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Desarrollo de un modelo de identificación de adulterantes para control de calidad en ajo en polvo

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*Dedicado a mis padres quienes con su amor
y apoyo incondicional me ayudaron a
alcanzar este logro.*

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Resumen

Desarrollo de un modelo de identificación de adulterantes para control de calidad en ajo en polvo

El fraude alimentario constituye una problemática global que no sólo afecta la economía sino también la salud y confianza del consumidor. El ajo en polvo es una especia susceptible a la adulteración con sustancias de bajo costo y apariencia similar: tiza blanca y almidón de maíz. Dado que los métodos existentes para identificar adulterantes en especias requieren equipos sofisticados y un elevado consumo de tiempo, es necesario recurrir a técnicas alternativas. El objetivo de esta investigación fue desarrollar modelos de predicción basados en espectroscopía en infrarrojo cercano, que identifiquen la presencia de tiza blanca o almidón de maíz en ajo en polvo y que cuantifiquen estos compuestos en las muestras adulteradas. Para este fin, se prepararon 626 muestras de dicha especia adulteradas en concentraciones entre 0 y 30% (w/w), se distribuyeron 500 muestras para calibración y 126 muestras para validación. Luego, se desarrollaron los modelos de clasificación por regresión de mínimos cuadrados parciales (PLS) con análisis discriminante y los modelos de cuantificación por PLS, con validación cruzada y externa, utilizando tratamientos de Variable Normal Estándar, Correlación de Dispersión Multiplicativa y las derivadas de Savitzky-Golay. El modelo de clasificación permitió identificar las muestras adulteradas y el tipo de adulterante, en tanto los modelos de cuantificación de cada adulterante permitieron conocer el porcentaje de adulteración en las muestras de ajo en polvo, con valores del error cuadrático medio de predicción (RMSEP) entre 0.6490% - 1.576%. Los resultados indicaron que es posible utilizar modelos espectrales para determinar la autenticación del ajo en polvo.

Palabras clave: autenticidad, fraude alimentario, seguridad alimentaria, especia

Abstract

Development of a model for identification of adulterants for quality control of garlic powder.

Food fraud is a global problem that not only affects the economy but also consumer health and confidence. Garlic powder is a spice that is susceptible to adulteration with low-cost substances of similar appearance: white chalk and corn starch. Since existing methods to identify adulterants in spices require high-tech and time-consuming equipment, alternative techniques are required. The objective of this research was to develop predictive models based on near infrared spectroscopy to identify the presence of white chalk or corn starch in garlic powder and quantify these compounds in adulterated samples. For this purpose, 626 samples of this spice adulterated in concentrations between 0 and 30% (w/w) were used, 500 samples were distributed for calibration and 126 samples for validation. Then, classification models were developed by partial least squares (PLS) regression with discriminant analysis and quantification models by PLS, with cross-validation and external validation, using Standard Normal Variable, Multiplicative Dispersion Correlation and Savitzky-Golay derivatives treatments. The classification model allowed the identification of adulterated samples and the type of adulterant, while the quantification models for each adulterant allowed knowing the percentage of adulteration in garlic powder samples, with root mean square error of prediction (RMSEP) values between 0.6490% - 1.576%. The results indicated that it is possible to use spectral models to determine the authentication of garlic powder.

Keywords: authenticity, food fraud, food safety, spice

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Lista de Símbolos y abreviaturas

Símbolos con letras griegas

Símbolo	Término	Unidad SI
β_i	Coeficiente de regresión	adimensional
λ	Longitud de onda	nm

Abreviaturas

ANMAT	Administración Nacional de Medicamentos, Alimentos y Tecnología Médica
ANN	Redes neuronales artificiales
DRIFTS	Espectroscopia de reflectancia difusa por transformada de Fourier infrarroja
EMA	Adulteración económicamente motivada
ESA	Asociación Europea de Especies
FDA	Administración de Alimentos y Medicamentos de los Estados Unidos
FT-IR	Infrarrojo por transformada de Fourier
FT-NIR	Infrarrojo cercano por transformada de Fourier
GC	Cromatografía de gases
GLC	Cromatografía gas-liquido
GSC	Cromatografía gas-solido
HCA	Análisis de agrupamiento jerárquico
HPLC	Cromatografía líquida de alta eficacia
INVIMA	Instituto Nacional de Vigilancia de Medicamentos y Alimentos.
LC-MS	Cromatografía líquida y espectrometría de masas
LDA	Análisis discriminante lineal

LOD	Límite de detección
MIPs	Polímeros impresos molecularmente
MIR	Infrarrojo medio
MLR	Regresión lineal múltiple
MSC	Corrección de dispersión multiplicativa
NIR	Infrarrojo cercano
NMR	Resonancia magnética nuclear
OPLS-DA	Análisis discriminante de mínimos cuadrados parciales ortogonales
PCA	Análisis de componentes principales
PCR	Regresión de componentes principales
PLS-DA	Análisis discriminante de mínimos cuadrados parciales
PLSR	Regresión de mínimos cuadrados parciales
R ²	Coefficiente de determinación
RMSE	Error cuadrático medio
RMSEC	Error cuadrático medio de la calibración
RMSEP	Error cuadrático medio de la predicción
RMSEV	Error cuadrático medio de la validación cruzada
SERS	
	Dispersión Raman mejorada en la superficie
SNV	Variable Normal Standard
SVMs	Máquinas de vectores de soporte
TLC	Cromatografía de capa fina
UV-VIS	Ultravioleta-visible

Introducción

El fraude alimentario se considera una problemática global que afecta la economía agroalimentaria, así como la salud y confianza del consumidor. En 2019 se presentaron 292 reportes de fraude en la Unión Europea, dentro de los cuales el 4% correspondían a especias, valor que ha incrementado en los últimos 3 años (European Commission, 2017, 2018, 2019). En general, la cadena de producción de especias es considerada como medianamente vulnerable al fraude, sin embargo, cada una de las etapas de producción presenta una alta vulnerabilidad a la adulteración (Silvis et al., 2017). El ajo en polvo es reconocido en la actualidad como especia debido a sus propiedades culinarias y se utiliza principalmente como saborizante y conservante de alimentos (Galvin-King et al., 2018; Reveles et al., 2009; Saif et al., 2020). Por otro lado, se destaca por sus propiedades medicinales, como antioxidante, antiinflamatorio, antimicrobiano, anticancerígeno, antidiabético, neuroprotector y protector renal (Navarro, 2007; Netzel, 2020; Ramírez et al., 2016; Saif et al., 2020; Tapsell et al., 2006). Sin embargo, se han encontrado otras sustancias en su composición como almidones y tiza, en concentraciones de 35% y 24%, respectivamente, con el propósito de aumentar el volumen e incrementar las ganancias (Lohumi, Lee, & Cho, 2015; Osman et al., 2019; Perkin Elmer, 2018).

Actualmente, 18 empresas colombianas importan el ajo en polvo para comercializarlo directamente o como insumo para la preparación de otros productos, sin realizar una inspección correcta del producto (connect2india, 2020; Suárez, 2016). En Colombia, el ente regulador de los productos alimenticios es el INVIMA, que realiza procesos de control en las diferentes cadenas alimenticias. Sin embargo, se pueden presentar algunos reportes de fraude cuando el producto ya ha llegado al consumidor. Esto se debe a que algunas inspecciones, principalmente de productos importados, no son exhaustivas y se basan solo en revisiones visuales del alimento y fechas de caducidad, sin indagar en la composición química de los productos (Instituto Nacional de Vigilancia de Medicamentos y Alimentos [INVIMA], 2019).

Dado que los métodos existentes de identificación de adulterantes en especias, como el análisis microscópico, la cromatografía de capa delgada y la cromatografía líquida, requieren de equipos de alta tecnología, un alto consumo de tiempo y el uso de reactivos contaminantes, no se realizan inspecciones continuas en las cadenas de producción y distribución (AOAC International, 2012a; Chaminda Bandara et al., 2019; Galvin-King et al., 2018). Las técnicas analíticas combinadas con la quimiometría representan una estrategia adecuada para la caracterización de sistemas biológicos complejos como alimentos, debido a que permiten la determinación simultánea de una gran cantidad de compuestos (Esslinger et al., 2014). Una de estas técnicas es la espectroscopía de Infrarrojo Cercano (NIR), que se ha utilizado en el análisis y control de calidad en la industria agroalimentaria, para sustituir o complementar los métodos analíticos tradicionales (Soldado et al., 2015). Debido a que esta tecnología no requiere de una preparación complicada de la muestra, no es invasiva y proporciona resultados de manera automática, es posible su incorporación dentro de las líneas de producción, sustituyendo los métodos analíticos tradicionales en laboratorio (Soldado et al., 2015).

Para utilizar esta tecnología, se requiere de la aplicación de la quimiometría para desarrollar modelos de predicción que relacionen la señal espectral con el analito de interés. En la construcción de un modelo con buena capacidad predictiva es necesario contar con un conjunto representativo de muestras en un intervalo adecuado de la propiedad que se desea medir y aplicar pretratamientos a los datos espectrales, para corregir el efecto de los componentes no relacionados con la información buscada (Burns & Ciurczak, 2008). La técnica ha demostrado ser efectiva en la autenticación de productos alimenticios, como la detección de melamina en la leche en polvo (Jeffrey Moore, Ganguly, et al., 2012), la detección de almidón en cebolla en polvo (Lohumi et al., 2014) y la determinación de almidón y contenido de humedad en cúrcuma (Thangavel & Dhivya, 2019). Además, proporciona resultados rápidos, eficientes, de bajo costo y sin generar residuos contaminantes (Oliveira et al., 2019).

Teniendo en cuenta lo anterior, el objetivo de esta investigación fue evaluar la espectroscopía de infrarrojo cercano (NIR) como técnica analítica rápida para el desarrollo de modelos de predicción que identifiquen la presencia de almidón de maíz y

tiza blanca como adulterantes en ajo en polvo y cuantifiquen la concentración de dichos adulterantes. Con este fin, primero se presenta un artículo de revisión para conocer las principales técnicas y avances en la identificación de adulterantes en especias; luego se observa el estudio de las características de la espectroscopía en infrarrojo cercano y sus aplicaciones como herramienta para la autenticación de alimentos.

A continuación, se muestra un artículo de investigación sobre el desarrollo de modelos de clasificación y cuantificación de adulterantes basados en espectroscopia NIR. Para ello, se prepararon 625 muestras de ajo en polvo: 500 muestras para la calibración (20 muestras sin adulterar, 240 muestras adulteradas con almidón de maíz y 240 muestras adulteradas con tiza blanca, en concentraciones entre 0.2 y 30% (w/w),) y 126 muestras adulteradas en concentraciones entre 0 y 30% (w/w) (63 muestras con cada adulterante), usadas en el proceso de validación. En el análisis espectral se estudiaron diferentes pretratamientos como la Variable Normal Estándar (SNV), la Correlación de Dispersión Multiplicativa (MSC) y las derivadas de Savitzky-Golay, para eliminar el ruido en los espectros y posteriormente construir los modelos de predicción a través de la Regresión de Mínimos Cuadrados Parciales (PLSR). Además, se presenta una validación final con marcas comerciales de ajo en polvo disponibles en el mercado local. Por último, el documento finaliza con las conclusiones generales de la investigación y algunas sugerencias para las investigaciones futuras.

1. Advances in Adulterants Identification Techniques in Spices

1.1 Abstract

Spices have an economic value in different industries such as food, medicines, and cosmetics. Their easy handling and little control encourage and promote economically motivated adulteration, thus incurring in food fraud. Some of the most common types of adulteration of powdered spices are partial or complete substitution of the product with a similar material, inclusion of common adulterants such as sawdust, starches, cereals, or talc, among others, and the addition of non-permitted dyes which in some cases have affected the health of consumers.

Therefore, different techniques for detection of adulterants in powdered spices have been developed, such as: chromatography, that separates the components of the sample in a moving and stationary state; aroma and flavor sensors, where specific smell or taste prints are recorded; DNA barcoding, which uses short stretches of standardized gene sequences to identify plant materials, and spectroscopic methods that employ electromagnetic radiation to interact with matter and obtain the characteristics of the sample. This review summarizes the use of these techniques, coupled with chemometric tools, for authentication of different spices and their potential for commercial application.

Keywords: fraud, powdered food, authentication, chemometrics.

1.2 Introduction

Spices are important ingredients of multiple industries, and their consumption has increased over the past decades due to consumer interest in new flavors and ethnic tastes. This demand has made them a multibillion-dollar industry, reaching a market value of USD 13.77 billion in 2019 (Black et al., 2016; Grand View Research, 2020; Heigi Library, 2019). Due to this growing demand and the food fraud scandals of recent years, it has been necessary to assess and understand food fraud vulnerability in production and supply chains. Food fraud involves all activities that have the objective of obtaining an

economic benefit through the violation of the law and that also constitute a risk to human, animal, or plant health, to animal welfare or to the environment (European Commission, 2019). The food sector is generally vulnerable to crime and spice industry is one of the most vulnerable ones, in addition to meat, fish, and oil industries (Silvis et al., 2017).

Some of the adulterants used in spices are corn starch, fruit seeds, peanuts, almond, and buckwheat (Galvin-King et al., 2020). The most concerning problems of spices adulteration are its health effects. In the case of these products, the price also depends on the intensity of the colors, therefore, one of the most common adulterations has been the addition of illegal dyes like Sudanese Dyes, which are group 3 genotoxic carcinogens (Black et al., 2016; Fonovich, 2013).

A wide variety of different detection methods have been developed and used to determine the authenticity of herbs, spices, and foods in general (Black et al., 2016; Galvin-King et al., 2018). The analytical methodologies used for adulterant identification include spectroscopic methods, chemical/biochemical methods, sensing methods (E-nose and E-tongue) and the most recent DNA barcoding tools (Osman et al., 2019). The aim of this review is to present the principal aspects of spice adulteration and the latest advances in the corresponding analytical techniques for detection of adulterants.

1.3 Types of adulteration in spices

Food fraud is the term used to refer to the intentional substitution, addition, handling and misrepresentation of food, food ingredients or food packaging; or misleading statements made about a product, with the objective of obtaining an economic benefit (Spink & Moyer, 2011). This term involves the concept of “economically motivated adulteration” (EMA) that is defined by the US Food and Drug Administration as “the fraudulent, intentional substitution or addition of a substance to a product for the purpose of increasing the apparent value of the product or reducing the cost of its production” (Food and Drugs Administration [FDA], 2009). Although the purpose of food fraud is financial gain, it has been found to have an impact on public health, due to adulteration or misreporting of potential risks to the consumer (Spink & Moyer, 2011). For instance, Sudan I, II, and III, colorants added to chili powder and curry, have been proved to be carcinogenic for humans (Fonovich, 2013); authentic ingredients substituted by allergenic

ingredients, such as nut protein from almonds in paprika (Galvin-King et al., 2020) or peanut and almond in cumin and meat products (Bennett, 2015), can cause anaphylaxis in susceptible individuals (Garber et al., 2016), and the addition of non-food substances such as chalk powder in turmeric (Nallappan et al., 2013), lead oxide for color in paprika (Everstine, 2013), and colored sawdust in red pepper powder may result in public health issues (Attrey, 2017).

Spices are parts of plants, either in the form of seeds, fruits, roots, or others, which are commonly employed to give flavor and color to a wide range of foods, such as processed foods or ready-to-eat products (Merieux NutriSciences, 2019). In the market, expensive products are the most attractive to food fraud. Spices are susceptible to adulteration due to their high economic value, high demand, and the limited capacity of the consumer to detect it, thus posing problems of quality and food safety (Lakshmi, 2012; Jeffrey Moore, Spink, et al., 2012). The annual report of the European Commission shows that herbs and spices ranked sixth in fraud cases reported in 2019, in which the most common non-compliance categories are related to mislabeling and adulteration (European Commission, 2019).

There are different ways to adulterate spices with the objective to increase their profit (Osman et al., 2019). The most common are:

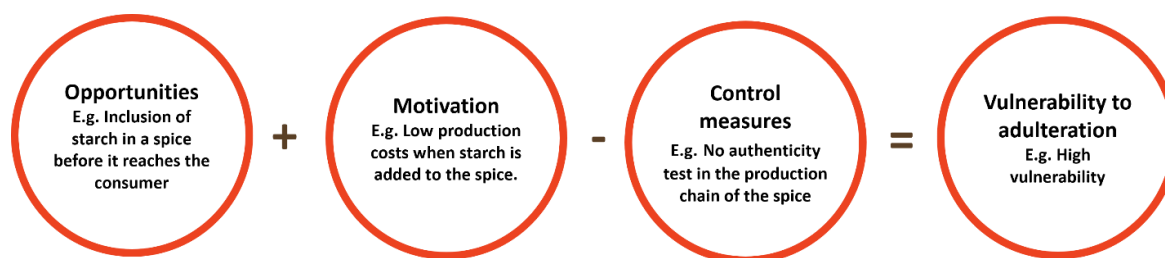
- Partial or complete substitution of the product with material from which a valuable constituent, such as essential oil, has already been extracted. For example, the material remaining after oleoresins are extracted from paprika is added to the powdered spice to increase the weight of the product (Galvin-King et al., 2020).
- Substitution of a part of the plant used as a spice by other parts of the same plant that do not offer the same active components or flavoring properties. In particular, saffron corresponds to the dried stigmas of the flowers of *Crocus sativus* and it is commonly adulterated with the stamen of the same flower (Petrakis & Polissiou, 2017).

-
- Adulteration with filler ingredients or lower quality products like sawdust, straw and different types of starches or cereals to increase weight. This is the case of the addition of cassava starch and corn flour to black pepper and cumin (Lima et al., 2020).
 - Inclusion of pure chemicals in spices adulterated with starch or straw to artificially impart the characteristic flavor of the spice, e.g., the addition of piperine in adulterated black pepper (Osman et al., 2019).
 - Addition of non-spice related plants to mimic the morphological characteristics of the original product. For example, safflower is often mislabeled as “saffron” or is used to adulterate this spice (Petraakis et al., 2015).
 - Use of inorganic substances such as talc, chalk, brick dust and metal salts. For example, yellow chalk powder is commonly used as an adulterant in turmeric (Dhanya & Sasikumar, 2010; Nallappan et al., 2013).
 - Addition of non-permitted colorants to impart stronger and more striking colors. Some colorants such as Sudan dyes are suspected to be carcinogenic, which may present health problems for consumers (Merieux NutriSciences, 2019). The addition of these dyes has been reported in paprika, turmeric, and saffron (Dhakal et al., 2019; Lohumi et al., 2017; Petraakis et al., 2017).
 - For spices, the geographical origin represents a higher price in their sale process. Geographical origin can be intentionally mislabeled for economic gain, as may be the case of Bourbon vanilla, Spanish saffron, Espelette pepper, among others (Merieux NutriSciences, 2019).

Food fraud has its opportunity when there is potential to carry it out and low risk of being detected. Multiple factors can increase the probability of fraud, and they have been organized into larger groups: opportunities, motivations, and inadequate control measures. By analyzing these three factors, the degree of vulnerability of a product can be estimated (Figure 1-1) (Hellberg et al., 2021; Silvis et al., 2017). Opportunities for the different types of adulteration emerge at each stage of the spice and herbs production

chain, since they can be committed by any individual or group involved in the chain, including suppliers, food manufacturers, retailers, and importers (Silvis et al., 2017). In cultivation, contamination by pesticides or fungicides may occur. During harvesting, non-functional parts of the plant or non-corresponding species may be harvested. In multi-product milling plants, cross-contamination may occur due to lack of proper cleaning. There could be inclusion or substitution of the product with other compounds in local markets, as well as in secondary processing and distribution stages (export and import) without the proper declaration in the label (British Retail Consortium [BRC] et al., 2016).

Figure 1-1: Factors to determine adulteration vulnerability



Source: Own elaboration

In 2019, the spice market was sized at USD 13.77 billion and is expected to grow at an annual rate of 6.3% from 2020 to 2027 (Grand View Research, 2020). Spice production has increased over the years with a global production of 2770000 tons in 2019, in which Asia is the largest producer with 86% of total production (FAOSTAT, 2019b). In the same period, a global exchange average of 553000 tons was reported, in which India was the largest exporter and Saudi Arabia the largest importer of these products (FAOSTAT, 2019a).

This increase is due to the multiple medicinal and cosmetic properties that most of the spices can offer, in addition to a growing consumer interest in new flavors and experiences within ethnic cuisine for flavoring, preserving, coloring, or highlighting the taste of the dishes (Carmona, 2013). Part of the production is destined for consumption in powder, while another part is used to obtain oleoresins and essential oils, which is why there is a higher production and consumption worldwide than that recorded in marketing statistics (Arizio & Curioni, 2014). Most of the spices consumed in the world originate in underdeveloped countries, where there may be political and economic instability that

leads to fraudulent activities (Hellberg et al., 2021). All these factors motivate fraud in the spice industry to maximize incomes and/or to reduce production costs.

Determining the authenticity of spices requires comprehensive monitoring with efficient analytical methods. Multiple safety associations have recommended some analytical methods to detect adulterants in food. They are based on density, physical properties, microscopic analysis, and chemical analysis (Lohumi, Lee, Lee, et al., 2015). These methods require the use of reagents such as chloral hydrate, ferric acetate, potassium iodide, potassium chlorate, among others, which lead to increased contamination due to the subsequent waste and a large cost for handling (AOAC International, 2012a). In addition, they consume time and energy that could be used to carry out other processes. For this reason, continuous inspections are not carried out in the distribution chains of these products (Chaminda Bandara et al., 2019). The development of analytical methods to authenticate these products is still at relatively early stages because specific knowledge of each spice and herb is required (Hellberg et al., 2021). The spice chain vulnerability assessment conducted in 2017 concluded that there is a medium perception of vulnerability in this chain. Opportunity and motivation factors scored high, while control measures varied by company size (Silvis et al., 2017).

1.4 Common adulterants in powdered spices

Spices are dried products used for flavoring, seasoning, imparting aroma and, in some cases, coloring food (Codex Alimentarius, 1995; Matthews & Jack, 2011). Besides their culinary properties, spices contain chemical compounds that produce health benefits for the consumer (Galvin-King et al., 2019), such as cholesterol-lowering and positive effects on mental health, diabetes, and arthritis (Tapsell et al., 2006). The spices supply chain is vulnerable to adulteration at different stages, for instance, in collection other parts of the plant or different plants are used to produce the spice, and at the grinding and packaging stage there might be addition or substitution with powder materials of lower price. For this reason, it is necessary to develop and implement a mitigation risk plan with verification measures and traceable information to guarantee the authenticity of the product (British Retail Consortium [BRC] et al., 2016; Silvis et al., 2017). The spice industry has been affected by EMA in multiple cases (Table 1-1), not only by inclusion of other materials, but also by the addition of different dyes that have shown to be harmful for the consumer as

they produce carcinogenic compounds when metabolized in the human body (Fonovich, 2013).

A study conducted in 2016 reported the analysis of 75 spice samples from different markets, where 24% presented one or more adulterants and three out of five spice samples were found to be adulterated with substances potentially harmful to health (Black et al., 2016). The 2019 report by the French General Directorate of Competition, Consumer Affairs and Fraud Control indicates that the spice with the highest number of adulterated samples is saffron (81%), followed by pepper (59%), paprika and chili (54%), and curry and turmeric (41%) (Direction générale de la concurrence, 2018). An analysis of authenticity using DNA-based methods of 5,957 commercial herbal products sold in 37 countries from all continents showed that 27% of the herbal products commercialized in the global marketplace are adulterated. The proportion of adulterated products varies among continents, being the highest in Australia (79%), followed by South America (67%), Europe (47%), North America (33%), Africa (27%) and the lowest in Asia (23%) (Ichim, 2019).

Depending on the type of spice, different concentrations of adulterants have been found. In saffron, levels varying between 5 and 20% (w/w) have been detected of turmeric or marigold (Petrakis et al., 2017); A range of 5 to 35% (w/w) of corn was found in garlic and onion (Lohumi et al., 2014; Lohumi, Lee, & Cho, 2015); while, high amounts of adulterants have been observed in pepper and cumin, mainly cassava starch and corn flour with concentrations of up to 50% (Lima et al., 2020). In turmeric, adulteration ranging between 2.8% and 17% (w/w) has been presented, mainly with rice flour and colorants (Chaminda Bandara et al., 2019) and in oregano, a scale adulteration between 30% to 70% was identified, even two of the samples analyzed had no oregano at all in their composition (Black et al., 2016). Recently, it was found that several commercial spices were prepared with donkey dung, artificial colorants and even acid and hay (Times of india, 2020).

Table 1-1: Adulterants in spices

Spice	Adulterant	Reference
Black pepper	Powdered papaya seed and exhausted black pepper	(Dhanya & Sasikumar, 2010; Lakshmi, 2012)
	Foreign berries, fruits seeds and artificial pepper	(Tremlová, 2001)
	Buckwheat and millet	(Galvin-King et al., 2019)
	Chili powder	(Parvathy et al., 2014)
Chili	Sudan I, III and IV	(Rani et al., 2015; Tarantelli, 2017)
	Orange G	(Sebaei et al., 2019)
	Talc powder, brick powder, almond shell dust and tomato wastes.	(Dhanya & Sasikumar, 2010)
Cumin	Sudan I and IV	(Sebaei et al., 2019)
	Peanut, almond, and Brazil nut	(Bennett, 2015; Garber et al., 2016)
Garlic	Chili and wheat	(M. Zhang et al., 2019)
	Maltodextrin	(Jessica Moore, 2020)
	Talcum powder and chalk	(Sherman, 2017)
	Corn starch	(Osman et al., 2019)
Onion	Wheat	(M. Zhang et al., 2019)
	Corn starch	(Mohiuddin, 2019)
Paprika	Sudan I and IV, Orange II and Acid Black I	(Tarantelli, 2017)
	Orange G	(Sebaei et al., 2019)
Saffron	Turmeric, paprika and calendula	(Dhanya & Sasikumar, 2010)
	Magenta III and rhodamine B	(Bhooma et al., 2020)
	Curcuma longa, Crocus vernus stigmas, Carthamus tinctorius and Calendula officinalis	(Torelli et al., 2014)
Turmeric	Sudan I, II, III and IV, metanil yellow and Orange II	(Rani et al., 2015; Sahu et al., 2020; Tarantelli, 2017)
	Corn, wheat, and chili	(M. Zhang et al., 2019)
	Chalk powder and saw dust	(Dhanya & Sasikumar, 2010)
	C. Zedoaria, cassava, barley, and rye	(Parvathy et al., 2015)

1.5 Economic impact of spice fraud

It has been identified that the global cost due to food fraud is approximately \$49 billion of dollars per year (McGrath et al., 2018). For a company, the cost of this kind of fraud is between 2% and 15% of its annual income. This includes the expenses of product recalls, investigation, lost sales, price drops, among others (PwC & SSAFE, 2016). This high loss of money is mainly due to the lack of detection and the increase in fraudsters (Bouzemrak & Marvin, 2016). Other factors contributing to this problem are the increase in the complexity of production chains, the development of technology and the rapid growth of refrigerated storage and transport systems (Elliott, 2014).

In response to the food fraud problem, multiple organizations have been created around the world to monitor food quality and establish processes to detect adulteration. Through rapid alerts, these organizations report unsafe, banned, or recalled products. The National Administration of Drugs, Food and Medical Devices (ANMAT) banned the commercialization of dehydrated oregano because it has violated the regulations of the Argentinian Food Code. In its composition, the product had more than 25% of colored wheat bran and more than 3% of leaves that did not correspond to oregano or wheat bran (Administración Nacional de Medicamentos Alimentos y Tecnología Médica [ANMAT], 2019). The Spanish Agency for Food Safety and Nutrition warned about the presence of undeclared sulfites in cinnamon powder, which pose a health risk to people allergic to sulfites (Agencia Española de Seguridad Alimentaria y Nutrición [AESAN], 2021).

Food fraud reports and product recalls have increased over the years due to the complexity of supply chains and strict regulations, which supports the fight against food fraud and demonstrates the importance of sharing information (Allianz Global Corporate and Speciality, n.d.; European Commission, 2019). Some manufacturers buy their ingredients or raw materials from fewer suppliers, as supply chains are becoming increasingly global. This has led to a “rippled effect”, where a single recall affects several manufacturers, brands, and countries, thus damaging their reputation and generating large economic losses (Allianz Global Corporate and Speciality, n.d.). For example, the Salmonella outbreak of 2009 resulted in at least 714 illnesses and contributed to 9 deaths. This caused a recall of 3900 products from more than 200 companies and triggered a reduction of 25% in peanut sales, which had a cost of USD \$1 billion for the

industry. Peanut Corporation of America, the company responsible for the outbreak, had to file for bankruptcy (Wittenberger & Dohman, 2010). In 2015 the recall of cumin spice contaminated with nuts affected 14 companies, 100 brands, 153 individual products, and 756 products in different packaging (Pollack, 2016). Food companies should have adequate controls and mitigation measures to prevent or detect product vulnerabilities and avoid recall processes. In addition, to have a managed product recall plan is necessary, since it can reduce the size of the recall, the potential litigation, loss of customers and the impact on brand value and reputation (Allianz Global Corporate and Speciality, n.d.; British Retail Consortium [BRC] et al., 2016).

1.6 Techniques for detection of adulterants in powdered spices

The interest in food quality and food safety has motivated the development of techniques for identifying adulterants in spices (Sasikumar et al., 2016). The adulteration of these products has become more sophisticated, making the product authentication process more difficult (Oliveira et al., 2019). Targeted methods based on identification of specific markers that indicate a particular property of the product allows quantifying the specific analyte and determine if it complies with the specified limit in the label or law (Esslinger et al., 2014). The control methods in spice adulteration reported in *Guidance on Authenticity of Herbs and Spices* includes visual inspection, microscopy, and analysis to meet the standards defined in the European Spice Association (ESA) Quality Minima Document (British Retail Consortium [BRC] et al., 2016). However, these analytical approaches include a highly trained analyst, complex laboratory activities, a large amount of time and product loss, since some of them are destructive (Oliveira et al., 2019). Therefore, research on new adulterant detection methods that are rapid, reliable, and competent is continually carried out.

Some of the new techniques need to be combined with chemometrics tools to improve the analysis of chemical data obtained by analytical instruments. This approach is based on the application of mathematical, statistical, and other methods to obtain an objective evaluation of a set of chemical data, to generate quantitative and qualitative measurements (Lang et al., 2014). The most common analyses applied to chemical information to identify compounds by correlation are principal component analysis (PCA),

partial least square discriminant analysis (PLS-DA), partial least square regression (PLSR) and principal component regression (PCR). The choice of one of them depends on the characteristics of the samples (Oliveira et al., 2019). These are the most common analyses used in the food industry, however other types have been studied to increase the accuracy of the developed models. In the following sections, the latest advances in identification techniques of adulterants will be described. Table 1-2 specifies the application of these techniques in the spice industry.

Table 1-2: Techniques of adulterants detection in spices

Spice	Adulterant	Chemometrics	Accuracy	Technique	Reference
Black pepper	Cassava starch	MLR	$R^2 = 0.97$	NIR	(Lima et al., 2020)
		PLS	$R^2 = 0.97$		
	Corn flour	MLR	$R^2 = 0.97$		
		PLS	$R^2 = 0.98$		
<i>Cinnamomum verum</i>	<i>Cinnamomum cassia</i>	PLS-DA PNN	D = 99.25% D = 100%	DRNIR	(Cantarelli et al., 2020)
Cumin	Cassava starch	MLR	$R^2 = 0.99$	NIR	(Lima et al., 2020)
		PLS	$R^2 = 0.99$		
	Corn flour	MLR	$R^2 = 0.99$		
		PLS	$R^2 = 0.99$		
	Coriander	PCA	D = 94.44% D = 100%	E-nose E-tongue	(Tahri et al., 2016)
Garlic	Cornstarch	PLSR-VIP	$R^2 = 0.95$	FT-IR	(Lohumi, Lee, & Cho, 2015)
Paprika	Sudan dye	HLA/GO	$R^2 = 0.98$	FT-IR	(Lohumi et al., 2017)
	Spent paprika	OPLS-DA	$R^2 = 0.85$	NIR	(Galvin-King et al., 2020)
			$R^2 = 0.94$	FT-IR	
	Sudan I	Linear regression	$R^2 = 0.98$	^1H NMR	(Hu et al., 2017)
$R^2 = 0.99$			SSNMR		
			$R^2 = 0.98$	SERS-TLC	(Gao et al., 2015)

MLR: multiple-linear regression; PLS: partial least square; PLS-DA: partial least square discriminant analysis; PNN: probabilistic neural network; PCA: principal component analysis; PLSR-VIP: partial least square regression with variable importance in projection; HLA/GO: Hybrid linear analysis Goicoechea & Olivieri; OPLS-DA: orthogonal partial least squares discriminant analysis; D: Discrimination; R^2 : coefficient of determination; NIR: near infrared; DRNIR: diffuse reflectance near infrared; FT-IR: Fourier transform infrared; NMR: nuclear magnetic resonance; SSNMR: solid state nuclear magnetic resonance; SERS-TLC: surface enhanced Raman spectroscopy with thin layer chromatography.

Table 1-2: Continuation

Spice	Adulterant	Chemometrics	Accuracy	Technique	Reference	
Saffron	Stamens Turmeric Buddleja Gardenia	SiPLSR	R ² = 0.94 R ² = 0.99 R ² = 0.99 R ² = 0.99	DRIFTS	(Petrakis & Polissiou, 2017)	
	Safflower	SiPLSR	R ² = 0.96	DRIFTS	(Petrakis & Polissiou, 2017)	
		OPLS-DA	R ² = 0.96	GC	(Farg et al., 2020)	
	Calendula	SiPLSR	R ² = 0.97	DRIFTS	(Petrakis & Polissiou, 2017)	
		OPLS-DA	R ² = 0.99	GC	(Farg et al., 2020)	
	Plant-derived agents	OPLS-DA	R ² = 0.97	¹ H NMR	(Petrakis et al., 2015)	
	Sudan I-IV	Linear regression		R ² = 0.99	¹ H NMR	(Petrakis et al., 2017)
				R ² = 0.99	HPTLC	(Rani et al., 2015)
	Safflower, turmeric powder, red 40 and yellow 5	PLS	R ² = 0.99	TLC-RS	(Dai et al., 2020)	
	Yellow styles, safflower, dyed corn stigma, tartrazine, and methyl orange	PCA-ANN	D = 100%	E-nose	(Kobra Heidarbeigi et al., 2015)	
PCA		D = 100%	E-tongue	(Kobra Heidarbeigi et al., 2016)		
PCA, HCA, SVMs, ANN-MLP		D = 89% -100%	E-nose -CV	(Kiani et al., 2017)		
Yellow turmeric	White turmeric, sudan red dye,	PLSR	R ² = 0.95 R ² = 0.97	FT-IR	(Dhakal et al., 2019)	
	metanil yellow and Sudan I	Linear regression	R ² = 0.99	1064 nm RSI	(Dhakal et al., 2018)	

SiPLSR: synergy interval partial least square regression; PCA-ANN: principal component analysis with artificial neural network; HCA: Hierarchical Cluster Analysis; SVMs: Support Vector Machines; ANN-MLP: multiplayer artificial neural network; RSI: Raman imaging system; D: Discrimination; R²: coefficient of determination; DRIFTS: Diffuse reflectance infrared Fourier transform spectroscopy; GC: Gas chromatography; HPTLC: high-performance thin layer chromatography; TLC-RS: thin layer chromatography with Raman spectroscopy; CV: computer vision.

1.6.1 Spectroscopic techniques

These techniques employ electromagnetic radiation to interact with matter and obtain the characteristics of a sample. This is a quantum phenomenon that depends on the properties of the radiation and the structure of the samples involved (Hofmann, 2010). Each atom or molecule in a material has a particular set of energy that provides a unique spectrum, based on adsorption or emission of light at specific wavelengths (Ravindran et al., 2018). This spectral information can be affected by noise, edge effects, changes in baseline or scattering of light. To reduce these effects, multiplicative scatter correction (MSC), standard normal variate (SNV) and Savitzky-Golay polynomial derivative filter techniques are applied (Rinnan et al., 2009). There are different spectroscopic analytical techniques combined with chemometrics that have been used in the spice industry for food authenticity, such as near-infrared (NIR), mid-infrared (MIR), Raman and Nuclear Magnetic Resonance (NMR) (Galvin-King et al., 2018). These techniques have proved to be efficient in authentication of spices, with their own particularities, advantages, and disadvantages (Oliveira et al., 2019).

Fourier transform infrared (FT-IR) and Fourier transform near-infrared (FT-NIR) spectroscopies have been used for quality and quantity analysis of different products to detect food fraud issues (Lohumi et al., 2014; McGrath et al., 2018). FT-IR focuses on the MIR spectrum region from 2500 to 25000 nm, thus providing the chemical profile of the sample, while FT-NIR works with the NIR spectrum in the wavelength range of 780-2500 nm in which the chemical and physical composition of the samples can be revealed (Galvin-King et al., 2018; Lee et al., 2015). Different studies have shown the efficiency of these two techniques combined with chemometrics analysis for authentication of powdered spices. FT-IR and PLSR was applied by Lohumi, Lee and Cho (2015) to identify adulteration with cornstarch in garlic powder, using variable importance in projection (VIP) as a variable selection technique, to improve the accuracy of the prediction model. In 2017, a rapid determination of Sudan dye in paprika powder was developed with the application of FT-IR and hybrid linear analysis (HLA) (Lohumi et al., 2017). In addition, Dhakal et al. (2019) applied this technique along with PLRS to detect the presence of white turmeric (*Curcuma zedoaria*) and Sudan Red dye as adulterants in yellow turmeric (*Curcuma longa*) powder. Diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) was applied by Petrakis and Polissiou (2017) to identify and quantify the

adulteration of saffron with six characteristic adulterants of plant origin; The authors used synergy interval PLS for variable selection and development of prediction models with improved performance.

In 2020, diffuse reflectance NIR spectroscopy was applied for a quick and direct analysis of cinnamon (*Cinnamom verum*) samples adulterated with common cinnamon (*Cinnamom cassia*) and showed an average discrimination of 99.25% for a model obtained with PLS-DA and 100% with Probabilistic neural network (PNN) (Cantarelli et al., 2020). The techniques NIR and FT-IR along with orthogonal partial least squares discriminant analysis (OPLS-DA) were compared in the identification of spent paprika as an adulterant of paprika. The models obtained had an R^2 of 0.853 for NIR and 0.943 for FT-IR (Galvin-King et al., 2020). Additionally, models based on NIR spectroscopy with multiple linear regression (MLR) and PLS techniques were developed for authenticity of black pepper and cumin powder adulterated with cassava starch and corn flour. These models were evaluated with commercial products, thus identifying 62% and 79% of black pepper and cumin adulterated, respectively (Lima et al., 2020).

Raman spectroscopy is an optical measurement technique used in the analysis of the inelastic scattered light of the sample material (Xu et al., 2020). The technique is not affected by water and is useful to analyze products inside packaging such as glass or plastic (Oliveira et al., 2019). Gao et al. (2015) developed a biosensor combining surface enhanced Raman spectroscopy (SERS), molecularly imprinted polymers (MIPs), and thin layer chromatography (TLC) to identify and quantify Sudan I dye in adulterated paprika samples. The authors developed a linear regression model relating the concentration to the intensity peak at 721 cm^{-1} . Raman spectroscopy coupled with TLC was applied by Dai et al.(2020), to estimate the adulteration level of saffron with safflower, turmeric powder, and red 40 and yellow 5 dyes, thus showing the potential application of these techniques in the authentication of saffron. Commercial spectroscopy techniques measure samples at a microscopic scale, but they are not capable to measure the entire surface of the sample. For this reason, Dhakal et al. (2018) developed a 1064 nm Raman chemical imaging system to identify adulterants with fluorescing compounds, such as Metanil yellow and Sudan I dye, which are azo compounds harmful to human health. The

adulterant concentration was determined by linear correlation with the number of pixels of each contaminant in the image.

Nuclear magnetic resonance (NMR) spectroscopy is based on the interaction of a magnetic field with the atom nuclei of a sample, which allows detecting components that are not related to the authentic product (Oliveira et al., 2019; Osman et al., 2019). In 2015, ^1H NMR with OPLS-DA was used for the detection of adulterated saffron with plant derived bulking agents, thus showing a successfully model capable of distinguishing the authentic saffron from the adulterated one at a minimum level of 20% (w/w) (Petrakis et al., 2015). This technology was used in 2017 to identify and determine the adulteration of saffron with Sudan I-IV dyes. Each dye was related to a specific proton signal for a quantitative analysis method that, compared with other spectroscopic techniques, allows detecting a lower concentration of Sudan III (Petrakis et al., 2017). ^1H solution and solid-state NMR were applied to identify adulteration with Sudan I dye in paprika, with an accuracy of 98% and 105%, respectively. The range of detection was 6.7 to 128.6 mg/kg, and the limits of quantification were 22.5 to 313.7 mg/kg (Hu et al., 2017).

1.6.2 Aroma and flavor sensors

One of the techniques used for the detection of adulterants in powdered spices is the E-nose technology. This technique is based on an instrument that allows the detection and discrimination of complex odors by using a sensor array (Bhattacharyya et al., 2005). It is composed of three elements: (I) sample handling system, (II) detection system or sensor array, and (III) a data processing system such as classification and prediction models (Bhattacharyya & Bandhopadhyay, 2010). The detection system is a nonspecific sensor, which is usually metal oxides treated with chemical materials. The sensor array is exposed to volatile molecules and a specific smell-print is generated from it. Databases are constructed with patterns of smell-prints from known aromas that are used to train a new pattern recognition system to identify unknown aromas (Shaffer et al., 1998).

An example of the application of this technique is the work by Heidarbeigi et al. (2015), who used an electronic nose system to detect the smell-print of saffron, saffron with yellow styles, safflower, and dyed corn stigma. These patterns along with chemometric analysis were used to developed models to detect adulterated samples of saffron (PCA)

and identify the adulterant (ANN) with a success rate between 86.87% and 100%. In 2017, a combined system based on computer vision and electronic noses were developed for the detection of artificially colored safflower and yellow styles of saffron as adulterants of saffron. Color and aroma features were analyzed with PCA, hierarchical cluster analysis (HCA) and support vectors machines (SVMs) techniques, thus showing a recognition rate between 89% and 100%. The capability of the system was also validated with two multilayer artificial neural networks (ANN-MLP) (Kiani et al., 2017).

Another technique used for the detection of adulterants in powdered spices is the electronic tongue (e-tongue) technology that measures and compares the chemical compounds responsible for taste (Bhattacharyya & Bandhopadhyay, 2010). These taste recognition systems, similar to human tongues, are composed of three elements: (I) the receptor or group of sensors, which will depend on the samples analyzed; (II) the circuit, in charge of quantifying, digitalizing, and recording the results, and (III) the software with the algorithm to process the information (Peris & Escuder-Gilabert, 2016). Each receptor has a spectrum of reactions, and their combination generates a specific signal, which is interpreted by the statistical software and recognized in the taste pattern database (Bhattacharyya & Bandhopadhyay, 2010).

A portable e-tongue was developed for analysis of saffron from Spain and Iran. The system was composed of an array of sensors based on screen printed electrodes (SPE) that identify the characteristic chemical compounds of quality saffron (K. Heidarbeigi et al., 2016). This technology was applied by Kobra Heidarbeigi et al. (2016) for the discrimination of adulterated saffron samples and the identification of adulterants in this product such as yellow styles, safflower, dyed corn stigma, tartrazine and methyl orange. Tahri et al. (2016) developed an electronic sensing system based on e-nose and e-tongue for geographical differentiation and adulterant detection in cumin samples. The study showed an accuracy of 100% of the e-tongue technology for origin classification and adulterated samples identification, while e-nose only work for the second purpose.

1.6.3 DNA Barcoding

There are many analytical approaches to the identification of adulterants in powdered spices and validation of food authenticity (Swetha et al., 2014). Nevertheless, DNA

markers are the most advanced traceability tools, and could be more ideal for adulteration detection in traded commodities of plant origin, especially when the adulterants are biological substances (Dhanya & Sasikumar, 2010). DNA barcoding uses short stretches of standardized gene sequences to identify plant materials. The variability of the gene sequences is analyzed to determine a unique “barcode” for each species (Parvathy et al., 2015). These methods of identification of adulterants are classified into three types based on: polymerase chain reaction (PCR), sequencing, and hybridization. PCR presents a high potential in adulterant detection and authentication of commodities due to its simplicity, sensitivity, specificity, as well as rapid processing time and low cost (Dhanya & Sasikumar, 2010).

A method based on DNA barcoding was developed in 2019 for authenticity of natural powdered spices, using ITS2 and *psbA-trnH* sequences. A total of fourteen out of sixteen types of spices were found to be adulterated with congeneric species, cheaper substitutes, and rice, corn, or wheat flour (M. Zhang et al., 2019). Parvathy et al. (2015) proved the technique with PCR amplification to identify plant-based adulterants in turmeric powder such as wheat, rye, barley, and cassava. Additionally, Bansal et al. (2018) used the technique for validating the authenticity of black cumin (*Kala Zeera*), which is usually adulterated with common cumin (*Cuminum cyminum*).

The European Food Fraud Detection Unit of the Joint Research Center in Geel, Belgium, uses different scientific equipment to detect fraud in samples of spices imported into Europe. Part of this evaluation is the DNA analysis, where the genetic map allows knowing if the spice samples have foreign biological elements. Liquid chromatography and tandem mass spectrometry (LC-MS / MS) are used along with this technique (Rosmino, 2020).

1.6.4 Chromatography

Chromatography separates the components (solutes or analytes) of a sample into a mobile phase (MP) and a stationary phase (SP). According to the physical state of the MP, the technique can be classified into gas chromatography or liquid chromatography, and according to the state of SP, sub-classifications can be made such as gas-solid chromatography (GSC), gas-liquid chromatography (GLC) and some variations of liquid

chromatography (Miller, 2009). These techniques have been widely used in the food industry for quality analysis of products. GC combined with mass spectrometry was used in 2020 to determine authenticity, freshness, and adulterants in saffron through the identification of its aroma. OPLS-DA models were developed with a dataset of volatile adulterants and showed good predictability (Farag et al., 2020). Headspace flash GC with flame ionization detection combined with chemometrics was evaluated to identify adulteration in saffron with turmeric and marigold. Linear discriminant analysis (LDA) and PLS-DA was used to develop models able to identify adulteration in concentrations of less than 30%, which cannot be identified by other methods such as UV-VIS Spectrometry (Morozzi et al., 2019). Liquid chromatography and its variations are the most common techniques for adulterant detection in spices (Galvin-King et al., 2018).

In 2019, high performance liquid chromatography (HPLC) with tandem mass spectroscopy was used to detect corn stigma, chrysanthemum, and safflower as adulterants in saffron, through the identification of the components of allantoin, chlorogenic acid (ChA) and hydroxysafflor yellow A (HSYA) (Kong et al., 2019). Using this technique, along with gel permeation chromatography (GPC), Sudan dyes were detected in Egyptian spices with a limit of quantification of 0.1 mg/kg (Sebaei et al., 2019). Characteristic fingerprints of phenolic compounds were obtained with HPLC-UV to identify adulterated paprika samples. LDA was applied for qualitative discrimination and PLS modelling for quantitative analysis, thus showing the high potential of this technique (Cetó et al., 2020). A reverse phase high performance liquid chromatography (RP-HPLC) was applied by Sahu et al. (2020) to determine adulteration in turmeric with artificial color metanil yellow (MET). The method was found to be robust with an R^2 of 0.99 and a coefficient of variation of 1.68%.

Thin layer chromatography (TLC) is often used for analysis of phytoconstituents of the plant and for plant identification. For this reason, it can be applied as a rapid method for quality control of spices (Danciu et al., 2018). It has also been applied to identify dyes adulteration of saffron in sixteen countries. The study found twenty adulterated samples with magenta and pink dyes, which are rarely found adulterants in this type of spice (Bhooma et al., 2020). Dai et al. (2020) developed a fast screening and quantification method based on TLC combined with Raman spectroscopy for the analysis of saffron

samples and the identification of colorants and adulterants that are not reported in the label. TLC was used to discriminate pure and adulterated samples and their level of adulteration, while Raman spectroscopy through PLS analysis was used to identify the adulterant.

Rani et al. (2015) used high-performance thin layer chromatography (HPTLC) to estimate the adulteration of turmeric and chili powder with Sudan dyes. The method exhibited a R^2 of 0.99 and an accuracy of 98%. Amirvaresi et al. (2020) combined this technique with multivariate image analysis (MIA) for authentication and adulterant detection of Iranian saffron. PCA was used to identify the saffron origin with an accuracy of 92%, then, PLS-DA identified the common plant-derived adulterants of this spice with an accuracy of 98.04%.

1.7 Conclusion

The spices industry has been affected by economically motivated adulteration due to its high commercial value and the vulnerability of the chain supply in the collection, grinding and package stages. The most common adulterants found in spices are plant-related materials, diverse types of starches, inorganic substances, and non-permitted colorants. Although the goal of adulteration is financial gain, it has posed a risk to the consumer's health with the inclusion of allergenic compounds, such as peanut or almond, without the correct specification on the label; or synthetic dyes, mainly Sudan I- IV, which are carcinogenic compounds unauthorized for human consumption.

It is important to develop risk mitigation strategies for the spice industry and manage product recall plans to reduce the impact of food fraud in the brand value and reputation. To effectively control the quality of spices, multiple analytical methods have been developed. These techniques make it possible to differentiate adulterated samples from pure samples and to identify and quantify the adulterants with the application of chemometric tools.

Spectroscopy-based methodologies have the highest number of reported studies due to their high precision and rapid response. These methods include FT-IR, FT-NIR, Raman and NMR spectroscopy. However, other techniques have shown to be reliable in the

authentication of spices, including chromatographic methods such as HPLC, HPTLC and GC-MS; DNA barcoding methods, useful for identification of plant origin and discrimination with closely related species, and sensing systems (e-tongue and e-nose) that have demonstrated to be accurate, although they require further research in the spice industry. Accuracy of the methodologies can be improved with the application of variable selection tools in the development of prediction models.

Adulteration procedures evolve with the advance in technology. For this reason, it is necessary to continue working in efficient control methodologies and expand them to other spice varieties, to reduce the fraud cases and guarantee quality products for the consumer.

2. Espectroscopía NIR como herramienta en la autenticación de alimentos

2.1 Introducción

El fraude alimentario corresponde a la sustitución, adición, manipulación e incorrecta representación de los alimentos, sus ingredientes y empaques, con el objetivo de obtener una ganancia económica, lo que sucede a través de la violación de la normativa y a expensas de los consumidores (European Commission, 2019). Esto puede generar riesgos para la salud y pérdida de la confianza en la marca y el producto (Bouzembrak & Marvin, 2016; Spink & Moyer, 2011). La adulteración de alimentos puede presentarse en diferentes etapas de la cadena de producción: se ha estimado un costo global para la industria de \$49 billones de dólares por año, correspondiente a los procesos de recolección, investigación, pérdida de ventas, entre otros (Oliveira et al., 2019).

En 2008, China reportó 6 muertes y 294.000 casos de niños con cálculos en el tracto urinario, debido al consumo de productos lácteos adulterados con melamina (Gossner et al., 2009); este incidente afectó a los productores de leche que perdieron el producto fresco debido al cierre de producción y el bajo consumo de lácteos (Jia et al., 2012). En 2013, la empresa Findus Nordic fue víctima de fraude cuando en su lasaña congelada se encontró carne de caballo en lugar de carne de vaca, lo que la obligó a invertir en una campaña de recuperación de confianza (Falkheimer & Heide, 2015). En 2015, más de 675 productos fueron retirados del mercado debido a que el comino usado en su preparación contenía trazas de maní y almendras, por lo que las personas sensibles a esos ingredientes podían correr el riesgo de sufrir una reacción alérgica grave o potencialmente mortal (Food and Drugs Administration [FDA], 2015; Garber et al., 2016).

El número de solicitudes de investigación por fraude alimenticio ha aumentado a lo largo de los años, siendo los incumplimientos más comunes los relacionados con adulteración. En el año 2019, el 47% de los reportes correspondieron a una incorrecta representación, debido a que la composición y cantidad en la etiqueta no reflejaban la realidad del alimento, y el 20% a alteraciones de los productos a través de reemplazos, diluciones, adiciones o remociones (European Commission, 2019). El incremento en las tendencias de adulteración y la falta de técnicas eficientes demuestran la necesidad de métodos analíticos rápidos, confiables y competentes para la autenticación de productos alimenticios (Lohumi, Lee, Lee, et al., 2015).

Múltiples técnicas instrumentales han sido estudiadas en los últimos años para el control de calidad de los alimentos. Entre ellas, la espectroscopia NIR que combinada con quimiometría, ha demostrado ser una técnica rápida, precisa, económica y útil en la detección de compuestos no deseados en alimentos (Lima et al., 2020). La quimiometría utiliza métodos matemáticos y estadísticos para establecer correlaciones entre parámetros de calidad o propiedades físicas de una muestra con datos instrumentales analíticos (Lohumi, Lee, Lee, et al., 2015; McGrath et al., 2018). Dado que los datos espectroscópicos se ven afectados por diferentes factores, es necesario aplicar pretratamientos para reducir las interferencias no deseadas sin afectar la información útil, y mejorar la posterior regresión multivariante, modelo de clasificación o análisis exploratorio a aplicar (Rinnan et al., 2009). En este documento se realizará una descripción de las principales características de la espectroscopía vibracional en infrarrojo cercano (NIR) como técnica de análisis composicional, así como de los métodos quimiométricos y su aplicación conjunta en la detección de adulterantes en alimentos.

2.2 Principios básicos de la espectroscopía NIR

Las técnicas espectroscópicas estudian la interacción de la radiación electromagnética con la materia y obtener las características de una muestra. Es un fenómeno cuántico que depende de las propiedades de la radiación y de la estructura de las muestras implicadas (Hofmann, 2010). Cada átomo o molécula de un material tiene un conjunto particular de energía que proporciona un espectro único, basado en la adsorción o emisión de luz a longitudes de onda específicas (Ravindran et al., 2018).

Al irradiar una muestra en las longitudes de onda del infrarrojo cercano (800-2500 nm) es posible predecir la concentración de un compuesto químico midiendo la energía reflejada o transmitida, que es inversamente proporcional a la energía absorbida. La irradiación genera vibraciones que cambian la distribución de la carga y el momento dipolar eléctrico de las moléculas, las principales vibraciones que generan este cambio son el estiramiento y la flexión (Nielsen, 2010).

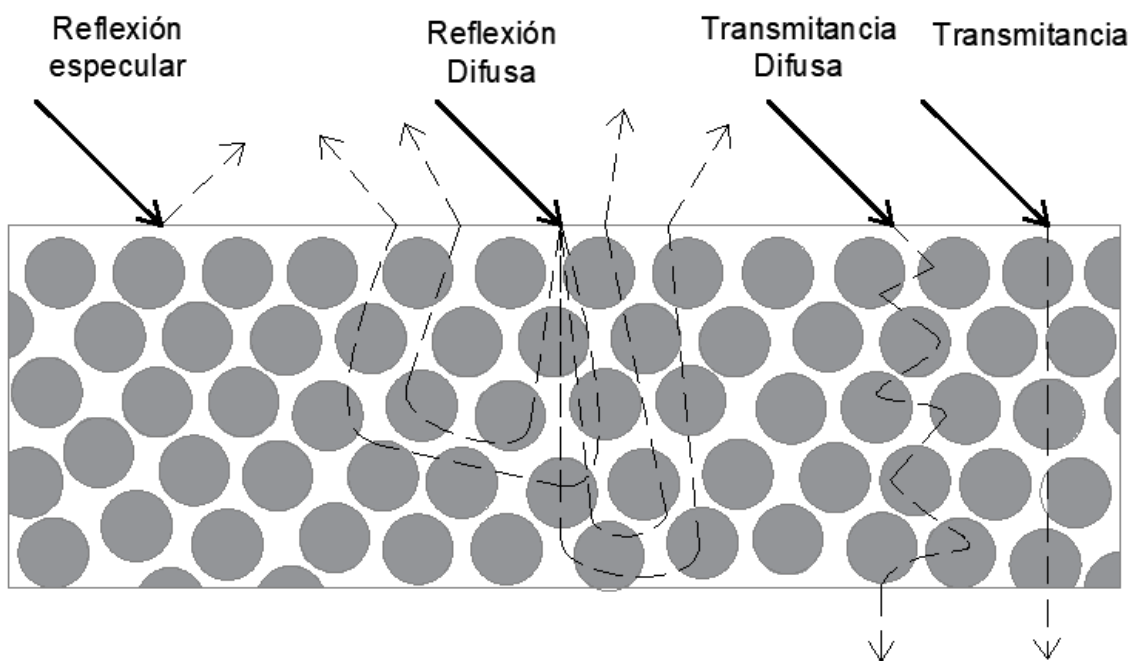
Los espectros obtenidos se observan debido a la excitación de las moléculas de la muestra, lo que genera sobretonos y combinaciones de vibraciones fundamentales (Burns & Ciurczak, 2008). Cuando la frecuencia de la energía irradiada es igual a la frecuencia de vibración del enlace, conocida como frecuencia fundamental, se genera la transición del estado básico al primer estado de excitación formando bandas de alta intensidad conocidas como bandas fundamentales (Aenugu et al., 2011). Adicionalmente, pueden presentarse transiciones a estados de excitación superiores (2 o 3), cuando la frecuencia de la radiación es dos o tres veces la de la frecuencia fundamental, esto genera bandas débiles conocidas como sobretonos. También se pueden presentar combinaciones cuando dos o más vibraciones ocurren de manera simultánea. (Aenugu et al., 2011; Nielsen, 2010)

Estas bandas corresponden a grupos funcionales que tienen enlaces químicos entre átomos de hidrógeno con carbono, nitrógeno u oxígeno, que son los grupos comunes en los principales constituyentes de los alimentos como el agua, las proteínas, los lípidos y los carbohidratos (Arévalo, 2013). Las bandas de absorción en esta región hacen que algunos picos de moléculas diferentes presenten superposición en el espectro, por ello es necesaria la aplicación de quimiometría para procesar los datos y así obtener información correcta sobre las propiedades de las muestras analizadas (Nielsen, 2010).

En la Figura 2-1 se observa la interacción de la luz con la materia. La radiación reflejada se conoce como *reflexión especular* y no proporciona información relevante sobre la muestra. Parte de la radiación que penetra a través de la superficie y refleja las partículas de la muestra hasta salir de ella, esto se conoce como *reflexión difusa*. La radiación es absorbida por los constituyentes químicos de las partículas y por tanto la *reflexión difusa*

contiene la información de la composición química de la muestra lo que se observa en la energía absorbida a longitudes de onda específicas (Nielsen, 2010). En algunos productos se puede aplicar la ley de Beer-Lambert, que relaciona la *transmitancia* con el producto de la concentración de una especie absorbente y una longitud de paso fija. Sin embargo, esta Ley no es aplicable cuando hay dispersión de la luz en la muestra, lo que se conoce como *transmitancia difusa*, cuya medición se realiza en el rango de 800 a 1100 nm, donde se obtiene datos útiles con muestras de 1 a 2 cm de espesor (Osborne, 2006).

Figura 2-1: Interacción de la luz con la materia



Fuente: Elaboración propia

Para garantizar el éxito de la técnica en la autenticación de alimentos es necesario seleccionar el instrumento y modo correcto de medición. La fuente de radiación empleada en los equipos es generalmente una lámpara halógena de tungsteno con envoltura de cuarzo. Los monocromadores se usan para medir el espectro de absorción y pueden utilizarse en transmitancia y reflectancia. Existen detectores de silicón (400-1100 nm) y detectores de sulfuro de plomo (1100-2500 nm); algunos instrumentos contienen uno o ambos detectores, lo que determina su campo de aplicación. Al usar

ambos detectores es posible cubrir el rango de infrarrojo (400 a 2500 nm), en tanto si el instrumento solo posee el detector de sulfuro de plomo, su uso se restringe a la medición de muestras granulares y en polvo por reflexión difusa (Nielsen, 2010; Osborne, 2006).

Los espectrómetros de diodos usan una matriz de emisión infrarroja que funciona como fuente de luz y sistema de selección de onda al mismo tiempo (400-1700 nm), siendo útiles en líneas de producción e inspección. Otros instrumentos utilizan entre seis y veinte filtros para representar la absorción deseada dependiendo su aplicación en la industria, como por ejemplo para la determinación de proteínas, contenido de humedad o contenido de aceite, entre otras. Estos son más simples, económicos y pueden utilizarse en mediciones en línea (Osborne, 2006).

2.3 Análisis de datos en la espectroscopía

Las determinaciones analíticas se basan en la aplicación de la quimiometría. Esta consiste en la aplicación de herramientas estadísticas, matemáticas y gráficas para reconocer patrones y establecer correlaciones entre los espectros infrarrojos de las muestras y las concentraciones obtenidas por métodos de referencia (McGrath et al., 2018). Adicionalmente, usa los planteamientos de optimización de experimentos en el análisis multivariado de los resultados para resolver sistemas químicos (Barros et al., 2010).

2.3.1 Pretratamientos

Antes de realizar el análisis matemático de los datos espectrales y la construcción de un modelo de predicción es necesario que se apliquen pretratamientos para eliminar la información no relevante y mejorar la relación lineal entre la señal espectral y la concentración del analito estudiado (Rinnan et al., 2009). Múltiples fenómenos físicos y químicos pueden causar una desviación en la relación lineal: la dispersión de la luz debido al tamaño de la partícula, interferencias, interacciones moleculares y el cambio de índice de refracción a altas concentraciones (Bjorsvik & Martens, 2007).

Entre los pretratamientos más usados en la espectroscopía vibracional se encuentran: la corrección de dispersión multiplicativa (MSC), el suavizado y la aplicación de derivadas. A continuación, se presenta una descripción de estos pretratamientos:

2.3.2 Corrección de dispersión multiplicativa (MSC)

El método de Corrección de Dispersión Multiplicativa (Multiplicative scatter correction – MSC) se utiliza para corregir los efectos aditivos, debido a los cambios en la línea base del espectro y los efectos multiplicativos, por la inclinación de la línea base. Consiste en la linealización de cada espectro a un espectro ideal, que teóricamente representa el promedio del espectro de las muestras. De esta forma es posible remover los efectos de dispersión debidos al tamaño de partícula y el índice de refracción (Figura 2-2b) (Wang et al., 2015).

2.3.3 Suavizado (Smoothing)

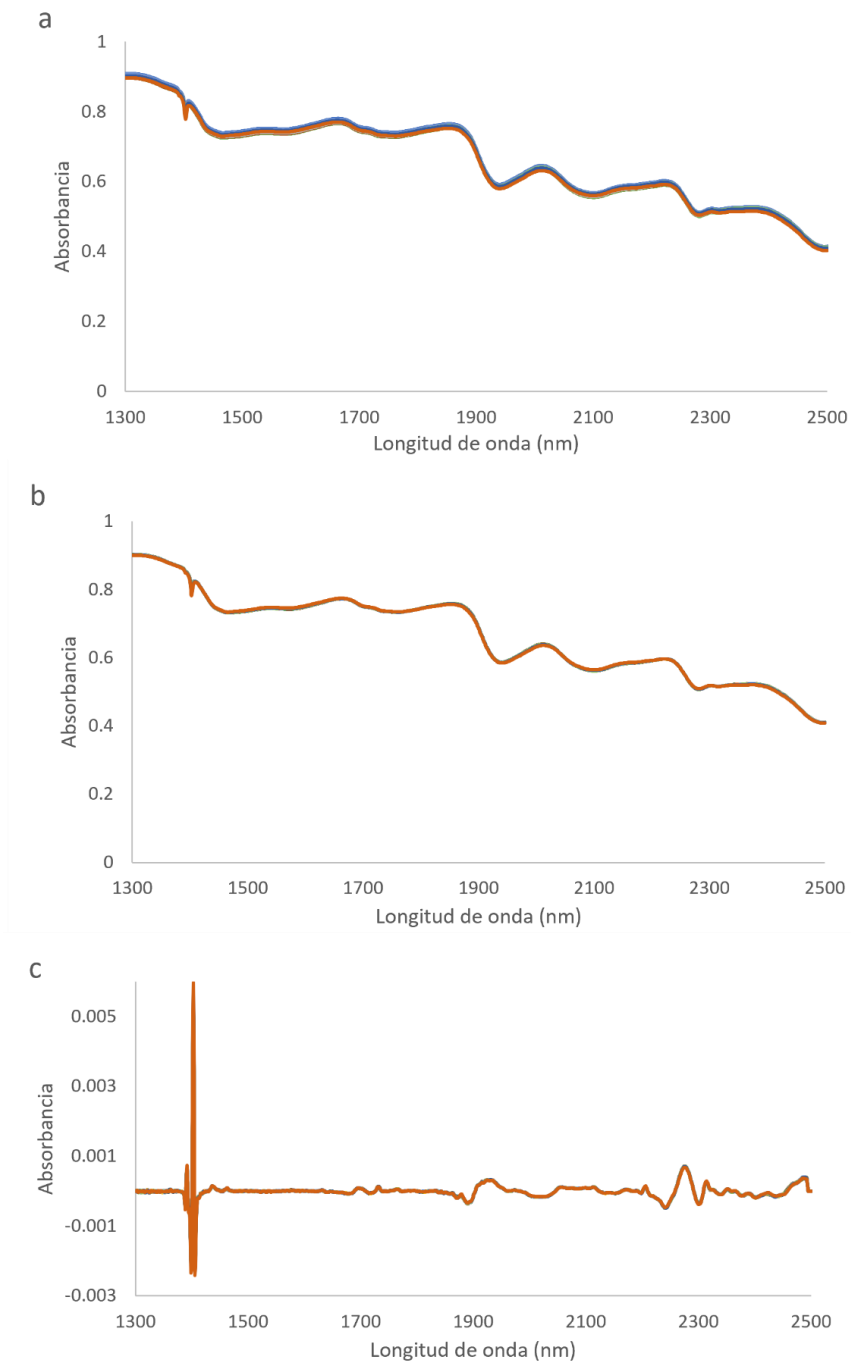
Esta técnica busca remover el ruido de alta frecuencia del espectro, mejorando la relación ruido-síñal. Existen variaciones en su aplicación, pero la más utilizada es el suavizado de Savitzky-Golay (Wang et al., 2015). Esta rutina consiste en filtrar los datos a través de ventanas que contienen $2g+1$ puntos (siendo g el número de puntos de cada lado) y ajustar estos datos a un polinomio de cierto orden. En ocasiones el ruido se acentúa con ventanas muy grandes, lo que conlleva a la pérdida de información importante. Se ha demostrado que 7 a 11 puntos y polinomios de segundo o cuarto grado tienen un mayor impacto en la relación señal-ruido (Rinnan et al., 2009).

2.3.4 Derivadas

Las derivadas se usan para remover o suprimir los efectos aditivos y multiplicativos en el espectro. Su efecto correctivo es fácil de entender gráficamente ya que cada derivada reduce el orden del polinomio, por lo tanto, elimina un desplazamiento constante. La segunda derivada transfiere los picos máximos en mínimos y viceversa, siendo particularmente útil para identificar picos que no son visibles en el espectro original (Figura 2-2c). Como en el suavizado, la rutina de Savitzky-Golay también puede ser usada para calcular derivadas con buenos resultados. Los métodos derivados reducen la sensibilidad multivariante, lo que da lugar a una mayor incertidumbre para la estimación

de la regresión y, en consecuencia, se generan errores de predicción altos (Rinnan et al., 2009; Siesler et al., 2006).

Figura 2-2: Espectro NIR a) Original. b) Preprocesado MSC. c) Preprocesado 2° derivada



Fuente: Elaboración propia

En la Figura 2-2, se observa el efecto de los pretratamientos en un espectro en infrarrojo de un grupo de muestras (Figura 2-2a). A través de la aplicación de la corrección de dispersión multiplicativa (MSC) se observa una reducción en el efecto aditivo y multiplicativo (Figure 2-2b) y a través de la aplicación de la segunda derivada se resaltan los picos en las longitudes de onda que contienen la información sobre la composición de la muestra analizada (Figure 2-2c).

2.3.5 Métodos de Regresión Multivariante

En el desarrollo de modelos de predicción es necesario el uso de técnicas de regresión multivariable, que permitan obtener una relación lineal entre los datos espectrales, conocidos como predictores y la variable dependiente o respuesta.

A continuación, se presentará una descripción de los procesos de regresión:

2.3.6 Regresión de Componentes Principales (PCR)

En este método se selecciona un número de componentes principales a partir de un análisis PCA (Principal Component Analysis) que posteriormente se utilizan como predictores en lugar de los datos espectrales originales (Wang et al., 2015). Esta técnica consiste en la transformación de una matriz de datos, a través de la construcción de un nuevo sistema de ejes conocidos como componentes principales, para que la naturaleza multivariada de los datos pueda representarse en pocas dimensiones. Este algoritmo está basado en la correlación entre las variables, agrupando aquellas que se encuentren altamente correlacionadas. Las nuevas variables se obtienen en orden descendente de la información estadística que describen, por lo tanto, cada componente principal describe un porcentaje de la varianza total de los datos (Ferreira, 2013; Morgano, 2005). Para el desarrollo del modelo de regresión se utilizan los componentes que representan al menos el 80% de la varianza (Artigue & Smith, 2019).

2.3.7 Regresión de Mínimos Cuadrados Parciales (PLSR)

La regresión por mínimos cuadrados parciales (Partial least squares regression – PLSR) busca una covarianza máxima entre la matriz de espectros X y el vector de respuesta Y,

de manera que se pueda obtener una relación lineal en la fase de calibración. Las combinaciones lineales de los datos espectrales (Matriz X) y de referencia (Matriz Y) generan el número de variables latentes necesarias para correlacionar espectros y concentraciones. La serie de variables latentes que producen el error de predicción más pequeño posible se usa para la creación del modelo (Morgano, 2005).

Se debe seleccionar una serie de variables latentes con información suficiente para modelar el sistema, de manera que no se sobreajuste y tampoco modele ruido. Usar pocas variables puede excluir información importante y usar muchas podría representar el ruido de los datos. Para hallar el valor adecuado de variable latentes, se utiliza la validación cruzada (Burns & Ciurczak, 2008; Morgano, 2005).

2.3.8 Validación del modelo

Para garantizar que el modelo funciona correctamente se utilizan dos métodos de validación: cruzada y externa. La validación cruzada consiste en dejar parte de la muestra fuera del *set* de calibración y predecir su concentración, repitiendo el proceso hasta que cada muestra ha hecho parte del proceso de calibración. Posteriormente, se calcula el coeficiente de determinación R^2 , que mide la proporción de la varianza de los datos predichos con los datos de referencia (Berrar, 2018).

Por otro lado, la validación externa se realiza usualmente con el 30% de las muestras totales diferentes a las de calibración. El modelo hace la predicción de la concentración en cada muestra y luego las compara con la referencia, calculando el error entre ellas como la raíz cuadrada de la varianza RMSE (Naes et al., 2004). El mejor modelo será aquel que proporcione un valor de R^2 más cercano a 1 y un RMSE más bajo (Ferreira, 2013).

2.4 Aplicaciones

En la industria alimentaria se puede presentar adulteración accidental como resultado de la negligencia, ignorancia o instalaciones de procesamiento inadecuadas (Garber et al., 2016); o adulteración intencional cuando se adicionan compuestos de bajo costo para obtener una mayor ganancia (Ravindran et al., 2018). El continuo desarrollo de

instrumentos y softwares de análisis ha hecho que la espectroscopia NIR sea considerada como una técnica confiable, eficiente y estándar para el control de calidad de un amplio rango de productos (Lohumi, Lee, Lee, et al., 2015).

En productos lácteos esta técnica se ha utilizado para detectar y cuantificar la presencia de melanina, sucrosa, proteína de soya e incluso urea en leche en polvo (Mazivila et al., 2020; Jeffrey Moore, Ganguly, et al., 2012). La técnica combinada con un algoritmo PLS se aplicó en la identificación y cuantificación de la adulteración de leche de cabra con leche de vaca, determinando simultáneamente los contenidos de grasa y proteína (Pereira et al., 2020). Temizkan et al. (2020) utilizaron esta técnica para el desarrollo de un modelo de regresión PLS que detectó la adulteración de grasa láctea en yogurt.

Dado que las especias han sido productos vulnerables a la adulteración se ha estudiado la aplicación de esta técnica para su autenticación. Lohumi et al. (2014) concluyeron que la generación de modelos con la espectroscopía NIR permite detectar la presencia de almidón en cebolla en polvo debido a su capacidad de absorción de la luz. En 2019, se desarrolló un modelo con análisis discriminante (PLS-DA) para la identificación rápida de pimienta negra adulterada con diferentes compuestos (Wilde et al., 2019). Un modelo de clasificación similar se utilizó en canela, para diferenciar el producto de una variedad más barata (*Cinnamon Casia*) que contiene un alto contenido de 1,2 benzopirona que afecta la salud del consumidor (Cantarelli et al., 2020).

Adicionalmente, se ha usado la técnica en la autenticación de productos cárnicos, con el desarrollo de métodos con PLS-DA para la clasificación de pollo fresco y congelado (Parastar et al., 2020). Para la detección y cuantificación de carne de cerdo y pato en otras muestras de carne, fueron desarrollados modelos PLS-DA para la discriminación de carne pura y adulterada y regresiones PLSR optimadas con selección de variables para la predicción del contenido de carne de cerdo y pato (Leng et al., 2020; Mabood et al., 2020).

Aún se presentan algunas restricciones para el uso industrial de estas técnicas, pero es probable que la creciente demanda de análisis de calidad y autenticidad de alimentos, y la investigación en curso sobre el desarrollo de métodos espectroscópicos, promuevan su implementación en la industria alimentaria (Lohumi, Lee, Lee, et al., 2015).

2.5 Conclusiones

Es evidente que las cadenas de producción de alimentos son susceptibles a la adulteración accidental o intencional, lo que conlleva graves afectaciones de salud para los consumidores y pérdidas económicas tanto para la marca como para los productores primarios. Esto hace que sea necesario contar con métodos rápidos y confiables para el control de calidad y la autenticación de alimentos.

La espectroscopía vibracional en infrarrojo cercano (NIR) ha demostrado ser una técnica analítica confiable y eficiente en la detección y cuantificación de compuestos no deseados en diferentes productos. Dada la complejidad de los espectros, es necesario aplicar diferentes técnicas de análisis quimiométrico para generar modelos de predicción y clasificación, siendo de gran importancia el pretratamiento de los datos para garantizar el éxito del método.

La aplicación industrial de esta técnica es limitada; por lo que es necesario continuar su investigación para que pueda ser implementada en las diferentes cadenas de producción agroalimentarias.

3. Development of models for the identification of adulterants in garlic powder based on near infrared spectroscopy.

3.1 Abstract

Food fraud is a global problem that causes unfair competition and negative effects not only on the nutritional quality of a product, but also on the consumers trust, health, and safety. Therefore, it is necessary to optimize the process of identification of foreign agents in food. Garlic powder is susceptible to adulteration with cornstarch and chalk due to its physical characteristics, which makes it difficult to identify by organoleptic procedures. In addition, the actual methods for detecting adulterants are destructive, time-consuming, and expensive. For this reason, it is necessary to appeal to alternative techniques such as near infrared spectroscopy, which has demonstrated satisfactory results in the agri-food industry. The objective of this study was to develop models based on this technology for the identification and quantification of cornstarch and chalk in garlic powder. A 3-class partial least squares regression model with discriminant analysis was developed to identify the type of adulterant on the samples. Subsequently, partial least squares regression models were developed for the quantification of each adulterant. The selected classification and quantification models were tested with commercial samples and the results indicated that it is possible to use spectral prediction models for the authentication of garlic powder.

Key words: quality, food safety, spices, food fraud, authenticity,

3.2 Introduction

One of the most common types of food fraud is food adulteration, which is the deliberate addition or subtraction of foreign substances to the product, in order to increase its volume and therefore its profit (Spink & Moyer, 2011). These sorts of actions have an impact on consumer health generating different symptoms such as diarrhea, nausea, and vomiting, among others, and issues in the gastrointestinal tract, liver, skin, and lungs (Galvin-King et al., 2018). The level of harm depends on the type of adulterant and the frequency of consumption. An adulterant may be considered as not harmful when it is for human consumption, as in the case of starch, however, most adulterants are unhealthy and can generate serious risks if ingested regularly. Some damage may be immediate, as in the case of toxic adulterants, others may be reflected in the long term by causing chronic effects, or there may be cases of allergies whose effect is independent of the amount ingested (Spink & Moyer, 2011). In addition, there is an impact on the economics of the agri-food industry, as one fraud report can cost up to \$49 billion to a company, due to the loss of products and sales, product recalls, research, price drops and image recovery processes, among others (PwC & SSAFE, 2016). These losses do not consider the impact on primary producers, who lose their production when sales are reduced due to the loss of consumer confidence, as in the case of dairy farmers when the melamine scandal in 2008 reduced the consumption of dairy products and stopped its production (Jia et al., 2012).

In recent decades, there has been an increase in the global production of spices with 2770000 tons produced in 2019 and Asia was the largest producer accounting for 86% of total production (FAOSTAT, 2019b). This increase is due to the multiple medicinal and cosmetic properties they can offer, in addition to the growing trend of consumers in the search of new flavors and ways to preserve, color or highlight the taste of their dishes (Carmona, 2013). Due to the high consumption of this product, since ancient times adulteration of spices has been present by adding other elements to the product to increase the production weight, with the main purpose of obtaining a higher profit (M. Zhang et al., 2019). The spice production chain is moderately vulnerable to fraud as there is a substantial risk of adulteration at each stage due to easy access, motivation and few control measures (Silvis et al., 2017). A study of seventy-five spice samples from different markets reported that 24% of them presented one or more adulterants and three out of

five spice samples were found to be adulterated with substances potentially harmful to health (Black et al., 2016). This is reflected on food fraud reports, where spices appear as the sixth group with most complaints with a total of 1136 tons seized in 2019 (European Commission, 2019; EUROPOL & INTERPOL, 2019).

Garlic (*Allium sativum* L.) is a widely used ingredient in many countries and cultures, not only for its characteristic flavor and culinary properties, but also for its potential health benefits, which have been reflected on the therapeutic and medicinal solutions in both traditional and modern medicine (Netzel, 2020; Singh et al., 2019). This product is attributed with numerous properties such as antioxidant, anti-inflammatory, antimicrobial, immune system modulator, cardiovascular system protector, anticancer, digestive system protector, antidiabetic, neuroprotective and renal protector (Navarro, 2007; Netzel, 2020; Ramírez et al., 2016; Tapsell et al., 2006). It is found in the world market in different presentations as a raw vegetable (fresh leaves or dried cloves) or after processing, as garlic oil, garlic extract and garlic powder. It is recognized as a valuable spice whose composition may vary depending on the type of processing (Singh et al., 2019).

Few studies have been conducted on adulterants in this spice. However, garlic powder is considered to be vulnerable to adulteration as it is easy to imitate. Different materials could be used in place of garlic such as cornstarch, which has been found to be in concentrations of up to 35%, and even inedible compounds such as chalk or talc in concentrations close to 23% have been recorded (Osman et al., 2019; Perkin Elmer, 2018). Cornstarch is a carbohydrate of high consumption in the world since it is found as a natural reserve in corn with an exceptionally low price (Zhu, 2018). It is one of the most commonly found adulterants in spices, because it has similar physical properties such as granulometry, it is easily accessible, it has no characteristic odor or flavor and its presence is difficult to distinguish visually since color variations are imperceptible with spices such as garlic or onion (Lee et al., 2015; Lohumi et al., 2014; Osman et al., 2019; Su & Sun, 2018; Sun, 2012). Chalk is obtained by pulverizing a non-clastic carbonate sedimentary rock (Fried & Mcdonnell, 2000). It has a white to grayish in color and the purest varieties contain up to 99% calcium carbonate with small amounts of silica, glauconite clay minerals and calcium phosphates. Studies conducted on commercially found chalk powders show that sulfur, calcium, and carbon are the main components,

although low amounts of magnesium have also been found (Y. Zhang et al., 2015). As an adulterant, this product has been found in garlic powder and saffron with a concentration of 23.2% and 2.5%, respectively (Perkin Elmer, 2018). Its presence has been shown to generate a significant impact on the health of consumers from coughing, digestive problems, fever, drowsiness, and irritability (Martínez, J.a et al., 2008) to the development of cancer, when consumed in large quantities (Attrey, 2017).

Determining the authenticity of spices requires comprehensive monitoring with efficient analytical methods. Multiple safety associations have recommended some methods to detect adulterants in food which are based on density, physical properties, microscopic probing, and chemical analysis (Lohumi, Lee, Lee, et al., 2015). These methods require the use of some reagents such as chloral hydrate, ferric acetate, potassium iodide, potassium chlorate, among others, which lead to increased contamination due to subsequent waste, in addition to involving a large cost for handling (AOAC International, 2012a) and being time-consuming. In consequence, there are no continuous inspections of the spice supply chains (Chaminda Bandara et al., 2019). Due to the growing trend in adulteration of spices, multiple studies have been conducted on rapid, reliable, and competent methods for authentication and assurance of products, so that there is no loss of sample during testing.

Near Infrared (NIR) spectroscopy combined with chemometrics has been widely accepted in the agri-food industry as a useful technique for the detection of undesirable compounds in food, since it is an environmentally friendly, non-invasive analytical method with reliable, fast, accurate, safe, and low-cost results (Jamshidi, 2020; Lima et al., 2020). The feasibility of the technique was shown in a study of 2019, where PLS model with discriminant analysis (PLS-DA) was developed for rapid identification of black pepper adulterated with different compounds (Wilde et al., 2019). A similar classification model was used in cinnamon to differentiate the product from a cheaper variety that has a high content of the health-affecting compound 1,2-benzopyrone (Cantarelli et al., 2020). The aim of this research was to show the application of near infrared spectroscopy (NIR) coupled with chemometric techniques in the identification and quantification of adulterants such as starch and chalk in garlic powder.

3.3 Materials and methods

3.3.1 Samples preparation

Dehydrated garlic powder (*Allium sativum* L) from China (Condimentos “Don Memo,” 100% purity) was purchased from the main distributor of the commercial brands of this product in the city. Corn starch and white chalk were purchased from a local distributor with 100% purity. For model calibration, 500 garlic powder samples were prepared: 20 unadulterated samples, 240 samples with corn starch and 240 garlic powder samples with white chalk as adulterants at randomly selected concentrations: 0.2%, 0.5%, 3%, 5%, 7%, 7%, 10%, 12.5%, 15%, 20%, 22.8%, 25% and 30%. For each concentration, 20 replicates of 40 g each were made. At the same time, 126 independent samples with known concentrations between 0% and 30% (w/w) of both adulterants were prepared and used in the validation process. In this study, garlic samples adulterated at the same time with corn starch and white chalk were not prepared. Prior to spectral measurements, samples were stored in airtight bags at room temperature and in a desiccator to prevent them from absorbing moisture from the environment.

3.3.2 Garlic powder analysis

The characterization of the chemical composition and quality of garlic powder was conducted at the Food Science and Technology Laboratory (ICTA). The compositional analysis was performed with standard procedures of the Association of Analytical Communities (AOAC): Moisture was determined with method AOAC 934.06 of Loss on drying (moisture) in dried fruit, in which the sample was dried for six hours at $70\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ under pressure of 13.3 kPa., crude protein was determined with method AOAC 920.176 (Kjeldahl Method): mercury oxide, potassium sulfate and sulfuric acid were used for digestion, then the ammonia obtained by the addition of NaOH was distilled off and titrate to obtained the total nitrogen content. Then crude protein was determinate using a conversion factor of 6.25 (AOAC International, 2012b). The amount of ash was determined with method AOAC 900.02, by carbonizing the sample at $525\text{ }^{\circ}\text{C}$, and the fat content was determined with method AOAC 920.177, in which fat was extracted with

diethyl ether to obtain the crude fat through the evaporation of the ether (AOAC International, 2012b).

The following methods were used in the microbiological analysis: The most probable number (MPN) of total coliforms was determined using ISO 9308 method, which was based on membrane filtration, culture in chromogenic coliform agar medium and calculation of the number of organisms (International Organization for Standardization [ISO], 2012). For the detection of salmonella the ISO 6579 method was used (International Organization for Standardization [ISO], 2017), the plate count of molds and yeasts was carried out using ISO 7954 method by incubating plates at 25 °C for 5 days and counting the number of colonies obtained per gram (International Organization for Standardization [ISO], 1987), and the foreign matter was determined using the *Norma Técnica Colombiana* (NTC) 2555, through mechanical separation of foreign matter from the sample (ICONTEC, 1989).

3.3.3 Spectral data

Near infrared spectra were obtained with a NIRFlex N-500 spectrometer (Buchi, Switzerland) with 8 cm⁻¹ spectral resolution and 0.2 cm⁻¹ wavenumber accuracy, measuring in the NIR region (1000-2500 nm/10000-4000 cm⁻¹) with a halogen light source. Spectral data was obtained through diffuse reflectance (R), which is the best measurement method for solid products. The signals were then transformed to absorbance using log 1/R. Each sample was poured and distributed in a 100 x 20 mm STERIPLAN Petri dish, soda-lime-glass (DURAN), then, the dish was placed in the equipment to perform the corresponding measurement. Spectra were recorded at room temperature and sixty-four scans were performed for each spectrum.

3.3.4 Chemometric analysis of spectral data

Since near-infrared spectral measurements may have noise and light scattering effects due to instrumental drift or particle size, it is necessary to apply pretreatments to the spectra to remove non-relevant information and improve the linear relationship between spectral signal and concentration (Rinnan et al., 2009). For this purpose, mathematical

techniques such as Standard Normal Variate (SNV), Multiple Scattering Correlation (MSC) and 1st and 2nd Savitzky-Golay derivatives were applied. On the one hand, scattering correction methods (SNV and MSC) remove high-frequency noise, reduce multiplicative and additive effects of dispersion and particle size, and reduce differences in overall signal intensities. Derivatives, on the other hand, correct the spectrum by removing trends and highlighting peaks of interest (Siesler et al., 2006).

Principal component analysis (PCA) is an unsupervised method that can serve as an indicator of the ability of spectral data to classify adulterated samples from unadulterated samples. It is a statistical technique that reduces the dimensionality of linearly correlated multivariate data, through an orthogonal transformation, into a set of nonlinearly correlated variables called principal components (S Kar et al., 2019; Lima et al., 2020).

Partial least squares (PLS) regression is the most popular multivariate tool for developing a calibration model that relates the information of interest in a sample to its spectral response (Chen et al., 2018). Partial least squares discriminant analysis (PLS-DA) was used to detect adulteration and identify the adulterant, while partial least squares regression (PLSR) was used for quantitative model building. A PLSR model seeks the maximum covariance between the independent variables in the matrix X and the response vector Y , so that a linear relationship can be obtained in the calibration phase. The linear combinations of the spectroscopic data (X matrix) and reference data (Y matrix) generate the necessary number of latent variables to correlate the two matrices (Morgano, 2005). In PLS-DA, the vector Y corresponds to a numerical code assigned to each class with values of -1, 0 and 1 to identify samples adulterated with corn starch, pure garlic samples and samples adulterated with white chalk, respectively. In PLSR, the vector Y corresponds to the adulterant concentration of each sample. This multivariate tool uses the equation (3-1) (Zossi et al., 2010):

$$Y = \beta_0 + \beta_{\lambda_1}X_{\lambda_1} + \dots + \beta_{\lambda_n}X_{\lambda_n} \quad (3-1)$$

where Y is the variable to be predicted, β_0 is the ordinate of origin, β_{λ_1} to β_{λ_n} are the regression coefficients for each wavelength and X_{λ_1} to X_{λ_n} are the absorbance values. It

is important to select the correct number of latent variables to include in the regression model, as it should have enough information to avoid over-fit and model noise. Usually, a larger number of latent variables is more appropriate, however, it may produce modeling noise. In contrast, using too few latent variables may exclude valuable information. To find the appropriate value of latent variables, cross-validation was used (Burns & Ciurczak, 2008).

External validation of the model was executed with the set of 126 samples considering the root mean square error (RMSE) between the values obtained through the calibration model and the reference data with known adulterant concentrations. All the processes mentioned above were performed with Unscrambler X 10.4 software (CAMO Software AS, Oslo, Norway). The statistical parameters considered to select the model were the coefficient of determination (R^2) and the root mean square error (RMSE) of the calibration and validation data.

To evaluate the PLS-DA classification model, the following values were determined: true positives (TP), corresponding to samples correctly classified within a category; true negatives (TN), corresponding to samples correctly classified outside a category; false positives (FP), corresponding to samples incorrectly classified within a category; and false negatives (FN), corresponding to samples incorrectly classified as outside a category. Taking these values into account, the parameters of Precision (2), Sensitivity (3), Error Rate (4), Accuracy (5) and Specificity (6) were calculated using the following equations (Monago-Maraña et al., 2021):

$$Precision = \frac{TP}{TP+FP} \quad (2)$$

$$Sensitivity = \frac{TP}{TP+FN} \quad (3)$$

$$Error\ rate = \frac{FN+FP}{TP+TN+FP+FN} \quad (4)$$

$$Accuracy = \frac{TP+TN}{TP+TN+FP+FN} \quad (5)$$

$$\text{Specificity: } \frac{TP+TN}{TN+FP} \text{ (6)}$$

3.3.5 Commercial samples analysis

In order to use the models developed with commercial products, thirty-five samples of seven commercial brands of garlic powder were purchased in the local market, five samples per brand. First the PLS-DA model was applied to identify adulterated samples and the type of adulterant, then the PLS model was applied to quantify adulteration in the adulterated samples.

3.4 Results and discussion

3.4.1 Garlic powder analysis

The compositional analysis of the garlic powder used in this research indicated that the moisture was of 5.93%, with a protein content of 15.7%, ash content of 4.85% and total fat content of 0.61%. Microbiological analysis showed less than 3 Most Probable Number (MPN) of fecal coliforms per gram, no presence of salmonella, less than 10 Colony Forming Units (CFU) of mold and yeast per gram, and 0.3% foreign matter. According to the *Norma Técnica Colombiana* (NTC) 4423 of the food industry spices and condiments, this product met the Colombian regulations since it has less than 7% moisture, less than 5.5% ash and less than 0.5% insoluble ash. In addition, it is considered an excellent quality spice because it has less than 11 NMP/g and less than 5000 UFG/g (ICONTEC, 1998).

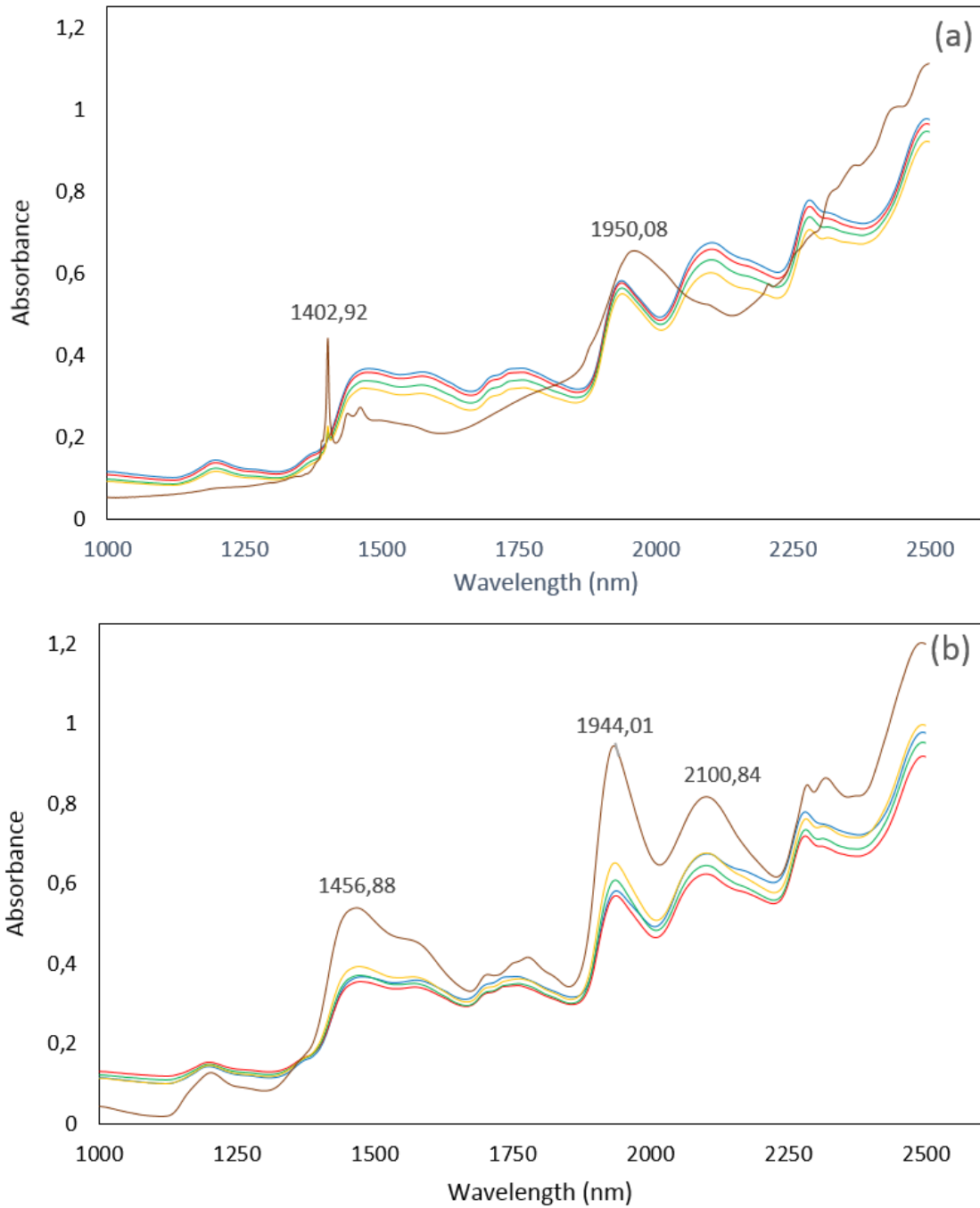
3.4.2 NIR spectra of garlic powder and adulterants

The spectra presented in Figure 3-1 correspond to the average absorbance of different concentration ranges and type of adulterant used in garlic powder. On the one hand, Figure 3-1(a) shows that the pure spice has a higher absorbance than white chalk in most of the range, which can be useful for the visualization of the addition of this adulterant in garlic powder, since it reduces the absorbance of the sample. However, specific peaks were identified at approximately 1402 nm and 1950 nm, whose intensity increases with

the amount of chalk. This corresponds to the C-H combination and the second overtone region of the C-O stretch, respectively (Aenugu et al., 2011). Lang et al. (2014) also identified these peaks in their study of the application of FT-IR NIR Spectrometry for the identification of talc in garlic powder, which may indicate that talc is part of the composition of the chalk used in this research, in addition to calcium carbonate or gypsum which are the most common components in commercial chalk (Goel et al., 2015; Y. Zhang et al., 2015).

On the other hand, Figure 3-1(b) shows that the spectrum of corn starch has a higher absorbance than the spectrum of pure garlic powder, presenting an opposite effect to that of the chalk, because a decrease in absorbance of garlic adulterated with corn starch is visualized. In this case, there are three peaks that allow the identification of this adulterant around 1456 nm, 1944 nm, and 2100 nm. This is consistent with data reported in the literature, since it has been found that the regions near 1450 nm, 1930 nm and 2100 nm correspond to the first overtone of O-H stretch, combination of O-H and H-OH deformation, and C-H stretch combination, respectively (Aenugu et al., 2011). O-H bonds represent starch, which allows considering these bands as representative of this adulterant in garlic powder samples (S Kar et al., 2019; Lohumi et al., 2014).

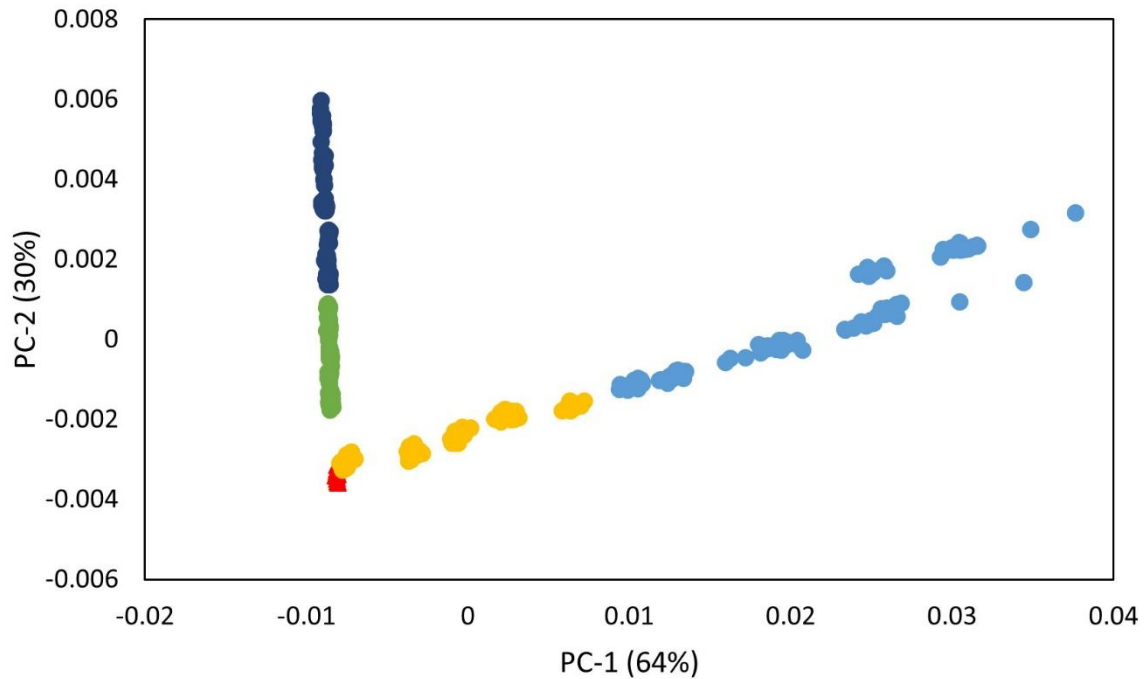
Figure 3-1: Mean absorbance spectrum of garlic powder and adulterated samples with white chalk (a) and cornstarch (b). Blue line: pure garlic powder; Red line: 0-10% adulterant; Green line: 11-20% adulterant; Orange line: 21-30% adulterant; and Brown line: pure adulterant.



3.4.3 Exploratory analysis

Figure 3-2 shows the principal component analysis (PCA) scores of the spectral data, previously pretreated with SNV and first derivative; only two principal components were necessary to explain 95.9% of the total variability in the data. It is observed the formation of groups according to the level of adulteration: 0-10% of adulterant and 11-30% of adulterant, it is clearly differentiated the samples of garlic adulterated with chalk from the samples of garlic adulterated with corn starch. However, pure garlic samples are not remarkably close to the samples with 0-10% chalk as adulterant. This classification can be improved during model development with the application of pretreatments that reduce noise in the spectroscopic signal.

Figure 3-2: PCA score plots of garlic powder. Red mark: Pure; Yellow: adulterated with chalk (0-10%); Light blue: adulterated with chalk (11-30%); Green: adulterated with corn starch (0-10%) and Blue: adulterated with corn starch (11-30%)



3.4.4 Multivariate classification model

For adulterant identification in garlic samples, a PLS-DA was developed. The model with the best performance was obtained with the complete spectrum range, a calibration set of 480 samples, 126 samples in the validation set and spectral data pre-processed with SNV and second-order second derivative with a window of six points. In this three-class PLS-DA model, the first five factors or latent variables explain 95.77% of the variance and eight factors were selected to explain 97.77% of the variance. The most negative values represent samples adulterated with cornstarch, while the most positive values are related to the presence of white chalk, and values near to zero identify unadulterated garlic samples. The model R² was 0.9777 and the prediction R² based on cross-validation was 0.9626 indicating good calibration and predictive ability. It correctly classified 60% of the total samples: 100% of the pure garlic powder samples, 56% of the samples adulterated with corn starch and 61% of the samples adulterated with white chalk. The samples that were not correctly classified correspond to the samples with the lowest concentration of adulterant.

Table 3.1 shows the model performance parameters for the adulterants white chalk and corn starch. Error rates were low for calibration and cross-validation but increased with external validation. The model showed higher accuracy for classification of samples with white chalk compared to classification of starch samples. For sensitivity and specificity, values close to one were obtained, showing a good ability of the model to classify samples in the correct category (white chalk or corn starch), indicating low false positives.

Table 3-1. Validation parameters of the classification model for each adulterant

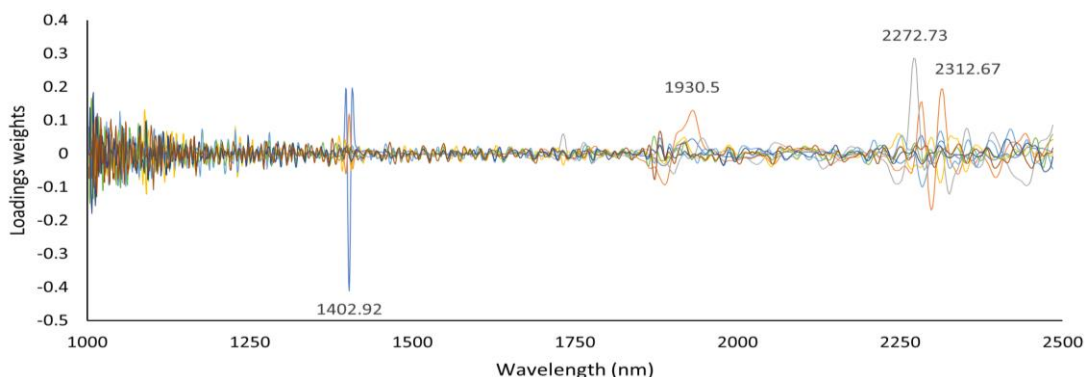
Parameter	White chalk			Corn starch		
	Cal	Val	Pre	Cal	Val	Pre
Precision	0.982	1.000	0.978	1.000	0.750	1.000
Sensitivity	0.991	0.460	0.982	0.996	0.393	0.975
Error rate	0.013	0.270	0.019	0.002	0.357	0.013
Accuracy	0.988	0.730	0.981	0.998	0.643	0.988
Specificity	0.984	1.000	0.981	1.000	0.877	1.000

Cal: Calibration, Val: Cross – validation, Pre: Prediction

From the loadings (Figure 3-3), it is identified that the important variables contributing to the response of the samples adulterated with white chalk are related to bands at 1402.92 nm, while for cornstarch the important variables were represented by 1930.5 nm, 2272.73 nm, and 2312.67 nm which corresponds to what has been reported in the literature as the O-H bonds related to starch are found around 1930 nm, 2280 nm, and 2322 nm (Aenugu et al., 2011; S Kar et al., 2019; Lohumi et al., 2014; Nallan et al., 2019).

PLS-DA has been previously used for the classification of adulterated and unadulterated samples in different spices. Petrakis & Polissiou (2017) applied a PLS-DA model that correctly classified 99% of saffron samples adulterated with different compounds. Cantarelli et al. (2020) developed a PLS-DA model based on diffuse reflectance NIR spectroscopy, showing 99.25% correct discrimination of cinnamon samples adulterated with a lower value spice (*C. cassia*). Lima et al. (2020) applied a PLS-DA model based on NIR spectroscopy to differentiate adulterated black pepper and cumin samples. This model showed a good ability to discriminate adulterated samples; however, it had a low ability to correctly classify pure samples, which was also observed in the model developed in this work. Additionally, a classification model for adulterated paprika with spent paprika using NIR spectroscopy showed good classification capacity of samples with more than 40% adulteration and 100% identification of pure paprika (Galvin-King et al., 2020). Galvin-King et al. (2021) also developed a classification model using NIR and FTIR for garlic powder adulterated with different adulterants, showing a good capability for authentic garlic detection in the range of 20-90%. The results obtained in this work and those reported by other authors show that PLS-DA is a supervised technique with good ability to differentiate between adulterated and pure samples. In this case it was also demonstrated that it is possible to identify the type of adulterant in the sample, which could be useful in the quality control of this product and identify possible adverse health effects.

Figure 3-3: Loadings of important variables for the PLS-DA model



3.4.5 Multivariate quantification model

Quantitative calibration models for each adulterant were generated with PLS regression by applying different pre-processing techniques, as shown in Table 3-2. Models were selected based on high values of the correlation coefficient for calibration (R^2), and low values of the root mean square error for calibration (RMSEC), and root mean square error of cross-validation (RMSEV) and prediction (RMSEP). The calibration models showed good correlation values (R^2) between 0.978 and 0.990 depending on the pretreatment applied and variations in RMSEP values were of 0.649% - 1.576%, also depending on the pretreatment applied.

The best model for white chalk quantification showed a R^2 of 0.990, an RMSEC of 1.020% and a RMSEP of 0.851% using SNV and a first derivative of Savitzky Golay with a seven-point window and third order polynomial, in this case due to the high peaks that were identified in the raw data, it is only required to use one factor. The LOD (mean + 3 standard deviations) was calculated from the 20 pure garlic powder samples and was found to be 0.11%. On the other hand, the best model for cornstarch quantification was obtained with MSC and second derivative of Savitzky Golay, with a seven-points window and a R^2 of 0.989, a RMSEC of 1.042% and a RMSEP of 0.649%, using two factors. The Limit of detection for this model was found to be 0.40%.

Table 3-2: Quantitative models generated for each adulterant

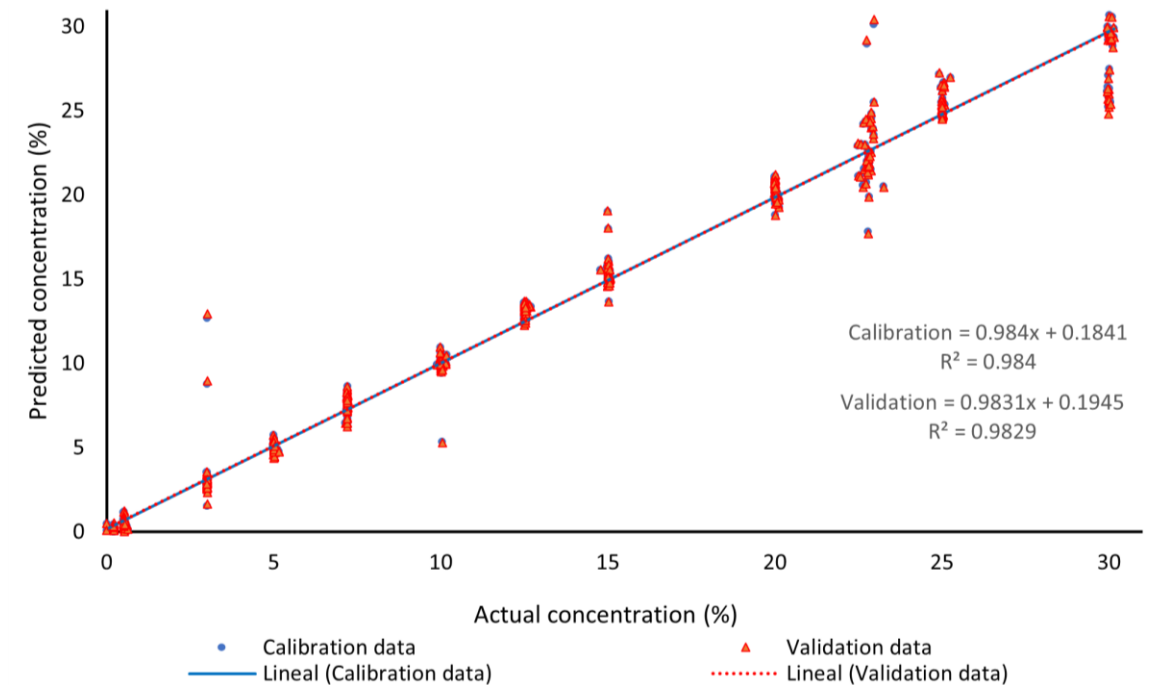
Adulterant		White chalk			Cornstarch		
		Raw data	1st Derivative	2nd Derivative	Raw data	1st Derivative	2nd Derivative
SNV	R ²	0.985	0.990	0.978	0.990	0.991	0.989
	RMSEC(%)	1.178	1.020	1.518	0.960	0.944	1.042
	RMSEV(%)	1.206	1.198	1.541	1.011	1.002	1.058
	RMSEP(%)	1.576	0.851	0.900	1.014	0.737	0.692
	Factors	3	1	1	4	3	2
	Window, Polynomial order		7, 3	7, 3		7, 3	7, 2
MSC	R ²	0.985	0.979	0.978	0.990	0.991	0.989
	RMSEC(%)	1.179	1.479	1.517	0.961	0.944	1.042
	RMSEV(%)	1.209	1.504	1.532	1.013	1.005	1.059
	RMSEP(%)	1.575	1.098	1.164	1.022	0.665	0.649
	Factors	3	1	1	4	3	2
	Window, Polynomial order		7, 3	7, 3	7, 3	7, 3	7, 3

The development of a single model quantifying both adulterants was also studied, the chemometric preprocessing parameters investigated are shown in Table 3-3. The selected model showed a R² of 0.984 with a RMSEC of 1.194%, a RMSEV of 1.235% and RMSEP of 0.690%. This model was obtained with four factors explaining 98.4% of the variance by applying SNV followed by the first derivative of Savitzky Golay with a seven-point window and second order polynomial. The Limit of detection of this model is 0.49%. These results show that it is possible to use a single model for the quantification of these two adulterants, which is evident in the cross validation (Figure 3-4) since a low dispersion of the data is observed, as well as high R² values for validation and calibration.

Table 3-3: Quantitative models developed for both adulterants

Parameters	SNV			MSC		
	Raw data	1st Derivative	2nd Derivative	Raw data	1st Derivative	2nd Derivative
R ²	0.982	0.984	0.983	0.982	0.986	0.982
RMSEC (%)	1.343	1.194	1.231	1.344	1.127	1.259
RMSEV (%)	1.369	1.235	1.261	1.379	1.196	1.281
RMSEP (%)	0.892	0.690	0.832	0.904	0.766	0.782
Factors	4	4	3	4	4	3
Window, Polynomial order		7, 2	5, 2		7, 3	7, 3

Figure 3-4: Actual concentration of the adulterant versus the predicted concentration of the selected adulterant quantification model (white chalk and corn starch) in garlic powder.



Lohumi, Lee, and Cho (2015) developed a FT-NIR-based quantification model for corn starch in garlic powder with an R² of 0.95, an RMSEC of 2.91%, and an RMSEP of 2.56%. Kar et al. (2018) developed models with FT-NIR for the quantification of corn starch in turmeric powder showing an R² of 0.99, an RMSEC of 0.233% and an RMSEP

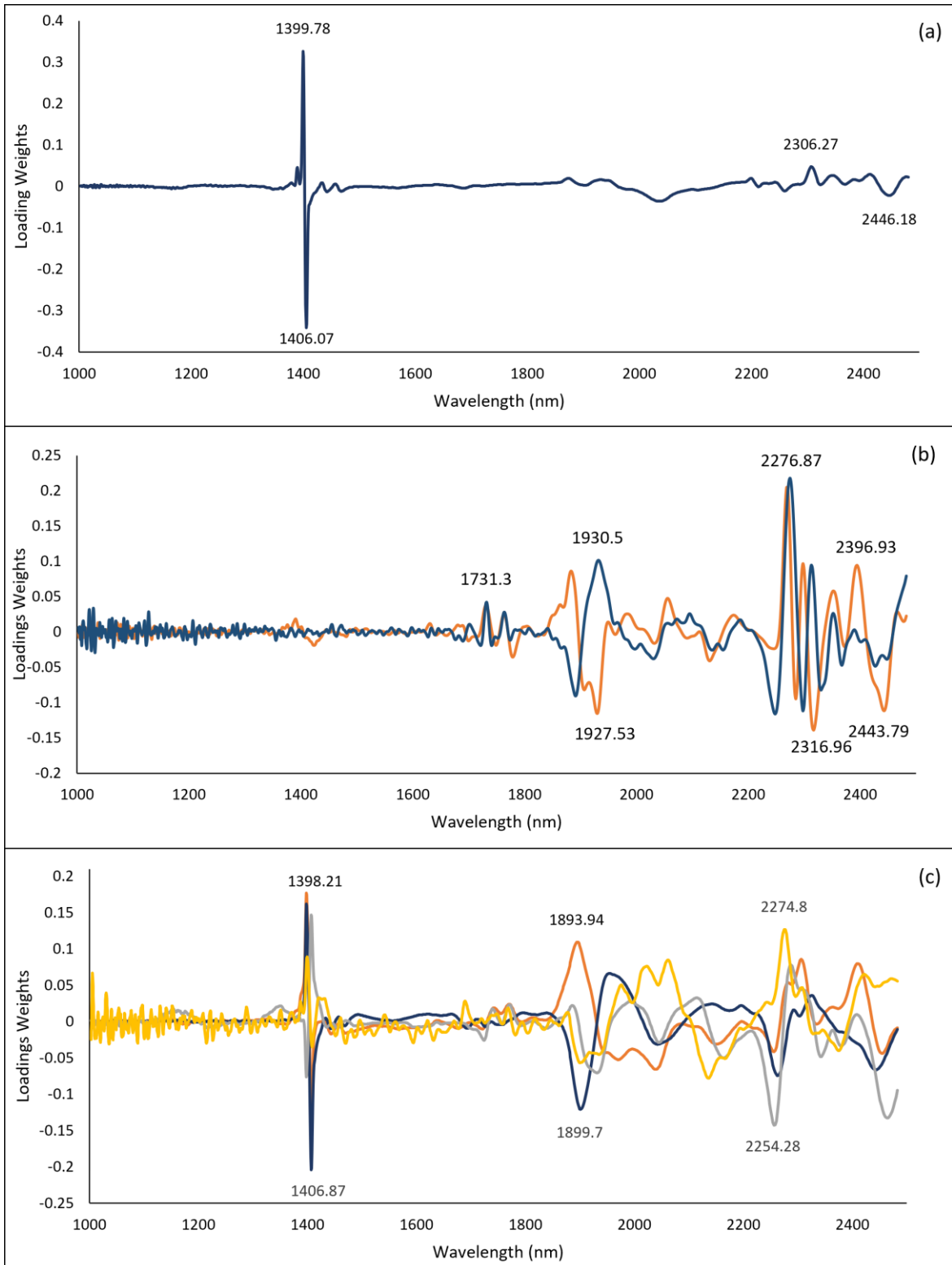
of 0.257%, in the range of 1400 to 1550 nm. Lima et al. (Lima et al., 2020) developed quantification models for cassava starch and corn flour, as adulterants of black pepper and cumin, showing R^2 values between 0.97 and 0.99 for calibration and between 0.95 and 0.99 for validation, thus requiring between 10 and 15 factors. PLS regression was also applied for the quantification of different plant-derived adulterants in saffron showing an R^2 of 0.99, RMSEC between 0.62% and 1.09%, and RMSEP 0.94% to 1.75% (Petrakis & Polissiou, 2017). These results showed that the models developed in this research have a high performance for the quantification of white chalk and corn starch in garlic powder.

Considering the structure of equation 3-1, Table 3-4 presents the equations of the three quantification models generated. Additionally, Figure 3-5 shows the representative wavelengths for the identification of the adulterant in each model. In the white chalk model, it is evident that the most important variables are 1399.78 nm and 1406.07 nm, related with the C-H combination, and the O-H first overtone. Followed by wavelengths of 2306.27 nm and 2446.18 nm corresponding to the C-H bend second overtone and, C-H combination which can be related with the composition of the chalk in the samples. In the corn starch model, the important variables are between 2276.87 nm and 2396.93 nm followed by 1927 nm to 1930 nm, corresponding to the C-H stretch region, the CH_2 deformation and the O-H stretch, which are related with the starch. In the quantification model of both adulterants the main variables are 1398 nm and 1406.87 nm related to white chalk, and the wavelengths of 2254.28 nm and 2274.8 nm related to corn starch.

Table 3-4: Equations of the quantitative models developed

Adulterant	Quantitative model
White chalk	$C_{wc}(\%) = 6.5452 + 0.2947X_{1002.81} + \dots - 4.4560X_{2482.62}$
Cornstarch	$C_{cs}(\%) = 18.9486 - 10.1589X_{1002.81} + \dots + 692.3785X_{2482.62}$
White chalk and cornstarch	$C_{wc\&cs}(\%) = -2.5299 - 8.1339X_{1002.81} + \dots - 4.7871X_{2482.62}$

Figure 3-5: Loadings of important variables in the developed models. (a) White chalk; (b) Cornstarch and (c) White chalk and cornstarch



3.4.6 Commercial samples

To validate the models with commercial samples, the main garlic powder brands distributed in Bogotá were selected and a total of thirty-five samples (7 brands) were acquired from different points in the city. The PLS-DA model was used to identify whether there were samples adulterated with white chalk or corn starch in the local market. The model identified fifteen samples with the presence of corn starch in their composition. Of these, ten samples belonging to two brands reported the presence of rice flour on the label, which is a carbohydrate allowed in mild spices (ICONTEC, 1998), but corn starch was not reported. In addition, the other five samples belonging to another brand reported the product as garlic powder without other additives. This constitutes food fraud since the consumer does not acquire a completely pure product and the label does not report the correct composition of it.

In addition, since the classification model only identified samples adulterated with corn starch, the quantification model for this adulterant was applied to estimate the concentration in the samples. The model predicted a content of $61.0 \pm 0.4\%$ and $56.5 \pm 0.8\%$, for the two brands claiming to contain rice flour and a concentration of $65.4 \pm 1.0\%$ for the samples claiming the pure product. On the other hand, the third quantification model was also applied with which a concentration of $67.3 \pm 0.4\%$, $63.7 \pm 0.7\%$ and $74.1 \pm 0.9\%$ was obtained for the three brands, respectively.

The difference between the concentrations predicted by the two models could be related with the sensitivity of each model, since the starch quantification model has a higher accuracy than the model for quantification of both adulterants. Additionally, it is important to consider that most of the commercial samples correspond to mixtures of garlic with salt, which may influence the response of the quantification models while the PLS-DA model showed a good classification of these samples

3.5 Conclusions

The results obtained indicate the efficiency of near infrared spectroscopy (NIR) combined with chemometric techniques to identify garlic powder samples adulterated with white chalk and cornstarch, as well as quantify the concentration of these adulterants in the

samples with a single model that presents an R^2 of 0.984 and an RMSEP of 0.690 % and a detection limit of 0.49%. Additionally, it proves to be a fast, simple, and effective method in the identification and quantification of food fraud in spices, thus it could replace traditional complex techniques that consume time and resources.

The region from 1300 to 1410 nm is related to the composition of white chalk, due to C-H combinations and the first O-H overtone, and the region from 2200 to 2300 nm corresponding to C-H bond stretching, CH₂ deformation and O-H stretching, is related to corn starch. Therefore, specific study of these regions will allow better development of future models related to these adulterants. On the other hand, to improve the robustness of the models, it is necessary to include a greater number of samples, other adulterants, and garlic powder from other sources to increase the variability, so that its effectiveness can be guaranteed in future analyses. Additionally, it would be necessary to study the use of portable elements so that the analysis can be conducted directly at the inspection points of entry of food into the country.

4. Conclusiones y recomendaciones

4.1 Conclusiones

Las cadenas de producción y distribución de especias son altamente susceptibles a la adulteración económicamente motivada, que puede tener repercusiones económicas no solo para la marca sino también para todos los involucrados en la producción y comercialización, desde el productor primario hasta el consumidor final. Entre los principales adulterantes encontrados en estos productos se encuentran compuestos comestibles como los almidones de diferentes fuentes, o materiales no aptos para el consumo que pueden presentar un riesgo para la salud humana como es el caso de la tiza. Por ello es necesario realizar planes de mitigación de riesgos que incluyan puntos de inspección y control continuo de la composición de los productos desde la recolección y procesamiento hasta el empaque, distribución y venta final.

Las técnicas espectroscópicas cuentan con un mayor número de estudios para el control de calidad de las especias, debido a su alta precisión y rápida respuesta para clasificar las muestras adulteradas de las puras, identificar el tipo de adulterante y cuantificar la concentración de estos. Dada la complejidad de los espectros que se obtienen a través estas técnicas, es necesario aplicar de manera correcta las herramientas de análisis quimiométrico para obtener modelos de predicción eficientes.

Así como avanza la tecnología para identificar adulterantes, también evolucionan los métodos de adulteración. Por ello, es necesario seguir investigando en metodologías de control eficaces aplicables a un nivel industrial para diferentes variedades de especias de manera que se reduzcan los casos de fraude y se garanticen productos de calidad para el consumidor.

Los resultados obtenidos en la presente investigación indican la eficacia de la espectroscopia de infrarrojo cercano (NIR) combinada con quimiometría para clasificar muestras de ajo en polvo con muestras adulteradas con tiza blanca y almidón de maíz, así como cuantificar la concentración de estos adulterantes. Además, demuestra ser un método rápido, sencillo y eficaz en la identificación de fraude en especias, por lo que podría sustituir a las complejas técnicas tradicionales que consumen tiempo y recursos.

4.2 Recomendaciones

Para mejorar la robustez de los modelos desarrollados en esta investigación, es necesario incluir una mayor cantidad de muestras, que incluyan otros adulterantes e incluso ajo en polvo de diferentes orígenes para aumentar la variabilidad, de forma que se pueda garantizar su eficacia en futuros análisis. Igualmente, la investigación puede ampliarse a otras especias en diferentes presentaciones de manera que pueda construirse una base datos con aplicabilidad comercial. Para ello, es necesario aplicar diferentes técnicas estadísticas para el procesamiento de los datos y mejorar la respuesta de los modelos de calibración y predicción.

Además, se recomienda estudiar el uso de elementos de medición portátiles para que el análisis se pueda realizar directamente en los puntos de inspección de entrada y salida de alimentos al país. Debido a que la complejidad de las cadenas de distribución de los productos importados y exportados, se presenta una alta vulnerabilidad a la adulteración.

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