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CINTHIA DE CARVALHO COUTO

TECNOLOGIAS CONVERGENTES PARA A DETECÇÃO DE ADULTERANTES EM CAFÉ TORRADO E MOÍDO

CONVERGING TECHNOLOGIES FOR DETECTION OF ADULTERANTS IN ROASTED AND GROUND COFFEE

Rio de Janeiro
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CINTHIA DE CARVALHO COUTO

CONVERGING TECHNOLOGIES FOR DETECTION OF ADULTERANTS IN ROASTED AND GROUND COFFEE

PhD thesis presented to the Graduate Program in Food and Nutrition at the Federal University of the State of Rio de Janeiro as partial requirement for title of PhD in Food and Nutrition.

Supervisor: Otniel Freitas-Silva
Co-supervisors: Edna Maria Morais Oliveira e Susana Casal

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BANCA EXAMINADORA

_____

Prof. Dr. Otniel Freitas-Silva

UNIRIO/EMBRAPA Agroindústria de Alimentos, Rio de Janeiro, Brazil

_____

Prof. Dra. Thais Matsue Uekane

UFF, Niterói, Brazil

_____

Prof. Dra. Alexandra Mara Goulart Nunes Mamede

Instituto Federal de Educação, Ciência e Tecnologia da Bahia (IFBA), Barreiras, Brazil

_____

Prof. Dr. Annibal Duarte Pereira Netto

UNIRIO, Rio de Janeiro, Brazil

_____

Prof. Dr. Renata Galhardo Borguini

UNIRIO/EMBRAPA Agroindústria de Alimentos, Rio de Janeiro, Brazil

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RESUMO

O café é uma das principais bebidas consumidas em todo o mundo, sendo o Brasil seu maior produtor e exportador. Devido ao seu alto valor comercial o café é um alvo constante de adulteração. O objetivo da tese foi avaliar o uso de diferentes técnicas na detecção de adulterantes em café torrado e moído. Assim, este trabalho foi composto por 1 artigo de revisão sistemática de literatura (RSL) e outros 3 artigos originais sobre métodos de detecção de fraudes. A RSL foi realizada com auxílio do software StArt em três etapas: Planejamento, Execução e Sumarização. Para a análise experimental foram preparadas amostras torradas e moídas: de café arábica e adulterantes puros; e cafés adulterados com milho, cevada, soja, arroz, cascas de cafés e Café Robusta, variando de 0,25 a 80% (p/p). As amostras foram submetidas às análises de Espectroscopia de Infravermelho Próximo (NIR) e microextração em fase sólida por cromatografia gasosa acoplada à espectrometria de massas (MEFS-CG-EM) e os resultados combinados com análises quimiométricas. Através da RSL foram selecionados um total de 83 estudos, os quais evidenciaram que a espectroscopia e a cromatografia foram as técnicas analíticas mais estudadas; entre os adulterantes mais analisados estão o café robusta, subprodutos do café e o milho. A análise experimental pela técnica de NIR aliada a quimiometria separou as amostras de café arábica puro dos múltiplos adulterantes; a identificação do tipo de adulterante só foi possível ≥10% de adulteração; foi verificado também o potencial para discriminação geográfica de cafés arábica. Os resultados da análise de metabólitos voláteis não alvo obtidos por MEFS-CG-EM mostraram através da análise de componentes principais (ACP) e análise discriminante por mínimos quadrados parciais (AD-MQP), a distinção das amostras puras de café e adulterantes torrados moídos, enquanto a clusterização hierárquica dos componentes principais (CHPC) e o mapa de calor mostram uma tendência de separação entre adulterantes; foram selecionados 26 compostos voláteis como candidatos a potenciais marcadores para detectar fraude em café. Cafés com adulterações múltiplas foram discriminados de acordo com os modelos de regressão por mínimos quadrados parciais (R-MQP), com uma tendência de identificação de dois grupos de percentuais de adulteração (0 a 1% e acima de 5%). Os compostos voláteis e os espectros obtidos por MEFS-CG-EM e pela técnica de NIR, respectivamente, evidenciaram ser ferramentas poderosas na discriminação de café arábica torrado e moído de café adulterado.

Palavras-chave: adulteração de café, fraude em alimentos, cromatografia, espectroscopia.

ABSTRACT

Coffee is one of the main beverages consumed around the world, with Brazil being its largest producer and exporter. Due to its high commercial value, coffee is constant target of adulteration. The objective of this thesis was to evaluate the use of different techniques in the detection of adulterants in roasted and ground coffee. Thus, this work consisted of one article with systematic literature review (SLR) article and three other original articles on fraud detection methods. The SLR was performed with the StArt software considering the stages of Planning, Execution and Summarization. For the experimental analysis, roasted and ground samples were prepared: Arabica coffee and pure adulterants; and coffees adulterated with corn, barley, soybeans, rice, coffee husks and Robusta Coffee, ranging from 0.25 to 80% (w/w). Samples were submitted to Near Infrared Spectroscopy (NIR) and solid phase microextraction by gas chromatography coupled to a sequential mass spectrometer (SPME-GC-MS) analyses and the results were combined with chemometric analyses. A total of 83 studies were selected through RSL, which showed that spectroscopy and chromatography were the most studied analytical techniques; among the most analyzed adulterants are robusta coffee, coffee by-products and corn. The experimental analysis by the NIR technique combined with chemometrics separated the samples of pure arabica coffee from the multiple adulterants; identification of the type of adulterant was only possible with ≥10% of adulteration; the potential for geographic discrimination of arabica coffees was also verified. The results of the analysis of non-target volatile metabolites obtained by SPME-GC-MS showed, through the analysis of principal components (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA), the distinction of pure coffee samples and ground roasted adulterants, while the Hierarchical Clustering of Principal Component (HCPC) and the heat map show a tendency of separation between adulterants; 26 volatile compounds were selected as potential marker candidates to detect coffee fraud. Coffees with multiple adulterations were well discriminated according to Partial Least-Squares Regression (PLS-R) models, with a tendency to identify two groups of adulteration percentages (0 to 1% and above 5%). The volatile compounds and the spectra obtained by SPME-GC-MS and the NIR technique, respectively, proved to be powerful tools in the discrimination of roasted and ground arabica coffee from adulterated coffee.

Keywords: coffee adulteration, food fraud, chromatography, spectroscopy.

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AAS	Atomic Absorption Spectroscopy
ABIC	Brazilian Coffee Industry Association
ANOVA	Analysis of Variance
AOAC	Association of Official Agricultural Chemists
FTLA	Fourier-transform near-infrared spectrometer
CLAE	Cromatografia Líquida de Alta Eficiência
GC-EM	Cromatografia-Espectometria de Massas
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
HCPC	Hierarchical Clustering of Principal Components
HPLC	High-Performance Liquid Chromatography
HS-GC-IMS	Headspace-Gas Chromatographic-Ion Mobility Sum Spectrum
ICO	International Coffee Organization
InGaAs	Indium-Gallium-Arsenide
KI	Kovats index
LaPES	Software Engineering Research Laboratory
LIBS	Laser Induced Breakdown Spectroscopy
LOD	Limits of Detection
LOQ	Limits of quantification
MEFS-CG-EM	Microextração em fase sólida por cromatografia gasosa acoplada à espectrometria de massas
MSC	Multiplicative Scatter Correction
NIR	Near Infrared Spectrometry
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PCs	Principal Components
PICOC	Population, Intervention, Comparison, Outcomes and Context
PLS-DA	Partial Least Squares Discriminant Analysis
PSL-R	Partial Least-Squares Regression
PPCPC	Permanent Coffee Purity Control Program

RCP	Reação em Cadeia da Polimerase
RDC	Resolution of the Collegiate Board of Directors
RMSEC	Root Mean Square Errors of Calibration
RMSECV	Root Mean Square Errors of Cross Validation
ROC	Operating Characteristic Curve
RT	Retention Time
SNV	Standard Normal Variate
SLR	Systematic Literature Review
SPME	Solid-Phase Microextraction
SPME-GC-MS	Solid-Phase Microextraction-Gas Chromatography-Mass Spectrometry
StArt	State of the Art through Systematic Review software
VOCs	Volatile Compounds
UFSCar	Computer Science Department of the Federal University of São Carlos
UV–VIS	Ultraviolet–Visible Spectrophotometry

SUMMARY

INTRODUÇÃO

A cadeia de produção de alimentos é um sistema complexo e extenso que começa desde a plantação, passando pela indústria, distribuidores/exportadores e comércio até a transformação do produto final para o consumidor. Cada etapa envolve uma série de normas e leis que determinam ações integradas a fim de garantir a padronização, rastreamento e por fim a segurança e qualidade dos alimentos durante todo o processo de produção, exigidos pelo consumidor e pelas agências regulatórias.

No entanto, apesar de toda a organização, integração e correção de possíveis falhas durante cada etapa de produção de alimentos, todo o empenho em atender os critérios de segurança alimentar estabelecidos pelos órgãos de fiscalização e agências reguladoras, a cadeia de produção de alimentos ainda é suscetível a muitos tipos de contaminação e adulteração.

A fraude de alimentos, não intencional e intencional, pode ocasionar problemas de saúde pública. Focando na adulteração intencional, esta pode ter basicamente a intenção de causar algum dano físico ou econômico, ou sem a intenção de causar algum prejuízo à saúde nem a detecção do adulterante, mas com o objetivo de vantagem econômica, este último tipo representa a maioria dos casos de adulteração.

Dentre os alimentos comumente adulterados, encontra-se o café torrado e moído. Sendo uma das bebidas mais consumidas mundialmente e por consequência representando um alto valor comercial, o café é constantemente alvo de ações fraudulentas. As matérias-primas vegetais adicionadas ao café torrado e moído apresentam um baixo custo e são geralmente cultivadas ou de fácil acesso no país ou região, alterando assim a sua qualidade. A adição desses vegetais ao café, além de acarretar um prejuízo de ordem econômica para o consumidor, também o expõe a possíveis alergias alimentares devido à ausência de informações precisas na rotulagem.

O Brasil é o maior produtor e exportador de café, e está no topo da lista dos países consumidores da bebida, porém o país ainda adota como método oficial de detecção de adulterantes em café a microscopia, cujas diversas limitações comprometem a confiabilidade, a precisão e exatidão dos resultados. Assim fica evidente a necessidade de controle não só da qualidade do café nacional, mas também nos diversos países consumidores de café torrado e moído.

A falta de técnicas e métodos de detecção de adulterantes em café torrado e moído que garantam a confiabilidade e rastreamento dos resultados impedem a elaboração e o aprimoramento de novas normas de qualidade do café no mundo inteiro, o que em suma limita as ações de fiscalização durante o processo de produção do café torrado e moído. Assim, há uma demanda urgente por métodos com maior sensibilidade e marcadores de qualidade para a detecção de contaminantes e adulterantes.

Desse modo, a disponibilidade de métodos analíticos mais específicos e sensíveis para a análise da qualidade do café torrado e moído poderá dar suporte aos laboratórios de análise de alimentos e às agências regulamentadoras no que se refere a elaboração / cumprimento da legislação. Dentre essas técnicas pode-se citar a Espectroscopia de Infravermelho Próximo (NIR), Cromatografia Gasosa-Espectrometria de Massas (CG-EM), Reação em Cadeia da Polimerase (RCP) em tempo real e a Cromatografia Líquida de Alta Eficiência (CLAE).

Dentro desse contexto, a tese foi desenvolvida a fim de ampliar as possibilidades de utilização de técnicas analíticas a serem aplicadas na detecção de adulterantes em café torrado e moído, bem como abordar os principais adulterantes utilizados no café torrado e moído em diferentes concentrações e múltiplas adulterações.

Por isso, o principal objetivo da tese foi avaliar o uso de diferentes técnicas na detecção de adulterantes em café torrado e moído. Além disso, os objetivos específicos foram definidos da seguinte maneira: i) produzir amostras de café torrado e moído adulteradas com os principais adulterantes de café; ii) detectar múltiplos adulterantes em café baseado na técnica de Espectroscopia de Infravermelho Próximo; iii) Detectar múltiplos adulterantes em café baseado na técnica de Cromatografia Gasosa.

A parte experimental da tese de doutorado foi realizada no Laboratório de Bromatologia e Hidrologia e no LAQV/REQUIMTE Porto da Faculdade de Farmácia da Universidade do Porto, na modalidade de "doutorado sanduíche no exterior" através do Programa de Doutorado Sanduíche no Exterior da CAPES (PDSE-CAPES – EDITAL Nº 47/2017). A revisão do tema da tese e os resultados das análises laboratoriais foram reportados através de 1 artigo de revisão sistemática e 3 artigos científicos originais:

I) Uma revisão sistemática sobre os estudos com técnicas analíticas aplicadas na detecção de adulteração de café torrado, intitulado "Fraud and adulteration in coffee: A comprehensive systematic review of analytical detection approaches";

II) Um artigo original com a aplicação da técnica de NIR em café torrado e moído adulterado, intitulado "Near-Infrared Spectroscopy Applied to the Detection of Multiple

Adulterants in Roasted and Ground Arabica Coffee;

III) Um artigo original com a aplicação da técnica de Cromatografia Gasosa em amostras puras de café torrado e moído e seus adulterantes mais comuns, intitulado "Analytical approach to the selection of untargeted and target SPME-GC-MS markers for fraud detection in roasted and ground coffee";

IV) Um artigo original com a aplicação da técnica de Cromatografia Gasosa em amostras de café torrado e moído adulterado com seus adulterantes mais comuns, intitulado "Analysis of volatile compounds from coffee adulterated with food matrices by headspace-solid phase microextraction-gas chromatography".

A estrutura da tese está representada na Figura 1.

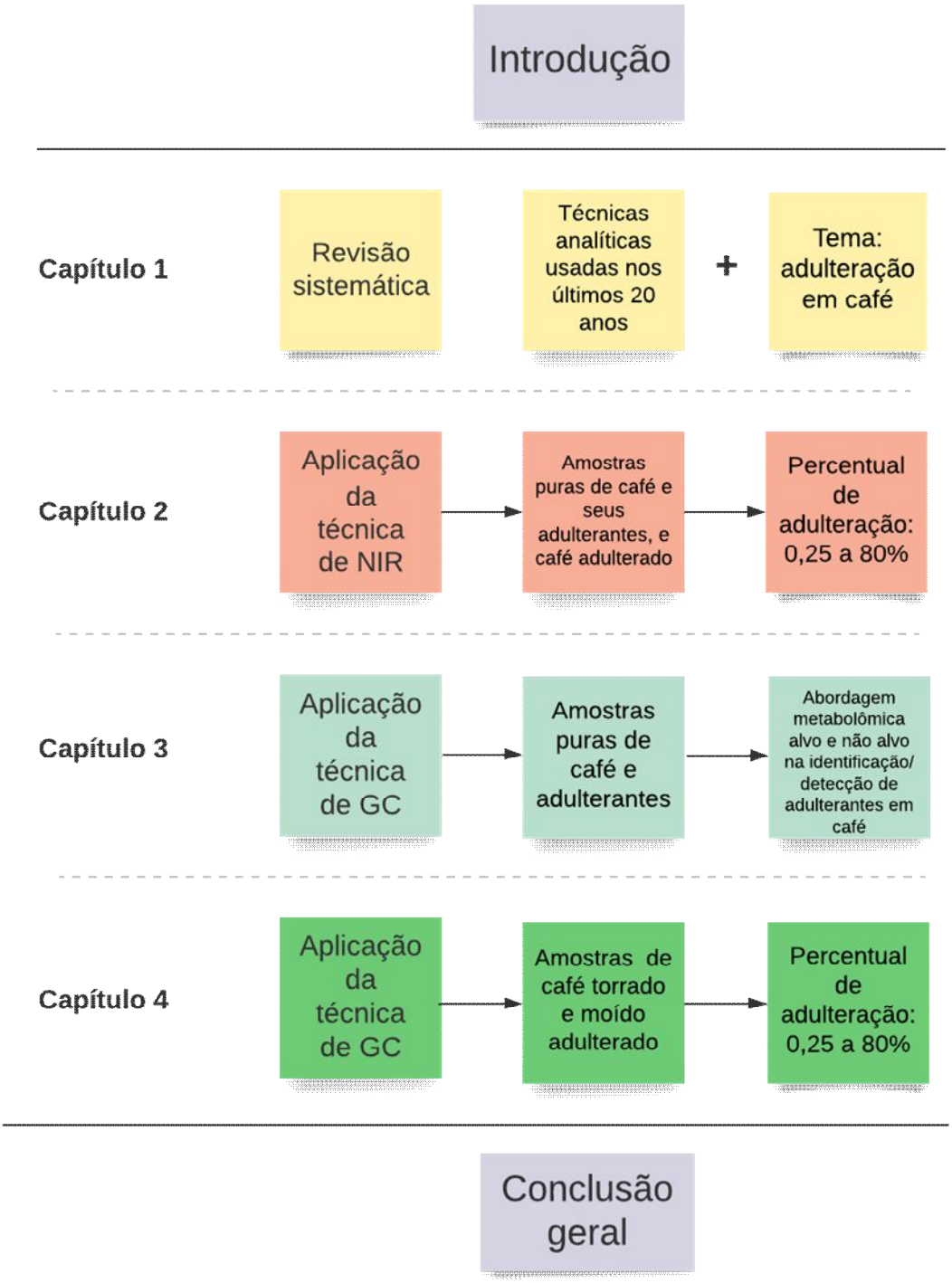

Figura 1. Estrutura da Tese.

INTRODUCTION

The food production chain is a complex and extensive system that starts from the plantation, through industry, distributors/exporters and commerce, until the transformation of the final product for the consumer. Each stage involves a series of norms and laws that determine integrated actions in order to guarantee the standardization, tracking and, finally, the safety and quality food throughout the production process, required by the consumer and by regulatory agencies.

However, despite all the organization, integration and correction of possible failures during each stage of food production, every effort to meet the food safety criteria established by inspection and regulatory agencies, the food production chain is still susceptible to many types of contamination and adulteration.

Food fraud, both unintentional and intentional, can lead to public health problems. Focusing on intentional adulteration, this may be basically intended to cause some physical or economic harm, or without the intention to cause any harm to health or the detection of the adulterant, but with the objective of economic advantage, the latter type represents the majority of cases of adulteration.

Among the commonly adulterated foods, there is roasted and ground coffee. As one of the most consumed beverages worldwide and therefore representing a high commercial value, coffee is constantly the target of fraudulent actions. Vegetable raw materials added to roasted and ground coffee have a low cost and are generally cultivated or easily accessible in the country or region, thus altering their quality. The addition of these vegetables to coffee, in addition to causing an economic loss, especially for the consumer, it also exposes them to possible food allergies due to the absence of accurate labeling information.

Brazil is the largest producer and exporter of coffee, and it is also at the top of the list of countries that consume the beverage, but the country still adopts microscopy as the official method of detecting adulterants in coffee, whose various limitations compromise the reliability, precision and accuracy of the results. Thus, the need to control not only the quality of national coffee is evident, but also in the different countries that consume roasted and ground coffee.

The lack of techniques and methods for the detection of adulterants in roasted and ground coffee that guarantee the reliability and tracking of results prevents the elaboration and

improvement of new coffee quality standards worldwide, which in short limits inspection actions during the roast and ground coffee production process. Thus, there is an urgent demand for methods with greater sensitivity and quality markers for the detection of contaminants and adulterants.

Thus, the availability of more specific and sensitive analytical methods for analyzing the quality of roasted and ground coffee may support food analysis laboratories and regulatory agencies in terms of the preparation/compliance with legislation. Among these techniques, we can mention the Near-Infrared Spectrometry (NIR), Gas Chromatography-Mass Spectrometry (GC-MS), real-time PCR (Polymerase Chain Reaction) and High-Performance Liquid Chromatography (HPLC).

Within this context, the thesis was developed in order to expand the possibilities of using analytical techniques to be applied in the detection of adulterants in roasted and ground coffee, as well as to approach the main adulterants used in roasted and ground coffee in different concentrations and multiple adulterations.

Therefore, the main objective of the thesis was to evaluate the use of different techniques in the detection of adulterants in roasted and ground coffee. In addition, the specific objectives were defined as follows: i) to produce samples of roasted and ground coffee adulterated with the main coffee adulterants; ii) to detect multiple adulterants in coffee based on the Near Infrared Spectroscopy (NIR) technique; iii) to detect multiple adulterants in coffee based on the Gas Chromatography (GC) technique.

The experimental part of the doctoral thesis was carried out at the Laboratory of Bromatology and Hydrology and at LAQV/REQUIMTE Porto of the Faculty of Pharmacy of the University of Porto, in the modality of “sandwich doctorate abroad” through the CAPES Sandwich Doctoral Program abroad (PDSE-CAPES - NOTICE No. 47/2017). The review of the thesis topic and the result of laboratory analyzes were reported through a systematic review article and other three original scientific articles:

I) A systematic review of studies with analytical techniques applied to detect adulteration of roasted coffee, entitled “Fraud and adulteration in coffee: A comprehensive systematic review of analytical detection approaches”;

II) An original article with the application of the NIR technique in adulterated roasted and ground coffee, entitled “Near-Infrared Spectroscopy Applied to the Detection of Multiple Adulterants in Roasted and Ground Arabica Coffee;

III) An original article with the application of the Gas Chromatography technique in pure samples of roasted and ground coffee and its most common adulterants, entitled "Analytical approach to the selection of untargeted and target SPME-GC-MS markers for fraud detection in roasted and ground coffee";

IV) An original article with the application of the Gas Chromatography technique in samples of roasted and ground coffee adulterated with its most common adulterants, entitled "Analysis of volatile compounds from coffee adulterated with food matrices by headspace-solid phase microextraction-gas chromatography".

The structure of the thesis is represented in Figure 1.

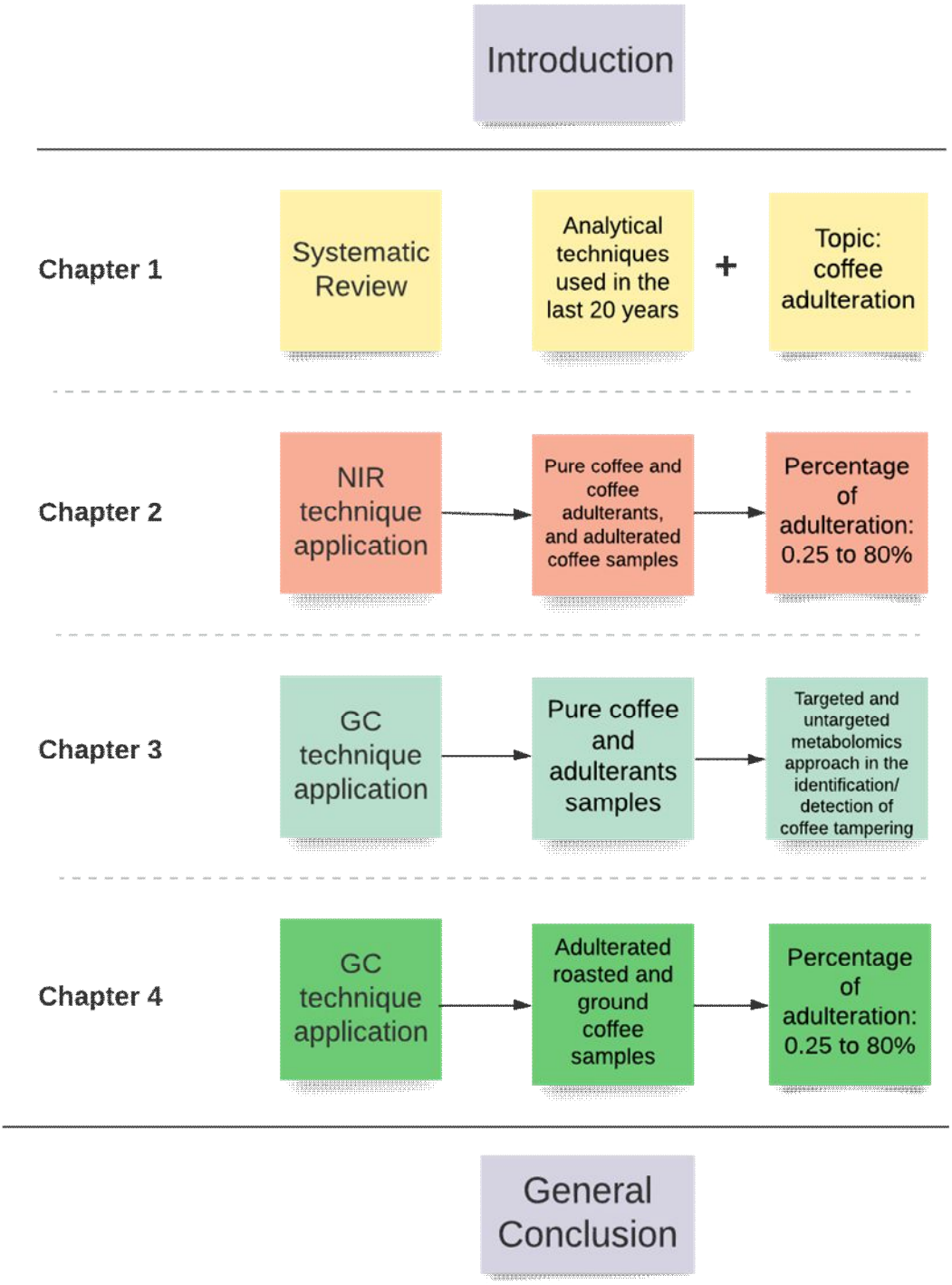

Figure 1. Thesis structure.

CHAPTER 1

Fraud and adulteration in coffee: A comprehensive systematic review of analytical detection approaches

Cinthia de Carvalho Couto[a], Caroline Corrêa de Souza Coelho[a], Edna Maria Moraes Oliveira[b], Susana Casal[c], Otniel Freitas-Silva[b].

[a]Food and Nutrition Graduate Program, the Federal University of State of Rio de Janeiro, Av. Pasteur, 296, 22290-240 Rio de Janeiro, Brazil;

[b]Embrapa Food Agroindustry, Av. das Américas, 29501, 23020-470 Rio de Janeiro, Brazil:

[c]LAQV/REQUIMTE, Laboratory of Bromatology and Hydrology, Faculty of Pharmacy, University of Porto, 4050-313, Porto, Portugal.

ABSTRACT

The presence of impurities in roasted ground coffee interferes with its quality. This Systematic Literature Review (SLR) focused on the different types of analytical techniques published in the last 20 years for the detection of adulterants in roasted coffee. The SLR was performed on StArt software in three stages: Planning, Executing, and Summarization. A total of 83 works were selected. The type of coffee most frequently studied was roasted ground coffee and *Coffea arabica,* while among the adulterants, *Coffea canephora*, coffee wastes, and corn. There is a trend of chromatographic spectroscopic, and multi-adulterant applications. The most sensible techniques were Gas Chromatography, Near Infrared Spectrometry, and vision system. Suitable techniques to detect/quantify adulterations in coffee, at different percentages and particularly as multi-detection approaches, are crucial to improve the coffee quality worldwide.

Keywords: food fraud, coffee adulteration, robusta coffee, arabica coffee, corn, chromatographic methods, spectroscopy methods, systematic review.

small percentages (>0-5%) of adulterants by scientific studies. In addition, the majority of the studies investigated only one adulterant at a time, showing the need adulterant multi-detection approach, considering the different types of adulterants and possible concentrations in coffee.

This systematic review is an important contribution to the investigation of coffee adulteration. Moreover, it is crucial the permanence of the investigation of a suitable technique (s) to detect the adulteration in coffee, mainly considering the most common adulterants, different percentages, and combinations of them in coffee.

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SUPPLEMENTARY MATERIALS

Table S1. Extraction Form for Selected Studies

Name	List items/text	
n	text	
Type of coffee	*C. arabica*	
	C. canephora	
	Coffee, roasted bean	
	roasted and ground	
	Peaberry coffee	
	Civet coffee	
	Gourmet coffee	
	Hybrid coffee	
Coffee origin	text	
Type of adulterant*	Barley	Brown sugar
	Corn	Drugs
	Soybean	*Coffea arabica*
	Rice	Chicory
	Wheat	Tamarind
	Coffee waste	Potato
	Açai	Beans
	Coffea canephora	Walnut
	Chickpea	Oak
	Geographic origin	Fig
	Mung beans	Cacao
	Cultivation systems	Pea
	Type of coffee bean	Oat
	Starch	Date palm
	Triticale	Rye
Analyte/Coffee waste/Other wastes	text	
Detection techniques	GC	UV-Vis
	HPLC/UPLC	1H NMR spectroscopy
	DNA	Spectrometry
	NIR	Physicochemical properties
	FTIR	Thermal analysis
	Microscopy	PTR-MS
	Ionization methods	Vision system
	LIBS	Sensory analysis
	ICP-OES	MID-infrared
	AAS	
	Electroanalytical methods	
Results	text	
Detection range of adulteration	>0-0.5	

CHAPTER 2

Near-Infrared Spectroscopy Applied to the Detection of Multiple Adulterants in Roasted and Ground Arabica Coffee

Cinthia de Carvalho Couto[1], Otniel Freitas-Silva[2], Edna Maria Morais Oliveira[2], Clara Sousa[3],*and Susana Casal[4]

[1]Food and Nutrition Graduate Program, Federal University of State of Rio de Janeiro, Av. Pasteur 296, Rio de Janeiro 22290-240, Brazil; cinthiaccouto@gmail.com

[2]Embrapa Food Agroindustry, Av. das Américas 29501, Rio de Janeiro 23020-470, Brazil; otniel.freitas@embrapa.br (O.F.-S.); edna.oliveira@embrapa.br (E.M.M.O.)

[3]CBQF—Centro de Biotecnologia e Química Fina, Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Diogo Botelho 1327, 4169-005 Porto, Portugal

[4]LAQV/REQUIMTE, Laboratory of Bromatology and Hydrology, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal; sucasal@ff.up.pt

*Correspondence: cssousa@ucp.pt

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ABSTRACT

Roasted coffee has been the target of increasingly complex adulterations. Sensitive, non- destructive, rapid and multicomponent techniques for their detection are sought after. This work proposes the detection of several common adulterants (corn, barley, soybean, rice, coffee husks and robusta coffee) in roasted ground arabica coffee (from different geographic regions), combining near- infrared (NIR) spectroscopy and chemometrics (Principal Component Analysis—PCA). Adulterated samples were composed of one to six adulterants, ranging from 0.25 to 80% (*w*/*w*). The results showed that NIR spectroscopy was able to discriminate pure arabica coffee samples from adulterated ones (for all the concentrations tested), including robusta coffees or coffee husks, and independently of being single or multiple adulterations. The identification of the adulterant in the sample was only feasible for single or double adulterations and in concentrations ≥10%. NIR spectroscopy also showed potential for the geographical discrimination of arabica coffees (South and Central America).

Keywords: coffee; adulteration; infrared spectroscopy; authenticity; chemometrics

1 INTRODUCTION

Coffee is among the most consumed beverages worldwide [1], having enormous economic relevance, and has a continuously growing market, expanding to different applications, such as the cosmetic and pharmaceutical industries [2]. According to the International Coffee Organization (ICO), the global coffee output achieved near 172 million bags in 2020/21, represented by the main commercialized species, *Coffea arabica* (59%) and *Coffea canephora* (robusta) (41%). Brazil is the main coffee producer and exporter worldwide, with a total production estimated in the crop year 2020/2021 of 69 million bags (arabica and robusta), followed by Vietnam (mainly robusta) and Colombia (arabica), with 29 and 14.3 million bags, respectively [3,4].

Due to its commercial value, arabica coffee has been the target of countless and increasingly complex adulterations over the years [5], mainly through the addition of roasted barley, corn, rice and coffee husks [6,7]. Robusta coffee, due to its lower market and compositional similarity, is also commonly used for arabica coffee adulterations [1,7,8].

A plethora of studies have been developed to tentatively detect adulterations in roasted ground coffee employing physical, chemical, and biological techniques. Some include DNA-based approaches [9–13], chromatographic analysis [14,15], ultraviolet–visible spectrophotometry (UV–VIS) [16], digital image processing [17], capillary electrophoresis tandem mass spectrometry [18], electrospray ionization mass spectrometry [19], etc. However, these techniques require sophisticated and expensive instrumentation, as well as skilled personnel, are generally time-consuming, include chemical pre-treatments that make them destructive [20,21] and allow for the detection of only a few contaminants [22]. Microscopic inspection, one of the oldest approaches, is still commonly applied, including in official laboratories [23], despite its recognized incapacity to distinguish accurately multiple and complex contaminations, together with its inherent subjectivity, highly based on the analyst's experience [7]. More expedite methods are deemed necessary to effectively support adulteration detection worldwide [24,25].

Some vibrational spectroscopic techniques, such as NIR spectroscopy and NMR, coupled with chemometrics have already proved to be reliable tools in the detection of particular coffee adulterations [7,19,24,26,27]. These techniques are well known for their high efficiency, fastness, reliability and easy use. They commonly do not demand sample pre-treatments nor reagents, showing to be green analytical tool alternatives [7,28,29]. NIR

spectroscopy has been widely used to discriminate arabica and robusta species, in both green and roasted coffee [30], and even to correlate with sensorial attributes in roasted coffee [31]. The detection of different adulterants in coffee through NIR, such as corn, barley and coffee husks, has also been reported [24,25,29], but not yet extensively tested for the detection of mixtures, increasingly used as coffee adulterants [13,21,26]. In most published works, only one or two adulterants per sample have been tested, which does not represent the reality of the actual market. Therefore, models should be more representative, composed of a wider variety of coffee origins and adulterants simultaneously in the same sample. Additionally, the most likely types of combinations of the different varieties and mixtures must be considered [21,24]. Recently, advances in NMR have been made, demonstrating the versatility of this technique for the detection of multiple adulterants [32] but not, as far as the authors know, for NIR spectroscopy.

Considering the lack of information on some of the most recent materials used for coffee fraud, and the increased use of multiple adulterations, this work aimed to study the feasibility to detect multiple coffee adulterants in roasted and ground coffee, in different combinations, based on NIR spectral information.

2 MATERIAL AND METHODS

Roasted coffee beans were kindly selected and provided by Nestlé roaster (Porto, Portugal). Sampling was representative of the main species commercialized, including different geographical origins as well as the main producers and exporters of coffee. Four arabica roasted samples were used: two from Brazil (both natural), and one each from Colombia and Honduras (both washed—"milds"). Two robusta roasted samples were used as adulterants, from Vietnam and Cameroon. All coffee beans were ground (Retsch, GM 200, Haan, Germany) and stored at room temperature under light and air protection until analysis using aluminum bags with one-way valves as usual in the coffee industry.

The remaining adulterants (corn, soybeans, rice seeds, barley and the dried residues from natural coffee processing, commonly known as coffee husks) were chosen considering the most recent trends in commercial roasted and ground coffee adulteration (Table 1) [6,11,27]. Two distinct batches of each adulterant were acquired (1 and 2), roasted to achieve a color similar to that of the coffees used (medium dark) in a laboratory oven

(WTC Binder, Tuttlingen, Germany) (Table 1) and ground (Retsch, GM 200, Haan, Germany), except barley which was already acquired roasted and ground in the local market.

The blends (adulterated arabica coffee) were prepared with a single adulterant up to all the six adulterants together, in different mass percentages (0.25, 0.5, 1, 5, 10, 20, 40, 60 and 80%) and combinations. All the blends were prepared in triplicate. Briefly, the 0.25% and 0.5% adulterations were only prepared with single adulterants, while the 40, 60 and 80% adulterations were only prepared with robusta coffee as adulterant. The adulterations between 1 and 30% resulted either from individual adulterations or from combinations of two to six adulterants. The 2% frauds, for example, resulted from the blend of two adulterants at 1% and from combination of 4 adulterants at 0.5%. The 5%, similarly, was the result of individual adulterations at 5% or from combination of five adulterants at 1%. Only a single adulteration at 25% and 30% was prepared, resulting from using five and six adulterants at 5%, respectively. Single adulterations at 20% were only prepared with corn, coffee husks and robusta coffee, although 20% fraud could result from a combination of two (at 10%) or four (at 5%) adulterations. Globally, a total of 73 combinations were prepared, in triplicate, totaling 219 adulterated samples. For details, please see Tables S1 and S2 (Supplementary Materials).

Table 1. List of the coffee samples and adulterants according to origin and degree of roasting.

Sample	Origin	Roasting Condition
Arabica B1	Brazil (natural)	medium-dark
Arabica B2	Brazil (natural)	medium-dark
Arabica C	Colombia (washed)	medium-dark
Arabica H	Honduras (washed)	medium-dark
Robusta 1	Vietnam	medium-dark
Robusta 2	Cameroon	medium-dark
Corn 1	Brazil	225 °C 30 min
Corn 2	Portugal	250 °C 45 min
Soybeans 1	Portugal	250 °C 15 min
Soybeans 2	Portugal	250 °C 15 min
Rice seeds (with chaff) 1	Brazil	250 °C 25 min
Rice seeds (with chaff) 2	Portugal	250 °C 30 min

Coffee husks 1	Brazil	220 °C 10 min
Coffee husks 2	Brazil	212 °C 14 min
Barley 1	Portugal	commercial
Barley 2	Portugal	commercial

2.1. NEAR-INFRARED SPECTROSCOPY

Near-infrared spectra of all the samples were acquired on a Fourier-transform near-infrared spectrometer (FTLA 2000, ABB, Québec, QC, Canada) equipped with an indium- gallium-arsenide (InGaAs) detector in diffuse reflectance mode. Each spectrum resulted from an average of 64 scans with a resolution of 8 cm^{-1} in the wavenumber interval of 4000–10,000 cm^{-1}. Bomen-Grams software (version 7, ABB, Québec, QC, Canada) was used to control the equipment. A total of five spectra per sample were acquired for each sample triplicate (meaning a total of 15 spectra for each plain sample of coffee and adulterant plus all the 291 blends prepared). All the analysis took place within 6 months after roasting.

2.2. DATA ANALYSIS

Due to the large amount of spectral data, the 5 spectra of each sample were averaged before data analysis. The mean spectra were pre-processed with standard normal variate (SNV) and Savitzky-Golay filter (15 smoothing points, 2nd order polynomial and 1st derivative) [33] to remove baseline drifts and further mean centered. Other data pre-treatments were tested as: (I) different combinations of SNV and SavGol filter (SNV + mean center; SavGol + mean center); (II) different windows of the SavGol filter (9–15) and also the second derivative; (III) multiplicative scatter correction (MSC) and (IV) autoscale. It should be stressed that the best results were obtained with the above-mentioned pre-treatment. Spectra were further modelled by Principal component analysis (PCA) [34]. Outliers were verified by Q Residuals versus Hotelling T^2. The root mean square errors of calibration (RMSEC) and cross validation (RMSECV) of all the PCA models developed in the current study were presented in Table S3 (Supplementary Materials). All chemometric models were performed in Matlab version 9.5 Release 2018b (MathWorks) and PLS Toolbox version 8.7 (2019) for Matlab (Eigenvector Research, Manson, WA, USA).

3 RESULTS AND DISCUSSION

3.1. DISCRIMINATION AMONG PURE SAMPLES AND ADULTERATED COFFEE

An exploratory PCA was performed to evaluate possible clusterization among all the analyzed samples (Figure 1A). The analysis was performed considering the whole spectral range (4000–10,000 cm^{-1}). Spectra were pre-processed prior to the analysis (for details, please see the Materials and Methods section).

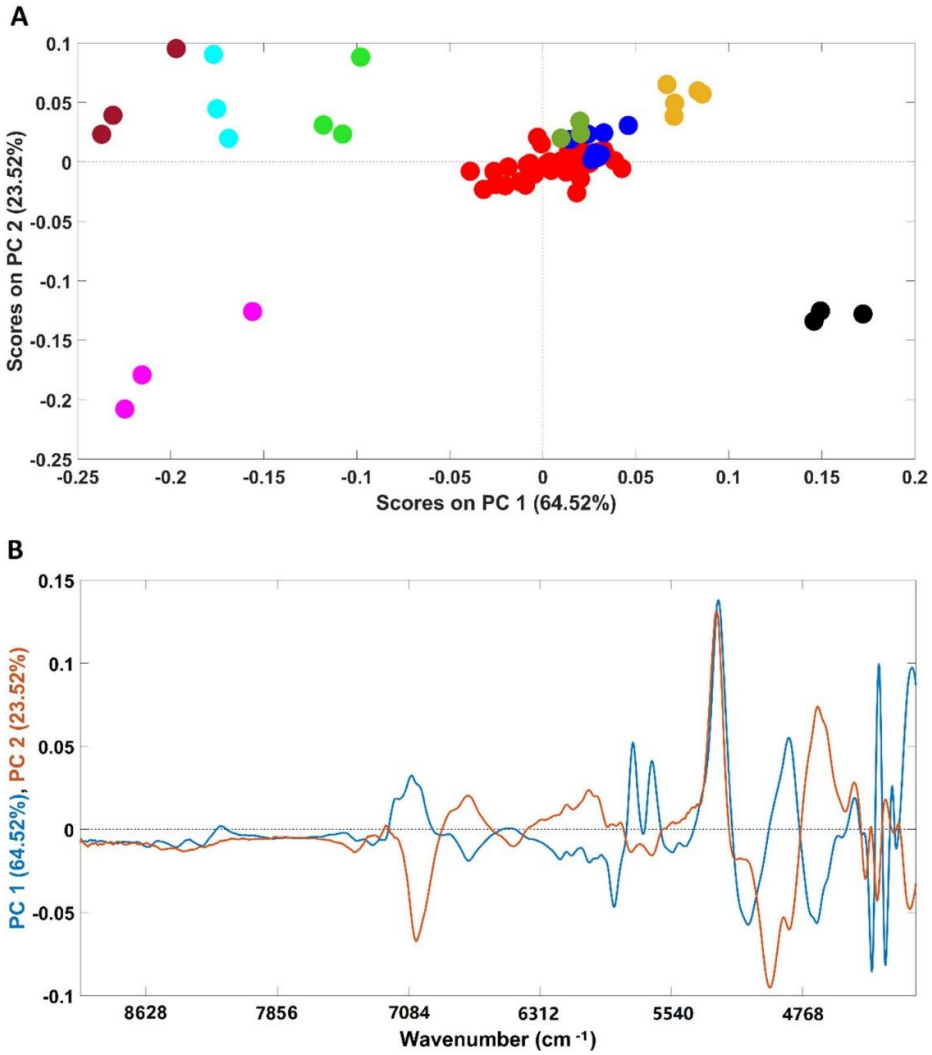

Figure 1. Scores plot of the PCA models developed with all the samples included in this study (A) and their corresponding loadings (B). Legend: • arabica; • robusta; • adulterated samples with robusta; • adulterated samples with rice/corn/soy/barley/coffee husks; • soy; • barley; • rice; • corn; • coffee husks.

NIR spectroscopy was able to clearly discriminate the pure adulterants (rice, barley, soybean, corn and coffee husks) from samples containing coffee (robusta, arabica and arabica adulterated with robusta). It should be stressed that the first PC (PC1) mainly accounts for the discrimination between corn, rice, barley and soybean samples (negative part of PC1) from coffee husks (positive part of PC1). According to the loadings plot (Figure 1B), the wavenumber regions/bands that mostly account for such discrimination (higher-intensity bands) were: (I) the region between 5800 and 5650 cm^{-1} which are due to S-H and C-H bonds in first overtone; (II) peaks around 4360 and 4270 associated with the C-H plus C=C combination and at 4324 cm^{-1}, a vibration attributed to lipids. It should be noted that, despite being high in intensity, the bands around 5200 and 7000 cm^{-1} are associated with the O-H combination and the first O-H overtones regions, respectively, due to the presence of water bands [35] and should not be taken into consideration for sample discrimination. Additionally, corn, rice and barley samples were closer in the scores map of PCA (Figure 1A) denoting a higher similarity when compared with soybean ones, discriminated across PC2. The spectral bands that seem to account for the discrimination are located at 4960 and 4671 cm^{-1}, corresponding to a spectral range dominated by C-H plus C=C vibrations, and at 4324 cm^{-1}, frequently attributed to lipid vibrations. Regarding the samples containing only coffee, they are closer in the scores map, with the four plain “arabica” samples being the most dissimilar ones. It is interesting to note that plain “robusta” and “arabica” sam- ples adulterated with “robusta” cluster together, with the remaining adulterated samples lying in the top of the cluster closer to the “arabica” samples. The results obtained with the PCA demonstrate the high potential of this technique to discriminate among pure and adulterated coffee samples. Previous studies already demonstrated the suitability of NIR spectroscopy to discriminate among “arabica” and “robusta” varieties, which are in accordance with the results herein obtained [8,29,30].

An additional PCA was performed solely with the spectra of coffee samples (arabica, robusta and arabica adulterated with robusta) due to its closeness in the first PCA (Figure 2A). Both pure “arabica” and pure “robusta” coffee samples are clearly discriminated from the adulterated samples (all adulterated samples were included in the analysis) in the first PC (PC1). According to the loadings plot (Figure 2B), the spectral region re-

sponsible for the discrimination was 5150–4920 cm^{-1}, a spectral region indicating the predominance of carbohydrates, proteins and chlorogenic acid vibrations in coffee samples [36]. Regarding the samples adulterated with "robusta" coffee, 4/8 samples were placed apart from the main cluster. These samples correspond to those with a higher "robusta" proportion (20/40/60/80%). Another interesting point is that the samples are positioned in the scores map according to their "robusta" proportion, e = with the sample with a higher content being closer to the pure "robusta" samples. Samples with lower "robusta" contents cluster together with the remaining adulterated samples. Regarding plain "arabica" samples (B1/B2/C/H and their blend X), it could be seen that samples from Brazil (B) and Colombia (C) are closer, lying mostly in the negative part of PC 3, while the sample from Honduras (H) is on the positive part of the PC 3. The loadings plot (Figure 1, panel IIB) shows that the regions between 5800 and 5650 cm^{-1} (vibration due to S-H and C- H bonds in first overtone) and between 4460 and 4270 cm^{-1} (dominated by carbohydrates, proteins and caffeine vibrations) are mainly responsible for the discrimination [36]. The green coffee processing method cannot be used to justify this separation since the Brazilian samples were processed by the natural method while the samples from Colombia and Honduras are washed coffees. Therefore, the relative location of the samples in the scores map could be related to their geographic origins. Colombia and Brazil are in South America, probably sharing many edaphoclimatic conditions, and Honduras is located in Central America. The geographic origin could justify the slightly different chemical composition suggested by the PCA. Previous studies on green coffee demonstrated the suitability of NIR spectroscopy to discriminate samples according to their geographical regions, while this work highlights a possible difference between roasted and ground coffees in terms of countries bases [37–39]. Precisely, following the findings of Giraudo and collaborators [40], the green samples from Honduras and Brazil showed a tendency towards separation. Since the "arabica" X sample corresponds to a balanced mix of all the four samples (B/B/C/H, 25% each) it is located closer to samples B and C due to their relative compositions (75% of B plus C and 25% of H).

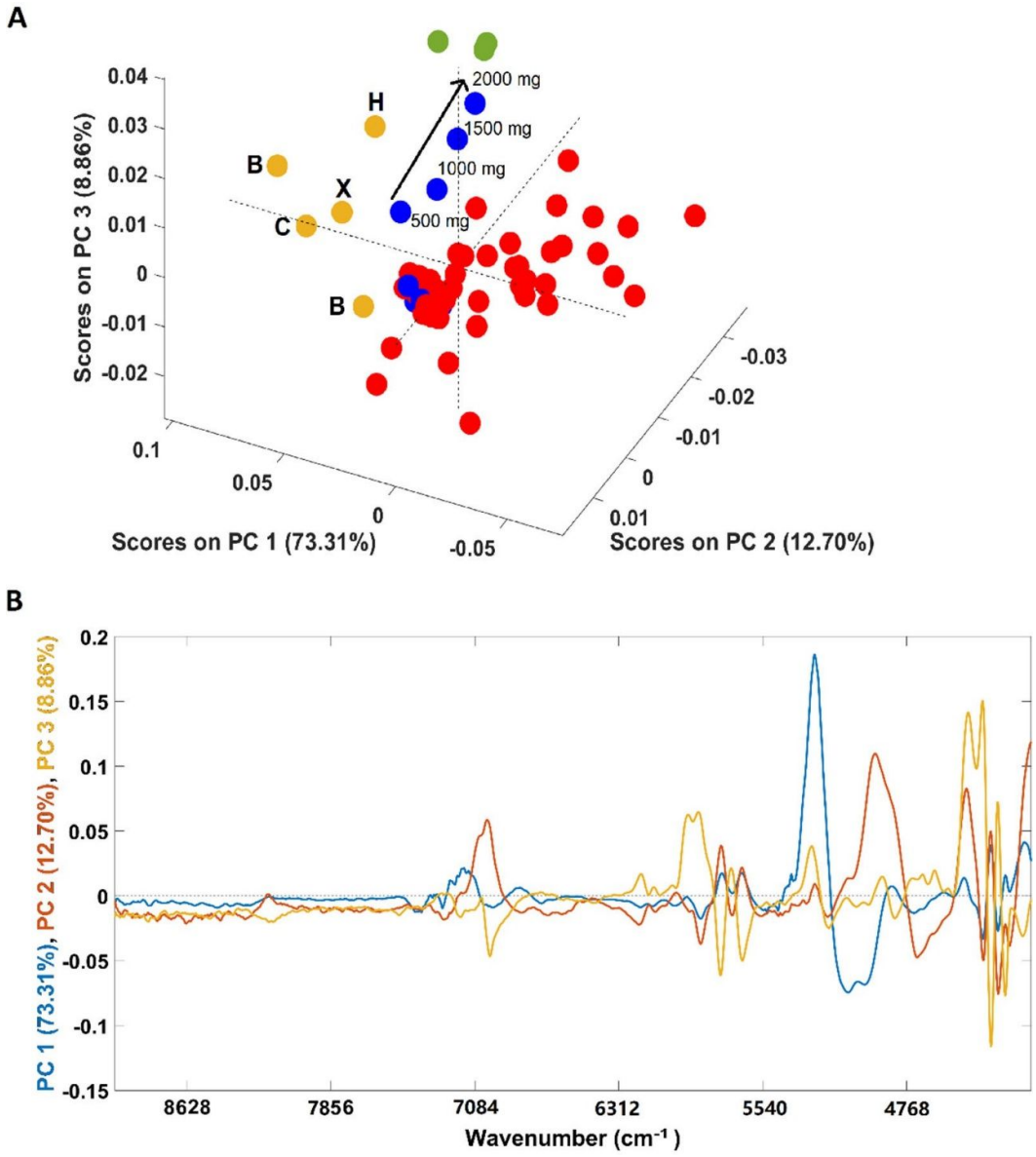

Figure 2. Scores plot of the PCA models developed solely with samples containing coffee (A) and its corresponding loadings (B). Legend: • arabica (B = Brazil, H = Honduras, C = Colombia, X = blend of the 4 arabica samples); • robusta; • adulterated samples with robusta; • adulterated samples with rice/corn/soy/barley/coffee husks.

3.2. DISCRIMINATION ACCORDING TO THE ADULTERANT

Due to the high ability to discriminate between pure and contaminated samples, the potential of NIR spectroscopy to discriminate between samples according to the adulterants present was also evaluated. A PCA model was developed with spectra of pure arabica and arabica samples adulterated with rice (rice alone + all the adulterations with rice, alone and in

combination with other adulterants). Figure 3 exhibits the scores plot of the first two PCs of the PCA model. The first PC (PC1), which captures 90.6% of the spectral variability, was responsible for the clear discrimination between arabica samples (cluster C1) and the contaminated ones (cluster C2 and C3) even in the presence of coffee husks and "robusta" coffee. The discrimination of these two clusters (C2 and C3) was related to the percentage of the adulterant present in the coffee sample and not with the kind of adulterant. Namely, samples with more than 10% of adulterants were in C3 and samples with less than 10% of adulterants were in C2, these last ones being closer to the arabica pure samples on the scores map of the PCA model. Included in C2 were only two samples' spectra, containing exactly 10% of adulterants, one corresponds to spectra "Z", with 5% of rice and 5% of coffee husks, and the second one with 10% of rice as the unique adulterant "Y". The spectrum from sample Z was quite apart from the remaining ones probably due to the presence of coffee husks in a high percentage. It should be noted that despite containing 10% of adulterant, sample Y contains only rice as the adulterant, which makes this sample more similar to the others present in C2 (where all the samples containing only alteration with rice appeared). Similar PCA models were developed for each of the remaining adulterants and the obtained results were quite similar (data not shown).

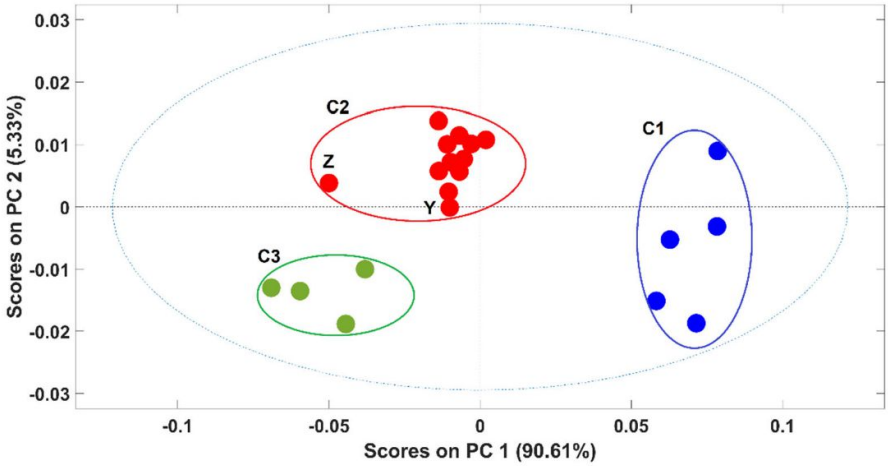

Figure 3. Scores plot of the first two principal components (PCs) of the PCA model. Legend: • arabica; • ≤10% of adulterants; • >10% of adulterants. Samples Z and Y contain 10% of adulterants (5% of rice + 5% of coffee husks and 10% of rice, respectively).

Globally, it arises that sample discrimination according to the adulterant present was not possible. Instead, the discrimination observed in the scores map seems to be highly related to the total percentage of adulterants in the samples.

It should be stressed that the above conclusion was based on PCA models devel-

oped with adulterated samples with up to six adulterants simultaneously. In this context, an additional study was undertaken to evaluate if the discrimination according to the adulterant was feasible when solely up to two adulterants were present. Fifteen PCA models were developed (C 6,2- combinations of six adulterants, two by two) to include all the combinations. Figure 4 corresponds to the PCA model developed with adulterated samples containing rice and coffee husks for example proposes. Pure arabica samples were discriminated from the adulterated ones across the PC1 (86.2% of the spectral variability), as stated previously. Regarding the adulterated samples, some appeared in the scores map in a very compact cluster and others quite disperse across it. Samples belonging to the compact cluster possess percentages of coffee between 95 and 99.75%, which makes them all very similar even if they were adulterated with rice; coffee husks or rice + coffee husks. The dispersed ones possessed percentages of coffee 90% enabling the discrimination according to the adulterant present (rice/coffee husks/rice + coffee husks).

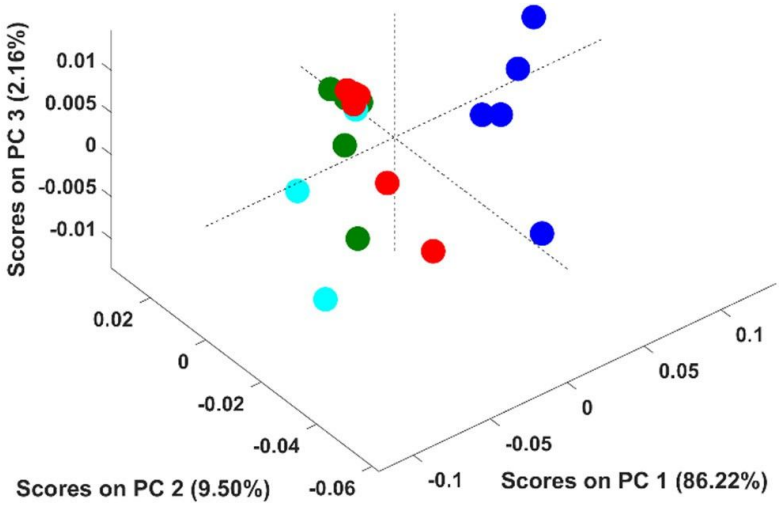

Figure 4. Scores plot of the first three principal components (PCs) of the PCA model. Legend: • arabica; • samples adulterated with coffee husks; • samples adulterated with rice; • samples adulterated with rice and coffee husks.

Similar results were obtained for the remaining PCA models developed (data not shown), meaning that the discrimination according to the adulterant present in the sample is only possible for percentages of adulterants ≥ 10% and with up to two adulterants. This result differs from the obtained previously because in the first attempt to discriminate samples according to the adulterant, some samples had very small amounts of 4 to 5 distinct adulterants.

3.3. DISCRIMINATION AT A CONSTANT ADULTERANT CONCENTRATION

Based on the previous approaches, samples discrimination according to the adulterant might be possible if only up to two adulterants are considered. However, even in such conditions, the discrimination ability was highly related to the adulterant concentration (only feasible for adulterant concentration 10%). In this context, an additional study was performed to evaluate the feasibility of the discrimination according to the adulterant present keeping their concentration constant. Three PCA models were developed, each including solely samples of a certain adulterant concentration, namely, 20%, 10% and 1%. These percentages were selected based on the available data in order to ensure a representative range of adulterant amounts and based on the number of available spectra for each amount to

develop robust PCA models. The scores plot of the PCA model developed with samples containing 20% of adulterant (Figure 5A) showed discrimination between samples containing just coffee (arabica and arabica adulterated with robusta) from adulterated coffee in the first PC (PC1 encompassing 88.3% of the spectral variability). Despite lying in the positive part of the PC1, plain arabica samples were discriminated from those adulterated with robusta. Adulterated samples with coffee husks and/or corn appear mostly on the negative part of PC2 while samples containing a mixture of adulterants and rice or soy plus coffee husks appear on the positive part of PC2. Even with a constant and quite high adulterant percentage in samples, when many adulterants were included, it seems to be not possible to discriminate samples according to the adulterants present.

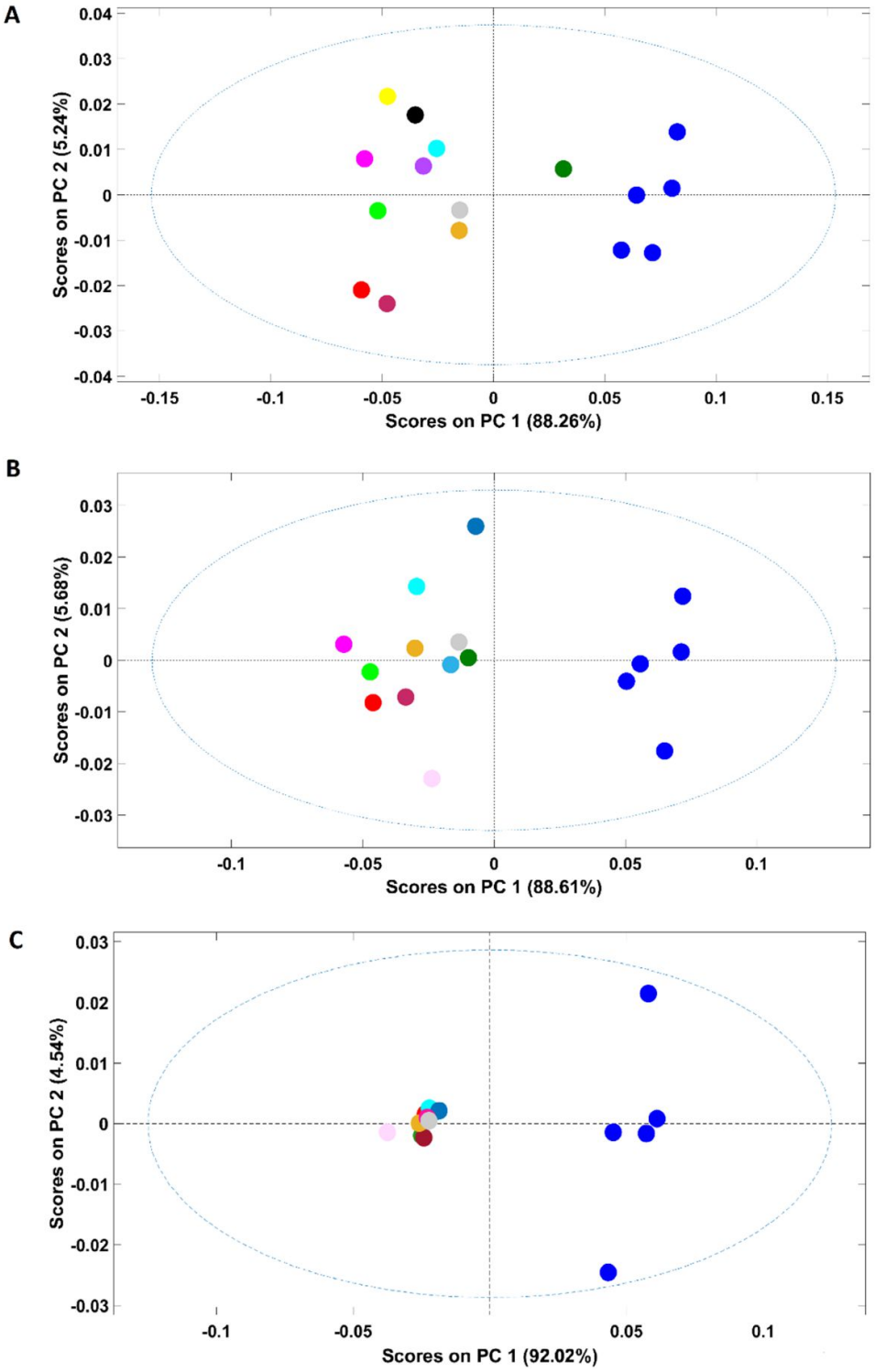

Figure 5. Scores plot of the PCA model developed with samples containing distinct percentages of adulterants: (A)—20%; (B)—10%; (C)—1%. Legend: • pure arabica; • robusta; • corn; • coffee husks; rice; • soy; • barley; • coffee husks + barley; • coffee husks + corn; • coffee husks + rice; • coffee husks + robusta; • soy + coffee husks; • barley + corn + soy + rice; • barley + corn + soy + coffee husks; • barley + corn + soy + robusta.

Regarding samples with 10% of adulterant (scores map of the model in Figure 5B), a clear discrimination between pure arabica samples and adulterated ones occurred on PC1. Contrary to samples with 20% of adulterant, the arabica sample adulterated with robusta is on the opposite part (negative) of PC1. This might have occurred due to the lower percentage of adulterants in these samples, which make them more similar (all of them possess a higher arabica content, 90% versus 80% in the first case). The discrimination between arabica and robusta coffees is important, particularly for products labelled as 100% arabica. Adulterations with robusta are frequent due to its lower price (<20–25%), and it is frequently used to reduce the costs of the product [8,30]. Figure 5C presents the scores map of the PCA model developed with samples containing just 1% of adulterant. It was interesting to note that NIR spectroscopy possessed the ability to discriminate between pure and adulterated arabica samples even with a low percentage of adulterant (1%) on the first PC. Winkler-Moser et al. [7], in a single approach for corn detection in coffee using NIR, showed that the model developed using partial least-squares regression (PSLR) analysis was not able to detect samples at the 1% level, but an accurate detection by NIR was possible at or above 5%. The detection of corn in coffee was also effective by micro NIR (the limits of detection, LOD, and of quantification, LOQ, were 1.6 and 5.2%, respectively) [29]. In an additional work, barley adulteration was detected at 2% in coffee using PLSR [24]. It is important to highlight that the legislation in Brazil that allowed up to 1% of foreign material in roasted ground coffee through Normative Instruction nº 16 [41] was revoked by Normative Instruction nº 7 [42]. The results obtained in this work, allowing discrimination of adulteration below 1% of contribute to imposing the strict regulation of coffee products due to their high commercial value. Additionally, all of the adulterated samples appear in a very compact cluster, highlighting their similarity.

4 CONCLUSIONS

NIR spectroscopy coupled with chemometrics proved to be able to distinguish all the pure samples included in this work (coffee, including the two species arabica and robusta, coffee husks, barley, soybean, rice and corn).

This technique was also able to discriminate the coffee varieties among each other,

namely, arabica, robusta and arabica contaminated with robusta from as low as 1%. Indeed, contaminated samples appeared positioned in the scores map according to their relative percentages. Additionally, pure arabica samples seem to be discriminated from each other according to their geographic origins.

The discrimination between pure and adulterated arabica coffee samples was also feasible for all the adulterants and independently of the concentration tested (from as low as 0.25%). However, the discrimination of the samples according to the adulterant present was only achievable if no more than two contaminants were present simultaneously and for adulterant concentrations ≥10%.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/foods11010061/s1, Table S1: blends composition, Table S2. Prevalence of each adulterant in the blends, Table S3. Root mean square errors of calibration (RMSEC) and cross-validation (RMSECV) of the PCA models developed in this study. PCA models were identified through their figure numbers in the manuscript.

Author Contributions: C.S. and S.C.—Conceptualization and methodology; C.S.—Data analysis; C.d.C.C.—Experimental work and writing: original draft preparation; C.S., S.C., C.d.C.C., O.F.-S. and E.M.M.O.—Manuscript final corrections/adjustments. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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SUPPLEMENTARY MATERIALS

Table S1. Blends composition.

Blend (%)						
Natural Arabica Coffee Brazil	**Natural Arabica Coffee Brazil**	**Washed Arabica Coffee Colombia**	**Washed Arabica Coffee Honduras**			
B1						
	B2					
		C				
			H			
	Arabica X					
Barley 1	**Barley 2**			**Rice 1**	**Rice 2**	
Barley Y				Rice Y		
Corn 1	**Corn 2**			**Coffee husks 1**	**Coffee husks 2**	
Corn Y				Coffee husks Y		
Soy 1	**Soy 2**			**Robusta 1**	**Robusta 2**	
Soy Y				Robusta Y		
Arabica X	**Barley Y**	**Corn Y**	**Soy Y**	**Rice Y**	**Coffee husks Y**	**Robusta Y**
99.8	0.25	-	-	-	-	-
99.8	-	0.25	-	-	-	-
99.8	-	-	0.25	-	-	-
99.8	-	-	-	0.25	-	-
99.8	-	-	-	-	0.25	
99.8	-	-	-	-	-	0.25
99.5	0.5	-	-	-	-	-
99.5	-	0.5	-	-	-	-
99.5	-	-	0.5	-	-	-
99.5	-	-	-	0.5	-	-
99.5	-	-	-	-	0.5	-
99.5	-	-	-	-	-	0.5
99.0	0.5	-	-	-	0.5	-
99.0	-	0.5	-	-	0.5	-
99.0	-	-	0.5	-	0.5	-
99.0	-	-	-	0.5	0.5	-
99.0	-	-	-	-	0.5	0.5
98.5	0.5	0.5	0.5	-	-	-
98.5	-	0.5	-	-	0.5	0.5
98.0	0.5	0.5	0.5	-	-	0.5
98.0	0.5	0.5	0.5	0.5	-	-
98.0	0.5	0.5	0.5	-	0.5	-
97.5	0.5	0.5	0.5	0.5	0.5	-
99.0	1	-	-	-	-	-
99.0	-	1	-	-	-	-
99.0	-	-	1	-	-	-

99.0	-	-	-	1	-	-
99.0	-	-	-	-	1	-
99.0	-	-	-	-	-	1
98.0	1	-	-	-	1	-
98.0	-	1	-	-	1	-
98.0	-	-	1	-	1	-
98.0	-	-	-	1	1	-
98.0	-	-	-	-	1	1
97.0	1	1	1	-	-	-
97.0	-	1	-	-	1	1
96.0	1	1	1	1	-	-
96.0	1	1	1	-	1	
96.0	1	1	1	-	-	1
95.0	1	1	1	1	1	-
95.0	5	-	-	-	-	-
95.0	-	5	-	-	-	-
95.0	-	-	5	-	-	-
95.0	-	-	-	5	-	-
95.0	-	-	-	-	5	-
95.0	-	-	-	-	-	5
90.0	5	-	-	-	5	-
90.0	-	5	-	-	5	-
90.0	-	-	5	-	5	-
90.0	-	-	-	5	5	-
90.0	-	-	-	-	5	5
85.0	5	5	5	-	-	
85.0	-	5	-	-	5	5
80.0	5	5	5	5	-	
80.0	5	5	5	-	-	5
80.0	5	5	5	-	5	-
75.0	5	5	5	5	5	-
70.0	5	5	5	5	5	5
90.0	10	-	-	-	-	-
90.0	-	10	-	-	-	-
90.0	-	-	10	-	-	-
90.0	-	-	-	10	-	-
90.0	-	-	-	-	10	-
90.0	-	-	-	-	-	10
80.0	10	-	-	-	10	-
80.0	-	10	-	-	10	-
80.0	-	-	10	-	10	-
80.0	-	-	-	10	10	-
80.0	-	-	-	-	10	10
80.0	-	20.0	-	-	-	-
80.0	-	-	-	-	20.0	-
80.0	-	-	-	-	-	20.0
60.0	-	-	-	-	-	40.0
40.0	-	-	-	-	-	60.0

20.0	-	-	-	-	-	80.0

All samples were prepared in triplicate.
X- 25% mixture of B1:B2:C:H; Y- 50% mixture of the adulterant batch 1 and 2.

Table S2. Prevalence of each adulterant in the blends

%	Barley	Corn	Soybean	Rice	Coffee husks	Robusta coffee
	Number of combinations prepared with each of the adulterants					
0,25	1	1	1	1	1	-
0,5	7	7	7	4	9	**4**
1	7	7	7	4	9	**4**
5	7	7	7	4	9	**4**
10	2	2	2	2	6	**2**
20	-	1	-	-	1	**1**
40	-	-	-	-	-	**1**
60	-	-	-	-	-	**1**
80	-	-	-	-	-	**1**

Table S3. Root mean square errors of calibration (RMSEC) and cross-validation (RMSECV) of the PCA models developed in this study. PCA models were identified through their figure numbers in the manuscript.

PCA model	RMSEC	RMSECV
Figure 1	3.8×10^{-5}	1.8×10^{-4}
Figure 2	9.1×10^{-5}	1.4×10^{-4}
Figure 3	1.1×10^{-4}	2.5×10^{-4}
Figure 4	1.7×10^{-4}	3.0×10^{-4}
Figure 5A	1.6×10^{-4}	3.5×10^{-4}
Figure 5B	1.8×10^{-5}	3.1×10^{-4}
Figure 5C	8.2×10^{-5}	3.3×10^{-4}

CHAPTER 3

Analytical approach to the selection of untargeted and target SPME-GC-MS markers for fraud detection in roasted and ground coffee

Cinthia de Carvalho Couto[a], Edna Maria Morais Oliveira[b], Davy William Hidalgo Chávez[c], Otniel Freitas-Silva[b]; Susana Casal[d*]

[a]Food and Nutrition Graduate Program, the Federal University of State of Rio de Janeiro, Av. Pasteur, 296, 22290-240 Rio de Janeiro, Brazil;

[b]Embrapa Food Agroindustry, Av. das Américas, 29501, 23020-470 Rio de Janeiro, Brazil;

[c]Graduate Program in Food Science and Technology, Federal Rural University of Rio de Janeiro (PPGCTA-UFRRJ), Rodovia Br 465, km 7, 23890-000, Seropédica, RJ, Brazil;

[d]LAQV/REQUIMTE, Laboratory of Bromatology and Hydrology, Faculty of Pharmacy, University of Porto, 4050-313, Porto, Portugal.

*Corresponding author

ABSTRACT

Roasted ground coffee has been the target of intentional adulteration for economic gain. To date, studies on volatile metabolites in coffee adulteration have not been reported extensively mainly on individual coffee species and their common adulterants. Solid-phase microextraction coupled with gas-chromatography used was used to determine the most important volatile compounds (VOCs) for the individual discrimination between roasted ground Arabica coffee and its six most common adulterants (barley, corn, rice, soybean, coffee husks, and Robusta coffee) by SPME-GC-MS associated with chemometric analysis. The Principal Component Analysis (PCA) showed the distinction of roasted ground coffee and its most common adulterants, while Hierarchical Clustering of Principal Components (HCPC) and heat map show a tendency of separation of the adulterants. The proposed non-targeted strategy showed satisfactory results with Partial Least-Squares Discriminant Analysis (PLS-DA) analysis, showing to reduce the classification error rate from 5497 to 7 components, which corresponded to the same categories of PCA analysis: Arabica coffee, corn, soybean, barley, rice, and Robusta coffee. Through the target approach, 26 VOCs were selected as candidates for potential markers to detect fraud in coffee. The Tukey and Kruskal-Wallis tests validated that such markers can be suitable to assess the authenticity of ground roasted coffee and thus work as a tool for the control and prevention of fraud in coffee.

Keywords: *Coffea arabica*, *Coffea canephora*, cereal, grain, chromatography, adulteration, authenticity

Data on VOCs of coffee and its adulterants samples could be used to develop an essential comprehension of the differences between them, as well as to indicate possible markers of coffee adulteration.

Further research could explore other variables given the complexity of the roasted and ground coffee sample, such as coffee and adulterants from other origins, different roasting conditions, especially targeting selected compounds in this study.

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CHAPTER 4

Analysis of volatile compounds from adulterated coffee with different food matrices by solid-phase microextraction-gas chromatography-mass spectrometry (SPME-GC-MS)

Cinthia de Carvalho Couto[a], Edna Maria Morais Oliveira[b], Davy William Hidalgo Chávez[c], Otniel Freitas-Silva[b]; Susana Casal[d*]

[a]Food and Nutrition Graduate Program, the Federal University of State of Rio de Janeiro, Av. Pasteur, 296, 22290-240 Rio de Janeiro, Brazil;

[b]Embrapa Food Agroindustry, Av. das Américas, 29501, 23020-470 Rio de Janeiro, Brazil;

[c] Pos-graduate Program in Food Science and Technology, Federal Rural University of Rio de Janeiro (PPGCTA-UFRRJ), Rodovia Br 465, km 7, 23890-000, Seropédica, RJ, Brazil;

[d]LAQV/REQUIMTE, Laboratory of Bromatology and Hydrology, Faculty of Pharmacy, University of Porto, 4050-313, Porto, Portugal.

*Corresponding author

ABSTRACT

This study aimed to identify possible volatile compounds (VOCs) as chemical markers in roasted ground arabica coffee blended with multiple adulterants (corn, barley, soybean, rice, coffee husks, and Robusta coffee) through the application of the solid-phase microextraction-gas chromatographic-mass spectrometric. Adulterated samples were composed of one to six adulterants, ranging from 0.25 to 80% (w/w). Applying the chemometric analysis to VOCs, coffees with multiple adulterations were successfully discriminated according to Partial Least-Squares Regression (PLS-R) models, with a trend of identification of two groups of percentages, from 0 to 1% and from above 5%. VOCs composition proved to be a powerful tool for discriminating roasted ground arabica coffee from adulterated coffee.

Keywords: coffee adulteration, authenticity, *Coffea arabica*, *Coffea canephora*, food quality, food safety, food fraud

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SUPPLEMENTARY MATERIALS

Table S1. Example of sampling from models with multiple adulterations

Sample Type		**Origin**			**Sample Code**	
Arabica blend		Natural coffee Brazil (1), Natural coffee Brazil (2) Washed coffee Colombia, and Washed coffee Honduras			CX	
Robusta blend		Natural coffee Vietnam and Natural coffee Cameroon			RX	
Corn blend		Brazil and Portugal			MX	
Soybean blend		Portugal (1) and Portugal (2)			SX	
Rice blend		Brazil and Portugal			AX	
Coffee husks blend		Brazil (1) and Brazil (2)			PX	
Barley blend		Portugal (1) and Portugal (2)			VX	
		Blend (%)				
CX	**VX**	**MX**	**SX**	**AX**	**PX**	**RX**
99.8	0.25	-	-	-	-	-
99.8	-	0.25	-	-	-	-
99.8	-	-	0.25	-	-	-
99.8	-	-	-	0.25	-	-
99.8	-	-	-	-	0.25	
99.8	-	-	-	-	-	0.25
99.5	0.5	-	-	-	-	-
99.5	-	0.5	-	-	-	-
99.5	-	-	0.5	-	-	-
99.5	-	-	-	0.5	-	-
99.5	-	-	-	-	0.5	-
99.5	-	-	-	-	-	0.5
99.0	0.5	-	-	-	0.5	-
99.0	-	0.5	-	-	0.5	-
99.0	-	-	0.5	-	0.5	-
99.0	-	-	-	0.5	0.5	-
99.0	-	-	-	-	0.5	0.5
98.5	0.5	0.5	0.5	-	-	-
98.5	-	0.5	-	-	0.5	0.5
98.0	0.5	0.5	0.5	-	-	0.5
98.0	0.5	0.5	0.5	0.5	-	-
98.0	0.5	0.5	0.5	-	0.5	-
97.5	0.5	0.5	0.5	0.5	0.5	-
99.0	1	-	-	-	-	-
99.0	-	1	-	-	-	-
99.0	-	-	1	-	-	-

CONCLUSÕES GERAIS

A revisão sistemática da literatura sobre as técnicas de detecção de adulteração em café torrado e moído aplicadas nos últimos 20 anos revelou dados concretos e tendências de pesquisas realizadas por diferentes grupos de pesquisa em todo o mundo, tanto em relação às técnicas analíticas quanto aos tipos de café alvo de adulteração, tipos de adulterantes de café, porcentagem de adulteração mais estudados. Destacam-se também entre os adulterantes mais estudados a espécie de café *C. canephora*, milho e resíduos de café. E entre as técnicas analíticas houve uma tendência para a aplicação da análise cromatográfica e espectroscópica, reconhecidamente mais sensíveis, precisas, confiáveis, não demorada, e no último caso também é uma ferramenta verde.

Nesse sentido, duas técnicas analíticas foram empregadas na discriminação de múltiplos adulterantes em café torrado moído, a saber, NIR e SPME-GC-MS. Primeiramente, a espectroscopia NIR foi capaz de distinguir as amostras puras (café de acordo com suas variedades, arábica e robusta, casca de café, cevada, soja, arroz e milho) quando acoplada à quimiometria. Além disso, arábica contaminado com robusta a partir de 1% também foi discriminado, assim como amostras de arábica puro pareciam estar separadas por origens geográficas. Em relação à amostra de café arábica puro e adulterado, sua discriminação foi viável para todos os adulterantes a partir de 0,25%. No entanto, o tipo de adulterante só foi possível distinguir se não mais do que dois contaminantes estivessem presentes simultaneamente e para concentrações de adulterantes ≥10%.

Para a técnica de MEFS-CG-EM, também acoplada a ferramentas quimiométricas, foram desenvolvidos dois manuscritos, para amostras puras e misturadas. No primeiro também foi possível distinguir o café moído torrado de seus adulterantes mais comuns pelo PCA e corroborado pelo PLS-DA. Enquanto isso, o HCPC parece classificar o tipo de grupos adulterantes. A abordagem não direcionada foi completada com o mapa de calor, que indicou os 30 tempos de retenção (expressos em porcentagem de abundância) que mais contribuíram para diferenciar as amostras. Além disso, a abordagem metabolômica-alvo mostrou 26 compostos voláteis supostamente identificados como possíveis marcadores de adulteração de café.

Na segunda abordagem da técnica de MEFS-CG-EM, o resultado das amostras adulteradas em diferentes percentagens e combinações no café torrado e moído, mostrou um bom ajuste dos modelos de PLS-R desenvolvidos, separando as amostras em componentes explicavam ≥ 80% para cada adulterante individualmente. A distribuição

geométrica das variáveis preditas e componentes confirmou os resultados dos modelos de PLS-R. O heatmap ilustrou os resultados descritos, mostrando uma tendência a dois grupos de amostras, um de 0 a 1% e outro de acima de 5%, para o arroz.

As duas técnicas demonstraram, através de métodos quimiométricos, resultados satisfatórios na discriminação entre amostras puras e adulteradas de café arábica. Destaca-se na técnica de NIR, a discriminação a partir de 0,25% de adulteração, a distinção entre adulterantes ≥10%, e ainda a distinção geográfica entre amostras de café arábica torrado e moído. Para a técnica de SPME-GC-MS ressalta-se a tendência de classificação por tipo de adulterantes, quando analisadas amostras puras de café e adulterantes, e ainda a distinção entre cafés arábica e robusta. Foi possível ainda indicar 26 compostos voláteis como possíveis marcadores de adulteração de café. Na abordagem de amostras adulteradas, a técnica de SPME-GC-MS mostrou uma boa separação entre os componentes, e ainda a identificação de dois grupos percentagens de adulteração (de 0 a 1% e acima de 5%).

GENERAL CONCLUSIONS

The systematic literature review on techniques for detecting adulteration in roasted and ground coffee applied in the last 20 years revealed concrete data and trends of research carried out by different research groups worldwide, to the analytical techniques as well as to the types of coffee targeted for adulteration, types of coffee adulterants, percentage of adulteration most studied. Also noteworthy among the most studied adulterants are the coffee species *C. canephora*, corn and coffee waste. And between the analytical techniques there was a trend for the application of chromatographic and spectroscopy analysis, well-known to be more sensitive, precise, reliable, not time-consuming, and in the last case it is also a green tool.

In this sense, two analytical techniques were employed in the discrimination of multiple coffee adulterants in roasted ground coffee, namely, NIR and SPME-GC-MS. Firstly, NIR spectroscopy was able to distinguish the pure samples (coffee according to its varieties, arabica and robusta, coffee husks, barley, soybean, rice and corn) when coupled with chemometrics. In addition, arabica contaminated with robusta from as low as 1% was also discriminated, as well as pure arabica samples seemed to be separated by geographic origins. Regarding the pure and adulterated arabica coffee sample, their discrimination was viable for all the adulterants from as low as 0.25%. Nonetheless, the type of adulterant was only possible to be distinguished if no more than two contaminants were present simultaneously and for adulterant concentrations ≥10%.

For the SPME-GC-MS technique, also coupled with chemometrics tools, two manuscripts were developed, for pure and blended samples. In the first, it was also possible to distinguish roasted ground coffee from its most common adulterants by PCA and corroborated by PLS-DA. Meanwhile HCPC seem to classify type of adulterant groups. The untargeted approach was completed with the heatmap, which indicated the 30 retention times (expressed as a percentage of abundance) that contributed the most to differentiating the samples. In addition, the target metabolomics method showed 26 VOCs putatively identified as possible markers of coffee adulteration.

On the second approach of the SPME-GC-MS technique, the result of the adulterated samples in different percentages and combinations in the roasted and ground coffee, showed a good fit of the developed PLS-R models, separating the samples into components explained ≥ 80% for each adulterant individually. The geometric distribution of the predicted variables and components confirmed the results of the PLS-R models. The heatmap illustrated the

results described, showing a tendency to two groups of samples, one from 0 to 1% and the other from above 5%, for rice.

The two techniques demonstrated, through chemometric methods, satisfactory results in the discrimination between pure and adulterated samples of arabica coffee. In the NIR technique, the discrimination from 0.25% of adulteration, the distinction between adulterants ≥10%, and the geographical distinction between samples of roasted and ground Arabica coffee stands out. For the SPME-GC-MS technique, the trend of classification by type of adulterants stands out, when pure samples of coffee and adulterants are analyzed, as well as the distinction between arabica and robusta coffees. It was also possible to indicate 26 volatile compounds as possible markers of coffee adulteration. In the approach of adulterated samples, the SPME-GC-MS technique showed a good separation between the components, and also the identification of two groups percentages of adulteration (from 0 to 1% and above 5%).

ATTACHMENTS

ATTACHMENT A - PUBLICATIONS

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Article

Near-Infrared Spectroscopy Applied to the Detection of Multiple Adulterants in Roasted and Ground Arabica Coffee

Cinthia de Carvalho Couto [1], Otniel Freitas-Silva [2], Edna Maria Morais Oliveira [2], Clara Sousa [3,*] and Susana Casal [4]

[1] Food and Nutrition Graduate Program, Federal University of State of Rio de Janeiro, Av. Pasteur 296, Rio de Janeiro 22290-240, Brazil; cinthiaccouto@gmail.com
[2] Embrapa Food Agroindustry, Av. das Américas 29501, Rio de Janeiro 23020-470, Brazil; otniel.freitas@embrapa.br (O.F.-S.); edna.oliveira@embrapa.br (E.M.M.O.)
[3] CBQF—Centro de Biotecnologia e Química Fina, Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Diogo Botelho 1327, 4169-005 Porto, Portugal
[4] LAQV/REQUIMTE, Laboratory of Bromatology and Hydrology, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal; sucasal@ff.up.pt
* Correspondence: cssousa@ucp.pt

Abstract: Roasted coffee has been the target of increasingly complex adulterations. Sensitive, non-destructive, rapid and multicomponent techniques for their detection are sought after. This work proposes the detection of several common adulterants (corn, barley, soybean, rice, coffee husks and robusta coffee) in roasted ground arabica coffee (from different geographic regions), combining near-infrared (NIR) spectroscopy and chemometrics (Principal Component Analysis—PCA). Adulterated samples were composed of one to six adulterants, ranging from 0.25 to 80% (*w*/*w*). The results showed that NIR spectroscopy was able to discriminate pure arabica coffee samples from adulterated ones (for all the concentrations tested), including robusta coffees or coffee husks, and independently of being single or multiple adulterations. The identification of the adulterant in the sample was only feasible for single or double adulterations and in concentrations ≥10%. NIR spectroscopy also showed potential for the geographical discrimination of arabica coffees (South and Central America).

Keywords: coffee; adulteration; infrared spectroscopy; authenticity; chemometrics

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1. Introduction

Coffee is among the most consumed beverages worldwide [1], having enormous economic relevance, and has a continuously growing market, expanding to different applications, such as the cosmetic and pharmaceutical industries [2]. According to the International Coffee Organization (ICO), the global coffee output achieved near 172 million bags in 2020/21, represented by the main commercialized species, *Coffea arabica* (59%) and *Coffea canephora* (robusta) (41%). Brazil is the main coffee producer and exporter worldwide, with a total production estimated in the crop year 2020/2021 of 69 million bags (arabica and robusta), followed by Vietnam (mainly robusta) and Colombia (arabica), with 29 and 14.3 million bags, respectively [3,4].

Due to its commercial value, arabica coffee has been the target of countless and increasingly complex adulterations over the years [5], mainly through the addition of roasted barley, corn, rice and coffee husks [6,7]. Robusta coffee, due to its lower market and compositional similarity, is also commonly used for arabica coffee adulterations [1,7,8].

A plethora of studies have been developed to tentatively detect adulterations in roasted ground coffee employing physical, chemical, and biological techniques. Some include DNA-based approaches [9–13], chromatographic analysis [14,15], ultraviolet–visible spectrophotometry (UV–VIS) [16], digital image processing [17], capillary electrophoresis tandem mass spectrometry [18], electrospray ionization mass spectrometry [19], etc. However, these techniques require sophisticated and expensive instrumentation, as well

Food Research International

Fraud and adulteration in coffee: A comprehensive systematic review of analytic detection approaches

--Manuscript Draft--

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Corresponding Author:	Otniel Freitas-Silva, Ph.D. Brazilian Agricultural Research Corporation's, EMBRAPA Food Technology Rio de Janeiro, Rio de Janeiro BRAZIL
First Author:	Cinthia de Carvalho Couto, MSc
Order of Authors:	Cinthia de Carvalho Couto, MSc
	Caroline Corrêa de Souza Coelho, PhD
	Edna Maria Moraes Oliveira, PhD
	Susana Casal, PhD
	Otniel Freitas-Silva, Ph.D.
Abstract:	The presence of impurities in roasted coffee interferes with its quality. This System Literature Review (SLR) focused on the different types of analytical techniques published in the last 20 years for the detection of adulterants in roasted coffee. Th SLR was performed on StArt software in three stages: Planning, Executing, and Summarization. A total of 83 works were selected. The type of coffee most frequently studied was roasted and ground and Coffea arabica, while among the adulterants, Coffea canephora , coffee wastes, and corn. There is a trend of chromatographic spectroscopic, and multi-adulterant applications. The most sensil techniques were GC, NIR, and vision system. Suitable techniques to detect/quanti adulterations in coffee, at different percentages and particularly as multi-detection approaches, are crucial to improve the coffee quality worldwide