Title:

A quick NIR based method for ascertaining Coffee and Chicory percentage in a mixture

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Highlights

- FT-Near infrared method for ascertaining coffee and chicory percentage accomplished
- Fast and non-invasive method for ascertaining coffee and chicory percentage
- Method applicable to Robusta, Arabica varieties and instant coffee along with chicory
- Facilitates customer for informed choices on coffee selection

Abstract:

Coffee is a widely consumed beverage of the human population for several centuries. In coffee consuming countries encompassing India, Brazil, France, and parts of the USA,

chicory is added to coffee as a substitute and to enhance the color of the beverage. There is hardly any non-destructive technique to ascertain the percentage of chicory and coffee in the solid mixture. Herein, we report a simple and quick near infrared spectroscopy (NIR) based method for quantification of coffee and chicory percentages in the solid mixture. The method has been developed for Arabica, Robusta variety coffee powder in addition to instant coffee powder. We evaluated a commercial coffee powder having reported values of 65 % of Coffee and 35 % of Chicory by employing the developed method. The achieved method revealed a result of 64.2 % of coffee and 35.8 % of Chicory. Results demonstrate the power of NIR spectroscopic method as a rapid technique for quantification of coffee and chicory percentage in the solid mixture which is expected to facilitate the consumer and coffee industry.

Keywords:

Coffee, Chicory, NIR, PLS, quantification

1. Introduction

Coffee is a popular beverage consumed on daily basis by the human population across the globe. In major coffee consuming countries including India, France, Brazil, and in few states of the USA, roasted chicory root powder is added to coffee as a substitute due to its

similar flavor attributes [1]. Chicory root beside the substantial number of flavors is also composed of prebiotic polysaccharide inulin [2]. Historically, chicory was added to coffee in France mainly to address the scarcity of coffee due to political turmoil leading to blockage of ports (Wild 2005). But the chicory addition practice has sustained to date mainly due to lower cost and comparable flavor attribute [1].

Fourier transform infrared (FT-IR) spectroscopy has found innumerable applications in food analysis [3,4]. FT-IR application in the coffee analysis is mainly facilitated by attenuated total reflection (ATR) technology. On the other hand, because of game changing development of commercial FT-NIR instruments [3,4], the FT-NIR technique has been at the forefront of food analysis including coffee [5-6]. FT-NIR based method has been developed for the detection of adulterant barley in the coffee powder [7]. Many researchers have actively pursued the identification/classification of Arabica and Robusta varieties and their discrimination study by utilizing FT-NIR based methods [8-11]. FT-NIR methods for the analysis of coffee components such as caffeine, theobromine, and theophylline have been accomplished by employing suitable wavelengths in NIR region [5,12-13]. Further, analysis of ash and lipid content has been achieved by Pizarro and the team utilizing FT-NIR based method [14]. Furthermore, caffeine content and roasting color were analyzed by employing FT-NIR in a wavelength range of 1100-2500 nm [15-16]. Besides the coffee composition analysis, the prediction of sensory attributes of coffee by employing FT-NIR has also been reported [9,17]. One of the rare studies is also reported on the prediction of the roasting degree of coffee beans by employing FT-NIR in the wavelength of 834-2500 nm [18].

Chicory powder has been reported to contain nearly tenfold higher acrylamide content compared to roasted coffee [19]. whereas, the instant coffee has a higher occurrence level of acrylamide in comparison to roasted coffee [19-20]. The addition of chicory to coffee as a substitute benefits the beverage acceptance concerning the color and also renders

the advantage of prebiotic molecule inulin [2]. Further, chicory addition brings economic benefits for a grower/manufacture. But the coffee drinker has the risk of consumption of nearly tenfold higher acrylamide as a result of chicory addition to coffee. In this context, a simple, rapid and user friendly method is warranted to ascertain the ratio of coffee and chicory to make informed choices for the coffee consumer and as well as for the coffee industry in general. There has been an effort to quantify chicory in ground coffee, employing high pressure anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) [21]. The HPAEC-PAD method is based on the analysis of D-mannose and D-galactose after acid hydrolysis of water extract of coffee and chicory mixture. This method is a laborious, destructive, and also indirect method to ascertain chicory concentration. Furthermore, it is based on the quantification of coffee via measurement of polysaccharide galactomannan using hydrolyzed D-mannose and D-galactose to ascertain chicory concentration. In comparison, NIR is a non-destructive technique and offers an advantage as it is applicable in solid state and also sample preparation is minimal. We present in this paper a detailed report on the NIR method development for ascertaining the percentage of coffee and chicory in the solid mixture.

2. Materials and Methods

2.1 Coffee and chicory samples procurement

Roasted and powdered Arabica and Robusta varieties of coffee were procured from the local Mysuru market. The procured coffee samples were kept in airtight containers at 25 °C till subjecting it to spectroscopic experiments. The instant coffee and commercial coffee samples (Coffee day brand, Cothas, Bayar’s, Kwality, Suma, Modern Coffee, etc) containing the various percentage of coffee and chicory were procured from Bengaluru supermarket. These samples were also stored in airtight containers at 25 °C.

2.2 Sample preparation for Arabica coffee and chicory mixture

Initially, clean 40 glass vials were dried in a hot air oven at 130° C for 5 hours. After that, Arabica coffee powder in an incremental way from 0.25 to 9.75 g along with chicory powder in decreasing order 9.75 to 0.25 g were weighed and mixed thoroughly in the glass vial. Besides, 10 g of pure Arabica and pure chicory powder also weighed separately and stored in the vial. In total, 40 samples containing various percentages of coffee and chicory were prepared.

2.3 Sample preparation for Robusta coffee and chicory mixture

The sample preparation concerning Robust coffee and chicory mixture was similar to Arabica coffee powder and chicory mixture samples. In total, 40 samples containing the various percentage of Robusta coffee powder and chicory were prepared.

2.4 Sample preparation for coffee Arabica, coffee Robusta and chicory mixture

Initially, about 500 g of Robust and Arabica varieties of coffee powder are thoroughly mixed in a glass jar. Subsequently, coffee powder (an equal mixture of Arabica & Robusta varieties) in an incremental way from 0.25 to 9.75 g along with chicory powder in decreasing order 9.75 to 0.25 g were weighed and mixed thoroughly in the glass vial. Further,10 g of coffee powder (an equal mixture of Arabica & Robusta varieties) and pure chicory powder were also weighed separately and stored in the vial In total, 40 samples containing the various percentage of coffee (an equal mixture of Arabica & Robusta varieties) and chicory were prepared.

2.5 Sample preparation for Instant coffee and Chicory mixture

The sample preparations were similar to Arabica coffee and chicory mixture samples wherein, instant coffee powder in an incremental way from 0.25 to 9.75 g along with chicory powder in decreasing order 9.75 to 0.25 g were weighed and mixed thoroughly in the glass vial. Additionally, 10 g of pure instant coffee powder and pure chicory powder also weighed

separately and stored in the vial. In total, 40 samples containing various percentages of instant coffee and chicory were prepared.

2.6 NIR Spectra acquisition

FT-NIR spectra were recorded by employing Bruker instrument (Model Tango) in the frequency range of 4000-11500 cm^{-1} (corresponding wavelength range 870-2500 nm) for every sample with 32 scans with resolution 8 cm^{-1}. Glass vial loaded with coffee powder sample was placed on the trans-reflectance accessory of Tango instrument to acquire the NIR spectra. FT-NIR spectra were recorded in triplicate for every sample.

2.7 Calibration and validation of the developed model for coffee content and chicory content

FT-NIR spectra for various ratios of coffee and chicory powder were recorded using the Bruker Tango instrument as mentioned in the previous section. Opus 7.5 (Bruker optics) software program was used for data pre-processing and method development. Method development employed the partial least square (PLS) regression analysis.

The partial least square regression (PLS) is the most commonly used chemometric method in analytical chemistry. In the OPUS software program, the number of PLS vectors employed is defined by the 'rank'. For accomplishing lower rank (Less number of PLS factors), a substantial number of samples are required (we employed 40 samples). The calibration model was developed by employing 40 samples having various concentrations of coffee and chicory (for sample preparation procedure please refer to previous sections) and its error was estimated by employing RMSEE

$$RMSSE = \frac{\sqrt{SSE}}{\sqrt{M-R-1}}$$

Where in SSE= $\sum_{i=1}^{M} (Resi)2$

The validation of the developed calibration model was carried out via a cross-validation technique. The steps of cross-validations are as follows

1) One sample was removed from the calibration step.

2) Calibration model was set up with the remaining sample.

3) Analysis of the removed test sample was carried out and further error of analysis this sample was estimated: Y_1^{meas}- Y_1^{pred}

4) Removed sample is added to the data set and another sample is removed. The new model is calculated and further error of analysis of this sample was estimated: Y_2^{meas}- Y_2^{pred}.

5) All the above steps were carried out for M samples of the calibration data set until all the M samples have been analyzed. The mean error of prediction RMSECV is calculated as

$$RMSECV = \sqrt{1/M} \sum_{i}^{M} (Yimeas - Yipred)2$$

Data pre-processing is an important task in the development of the calibration model and as well in the subsequent cross-validation. By selection of appropriate pre-processing conditions, (first derivative, second derivative, etc. more information is reported in Table 2) excellent correlation of NIR spectra and concentration of chicory can be established.

3. Results and Discussion

3.1 NIR method for coffee Arabica and chicory mixture.

The first and crucial step in the NIR based method development comprises the analysis of the concentration parameter of an analyte using primary techniques such as volumetric method, gravimetric method, HPLC, GC, MS, etc, Chicory is a complex material and a single analytical method is reported for its analysis in chicory containing coffee. Chicory content in coffee has been previously analyzed based on high pressure anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) [21]. The HPAEC-PAD based method involves the analysis of D-mannose and D-galactose after acid hydrolysis of water extract of coffee and chicory mixture. This method is laborious and also not a reliable primary technique. It does not quantify chicory by direct measurement instead it relies on the quantification of coffee via measurement of hydrolyzed D-mannose and D-galactose of coffee polysaccharide galactomannans. Due to the lack of a primary technique for the direct analysis of chicory content in coffee mixture, we were challenged initially itself before attempting NIR method development. However, as the gravimetric method is one of the accepted primary technique (E.g. moisture content in green coffee) which relies on the amount of mass measurement [22-24] we opted for mass measurement of chicory during sample preparation itself as the primary technique of analysis of chicory content before subjecting it to NIR method development.

NIR method development started with spectral data acquisition followed by preprocessing of the spectra and subsequent application of the chemometric software package (Opus quant). The NIR spectra (figure 1) was recorded in the frequency range of 4000-11500 cm^{-1} (corresponding wavelength range 870-2500 nm) for various composition mixture of Arabica coffee and chicory to develop a calibration model. For the NIR method development, a wavelength range 1099-2381 nm was employed.

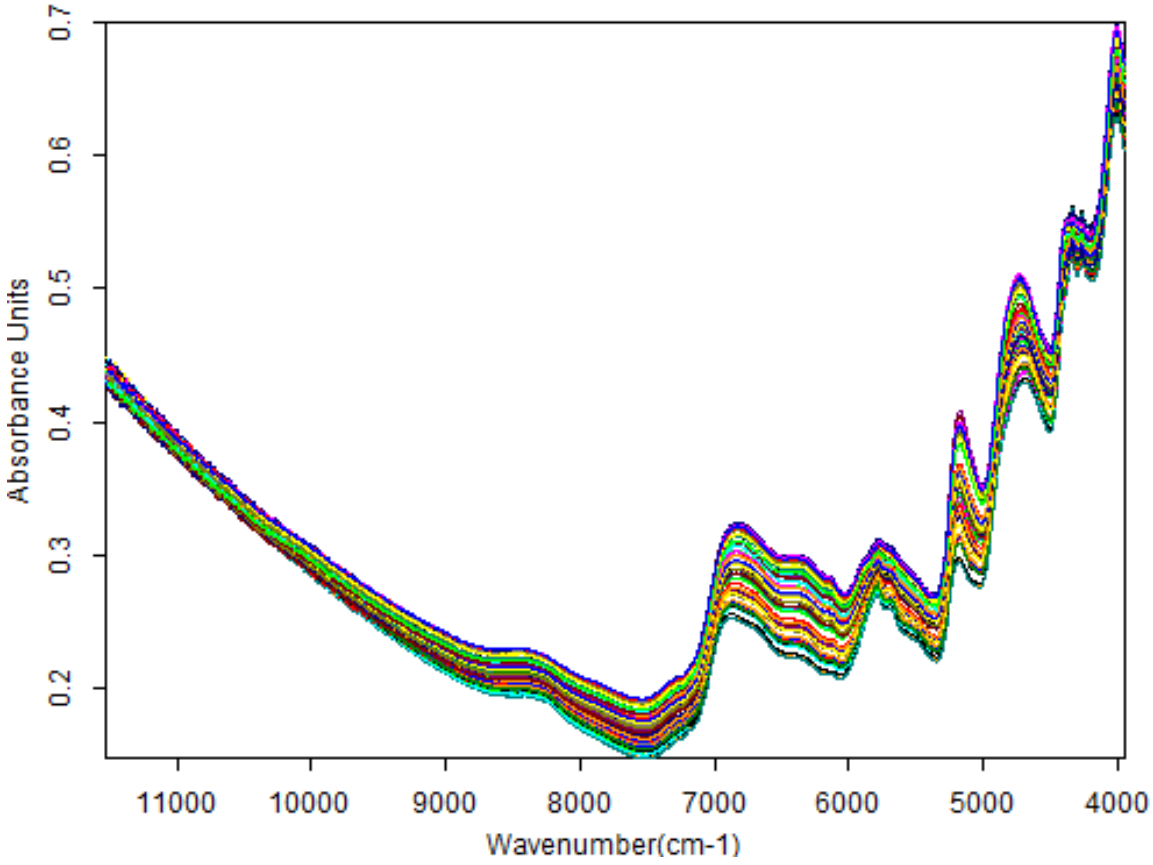

Figure 1: NIR spectra of the various compositional mixture of Arabica coffee and chicory

The wavelength range deployed for the method development mainly encompasses combination and overtone hands of methyl (CH_3), methylene (CH_2), aromatic CH, alcoholic OH, CHO groups (Table 1).

Table 1:.Representative functional groups in the frequency range 4100-9100 cm^{-1}

Groups	Nature of bands	Frequency (cm^{-1})	Assignment
CH_3 (methyl)	Combination	4380-4400	CH stretching +CH bending
		7330-7380	2x CH stretching+ CH bending
	1^{st} overtone	5850-5780	1^{st} overtone of anti-symmetric stretching
		5600-5650	1^{st} overtone of symmetric stretching
	2^{nd} overtone	8580-8700	2^{nd} overtone of anti-symmetric stretching
		8330-8400	2^{nd} overtone of symmetric stretching
CH_2	Combination	4290-4310	CH stretching +CH bending

(Methylene)		7040-7090	2X CH stretching+ 3x CH bending
	1st overtone	5710-5760	1st overtone of anti-symmetric stretching
		5570-5620	1st overtone of symmetric stretching
	2nd overtone	8470-8550	2nd overtone of anti-symmetric stretching
		8260-8330	2nd overtone of symmetric stretching
CH (aromatic)	Combination	6900-6940	2x CH stretching+ CH bending
		7040-7090	2x CH stretching+ 3x CH bending
		9220-9350	2x CH stretching+ 3x CH bending
	1st overtone	5920-5950	None
	2nd overtone	8770-8850	None
C=O (Ketone)	2nd overtone	5110-1230	None
CHO aldehyde	Combination	4520-4570	CH stretching +C=O streching
Free OH(alcohol)	Combination	4780-4850	OH stretching +OH bending
	1st overtone	7020-7170	
	2nd overtone	4180-4220	2nd overtone of OH bending

Details of pre-processing techniques employed are mentioned in table 2. The spectra of various compositions of Arabica coffee and chicory are displayed in figure 1. The optimization of the method was achieved by carrying out PLS regression for 40 samples with various concentrations of Arabica coffee and chicory powder. To figure out suitable pre-processing conditions, the calibration model and cross validation process was attempted by employing all the available preprocessing conditions as depicted in table 2. Data pre-processing is an important task in the development of the calibration model and as well in the subsequent cross-validation. By selection of appropriate pre-processing conditions, an excellent correlation of NIR spectra and concentration of the analyte (chicory) can be established. In this regard, we employed various standalone and also a combination of pre-processing techniques for the spectral data leading optimization of the method.

Table 2: Effect of different preprocessing techniques on calibration and validation of method for Arabica coffee and chicory mixture

Preprocessing technique	R^2 calibration	RMSEE	R^2 validation	RMSCV	PLS factor
No preprocessing	99.96	0.65	99.91	0.94	9
First derivative	99.97	0.54	99.86	1.15	7
Second derivative	99.95	0.59	99.86	0.97	3
First derivative + Straight line substation	99.92	0.94	99.81	1.4	6
First derivative +Vector normalization	99.99	0.38	99.87	1.06	7
First derivative +MSC	99.91	1.06	99.83	1.31	8
Vector normalization	99.99	0.32	99.97	0.61	9
Multiplicative scattering correction	99.94	0.86	99.87	1.18	9
Straight line substation	99.96	0.70	99.83	1.31	9
Min-Max normalization	99.99	0.39	99.89	1.02	10

Among the pre-processing techniques employed for the method development, the second derivative furnished the best result with 3 PLS factor for the optimization of the method with a coefficient of determination (R^2) 99.86 with RMSCV 0.9, followed by the first derivative and incorporating straight line subtraction requiring 6 PLS factor with R^2 of 99.86 with RMSCV 1.4. Further, the employment of the first derivative and combination of the first derivative with vector normalization required decent 7 PLS factors with a comparable coefficient of determination.

Then commercial coffee samples blended with chicory were tested for ascertaining the percentage of coffee and chicory. One spectrum was enough to get the result after dropping the spectrum in the graphical interface of the model developed. The prediction of

commercial samples for the percentage of coffee and chicory was in agreement with the methods mentioned in table 2, best being the one with the least PLS factors.

3.2 NIR method for Robusta coffee and chicory mixture.

The NIR method development for chicory content in Robusta coffee containing chicory was developed similar to Arabica coffee and chicory mixture. The spectra of the various compositions of Robusta coffee and chicory mixture are displayed in supporting information. The spectral input for the method involved 1099-2381 nm. Among the pre-processing techniques utilized for the method development second derivative was the best with 5 PLS factors employing R^2 of 99.82 with RMSCV 1.29. On the other hand, three pre-processing techniques consisting of the first derivative, first derivative plus vector normalization, and first derivative plus MSC required 6 PLS factors with comparable R^2 and RMSCV values.

3.3 NIR method for Robusta coffee Plus Arabica coffee and chicory mixture.

Before the NIR method development, an equal amount of Arabica and Robusta coffee powder was mixed and it was homogenized thoroughly. Subsequently, experimental samples were prepared by drawing a known mass from coffee mixture and chicory in various amounts as discussed in materials and methods. The optimization of the method was very similar to that of coffee Arabica and chicory mixture. The spectral input for the method involved a wavelength range of 1099-2381 nm. Among the pre-processing techniques utilized for the method development (supporting information) the second derivative was the best with 4 PLS factors having R^2 of 99.68 with RMSCV 1.62. Few other pre-processing techniques required 7 PLS factors with comparable R^2 and RMSCV.

This method was applied to predict the percentage of coffee and chicory for the commercial samples when the variety of coffee was not mentioned on the label.

3.4 NIR method for Instant coffee and chicory mixture

The NIR method for chicory content in instant coffee containing chicory was developed similar to Arabica coffee and chicory mixture. The spectral input for the method involved a wavelength range of 1099-2381 nm. Various pre-processing techniques utilized for the method development are depicted in supporting information. The first derivative plus vector normalization and first derivative with MSC required 3 PLS factors with comparable R^2 and RMSCV followed by the second derivative with R^2 of 99.75 and RMSCV of 1.5.

3.5 Screening of commercial samples utilizing the developed method.

Table 3: Screening of commercial coffee samples for ascertaining coffee and chicory percentage

Commercial samples	NIR model applied	Preprocessing	% coffee according to label	% chicory according to label	% coffee from NIR method	% chicory from NIR method
Coffee day	Arabica+Chicory model	Second derivative	65	35	64.20	35.80
	Arabica+Chicory model	Second derivative	85	15	85.05	14.95
Bayar's Coffee	Robusta+Chicory model	Second derivative	70	30	69.39	30.61
	Robusta+Chicory model	Second derivative	80	20	80.27	19.73
Cothas	Robusta+Chicory model	Second derivative	60	40	59.53	40.47
	Robusta+Chicory model	Second derivative	80	20	79.84	20.16
Kwality Coffee	Robusta+Chicory model	Second derivative	60	40	60.30	39.70
	Robusta+Chicory model	Second derivative	80	20	79.66	20.34
Suma Coffee	Robusta+Chicory model	Second derivative	90	10	90.28	9.72

Modern Coffee	Robusta+Chicory model	Second derivative	85	15	84.92	15.08

Various commercial samples were screened by the application of developed models for ascertaining the coffee and chicory percentage. The Arabica coffee NIR method with second derivative preprocessing was applied to a couple of samples that had labeling Arabica as coffee contents. On the other hand, many samples that did not specify the variety used were screened with the Robusta coffee NIR method with second derivative preprocessing and the results were in excellent agreement as shown in table 3.

4. Conclusion

We have devised and established quick and convenient NIR based methods for the authentication chicory percentage in the coffee chicory solid mixture. It is a fast and non-destructive method that allows the determination of the percentage of coffee and chicory without the intervention of wet chemistry. This technique may lead to a wide application in industry and among coffee customers to make informed choices concerning the composition of coffee with coffee substitute chicory.

Disclosure Statement

The authors report no conflict of interest.

Supporting information

The supporting information is available free of charge in the online version of the journal. The supporting information is composed of NIR spectra various compositional mixture of coffee with chicory and PLS regression plots.

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