FoodIntegrity Handbook

A Guide to Food Authenticity Issues and Analytical Solutions

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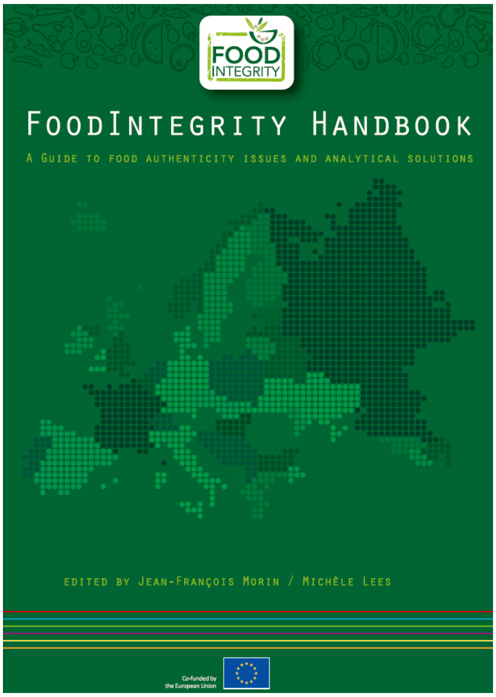

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Cocoa, cocoa preparation, chocolate and chocolate-based confectionery

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General overview of the product

Although there are many products of cocoa, most of the cocoa production is used to manufacture a product that is often associated with many positive feelings, i.e. chocolate [1]. Nowadays, chocolate represents one of the most popular and widely consumed confectionery products. In 2017 its consumption reached 8.8 kg per person and per year in Switzerland, the biggest European consumer [2]. Chocolate is not only a common confectionery product, it also plays an important social role being an inherent part of many celebrations. Currently, an increasing number of various specialties and gourmet products is appearing on the market and they are in high demand. Some of them are even crossing the confectionery industry line by aiming at consumer interest in health. The products benefit from the positive effect of cocoa (e.g. chocolate with 100 % cocoa solids) as well as from various additional ingredients (e.g. dried berries, herbs, seeds, nuts, dietary fibre or probiotics) [3,4]. Another group of products focuses on the ethical and ecological aspects of production (Fairtrade and/or organic products). By introducing various novel products, the cocoa industry is responding promptly to modern trends in order to keep enticing its consumers. However, these dynamic changes may be extremely challenging for producers who are pushed to offer items that are acceptable both in terms of quantity as well as quality [5].

An enormous growth in cocoa production has occurred since the second half of the 20th century, from 1 695 in 1980/1981 to about 4 587 thousand tonnes in 2017/2018. This corresponds to a steady increase in cocoa and chocolate confectionery consumption. Global demand is still growing annually by 2-3 % [6–9].

The cocoa value chain faces a number of challenges in growing and selling this crop. Cocoa production is located in a limited geographical area around the equator, mostly in developing countries, while its consumption is focused almost exclusively in developed countries. For many of the producers, cocoa is a vital part of their economic income. Farmers often have limited knowledge of modern farming techniques and farm management skills as well as limited access to finance that would enable them to purchase input supplies and quality planting material. Other challenges that impede productivity include aging trees, decline in soil fertility and the struggle with pests and diseases that attack cocoa trees [6].

Due to the increasing importance of social, environmental and economic issues, more focus is aimed at the traceability and sustainability of cocoa production [10]. Cocoa is predominantly a smallholder crop, as more than 90 % of world cocoa production originates from small farms. In

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Africa and Asia, a typical smallholder cocoa farm covers only 2 to 5 hectares of land. In a meaningful concept of sustainability, consumption is of equal importance to production. A sustainable world cocoa economy implies an integrated value chain in which all stakeholders develop and promote appropriate policies to achieve levels of production, processing and consumption that are economically viable, environmentally sound and socially responsible for the benefit of present and future generations, with the aim of improving productivity and profitability in the cocoa value chain for all stakeholders concerned, in particular for the smallholder producers. Basic principles are given in the Cocoa Agreement by the International Cocoa Organisation describing arrangements between producing and consuming countries to safeguard markets and raise average prices to stabilise trade, supplies and prices of cocoa [11]. CAOBISCO (Association of Chocolate, Biscuit and Confectionery Industries of Europe), the European Cocoa Association (ECA) and the Federation of Cocoa Commerce (FCC) are committed to working towards more sustainable cocoa which complies with such requirements for benefit of the consumer, the manufacturer and the farmer [10].

To support the sustainability of cocoa production, independent certification schemes have been established to provide increased transparency and responsibility in cocoa supply chains, providing farmers with the resources they need and helping them to manage their farms professionally, and in turn be rewarded for sustainable production and for providing consumers with products they can enjoy and trust. Examples of these certifications are Fairtrade [12], UTZ Certified [13] and Rainforest Alliance Certified [14].

1. Product Identity

1.1. Definition of the product and manufacturing process

A wide range of cocoa products originates from the seeds of the cocoa tree, *Theobroma cacao* L. Cocoa trees are grown in a narrow band around the equator (approximately 20° north and 20° south), which goes through four continents: Africa, Asia, Australia and Oceania, and South America. Africa produces 73 % of the world production, followed by America with less than 17 %, and Asia and Oceania at about 10 % [15]. For a long time, most cocoa production has been concentrated in 7 countries: Ivory Coast, Ghana, Indonesia, Cameroon, Nigeria, Brazil and Ecuador.

The cocoa tree has four main varieties (some of which are bound to a particular geographical region) and several hybrids, each of which possesses a unique potential for flavour development. In terms of world trade, the quality of cocoa beans is divided into two categories (i) ‘fine’ or ‘flavour’ and (ii) ‘bulk’ or ‘ordinary’. The difference between ‘fine’ or ‘flavour’ cocoa and ‘bulk’ cocoa is in the flavour rather than in other quality factors [16]. Forastero accounts for the most of ‘bulk’ cocoa production and is referred to as a basic variety. Criollo, Trinitario and a rare variety Nacional (last producing well-known Arriba beans) are considered ‘fine or flavour’ cocoas and are used for gourmet chocolates [1,5,17]. However, it is not only variety that influences flavour development, which is also affected by other factors such as a growing locality and conditions during growth and harvesting. Moreover, the final flavour and taste of cocoa products are highly dependent on individual processing stages and conditions. The processing is thus very important for final product quality, though it may not be necessarily related to its authenticity. While the term quality has different associations, authenticity is always strictly related to true product identity. Especially in the case of chocolate, consumers may have different preferences and expectations, often in relation to their geographical regions. When assessing chocolate authenticity, tracing the initial ingredients may be much harder due to these differences in manufacturing processes and their complexity [5].

To understand the identity of individual cocoa products, it is important to firstly explain the cocoa manufacturing process. This can be divided into two stages, firstly cocoa processing, and, secondly, chocolate manufacturing. However, both of them are often directly connected since most cocoa is used to make chocolate. After harvest, **cocoa beans** are released from **cocoa pods** (fruit) and then cleaned of any extraneous matter by blowers and sieves. The fresh cocoa beans are then left to ferment under the action of naturally present yeasts. During this process, which is especially important for the development of main flavour precursors, the beans change colour from purple to brown. To prevent the growth of moulds and reduce microbial contamination, the fermented beans are dried and then roasted. During roasting, the beans also gain additional flavour. Loosened hard shells are then removed from the beans (winnowing) to reveal **cocoa nibs** (or, alternatively, deshelling may be performed prior to the roasting). The nibs are ground to a homogenous paste called **cocoa liquor** (or by a number of other terms, such as cocoa mass, cocoa paste, chocolate paste). This paste can be used directly in products such as chocolate or pressed to separate **cocoa butter** (fat) from cocoa solids (**cocoa cake**). Crushing the cocoa cake will result in natural **cocoa powder**. An optional process, typical for Dutch cocoa powder, contains an alkalising step using potassium or sodium carbonate, which leads to lower acidity, a darker colour, more intense flavour, milder taste and better dispersibility in water.

Cocoa is traded at different stages of this process and intermediates/products may differ significantly in composition; thus, the process of authentication is a complex procedure involving various steps for different products.

In chocolate manufacturing, the first step is to mix all its ingredients together while applying moderate heat to melt the cocoa butter. Additional steps, such as refining and conching, are carried out to achieve a smooth texture and intense flavour. Finally, a tempering step occurs which is important to obtain good surface gloss, a snap and a stable structure resistant to fat bloom. All manufacturing processes have a strong influence on the final product quality and, due to their complexity, can make authentication very difficult [5,17,18]. Regarding the ingredients used within the manufacturing process, there are three basic types of chocolate: dark, milk, and white. Dark chocolate is a complex food product in which sugar crystals and non-fat cocoa particles are surrounded by a continuous phase of crystalline and liquid cocoa butter. Milk chocolate is a complex rheological system having solid particles (non-fat cocoa, milk and sugar particles) dispersed in cocoa butter, which represents the fat phase [19]. White chocolate has a similar composition to that of milk chocolate, but the cocoa is represented exclusively by cocoa butter.

1.2. Current standards of identity or related legislation

Various standards of identity and legislation are related to cocoa and cocoa products with some minor differences, often due to different habits in various geographical regions [20]. Most of them define compositional requirements, whereas product processing is not specified in detail.

1.2.1. In the European Union

In the EU, the main legal document related to cocoa and chocolate products is Directive 2000/36/EC [21]. It specifies the categories of the products (sales names) and requirements for their composition and labelling. This latest Directive is much simpler than the earlier Directive 73/241/EEC and its main role has been to harmonise legislation all over the EU. The main difference, though, is that it has authorised the use of other vegetable fats in chocolate, up to a level of 5 %, which previously had only been acceptable in seven Member states, such as the United Kingdom or Austria. However, only six specified fats, the so-called cocoa butter equivalents (CBEs), without any enzymatic modifications, can be used (illipe, palm-oil, sal, shea, kokum gurgi and mango kernel), together with the mention "contains vegetable fats in addition to cocoa butter" on the product label. The list of specified products has also been reduced together with their detailed descriptions, such as "cocoa beans", "cocoa nibs", "cocoa mass" or "cocoa press cake". Products specified in the new Directive are "cocoa butter", "cocoa powder", "drinking chocolate", "milk chocolate", "family milk chocolate", "white chocolate" among others. According to the definitions, for instance, cocoa butter is described as the fat obtained from cocoa beans or parts of cocoa beans with the specified content of free fatty acids and unsaponifiable matter; and chocolate is defined simply as the product obtained from cocoa products and sugars which contains not less than 35 % total dry cocoa solids, including not less than 18 % cocoa butter and not less than 14 % dry non-fat cocoa solids. There are slight differences when the name is supplemented by any of the specified words (such as vermicelli, flakes, couverture, gianduja nut). For most cocoa and chocolate products, their labelling must indicate their total dry cocoa solids content. Moreover, the Directive authorises the addition of other edible substances (with the exception of flour, starch or animal fat other than milk fat) up to 40 % of the total weight of finished chocolate products, while the content of cocoa butter and cocoa solids still has to be calculated after deducting these substances. Various flavourings, if they do not imitate the taste of chocolate or milk, may also be added to several cocoa/chocolate products. The use and amount of sugar in chocolate products are no longer restricted; any sugars intended for human consumption can be used. Additives that are applicable for cocoa and chocolate products are specified in a separate, general document on food additives, Regulation (EC) No. 1333/2008 [22] as amended. This document lists various additives authorised for certain foods and specifies their maximum levels in the products (or their use according to the principle of *quantum satis (*q.s.*)*, meaning the minimum level for achieving the desired effect). For cocoa and chocolate products, common additives are emulsifiers and acidity regulators. Of emulsifiers, lecithins or mono- and di-glycerides of fatty acids are applicable at q.s. levels, while polyglycerol polyricinoleate has a maximum level of 5 000 mg/kg and ammonium phosphatides of 10 000 mg/kg. Acidity regulators include carbonates, hydroxides, magnesium oxide and citric acid. More attractive surface gloss can be achieved by the use of glazing agents, such as gum arabic, carnauba wax, shellac or pectins. Products with reduced energy or no added sugar can contain various polyols (e.g. sorbitol, mannitol, maltitol) or sweeteners (e.g. aspartame, acesulfame K, saccharin, sucralose and steviol glycosides). No food colour is permitted in cocoa and chocolate products. The EU Directive does not recommend any methods of analysis.

1.2.2. In the Codex Alimentarius

The globally accepted Codex Alimentarius contains four standards related to cocoa and chocolate products: 86-1981 for cocoa butter; 87-1981 for chocolate and chocolate products; 105-1981 for cocoa powders (cocoas) and dry mixtures of cocoa and sugars; and 141-1983 for cocoa mass (cocoa/chocolate liquor) and cocoa cake. This latter standard introduces some more terms compared to the European "Chocolate Directive" 2000/36/EC in force and specifies that cocoa mass (cocoa/chocolate liquor) must not contain more than 5 % of cocoa shell and/or germ. Some other terms are also more specific. According to Codex, chocolate is described as a homogenous product complying with the stated description (cocoa butter content, fat-free cocoa solids, total cocoa solids, milk fat, total milk solids), obtained by an adequate manufacturing process from cocoa materials, which may be combined with milk products, sugars and/or sweeteners, and other additives listed in the Standard. Following this general description, Codex specifies chocolate types and their composition (chocolate or, alternatively, bitter sweet chocolate, semi-sweet chocolate, dark chocolate, chocolate fondant; sweet chocolate; couverture chocolate; milk chocolate; etc.). The addition of other edible foodstuffs is limited to 40 % and other vegetable fats to 5 %, as in the EU Directive, but the nature of these fats is not further specified. Furthermore, Codex recommends some internationally recognised analytical methods (for example, for the determination of fat content, cocoa shell, free fatty acids or moisture) published by the Association of Official Analytical Chemists (AOAC), the International Union of Pure and Applied Chemistry (IUPAC) or the International Confectionery Association (ICA, formerly International Office of Cocoa, Chocolate and Sugar Confectionery, IOCCC).

1.2.3. In the United States

When compared to the EU Directive and Codex, the US Code of Federal Regulations (CFR) defines cocoa and chocolate products slightly differently. The main parameter for describing the composition of most of the products is cocoa fat content and/or chocolate liquor content. Notice also the difference in this terminology; cocoa fat instead of cocoa butter, and chocolate liquor instead of total cocoa solids. In the CFR, there are no specifications for cocoa fat, in contrast to cocoa butter in the EU Directive or Codex. For the product prepared by finely grinding cacao nibs (and, eventually, the addition of cocoa fat and/or cocoas) containing between 50 and 60 % of cacao fat, there are several appropriate terms listed: “chocolate liquor”, “chocolate”, “unsweetened chocolate”, “bitter chocolate”, “baking chocolate”, “cooking chocolate”, “chocolate coating” or “unsweetened chocolate coating”. In contrast to the EU Directive or Codex, no other vegetable fats may be added to these products, and no limitation for the added amount of edible foodstuffs is specified. Among other product categories listed, some are very similar to those in the EU, such as “white chocolate” and “semisweet/bittersweet chocolate”, whereas “sweet chocolate”, for instance, must contain only at least 15 % chocolate liquor and, thus, could not be labelled as chocolate in the EU. The CFR also recommends methods of analysis, particularly those published by the AOAC, for the determination of shell content and fat content.

1.2.4. Other standards

Many more standards for cocoa, mostly cocoa beans, are recognized by various international organizations. The International Organization for Standardization has published the standard ISO 2451:2017, which specifies the requirements, classification, sampling, test methods, packaging and marking for cocoa beans, as well as recommendations for their storage and disinfestation.

The Federation of Cocoa Commerce (FCC), which aims at promoting, protecting and regulating the trade of cocoa beans and some cocoa products, has developed contract standards and rules for cocoa beans, defining their quality, sampling and weighing, and contract rules for liquid cocoa products and packed cocoa products. In FCC Quality Rules [23], cocoa beans are described according to their size, observed defects, fermentation, dryness, the presence of any foreign matter, contamination, insects, off-flavours and free fatty acid content. Some standards are still being developed, such as International Standards on Cocoa Quality and Flavour Assessments. A draft of this document was prepared in November 2017 under the coordination of the Cocoa of Excellence (CoEx) Programme [24] (Working Group on the Development of International Standards for the Assessment of Cocoa Quality and Flavours, the members of which represent various stakeholders, from associations of cocoa producers to traders, chocolate manufacturers and research organisations). The document aims at standardising the procedures and terminology for high quality cocoa and chocolate products, and includes also the sampling, assessment of physical quality and flavour of cocoa beans, manufacturing procedures, or storage conditions [25].

For the use by Customs authorities, statistical agencies and other regulatory bodies, all commodities are classified and coded by the Harmonised Commodity Description and Coding System ("Harmonised System") developed by the World Customs Organization [26]. The Harmonised System is applied worldwide to facilitate the international trade and monitor and control the import and export as well as for the purposes of customs tariffs and taxes. The Combined Nomenclature was established by Council Regulation (EEC) No 2658/87 on tariffs and statistical nomenclature and is updated every year; the latest version is now available as EU Regulation No 2017/1925 [27]. In the Combined Nomenclature, the codes of the Harmonised System are used. There are different categories for cocoa and cocoa preparations, beginning with the number 18 (e.g. 18 06 10 for cocoa powder, containing added sugar or other sweetening matter), while white chocolate is classified as sugar confectionery and coded as 17 04 90 30.

At the end of this chapter, it is worth mentioning the famous Swiss and Belgian chocolate. Both these terms are related to the product manufacturing country. The term 'Belgian chocolate' was introduced in 2008 in the 'Belgian Chocolate Code' of The Royal Belgian Association of the Biscuit, Chocolate, Pralines and confectionary (Choprabisco) [28] which is an agreement between Belgium manufacturers and has no legal weight. The only criterion for chocolate to be called Belgian is that the complete process of mixing, refining and conching is carried out in Belgium. 'Switzerland', 'Swiss' or 'Suisse' chocolate are the trademarks registered by the Association of Swiss Chocolate Manufacturers (Chocosuisse) [29] which are used for products manufactured in the Switzerland under specific technical guidelines.

2. Authenticity issues

2.1. Identification of current authenticity issues

In ISO standard 2451:2017 and FCC Quality Rules[23] related to cocoa beans, the term adulteration is defined as the "alteration of the composition of a lot of cocoa by any means whatsoever"; a lot is defined as "quantity of cocoa beans in bags or in bulk established at any point in the cocoa supply chain and from which primary samples and/or incremental samples are to be drawn for quality analysis purposes". For whole beans, the possibilities of adulteration are rather limited, involving the presence or addition of foreign matter or of cocoa beans of poor quality (insufficient deshelling, defective beans). These issues can be quite easily recognized by simple visual inspection or other simple tests. On the other hand, the authentication of different complex products can become a highly demanding task. The most straightforward examples of product adulteration are inappropriate labelling, substitution of valued materials by cheaper ones, and the addition of undeclared or unauthorised materials/substances.

Among various cocoa materials, cocoa butter is considered the most important by-product of cocoa beans due to its unique physical and chemical characteristics and to its specific functional properties compared to other fats, such as brittleness at room temperature, fast melting at body temperature [30]. Since fat, especially its amount, is very important for the sensory properties of a product, cocoa butter may be "diluted" by other fats rather than used in lower amounts than declared. In addition to the economic reasons, such dilution may be motivated by certain technological advantages, such as increased stability [31]. Among various fats, cocoa butter equivalents (CBEs) are most suitable for mixing with cocoa butter in unlimited quantities due to their similar physical and chemical properties [30]. As mentioned above, in the EU only six CBEs can be added in a limited amount and declared on the product label. Such addition has to be declared by an informative statement on the labelling, in addition to the listing among the ingredients. For other products, any presence of undeclared fat is considered adulteration. This is not limited to vegetable fats, since animal fats, lard or tallow, are used in the adulteration of cocoa butter, mostly in developing countries. This type of adulteration is also of religious concern [32]. Moreover, the quality of cocoa butter intended for the human consumption is strictly defined in various standards; any use of cocoa butter or cocoa fat of poor quality would be categorised as adulteration. For instance, a higher level of free fatty acids in cocoa butter indicates that cocoa beans or cocoa butter have not been handled properly (cocoa beans are diseased or damaged, stored or transported in poor conditions). Such cocoa butter can negatively affect a flavour as well as crystallisation properties (snap, melting properties). In both the EC Directive 2000/36 [21] and the Codex Alimentarius, a level of free fatty acids of 1.75 % is specified as the maximum amount. Unsaponifiable matter is another parameter that is often used to assess fat quality and purity. This includes all compounds that, after saponification, are insoluble in water but soluble in fat. These are those compounds frequently found dissolved in fats and oils that cannot be saponified by the usual caustic treatment but are soluble in ordinary fat and oil solvents. They are mainly various natural components of fat (e.g. sterols, pigments, terpenic alcohols, higher aliphatic alcohols, hydrocarbons), the amount of which is characteristic for particular fat; however, unsaponifiables also include contaminants, such as mineral oil hydrocarbons, which come from transport materials, lubricants, fuels, exhaust fumes or debris from tyres [10,33].

Various cocoa and chocolate products may be adulterated by the use of improperly processed cocoa beans, which contain higher amounts of cocoa shell or germ, or other plant materials than those that occur naturally, or have higher moisture content. Interestingly, not all standards or legislation include the limitation of cocoa shell content; nevertheless, Codex limits the shell content in cocoa mass and cocoa cake to a maximum of 5 % by weigh calculated on the fat-free dry matter. Additional dilution of cocoa and cocoa products may be achieved by the intentional admixture of starch, flour, dextrins or various powdered materials, such as peanut shells, chestnut shells, soybean meal, sesame meal, carob and non-fermented cocoa beans [34].

Since chocolate is a more complex product, possibilities of its adulteration are increasing. Although the presence of some undeclared components (milk, peanuts, and nuts) may be unintentional, other ones may be added to reduce production costs or to increase the palatability of the product. The latter, for instance, may be achieved by the addition of milk. Milk fat not only influences the sensory properties (taste, softer texture) of products, its addition significantly improves resistance to bloom [35]. Improved stability, thus appearance and attractiveness, can be also achieved by the admixture of some foreign vegetable fats into cocoa butter [30,36]. In addition to the alteration of fat content or composition, the content of non-fat cocoa solids may decrease, resulting in a value which is non-compliant either with the legislative limit or, when summed together with cocoa butter, with total dry cocoa solids on product label. The lower cocoa content can then be easily adjusted by increasing the major and cheapest chocolate ingredient, sugar or any of the materials mentioned above.

In the EU [21], the addition of up to 40 % of other edible substances to chocolate brings the possibility of decreasing the mass of cocoa needed for the production of chocolate without affecting the cocoa percentage on the labelling (this value, and also the minimum requirement for cocoa butter and dry non-fat cocoa solids, are calculated after deducting the weight of such substances). If these substances are not clearly visible and do form a homogenous matter with the chocolate, consumers may not be aware of the difference between such a product and a common chocolate with an identical cocoa percentage on the label. Examples of products which benefit from the rule of calculating cocoa solids for the label are those with the addition of dietary fibre (e.g. inulin, used also as an alternative to sucrose as a sweetener to develop sugar reduced chocolate products) [37].

Particularly vulnerable to adulteration are the various specialty (or premium) cocoa and chocolate products, which are characterised by a higher market price. Recently, the rapid increase in consumer demand for such products has opened a new, very lucrative area for fraudulent practices. Consumers have become especially interested in premium chocolates with a variety of exotic ingredients, chocolates made from single-origin cocoa beans, such as those from Ghana, Ecuador or Venezuela, products that have declaration of 'fine and flavour' cocoa content, or products with a certain type of certification (e.g. Fairtrade, UTZ, organic), or production (e.g. raw, with reduced sugar, glycaemic index or cariogenicity) [38]. Geographical Indications (GIs), first introduced in the EU, are also increasingly used as a marketing tool to differentiate agri-food products, including cocoa beans, in the globalised marketplace. While the majority of origin-specific products are produced in EU countries, some of the developing countries where the cocoa beans are produced have also successfully implemented GIs and origin-based and quality differentiation strategies. Examples from the international cocoa markets for 'fine and flavour' cocoa, vulnerable to fraudulent practices, include Arriba from Ecuador, and Chuao from Venezuela [39,40].

2.2. Potential threat to public health

The adulteration of cocoa products is generally not a serious threat to public health. Most of the fraudulent practices involve the addition of edible ingredients that are safe for human consumption. However, for a small part of the population, the undeclared presence of allergens, such as nuts, peanuts, soya or milk, might be highly dangerous.

3. Analytical methods used to test for authenticity

3.1. Officially recognised methods

As for all raw materials and foods, sampling is the first critical step to obtain the results that reflect the composition of a whole product. Representative sampling of cocoa and chocolate products is described in several standard operating procedures as well as in ISO 2292:2017, ISO 2451:2017, FCC Sampling Rules or the Cocoa Merchants' Association of America (CMAA) guidelines (for cocoa beans), ISO 5555 (for animal and vegetable fats and oils), and AOAC 970.20 or International Confectionery Association (ICA, formerly International Office of Cocoa, Chocolate and Sugar Confectionery, IOCCC) Analytical Method 3 (for cocoa and chocolate products).

For raw cocoa beans, various procedures are described in FCC Quality Rules, ISO 2451 or the FDA Macroanalytical Procedures Manual V-4. Physical testing is fundamental involving the assessment of bean count (size), sievings (extraneous matter), cut test (slaty and defective beans) and visual examination for the presence of defective beans (e.g. mouldy, slaty, insect-damaged, germinated), insects or any extraneous material. Additionally, beans are assessed for the presence of off-flavours by a panel of assessors (ICA Analytical Method 44) as well as for free fatty acids (alkalimetry, ICA Analytical Method 44) and moisture (oven drying, ICA Analytical Method 43).

Other attributes considered for the authenticity testing of cocoa and chocolate products are related to their essential composition and quality factors [41]. For most cocoa and chocolate products, analytical methods are then related to the two main components of cocoa beans: cocoa butter, and non-fat cocoa solids.

3.1.1. Cocoa butter content

To determine cocoa butter content, the first step is the analysis of total fat content (acid hydrolysis followed by Soxhlet extraction and gravimetry of the obtained fat). For this task, standard procedures have been adopted by AOAC (963.15-1973) or ICA (14-1972). However, fats other than cocoa butter can be present, thereby overestimating such results. It is therefore necessary to investigate the actual composition of the fat. Such methods have moved on since the EU permitted the use of CBEs in chocolate in 2003. The most reliable approach is to investigate triacylglycerol (TAG) profiles. In this chapter, TAGs are labeled according to their fatty acid composition, e.g. POS being 1-palmitoyl-2-oleoyl-3-stearoyl-glycerol. Fatty acid abbreviations are: P – palmitic acid, O – oleic acid, S – stearic acid, B – butyric acid.

The pioneering work in this field was carried out, independently by Padley and Timms [42] and by Fincke [43], back in 1980 [44]. They reported that cocoa butter has a characteristic TAG composition with a linear relationship between the content of C50 and C54 TAGs ('CB-line'), and any deviation from that line caused by a higher C50 content means that the tested sample contains other fats. However, since 2003, the need for the reliable quantification of CBEs has led to more comprehensive studies. Based on the work of Buchgraber et al. [45,46], a method for the detection and quantification of CBEs in chocolate was introduced in international standards ISO 23275:2006, and ISO 11053:2009 for milk chocolate. Similar procedures are described in American Oil Chemists' Society (AOCS) Official Methods Ce 11-05 (2005) and Ce 11a-07 (2007). According to these standards, the TAG profiles (POP, POS, SOS, POO, SOO) determined by gas chromatography with a flame ionisation detector (GC-FID) provide the basis for calculating the CBE content. In milk chocolate, in addition to the main cocoa TAGs, PSB has to be determined (by the same method) to correct for any interferences caused by the considerable POP content of milk fat.

3.1.2. Non-fat cocoa solids

The most widely accepted approach for estimating dry non-fat cocoa solids content is to calculate it from the content of the cocoa alkaloids, theobromine (3,7-dimethylxanthine) and caffeine (1,3,7-trimethylxanthine). Although the natural levels of these compounds in cocoa can vary slightly according to cocoa variety, geographical origin, soil factors, climatic conditions, cultivation or post-harvest technology including processing [47], this approach has been routinely used for decades and has been standardized in AOAC 980.14. Since the alkaloid content is influenced by the factors listed above, the main issue has been the inconsistent value of the conversion factor to be used in the calculation for the content of dry non-fat cocoa solids. In 2012, Richards and Wailes presented the results of a large project analysing almost 200 cocoa liquor samples collected from various geographical areas over a two-year period [48]. Based on their data, they suggested new conversion factors of 40.7 and 36.1 for calculations using theobromine and the sum of theobromine and caffeine, respectively, with confidence intervals of ±1.7 % ($p=0.95$). Caffeine and theobromine content can be determined by various methods. In the most common approach, extraction by hot water (or by hot water with the addition of an acid/alkali) is followed by liquid chromatography (LC) separation and UV detection [47,48]. Different approaches, such as titrimetric, spectrophotometric or gravimetric, are mostly no longer applied, although the gravimetric method AOAC 931.05 (non-fat cacao mass of chocolate liquor) is still listed in Codex.

For chocolate, another basic parameter is total dry cocoa solids content, which is then calculated as a sum of cocoa butter content and non-fat cocoa solids content.

3.1.3. Free fatty acids content and unsaponifiable matter

For the analysis of pure cocoa butter, there are two additional parameters to be analysed: free fatty acids (acidity) and unsaponifiable matter. Acidity is determined by titrimetry (alkalimetry) and expressed as the content of oleic acid in the sample (in %). A closely related and frequently used term is acid value, for which the results of the identical procedure are just expressed differently, as milligrams of KOH necessary to neutralize 1 g of sample. Standard procedures to determine the acidity and acid value are described in IUPAC 2.201 (1987), ISO 660:2009, ICA Analytical Method 42, AOCS Ca 5a-40 (acidity) and AOCS Cd 3d-63 (acid value). Another parameter is unsaponifiable matter, whose quantification method is standardised in ICA Analytical Method 23, IUPAC 2.401 (1987), AOAC 933.08 and AOCS Ca 6b-53.

3.1.4. Other authenticity parameters

Other common methods are the same as those being performed for many foods with slight modifications. For moisture content, titration using Karl Fischer method is standardised in ICA Analytical Method 26, AOAC 977.04 and AOAC 977.10 (cacao bean and its products/confectionary coatings, cocoa bean and its products/milk chocolate); and a gravimetric method is described in AOAC 931.04 (cacao products) or ICA Analytical Method 1 or 43.

Although the amount of cocoa shells present in cocoa products is not specified by most standards, there are standardised methods for the determination of this ‘foreign’ material. The so-called “blue value” is analysed according to ICA Analytical Method 29. The approach recommended in Codex is described in AOAC 968.10 and 970.23.

In milk chocolate, the procedure for protein (fat-free milk solids) determination by the Kjeldahl method is described in AOAC 939.02.

Starch in cocoa mass, cocoa and cocoa products, being considered as a fibre-related substance in fibre-rich products, or as a substance enabling a reduction in the mass of cocoa needed for the production of chocolate, can be determined using method AOAC 920.84, Section 12.043.

For chocolate and sugar confectionery products where various syrups (e.g. agave or date) are used as sweeteners or where no sucrose content is declared among the list of ingredients, methods for the determination of glucose, fructose and sucrose by means of enzymes (e.g. IOCCC 33:1989) or by high performance liquid chromatography (e.g. IOCCC 34:1989) can be used to assess a product authenticity.

3.2. Other commonly used methods

For many of the standard methods mentioned in the previous section, various alternative procedures have been proposed.

3.2.1. Substitution with non-cocoa fats or shell content

To assess fat composition (cocoa butter purity), the approach that has been described in chapter 3.1 can be applied for TAG profiles obtained by different techniques. M. Buchgraber et al. [49] and C. Simoneau et al. [50] showed a good suitability of non-aqueous reversed-phase LC (NARP-LC) for

TAG determination by comparing with GC-FID. Other techniques that have been reported as appropriate for this task are MALDI-TOF-MS (Matrix Assisted Laser Desorption/Ionisation coupled with mass spectrometry) [51,52], silver ion LC-MS [53] and Fourier transform infrared spectroscopy (FTIR); the latter for the quantification of lard content [32] or other vegetable fats in cocoa butter [54]. The potential of different analytical approaches for the reliable quantification of foreign fats in real samples in food control laboratories is still being investigated. Indeed, such quantification is complicated due to a high variability in cocoa butter composition (caused for example by differences in geographical origin and processing) as well as by the variability in admixed fats.

To determine cocoa shell content, the standard photometric method has been criticised for its low sensitivity and selectivity. Alternatively, an HPLC-FLD method for the determination of fatty acid tryptamides (behenic acid tryptamide, lignoceric acid tryptamide) as indicators for cocoa shell was first described by Münch and Schieberle in 1999 [55] and in 2000 published as optimised for routine analyses. Using a high number of various cocoa products (cocoa nibs, cocoa shells, cocoa liquors, cocoa powders, cocoa butters, cocoa pods), the use of this method was further evaluated by Janßen and Matissek [56]. Another detection technique, GC-FID, has been shown to be suitable for these indicators [57], thereby making this approach applicable in a wide range of laboratories.

Colorimetry and photoacoustic spectroscopy have been reported as a suitable tool for the determination of non-fat cocoa solids in dark chocolates [58], thermogravimetry for the characterization of milk and dark chocolates [19,59].

3.2.2. Geographical or botanical origin

Other methods focus particularly on geographical or botanical origin of the cocoa beans, and on the influence of processing on product composition and its characteristic markers. Typical features of a product and verification of its authenticity are then influenced by the intrinsic characteristics of cocoa and cocoa-based products and the complex technological process, making the whole analytical strategy challenging. Various analytical approaches can be applied for this purpose using specific markers and appropriate analytical techniques.

To assess botanical origin, geographical origin and brand of dark chocolates, volatile organic compounds composition responsible for characteristic aroma, analysed by GC-MS [60] and HS-PTR-MS (head space-proton transfer reaction-mass spectrometry [61] followed by chemometrics for further discrimination the samples can be used.

Other approaches using stable isotope composition by IRMS (isotope ratio mass spectrometry) [62], ^{1}H-NMR (proton nuclear magnetic spectroscopy) [63] and multielemental composition by ICP-MS (inductively coupled plasma-mass spectrometry) [64] combined with chemometrics can be applied to differentiate production areas or cocoa beans.

NIR (Near Infrared) spectroscopy has been also reported as a potential analytical method to classify different varieties and predict the chemical composition of cocoa [65]. It can be also used to detect cocoa adulterated with carob flour [66].

The analysis of cocoa proteins and oligopeptide profiles in beans from various geographic origins by UHPLC-ESI-QTOF (ultra-high performance liquid chromatography coupled with electrospray ionisation-quadrupole-time of flight-mass spectrometry) [61], GCxGC-FID [67], UPLC-ESI-MS (ultra-performance liquid chromatography-electrospray ionisation-mass spectrometry) [68] or different cocoa hybrids by MALDI-TOF-MS [69] allows the assessment of differences with respect to cocoa origin as well as its fermentation status (non-fermented vs. fermented), respectively.

Finally, a modern analytical approach, metabolomics, is focused on obtaining very comprehensive information about the samples by either non-targeted fingerprinting or targeted profiling [70]. For this purpose, various instruments are used, such as MS or NMR, and a large amount of generated data are processed using sophisticated statistical methods. Metabolomics employing LC-TOF-MS coupled with a partial least squares discriminant analysis model and phenolic compounds as biomarkers for construction of the predictive model can be applied for the discrimination of cocoa beans based on their geographical origins for effective quality assurance [71]. Profiling based on bioactive compounds present in cocoa beans, such as proanthocyanidins, represented by flavanols and procyanidins, and attributed to the antioxidation activity of cocoa, can be also performed by LC-FLD (AOAC 2012.24).

3.2.3. Sensory analyses

Sensory analysis is a possible option for the assessment of cocoa product quality. Although it does not provide information on product authenticity, it is the only method related directly to human experience with the product. When using a well-trained panel, sensory analysis can be applied to assess the quality of premium products (with high cocoa content where non-chocolate substitutes are inadequate) or specialty products (sucrose-free, containing various fillings etc.). In addition, assessing the changes in sensory attributes (e.g. flavour by volatile compounds) [72] during cocoa beans processing and chocolate manufacturing can help in the monitoring of technological processes.

4. Overview of methods for authenticity testing

The following table provides a summary of the methods and the authenticity issues they address.

Analytical technique	Indicative data or analyte	Authenticity issue / information
Physical testing	Physical properties of cocoa beans	Substitution of high quality raw material, cocoa beans
Sensory analysis	Off-flavour, aroma profile	Substitution of high quality raw material, cocoa beans
Gravimetry (acid hydrolysis followed by Soxhlet extraction)	Total fat in all cocoa products	Decreased fat content: substitution of cocoa butter with non-fat components
GC-FID	Triacylglycerols	Cocoa butter equivalents in cocoa butter or chocolate
Titrimetry (alkalimetry)	Total free fatty acids	Substitution of cocoa butter
GC-FID	Fatty acids	Substitution of cocoa butter
GC-FID, MALDI-TOF-MS, NARP-LC-MS, silver ion LC-MS, FTIR	Fat composition	Substitution of cocoa butter
LC-UV, LC-MS	Non-fat cocoa solids in cocoa products based on theobromine and caffeine content	Substitution (lower content of cocoa solids)
Gravimetry (saponification followed by extraction)	Unsaponifiable matter	Substitution
Enzymatic (diastase)	Starch	Substitution
LC-FLD, GC-FID	Behenic acid tryptamide, lignoceric acid tryptamide	Substitution (cocoa shell / shell processing contamination in cocoa products)
GC-MS, HS-PTR-MS	Volatile organic compounds	Geographical or botanical origin Substitution (mono-variety/region products)
IRMS, ^{1}H-NMR	Stable isotope composition	Geographical or botanical origin Substitution (mono-variety/region products)
ICP-MS	Trace elements	Geographical or botanical origin Substitution (mono-variety/region products)
LC-ESI-Q-TOF, GCxGC-FID, LC-ESI-MS, MALDI-TOF-MS	Proteins and oligopeptides profiles	Geographical or botanical origin Substitution (mono-variety/region products)
NIR	Spectral information	Geographical or botanical origin Substitution (mono-variety/region products)
LC-MS	Phenolic compounds	Geographical or botanical origin Substitution (mono-variety/region products)

5. Conclusion

Food fraud in the cocoa beans processing sector is influenced by the increasing price of cocoa as a consequence of the production size, the influence of weather conditions, pests or diseases and a higher demand for cocoa. In addition to social and ethical aspects for less developed countries where cocoa beans are primarily produced, government support for the production and certification of premium products (e.g. geographical indication) must be sustained for this sector to continue developing.

The main challenge to the authentication of cocoa beans and cocoa-based products is the inherent compositional variability due to differences in variety, geographical origin, and also processing techniques.

Nowadays, non-targeted analysis (fingerprinting) to assess the authenticity of a suspicious sample by comparing it to an authentic one is a novel approach that is particularly useful in differentiating geographical origin, genotype and production technology by using fingerprints of cocoa and cocoa-based products obtained by various analytical approaches, such as LC-MS, GC-MS, proteomic, peptidomic, elemental, and combined with appropriate chemometric tools. However, the availability of well-designed, specific and extensive compositional databases, reference materials and reliable, validated analytical protocols are needed.

Due to the large number of different fats, especially artificially prepared mixtures, which can be used as alternatives to cocoa butter, it is difficult to assess fat composition based on only a few physical or chemical parameters. Moreover, the use of several analytical procedures to assess the quality of cocoa butter is time consuming. Thus, lipidomic fingerprinting appears to be an interesting approach for the future.

Analytical methods enabling the determination of various parameters according to the legislation within a single analytical run are desirable, with the aim of increasing sample throughput in control labs. Supercritical fluid chromatography (SFC), coupled with UV detection, refractometric detection (RID), evaporative light scattering detection (ELSD), FID or mass spectrometric (MS) detection for fat composition, saccharides profile and purine alkaloids content might be a solution.

Instead of conventional analytical methods, the application of ‘omic’ technologies also represents new trends for the future. Plants produce a considerable amount of chemically diverse metabolites. Differences observed in the composition of metabolites of a particular species or cultivar of the plant are determined by various genetic and environmental factors. They cause small variations, such as within one fruit tree, between fruit from the marginal and inner parts of the crown, as well as medium or larger differences (due to different soil types and other climatic conditions given by the region). Geographic origin has a significant influence on metabolite composition and is an important attribute for determining the quality and price of many foods including cocoa-based products. The natural variability of metabolites thus provides reliable information on the origin and authenticity of food. Another important factor for the quality of food of plant origin are the conditions of harvesting and storage of crops. Even after the harvest, intense metabolic processes are underway, and plant materials can be degraded by a misuse. Other changes can occur during cocoa beans processing. Metabolomics might be a useful tool for finding the conditions that will be optimal for maintaining quality.

6. Bibliographic references

1. International Trade Centre UNCTAD/WTO, ed. (2001). – *Cocoa: a guide to trade practices*. International Trade Centre UNCTAD/WTO, Geneva.
2. Worldwide chocolate consumption by country *Stat. Portal*. Available at: https://www.statista.com/statistics/819288/worldwide-chocolate-consumption-by-country/.
3. Ackar D., Valek Lendić K., Valek M., Šubarić D., Miličević B., Babić J. & Nedić I. (2013). – Cocoa Polyphenols: Can We Consider Cocoa and Chocolate as Potential Functional Food? *J. Chem.*, **2013**, 1–7. doi:10.1155/2013/289392.
4. Konar N., Toker O.S., Oba S. & Sagdic O. (2016). – Improving functionality of chocolate: A review on probiotic, prebiotic, and/or synbiotic characteristics. *Trends Food Sci. Technol.*, **49**, 35–44. doi:10.1016/j.tifs.2016.01.002.
5. Afoakwa E.O. (2010). – *Chocolate Science and Technology*. Available at: http://ssu.ac.ir/cms/fileadmin/user_upload/ivfen/ensite/lib/075-Chocolate_Science_and_Technology-Emmanuel_Ohene_Afoakwa-1405199067-Wiley_Blackwell-2010-310-_.pdf.
6. World Cocoa Foundation Available at: http://www.worldcocoafoundation.org/.
7. The Statistics Portal Available at: https://www.statista.com/statistics/262620/global-cocoa-production/.
8. Dand R. (2011). – *The international cocoa trade*. 3rd ed., Woodhead Publishing Limited, Cambridge, UK.
9. Kongor J.E., Hinneh M., Walle D.V. de, Afoakwa E.O., Boeckx P. & Dewettinck K. (2016). – Factors influencing quality variation in cocoa (Theobroma cacao) bean flavour profile — A review. *Food Res. Int.*, **82**, 44–52. doi:10.1016/j.foodres.2016.01.012.
10. End M.J. & Dand R., eds. (2015). – *CAOBISCO/ECA/FCC Cocoa Beans: Chocolate and Cocoa Industry Quality Requirements*. ECA-Caobisco-FCC Cocoa Research Fund. Available at: http://www.cocoaquality.eu/data/Cocoa%20Beans Industry%20Quality%20Requirements%20Apr%202016_En.pdf.
11. International Cocoa Organization *Int. Cococa Organ.* Available at: www.icco.org.
12. Fairtrade International *Fairtrade Int.* Available at: https://www.fairtrade.net/.
13. UTZ *UTZ - Label Program Sustain. Farming*. Available at: https://utz.org/.
14. Rainforest Alliance *Rainfor. Alliance*. Available at: https://www.rainforest-alliance.org/.
15. International cocoa organisation. Production - Latest figures from the Quarterly bulletin of cocoa statistics Available at: http://icco.org/about-us/international-cocoa-agreements/cat_view/30-related-documents/46-statistics-production.html.
16. FAOSTAT - FAO's corporate database *Food Agric. Organ. U. N.* Available at: www.fao.org/faostat/en.
17. Talbot G. (2009). – *Science and technology of enrobed and filled chocolate, confectionery and bakery products*. 1st ed., Woodhead Publishing, Cambridge, UK.
18. Beckett S.T. (2008). – *The Science of Chocolate*. 2nd ed., Royal Society of Chemistry.
19. Ostrowska-Ligęza E., Górska A., Wirkowska-Wojdyła M., Bryś J., Dolatowska-Żebrowska K., Shamilowa M. & Ratusz K. (2018). – Thermogravimetric characterization of dark and milk chocolates at different processing stages. *J. Therm. Anal. Calorim.* doi:10.1007/s10973-018-7091-4.
20. Cidell J.L. & Alberts H.C. (2006). – Constructing quality: The multinational histories of chocolate. *Geoforum*, **37** (6), 999–1007. doi:10.1016/j.geoforum.2006.02.006.
21. Directive 2000/36/EC of the European Parliament and of the Council of 23 June 2000 relating to cocoa and chocolate products intended for human consumption
22. Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives
23. Quality Rules
24. Cocoa of Excellence Programme *Cocoa Excell. Programme*. Available at: http://www.cocoaofexcellence.org.
25. Working Group on the Development of International Standards for the Assessment of Cocoa Quality and Flavours (2017). – Elements of harmonized international standards for cocoa quality and flavour assessment. Available at: https://static1.squarespace.com/static/56680247841abadb3a819e1c/t/59c8eadfcf81e018692d611f/1506339558874/WG-Quality-Flavour-Standards-ENGLISH-11Set2017.pdf.
26. What is the Harmonized System (HS)? *World Cust. Organ.* Available at: http://www.wcoomd.org/en/topics/nomenclature/overview/what-is-the-harmonized-system.aspx.

27. Commission Implementing Regulation (EU) 2017/1925 of 12 October 2017 amending Annex I to Council Regulation (EEC) No 2658/87 on the tariff and statistical nomenclature and on the Common Customs Tariff

28. The Royal Belgian Association of the Biscuit, Chocolate, Pralines and Confectionary Available at: http://www.choprabisco.be/engels/choprabisco_frameset.htm.

29. The Association of Swiss Chocolate Manufacturers Available at: https://www.chocosuisse.ch/en/.

30. Lipp M. & Anklam E. (1998). – Review of cocoa butter and alternative fats for use in chocolate - Part A. Compositional data. *Food Chem.*, **62** (1), 73–97.

31. Kurvinen J.P., Sjövall O., Tahvonen R., Anklam E. & Kallio H. (2002). – Rapid MS method for analysis of cocoa butter TAG. *J. Am. Oil Chem. Soc.*, **79** (7), 621–626. doi:10.1007/s11746-002-0534-2.

32. Cheman Y. (2005). – Analysis of potential lard adulteration in chocolate and chocolate products using Fourier transform infrared spectroscopy. *Food Chem.*, **90** (4), 815–819. doi:10.1016/j.foodchem.2004.05.029.

33. Beckett S.T. (2009). – *Industrial Chocolate Manufacture and Use*. 4th ed., Wiley-Blackwell, UK.

34. Yang W. li, Hu M. hua, Chen S. wei, Wang Q., Zhu S., Dai J. & Li X. zhong (2015). – Identification of Adulterated Cocoa Powder Using Chromatographic Fingerprints of Polysaccharides Coupled with Principal Component Analysis. *Food Anal. Methods*, **8** (9), 2360–2367. doi:10.1007/s12161-015-0126-6.

35. Sonwai S. & Rousseau D. (2008). – Fat Crystal Growth and Microstructural Evolution in Industrial Milk Chocolate. *Cryst. Growth Des.*, **8** (9), 3165–3174. doi:10.1021/cg070503h.

36. Walter P. & Cornillon P. (2002). – Lipid migration in two-phase chocolate systems investigated by NMR and DSC. *Food Res. Int.*, **35** (8), 761–767.

37. Bolenz S., Amtsberg K. & Schape R. (2006). – The broader usage of sugars and fillers in milk chocolate made possible by the new EC cocoa directive. *Int. J. Food Sci. Technol.*, **41** (1), 45–55. doi:10.1111/j.1365-2621.2005.01023.x.

38. Aidoo R.P., Depypere F., Afoakwa E.O. & Dewettinck K. (2013). – Industrial manufacture of sugar-free chocolates – Applicability of alternative sweeteners and carbohydrate polymers as raw materials in product development. *Trends Food Sci. Technol.*, **32** (2), 84–96. doi:10.1016/j.tifs.2013.05.008.

39. White A. (2016). – The potential and pitfalls of geographical indications for cacao. *Choc. Cl. - Multimed. Essays Choc. Cult. Polit. Food*.

40. Branding matters: The success of Chuao cocoa beans Available at: http://www.wipo.int/ipadvantage/en/details.jsp?id=2618.

41. Afoakwa E.O., Paterson A., Fowler M. & Ryan A. (2008). – Flavor formation and character in cocoa and chocolate: a critical review. *Crit. Rev. Food Sci. Nutr.*, **48**, 1–18.

42. Padley F.B. & Timms H.P. (1980). – The Determination of Cocoa Butter Equivalents in Chocolate. *J. Am. Oil Chem. Soc.*, **57** (9), 286–293.

43. Fincke A. (1980). – Moglichkeiten und Grenzen einfacher gaschromatographischer Triglyceridanalysen zum Nachweis fremder Fette in Kakaobutter und Schokoladefetten. 2. Mitteilung: Verteilung der nach C-Zahlen klassifizierten Triglyceride in Kakaobutter. *Dtsch. Lebensm. Rundsch.*, **76**, 187–192.

44. Ulberth F. & Buchgraber M. (2003). – Analytical platforms to assess the authenticity of cocoa butter. *Eur. J. Lipid Sci. Technol.*, **105**, 32–42.

45. Buchgraber M., Ulberth F. & Anklam E. (2004). – Method validation for detection and quantification of cocoa butter equivalents in cocoa butter and plain chocolate. *J. AOAC Int.*, **87**, 1164–1172.

46. Buchgraber M., Senaldi C., Ulberth F. & Anklam E. (2004). – Detection and quantification of cocoa butter equivalents in cooca butter and plain chocolate by gas liquid chromatography of triacylglycerols. *J. AOAC Int.*, **87**, 1153–1163.

47. Matissek R. (1997). – Evaluation of xanthine derivatives in chocolate–nutritional and chemical aspects. *Z. Für Leb. - Forsch. A*, **205** (3), 175–184.

48. Richards A. & Wailes B. (2012). – Estimation of fat-free cocoa solids in chocolate and cocoa products—global survey of typical concentrations of theobromine and caffeine determined by HPLC. *J Assoc Public Anal*, **40**, 1–12.

49. Buchgraber M., Ulberth F. & Anklam E. (2000). – Comparison of HPLC and GLC Techniques for the Determination of the Triglyceride Profile of Cocoa Butter. *J. Agric. Food Chem.*, **48** (8), 3359–3363. doi:10.1021/jf991000p.

50. Simoneau C., Lipp M., Ulberth F. & Anklam E. (2000). – Quantification of cocoa butter equivalents in mixtures with cocoa butter by chromatographic methods and multivariate data evaluation. *Eur. Food Res. Technol.*, **211** (2), 147–152.

51. Bono L., Seraglia R., Roverso M., Di Carro M. & Magi E. (2014). – Triacylglycerol profile in cocoa liquors using MALDI-TOF and LC-ESI tandem mass spectrometry: Nine TAGs identified in Ecuador cocoa liquor. *J. Mass Spectrom.*, **49** (9), 894–899. doi:10.1002/jms.3439.

52. Guyon F., Absalon C., Eloy A. & et al. (2003). – Comparative study of matrix assisted laser desorption/ionization and gas cromatography for quantitative determination of cocoa butter equivalent triacylglycerol composition. *Rapid Commun. Mass Spectrom.*, **17** (20), 2317–2322.

53. Segall S.D., Artz W.E., Raslan D.S., Ferraz V.P. & Takahashi J.A. (2005). – Analysis of triacylglycerol isomers in Malaysian cocoa butter using HPLC-mass spectrometry. *Rood Res. Int.*, **38** (2), 167–174.

54. Goodacre R. & Anklam E. (2001). – 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** (10), 993–1000.

55. Münch M. & Schieberle P. (1999). – A sensitive and selective method for the quantitative determination of fatty acid tryptamides as shell indicators in cocoa products. *Z. Für Leb. -Forsch. A*, **208** (1), 39–46.

56. Janßen K. & Matissek R. (2002). – Fatty acid tryptamides as shell indicators for cocoa products and as quality parameters for cocoa butter. *Eur. Food Res. Technol.*, **214** (3), 259–264. doi:10.1007/s00217-001-0433-6.

57. Hug B., Golay P.A., Giuffrida F., Dionisi F. & Destaillats F. (2006). – Development of a Gas−Liquid Chromatographic Method for the Analysis of Fatty Acid Tryptamides in Cocoa Products. *J. Agric. Food Chem.*, **54** (9), 3199–3203. doi:10.1021/jf0527044.

58. Dóka O., Prágai E., Bicanic D., Kulcsár R. & Ajtony Z. (2013). – Colorimetry and photoacoustic spectroscopy as a suitable tool for determination of fat-free cocoa solids in dark chocolates. *Eur. Food Res. Technol.*, **236** (6), 963–968.

59. Materazzi S., De Angelis Curtis S., Ciprioti S.V., Risoluti R. & Finamore J. (2014). – Thermogravimetric characterization of dark chocolate. *J. Therm. Anal. Calorim.*, **116** (1), 93–98. doi:10.1007/s10973-013-3495-3.

60. Cambrai A., Marcic C., Morville S., Sae Houer P., Bindler F. & Marchioni E. (2010). – Differentiation of Chocolates According to the Cocoa's Geographical Origin Using Chemometrics. *J. Agric. Food Chem.*, **58** (3), 1478–1483..

61. Acierno V., Yener S., Alewijn M., Biasioli F. & Ruth S. van (2016). – Factors contributing to the variation in the volatile composition of chocolate: Botanical and geographical origins of the cocoa beans, and brand-related formulation and processing. *Food Res. Int.*, **84**, 86–95. doi:10.1016/j.foodres.2016.03.022.

62. Perini M., Bontempo L. & Ziller L. (2016). – Stable isotope composition of cocoa beans of different geographical origin. *J Mass Spec*, **51** (9), 684–689.

63. Marseglia A., Acquotti D. & Consonni R. – HR MAS 1H NMR and chemometrics as useful tool to assess the geographical origin of cocoa beans – Comparison with HR 1H NMR. *Food Res. Int.*, **85** (2016), 273–281.

64. Bertoldi D., Barbero A. & Camin F. (2016). – Multielemental fingerprinting and geographic traceability of Theobroma cacao beans and cocoa products. *Food Control*, **65**, 46–53.

65. Barbin D.F., Maciel L.F., Bazoni C.H.V., Ribeiro M. da S., Carvalho R.D.S., Bispo E. da S., Miranda M. da P.S. & Hirooka E.Y. (2018). – Classification and compositional characterization of different varieties of cocoa beans by near infrared spectroscopy and multivariate statistical analyses. *J. Food Sci. Technol.*, **55** (7), 2457–2466.

66. Quelal-Vasconez A., Perez-Esteve E. & Arnau-Bonachera A. (2018). – Rapid fraud detection of cocoa powder with carob flour using near infrared spectroscopy. *Food Control*, **92**, 183–189.

67. Oliveira L.F., Braga S.C.G.N., Augusto F., Hashimoto J.C., Efraim P. & Poppi R.J. (2016). – Differentiation of cocoa nibs from distinct origins using comprehensive two-dimensional gas chromatography and multivariate analysis. *Food Res. Int.*, **90**, 133–138. doi:10.1016/j.foodres.2016.10.047.

68. Caligiani A., Marseglia A., Prandi B., Palla G. & Sforza S. (2016). – Influence of fermentation level and geographical origin on cocoa bean oligopeptide pattern. *Food Chem.*, **211**, 431–439. doi:10.1016/j.foodchem.2016.05.072.

69. Moreira I.M. da V., Vilela L. de F., Santos C., Lima N. & Schwan R.F. (2018). – Volatile compounds and protein profiles analyses of fermented cocoa beans and chocolates from different hybrids cultivated in Brazil. *Food Res. Int.*, **109**, 196–203. doi:10.1016/j.foodres.2018.04.012.

70. Castro-Puyana M. & Herrero M. (2013). – Metabolomics approaches based on mass spectrometry for food safety, quality and traceability. *TrAC Trends Anal. Chem.*, **52**, 74–87. doi:10.1016/j.trac.2013.05.016.

71. Hori K., Kiriyama T. & Tsumura K. (2016). – A Liquid Chromatography Time-of-Flight Mass Spectrometry-Based Metabolomics Approach for the Discrimination of Cocoa Beans from Different Growing Regions. *Food Anal. Methods*, **9** (3), 738–743. doi:10.1007/s12161-015-0245-0.

72. Moreira I.M. da V., Miguel M.G. da C.P., Ramos C.L., Duarte W.F., Efraim P. & Schwan R.F. (2016). – Influence of Cocoa Hybrids on Volatile Compounds of Fermented Beans, Microbial Diversity during Fermentation and Sensory Characteristics and Acceptance of Chocolates. *J. Food Qual.*, **39** (6), 839–849. doi:10.1111/jfq.12238.