink et al. 2015).

Numerous studies have reported inulin extraction procedures from different sources, mostly applying water as the extraction solvent (Brkljača et al. 2014; Başaran et al. 2017; Iraporda et al. 2019). High-performance liquid chromatography (HPLC) seems to be the most applied technique for inulin quantification from plants (Milani et al. 2011; Başaran et al. 2017), cookies, and cereal bars (Zuleta and Sambucetti 2001).

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Cation exchange resins have been the most commonly used type of adsorbents for saccharide separation with a wide range of degree of polymerization (DP). Fabersani et al. (2018) used the cation exchange (Ag+) column to analyze higher fructooligosaccharides (FOSs, DP2–DP $\geq$ 10) in yacon flour, caprine milk, and goat yogurt, while Wiśniewski et al. (2013) employed this chromatographic column in galactooligosaccharide analysis.

Many studies devoted to inulin determination required its hydrolysis and quantification as a sum of fructose and glucose units (Wang et al. 2010; Li et al. 2018). Hydrolysis was an unavoidable step in inulin quantification when hydrogen ionic, calcium-loaded, calcium-type sulfone group, and hydrophilic polymeric gel resin columns were applied (Wang et al. 2010; Kristo et al. 2011; Xu et al. 2016; Li et al. 2018). Nevertheless, Jurková et al. (2014) explained that 100% transformation of polymers and oligomers of carbohydrates to monohydrates during the enzymatic reaction is hard to achieve. They explained utilization of cation exchange polymeric column in Ag + cycle mode (Rezex™ RSO Oligosaccharide), which is able to separate oligomers of glucose with DP 2–DP 10 and over DP 10, instead of Ca2 + column which was not efficient enough to separate carbohydrates and their oligomers formed by enzymatic cleavage. Chromatographic analysis in Ca + mode used in their study resulted in some non-separated compounds and an error of quantification. Additionally, Ag + resin columns perform stronger complexes with carbohydrates resulting in greater retention and better resolution when compared to Ca + resin columns (Huber and Bonn 1995).

Evaporative light-scattering detector (ELSD) is compatible with the detection of wide molecular weight carbohydrates, including polysaccharides and oligosaccharides, with a lower limit of detection for carbohydrates and the ability to work in gradient regime compared to RID (Condezo-Hoyos et al. 2015). ELSD detector was used in inulin determination from Jerusalem artichoke in the study of Li et al. (2018).

This study aimed to develop and validate an HPLC-ELSD method for quick and simple inulin quantification, without hydrolysis, using an analytical ion exchange Ag + mode (Rezex™ RSO Oligosaccharide Ag +, Phenomenex®, Aschaffenburg, Germany) column which was not used earlier for inulin determination. Special attention was paid to the optimization of inulin extraction and purification steps. Even though the sample preparation was a relatively long process, it allows adequate separation of analytes from the matrix. Furthermore, the sample preparation in chromatography is an important step for extraction of target analytes and removing the interferents (Ma et al. 2014); otherwise, the chromatograms would not be clear enough and the HPLC analysis itself would be more complicated. Additionally, optimization of inulin extraction, as well as optimization of detector parameters, has been performed. By applying this method, it is possible to quantify inulin content in chicory (*Chicorium intybus* L.) root and chicory-based food.

Material and Methods

Chemical and Reagents

Inulin from dahlia tubers was used as an internal standard (CAS 9005–80-5, Sigma-Aldrich, Darmstadt, Germany). Bidistilled water was produced by a Simplicity UV system from Millipore (Bedford, MA, USA). Ethanol (Zorka Pharma-Hemija DOO, Serbia) and acetone (Avantor®, USA) were of analytical grade. All chicory-based products available on the market in Serbia were collected and analyzed: three chicory roots (one was produced by the Institute "Dr. Josif Pančić", Belgrade, Serbia, while two others were purchased from local markets), four chicory root coffees (two pure chicory root coffees and two blends) and chicory inulin dietary supplement were purchased from the local markets and analyzed by established method. Rice-based and corn-based chicory snacks with 30% of chicory root were produced on a laboratory single-screw extruder (Model GNF 1014/2, Type 110,513, Brabender, Duisburg, Germany) at 14% moisture, 250 rpm screw speed, and 140 °C die temperature for a rice-based snack, and at 18% moisture, 250 rpm screw speed, and 150 °C die temperature for a corn-based snack. All samples were milled on a cooling mill (Knifetec™ 1095, FOSS, Denmark) prior to analysis.

Inulin Extraction and Precipitation

Ground and homogenized sample (1 g) was suspended in 10, 15, 20, or 30 ml of hot water, set in a water bath (WB OS-47, Memmert, Germany) for 1 h at 80 °C with constant agitation, and then sonicated 30 min in an ultrasonic bath (ATU Ultrasonidos, Valencia, Spain). The samples were centrifuged for 10 min at 4835 g-force (Eppendorf Centrifuge 5804R, Eppendorf, Wien, Austria). Supernatants were precipitated with two, three, or four volumes of acetone or ethanol in the refrigerator (4 °C) during one, two, or three nights. The samples were then centrifuged at 19,341 g-force for 15 min, and precipitates were dried at 40 °C in the laboratory dryer (ST-05, Instrumentaria, Zagreb, Croatia). The precipitates obtained under optimal extraction and precipitation conditions were then dissolved according to one of the following procedures:

1. The precipitates were resuspended in hot water and ultrasonicated for 30 min.
2. The precipitates were resuspended in hot water, placed in a water bath (80 °C) for 30 min, and then ultrasonicated for 30 min.

3. The precipitates were resuspended in hot water, placed in a water bath (80 °C) for 60 min, and then ultrasonicated for 30 min.

These processes differ according to whether the samples were placed in a water bath or not, and how long it took to dissolve them in the water bath (30 or 60 min).

The samples were filtrated through a membrane filter (regenerated cellulose, pore size 0.45 µm, diameter 25 mm, Agilent Technologies, Santa Clara, USA) prior to HPLC-ELSD analysis.

HPLC-ELSD Analysis

Inulin analysis was performed with an Agilent 1260 Infinity Series HPLC system (Agilent, Santa Clara, CA, USA) equipped with an autosampler, provided with a temperature controller, a quaternary pump, Rezex™ RSO-Oligossacharide Ag + (Phenomenex, Aschaffenburg, Germany) column (4%, 12 µm particle size, 200 × 10 mm), and ELSD (1290 Infinity ELSD, Agilent Technologies, USA). Applied chromatographic conditions were 0.3 ml/min flow rate with ultrapure water as a mobile phase, isocratic regime, 10 µl injection volume using autosampler injection mode, and 80 °C continuous column temperature. The total run time was 16 min. The following investigated detector parameters were included: evaporator temperature (Te) 80–90 °C, nebulizer temperature (Tn) 80–90 °C, gas flow rate 0.9–1.3 standard liter per minute (SLM), and a photomultiplier tube (PMT) detector gain 1.0–7.0.

Method Validation

Method performances were tested in terms of linearity, the limit of quantification and detection, intra- and inter-day precision and accuracy, and recovery.

Linearity

The linearity was determined by using spiked blank samples (rice flour spiked with different standard concentration range 0.1–0.25 mg/ml). The linearity of instrument response was expressed by the coefficient of determination (r^2).

According to Eurachem Guide (2014) the linearity should be determined by measuring plot response against concentration, by analyzing blank samples spiked with 6 to 10 different concentration of standard spaced in the range of interest. Since the chicory root contains appreciable amount of analyte (inulin), it was not considered as a blank sample and therefore not applicable for linearity determination.

The Limit of Quantification and the Limit of Detection

The limit of quantification (LOQ) is the lowest non-zero standard concentration. Rice flour was used as "the blank sample" (Eurachem Guide 2014) and was spiked with the reference standard in ten different concentrations (0.01–2.5 mg/ml) in order to define LOQ. Each concentration level was injected six times and relative standard deviation (RSD) was calculated. According to the Eurachem Guide (2014), if the standard deviation is approximately constant at low concentrations than a 10% relative standard deviation corresponds to LOQ. The LOQ may also be determined as the minimum concentration of the analyte (mg/ml) giving a signal-to-noise ratio of 10 (S/N) (Jiang et al. 2014; Weng et al. 2019), and this methodology was used for LOQ determination in other matrices (chicory root, rice-based chicory snack and chicory coffee).

On the other hand, the limit of detection (LOD) is the level of analyte at which detection becomes problematic (Eurachem Guide 2014) and was estimated as the minimum concentration giving a signal-to-noise ratio 3, for the analyte in the different matrices including rice flour, chicory root, rice-based chicory snack, and chicory coffee.

Repeatability and Reproducibility

Repeatability and reproducibility were evaluated through precision and accuracy tests. Measurement precision as a qualitative descriptor represents the closeness of compared results (Eurachem Guide 2014). On the other hand, the closeness of a single result to a reference (true) value is known as measurement accuracy and is typically expressed as a percentage of the true value. The accuracy was assessed as percent ratio of the standard concentration calculated from the calibration curve versus nominal concentration was determined as accuracy (Condezo-Hoyos et al. 2015). Intra- and inter-day variation were chosen to determine the precision and accuracy of the method, and the seven different standard solutions (0.05–0.25 mg/ml) were analyzed for six replicates within one and six consecutive days.

Recovery

The recovery trial was performed by adding known amounts of the standard at low (80% of the known amounts, I level), medium (the same as the known amounts, II level), and high (120% of the known amounts, III level) concentrations into rice flour and chicory-based products. The spiked samples were then extracted, processed, and quantified in accordance with the method mentioned above.

The recovery (R) was calculated according to the following formula:

$$R = ((\%found - \%sample)/\%added) \times 100$$

where %found is the percent of inulin measured in the spiked sample, %sample is the inulin content in a sample without spiking, and %added presents the percent of added inulin.

Statistical Data Processing

The optimization of HPLC parameters was performed using a standard score (SS). Analysis of variance (ANOVA) and Tukey's HSD test were used to compare differences between inulin content during extraction and detector optimizations. Statistical data processing was performed using the software package STATISTICA 13.0 (StatSoft Inc., Tulsa, OK, USA).

Results and Discussion

Optimization of Sample Extraction and Precipitation

Water is the most common solvent for inulin extraction, especially at higher temperatures (about 90 °C) which increases inulin solubility in water (Apolinário et al. 2017). Furthermore, the solid-to-solvent ratio (SSR) also manifests an important impact on the extraction yield. The investigated SSR included 1:11, 1:16, or 1:20 (w/v) for inulin extraction from Jerusalem artichoke (Lingyun et al. 2007; Abozed et al. 2009; Iraporda et al. 2019), 1:8, 1:16, 1:24, and 1:32 (w/v) ratio for ultrasonic-assisted inulin extraction from chicory root (Liu et al. 2011). One-step inulin extraction is mostly applied for inulin isolation (Milani et al. 2011; Başaran et al. 2017), but three-step extraction is also often used (Li et al. 2012; Mensink et al. 2015). To obtain inulin in solid form from liquid extract, it is necessary to precipitate it by cooling/freezing and then spray-drying which requires high-energy consumption, or by using different organic solvents in which inulin is hardly soluble such as methanol, ethanol, propanol, acetonitrile, and acetone (Mensink et al., 2015). Moreover, ethanol and acetone seem to be the most appropriate solvents for inulin precipitation, with an accent on acetone as a solution for long-chain inulin precipitation (Apolinário et al. 2014). According to these facts, different impacts were evaluated in the process of optimization of inulin extraction and precipitation, as it is presented in the Supplementary Information (Table S1).

Our preliminary study conducted in order to investigate the following inulin extraction parameters (under constant precipitation conditions: overnight precipitation at 4 °C with four volumes of acetone) showed that optimum conditions include 1:10 SSR and one-time extraction repetition. These conditions were applied for further inulin precipitation optimization. Optimized extraction results obtained in our study (SSR 1:10, 1 h at 80 °C extraction in water bath continued for 30 min in ultrasound bath) are in agreement with Lingyun et al. (2007) who established SSR 1:10.56 and 20 min extraction at 76.6 °C for inulin isolation from Jerusalem artichoke. Similarly, Baldini et al. (2004) extracted inulin from Jerusalem artichoke at SSR 1:10, at 85 °C for 3 h with mild shaking. Milani et al. (2011) also concluded that the ultrasonic technique will increase the extraction of inulin from Burdock root, indicating higher inulin yield after 25 min sonication compared to the conventional method. In our study, the optimization of inulin extraction (Table 1) was investigated in terms of inulin content, inulin peak characteristics, and SS.

Monitored output parameters for inulin precipitation were inulin content, peak resolution, and signal-to-noise value (S/N), as well as a peak shape (1, good shape; 0, bad shape) for inulin extraction (Table 1). The normal scores were calculated for each of these variables and were used for complex comparison of observed experimental runs. The ranking procedure between these runs was performed, based upon the ratio of raw data and extreme values for each applied assay (Brlek et al. 2013) according to the Eq. (1):

Table 1 The results (peak shape, inulin content, average peak resolution, average S/N, SS) obtained during optimization (solid-to-solvent ratio (SSR), number of extraction) of inulin extraction

SSR	Number of extraction	Peak shape	Inulin content %	Average peak resolution	Average S/N	SS
1:10	1	1	3.25 ± 0.79^{a}	2.03	56.00	0.729
1:10	3	0	3.10 ± 0.79^{ab}	2.01	22.15	0.00
1:15	1	1	3.27 ± 0.23^{a}	2.01	49.15	0.534
1:15	3	0	2.82 ± 0.37^{b}	2.02	50.05	0.00
1:20	1	1	3.03 ± 0.40^{ab}	2.00	43.30	0.546
1:20	3	0	2.76 ± 0.30^{b}	2.05	32.70	0.00
1:30	1	1	3.01 ± 0.28^{ab}	1.65	58.25	0.428
1:30	3	0	2.92 ± 0.19^{ab}	1.70	42.63	0.00

Data are shown as mean ± standard deviation of three replicates. Values in the column followed by different lowercase letters are significantly different ($P < 0.05$)

US ultrasonication

$$\bar{x}_i = \frac{x_i - \min_i x_i}{\max_i x_i - \min_i x_i}, \ \forall i \quad (1)$$

where x_i represents the raw data.

An optimization procedure was performed according to the standard score algorithm, to determine the workable optimum conditions for inulin precipitations (Table 2).

SS was calculated according to Eq. (2):

$$SS = w_1 \cdot \overline{Inulin\ content} + w_2 \cdot \overline{Resolution} + w_3 \cdot \bar{S/N} \quad (2)$$

The highest SS for inulin extraction and precipitation (0.729 and 0.532, respectively) are assigned to the optimum conditions with the following results: 1:10 solid-to-solvent ratio and 1 step extraction with hot water, 4 volumes of acetone during the night for precipitation at 4 °C, and 80 °C/60 min in a water bath and 30 min ultrasonicated for dissolving of the precipitated inulin. Precipitation with acetone gave higher inulin content (2.15–2.57%), due to the fact that acetone seemed to be better precipitation solvent for carbohydrates with higher DP including inulin, than ethanol (1.42–1.83%). Higher volumes of precipitate reagent are more effective for an increase of precipitated inulin weight than lower volumes (Abozed et al. 2009). The same conclusion was reached in our experiments, where 4 volumes of reagents were more effective for inulin precipitation (2.57% content) compared to lower volumes (2 and 3 reagent volumes gave 1.72% and 2.15% inulin content, respectively). The highest inulin content was achieved at the dissolving process under conditions optimized for inulin extraction (3.25%). Obtained results indicate that longer inulin dissolution shows greater efficacy compared to a shorter dissolving time (2.66% content) or the application of ultrasonication alone (2.57% content). Additionally, the increase in precipitation duration did not show a significant impact on inulin content, indicating that the most inulin precipitates during the first 12 h (López-Molina et al. 2005). The same conclusion was achieved in our study, distinguishing overnight precipitation (2.15%) as more effective in terms of inulin content when compared to two (1.21%) and three (1.31%) nights precipitation duration. Optimized precipitation results obtained in our study (precipitation of extracted supernatant with four volumes of acetone during the night at 4 °C) are in agreement with Abozed et al. (2009) who also performed overnight inulin precipitation at 4 °C. They noted that acetone (68.87%) and ethanol (70.25%) were better precipitation reagents compared to propanol (62.82%) and acetonitrile (65.21%) in terms of precipitated inulin weight, as well as higher volumes of precipitate reagent. Figure 1 shows the promotion of inulin separation and purification during precipitation optimization.

Optimization of ELSD Parameters

Independent variables used in central composite experimental design (Supplementary Information, Table S2) were Tn (80–90 °C), Te (80–90 °C), gas flow rate (0.9–1.3 SLM), and detector gain (1–7). Investigation parameters were chosen in accordance with Agilent ELSD recommendations (Agilent Technologies 2013), explaining that evaporator temperature for non-volatile compounds (such as sugars) should be set to 80–90 °C, while higher nebulizer temperature increases peak response and should not exceed the boiling point of the mobile phase. Gas flow rate should be higher for aqueous mobile phase compared to organic solvents (e.g., 1.6 SLM), but for the higher evaporator temperature, the lower

Table 2 The optimization (precipitation solvent, extract-to-solvent ratio, precipitate dissolving) of inulin precipitation and obtained results (inulin content, average peak resolution, average S/N, SS)

Precipitation solvent	Extract-to-solvent ratio	Precipitation duration	Precipitate dissolving	Inulin content %	Average peak resolution	Average S/N	SS
Acetone	1:2	Overnight	30 min US	1.72 ± 0.07^{ab}	1.93	135.91	0.311
Acetone	1:3	Overnight	30 min US	2.15 ± 0.08^{ab}	1.64	92.25	0.358
Ethanol	1:3	Overnight	30 min US	1.83 ± 0.40^{ab}	1.53	114.40	0.269
Acetone	1:4	Overnight	30 min US	2.57 ± 1.23^{ab}	2.02	100.68	0.346
Ethanol	1:4	Overnight	30 min US	1.42 ± 0.11^{a}	1.83	91.45	0.420
Acetone	1:3	2 nights	30 min US	1.21 ± 0.14^{a}	2.02	216.55	0.346
Acetone	1:3	3 nights	30 min US	1.31 ± 0.06^{a}	2.21	167.45	0.341
Acetone	1:4	Overnight	30 min/80 °C + 30 min US	2.66 ± 0.44^{ab}	1.76	119.50	0.432
Acetone	1:3	Overnight	60 min/80 °C + 30 min US	2.76 ± 0.48^{ab}	1.47	164.28	0.316
Acetone	1:4	Overnight	60 min/80 °C + 30 min US	3.25 ± 0.18^{c}	1.25	100.97	0.532

Data are shown as mean ± standard deviation of three replicates. Values in the column followed by different lowercase letters are significantly different ($P < 0.05$)

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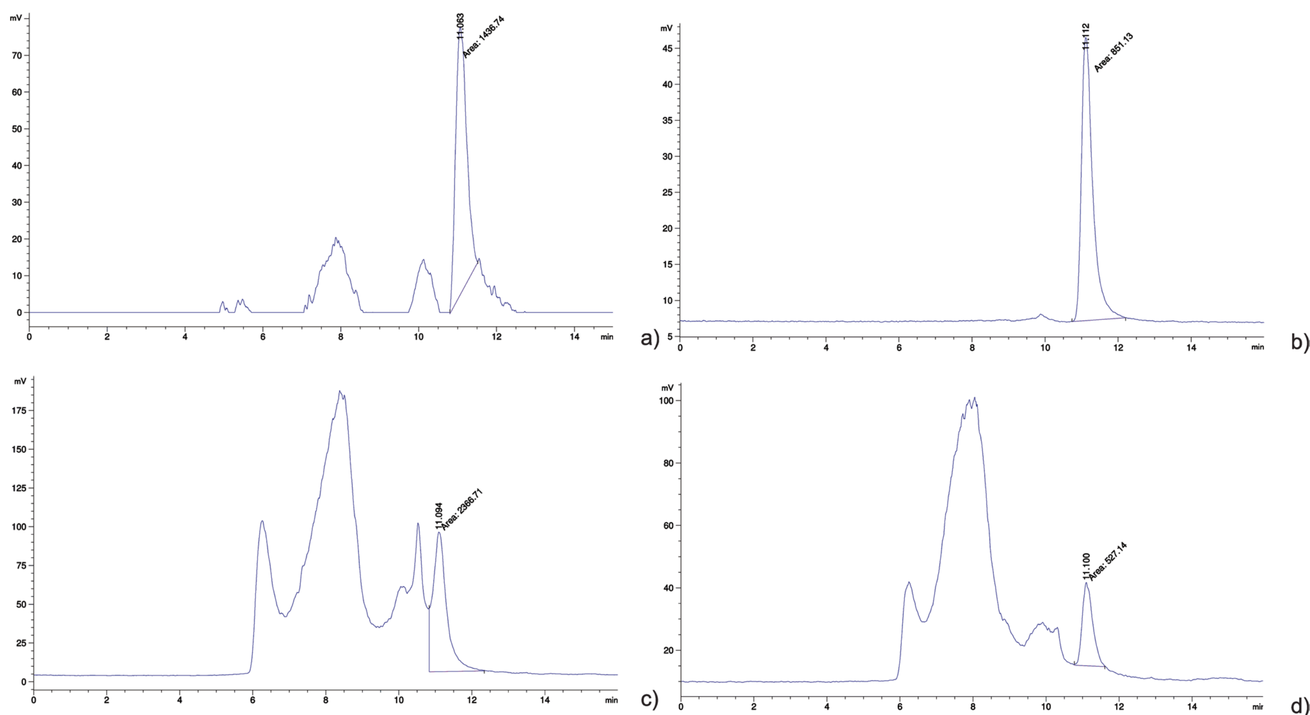

Fig. 1 Chromatograms during optimization of inulin separation without/with precipitation from chicory root (**a**, **b**) and rice-based snack (**c**, **d**)

gas flow is required (e.g., 1.0–0.9 SLM). The detector gain was investigated in the range of 1 to 7, according to scientific publications devoted to ELSD parameters (Dvořačkova et al. 2014; Condezo-Hoyos et al. 2015; Koh et al. 2018). Response variables were peak shape, peak symmetry, and S/N, which correspond to peak properties, and a number of theoretical plates that indicate column efficiency and impact of certain set conditions (such as temperature). The optimization of ELSD was performed for 0.15 mg/ml standard solution. Optimization of detector parameters was evaluated through SS obtained on the basis of maximum values for a good peak shape and peak symmetry, number of theoretical plates, and S/N ratio. The obtained values of these variables are shown in the Supplementary Information (Table S2).

Weight coefficients were given to response variables according to their significance, in the following order: S/N ($w_1 = 0.5$), peak symmetry ($w_2 = 0.25$), and the number of theoretical plates ($w_3 = 0.25$), while polarity was also positive.

SS values were calculated according to the Eq. (3):

$$SS = Peak\ shape \cdot \left(w_1 \cdot \overline{Simmetry} + w_2 \cdot \overline{Number\ of\ theoretical\ plates} + w_3 \cdot \bar{S/N} \right) \quad (3)$$

The peak shape directly eliminated those detector conditions that gave bad peak shapes (Supplementary Fig. S1), while peak symmetry, number of theoretical plates, and S/N values should be as high as possible. Finally, the best SS (0.849) was assigned for 80 °C Tn, 80 °C Te, 1.3 SLM, and gain 1. Similar detector parameters were adjusted for carbohydrate analysis with HPLC-ELSD (Tn 79.9 °C, Te 88.9 °C, gas flow rate 1.1 SLM, and gain 1) in the study of Condezo-Hoyos et al. (2015).

Method Validation Parameters

Linearity

The linearity was defined with line $Y = 3078x - 252.52$, where Y presents the peak area and x is the concentration of inulin (mg/ml). An excellent linearity was confirmed through the correlation coefficient of $r^2 = 0.9982$ ($n = 9$).

LOQ and LOD

Exponential function with good correlation (coefficient of determination 0.9667) gave Eq. (4) for determination of LOQ:

$$y = 14.291e^{-9.509x} \quad (4)$$

where y is precision (%) and x is the concentration of inulin (mg/ml). LOQ was 0.037 mg/ml from this equation as it is presented in Supplementary Fig. S2. The LOQ was determined as the minimum concentration of the analyte (mg/ml) giving a signal-to-noise ratio of 10 (S/N) (Jiang

et al. 2014; Weng et al. 2019). LOQ values were 0.018 mg/ml, 0.031 mg/ml, and 0.11 mg/ml for chicory root, rice-based chicory snack, and chicory coffee, respectively. The LOQ values for spiked blank sample (based on rice flour, 0.037 mg/ml) and rice-based chicory snack (0.031 mg/ml) gave approximately the same values, which confirms the consistency of determining the LOQ by applying two different methods (exponential dependence of RSD and inulin concentration, and when S/N ration is 10). Similarly, LOQ values for HPLC-ELSD analysis of inulin-type oligosaccharides in traditional Chinese medical plant ranged from 0.0072 to 0.0493 mg/ml (Yang et al., 2011a, b). The LOD was 0.01 mg/ml for rice flour, 0.006 mg/ml for chicory root, 0.0103 mg/ml for rice-based chicory snack, and 0.036 mg/ml for chicory coffee.

Such a low LOD and LOQ values were also reported in the study of Li et al. (2013) who established LOD at 0.00363 mg/ml and LOQ at 0.02482 mg/ml for the HPLC-ELSD method for determination of fructooligosaccharides in burdock. Additionally, Yang et al. (2011a, b) reported LOD at 0.0021–0.0148 mg/ml and LOQ at 0.0072–0.0493 mg/ml during validation of the HPLC-ELSD method for inulin-type oligosaccharide quantification.

Repeatability and Reproducibility

Accuracy ranged from 88.28 to 102.51% and 88.48 to 100.07% for intra- and inter-day, respectively. Precision ranged from 2.55 to 7.93 for intra-day and 1.84 to 5.81 for inter-day test. Obtained values (Table 3) are within the acceptable ranges. Acceptability range refer both for the precision and accuracy because acceptance criteria for accuracy should be ± 15% of nominal concentration, and for precision, it should be ± 15% CV (Guidance for Industry, Bioanalytical Method Validation 2001).

Table 3 Intra- and inter-day precision and accuracy

Inulin	Nominal conc. (mg/ml)	Measured conc. (mg/ml)	Precision (% RSD)	Accuracy (%)
Intra-day ($n = 7$)	0.1	0.0981	5.67	98.14
	0.125	0.1281	4.57	102.51
	0.15	0.1487	7.93	99.16
	0.175	0.1545	3.43	88.28
	0.2	0.2007	2.82	100.38
	0.225	0.2239	3.46	99.54
	0.25	0.2479	2.55	99.16
Inter-day ($n = 7$)	0.1	0.0972	5.17	97.27
	0.125	0.1248	5.81	99.84
	0.15	0.1462	1.84	97.46
	0.175	0.1531	3.29	87.48
	0.2	0.2002	2.45	100.07
	0.225	0.2245	3.22	99.77
	0.25	0.2447	2.63	97.86

Lin et al. (2010) reported RSD of 2.59–11.43% for intra-day and 7.12–11.9% for inter-day precision in LC–MS inulin quantification. Similar results were reported in the study of Zhuang et al. (2019) where they obtained intra-day precision of 0.29–4.43% and inter-day precision of 5.74–10.91% for three different concentrations ($n = 6$), and accuracy of 83.16–99.6% ($n = 3$) in HPLC-ELSD analysis of inulin-type fructooligosaccharides.

Recovery

Recovery test showed the recovery range of 81.02–97.38% for chicory coffee, 96.19–104.23% for a rice-based chicory snack, and 96.20–99.19% for the rice flour (Table 4). Retnaningtyas (2012) validated a method for inulin determination in syrup product and obtained recovery for the proposed method ranging 98.33–100.28%. Obtained inulin recovery was 95.93–100.90% in the study of Weiß and Alt (2017) who developed a method for the detection of fructans.

Inulin Content in Chicory-Based Food

The established method was confirmed through determination of inulin content in four different product groups available at Serbian market: chicory roots (3) and chicory-based coffees (4), snacks (2), and dietary supplement (1). Table 5 summarizes obtained results calculated on the dry weight (d.w.).

Chicory root contains 7.92 to 13.99% of inulin calculated on the dry weight. These results are similar to study of Başaran et al. (2017) who reported 9.65 to 14.39% of inulin content after its determination in 20 chicory root samples. On the other hand, Figueira et al. (2004) noted 11.85 to 14.93% of inulin in chicory roots. Variation in inulin content may be due to factors that can affect the inulin amount in plants including growing conditions, maturity at harvest date, and storage duration after harvest (Apolinário et al. 2014).

The intensity of roasting process may affect inulin content in chicory root coffees, causing inulin conversion into fructose and reducing inulin content depending on roasting duration in order to obtain “dark” (48% inulin degradation) or “normal” (21.5% inulin degradation) roasted product (Loo et al. 1995). Analyzed coffee samples did not contain information about the roasting temperature and duration, neither the share of chicory root in coffee mixtures.

Table 4 Recovery test results

Sample	Inulin content (%)	Level of standard inulin added (%)	Recovery (%)
Chicory coffee III (mixture)	3.77	I (80%) = 3.01	97.38 ± 1.88
		II (100%) = 3.77	91.01 ± 0.28
		III (120%) = 4 .52	81.2 ± 0.51
Rice-based chicory snack	3.51	I (80%) = 2.81	96.2 ± 1.73
		II (100%) = 3.51	104.23 ± 4.63
		III (120%) = 4.21	103.003 ± 2.99
Rice flour	0	I (80%) = 1.60	97.13 ± 1.47
		II (100%) = 2.00	99.19 ± 2.85
		III (120%) = 2.40	96.20 ± 1.73

Data are shown as mean ± standard deviation of three replicates

Table 5 Inulin content in analyzed chicory roots and chicory-based food samples

Sample	Inulin content % (d.w.)
Chicory root I	11.72 ± 0.67
Chicory root II	7.92 ± 0.15
Chicory root III	13.99 ± 0.32
Chicory coffee I (100% chicory root)	0.87 ± 0.04
Chicory coffee II (100% chicory root)	1.43 ± 0.12
Chicory coffee III (mixture)	3.77 ± 0.12
Chicory coffee IV (mixture)	8.19 ± 0.76
Rice-based chicory snack (30% chicory root)	3.51 ± 0.17
Corn-based chicory snack (30% chicory root)	3.43 ± 0.13
Dietary supplement of chicory inulin	94.95 ± 0.75

Data are shown as mean ± standard deviation of three replicates

The chicory coffee I (100% chicory root) is the darkest among analyzed coffees, followed by the chicory coffee II (100% chicory root), while the coffee mixtures were much brighter, which is in accordance with determined inulin contents of 0.87–8.19% (Table 5). It can be assumed that milder thermal treatment during the roasting process gave lighter coffee samples (b, c, and d) with higher inulin content (1.43%, 3.77%, and 8.19%, respectively), compared to the darkest coffee sample (a, 0.87%). Although, it may be supposed that the share of chicory root in the mixtures was larger in coffee sample (d) with higher inulin amount (8.19%).

Rice- and corn-based snacks enriched with 30% of chicory root I contained 3.51 and 3.43% of inulin. These results are in agreement with the inulin amount present in raw material, confirming the fact that extrusion-cooking does not affect inulin stability importantly (Peressini et al. 2015). Dietary supplement containing powdered plant fibers from chicory root is in accordance with product declaration and contains 94.95% of inulin.

Conclusion

The HPLC-ELSD method for inulin quantification in chicory root and rice-based-chicory snack was successfully developed. This HPLC analytical method is quick (16 min) and simple with isocratic elution and inulin separation achieved by ion exchange Ag + column with water as mobile phase (0.3 mL/min). The method suitability for inulin determination is reflected through method validation parameters that demonstrated satisfactory results in terms of the linearity, precision, recovery percentage, detection limits, and quantification. Optimal extraction and chromatographic conditions were determined by standard scores in order to provide the highest inulin content and the best chromatographic peak properties. Thus, the relatively long process of sample preparation is compensated with the rapid and inexpensive HPLC-ELSD method developed for inulin quantification in chicory root and chicory-based food which can be easily applied in research and industry to analyze inulin content in raw materials and final products.

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Declarations

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