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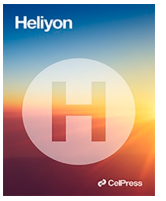

Review article

Chromatographic and spectroscopic methods for the detection of cocoa butter in cocoa and its derivatives: A review

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ABSTRACT

Currently, there is fierce competition in the cocoa industry to develop products that possess distinctive sensory characteristics and flavours. This is because cocoa and its derivatives provide numerous health and functional advantages, which is essential to their economics. The fatty acid and triglyceride composition of cocoa determines its quality. This review emphasises the necessity of developing precise, adaptable analytical techniques to identify and quantify cocoa butter in cocoa and its derived products, from cocoa beans to chocolate bars. Key chromatographic and spectroscopic techniques play crucial roles in understanding the fundamental principles underlying the production of cocoa with desirable flavours. This significantly impacts the sustainability, traceability, and authenticity of cocoa products while also supporting the battle against adulteration.

Specifications table.

Subject area	Food Science
More specific subject area	Analytical, Quantifying, Traceability and authenticity
Name of the reviewed methodology	Semi-systematic
Keywords	Cocoa, Cocoa butter, Chocolate, Infrared, NIR, NMR, Chromatography, Authenticity, Quality, Fatty acids.
Review question	What is the chemical composition of fats in cocoa and its derivatives? Which chromatographic techniques are suitable for quantifying the fat content of cocoa and its derivatives? What spectroscopic methods are used to monitor the authenticity and distinctiveness of cocoa fats?

1. Introduction

Theobroma cacao L., which grows in Central and South America, as well as West Africa [1], is processed to produce cocoa and its derivatives (cocoa powder, cocoa liquor, and chocolate) [2], with Southern Bahia becoming as Brazil's second largest cocoa producing region until 1989 [3]. The profits generated from cocoa production in 2015 reached $267 million, which prompted these countries to

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regard cocoa as an important global economic good. Exports of cocoa and its derivatives amounted to $294 million in 2019 [4].

Chocolate is one of the most widely consumed food and beverage products worldwide [5]. Over the last decade, cocoa production has increased by approximately 30 % worldwide [6]. Switzerland, whose economy depends on the chocolate industry, is a primary consumer of cocoa-related goods [7]. White chocolate, milk chocolate, and dark chocolate are distinguished by the amounts of milk, CB, and cocoa mass added during production [1]. The percentage of cocoa in chocolate determines its nutrient content; as the percentage of cocoa solids increases, the percentage of carbohydrates decreases and the amount of total fat increases [7]. The amount of fat varies depending on the type of chocolate, with dark chocolate having approximately 43 % fat and CB having 40–50 % fat [1] (comprising 25 % palmitic acid, 33 % stearic acid, and 33 % oleic acid) [8]. Dark chocolate is the variety with the lowest amount of added fats and the lowest percentage of milk compared to milk and white chocolate When it comes to cocoa mass, dark chocolate has the highest amount whereas white does not contain any. White and milk chocolate contain similar components however, and have similar properties in terms of taste and health benefits, including lowering the risks of diabetes, cancer, cardiovascular disease, hypertension, and arterial disease [6].

Producers strive to separate the fat from cocoa beans without introducing excessive moisture to guarantee the high quality of the end product [9]. It is essential for chocolate manufacturers to have accurate knowledge of the fat content of the cocoa mass to effectively determine the appropriate ratios of other ingredients and achieve the desired chocolate quality [10]. Imbalanced fat ratios may result in complications during the chocolate production process, leading to deterioration of the chocolate quality. Understanding the fat percentage in cocoa mass allows the selection of various grades of cocoa with variable fat levels and prices [11–13].

Unauthorised additives such as artificial sweeteners, vegetable oils, and synthetic colouring are sometimes incorporated into chocolate products to enhance their visual appeal and lower manufacturing expenses [14]. These fraudulent practices have been reported in various countries, including Pakistan, India, Spain, Turkey, Cameroon, and Germany [15]. The rising global demand for cocoa and CB, combined with their escalating prices, increases the potential for such fraud [16]. Identifying adulteration and determining the authenticity of CB are significant problems in the food sector that are raising concerns among customers and demanding the attention of food producers [5]. CB offers several health benefits; however, its high saturated fat content requires moderation. Excessive intake of saturated fat raises LDL cholesterol levels and increases the likelihood of developing cardiovascular disease due to its elevated caloric content; therefore, an inadequate diet and unhealthy eating patterns may result in weight imbalance [16]. CB fraud impacts all stakeholders in the food supply chain, with merchants being particularly susceptible. The ramifications of CB fraud include harm to a company's reputation, diminished sales and impacting financial stability. Consumers purchasing adulterated or mislabelled CB face risks related to health, financial, and ethical considerations. In addition, agricultural producers may face unfair competition, lower market values for their original commodities, and financial losses [17]. Fraudulent practices are also widespread in the fat and oil processing sectors. Animal fats, such as lard and cattle and mutton tallow, have been used as adulterants in the food industry [18]. Given that lard is currently the most affordable and commonly available fat in the food industry, food manufacturers in some nations use it to adulterate CB [5]. Cocoa butter equivalents (CBEs), cocoa butter replacers (CBRs), and cocoa butter substitutes (CBSs) can also be used to partially or completely replace expensive CB. Additionally, natural oils rich in C16 and C18 fatty acids are subjected to hydrogenation to create fat substitutes for CB. Due to their similar qualitative composition to CB, these fats seamlessly integrate with chocolate products, particularly when utilised in the production of cocoa powder with high CB content (Table 1) [19]. As such fraudulent practices are rife within the food industry, it is essential to develop mechanisms to check the quality of food at various points during production, processing, and distribution.

Understanding the chemical composition of cocoa and its fat derivatives will promote the production of health-conscious products and prevent adulteration [20,21]. Advanced analytical instruments can be used to measure and compare a range of complex CB products, control quality, and provide uniformity [22]. Some of the analytical instruments implemented are nuclear magnetic resonance (NMR), infrared analysis [18], and chromatography, which are characterised by a high degree of sensitivity.

This review focuses on the analysis of CB and its derived products, as well as the impact of different types of added fats on the quality and characteristics of these goods. The aim is to provide relevant authorities with valuable knowledge that can be used both to produce high-quality products and to detect fraud [16]. This is achieved by presenting the fundamental techniques of infrared spectroscopy, Raman spectroscopy, nuclear magnetic resonance spectroscopy, chromatography, particularly high-pressure liquid chromatography (HPLC), and gas chromatography (GC). These techniques are utilised to assess the genuineness and distinctiveness of

Table 1
Classification of cocoa butter solids according to their properties and the specific raw ingredients used in their production [17–19].

Classification	Primary components utilised in manufacturing	Particulars and properties
Cocoa butter (CB)	Cocoa beans	This is chocolate's most expensive and also most vital ingredient. It consists primarily of symmetrical triacylglycerols, approximately 75 % of which contain 2-oleic acid.
Cocoa butter replacements (CBRs)	Fats from tropical plants, such as shea, kokum, illipe, or mango kernels	Lauric cocoa butter replacements. These fats are incompatible with cocoa butter, yet have physical qualities that are similar to it.
Cocoa butter substitutes (CBSs)	Palm kernel oil (PKO) is widely utilised	These do not include lauric acid. These fats are partially compatible with cocoa butter.
Cocoa butter equivalents (CBE)	High stearic-high oleic sunflower oil fractionation The enzymatic, solvent-free interesterification of palm oil mid fraction (POMF) with stearic acid	Cocoa butter replacements and additions. These fats are chemically and physically compatible with cocoa butter.

samples and are highly valuable because of their ability to identify the molecular characteristics of the primary fatty acids found in the samples [2,23,24].

2. Chemical composition of cocoa butter and its derivatives

CB is an important component in chocolate recipes, and also the most expensive ingredient [14]. A mature cocoa bean contains approximately 700 mg of CB. When measured dry, the whole bean has a total fat content of approximately 48–49 %. The main constituents of CB are glycerolipids, with triacylglycerols being predominant, comprising 97 % of its composition [20]. This substance contains significant constituents such as free fatty acids and sterols, and fat-soluble oxidation products such as hydrocarbons, alcohols, and ketones [15]. Additionally, it contains trace quantities of di- and monoglycerides and phospholipids, as well as 3 % glycolipids and unsaponifiable materials [20]. Despite being such minor components, the presence of phospholipids and sterols in CB significantly influence its distinct features and chemical composition, rendering it a valuable ingredient that is extensively used in the chocolate industry [10]. Each of these lipids presents novel issues regarding the fat chemistry and physical characteristics for cocoa and chocolate technologists. In addition, they account for distinct and intricate flavour attributes that are unique to chocolate [21,22].

2.1. Triglycerides

The triglycerides (TG) comprise three specific molecules: 2-oleoyl-1-palmitoyl-3-stearoylglycerol (POS), 2-oleoyl-1,3-distearoyl-glycerol (SOS), and 2-oleoyl-1,3-dipalmitoylglycerol (POP) [25]. Monosaturated dioleoyl-glycerols (SOO) and disaturated-2-linoleoyl-glycerols (SLS) are also present in appreciable amounts [23,24,26]. The mean compositions are 40.2–44 % POS, 25.7–28 % SOS, 17.6–21 % POP, and 5 % other forms [23,24,26]. The melting properties of CB are associated with the melting points of its main TG, i.e., POS, SOS, and POP [27]. The distinctive triacylglycerol composition, combined with the exceptionally low concentration of diacylglycerols, gives CB its attractive physical characteristics and its capacity to undergo stable crystal modification during processing [17,28]. The crystallisation of CB is a complex process owing to the ability of triacylglycerols to form various crystal-like structures. This is influenced by the triacylglycerol contents and the specific circumstances of the manufacturing and storage processes, such as crystallisation and tempering. The average concentration of trisaturated triglycerides in CB that has undergone normal processing is 1.4 %. However, for heat-damaged fat, this value can increase to 3.6 %. This suggests that interesterification may take place as a result of the high-temperature treatment [9].

2.2. Fatty acids

Fatty acids (FAs(are primarily hydrocarbon biomolecules with an even number of carbon atoms and a carboxylic functional group at one end of the chain. Saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs) can be identified based on the quantity of bonds present in the acyl chain [29,30] (Fig. 1). In chocolate, few non-esterified or free fatty acids (FFAs) are present; the majority of FAs are bonded as esters. The fatty acid (FA) composition of chocolate includes the following major FAs: palmitic (C16:0, 25–30 %), stearic (C18:0, 3–37 %), oleic (C18:1, 31–38 %), linoleic (C18:2, 2–5%), and C18:3 linolenic acid, accompanied by small amounts of other saturated and PUFA C14–C22 acids, such as C20:0 arachidic acid and C22:0 behenic acid [3,31,32]. Long-chain acids, including stearic, palmitic, and oleic acids, make up more than 90 % of the FAs [33]. It is crucial to emphasize that food is the source of most circulating and stored FAs in the blood. Therefore, it is vital to undertake a thorough examination of how much and what kind of dietary FAs actually contribute to disease [34]. Recommendations to restrict dietary SFA intake are well-established. It is also clear that the health consequences of consuming foods cannot be predicted based on their content of any one nutrient group, and that the distribution of all macronutrients must be taken into account. Dark chocolate is an SFA-rich food with a complex matrix that is not associated with an elevated CVD risk [35]. CB contains stearic acid, a saturated fat that does not affect LDL cholesterol levels. Moreover, CB contains antioxidants, which improve cardiovascular health and reduce inflammation [16]. It also contains linoleic acid (LA, C18:2) and linolenic acid (ALA, C18:3), which are classified as essential FAs that humans cannot

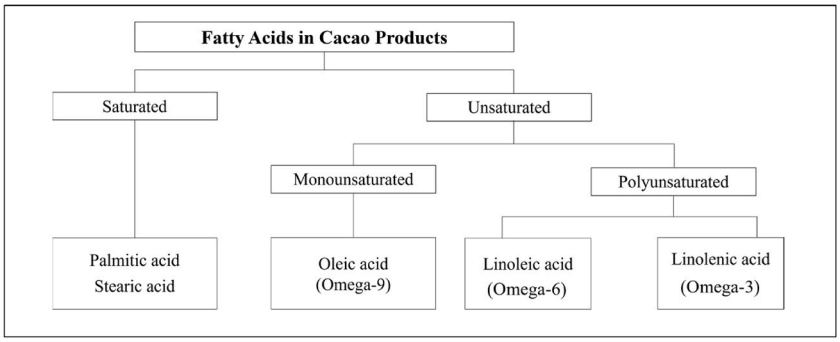

Fig. 1. Fatty acids in cocoa products.

synthesise within the body and thus must be obtained through diet alone [36,37].

2.3. Phospholipids

Phospholipids are lipid molecules comprising a glycerol backbone, two fatty acid chains, and a phosphate group [38]. Phospholipids are found in different levels in CB, with a higher concentration in the seed crystal fraction, which has a high melting point [38]. The phosphorus levels in CB range from 2.3 ppm to 63 ppm, implying a phospholipid range of 0.006–0.16 [9]. Phospholipids are essential for determining the characteristics of CB and for chocolate synthesis [39]. They affect the shape of the butterfat crystals, which in turn affects their structure and ability to change form. This eventually improves the functional qualities of CB [40]. Seed crystals derived from CB have considerably elevated levels of phospholipids compared to the original butter, which has a notable influence on the crystallisation of lipids and the growth of seed crystals [41].

2.4. Sterols

Sterols are a group of lipids characterised by a distinct structure consisting of four linked rings [38]. Sterols fulfil many physiological roles and offer numerous health benefits, including reducing cholesterol and inflammation, as well as serving as precursors to various hormones and vitamin D [19]. Lipids are a class of chemicals; this class includes cholesterol. Trace amounts of these compounds can be detected in CB [9]. The presence of sterols in CB has a notable impact on its composition and overall quality [42]. CB consists of various sterol fractions, such as free sterols, steryl esters, steryl glucosides, and acylated steryl glucosides [43]. The primary sterol components in CB are free sterols, although esterified sterols and glucosidic sterols are also present. Moreover, the sterol composition of CB plays a vital role in identifying CBSs in chocolate goods [44].

3. Cocoa butter alternatives in the form of other oils and fats

CB is sometimes replaced by adulterants, such as vegetable oils and fats, which are less expensive and can modify the texture and taste of chocolate [17]. Shea butter and coconut oil are also used to adulterate CB, as well as fats from alternative sources, such as fully hydrogenated oils extracted from palm kernels or palm oil [45]. Additionally, vegetable fats such as kokum gurgi and mango kernel stearine can be used to adulterate CB because they have similar fatty acid compositions [9]. Several companies employ animal fats, such as hog lards [46].

The synthesis of CBRs, CBEs, and CBSs obtained from diverse natural sources has been investigated in depth. PKO, PO, kokum butter, sal fat, shea butter, illipé butter, soya oil, rapeseed oil, cotton oil, ground nut oil, and coconut oil are derived from fats found in plants. Experimentation with replacing CB with alternative natural fats, either partially or completely, has been motivated by the potential technological and economic benefits that doing so may offer [28] (see Table 2).

Chocolate produced in the European Union may contain vegetable fats other than CB [9], such as shea fat and kokum gurgi fat [46], and the manufacturing process is confined to fractionation and refinement [9]. Chocolate produced in the European Union may contain no more than 5 % CBSs, which are also known as CBEs [45]. However, it is imperative that chocolate contain a minimum of 25

Table 2
Most significant fats used as replacements for cocoa butter, entirely or partially [19,47,46].

Type of fat	Similarities to cocoa butter	Differences from cocoa butter
Palm kernel oil (PKO), Palm oil (PO)	The presence of similar fatty acids, such as palmitic acid, stearic acid, and oleic acid. Moreover, both palm oil and cocoa butter possess triglyceride compositions that can be modified to imitate each other.	PKO contains a substantial quantity of lauric acid (C12) and myristic acid (C14), while PO has a higher proportion of oleic acid (C18:1) compared to cocoa butter.
	Properties such as solidity at room temperature.	Differences in the slip melting point, solid fat content, iodine value, saponification value, and acid value of the final product, compared to those of cocoa butter.
Sunflower oil	They can readily undergo processing in order to modify their properties, such as altering their melting points and modifying their fatty acid makeup. (CBEs can be made from high-oleic sunflower oil using enzymes to optimise the triacylglycerol percentage in order to create cocoa butter-like properties.)	Sunflower oil is liquid at room temperature because it contains unsaturated fats, but cocoa butter hardens because it contains saturated fats.
Mango kernel	Both have comparable triglyceride compositions; therefore, they can be blended without being observed. The presence of palmitic, stearic, and oleic acids in both fats, contributing to their similar physicochemical properties.	Different triglyceride compositions, with specific triacylglycerols such as 1,3-distearoyl-2-oleoyl-glycerol.
Coconut oil	Coconut oil can be solid like cocoa butter; both contain lauric acid. Coconut oil has a slip melting point (SMP) of 25–33 °C, which is lower than that of palm stearin and closer to that of cocoa butter, making it suitable as an adulterant.	Coconut oil feels waxy in chocolate, unlike cocoa butter. Cocoa butter and coconut oil have distinct fat compositions; thus, they do not mix well in chocolate.
Shea butter	Shea butter must be modified to contain stearic acid in order to resemble cocoa butter.	Cocoa butter exhibits polymorphism, a unique property that allows it to undergo many solid-state transformations, unlike shea butter.

% fat by weight [48]. In the United States, stringent restrictions prohibit the inclusion of any non-cocoa vegetable fat in chocolate, such as sweet, semisweet, milk, and white chocolate. While products that do permit vegetable fats, such as "milk chocolate and vegetable fat coating", are permitted to contain specific vegetable oils, fats, and stearins, these must still meet the minimum requirements for cocoa solids and milk solids [9]. CB can be chemically modified by blending it with fats that have comparable physical and chemical characteristics. These fats are occasionally altered using techniques such as enzymatic interesterification or chemical catalysis to ensure that they precisely imitate the features of CB [49].

Distinguishing these adulterants from authentic CB may prove challenging unless specific tests are applied. Adulterated CB may contain different levels of triacylglycerols (TAGs) and fatty acids; this fact is used to determine the authenticity of the product. Other techniques for identifying adulteration include quantifying the solid fat content (SFC) and measuring the extinction coefficient. These measures can be used to detect differences from the expected values of pure CB [44].

4. Spectroscopic methods for analysis of cocoa butter and its derivatives

Spectroscopic techniques enable the ongoing assessment of the fat and fatty acid contents of CB and its derivatives for quality control and adulteration detection.

4.1. UV-VIS spectroscopy

A UV spectrophotometer is a precise analytical device used to quantify the absorption of ultraviolet and visible light by a sample [45]. UV spectrophotometers operate by transmitting a light beam through a sample and quantifying the degree of light absorption at various wavelengths. The degree of light absorption is directly proportional to the concentration of the solute in the solution and the thickness of the solution under analysis in the sample [48]. The quantification of analytes using UV–Vis spectroscopy relies on Beer's law, which states that the amount of light absorbed by an analyte in a consistent, unchanging medium is directly proportional to the concentration of the analyte in the sample [49]. Ultraviolet and visible absorption spectroscopy enables the identification of tainted and forged food products, differentiation between various food origins, and categorisation of food items. A reliable screening technique for identifying tainted food using UV-VIS spectroscopy was developed based on measurement of the absorbance loss in commercial samples [50]. In this study, fluorescence and ultraviolet light were used to detect fake CB. Data from multiple spectral analyses were combined to improve the classification accuracy. CB adulteration was tested using eight cocoa paste samples and eight local chocolate samples. CB samples infused with 0–100 % CBEs were prepared in 10 % (w/w) intervals. The mixtures were heated at 50 °C to create 144 CB-CBEs combinations. The results showed that CB and CBEs exhibited different synchronous fluorescence and ultraviolet (UV) spectra. A comparison of applying PCR and PCA-LDA to the data from the individual spectroscopy techniques and a combination of the data from both techniques demonstrated that UV spectroscopy provided additional and complementary information to fluorescence. Combining the UV and fluorescence intensities within 10 nm wavelengths gave the PCR models maximum prediction accuracy. The RMSEC was 3.7 and the RMSEV was 4.7. The data fusion outperformed the individual spectroscopies with regard to classification accuracy, with 100.0 % and 95.2 % accuracy for the calibration and validation datasets, respectively. An LDA model with fluorescence intensities at 10 and 30 nm wavelengths yielded these results. Synchronous fluorescence and UV spectroscopy, along with chemometric analyses, can be used to detect CBRs replacements. These findings suggest that synchronous fluorescence, UV, and chemometric analyses can be used to detect CB adulteration [51]. Our results indicate that UV-VIS spectroscopy is a cheaper and faster alternative for fat profiling than conventional time-consuming methods such as gas chromatography and infrared spectroscopy. However, cocoa fats are rarely analysed using this approach.

4.2. Infrared spectroscopy

Infrared (IR) radiation spans the spectrum of electromagnetic radiation frequencies from 14,300 to 20 cm^{-1}. The mid-infrared spectrum, which is crucial for the majority of molecules, ranges from 4000–400 cm^{-1}. Within this specific range, there are four clearly defined regions. The spectrum is divided into these four regions based on the type of bonds present. The first region corresponds to single bonds (2500–4000 cm^{-1}), the second to triple bonds (2000–2500 cm^{-1}), the third to double bonds (1500–2000 cm^{-1}), and the fourth to the fingerprint region (600–1500 cm^{-1}) [52,53]. The proposed methods offer efficient and accurate approaches for analysing the chemical composition of CB. This involves analysing the composition of fatty acids and quantities of isotopes in glycerol, which can provide valuable data for evaluating the quality of CB [54]. The various IR-spectroscopy-based approaches are described in the following sections.

4.2.1. Raman spectroscopy

Raman spectroscopy (RS) is a vibrational spectroscopic method that relies on the phenomenon of Raman scattering [55]. RS is commonly employed in various domains, including food safety assessment, quality control, and detecting the adulteration control of edible fats and oils, because of its non-destructive nature, high degree of sensitivity, and real-time detection capability [56–58]. Spectral analysis conducted by RS revealed a distinct, noteworthy fingerprint region spanning 945–1600 cm^{-1} [59]. This finding proved helpful for accurately identifying and quantifying free fatty acids (FFA). This is a critical aspect for assessing the quality of edible fats and oils. Raman spectroscopy can be used to rapidly and effectively identify FA adulteration by monitoring the level of FA saturation [60]. Raman spectroscopy was successfully used in one study to monitor the extraction and processing stages of cocoa seeds; the results showed that the (CO) and (CC) stretch modes are representative of fatty substances, mainly C18:1 (oleic acid), and thus can

be used as a key marker for this chemical substance classification. This indicates that Raman spectroscopy can be adopted as a non-destructive and relatively fast method for monitoring cocoa bean processing and quality control [61]. In another study, primary FAs were identified and quantified to evaluate the fat profile of different types of Brazilian chocolate using FT-Raman spectroscopy. The presence of SFAs or UFAs was the primary spectroscopic difference between the chocolate samples examined. Well-defined bands were found in the spectra of the samples with CB in their composition in accordance with the label specifications, primarily in the dark chocolate samples, at approximately 1660 cm^{-1}, related to the C═C stretching mode, and at 1267 and 1274 cm^{-1}, related to the in-plane = C–H deformation, all of which were associated with UFAs [62]. Vegetable fats are emerging as the next frontier in chocolate manufacturing, owing to the abundance of nutritious additives that can be used in the final product. The objective of this study is to examine the crystallisation behaviour of two combinations of vegetable fats using diverse analyses. One of the fats was obtained from palm and shea and was non-lauric, whereas the other was a vegetable substitute for milk fat. Various techniques were employed to purify the fat samples, which were subsequently analysed again in combination. Among these techniques, RS proved to be the most effective. This study compared the results for vegetable and animal milk fats by employing different quantities of CB. The significant regions of the RS spectrum made it valuable for identifying CB polymorphs. Two overlapping peaks were present in the range of 3050–2700 cm^{-1}, however, which were linked to the symmetric and asymmetric expansion of the CH_2 group. These peaks occur at 2845 and 2882 cm^{-1}. Owing to the reduced number of side chains and interactions in the liquid phase, isotropic stretching played a key role in the overall Raman signal. Conversely, the solid-state induces an increase in the asymmetric stretching peak owing to the heightened influence of the adjacent methylene groups. The utilisation of Raman spectroscopy has enabled the chocolate industry to successfully create different combinations of fats with specific physical and organic characteristics, which is a significant achievement. This capability allows for differentiation between different crystalline structures [63].

4.2.2. Near-infrared (NIR) spectroscopy

NIR spectroscopy is a fast and non-destructive technique suitable for routine analysis. It has been a known method for fat analysis in the industry for several years [64]. This method is based on the interaction between electromagnetic radiation and the molecular vibrations of chemical compounds [65]. When infrared light strikes a chemical compound, it excites molecular vibrations at specific frequencies corresponding to the transition energies of covalent bonds. The presence of specific functional groups and the environment surrounding them causes the absorption of light at specific energy levels. This absorption results in the transition of the molecular vibrations from low to high energy levels [66]. Transmission-mode NIR spectroscopy can be used to determine both the internal and external properties of a sample because the light passing through it reveals information about its internal properties, which specifies the parameters for the ranges of fat and protein [21]. When evaluating the spectral data, the region between 7600 and 8000 cm^{-1}, which corresponds to the saturated and unsaturated triglycerides present in cocoa beans, may be helpful. Cocoa beans are known to contain approximately 60 % SFAs and 35.4 % UFAs. The spectral peaks typically correspond to proteins, fats, moisture, and certain aromatic compounds in the cocoa beans. Other useful peaks can be found at approximately 8235 cm^{-1}, which is associated with the –CH═CH second overtone; at 7030 cm^{-1}, which is associated with the C–H first overtone; and at 5798/5421 cm^{-1}, which are associated with the C–H first overtone. Therefore, a comprehensive analysis of the NIR spectral fingerprints of cocoa beans and cocoa bean products provides essential background data for qualitative and quantitative analysis [67]. In another study, NIR technology was utilised to examine the CB of seeds originating from various Brazilian regions. The results indicated that the fermentation process strongly affected the ability to distinguish between cocoa samples from different regions and that the removal of fat from the fermentation samples made it difficult to categorise them according to their region of origin. A total of 189 cocoa samples were obtained from three geographical regions: Tocuma, Medicilandia, and Tomi Aso. The samples were divided into two groups: fermented (1-with fat and 2-fat-free) and non-fermented (3-with moisture and 4-dried). The conclusion of Fabelle Ferreira et al. (2022) emphasised the importance of the fats found in fermented cocoa seeds and their impact on the quality of the food items. Based on the lack of identical findings in the defatted samples, it was argued that different regions have varying effects on the fatty acids of CB. This also highlights the importance of geography in determining cocoa quality and the fermentation process, as well as the use of NIR technology to determine geographical origin [68].

4.2.3. Fourier transform infrared (FTIR) spectroscopy

FTIR spectroscopy relies on the vibrational behaviour of the functional groups contained in a sample upon exposure to infrared light. Molecules selectively absorb infrared light that matches the vibrational frequencies of the chemical bond [69,70]. C–H, O–H, and N–H bonds exhibit distinct vibrational frequencies, leading to varied absorption infrared radiation patterns. The sample spectra were obtained by quantifying the light absorption strength at various wavelengths using an FT-IR spectrometer. This spectrum can be used to ascertain the functional groups and chemical composition of a sample [71,72], offering a distinct method for qualitative and quantitative analyses [6]. Incredibly distinct and reproducible fingerprints for primary vibrations belonging to the C–H and C═H stretches or the C═O vibrations of triglycerides can also be obtained using FTIR [73]. Assessment ofthe biochemical quality of cocoa has been conducted using this method, as has evaluation of chocolate and CB adulteration [2]. An Italian study used samples consisting of 14 CBs, 18 CBEs, 12 blends, and 6 pure CBs from Kokum, Elliptical, and part of the central palm, as well as 154 mixtures of CB with CBEs at various concentrations (ranging from 5 % to 20 %). The analysis was performed in triplicate on 192 samples. CB and CBE were demonstrated to have highly distinctive FTIR spectra that provided highly reproducible fingerprints. The primary vibration modes were also described. The differences between pure CB and pure CBE were clearly distinguished using FTIR spectroscopy. FTIR was used to distinguish between CB mixed with CBE at concentrations of 10 % and 20 % (corresponding to approximately 2 % and 5 % CBE in chocolate), based on prior knowledge of the amount of CB contained in the mixtures. Although these supervised algorithms detected distinctions within the training set, it was simpler to recognise the CB with 10 % CB than the CB with 20 % CB. However, it proved

impossible to categorise the test set accurately [73]. Additionally, two studies have demonstrated that FTIR spectroscopy can be used extensively to examine adulteration in food and pharmaceutical analyses; ATR and PLS regression were used to determine the content of lard when mixed with CB and chocolate, respectively. This is because the fingerprints of the functional groups in pure lard and CB can be used to identify adulterants within substances through capturing, analysing, and identifying the accompanying spectral bands and combinations (ranging from 0 % to 15 % lard in CB and approximately 0 %–10 % lard in chocolate). Based on spectral data in the frequency range 4000–400 cm^{-1}, a semi-quantitative technique was used to calculate the proportion of lard in the mixtures [5,74].

4.3. Nuclear magnetic resonance (NMR) spectroscopy

Nuclear magnetic resonance (NMR) is an effective technique for the analysis of edible fats and oils. It can be used to accurately measure important properties without matrix derivatisation. This technology has been successfully applied to many different types of oils and fats [75]. According to Elina Zailer (2018), ^{13}C NMR spectroscopy can be used to precisely examine the distribution of fatty acids in oils and fats. This technique can identify distinct signals for each carbon atom, making it a versatile method for determining the molecular weight of vegetable oils and tracking the formation of oxidation products. The ^{1}H NMR technique exhibits a strong correlation with conventional titration methods for most substances, with the exception of hard fats. The anisotropy of the double bonds in fatty acids influences the NMR signals, enabling the differentiation of various types of fatty acids. NMR spectroscopy was reported to be a flexible technique for general examination of the chemical composition of oils and fats [76]. In this study, for the first time, vegetable fats were directly extracted from a sample of natural CB using a method that modifies the distribution of FAs but leaves their nature unaltered. The chemical–physical properties of the CB derivatives appeared to be enhanced, even when the initial proportion of each FA in the combination remained unchanged. Macroscopic and molecular analyses, including ^{1}H and ^{13}C nuclear magnetic resonance (NMR, 1D, and 2D) spectroscopy, were performed to quickly analyse the FA composition, including regioisomeric distribution, and assess whether the new matrices obtained might be considered legitimate substitutes for other vegetable fats (e.g., palm oil (PO)). The spectra of the fats were assigned based on bi-dimensional (2D) homo- and heteronuclear correlation NMR experiments (^{1}H COSY, ^{1}H–^{13}C HMQC) and one-dimensional (1D) ^{1}H, ^{13}C, and ^{13}C–^{1}H NMR spectra. These results were then compared with findings in the literature. This enabled a qualitative and quantitative description of the percentage of TAGs, and GC/MS confirmed and combined these results. According to this hypothesis, the treatment to which the native fats were subjected affected the how the acyl chains were distributed on the TAGs. Finally, molecular analysis using ^{13}C NMR supported the theory that the acyl chain positions were rearranged during the modification process and simultaneously allowed for the quantification of the degree of conversion for each sample. The relative degree of conversion was calculated by integrating the relevant peaks. It should be highlighted that neither the ^{1}H nor the ^{13}C spectra of the changed butters displayed any experimental evidence of the creation of trans FAs or other substances. These findings may be explained by the fact that natural and modified butters have distinct positional distributions, but are identical in terms of the fraction of single chains of FAs. This is crucial to the food industry, as it only modifies the distribution of FAs, rather than their type, so that the initial proportion of each FA remains constant [29]. The viability of using NMR for regulating chocolate quality was also assessed for the first time. The results clearly show that the combination of ^{1}H NMR spectroscopy and multivariate models is a promising technique for detecting and measuring the addition of CBEs to chocolate above the permitted range specified by European law. ^{1}H NMR spectroscopy was used to prepare and examine mixtures of chocolate fats with CBE contents ranging from 0 to 50 %. Multivariate statistical models were developed using datasets consisting of the peak regions or spectral variables (fingerprints) in the glycerol region. The CBE and its concentration were successfully identified using partial least-squares discriminant analysis (PLS-DA) and regression (PLS-R), respectively. The effectiveness of the models constructed using the two datasets was assessed and compared using chemometric markers. The resilience of the models was evaluated by examining the test sets and conducting random permutation tests. The usefulness of NMR in assessing chocolate quality was demonstrated by the successful classification and quantification of CBEs using the fingerprinting models. Compared to the area technique, the fingerprinting-based strategy had a marginally superior prediction performance with regard to both classification and regression. Therefore, the developed technology is a new contender for chocolate quality control. ^{1}H NMR spectroscopy does not require sample manipulation like that in chromatographic methods, making it an incredibly rapid, reproducible technique. Obviously, a large library of ^{1}H NMR spectra must be created in order to use this technology for chocolate quality control, as well as to improve model performance. Additionally, to account for the compositional changes in the glycerides and unsaponifiable fractions, models should be developed to assess a wide number of combinations of cocoa fats and CBEs gathered from various geographical origins using different extraction techniques. Owing to the robustness and accuracy of this spectroscopic technique, chocolate can be authenticated in several laboratories with the same findings following the construction of a spectral library and appropriate setup of the NMR spectrometer [77]. In addition, the findings produced using the NMR technique can be used to authenticate various food matrices containing these types of vegetable fats. Didier Diomandé et al. (2022) evaluated the fatty acid composition of CB using ^{13}C NMR spectra. Their investigation also analysed the isotopic composition of glycerol in CB and ascertained the geographical provenance of cocoa. Saturated fatty acids, particularly stearic and palmitic acids, constitute the majority of CB. Monounsaturated fatty acids, such as oleic acid, constituted a smaller percentage. The fatty acids comprised predominantly stearic acid and palmitic acid, which make up approximately 63–68 % of the total. Monounsaturated fatty acids accounted for approximately 30–32 %. This study involved analysing samples from various cocoa-producing countries and extracting CB using cyclohexane. The fatty acid chains in CB varied in both length and position along the glycerol backbone. This study aimed to distinguish between various types of cocoa and determine the geographical origin of cocoa beans by evaluating their fatty acid composition. Examination of various types of cocoa could enhance the ability to identify the most discernible characteristics while eliminating the influence of the specific cocoa variety [78].

Near-infrared spectroscopy can be used to quickly and accurately analyse cocoa, chocolate fats, and fatty acids without the need for

fat extraction, purification, or chemical analysis. This provides an opportunity for further exploration. Researchers can accurately measure the fat content of food products without changing their composition. Near-infrared spectroscopy is easily calibrated, making it accessible to many analysts and researchers. Raman spectroscopy is a precise and non-invasive technique. Because the sample can be directly loaded into the instrument, sample preparation is simple. Research in the field of green analytical chemistry has led to reduced solvent and reagent use, shortened analysis time, and improved efficiency. These techniques are distinguished by their sensitivity and repeatability. Nuclear magnetic resonance (NMR) is a complex and effective composition analysis technology, unlike ultraviolet (UV) spectroscopy, which is rarely used because of its low sensitivity. It can precisely evaluate attributes without deriving a matrix. Thus, its use is the focus of the current research. A synopsis is presented in Table 3.

5. Chromatographic methods for the analysis of cocoa butter and its derivatives

Chromatographic procedures encompass a range of techniques that are employed to separate and analyse the constituent elements of intricate mixes. The extensive use of chromatographic methods, specifically gas chromatography and high-performance liquid chromatography, for the examination of fats in recent years has resulted in a significantly enhanced understanding of the composition and arrangement of fats in both uncomplicated and intricate systems.

5.1. High-performance liquid chromatography (HPLC)

High-performance liquid chromatography (HPLC) is used to separate and purify substances in several industries, including medicine, biotechnology, environmental science, polymer science, and food science. This process involves introducing a small liquid sample into a flowing liquid stream (mobile phase) that moves through a column packed with a stationary phase. The column selectively retains the different components of the mixture to varying extents. Liquid chromatography techniques such as HPLC employ a mobile phase consisting of a liquid. In reversed-phase chromatography, which is the most common method, the stationary phase is non-polar, whereas the mobile phase is polar. HPLC can detect, measure, and separate chemicals [79]. Non-fatty cocoa solids make up approximately half of the chocolate liquor produced when shelled cocoa beans are roasted and ground into powder, whereas fat-containing CB makes up the other half [80]. Melania Gracia et al. studied the nutritional profiles and health benefits of cocoa products and cocoa beans from various sources by analysing the proportion of branched fatty acids in each sample. Three cocoa bean samples were showcased, each originating from a distinct location: Peru, Ecuador (Criollo), or Ghana (hybrid). Five samples of Criollo cocoa products with different proportions of cocoa solids (ranging from 70 to 100 %) were analysed. Chocolate samples with a cocoa solids content of 70 % were prepared using Ecuadorian cocoa beans from the same batch that had undergone processing without grinding. The Criollo cocoa beans had a fat level of approximately 50 %, which exceeded that of the hybrid beans by approximately 17 %. The fatty acid profiles of items containing cocoa and cocoa seeds were remarkably similar. Oleic (C18:1), stearic (C18:0), and palmitic (C16:0) acids are the three primary nutrients present in cocoa beans and cocoa products. The levels of fatty acids, specifically C18:0 and C18:1, in uncertified cocoa beans of various origins exhibited comparable results. Researchers have demonstrated that the presence of saturated fatty acids in chocolate can increase the likelihood of cardiovascular disease [7]. The following order was observed when the entire FA profile composition was considered: SFA > UFA > MUFA > PUFA. The UFA fat was represented by SFA levels ranging between 59.30 % and 62.72 %. Acids (UFA = MUFA + PUFA) account for 37.28–40.70 % of the total FAs, MUFAs for 33.03%–37.97 %, and PUFAs for 2.73%–4.49 %. The range for the UFA/SFA ratio in CB made from commercial Peruvian cocoa beans was 0.59–0.69. Additionally, it was discovered that the Malaysian cocoa beans had UFA/SFA ratios of 0.55 with levels of 64.5 % SFA, 33.1 % MUFA, and 2.5 % PUFA [81]. An untargeted metabolomic technique based on HPLC-MS was developed to identify the adulteration of cocoa powder with chicory, carob, and soybean flour. The extraction methods and colorimetric parameters were optimised to extract as many molecular characteristics as possible. By comparing the isotopic profile and MS/MS fragmentation pattern of each compound with patterns found in published works, cocoa processing databases, and the literature, 16 compounds were found in the negative mode and four in the positive mode, suggesting that these may serve as possible disease markers. Most of these compounds are terpenoids, flavonoids, FAs, and lysophospholipids. FAs also suggested adulteration in carob flour, while terpenoids, lysophospholipids, and galactolipids indicated adulteration in soy flour. In terms of flavonoids, chicory and apigenin were added to quercetin and 7-*O*-methylisoflavone for carob flour. Luteolin was unable to distinguish between dandelion flour and carob flour; however, it has been utilised as an adulterant [82]. This demonstrates how successfully this technology can detect fraud in several cocoa powder categories by measuring fatty acids.

5.2. Gas chromatography (GC)

Gas chromatography (GC) is an essential analytical method that is employed in the examination of CB. It assists in ascertaining the fatty acid composition and flavour characteristics of CB obtained from cocoa bean shells [83]. GC analysis has also been used to determine the fatty acid components present in both fermented and non-fermented CB. This analysis revealed the presence of dominant acids such as oleic, heptadecanoic, and palmitic acid [84]. In addition, high-temperature gas chromatography has been employed to examine the TG composition of different lipids found in the chocolate products. This method makes it possible to differentiate between natural CB, CBRs, and milk fat based on their distinct TG profiles [85]. In summary, GC is crucial for analysing the chemical characteristics and fatty acid composition of CB. It aids in evaluating the quality of and distinguishing between various types of fats derived from cocoa.

Table 3
Summary of the spectroscopic analysis methods discussed, categorised by the type of products and the technology employed for the analysis

Spectroscopic method	Operating conditions/ parameters	Objective	Advantages of the analysis	Limitations of the analysis	Reference
UV-VIS spectroscopy	Fat spectra were taken at 190–320 nm, with a 1 nm interval.	To discriminate real cocoa butter from fake CB using inferior oils quickly and cheaply.	It identified cocoa butter combined with cheaper oils, ensuring high-quality chocolate.	It measures light absorption, rather than molecular types, and therefore cannot discriminate between similar substances.	
	Fat samples were diluted (1 % v/v) in *n*-hexane.		This affordable test is perfect for routine cocoa butter purity testing.	When exposed to light, cocoa components may react or break down, thereby altering the test results.	
	All of the analyses were triplicate in nature.		UV light allows researchers to detect trace oils in cocoa butter.	This approach may fail if the content of the fake oil is small and hard to detect.	
Raman spectroscopy	A Bruker IFS 66 with a 1064 nm near-infrared Nd3YAG laser and liquid nitrogen-cooled InGaAs detector was used. A 4 cm^{-1} spectral resolution was used to collect 2000 scans in 30 min.	The cocoa seed processing and quality control steps involved identifying fats.	It determined the chemical components of cacao seeds without damaging the sample and promoted industrial quality control.	It may be less sensitive to substances in low concentrations or with weak Raman scattering. Sample fluorescence may obscure the Raman signal analysis.	[61]
	The Raman signal was applied 1000 times to improve the quality of the data, with 512 scans and 30 mW of laser power, but the same spectral resolution of 4 cm^{-1}.	To evaluate the fat composition of different types of Brazilian chocolate by detecting both saturated and unsaturated fatty acids.	This method delivered fast, reliable qualitative information on the chemical makeup of chocolate, thus making it possible to assess its quality.	Some chocolate is hard to analyse, which can obscure its chemical components.	[62]
			It detected saturated and unsaturated fatty acids in chocolate, exposing the fats and thus the product validity.	The approach may be unsuitable for quantitative analysis and may need to be supplemented with additional methods such as CZE to confirm the qualitative results.	
	Fat analysis utilizing a 250 mW 785 nm laser. The spectrometer readings covered a 2.4 mm^2 region. The range of the spectra was 3200–200 cm^{-3}.	To analyse the crystallisation patterns of two types of vegetable fats as potential substitutes for animal oils in the chocolate business.	This food quality evaluation and hazardous component detection technology was faster and used fewer chemicals.	It can be hard to tell different compositions apart if they have similar Raman signals, which can make the results less clear.	[63]
Near-infrared spectroscopy	Focused on dry unfermented cocoa spectrum regions, like 4300–9300 $cm^{-1.}$	To assess the significance of location in assessing cocoa quality by estimating its fat percentage.	Once the prediction model was built, the analysis of the cocoa sample by acquiring the spectra and applying the model was straightforward, fast, and affordable.	The capacity of the technique to distinguish without the fermentation process was limited by overlapping spectra, making model construction challenging.	[68]
	To evaluate the 780–2500 nm and 3600–12500 cm^{-1} spectral areas, R^2, RMSECV, and RMSEP were used as the statistical measures pre-processes using SNV, MSC, and derivatives.	To measure the amount and quality of the fat in cocoa beans.	It measured the quality, and chemical composition quickly, thus saving time and avoiding the use of harmful substances.	If the data relationships are complicated, constructing accurate models is difficult, and requires a wide range of samples, which can be difficult to obtain.	[67]

5.2.1. Gas chromatography - mass spectroscopy (GC-MS)

Gas chromatography has a diverse array of applications. This approach is mainly employed to isolate and screen intricate assemblages, such as essential oils, hydrocarbons, and solvents [86,87]. Gas chromatography offers the added capability of precisely quantifying substances that exist in extremely small amounts by utilizing a flame ionization detector and an electron capture detector, in addition to calculating thermochemical constants. Therefore, it has been utilised in the fields of analytical research and development. Gas chromatography is an important technology in the field of chemistry because of its simplicity, sensitivity, and efficiency [88,89]. GC-MS analysis has been used to identify and measure various cocoa and chocolate samples. The most significant FAs in CB were found to be C16:0, C18:0, and C18:1. These results demonstrate that the FA composition of CBs is influenced by their geographical origin. Dark chocolate and cocoa beans can be viewed as nutrient-dense foods compared to other cocoa products because of their high quantities of fat and carbohydrates. Their origin has a considerable impact on their composition, whereas their processing conditions appear to have little effect. The most significant differences in the FA profiles were detected in C12:0, C14:0, C16:0, C16:1, C17:0, C17:1, and C18:0, whereas only minor alterations were observed for chocolate in C16:0, C18:0, and C18:1. The most significant values for all samples in quantitative FA determination were C16:0, C18:0, C18:1, and C18:2. In terms of the SFA/USFA ratio and FA concentration, Ecuadorian chocolate was shown to have a healthy balance between *trans*- and SFAs. Although chocolate can be regarded

Table 4
Summary of the chromatographic analysis methods discussed, categorised by the type of product and the technology employed for the analysis

Chromatographic method	Operating conditions/ parameters	Detection objective	Advantages of analysis	Limitations of the analysis	Reference
HPLC-MS	HPLC analysis employed a stationary phase C18 column. The mobile phase was a binary system of solvent A (water with a little phosphoric acid) and solvent B (water, acetonitrile, and phosphoric acid).	To determine the fatty acid composition of products that contain cocoa beans from diverse geographical origins.	The analysis can identify important nutrients, i.e., fatty acids, in cocoa beans and products.	The analysis ignores the complicated chemical interactions in cocoa.	[7]
HPLC-Q-TOF-MS	The column used for the analysis was an C18 column.	To develop a method for identifying the adulteration of cocoa powder with chicory.	The approach can detect signs of cocoa powder adulteration.	The approach may not detect all possible adulterants, as it is based on the identification of known markers.	[82]
	The mobile phase employed was a mixture of water with a small amount of formic acid and acetonitrile with a small amount of formic acid.		It detects many chemicals using high-resolution mass spectrometry.	The accuracy of the method can be affected by analytical drift during the metabolomics sequence, which can impact the quality control samples.	
GC-MS	The column used was a fused-silica polyethylene glycol capillary column, which helped to separate different substances based on their properties.	To identify and measure various cocoa and chocolate samples.	The nutritional composition and fatty acid profile of cocoa and chocolate were determined by the analysis.	The investigation shows no substantial effect of processing conditions on chocolate's fatty acid profile; therefore, it may not capture all of the nuances.	[90]
	Helium gas was used as the mobile phase in the gas chromatograph at 250 °C.		It can identify how geographical origin affects the composition of cocoa and chocolate, providing insights into quality.	The study only included quantitatively important fatty acids.	
	The stationary phase was a column coating that separates compounds, although the sources do not specify its composition.	To measure chocolate samples that had been adulterated with lard.	This fast, accurate analysis did not require a standard sample, making it more convenient and faster.	The minimum detection limit for lard in chocolate is 4 %, which may not be sufficiently low for all applications.	[91]
	The mobile phase, commonly helium or nitrogen, transported compounds through the column. The sources do not specify the gas.		The sensitive approaches may identify substances even with noise in the analysis, simplifying the interpretation of the results.		
GC-FID	The column used was an HP-88 capillary column.	To evaluate the overall fat content and fatty acid composition.	GC-FID properly characterized dietary fatty acids.	If trans fatty acids are below the detection limit, the analysis may fail to identify them.	[94]
	Helium was used as the carrier and make-up gases.		This method accurately examined food labels for fatty acids.		
	The initial temperature of the oven was set at 130 °C and then increased to 170 °C, and finally to 240 °C.				
	The column used in the gas chromatograph for fatty acid analysis was a fused silica capillary column. Helium gas was used as the mobile phase, which carries the sample through the column and detector, which were kept at 250 °C.	To analyse the fatty acid contents of the cocoa beans used to make commercial cocoa butter.	The primary cocoa butter fatty acids were accurately identified and quantified by GC-FID. Comparing fatty acid profiles across cocoa cultivars was possible with this approach.	The technique may not detect minor fatty acids as effectively as the major ones.	[81]

(*continued on next page*)

Table 4 (*continued*)

Chromatographic method	Operating conditions/ parameters	Detection objective	Advantages of analysis	Limitations of the analysis	Reference
	A 120 m, 0.25 mm silica capillary column, N_2 gas mobile phase, and 250–280 °C detector temperatures were used. The injection volume was 1.5 μL.	To study the composition of fatty acids in cocoa shells post-fermentation.	GC-FID can test various fat types in a sample and track the changes in the fat levels after processing the cocoa shells.	GC-FID may miss small levels of certain fats, producing an incomplete picture. Without proper temperature management, the fats may fail to separate.	[1]
	Polar capillary columns with a 0.32 mm internal diameter, 30 m length, and 0.25 mm film thickness were used. Inert gas was used as the mobile phase.	To detect the presence of lard in cocoa butter by examining changes in the composition of the fatty acids and the profile of the triacylglycerols.	GC-FID accurately identified and quantified the fatty acid content in oils and fats, ensuring product purity and authenticity. The sensitive approach detected modest changes in the fatty acid profiles, such as increased oleic acid and decreased palmitic and stearic acids in lard–cocoa butter mixtures.	GC-FID requires the additional step of converting fatty acids into FAME prior to analysis. This approach may struggle to detect adulteration for fats with identical fatty acid contents.	[95]
	Starting at 90 °C, the oven was programmed to rise to 220 °C at varying rates, while the injector and detector were kept at 240 °C.				
	A 100-m capillary column with a 0.25-mm internal diameter and 0.20-mm film thickness were used. Helium was used for transport to the flame ionization detector. The oven was started at 130 °C–170 °C at 4° per minute, and reached 240 °C.	To determine the amounts of SFA, MUFA, and PUFA in a product.	GC-FID accurately detected dietary fatty acids. This method is reliable for examining food labels for fatty acid content. Low quantities of harmful trans fatty acids can be detected.	The method might not always distinguish between fats from different sources if they have overlapping fatty acid profiles. The preparation and analysis can take time.	[96]

as a significant source of SFAs, particularly stearic acid, because of its FA concentration, it should be highlighted that it may have a neutral effect on human health. Dark chocolate consumption should be moderate in the context of a healthy diet because of its chemical composition [90]. Another study explored the importance of ensuring the availability of halal food commodities in Indonesia, especially regarding the inclusion of lard in chocolate products. The results of this study revealed the presence of specific fatty acids, including eicosadienoate 11.4 acid (C20:2), which indicated the presence of lard in six imported chocolate samples. The results revealed the presence of three specific fatty acids: *trans*-9,12,15-octadecadetrionic acid (C18:3); 11,14,17-eicosatrienoate (C20:3); and 11,14,17-eicosatrienoate (C20:2). According to a typical chromatographic composition, 100 % lard oil contains eicosapienoic 11.14 acid compounds and is included in additional formulations that are added to lard. GC-MS analysis also yielded a positive result, indicating the presence of the chemical in all six samples, with a retention time of 38.8 min. This retention time indicates the presence of fats, namely eicosadienoate 11.14 acid chemicals [91].

5.2.2. Gas chromatography -flame Ionization detector (GC-FID)

The GC technique offers more details on specific trans isomers and the total FA makeup [92]. FA content was found to be affected by the type of cocoa used in the process [93]. All chocolate samples exhibited similarity in terms of their FA profiles. For the chocolate samples, C16:0 (>23.91 %), C18:0 (>30.25 %), and C18:1 (>32.24 %) were the most significant FAs in terms of quantity. C16:0, C18:0, and C18:1 were the three most significant FAs present in chocolate with high cocoa content. Given that Pará-parazinho and Ipiranga contained more UFAs and fewer SFAs than the other samples, these chocolates appear to have a better FA profile. The concentration of oleic acid, a USFA, was the highest among the samples. Oleic acid is thought to be the reason for the decrease in LDL cholesterol levels [3]. There was a low proportion of total fat, which is affected by the location of origin, in the fine-aroma cocoa beans

Table 5
Summary of the chemical techniques used to check for the presence of cocoa butter and its derivatives in cocoa products

Product	Analytical technique	Objective	Reference
Cocoa beans and cocoa products	ICP and HPLC	To explore the nutritional profile and some health benefits.	[7]
Chocolate bars	AAS	To determine the presence of nickel in hydrogenated fats.	[101]
Chocolate bars and chocolate snack foods	LC-IRMS	To explore the use of the simple extraction method for liquid chromatography and isotopic ratio mass spectrometry evaluation of $\delta^{13}C$ vanillin.	[102]
Cocoa beans	GC-FID	To identify variations in commercial cocoa beans sourced from Peru by measuring the level of fatty acid.	[81]
Chocolate	ICP-MS	To determine chocolate digestion and further toxic elements.	[103]
Chocolate	HT-GC	To compare the traditional and non-traditional extraction techniques to remove fat quantitatively from plain chocolate.	[104]
Chocolate	LC-MS/MS	To determine the free FAs in chocolate.	[31]
Chocolate	Iodine coefficient and saponification coefficient	To establish the adulteration of chocolate by determining the iodine and saponification coefficients of the extracted fat.	[33]
Dark, milk and white chocolate	Microstructure, rheology and modelling	To distinguish between three different types of chocolate: dark, milk and white and compare their microscopic and rheological properties (basic and experimental).	[105]
Chocolate	HR-GC	To detect and quantify the CB equivalents in chocolate model systems.	[106]
Milk chocolate	GC and HPLC	To determine conjugated linoleic acid (CLA) concentrations in milk chocolate	[107]
Chocolate	GC-FID	To explore the physico-chemical, rheological, textural, and organoleptic characteristics of chocolate.	[108]
Chocolate milk drink	Low-pressure cold plasma and pH	To employ low-pressure cold plasma technology to process chocolate milk drinks.	[109]
Cocoa shell	HPLC-QTOF-MS	To determine the presence of cocoa shells by using mass spectrometry.	[110]
Cocoa beans	Hyperspectral imaging	To estimate accurately the total lipid content in individual intact cocoa beans using hyperspectral chemical imaging.	[111]

of 30 cocoa ecotypes from northeastern Peru that were assessed for total fat content and FA profiles. The ecotypes under study had an average total fat level ranging from 17.51 to 30.87 %. According to the findings, the ecotypes SJJ-1 and ACJ-11 from the San Martin and Amazonas regions, respectively, had the highest average total fat content (30.49 %). Depending on the size of the vectors, PCA showed that the most significant FAs in the CB were C16:0, C18:0, C20:0, C18:1, C18:2, and C18:3 [94]. Another study examined the FA composition of CBs made from commercial cocoa beans from Peru. The profiles of the major and minor FAs were extremely similar across all samples. The distribution and content of FAs were as follows: stearic acid (C18:0) (between 28.83 % and 33.44 %), oleic acid (C18:1) (between 33.03 % and 37.97 %), linoleic acid (C18:2) (between 2.49 % and 4.21 %), and linolenic acid (C18:3) (between 0.21 % and 0.31 %). The commercial cocoa beans from Peru that were used to make the CB contained high amounts of oleic, palmitic, and stearic acids, while it was revealed that CB from Ghana and Ecuador included the following amounts of FAs in unroasted cocoa beans: oleic acid (34.30–34.73 %), stearic acid (33.75–36.40 %), palmitic acid (25.02–27.61 %), linoleic acid (2.02–2.43 %), and linolenic acid (0.13–0.14 %) [81]. A different study attempted to identify lard in CB based on alterations in the FA content and triacylglycerol profile. CB was combined with 1–30 % (v/v) lard and analysed using a gas chromatography flame ionization detector. The results revealed that combining this fat and CB resulted in an increase in oleic acid (C18:1) and a decrease in palmitic acid (C16:0) and stearic acid (C18:0) levels as the fat concentration increased from 1 % to 30 %. Triglyceride LLLn, LLL, and OOL concentrations increased with the addition of lard [95]. Regarding the FA composition of bakery products, the majority of the foods examined had a total SFA content that represented more than 40 % of the total FA content. The two main FAs were palmitic and oleic acid, with the former constituting the majority of the FA in 14 of the 19 different varieties of biscuits. A chocolate sandwich with cream filling had a palmitic acid content of 22.3 %, whereas a fine herb cookie had a palmitic acid content of 50.6 %. The amounts of SFA, MUFA, and PUFA in a product depend on the type of vegetable oil or fat used in its formulation, which is generally chosen based on the sensory and textural expectations of consumers. Additionally, the main FAs in chocolate, palmitic and stearic acids, altered the qualitative attributes of the final product when the growing conditions of the cocoa beans was changed [96]. The total TFA values for biscuits, cakes, croissants, and wafers ranged between 0.7 and 25.8 g/100 g fat. More than 2 g TFA/100 g fat was present in 83.3 % of the items. Given that the ratio of *trans*-C18:1/(*trans*-C18:2 + *trans*-C18:3) was greater than 1, the source of TFA was partly hydrogenated oils [97]. Finally, when studying the composition of FAs in cocoa shells post-fermentation, it was found that the concentration of the SFAs and stearic acid (18:0), which were present in higher amounts in the non-fermented cocoa shells, had decreased by approximately 42 %, and that the concentration of the MUFAs, particularly oleic acid (18:1n9c), which was the predominant FA at that time, had increased by approximately 25 %. Oleic acid can lower LDL cholesterol levels and is also a potential preventative agent in food. The UK Department of Health states that values below 0.45 are harmful and that 0.40 is the ideal FAPU ratio for FAs in food. The FAPU ratios in Ghanaian and Ecuadorian cocoa are 1.72 and 1.65, respectively, which clearly represent a high FAPU-to-FA ratios. Because the FAPU ratios in this instance ranged from 0.09 to 0.32, the cocoa shell was found to be healthier than the cocoa bean [1].

Chromatographic methods offer a significant benefit because they allow the separation of mixed components without requiring prior knowledge of the chemical structures of the compounds. In contrast, spectroscopic methods such as IR and NMR rely heavily on the accuracy of theoretical spectra for interpreting the chemical information obtained from experimental data. A synopsis is presented in Table 4.

6. Other approaches for analysing cocoa butter: cocoa and its derivatives

Recently, chocolate has garnered attention because of its key ingredient, cocoa, and its high fat content, which typically surpasses 30 g of fat per 100 g of food. The presence of fats and antioxidants in cocoa is widely acknowledged. Many studies conducted over the last several years suggest that there is a connection between the antioxidant activity of procyanidins and the potential health benefits of consuming cocoa derivatives, particularly dark chocolate, with regard to degenerative and cardiovascular disorders [98,99]. Analysis of the fat in cocoa and its derivatives has generated considerable research over the past few decades, ranging from traditional procedures to more complex automated techniques. The bulk of previous research summarized in Table 5 recommends using tests to measure FAs, triacylglycerol components, minor components, and their spectroscopic properties, because these are already useful for detecting dietary fats [100].

7. Conclusion

This review focused on several spectroscopic and chromatographic techniques that have been employed to analyse the fat content of CB and its derivatives. The methodologies outlined in this review can be categorised into various groups based on the particular electromagnetic radiation or analyser employed. It is essential to understand the fundamental principles and constraints of these methods and to develop the capacity to select the most suitable method for a specific research objective in order proficiently analyse cocoa fats and their characteristics. Fats are obtained from many different sources of cocoa, such as cocoa shells, cocoa beans, and cocoa-based products, such as CB, chocolate, and baked goods. These fats originate from different geographical regions. To reduce the harmful effects of high levels of fatty acids and triglycerides on cardiovascular health and the resulting economic costs, it is crucial to emphasize the significance of identifying the source of the raw materials, assessing the purity of the various types of materials, and analysing their composition, chemical characteristics, and sensory attributes. Determining the attributes of the products using these various methods allows for the detection and deterrence of fraudulent behaviour. This phenomenon is observed in different chemical analysis methods, particularly spectroscopic techniques such as NIR and FTIR, which have demonstrated their efficacy and reliability in analysis but still require further improvement. Alternatively, ^{13}C NMR spectroscopy is a reliable method for identifying the primary FAs present in various oils, particularly CB. Significant advancements in extraction analysis techniques have mainly focused on improving the efficiency, precision, and responsiveness of the procedures, along with their application to multivariate data analysis. Typically, multivariate data analysis is employed to establish a structure for evaluating cocoa quality. On the other hand, chromatographic techniques, such as HPLC, GC-MS, and GC-FID, are the most sophisticated methods available for evaluating sensitivity and accuracy.

Ethics statements

Ethics approval for this review study is not required.

Data availability

Saudi Food and Drug Authority - King Saud University, Saudi Arabia.

CRediT authorship contribution statement

Razan F. Alotaibi: Writing – original draft, Methodology, Investigation, Formal analysis. **Hissah H. AlTilasi:** Writing – review & editing, Supervision. **Adibah M. Al-Mutairi:** Writing – review & editing, Supervision. **Hibah S. Alharbi:** Writing – review & editing, Supervision.

Declaration of competing interest

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

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References

[1] O.A. Lessa, N.d.S. Reis, S.G.F. Leite, M.L.E. Gutarra, A.O. Souza, S.A. Gualberto, J.R. de Oliveira, E. Aguiar-Oliveira, M. Franco, Effect of the solid state fermentation of cocoa shell on the secondary metabolites, antioxidant activity, and fatty acids, Food Sci. Biotechnol. 27 (2018) 107–113.
[2] N.N. Batista, D.P. de Andrade, C.L. Ramos, D.R. Dias, R.F. Schwan, Antioxidant capacity of cocoa beans and chocolate assessed by FTIR, Food Res. Int. 90 (2016) 313–319.

[3] C.W.B.d. Melo, M.d.J. Bandeira, L.F. Maciel, E.d.S. Bispo, C.O.d. Souza, S.E. Soares, Chemical composition and fatty acids profile of chocolates produced with different cocoa (Theobroma cacao L.) cultivars, Food Sci. Technol. 40 (2020) 326–333.
[4] Y.M. López Cuadra, M.Y. Cunias Rodríguez, Y.L. Carrasco Vega, El cacao peruano y su impacto en la economía nacional, Revista Universidad y Sociedad 12 (2020) 344–352.
[5] Y.C. Man, Z. Syahariza, M. Mirghani, S. Jinap, J. Bakar, Analysis of potential lard adulteration in chocolate and chocolate products using Fourier transform infrared spectroscopy, Food Chem. 90 (2005) 815–819.
[6] J.E. Villa, C.D. Pereira, S. Cadore, A novel, rapid and simple acid extraction for multielemental determination in chocolate bars, Microchem. J. 121 (2015) 199–204.
[7] M. Grassia, G. Salvatori, M. Roberti, D. Planeta, L. Cinquanta, Polyphenols, methylxanthines, fatty acids and minerals in cocoa beans and cocoa products, J. Food Meas. Char. 13 (2019) 1721–1728.
[8] G.R. Caponio, M.P. Lorusso, G.T. Sorrenti, V. Marcotrigiano, G. Difonzo, E. De Angelis, R. Guagnano, A.D. Ciaula, G. Diella, A.F. Logrieco, Chemical characterization, gastrointestinal motility and sensory evaluation of dark chocolate: a nutraceutical boosting consumers' health, Nutrients 12 (2020) 939.
[9] G. Talbot, Chocolate and Cocoa Butter—Structure and Composition, Cocoa Butter and Related Compounds, Elsevier, 2012, pp. 1–33.
[10] S. Beegum Pp, R. Pandiselvam, R. Sv, S. P, A. Nooh, N. S, A. Gupta, E. Varghese, D. Balasubramanian, E.S. Apshara, Sensorial, textural, and nutritional attributes of coconut sugar and cocoa solids based "bean-to-bar" dark chocolate, J. Texture Stud. 53 (2022) 870–882.
[11] N.E. Maurer, L. Rodriguez-Saona, Rapid assessment of quality parameters in cocoa butter using ATR-MIR spectroscopy and multivariate analysis, J. Am. Oil Chem. Soc. 90 (2013) 475–481.
[12] O.S. Toker, I. Palabiyik, N. Konar, Chocolate quality and conching, Trends Food Sci. Technol. 91 (2019) 446–453.
[13] M. Forte, S. Currò, D. Van de Walle, K. Dewettinck, M. Mirisola, L. Fasolato, P. Carletti, Quality evaluation of fair-trade cocoa beans from different origins using portable near-infrared spectroscopy (NIRS), Foods 12 (2022) 4.
[14] N. Akramzadeh, H. Hosseini, Z. Pilevar, N. Karimian Khosroshahi, K. Khosravi-Darani, R. Komeyli, F.J. Barba, A. Pugliese, M.M. Poojary, A.M. Khaneghah, Physicochemical properties of novel non-meat sausages containing natural colorants and preservatives, J. Food Process. Preserv. 42 (2018) e13660.
[15] M. Momtaz, S.Y. Bubli, M.S. Khan, Mechanisms and health aspects of food adulteration: a comprehensive review, Foods 12 (2023) 199.
[16] K. Havriushenko, F. Gladkiy, Analysis of the ethyl stearate properties as a new alternative to cocoa butter, Technol. Audit Prod. Reserves 6 (2020) 56.
[17] V.K. Shukla, Confectionery lipids, Bailey's industrial oil and fat products 4 (2005) 159–173.
[18] B. Naik, V. Kumar, Cocoa butter and its alternatives: a review, Journal of Bioresource Engineering and Technology 1 (2014) 7–17.
[19] M. Jahurul, I. Zaidul, N. Norulaini, F. Sahena, S. Jinap, J. Azmir, K. Sharif, A.M. Omar, Cocoa butter fats and possibilities of substitution in food products concerning cocoa varieties, alternative sources, extraction methods, composition, and characteristics, J. Food Eng. 117 (2013) 467–476.
[20] A. Servent, R. Boulanger, F. Davrieux, M.-N. Pinot, E. Tardan, N. Forestier-Chiron, C. Hue, Assessment of cocoa (Theobroma cacao L.) butter content and composition throughout fermentations, Food Res. Int. 107 (2018) 675–682.
[21] S. Lohumi, S. Lee, H. Lee, B.-K. Cho, A review of vibrational spectroscopic techniques for the detection of food authenticity and adulteration, Trends Food Sci. Technol. 46 (2015) 85–98.
[22] R. Karoui, Food Authenticity and Fraud, Chemical Analysis of Food, Elsevier, 2020, pp. 579–608.
[23] T. Suri, S. Basu, Heat resistant chocolate development for subtropical and tropical climates: a review, Crit. Rev. Food Sci. Nutr. 62 (2022) 5603–5622.
[24] M.D. Alvarez, S. Cofrades, M. Espert, A. Salvador, T. Sanz, Thermorheological characterization of healthier reduced-fat cocoa butter formulated by substitution with a hydroxypropyl methylcellulose (HPMC)-based oleogel, Foods 10 (2021) 793.
[25] Z. Piravi Vanak, S. Abedinzadeh, S. Azadmard-damirchi, M. Gharachorloo, Analyzing the Chemical Composition and Quality Attributes of Cocoa Butter from Different Producers: A Comparative Study, Innovative Food Technologies 11 (2023) 35–45.
[26] N. De Clercq, S. Kadivar, D. Van de Walle, S. De Pelsmaeker, X. Ghellynck, K. Dewettinck, Functionality of cocoa butter equivalents in chocolate products, Eur. Food Res. Technol. 243 (2017) 309–321.
[27] A. Bertucco, G. Vetter, High Pressure Process Technology: Fundamentals and Applications, Elsevier, 2001.
[28] A. Bahari, C.C. Akoh, Synthesis of a cocoa butter equivalent by enzymatic interesterification of illipe butter and palm midfraction, J. Am. Oil Chem. Soc. 95 (2018) 547–555.
[29] M.F. Colella, N. Marino, C. Oliviero Rossi, L. Seta, P. Caputo, G. De Luca, Triacylglycerol composition and chemical-physical properties of cocoa butter and its derivatives: NMR, DSC, X-ray, rheological investigation, Int. J. Mol. Sci. 24 (2023) 2090.
[30] L. Jarukas, G. Kuraite, J. Baranauskaite, M. Marksa, I. Bezruk, L. Ivanauskas, Optimization and validation of the GC/FID method for the quantification of fatty acids in bee products, Appl. Sci. 11 (2020) 83.
[31] D. Perret, A. Gentili, S. Marchese, M. Sergi, L. Caporossi, Determination of free fatty acids in chocolate by liquid chromatography with tandem mass spectrometry, Rapid Commun. Mass Spectrom. 18 (2004) 1989–1994.
[32] D. Bergenholm, M. Gossing, Y. Wei, V. Siewers, J. Nielsen, Modulation of saturation and chain length of fatty acids in Saccharomyces cerevisiae for production of cocoa butter-like lipids, Biotechnol. Bioeng. 115 (2018) 932–942.
[33] B.M. Gabriela, Tracking down the chocolate adulteration by replacing the cocoa butter, Annals: Food Sci. Technol. 13 (2012) 15–18.
[34] F. Visioli, A. Poli, Fatty acids and cardiovascular risk. Evidence, lack of evidence, and diligence, Nutrients 12 (2020) 3782.
[35] A. Astrup, F. Magkos, D.M. Bier, J.T. Brenna, M.C. de Oliveira Otto, J.O. Hill, J.C. King, A. Mente, J.M. Ordovas, J.S. Volek, Saturated fats and health: a reassessment and proposal for food-based recommendations: JACC state-of-the-art review, J. Am. Coll. Cardiol. 76 (2020) 844–857.
[36] T. Jeyarani, T. Banerjee, R. Ravi, A.G. Krishna, Omega-3 fatty acids enriched chocolate spreads using soybean and coconut oils, J. Food Sci. Technol. 52 (2015) 1082–1088.
[37] U. Bhagat, U.N. Das, State of the art paper Potential role of dietary lipids in the prophylaxis of some clinical conditions, Arch. Med. Sci. 11 (2015) 807–818.
[38] M.A. Quelal-Vásconez, M.J. Lerma-García, É. Pérez-Esteve, P. Talens, J.M. Barat, Roadmap of cocoa quality and authenticity control in the industry: a review of conventional and alternative methods, Compr. Rev. Food Sci. Food Saf. 19 (2020) 448–478.
[39] J.A. Stobbs, E. Pensini, S.M. Ghazani, A.F. Leontowich, A. Quirk, K. Tu, S. Prévost, N. Mahmoudi, A.-L. Fameau, A.G. Marangoni, Phospholipid self-assembly in cocoa butter provides a crystallizing surface for seeding the form V polymorph in chocolate, Cryst. Growth Des. 24 (2024) 2685–2699.
[40] L. Metilli, A. Lazidis, M. Francis, S. Marty-Terrade, J. Ray, E. Simone, The effect of crystallization conditions on the structural properties of oleofoams made of cocoa butter crystals and high oleic sunflower oil, Cryst. Growth Des. 21 (2021) 1562–1575.
[41] B. Panchal, T. Truong, S. Prakash, N. Bansal, B. Bhandari, Influence of emulsifiers and dairy ingredients on manufacturing, microstructure, and physical properties of butter, Foods 10 (2021) 1140.
[42] R. Mohamad, B.A.P. Agus, N. Hussain, Changes of phytosterols, rheology, antioxidant activity and emulsion stability of salad dressing with cocoa butter during storage, Food Technol. Biotechnol. 57 (2019) 59.
[43] F. Zarabadipour, Z. Piravi-Vanak, M. Aminifar, Evaluation of sterol composition in different formulations of cocoa milk as milk fat purity indicator, Food Sci. Technol. 41 (2020) 519–523.
[44] D. Gegiou, K. Staphylakis, Detection of cocoa butter equivalents in chocolate, J. Am. Oil Chem. Soc. 62 (1985) 1047–1051.
[45] M.H. Rahuman, S. Muthu, B. Raajaraman, M. Raja, H. Umamahesvari, Investigations on 2-(4-Cyanophenylamino) acetic acid by FT-IR, FT-Raman, NMR and UV-Vis spectroscopy, DFT (NBO, HOMO-LUMO, MEP and Fukui function) and molecular docking studies, Heliyon 6 (2020) e04976.
[46] J. Rizikiana, O.P. Pratama, D. Lestari, Statistical mixture design for modelling and optimization of feed mixture in the chemical interesterification to produce cocoa butter alternatives, in: IOP Conference Series: Materials Science and Engineering, IOP Publishing, 2021 012036.
[47] K. Yamada, M. Ibuki, T. McBrayer, Cocoa Butter, Cocoa Butter Equivalents, and Cocoa Butter Replacers, Healthful Lipids, AOCS Publishing, 2019, pp. 642–664.
[48] R.A. Pratiwi, A.B.D. Nandiyanto, How to read and interpret UV-VIS spectrophotometric results in determining the structure of chemical compounds, Indonesian Journal of Educational Research and Technology 2 (2022) 1–20.

[49] M.S.H. Akash, K. Rehman, M.S.H. Akash, K. Rehman, Ultraviolet-visible (UV-VIS) spectroscopy, Essentials of pharmaceutical analysis (2020) 29–56.
[50] N. El Darra, H.N. Rajha, F. Saleh, R. Al-Oweini, R.G. Maroun, N. Louka, Food fraud detection in commercial pomegranate molasses syrups by UV–VIS spectroscopy, ATR-FTIR spectroscopy and HPLC methods, Food Control 78 (2017) 132–137.
[51] A. Dankowska, Data fusion of fluorescence and UV spectroscopies improves the detection of cocoa butter adulteration, Eur. J. Lipid Sci. Technol. 119 (2017) 1600268.
[52] A.B.D. Nandiyanto, R. Oktiani, R. Ragadhita, How to read and interpret FTIR spectroscope of organic material, Indonesian Journal of Science and Technology 4 (2019) 97–118.
[53] S.K. Pirutin, S. Jia, A.I. Yusipovich, M.A. Shank, E.Y. Parshina, A.B. Rubin, Vibrational spectroscopy as a tool for bioanalytical and biomonitoring studies, Int. J. Mol. Sci. 24 (2023) 6947.
[54] M. Kamal, A. Munawar, M. Sulaiman, Comparison of principal component and partial least square regression method in NIRS data analysis for cocoa bean quality assessment, in: IOP Conference Series: Earth and Environmental Science, IOP Publishing, 2021 012058.
[55] C. Berghian-Grosan, D.A. Magdas, Raman spectroscopy and machine-learning for edible oils evaluation, Talanta 218 (2020) 121176.
[56] H. Wang, Y. Xin, H. Ma, P. Fang, C. Li, X. Wan, Z. He, J. Jia, Z. Ling, Rapid detection of Chinese-specific peony seed oil by using confocal Raman spectroscopy and chemometrics, Food Chem. 362 (2021) 130041.
[57] L. Hasanah, C. Julian, B. Mulyanti, A. Aransa, R. Sumatri, M. Johari, J. David, A. Mohmad, Photoluminescence and Raman scattering of GaAs, Sains Malays. 49 (2020) 2559–2564.
[58] C.L. Jahncke, W. Zhang, B.M. DeMuynck, A.D. Hill, Exploring resonance Raman scattering with 4-nitrophenol, J. Chem. Educ. 99 (2022) 3233–3241.
[59] R.C. Castro, D.S. Ribeiro, J.L. Santos, R.N. Páscoa, The use of in-situ Raman spectroscopy to monitor at real time the quality of different types of edible oils under frying conditions, Food Control 136 (2022) 108879.
[60] A. Windarsih, L. Arsanti Lestari, Y. Erwanto, A. Rosiana Putri, Irnawati, N. Ahmad Fadzillah, N. Rahmawati, A. Rohman, Application of Raman spectroscopy and chemometrics for quality controls of fats and oils: a review, Food Rev. Int. 39 (2023) 3906–3925.
[61] H.G. Edwards, S.E.J. Villar, L.F.C. de Oliveira, M. Le Hyaric, Analytical Raman spectroscopic study of cacao seeds and their chemical extracts, Anal. Chim. Acta 538 (2005) 175–180.
[62] L.N. de Oliveira, J.C.d.R. Castro, M.A.L. de Oliveira, L.F.C. de Oliveira, Lipid characterization of white, dark, and milk chocolates by FT-Raman spectroscopy and capillary zone electrophoresis, J. AOAC Int. 98 (2015) 1598–1607.
[63] H. Yan, M.D. Neves, B.M. Wise, I.A. Moraes, D.F. Barbin, H.W. Siesler, The application of handheld near-infrared spectroscopy and Raman spectroscopic imaging for the identification and quality control of food products, Molecules 28 (2023) 7891.
[64] F. Tao, M. Ngadi, Recent advances in rapid and nondestructive determination of fat content and fatty acids composition of muscle foods, Crit. Rev. Food Sci. Nutr. 58 (2018) 1565–1593.
[65] C. Hernández-Hernández, V.M. Fernández-Cabanás, G. Rodríguez-Gutiérrez, Á. Fernández-Prior, A. Morales-Sillero, Rapid screening of unground cocoa beans based on their content of bioactive compounds by NIR spectroscopy, Food Control 131 (2022) 108347.
[66] V.A. Lorenz-Fonfria, Infrared difference spectroscopy of proteins: from bands to bonds, Chem. Rev. 120 (2020) 3466–3576.
[67] E. Teye, E. Anyidoho, R. Agbemafle, L.K. Sam-Amoah, C. Elliott, Cocoa bean and cocoa bean products quality evaluation by NIR spectroscopy and chemometrics: a review, Infrared Phys. Technol. 104 (2020) 103127.
[68] F. Negrão Ferreira, G.C. Albuquerque Chagas-Junior, M. Santana de Oliveira, J. Rodrigues Barbosa, M.E. Chaves Oliveira, A. Santos Lopes, Geographical discrimination of ground amazon cocoa by near-infrared spectroscopy: influence of sample preparation, J. Food Qual. 2022 (2022).
[69] P. Koczoń, J.T. Hołaj-Krzak, B.K. Palani, T. Bolewski, J. Dąbrowski, B.J. Bartyzel, E. Gruczyńska-Sękowska, The analytical possibilities of FT-IR spectroscopy powered by vibrating molecules, Int. J. Mol. Sci. 24 (2023) 1013.
[70] A. Weber, B. Hoplight, R. Ogilvie, C. Muro, S.R. Khandasammy, L. Pérez-Almodóvar, S. Sears, I.K. Lednev, Innovative vibrational spectroscopy research for forensic application, Anal. Chem. 95 (2023) 167–205.
[71] A.A. Câmara, T.D. Nguyen, R. Saurel, C. Sandt, C. Peltier, L. Dujourdy, F. Husson, Biophysical stress responses of the yeast Lachancea thermotolerans during dehydration using synchrotron-FTIR microspectroscopy, Front. Microbiol. 11 (2020) 521135.
[72] B. Feng, H. Shi, F. Xu, F. Hu, J. He, H. Yang, C. Ding, W. Chen, S. Yu, FTIR-assisted MALDI-TOF MS for the identification and typing of bacteria, Anal. Chim. Acta 1111 (2020) 75–82.
[73] R. Goodacre, E. Anklam, Fourier transform infrared spectroscopy and chemometrics as a tool for the rapid detection of other vegetable fats mixed in cocoa butter, J. Am. Oil Chem. Soc. 78 (2001) 993–1000.
[74] S.B. Sa'ari, Y. Che Man, Rapid detection of lard in chocolate and chocolate-based food products using Fourier transform infrared spectroscopy, J. Trop. Agric. Food Sci. 44 (2016) 253–263.
[75] E. San Martín, A. Avenoza, J.M. Peregrina, J.H. Busto, Solvent-based strategy improves the direct determination of key parameters in edible fats and oils by 1H NMR, J. Sci. Food Agric. 100 (2020) 1726–1734.
[76] E. Zailer, Holistic Control of Fats and Oils by NMR Spectroscopy, 2019.
[77] E. Truzzi, L. Marchetti, A. Fratagnoli, M.C. Rossi, D. Bertelli, Novel application of 1H NMR spectroscopy coupled with chemometrics for the authentication of dark chocolate, Food Chem. 404 (2023) 134522.
[78] D. Diomandé, T.T. Dro, J.S. Akpa, S. Virginie, I. Tea, G.S. Remaud, Quantitative measurement of the chemical composition of fatty acid of cocoa butter and the isotopic content of glycerol contained in cocoa butter by the NMR 13C from the INEPT sequence and characterization of the geographical origin of the cocoa, Am. J. Anal. Chem. 13 (2022) 79–95.
[79] A.H. Ali, High-performance liquid chromatography (HPLC): a review, Ann. Adv. Chem 6 (2022) 10–20.
[80] L. Gu, S.E. House, X. Wu, B. Ou, R.L. Prior, Procyanidin and catechin contents and antioxidant capacity of cocoa and chocolate products, J. Agric. Food Chem. 54 (2006) 4057–4061.
[81] F. Ramos-Escudero, S. Casimiro-Gonzales, Á. Fernández-Prior, K.C. Chávez, J. Gómez-Mendoza, L. de la Fuente-Carmelino, A.M. Muñoz, Colour, fatty acids, bioactive compounds, and total antioxidant capacity in commercial cocoa beans (Theobroma cacao L.), LWT 147 (2021) 111629.
[82] M. Greño, M. Plaza, M.L. Marina, M.C. Puyana, Untargeted HPLC-MS-based metabolomics approach to reveal cocoa powder adulterations, Food Chem. 402 (2023) 134209.
[83] E. Sahin, E. Capanoglu, A.C. Karaca, A.B. Demirkoz, Extraction of Cocoa Butter from By-Product Cocoa Bean Shells by Using SC-CO2 Extraction and Investigation of Components and Antioxidant Capacities, Agric. Food, Chem 2 (2022) 27–40.
[84] N. Andriani, R. Yenrina, N. Nazir, Physicochemical, fatty acid and sensory profile of cocoa butter produced from fermented and non-fermented cocoa butter, AJARCDE (Asian Journal of Applied Research for Community Development and Empowerment) 4 (2020) 6–14.
[85] S. Kadivar, N. De Clercq, B.P. Nusantoro, T.T. Le, K. Dewettinck, Development of an offline bidimensional high-performance liquid chromatography method for analysis of stereospecific triacylglycerols in cocoa butter equivalents, J. Agric. Food Chem. 61 (2013) 7896–7903.
[86] M.J. Kadhim, A.A. Sosa, I.H. Hameed, Evaluation of anti-bacterial activity and bioactive chemical analysis of Ocimum basilicum using Fourier transform infrared (FT-IR) and gas chromatography-mass spectrometry (GC-MS) techniques, J. Pharmacogn. Phytotherapy 8 (2016) 127–146.
[87] M. Hussein, Characterization of Bioactive Chemical Compounds from Aspergillus terreus and Evaluation of Antibacterial and Antifungal Activity, International Journal of Pharmacognosy and Phytochemical Research 8 (2016) 889–905.
[88] H.J. Al-Tameme, M.Y. Hadi, I.H. Hameed, Phytochemical analysis of Urtica dioica leaves by fourier-transform infrared spectroscopy and gas chromatography-mass spectrometry, J. Pharmacogn. Phytotherapy 7 (2015) 238–252.
[89] G.J. Mohammed, A.M. Omran, H.M. Hussein, Antibacterial and phytochemical analysis of Piper nigrum using gas chromatography-mass Spectrum and Fourier-transform infrared spectroscopy, International Journal of Pharmacognosy and Phytochemical Research 8 (2016) 977–996.
[90] M. Torres-Moreno, E. Torrescasana, J. Salas-Salvadó, C. Blanch, Nutritional composition and fatty acids profile in cocoa beans and chocolates with different geographical origin and processing conditions, Food Chem. 166 (2015) 125–132.

[91] S. Suparman, The use of Fourier transform infrared spectroscopy (FTIR) and gas chromatography mass spectroscopy (GCMS) for halal authentication in imported chocolate with various variants, Journal of Food and Pharmaceutical Sciences 3 (2015).
[92] M.M. Mossoba, J. Moss, J.K. Kramer, Trans fat labeling and levels in US foods: assessment of gas chromatographic and infrared spectroscopic techniques for regulatory compliance, J. AOAC Int. 92 (2009) 1284–1300.
[93] W.H. Organization, Report of the WHO expert consultation on the WHO protocol for measuring trans-fatty acids in foods, held virtually on 27 and 30 June 2022, World Health Organization (2023).
[94] M. Oliva-Cruz, P.L. Mori-Culqui, A.C. Caetano, M. Goñas, N.C. Vilca-Valqui, S.G. Chavez, Total fat content and fatty acid profile of fine-aroma cocoa from northeastern Peru, Front. Nutr. 8 (2021) 677000.
[95] M. Azir, S. Abbasiliasi, T.A. Tengku Ibrahim, Y.N.A. Manaf, A.Q. Sazili, S. Mustafa, Detection of lard in cocoa butter—its fatty acid composition, triacylglycerol profiles, and thermal characteristics, Foods 6 (2017) 98.
[96] P.Y. Omeroglu, T. Ozdal, Fatty acid composition of sweet bakery goods and chocolate products and evaluation of overall nutritional quality in relation to the food label information, J. Food Compos. Anal. 88 (2020) 103438.
[97] C. Saadeh, I. Toufeili, M.Z. Habbal, L. Nasreddine, Fatty acid composition including trans-fatty acids in selected cereal-based baked snacks from Lebanon, J. Food Compos. Anal. 41 (2015) 81–85.
[98] C. Paz-Yépez, I. Peinado, A. Heredia, A. Andrés, Lipids digestibility and polyphenols release under in vitro digestion of dark, milk and white chocolate, J. Funct.Foods 52 (2019) 196–203.
[99] S. Davinelli, G. Corbi, S. Righetti, B. Sears, H.H. Olarte, D. Grassi, G. Scapagnini, Cardioprotection by cocoa polyphenols and ω-3 fatty acids: a disease-prevention perspective on aging-associated cardiovascular risk, J. Med. Food 21 (2018) 1060–1069.
[100] J. Marikkar, M. Mirghani, I. Jaswir, Application of chromatographic and infra-red spectroscopic techniques for detection of adulteration in food lipids: a review, Journal of Food Chemistry and Nanotechnology 2 (2016) 32–41.
[101] L. Dohnalova, P. Bucek, P. Vobornik, V. Dohnal, Determination of nickel in hydrogenated fats and selected chocolate bars in Czech Republic, Food Chem. 217 (2017) 456–460.
[102] M. Bononi, G. Quaglia, F. Tateo, Easy extraction method to evaluate δ13C vanillin by liquid chromatography–isotopic ratio mass spectrometry in chocolate bars and chocolate snack foods, J. Agric. Food Chem. 63 (2015) 4777–4781.
[103] C.A. Hartwig, R.M. Pereira, F.S. Rondan, S.M. Cruz, F.A. Duarte, E.M. Flores, M.F. Mesko, The synergic effect of microwave and ultraviolet radiation for chocolate digestion and further determination of As, Cd, Ni and Pb by ICP-MS, J. Anal. Atomic Spectrom. 31 (2016) 523–530.
[104] C. Simoneau, C. Naudin, P. Hannaert, E. Anklam, Comparison of classical and alternative extraction methods for the quantitative extraction of fat from plain chocolate and the subsequent application to the detection of added foreign fats to plain chocolate formulations, Food Res. Int. 33 (2000) 733–741.
[105] V. Glicerina, F. Balestra, M. Dalla Rosa, S. Romani, Microstructural and rheological characteristics of dark, milk and white chocolate: a comparative study, J. Food Eng. 169 (2016) 165–171.
[106] C. Simoneau, P. Hannaert, E. Anklam, Detection and quantification of cocoa butter equivalents in chocolate model systems: analysis of triglyceride profiles by high resolution GC, Food Chem. 65 (1999) 111–116.
[107] W.J. Hurst, S.M. Tarka, G. Dobson, C.M. Reid, Determination of conjugated linoleic acid (CLA) concentrations in milk chocolate, J. Agric. Food Chem. 49 (2001) 1264–1265.
[108] D. Żyżelewicz, G. Budryn, J. Oracz, H. Antolak, D. Kręgiel, M. Kaczmarska, The effect on bioactive components and characteristics of chocolate by functionalization with raw cocoa beans, Food Res. Int. 113 (2018) 234–244.
[109] N.M. Coutinho, M.R. Silveira, L.M. Fernandes, J. Moraes, T.C. Pimentel, M.Q. Freitas, M.C. Silva, R.S. Raices, C.S. Ranadheera, F.O. Borges, Processing chocolate milk drink by low-pressure cold plasma technology, Food Chem. 278 (2019) 276–283.
[110] N. Cain, O. Alka, T. Segelke, K. von Wuthenau, O. Kohlbacher, M. Fischer, Food fingerprinting: mass spectrometric determination of the cocoa shell content (Theobroma cacao L.) in cocoa products by HPLC-QTOF-MS, Food Chem. 298 (2019) 125013.
[111] N. Caporaso, M.B. Whitworth, I.D. Fisk, Total lipid prediction in single intact cocoa beans by hyperspectral chemical imaging, Food Chem. 344 (2021) 128663.