Full title: Changes in cocoa properties induced by the alkalization process: a review

Name(s) of Author(s): Damián Valverde García, Édgar Pérez-Esteve and José Manuel Barat Baviera

Author Affiliation(s): Departamento de Tecnología de Alimentos. Universitat Politècnica de València, Camino de Vera, s/n, 46022, Valencia, Spain

Contact information for Corresponding Author: edpees@upv.es

Word count of text: 10881 words

Short version of title: Changes in cocoa induced by alkalization

Choice of journal/topic: *Comprehensive Reviews in Food Science and Food Safety*

ABSTRACT

Alkalization, also known as “Dutching”, is an optional, but very useful, step taken in the production chain of cocoa to darken its colour, modify its taste and increase natural cocoa solubility. Over the years, various attempts have been made to design new and more effective alkalization methods. Moreover, different authors have attempted to elucidate the impact of alkalization on the physico-chemical, nutritional, functional, microbiological and sensory characteristics of alkalised cocoa. The aim of this review is to provide a clear guide about not only the conditions that can be applied to alkalize cocoa, but also the reported effects of alkalization on the nutritional, functional, microbiological and sensory characteristics of cocoa. The first part of this review describes different cocoa alkalization systems and how they can be tuned to induce specific changes in cocoa properties. The second part is a holistic analysis of the effects of the alkalization process on different cocoa features, performed by emphasising the biochemistry behind all these transformations.

1 Introduction

Cocoa is one of the most distributed and well-known products worldwide. The colour and flavour that it confers to the formulations in which it is included, together with its richness in functional compounds and demonstrated health benefits, make cocoa a well appreciated food by consumers, a relevant ingredient for industry and an exceptional research source for scientists.

From the cocoa pods collected from *Theobroma cacao*, different kinds of natural products can be obtained: nibs, liquor, butter, cake and powder. The word “nib” refers to unshelled and fermented cocoa seeds, which are the final product of primary cocoa production chain processing (Figure 1). After obtaining nibs, they are ground to produce a paste called cocoa liquor, which is employed to produce chocolate, ice cream, bakery products, drinks and desserts. Apart from this cocoa mass being directly used, it can be pressed and divided into cake (solid part) and butter (oily part). Cocoa butter is employed, in combination with liquor and sugar, to produce chocolate, and can also be used in confectionery fillings and different skin products. This product is introduced into the formulations of an assortment of cosmetic skin care products, and also, food products like frozen desserts, bakery products, confectionery coatings, dairy products and instant premixes (Beg, Ahmad, Jan and Bashir, 2017; De Zaan, 2006).

In essence, the described cocoa production chain has not changed in the last 150 years, except for the used equipment that has been renovated and automated to not only improve efficiency, but to also cut process times (Beg et al., 2017).

With the described flow chart, cocoa processing yields natural cocoa powder. This product is characterised by having a light colour, low solubility, and an acidic, astringent and bitter taste. To darken its colour, reduce negative sensory characteristics and improve cocoa solubility, an alkalization step can be incorporated (De Zaan, 2006).

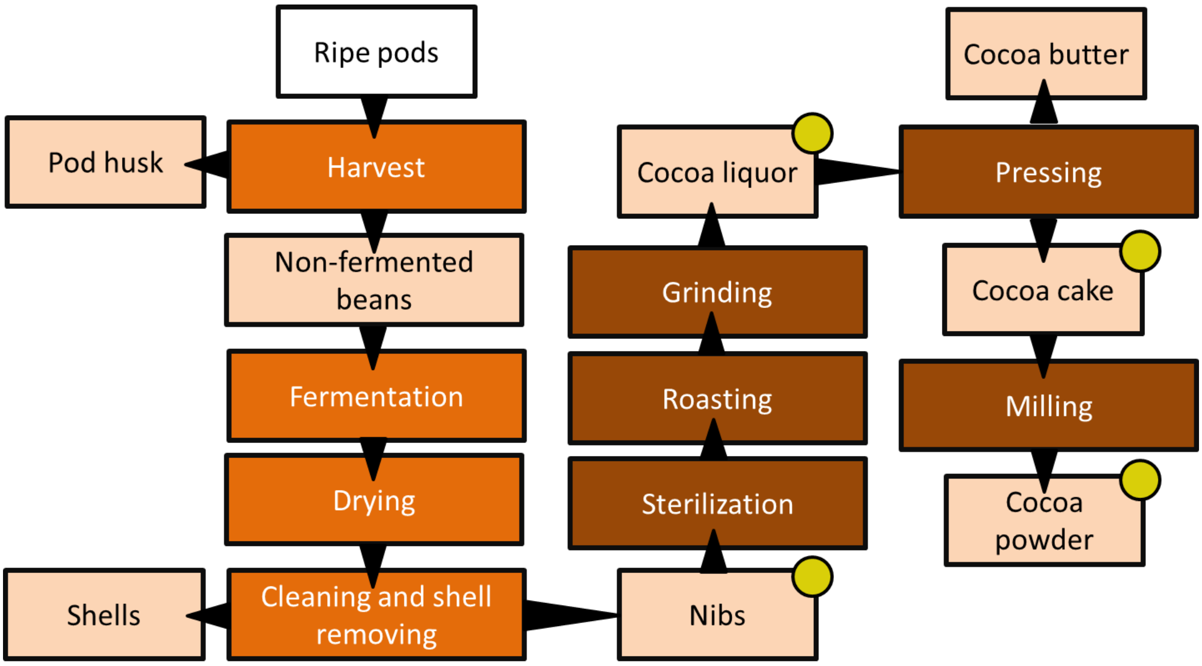

Figure 1. General scheme of natural cocoa powder production. The color of the primary process is dark orange, the secondary one is brown, while the different obtained products are depicted in light orange. Yellow circles indicate the products that can be alkalized.

Alkalization, also known as "Dutching", was a treatment firstly conceived by Coenraad Johannes van Houten in the 19th century to enhance cocoa powder solubility. However after its implementation, industry showed its capacity to modify colour and flavour, and started using it with cocoa nibs, liquors and cakes (De Zaan, 2006).

Alkalization generally consists of mixing natural cocoa material with an alkali solution, and treating this mixture with the combined effects of temperature and pressure. Once a product is alkalised, it is no longer considered natural. Then according to its pH, it is classified as dark natural (pH 5-6), light (pH 6-7.2), medium (pH 7.2-7.6) or strong alkalised cocoa (pH > 7.6) (Miller et al., 2008). As expected, strong alkalised products possess darker colours, lower astringent, bitter and acidic notes, and more solubility. In general, natural and light alkalised powders are used to prepare chocolates, milk chocolate, ice-creams, instant drink mixes, coatings and fillings. Medium and strong alkalised cocoas are used overall in ice-creams, cookies, cakes, coatings and truffles. Finally, black powders are used to prepare products with specific sensory characteristics, such as Oreo-type biscuits. The selection of specific cocoas to prepare certain products is important as consumer studies have demonstrated that colour has a strong impact on perceived flavour (Shankar et al., 2009).

Cocoa is so complex, but important, for the food industry that different scientific works have focused on improving and revealing the effects of different cocoa production chain steps on the product. In line with this, several authors have reviewed all existing information on a wide range of topics related to cocoa. Beg et al (2017) focused their work on providing an overview of the status, supply chain and processing of cocoa. Aprotosoaie, Luca and Miron (2016) described the cocoa production chain, the health effects of cocoa, and how flavour compounds and precursors are affected by different production chain steps. Saltini, Akkerman and Frosch (2013) reported how farming practices affect cocoa bean quality. Wollgast and Anklam (2000) conducted their work about alterations of polyphenols during general chocolate processing, and the methods used to identify, isolate, purify and quantify polyphenols. Kongor et al (2016) reviewed the factors influencing the bean flavour profile in cocoa beans. De Vuyst and Weckx (2016) focused on the microorganisms that participate in fermentation and on the changes that take place in cocoa beans. The review by Fei et al (2018) showed strategies for valorising cocoa pod husks and their fractions. Okiyama, Navarro and Rodrigues et al (2017) centred their work on cocoa shell applications in the food industry. Finally, Quelal et al (2020) proposed a roadmap to analyse the quality and authenticity of cocoa products and their derivatives by conventional and alternative analysis methods (2020).

Despite a considerable number of reviews dealing with cocoa transformation, no author has focused on alkalization in detail to date. Hence the present work aims to collect and evaluate all the information found in the scientific literature about the alkalization process. With this knowledge, it aims to describe the most widely used technology and parameters by industry for cocoa alkalization, as well as the physico-chemical, nutritional, functional, microbiological and sensory changes to cocoa caused by Dutching.

2 Alkalization from a technological point of view

From a technological point of view, alkalization consists of mixing cocoa material with an alkali solution and treating the mixture with a combination of pressure and temperature. The process is generally carried out in closed pressurised reactors by a continuous kneading system (Wissgott, 1988; Trout, 2001; Wiant, William, Lynch and LeFreniere., 1989), although some authors have described unpressurised versions of this process (Tanaka and Terauchi, 1999; Ellis, 1990; and Terink and Brandon, 1984). In an attempt to describe all the variables that can be combined during an alkalization process to develop a cocoa product with specific properties (colour, flavour, etc.), patents related to alkalization processes were reviewed. After analysing them all (Table 1), seven treatment variables were identified as the most important ones for bringing about the desired changes: alkali type and concentration temperature, aeration, water content, pressure and duration. In this section, the most frequent and most recommended conditions are presented to gain detailed insight into alkalization processing and its effects.

Novel systems that apply other heating technologies, such as extrusion (Chalin, 1972; Bandi, Kubicek and Raboud, 1984; and Bauermeister, 1989), can also be found in the literature. However, as far as we know, their use does not extend to industry.

Table 1. Patents related to the alkalization of cocoa samples. The table specifies the procedure followed for the treatment, the conditions (temperature (T), type and alkali concentration, water content, pressure (P) and duration (t)) and the characteristics of the final product

Author	**Procedure**	**T (°C)**	**Alkali (%)**	**Water content (%)**	**P (atm)**	**t (min)**	**Developed product**
Bandi, Kubicek and Raboud, 1984	Vapor injection and pressurized alkalization in a tube	120-130	K_2CO_3 (6-12%)	-	3-5	0.5-1.2	Alkalized cocoa
Bauermeister, 1989	Extrusion for disagglomerating and alkalizing cocoa	60-90	K_2CO_3 (0.5-5%)	-	Depends of the cocoa	Depends of the cocoa	Alkalized cocoa
Chalin, 1972	Cooking process, pressurized extrusion and mass granulation	87.77	Different alkalis (3-12%)	25-35	47-75	5	Homogeneous, dark and sterile cocoa with a good suspension capacity
Ellis, 1990	Cocoa cake alkalization in an unpressurized vessel and spray drying. No aeration.	85-87	K_2CO_3/NaOH 50:50 (1-6%)	>50	-	30-60	Brown cocoa
Ellis, 1990	Cocoa cake alkalization in an unpressurized vessel with the addition compressed air (1-5 bar/min) and spray drying	74-79	K_2CO_3 (1-6%)	>50	-	30-60	Red cocoa
Ellis, 1990	Cocoa cake alkalization in an unpressurized vessel with the addition of hydrogen peroxide, compressed air (3.5-5 bar/min) and spray drying	82	NaOH (4-6%)	>50	-	60	Dark red cocoa
Ellis, 1990	Cocoa cake alkalization in an unpressurized vessel with the addition of compressed air (1-3.5 bar/min) and spray drying	88	K_2CO_3/NaOH 85:15 (4-6%)	>50	-	60	Black cocoa
Kopp et al., 2010	Nibs alkalization in pressurized and heated mixer with direct steam injection (1) plus aeration (2).	- (1) 85 (2)	NaOH (2.4%) $(NaH_4)_2CO_3$ (12%)	10-50	2.5 (1) 2 (2)	90 (1) 30 (2)	Dark black cocoa
Kopp et al., 2010	Nibs alkalization in pressurized and heated mixer with direct steam injection (1) plus aeration (2).	- (1) 85 (2)	K_2CO_3 (2%) NaOH (1.2%) $(NaH_4)_2CO_3$ (12%)	10-50	2.5 (1) 2 (2)	90 (1) 30 (2)	Dark black cocoa
Kopp et al., 2010	Nibs alkalization in pressurized and heated mixer with direct steam injection (1) plus aeration (2).	124 (1) 85 (2)	K_2CO_3 (3.2%)	10-50	1.5 (1) 2 (2)	10 (1) 60 (2)	Bright red cocoa

Wiant, Lynch and LeFreniere, 1989	Cocoa cake alkalization in a vessel under pressure	65-100	$CaCo_3$, $Ca(OH)_2$, NaOH, KOH, $KHCO_3$, $NaHCO_3$ (1-12%)	5-60	5-12	5-60	Dark red cocoa
Wiant, Lynch and LeFreniere, 1989	Cocoa cake alkalization in a vessel under pressure	120-135	$CaCo_3$, $Ca(OH)_2$, NaOH, KOH, $KHCO_3$, $NaHCO_3$ (1-12%)	5-60	5-12	60-90	Dark black cocoa
Tanaka and Terauchi, 1999	Alkalization of nibs in a vessel with an agitation system	50-100	K_2CO_3 and Na_2CO_3 (<2%)	3-10	-	5-30	Cocoa rich in polyphenols and with a traditional taste
Terink and Brandon, 1981	Temperature controlled reactor with a kneading machine, where the water is being replaced until reaching a final pH lower or equivalent to 7.5	65-90	KOH (4.1%)	-	-	240-1140	Dark cocoa without the unpleasant taste of the strong alkalized cocoas
Trout, 2001	Alkalization after fat elimination from liquor or powder in a closed vessel (1) and its drying (2)	110	$(NaH_4)_2CO_3$ and NaOH	-	-	120-240 (1) 300-360 (2)	Cocoa with improved taste and handling characteristics
Wissgott, 1988	Alkalization in aqueous phase of cocoa liquor in two steps: treatment under pressure in a closed vessel (1) and water evaporation (2)	60-100 (1) 70-120 (2)	K_2CO_3 (1-3%) (1)	10-50	1-3 (1)	30-240 (1) - (2)	Dark brown-red cocoas
Wissgott, 1988	Alkalization in aqueous phase of non-roasted green cocoa in two steps: treatment under pressure in a closed vessel (1) and water evaporation (2)	60-100 (1) 70-120 (2)	K_2CO_3 (2-2.5%) (1)	10-100	1-3 (1)	120 (1) - (2)	Brown-red cocoas

2.1 Alkali type and concentration

As we see throughout this review, alkali type and concentration are some of the most important parameters to exert an effect during alkalization. The alkali media generated by them and their interactions with different cocoa components lead to the desired colour, flavour and solubility changes, which are the modifications that alkalization aims to make.

Several kinds of alkalis have been reported to be employed during alkalization, namely: NaOH, Na_2CO_3, $NaHCO_3$, KOH, K_2C0_3, $KHCO_3$, $(NaH_4)_2CO_3$, $Ca(OH)_2$, $CaCO_3$. All these salts are included in the Codex Alimentarius as authorised acidic regulator additives, whose maximum doses are limited by good manufacturing practices (Codex Alimentarius, 1981). Alkalis can be used alone or combined with others to induce the production of a given colour. Combinations and concentrations depend on their basicity, the final desired colour to be obtained and the alkali off-flavour that they confer. In line with all this, with their patent Kopp, Hennen, Seyller and Brandstetter (2010) provided two examples of obtaining dark black cocoas using different combinations of alkali. In one example, these authors used 2.4% NaOH and 12% $(NaH_4)_2CO_3$, and they replaced half NaOH with 2% K_2CO_3 in the other example. This replacement did not affect the final colour, but significantly reduced the alkali off-flavour of cocoa. This example stresses the importance of combining alkali agents to avoid this negative perception of alkalinity and to make the desired colour modification. Wiant et al. (1989) showed that potassium salts are very desirable to gain red powders, while a combination of potassium and ammonium compounds yields the most intense black colours while preserving a superior flavour. Besides colour, it is worth mentioning that different combinations of alkali agents, besides colour, may affect other characteristics (i.e. the solubility and formation of acrylamide), which must be taken into account when designing a new alkalization process.

By bearing this information in mind, it can be generally established that K_2CO_3 and NaOH are the most widely employed alkali agents during alkalization, although NaOH is reported as as being that which is best able to darken cocoa (Rodríguez, Pérez and Guzmán, 2009). Due to its effects, K_2CO_3 is considered the best alkalising salt to produce light and red colours without spoiling the product's taste, while NaOH (or its combination with ammonium salts) is recommended to obtain dark colours.

Apart from the employed types, their most frequent concentration ranges go from 1% to 6%. This concentration may vary depending on the nature of the employed alkali, the combination of alkali agents and the defects induced to taste. Additionally, the alkali concentration might affect final product properties. For example, baking products can be affected by introducing certain alkalised cocoas due to the leavening effect of the alkalising agent, while the protein stability of dairy products may reduce given a change in the pH of milk (De Zaan, 2006).

2.2 Water content

In general, water within the 10-50% range is added to the alkalization mixture. The importance of this variable lies in its ability to transport the alkali agent to the colour precursors that will be oxidised during alkalization. To ensure that alkali is well distributed, the amount of added water is necessary to wet the cocoa material, but we must remember that adding more water than necessary entails longer drying times (Wiant et al., 1989). In general, it is difficult to provide recommendations about the percentage of water that must be added to the alkalization mixture to confer cocoa powder specific colour characteristics. Thus in order to set a suitable water content, employing different concentrations of the same alkali and analysing changes in colour and the drying necessity are recommended to alkalise cocoa.

2.3 Temperature

Temperature is another important variable that determines colour and treatment duration. Generally increasing temperature leads to darker colours and faster reactions. The most frequently used range of temperatures goes from 60ºC to 130ºC. These temperatures must be tuned at will to gain cocoas with specific colours as temperature plays a crucial role during colour development.

Lower temperatures are generally needed to produce red cocoas, while higher ones must be employed to obtain darker ones (Wissgott, 1988; Wiant et al., 1989). For dark black cocoas, the maximum recommended temperature is 135ºC because, despite the fact that high temperatures will produce very black cocoas, they may adversely impact flavour (Wiant et al, 1989). Indeed Ellis (1992) reported two methods for producing red and dark red cocoas by treating samples at 74-79ºC and 82ºC, respectively. This inventor also reported two other processes for producing brown and black cocoa, in which the material was treated at 85-87ºC and 88ºC, respectively. An increasing temperature allows samples to darken, but raising temperatures too much results in loss of red chromophores and the formation of other colour compounds. Regarding this change in the coloured compounds' composition profile, another section explains how alkalization leads to the formation of red chromophores, but also to agglomeration, which leads brown high-molecular-weight compounds to appear (Germann, Stark and Hofmann, 2019a).

In another work, Wiant et al. (1989) patented two processes to produce dark red and black cocoa in a pressurised reactor, with differences in aeration, duration and temperature. These authors treated cocoa at 65-100ºC to produce dark red powders and at 120-135ºC to obtain dark black ones. Once again, the requirement to apply milder temperatures to produce red products is emphasised.

2.4 Pressure

Applying pressure is another parameter that can be used to shorten treatment duration. For example, when comparing the different works that describe a process to produce black cocoa (Terink and Brandon, 1981; Kopp et al., 2010; Wiant et al., 1989; Ellis, 1990; Chalin, 1972), the traditional system (pressure between 1 and 12 atm) requires a reaction time of between 30 and 120 minutes. During extrusion however, which applies pressure at between 47 and 75 atm, duration is below 5 minutes. In addition to speeding up the process, pressurisation has been associated with cocoa red coloration intensity (Wissgott, 1988). This effect on colour may be related to the ability of pressure to avoid the degradation or agglomeration of coloured compounds. By way of example, Wang et al (2012) studied the effect of high hydrostatic pressure on spinach purée and observed better colour preservation after applying pressure than after conventional treatment. They related colour preservation to protect pigments and to reduce browning reactions.

2.5 Treatment duration

Regarding treatment duration, Wiant et al. (1989) recommended that general exposure lasted between 5 and 180 minutes, and specified duration to range from 60 to 180 minutes for black products. So it is worth underlining the importance of treatment duration in relation to colour generation and the appearance of off-flavours. As stated in Section 2.3 about temperature, temperatures higher than those recommended can mean loss of red chromophores and the appearance of undesired off-flavours. These same defects can happen if exposure lasts longer than the recommended times.

2.6 Aeration

Dutching is based on the oxidative reactions that take place in basic media and contribute to colour formation. During these chemical reactions, injecting oxygen is necessary, which is why aeration is crucial for changes in cocoa colour to take place. Specifically, greater aerations have been reported as being necessary for producing red cocoas rather than obtaining black ones (Ellis, 1992; Kopp et al., 2010; Wiant et al., 1989; Kopp et al., 2010; Trout, 2001).

One example of the importance of aeration is that described by Ellis (1992), whose followed a method to produce brown, red, dark red and black cocoas by adding compressed air during treatment. The aeration injected by the inventor during the process increased from brown powders (no aeration needed) to dark red cocoa, which exhibited the greatest need. Ellis (1992) applied between 0, 1-3.5, 1-5 and 3.5-5 bar/min of air to produce brown, black, red and dark red cocoa, respectively.

Apart from increasing the amount of added air, other authors have extended treatment duration. One example of such is Kopp et al (2010), who applied air injection for 30 minutes to produce a dark back cocoa powder, while applying the same injection for 60 minutes to obtain a bright red one. Other inventors (Ellis, 1992) have applied hydrogen peroxide as an oxidiser to enhance the change in cocoa colour from light brown to red, which consequently reduced treatment duration.

In general, the preferred air flow varies from 0 to 5 bar/min, depending on the final desired product colour.

2.7 Summary

It is generally established that the most widely employed and recommended conditions are: temperatures from 60ºC to 130ºC; pressure between 1 and 12 atm; alkali concentrations of 1-6%; water contents between 10% and 50%; aeration rates from 0 to 5 bar/min. The most widely used alkalis are K_2CO_3 and NaOH, which can be used alone or combined with others to produce different cocoa colours. Aeration and temperature also play a key role in cocoa colour formation and must be properly controlled to produce correct tonalities. Finally, treatment duration markedly differs between works because it depends on technology, other conditions and pursued objectives. One general recommendation of traditional treatment is that exposure time lasts between 5 and 180 minutes. Bearing this information in mind, Table 2 indicates general guidelines to produce two of the most desired alkalised cocoa powders: red and black. These conditions may vary according to the origin of cocoa and, overall, to alkalising plant facilities.

Table 2. Recommended conditions for the production of red and black powders.

	Red powder	Black powder
Alkali (Type - Kg/100Kg)	K_2CO_3 – 3%	NaOH + $(NH_4)OH$ – 6%
Water (Kg/100Kg)	10-50	10-50
Temperature (°C)	60-100	90-130
Pressure (atm)	+++ (i.e. 10)	+ (i.e. 5)
Duration (min)	>10	>60
Aeration (bar/min)	+++ (i.e. 5)	+ (i.e. 3)

3 Desired changes induced by alkalization in cocoa products

Before this section, the most widespread technology and treatment conditions to alkalise cocoa are reviewed. They all have three main missions: improve the solubility of powder, darken cocoa colour and modify the product's flavour profile.

3.1 Solubility

One of the main problems of incorporating cocoa powders into the formulations of different food products, such as milk beverages, is solubility. If it is low, it consequently leads to floc formation, layer creation and sedimentation (Holkar, Jadhav and Pinjari, 2019).

Different strategies have been implemented in industry to correct cocoa-related solubility issues; e.g., using different stabilisers and emulsifiers. Another approach is to reduce the proportion of cocoa powder included in formulations. To do so however, a material richer in soluble components must be produced in advance (Holkar et al., 2019).

To increase cocoaac powder solubility, in the 19^{th} century Coenraad Johannes van Houten developed a method known as alkalization (De Zaan, 2006). The improvement in solubility produced by this technique is undeniable, but no study has been found that has attempted to unveil the chemical changes responsible for this change.

Regarding cocoa solubility, cell structures of this material are characterised by being insoluble and difficult to discompose. In the cocoa production chain, especially after roasting, cocoa proteins and polyphenols are linked to these structures, which makes them thicker and more resistant to degradation. Releasing these compounds and destroying these complexes are essential objectives to increase powder solubility (Holkar et al., 2019).

Some authors have reported NaOH, one of the most widely employed alkalis in alkalization, to break ester links and to hydrolyse cell walls in other matrices. These effects, combined with other alkalization conditions (temperature, pressure, etc.), along with reducing fat content, can be responsible for the increased solubility caused by Dutching (Domínguez-Rodríguez, Marina and Plaza, 2017). In line with alkali type, the application of high temperatures, pressures, alkali concentrations and water contents during alkalization will induce a more marked increase in solubility through the destabilisation and destruction of different complexes and cell structures.

3.2 Colour

Cocoa colour is one of the most appreciated characteristics of a cocoa product (liquor or powder) given the current trend to restrict using artificial colours in food preparations. During fermentation, cocoa beans pass from a slaty colour (unfermented), to violet (partly fermented) and brown (totally fermented). This colour is maintained in nibs, liquor, cake and powder. In this way, the powders obtained from well fermented cocoa beans present a light brown colour (see Fig. 2a). As mentioned earlier, the typical natural cocoa colour can be transformed during alkalization by the combined effect of alkali agents, water content, aeration, temperature, time and pressure to yield a cocoa colour that goes from light to dark, and with red or black hues (see Figs. 2b to 2e), depending on operational conditions (see Table 2).

In order to understand the contribution of each processing variable to colour development, Rodríguez, Pérez and Guzmán (2009) studied the effect of three alkali types (sodium bicarbonate, sodium carbonate, sodium hydroxide) at three concentrations (1, 2, 3 g/100g of liquor) on the colour of a final liquor made with beans from Venezuela. These authors found that all the salts lowered the three colour coordinates, and that reduction was more severe at the highest alkali percentage (3 g/100g). Regarding alkali type, NaHCO3 and NaOH were the alkalis to show the most marked reduction in the L* coordinate, which went from ca. 40 (natural cocoa) to ca. 27. In terms of C* and h* coordinates, NaOH was the salt that contributed the most colour change, with values changing from ca. 11 and 12 to ca. 7 in the C* and h* values, respectively.

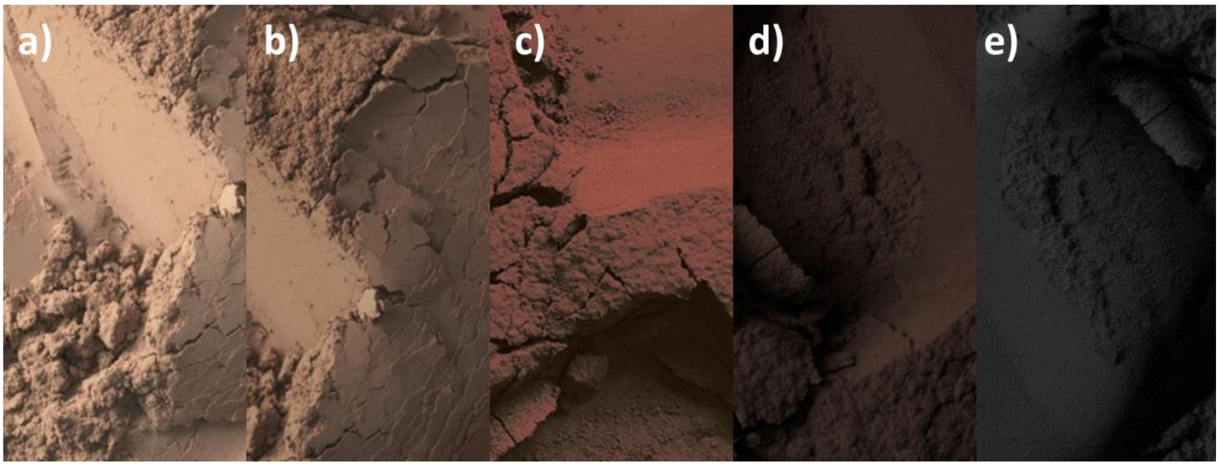

Figure 2. Cocoa powders submitted to different alkalization levels. a) natural; b) light alkalized, c) medium alkalized with reddish hue; d) strong alkalized; e) black powder.

Regarding the assessment of how colour develops, Li et al. (2013) studied the evolution of different colorimetric fractions of cocoa submitted to several alkalization levels. These authors realised that cocoa powder became darker with increased pressure, alkali concentration or reaction time. These colour transformations were associated with, on the one hand, the formation of polymers as a result of sugar degradation (caramelisations) and, on the other hand, with reactions between amino compounds and carbohydrates (Maillard reactions). The authors also observed that, in agreement with studies on caramelisation and Maillard reaction kinetics in model systems, which evidenced how the brown colour development of sugar and amino acid rich products (e.g. cocoa) intensified at high temperature (100ºC and above) and basic pH (> pH 8) (Ajandouz et al., 2001; Lan et al., 2010), these transformations intensified with increases in pH and temperature values.

Li and coworkers (2013) observed that in addition to Maillard reactions, dark colours were produced by a combination of anthocyanins and sugar. To reach this conclusion, the authors followed the evolution of anthocyanin absorbance (525 nm) and total colour difference (ΔE). As expected, with alkali (NaOH) concentrations increasing from 1% to 3%, the absorbance value at 525 nm lowered at the same time as ΔE increased, which suggests that darker cocoa powder contains less anthocyanin.

The characteristic colour of alkalised cocoa has been associated with the enzymatic activity of polyphenol oxidase (Rodríguez, Pérez and Guzmán, 2009). This enzyme, whose activity is optimal at pH 8, acts by oxidising polyphenolic compounds from cacao to produce melanoidines (brown-coloured pigments), and thus degrades and reduces polyphenolic substances (Biehl, 1986; Razzaque et al., 2000) As pH increases, phenolic compounds develop a reddish-brown to black colour. The higher the pH, the darker cocoa should be.

More recently, Germann et al. (2019a) studied the generation of chromophores from major polyphenols in cocoa (catechin and epicatechin). They based their work on the premise that changes in polyphenols in alkaline media may be responsible for part of cocoa's colour (Stark and Hofmann, 2006; Totlani and Peterson, 2005, 2007; Germann et al., 2019a, 2019b). Germann et al. (2019a) discovered that oxidation and chemical rearrangements transformed catechin and epicatechin into catechinic acid, which is an intermediate product of the chemical route to form different red and yellow chromophores. These authors also reported an increase in the high-molecular-weight products that exhibited a reddish-brown colour, which they assumed were the major contributors of cocoa darkening. None of the aforementioned molecules has been found in non-alkalised cocoas.

In a second work about the characterisation of unpolar chromophores deriving from catechin and epicatechin, Germann et al. (2019b) found that xanthenocatechins and xantheno-derived chromophores, a newly detected group of compounds, contributed to the red colour of alkalised cocoa. Figure 3 shows the chemical structures of colour precursors (catechin and epicatechin), and yellow and red molecules formed through different alkalization routes during cocoa alkalization.

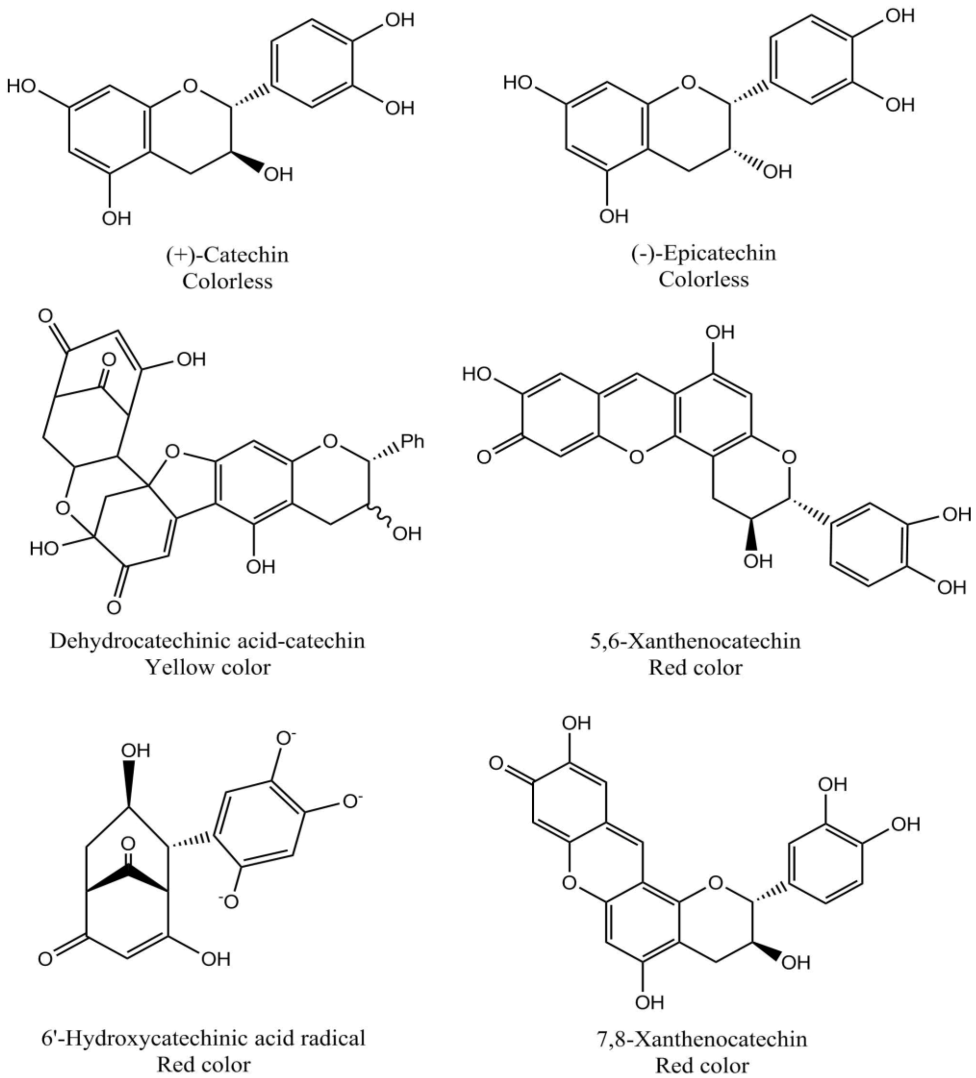

Figure 3. Examples of catechin and epicatechin-derived chromophores generated during alkalization.

In summary, colour changes occur by several chemical reactions, which are enhanced by the alkaline media generated by alkali agents, fed by oxygen from injected air, accelerated by temperature and facilitated by pressure. The formation of Maillard reaction products, the oxidation and polymerisation of polyphenols, their interactions with other molecules, and sporadic polyphenol oxidase activity, which works more efficiently under basic conditions, are some examples of the reactions that take place during alkalization and lead to visual colour changes.

3.3 Flavour

Along with colour, the characteristic cocoa flavour, which is a combination of taste and aroma, is one of its main hallmarks. Cocoa flavour originally depends on the cocoa genotype, farming conditions and environment factors, but is considerably modified by different post-harvesting operations (e.g. alkalization).

The basic taste sensations of cocoa are acidity, bitterness and astringency. As for taste sensation, it is well-known that citric, lactic acid, oxalic and succinic acid are major contributors to acidity in well-fermented natural cocoa. However, defects in cocoa fermentation can markedly increase acetic acid, which strongly impacts perceived cocoa acidity (Baigrie, 1994). During alkalization, acids are totally or partially neutralised and, thus, both their content and acidity perception lower in samples. Specifically in a study carried out by Li and coworkers (2012), the authors found that acids went from representing 60% of the volatile fraction to one below 30% after the alkalization process.

Cocoa bitterness is associated mainly with the presence of methylxanthines caffeine and theobromine, as well as diketopiperazines (formed by the thermal decomposition of proteins during cocoa roasting and alkalization), and monomeric and oligomeric flavan-3-ols (Baigrie, 1994; Stark & Hofmann, 2006). Catechin and epicatechin, and their epimerisation, isomerisation and non-enzymatic glycosylation modifications during alkalization, can affect sensory perception. In a study by Stark and Hofmann, (2006), they identified different glycosylated flavanols generated by alkalization, and also discovered that this modification eliminated the associated bitterness. It produced a velvety mouth-coating feeling and changed astringency from being puckering to smooth. Glycosylation also lowered the detection threshold of the astringency of modified molecules. For example, (-)-epicatechin and (-)-catechin have a threshold of 600 and 1,000 µmol/L, respectively, while those of their flavan-3-ol-3-glycosides range from 1.1 to 99.5 µmol/L. These authors also found that incorporating sugar into the structure had a significant effect on astringency depending on the position and type of sugar. Besides glycosylation, flavan-3-ols can also be modified by their interaction with Maillard reaction products (Totlani and Peterson, 2005, 2007). In order to establish whether these modifications can be associated with the generation of compounds capable of modulating the bitterness of cocoa, Zhang ent al. (2014) evaluated the formation of Maillard-catechin interaction products. To do so, they simulated simple Maillard reactions by mixing a reducing sugar (glucose, galactose or xylose), glycine and catechin under specific conditions. These researchers identified different Maillard-catechin interaction products that formed during simulations and evaluated if they were able to modulate the bitterness of caffeine. The results showed that of all the modified catechins, one was able to significantly reduce the bitterness generated by caffeine. This finding stresses that bitterness is not only related to bitter compounds, but also to the compounds capable of modulating it.

Concerning astringency, polyphenols such as phenolic acids, flavonol glycosides, anthocyanins, catechins and procyanidins, are mainly responsible for this mouth feeling (Baigrie, 1994). As indicated in-depth in Section 4.5, polyphenols are considerably affected during cocoa alkalization because alkalised cocoas tend to be less astringent than natural ones.

Besides acitidy, bitterness and astringency, cocoa specific flavour lies in the volatile or aroma faction, which is primarily detected by nasal receptors than by oral taste buds. The specific cocoa aroma arises from complex biochemical and chemical reactions that take place during the postharvest processing of raw beans, and are caused by many influences of the cocoa genotype, chemical make-up of raw seeds, environmental conditions, farming practices, processing and manufacturing stages (roasting and alkalization) (Baigrie, 1994). In recent decades, the different authors have attempted to describe all the chemical compounds that contribute to the cocoa aroma profile, with more than 600 identified volatiles (Ziegleder 2009). As previously mentioned, they include several chemical classes that provide some sensory perceptions; such as aldehydes (i.e. vanillin, chocolate, (sweet) vanilla), ketones (i.e. 2-pentanone -fruity), esters (ethyl acetate; fruity), alcohols (i.e. 1-propanol – sweet chocolate), acids (i.e. 2-methylpropionic acid; floral), terpenoids (i.e. geraniol; floral, fruity), nitriles (i.e. benzonitrile; nutty), pyrazines (2,5-dimethylpyrazine, sweet chocolate; nutty), furans (i.e. 5-methyl-2-furfural, sweet chocolate), lactones (i.e. δ-octenolactone; nutty), pyrroles (i.e. pyrrole; nutty) and diketopiperazines (cyclo (-Pro-Gly) - cyclo (-Pro-Gly)) (Bonvehí and Coll, 2000; Aprotosoaie et al., 2016; Borthwick and Costa, 2017).

How these compounds are modified during alkalization has rarely been studied. Nevertheless, a study by Li et al. (2012), in which the authors followed the evolution of 80 compounds in different alkalization stages, stated that some flavour compounds in the cocoa mass disappeared, while some appeared. Some new chemical structures include nonanal, ethanol, 2-propanol,1-methoxy-, and 2(s)-acetoxysuccinic anhydride. Most of these compounds stem from Strecker degradation, which is a minor pathway of the Maillard reaction of amino acids, and demonstrates the importance of Maillard reactions in flavour changes during alkalization (Cremer and Eichner, 2000).

Both the fractions that contribute to cocoa flavour, i.e., non-volatile (proteins, carbohydrates, alkaloids, methylxanthines and polyphenols) and volatile (alcohols and phenols, carboxylic acids, aldehydes, ketones, esters, amines, amides, nitriles, lactones, terpenoids, furans, furanones, pyrans, pyrones, pyrroles and pyrazines), are markedly transformed during cocoa alkalization. By bearing all these chemical transformations in mind, we can easily understand that, via the degradation and modification of the aforementioned compounds, alkalization has been reported to positively reduce the bitter, acidic and astringent tastes of natural cocoa and to generate new chemical structures to confer chocolate notes. Although it is true that new or derived compounds with bitter and astringent tastes can be formed or released from the matrix as a result of treatment, a reduction in the aforementioned tastes is generally perceived. It can be stated that: natural (non-alkalised powders) are acid and exhibit cocoa notes; light alkalised powders are mild and exhibit cocoa notes with mild alkali undertones; red powders exhibit moderate to strong alkali notes; strongly alkalised powders with brown dark products exhibit from milky to moderate alkali notes; black powders are characterised by an intensely unique alkali flavour (Dyer, 2003) provoked by the harsh treatment described in previous sections. Figure 4 graphically summarises flavour transformations, and illustrates the main flavour changes among a natural, a medium and a strong alkalised cocoa.

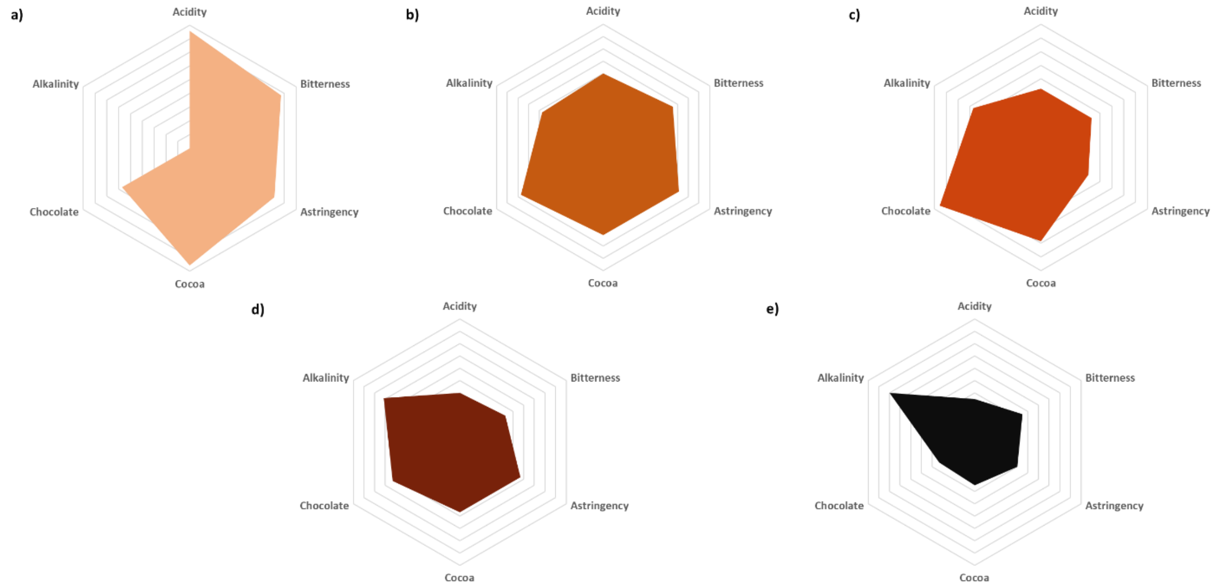

Figure 4. Sensory profile of cocoa powders submitted to different alkalization levels. a) natural; b) light alkalized, c) medium alkalized with reddish hue; d) strong alkalized; e) black powder.

4. Composition and chemical changes produced in cocoa by alkalization

Cocoa powder is known for being a nutritive ingredient with high diverse functional content. Although composition depends not only on the bean's origin, but also on the way that cocoa is treated, natural cocoa powder composition can be decomposed into complex carbohydrates (58%), proteins (20%) and fats (11%) that are its main constituents (Martín and Ramos, 2017). Cocoa powder also contains polyphenols, mainly flavanols (catechin, epicatechin and procyanidins (B1 and B2)), but also other families like flavonols (quercetin, isoquercetin), flavones (luteolin, apigenin), flavanones (naringenin), anthocyanins and phenolic acids, methylxanthines (theobromine, theofilline and caffeine), and a wide range of minerals (potassium, sodium, calcium, magnesium, phosphorus, chloride, iron, zinc, copper) and vitamins (retinol, thiamine, riboflavin, niacin, ascorbic acid, tocopherol and pantothenic acid). Cocoa also contains all the essential (isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, arginine and histidine) and non-essential (cysteine, tyrosine, alanine, aspartic acid, glutamic acid, glycine, proline and serine) amino acids (Martín and Ramos, 2017; Maleyki and Ismail, 2010; Tomas-Barberán et al., 2007; De Zaan cocoa, 2006; Holkar et al., 2019).

During natural powder alkalization, numerous complicated chemical reactions take place and are responsible for not only colour and flavour development, but also improved solubility. The same chemical reactions can produce desirable or undesirable changes in the nutritional, functional and microbiological characteristics of cocoa. Tables 3 and 4 briefly present the reported modifications to the nutritional, functional, microbiological and sensory features that may occur during cocoa alkalization.

4.1 Carbohydrates

One of the macromolecules whose evolution has been studied during alkalization is carbohydrates. Adeyeye (2016) compared the content of this group of compounds between non-alkalised and alkalised cocoa powders to find that total carbohydrate content did not change.

However, a detailed analysis of single molecules confirmed that the carbohydrate profile changed. Rodríguez, Pérez and Guzmán (2009) evaluated the effect of three different alkali agents at three concentrations on the amount of reducing sugars. These researchers found a reduction of more than 80% in sugars with NaOH and $NaHCO_3$, but observed no change with Na_2CO_3. Li et al (2012) studied changes in fructose in cocoa powder during alkalization. They found that this sugar sharply dropped during Dutching, which indicates that it can be the main sugar to interact with amino acids during Maillard reactions. Taş and Gökmen (2016) analysed the concentration of reducing sugars (sucrose, glucose and fructose). They did not observe any significant reduction in glucose and fructose, but noted a significant drop in sucrose content, which they associated with dipping cocoa beans in alkaline solution.

4.2 Proteins, peptides, amino acids and Maillard reaction products

Due to the temperature, enzymes and the media generated by alkali during alkalization, proteins are degraded by deamination and oxidation reactions (Rodríguez, Pérez and Guzmán, 2009; Oduns and Longe, 1998; Méndez-Albores, Campos-Aguilar, Moreno-Martínez, and Vázquez-Durán, 2013). Rodríguez, Pérez and Guzmán (2009) observed a reduction in crude protein due to thermal processing. Of all the tested alkalis, NaOH was the most aggressive one for proteins and led to a 12% loss.

Other authors have studied the degradation of proteins and observed a 45.5% reduction after alkalising with an equivalent cocoa pod ash concentration to 50 g/kg of NaOH (Oduns and Longe, 1998). In agreement with these authors, Adeyeye (2016) reported a diminished protein content of 55%. Méndez-Albores et al (2013) studied the effects of alkalization on the crude protein content of cocoa liquors and detected a 3.5% reduction in this parameter. These results all differ from those reported by Rodríguez, Pérez and Guzmán (2009).

To explain all the previous differences in protein content, comparisons were made of the protein estimation methodology, alkalization treatments and the conditions employed by different authors. The main differences found in these works seemed to be based on the employed conditions. Adeyeye (2016) did not define the alkalization process, while Oduns and Longe (1998) alkalised cocoa at room temperature with up to 50g/kg of cocoa-pod ash, the equivalent to NaOH for 6 h. Long duration and a high alkali concentration could explain the marked losses observed by these authors compared to the less marked losses reported by Méndez-Albores et al (2013) and Rodríguez, Pérez and Guzmán (2009). Both these works alkalised cocoa liquors under similar conditions, but with different cocoa:water proportions. Méndez-Albores et al (2013) used a proportion 1:2 and Rodríguez, Pérez and Guzmán (2009) applied 1:4. The higher water content employed by Rodríguez's group could explain why they observed a more marked degradation (12%) than that of Méndez-Albores' group (3.5%).

With peptides, several authors have reported the generation of bioactive peptides with antioxidant activity and inhibitory capacities via the induction of endogenous cocoa bean enzymes. Bioactive peptides are amino acid chains that remain inactive while they form part of their mother protein, but exhibit a functional activity when released (Sarmadi et al., 2012; Sarmadi, Ismail and Hamid, 2011). Although changes in the peptide profile and its associated functional activities have not being studied in relation to cocoa alkalization, the reported reduction in protein content brought about by treatment might lead to the formation of such peptides, and also to the consequent increase in cocoa functionality.

In addition to bioactive peptides, other structures have been documented to form during cocoa processing: diketopiperazines. These compounds are cyclic dipeptides that have been partially associated with the bitter taste of cocoa, and also with different functional effects

(Andruszkiewicz D'Souza, Altun, Corno, and Kuhnert, 2019). Although these molecules have not been studied during alkalization, they might form as a result of treatment, and may contribute to the flavour and functional effects of cocoa.

Apart from proteins and peptides, other researchers have studied the evolution of amino acids during alkalization (Taş and Gökmen, 2016; Li et al., 2012). These compounds, together with sugars, are consumed in Maillard reactions, and are specific precursors of cocoa aroma formation. Taş and Gökmen (2016) analysed lysine concentration during the Dutching of beans and reported no reduction. Li et al (2012) studied changes taking place during alkalization in all free amino acids. They observed that most were dramatically degraded and these losses were more substantial when glucose was added. This indicates that amino acids are lost through their participation in Maillard reactions, oxidative deamination, and by the interaction of peptides and polyphenols.

The aforementioned works report contradictory results as regards losses of amino acids and sugars. This contradiction can be associated with the different employed raw materials and alkalising methods. Li et al observed a significant degradation for lysine and fructose, while Taş and Gökmen did not notice any change. Li's group alkalised 25 g of cocoa powder using 20 mL of NaOH solution to treat the mixture with different pressures (from 0.02 to 0.1 MPa), alkali concentrations (from 1% to 3% NaOH) and treatment durations (from 20 to 30 minutes), which they chose in accordance with the desired degree of alkalization. Taş's group alkalised 160 g of cocoa beans with 7.5% Na_2CO_3 for 30 minutes. The differences in raw material, alkalization agent and alkalising method between both works apparently explain their contradictory conclusions.

Furthermore, the two previous groups followed different methods to determine amino acid content. Li et al (2012) measured amino acids content using HPLC equipment and measured at 338 nm. Taş and Gökmen (2016) carried out acid hydrolysis with the sample before analysing lysine content in a UPLC system coupled to a TQ detector. Acidification, degradation of proteins and the consequent release of amino acids, particularly lysine, could explain why no reduction in this compound was reported by Taş and Gökmen (2016).

Products of Maillard reactions are molecules formed by the interaction of amino acids and sugars that contribute to major cocoa sensory characteristics, i.e. flavour, taste and colour. Despite their desired effects, these compounds have been reported to be mutagenic, cytotoxic and carcinogenic. On the formation of Maillard reaction products, Taş and Gökmen (2016) studied the effect of alkalidation (cocoa beans treated with an alkaline solution of 7.5% Na_2CO_3 for 30 min) on the generation of these compounds. For this purpose, they monitored changes in the concentration of α-Dicarbonyls (3-deoxyglucosone, glucosone, glyoxal, methylglyoxal, diacetyl and 5-hydroxymethylfurfural) before and after alkalization by HPLC-ESI-MS. In their research, the authors found that alkaline treatment favoured sugar degradation and the formation of Maillard reaction products (α-dicarbonyl compounds), which could have a positive impact on cocoa taste, colour and flavour, but could also have a negative toxic impact. This increase in the previous Maillard reaction products could be associated with an increment in the oxidation of glucose and Amadori products, and also with the fragmentation of deoxyosones (Gobert and Glomb, 2009), reactions that alkalization increases.

4.3 Fat

Total fat content has been reported to be modified by alkalization. The interaction between the alkali agent and triglycerides leads to the hydrolysis and saponification of these compounds, and also to the formation of salts (Oduns and Longe, 1998; Adeyeye, 2016; Méndez-Albores et al., 2013). Excess alkali has been indicated to produce a soapy flavour through its interaction with fatty acids, which leads to their hydrolisation and saponification.

Rodríguez, Pérez and Guzmán (2009) observed a 16% decrease in total fat, but did not detect any soapy flavour in their samples. In line with them, Méndez-Albores et al (2013) also detected a 16% reduction of the crude fat content in the cocoa liquors alkalised with different alkalis.

Another author who studied the effects of alkalization on the fat content of cocoa was Adeyeye (2016), who reported a 66% loss and a 65.9% loss in crude fat and in fatty acid content, respectively. These losses were significantly more marked than those reported by Rodríguez, Pérez and Guzmán (2009) and Méndez-Albores, De Jesús-Flores, Castañeda-Roldan, Arámbula-Villa and Moreno-Martínez et al (2004).

The differences in fat content found by the above-cited researchers can be explained by the employed raw material. Rodríguez and Méndez-Albores' teams alkalised cocoa liquor (fat content ≈50%) and Adeyeye (2016) treated cocoa cake (fat content ≈10%). Cocoa cake has a lower fat content than liquor, because it is obtained after pressing liquor and partly removing fat.

In general, all the researchers agree that alkalization induces the hydrolysis of triglycerides and saponification of fatty acids. These degradation reactions may be taken into account during alkalization because, as documented, excess alkali concentrations lead to a soapy flavour developing in cocoa, which is certainly undesirable. These studies evidence that cocoa should be alkalised in the form of cake or powder to preserve cocoa's fat quality.

4.4 Minerals

Some authors have also studied changes in the mineral content produced by alkalization. It is generally assumed that some minerals increase during alkalization given the mineral contribution of the employed alkali.

Accordingly, Adeyeye (2016) analysed the mineral content of cocoa powders alkalised in a factory, and did not found any significant increase in mineral content. Sodium slightly increased in alkalised samples *versus* non-alkalised ones, which may be due to NaOH being used as an alkali agent.

In a different approach, Oduns and Longe (1998) studied the effect of alkalization on minerals (calcium, phosphorus, potassium, magnesium, sodium, copper, zinc, manganese and iron). These authors alkalised samples with a growing cocoa pod ash concentration that was the equivalent to a given NaOH concentration. Except for sodium and copper, the alkali agent and its different concentrations increased mineral content.

The increments in mineral content reported by Oduns and Longue can be related to the composition of the cocoa pod ash that they used for alkalization. Their work did not show any mineral content data about cocoa pod ash, which means that the observed increases in minerals could stem from this material.

In general, and as previously mentioned, alkalization increases the content of some minerals and, consequently, ash content. By way of example, Rodríguez, Pérez and Guzmán (2009) observed that ashes increased up to 113% with 30 g of NaOH/kg. In the work by Oduns and Longue (1998), a 34% increase took place with 50 g of NaOH equivalent/kg, while Rodríguez, Pérez and Guzmán (2009) noted a 3-fold higher increase with only 30 g of NaOH/kg. The difference in ash content between both these works might be related to the alkalising agent employed by Oduns and Longue (1998), whose concentration was not indicated.

4.5 Polyphenols

Polyphenols are secondary plant metabolites that play a relevant role in their defence against pathogenic diseases and infections, and also in their different maturation processes. More than 8,000 compounds have been identified in plants and divided into different groups according to their number of phenol rings and substitutions. The main groups of polyphenols include phenolic acids, flavonoids, stilbenes and lignans (Singh, Kesharwani and Keservani, 2017).

In dry cocoa beans, polyphenols, composed mainly of flavanols, represent approximately 10-15% of dry bean weight, which means that cocoa is the food with the highest flavanol content based on its dry weight (Martín, Goya and Ramos, 2013; Martín and Ramos, 2017; Aprotosoaie et al., 2016).

Of all the different types of polyphenols that exist in cocoa, three main groups appear: flavanols (catechin, epicatechin, gallocatechin, etc.), anthocyanins (leucoanthocyanins, cyanidins, etc.) and proanthocyanins (dimers, trimers and other polymers of flavan-3-ols). Apart from these groups, other compounds like flavones (apigenin, luteolin, kaempferol, etc.) and phenolic acids (caffeic acid, chlorogenic acid, etc.) can be found at low concentrations in cocoa (Aprotosoaie et al., 2016).

According to their structures and chemical modifications, and as seen later in this section, polyphenols are reported to exhibit different sensory and functional characteristics. For example, they have been identified as pigments, astringent and bitter compounds, and molecules able to modulate flavour, among others (El Gharras, 2009). Given their antioxidant capacity, they have been shown to have different *in vitro* beneficial effects, such as protection of neurons, stimulation of vasodilation, improvement of insulin secretion and inhibition of cancer cell proliferation (Del Rio, Costa, Lean and Crozier, 2009).

During alkalization, in exchange for product darkening, increased solubility and reduced acidity, astringency and bitterness, cocoa polyphenols have been reported to dramatically degrade. Gültekin-Özgüven, Berktaş and ÖzÇelik (2016) analysed total polyphenol, flavanol content and antioxidant activity of alkalised cocoa liquors. These researchers reported that the previous characteristics reduced by 87%, 83% and 50%. These losses were similar to those reported by Miller et al (2008) in commercial cocoa powders, which were 89% for flavanols in highly alkalised cocoa, by Gu, House, Wu, Ou, and Prior (2006) in commercial cocoa powders, which were 51% for antioxidant activity and 78% for procyanidins, and by Jolić, Redovniković, Marković, Šipušić, and Delonga (2011) in commercially alkalised cocoa nibs, which were 64% for total phenolic content, 59% for total procyanidins and 39% for antioxidant activity. In another work, Zhu et al (2002) simulated the alkaline conditions of the lower gut and found an 85% loss in flavanols and procyanidins at pH 7.4 after 24 h, and a 100% loss at pH 9 after 4 h. Recently, Quelal-Vascónez et al. (2019) in a study with more than 80 samples from different origins and alkalization levels found that among flavanols, epicatechin was the most abundant analyte. The highest content (536.59±0.08 mg/100 g) was found in a natural cocoa sample from Equator. Contents statistically lowered as the alkalization process became more intense, and reached average values of ca. 80, 33, 28 mg/100 g in light, medium and strong alkalised cocoas, respectively. In black cocoas, epicatechin content was under the limit of detection. The same pattern was found for catechin contents. In natural cocoas, catechin content ranged from 15.2±0.5 to 167.5±1.2 mg/100 g, and decreased until it was no longer detected in black cocoas.

All these researchers indicate the notion that alkalization strongly affects total polyphenol content and the antioxidant activity of cocoa, two characteristics that seem to be strictly correlated. Actually, an increased pH is responsible not only for the oxidation and interaction of polyphenols with polysaccharides, proteins, other polyphenols, Maillard products and pyrazines and their precursors, but also increases polyphenol oxidase activity (Rodríguez, Pérez and Guzmán, 2009; Misnawi, Jamilah and Nazamid , 2003). Such effects reduce the amount of polyphenols (Li et al., 2012).

Two compounds, epicatechin and catechin, are major polyphenols in cocoa. Their common stereoisomers are (-)-epicatechin and (+)-catechin, with (+)-epicatechin and (-)-catechin being rare conformations that are lacking in natural cocoa. In general, (-)-epicatechin content is higher than (+)-catechin content (Gültekin-Özgüven et al., 2016; Andres-Lacueva et al., 2008). When cocoa is alkalised, the epicatechin/catechin ratio is inversed by the generation of (-)-catechin (Gültekin-Özgüven et al., 2016; Hurst et al., 2011; Kofink, Papagiannopoulos and Galensa, 2007; Ortega et al, 2008). The appearance of this phenol is due to the isomerisation of (-)-epicatechin to (-)-catechin, the epimerisation of (+)-catechin to (-)-catechin, and the monomerisation of procyanidins (Gültekin-Özgüven et al., 2016; Hurst et al., 2011; Andres-Lacueva et al., 2008; Jolić et al., 2011). Unlike the findings reported by previous authors, other groups of researchers have found that epicatechin continues to be the major flavanol after alkalization (Todorovic, 2017; Jolić et al., 2011; Andres-Lacueva et al., 2008; Li et al., 2012). Nevertheless, if the analyses of these research teams are carefully studied, we can see that they simply quantified (+)-catechin content, and did not take into account the amount of (-)-catechin present in their samples. This would explain why these researchers reported how epicatechin continued to be a major flavanol after alkalization.

Of all the above polyphenols, (-)-epicatechin has the most bioaccessibility, followed by (+)-catechin and lastly by (-)-catechin (Gültekin-Özgüven et al., 2016; Rimbach, Melchin, Moehring and Wagner, 2009). As alkalization increases the (-)-catechin concentration, it can be stated that this cocoa production chain step reduces the bioaccessibility of cocoa polyphenols. Gültekin-Özgüven et al. (2016) defined this term as the percentage of procyanidins solubilised in chyme (water phase) after each digestion step (gastric and duodenal). They also found that alkalization significantly reduced this characteristic.

All these modifications in polyphenol content and profile are responsible for all the desired changes in the product. They are responsible, at least in part, for colour and change in flavour, and are also connected to cocoa solubility because their interaction with other compounds may be responsible for insoluble complexes appearing. Moreover, some authors have paid attention to the antimicrobial capacity of cocoas after alkalization. Cocoa contains a vast variety of polyphenols, among other functional compounds, that can destroy bacteria by reducing their fluidity, inhibiting the enzymes responsible for their growth and disrupting their cell membranes (Ariza et al., 2016). After alkalization, polyphenols have been reported to dramatically degrade, which could affect their antimicrobial properties.

In order to unveil the effect of alkalization on cocoa antimicrobial activity, Todorovic et al (2017) tested 11 extracts (six natural, five alkalised) of different commercial cocoa powders against three different Gram-positive bacteria strains (Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis), four Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella abony*) and one yeast (*Candida albicans*). The results showed that regardless of their degree of alkalization, the different cocoa extracts had a similar antimicrobial effect to those found in some herbal extracts like oregano, rosemary and celery. These authors also tested the effect of alkalization of different cocoa powders on antimicrobial activity. They found that Dutching did not significantly affect activity against Gram-positive bacteria, but significantly enhanced action against Gram-negative bacteria compared to the non-alkalised samples. These results suggest that losses in polyphenols do not imply diminished cocoa antimicrobial activity, which could be related to the formation of new bioactive compounds with enhanced antioxidant and antibacterial activities.

For all these reasons, it can be stated that cocoa is actually what it is thanks mainly to these compounds.

4.6 Methylxanthines

In addition to polyphenols, other compounds produced by plants that have been studied in cocoa are methylxanthines. These molecules are a group of alkaloids with a purine-based structure. Different methylxanthines can be distinguished by the presence of methyl groups in various numbers and positions (Bartella et al., 2019).

Methylxanthines are considered to have significant physiological and psychological effects on humans, which they perform via different mechanisms, such as the blockade of adenosine receptors, the regulation of intracellular calcium and the inhibition of phosphodiesterases (Franco, Oñatibia-Astibia and Martínez-Pinilla, 2013).

The effects of these compounds can include psycho-stimulation, modulation of sleep duration (theobromine has, for example, been reported to be the biggest contributor to sleeping time), neuroprotection, bronchodilatation, diuresis, gastric secretion stimulation, cardiovascular and metabolic effects, among others (Aprotosoaie et al., 2016; Franco et al., 2013). Apart from positive effects, methylxanthines have been reported to have several acute adverse effects, such as tachycardia, feeding intolerance, seizures and cardiac dysrhythmias. These effects are commonly associated with caffeine at usual therapeutic levels (Gauda and Martin, 2012).

In cocoa, theobromine is the methylxanthine at the highest concentration, followed by caffeine and lastly by theophylline. All these compounds have been reported to degrade during alkalization. Oduns and Longue (1998) reported how Dutching led to an 85.4% reduction in theobromine, while Li et al (2012) indicated reductions of 23% and 30.8% of theobromine and caffeine, respectively. As previously stated, the differences reported by researchers stem from their distinct alkalization methods, conditions and detection techniques. Oduns and Longue (1998) followed an especially complicated detection method with many steps, and also included filtering steps and chemical reagents for titration testing purposes. With Li et al (2012), the determination of theobromine was done more easily using a mass spectrophotometer.

The reported degradations of methylxanthines related to alkalization have been associated with not only the interaction of these compounds with the employed alkali agent, but also with their conversion into salts. Despite their degradation leading to lesser functional effects, it has been correlated with a desired reduction in bitterness and astringency (Stark and Hofmann, 2006; Aprotosoaie et al, 2016).

4.7 Volatile flavour compounds

Apart from colour, the other most well appreciated cocoa feature is its flavour. Its formation starts with the fermentation step where, under anaerobic conditions, essential peptides, required to produce flavour compounds are generated through protein degradation. Then during roasting, Maillard reactions that involve these peptides lead to the creation of characteristic cocoa flavour compounds (Scalone et al., 2019).

Cocoa flavour is composed of non-volatile flavours (see previous sections) and by the volatile flavour compounds studied by Li et al (2012). These researchers identified more than 80 volatile compounds that belong to different groups related to flavour (aldehydes, ketones, alcohols, acids, esters, alkanes, pyrazines, benzenes, furans, and others). They also evaluated the losses caused by distinct degrees of alkalization and found that the main volatile molecules present in cocoa were alcohols, acids and D-limonene, which accounted for almost 65% of all flavour volatile compounds. In general, alkalization did not affect alcohol concentrations, significantly reduced the concentration of acids and increased the presence of D-limonene, which incremented from 2% before alkalization to 31% after it. An increase in D-limonene enhances the citric flavour of cocoa.

These authors also observed that ketones, alkanes, furans and benzene-containing compounds incremented in the concentration after light alkalization, and that some significantly reduced after strong alkalization treatment.

Pyrazines, which are highly relevant nitrogen-containing heterocycles for flavouring cocoa, also decrease by alkalization. The proposed mechanism leading to the observed reductions in pyrazines, and in other volatile aroma compounds, is their interaction with polyphenols, apart from the effect of alkalising conditions. Polyphenols have hydroxyl groups that can form hydrogen bonds with amide carbonyls (Hagerman, 1992). With pyrazines, an increase in pH due to alkalization may allow these compounds to bind polyphenols.

In another step, Li et al (2012) also alkalised some samples by adding reducing sugar (glucose), which ended up with the formation of more flavour compounds during alkalization via different chemical processes, such as the production of Schiff's bases, Amadori rearrangements and Strecker degradations.

4.8 Acrylamide and furan

Acrylamide is a carcinogenic molecule that also has neurotoxic effects and is formed through the decomposition of L-asparagine and its interaction with carbonyl groups of reducing sugars during high-temperature treatments (>100ºC). Similarly, furan is formed as a result of the thermal processing of food from a wide range of precursors like amino acids, sugars, polyunsaturated fatty acids and carotenoids.

In order to study the formation of acrylamide during cocoa alkalization, Ofosu et al (2019) determined the appearance of this compound in alkalised roasted nibs. These researchers observed that, after alkalization, acrylamide content was lower than that found in roasted and non-alkalised nibs. The authors associated this inhibition of acrylamide formation with the presence of both antioxidants and alkalising salts in cocoa, which are believed to alter the formation course of the intermediate molecules responsible for Maillard reaction products.

More recently, Kruszewski and Obiedziński (2020) studied the formation of acrylamide and furan in cocoas with different degrees of alkalization to find that there were no statistical differences in acrylamide content between cocoas with different degrees of alkalization. Regarding furan content, the less alkalised cocoa had 3-fold more furan than the strongest alkalised powder. Therefore, it would seem that with cocoa powders, a higher degree of alkalization limits furan formation. So unexpectedly, alkalization either does not contribute to the formation of a significant amount of acrylamine or destroys the acrylamide generated in previous cocoa process stages.

4.9 Anti-nutritional factors

Anti-nutrients are a group of natural compounds present in food that hinder or have negative effects on the absorption and metabolism of other nutritional molecules. Examples of anti-nutritional factors are: tannins, which are able to combine with proteins, cellulose and other molecules to form insoluble structures; hydrocyanate, which are reported to inhibit cytochrome oxidase activity; phytic acid, with a strong binding capacity to different minerals that prevents their absorption; oxalates and oxalic acid, which capture calcium and reduce its absorption (Emire, Jha and Mekam, 2015; Astley and Finglas, 2016).

In cocoa, the reported anti-nutrients are hydrocyanate, oxalates, tannins and phytin (Aremu, Agiang and Ayatse, 1995; Adeyeye, 2016). Adeyeye (2016) studied the effect of alkalization on phytin, oxalate and tannin levels. Of them all, tannins, which are usually formed by the polymerisation of catechins, lowered by 38.6%. This agrees with the observations made in previous sections because polyphenols and their polymers are degraded by alkalization. The above-cited author observed an increment in phytin and oxalate of 39.9% and 69%, respectively.

Schroderm, Vanhanen and Savage (2011) evaluated oxalates content in 15 commercial cocoa powders of different origins and at several alkalization levels. These authors concluded that no relation appeared between oxalates content and Dutching because the level among the different powders was generally similar. Although these results apparently go against the augmentation in oxalated observed by Adeyeye (2016), Schroder et al (2011) used a group of natural and alkalised cocoas of different brands whose origins and producers were not related, which makes drawing conclusions about the effect of Dutching difficult.

4.10 Spores

Another important aspect associated with the bacterial quality of cocoa is the effect that alkalization has on spores. During fermentation, different bacteria and yeasts are allowed to grow and create the different precursors required to produce the desired cocoa flavour and colour. After fermentation, bacterial content significantly lowers by a sterilisation step.

However some bacteria, mainly of the genus *Bacillus*, survive and remain in cocoa thanks to their thermoresistant spore-forming abilities. The fact that they remain in powder does not affect its quality given their poor water activity, but this compromises the quality of the products containing this powder (Lima, Kamphuis, Nout and Zwietering, 2011).

The effects of alkalization on spores have been studied by several authors. Lima et al (2011) analysed microorganism content in a set of commercial cocoa powders and only detected thermoresistant spores in alkalised cocoa powders, but not in non-alkalised ones. Although a rising pH apparently leads to bacteria sporulation, the above authors indicated that further studies must be conducted to determine if pH positively influences the appearance of thermoresistant spores or if it is merely a coincidence. Pia et al (2019) applied alkalization to pre-roasted nibs and studied the evolution of *Bacillus cereus* and *Geobacillus stearothermophilus* populations. These authors concluded that alkalization neither enhanced nor reduced the effect of these spore-forming bacteria on spores, which showed that the observation made by Lima et al (2011) was probably more a coincidence than an effect of the pH of media.

4.11 Mycotoxins

Another factor that has been studied is the effect of treatment on mycotoxins. Fungi contamination is inevitable during storage and processing. In the cocoa production chain, beans, which are especially susceptible to contamination, can be affected by fungal spoilage during and after the fermentation step. One study detected several fungi species in beans that had already been fermented and dried, in which mycotoxin-producer species like *Aspergillus* and *Penicillium* were detected (Méndez-Albores et al., 2004).

In general, heat treatment over 250ºC can effectively lower the concentration of mycotoxins. For example, aflatoxins content in corn lowered by 81% after being roasted at 285ºC for 7 minutes (Méndez-Albores et al., 2013). In cocoa, Méndez-Albores et al (2013) roasted cocoa beans at 250ºC for 15 minutes and found that aflatoxin content lowered to 63.9 ng/g (71% fewer aflatoxins). Similarly, and by way of example, the Mexican regulation for aflatoxins sets a maximum of 20 ng/g by considering their hepatotoxic, teratogenic, mutagenic and carcinogenic properties. As shown, that limit was not reached after cocoa roasting.

After roasting, the authors alkalised samples using three different alkalis (NaOH, KOH, $Ca(OH)_2$) at three concentrations (10, 20, 30 g/kg). The results showed that the lowest concentration of alkalis (10 g/kg) reduced aflatoxins by 87.5% with KOH and by 92.2% with the other two agents. When high alkali concentrations were applied, reductions in aflatoxins above 98% were achieved by all the employed alkali agents. After the alkalization treatment, cocoa met legal Mexican specifications.

Other authors have studied the concentrations of aflatoxins (B_1, B_2, G_1, G_2) and ochratoxin in cocoa products (Turcotte, Scott and Tague, 2013). These researchers found out that when natural cocoas were alkalised, the concentration of aflatoxins B_1, B_2, G_1 and G_2, and ochratoxin, lowered by 67.7%, 70.5%, 44%, 100% and 68.2%, respectively. The concentrations of all the analysed mycotoxins were lower than the maximum level set by law.

Table 3. Reported changes in nutritional and functional compounds induced in cocoa by alkalization.

Attribute	Reported changes	Mechanisms	References
Carbohydrates	• No change of total carbohydrates • Decreasing of reducing sugars	• Maillard reactions	Li et al., 2012; Rodríguez, Pérez and Guzmán, 2009; Taş and Gökmen, 2016; Adeyeye, 2016
Protein	• Diminution of protein content	• Oxidative destruction by deamination	References: Rodríguez, Pérez and Guzmán., 2009; Adeyeye, 2016; Méndez-Albores et al., 2013; Oduns and Longe, 1998
Amino acids	• Reduction in 17 amino acids	• Maillard reactions, oxidative deamination and interaction with polyphenols	Li et al., 2012; Taş and Gökmen, 2016
Fat	• Reduction of total fat content	• Hydrolysis and saponification of triglycerides	Rodríguez, Pérez and Guzmán, 2009; Oduns and Longe, 1998; Adeyeye, 2016; Méndez-Albores et al., 2013
Ash	• Total ash content is increased	• By the addition of the alkalizing salts	References: Rodríguez, Pérez and Guzmán, 2009; Adeyeye, 2016; Oduns and Longe, 1998
Minerals	• Increase of the cation (Na^{+}/K^{2+}) present in the alkalization salt	•	Oduns and Longe, 1998; Adeyeye, 2016
Polyphenols	• Total phenol content is reduced • Antioxidant activity is reduced • Flavanols content is reduced • Relation epicatechin/catechin is inversed	• Oxidation of polyphenols and their interaction with amino acids, proteins, peptides, other flavonoids and Maillard products • Epimerization of (+)-catechin to (-)-catechin • Monomerization of procyanidins	Li et al., 2012; Andres- Lacueva et al., 2008; Rodríguez, Pérez and Guzmán, 2009; Miller et al., 2008; Gültekin-Özgüven, Berktaş and ÖzÇelik, 2016; Jolić et al., 2011; Zhu et al., 2002; Gu et al., 2006; Todorovic et al., 2017; Hurst et al., 2011; Kofink, Papagiannopoulos and Galensa, 2007; Ortega et al., 2008; Stark and Hofmann, 2006; Totlani and Peterson, 2005, 2007; Zhang, Xia and Peterson, 2014; Germann, Stark and Hofmann., 2019a and 2019b; Todorovic et al., 2017

	• Alkalization negatively affects bioaccessibility • Reduction of self-bitterness and self-astringency of catechin and epicatechin • Chemical modifications that modulate bitterness • Formation of red chromophores during alkalization • Alkalization increased the antimicrobial activity against *Gram-negative* bacteria	• Isomerization of (-)-epicatechin to (-)-catechin • Glycosilation of flavanols • Modifications induced by Maillard reaction products	
Methylxanthines	• Theobromine and caffeine are reduced	Theobromine interacts with bases and forms salts	Oduns and Longe, 1998; Li et al., 2012

Table 3. Reported changes in attributes related to sensory properties, toxicity and microbial quality

Attribute	• Reported changes	• Mechanisms	References
Volatile and flavor compounds	• Alcohols unchanged • Acids importantly reduced • D-Limonene highly increased • Pyrazines are reduced	• Degradation • Interaction with polyphenols due to the change in the pH	Li et al., 2012
Maillard reaction products	• Degradation of 3-deoxyglucosone • Increase of glucosone and diacetyl • Glyoxal, HMF and methylglyoxal unchanged	• Oxidation of Amadori compounds and glucose	Taş and Gökmen, 2016
Acrilamide	• Alkalization reduces acrylamide formation	• Presence of antioxidant molecules • Alkalis inhibit the formation of these compounds	Ofosu et al., 2019; Kruszewski and Obiedziński, 2020
Anti-nutritional factors	• Reduction in tannins and increase in phytins and oxalates	• Degradation • Releasing from cells due to the treatment	Adeyeye, 2016; Schroder et al., 2011
Spores	• Alkalization does not affect spores		Pia et al., 2019; Lima et al., 2011
Mycotoxins	• Reduction in aflatoxins and ochratoxin	• Thermal and basic degradation	Méndez-Albores et al., 2013; Turcotte, Scott and Tague, 2013

5 Conclusion

On the whole, this review offers a comprehensive overview of cocoa alkalization in terms of the most frequently used conditions and changes in nutritional, physicochemical, functional, microbiological and sensory characteristics.

In general, despite newly developed and alternatives techniques, traditional alkalization continues to be the most method widely used by industry. The data collected and shown in this review can be employed as a guide to design new alkalization methods, and to even optimise existing ones.

This review also provides insights into the changes that take place during alkalization by showing that producing the desired changes in cocoa involves nutritional and functional costs, but not all induced changes are negative. So via alkalization, cocoa safety increases by reducing microorganisms and their produced toxins.

After analysing all the published information, we conclude that polyphenols are responsible, at least in part, for the colour, flavour and functional properties of cocoa. Cocoa is a rich complex matrix but, notwithstanding, all cocoa features seem dependent on, or related to, the effects of polyphenols. All in all, cocoa is what it is given its richness and variety in this class of compounds.

Acknowledgements

This work was supported by the Ministerio de Economía, Industria y Competitividad (Spanish Government) and the European Regional Development Fund (Project RTC-2016-5241-2). Damián Valverde would like to thank the Regional Ministry of Education, Culture and Sports of Generalitat Valenciana for his PhD grant.

Author Contributions

D. Valverde collected, drafted and wrote the review. É. Pérez-Esteve and J. M. Barat helped to design the framework of this review and critically revised different sections of the draft. J. M. Barat performed the final revision and authorised the publication.

Conflict of interest

The authors declare no conflict of interest.

References

Adeyeye, E. I. (2016). Proximate, mineral and antinutrient compositions of natural cocoa cake, cocoa liquor and alkalized cocoa powders sourced in Nigeria. *Journal of Advance Pharmaceutical Science and Technology*, *1*, 12-28. https://doi.org/10.14302/issn.2328-0182.japst-15-855

Ajandouz, E. H., Tchiakpe, L. S., Ore, F. D., Benajiba, A., & Puigserver, A. (2001). Effects of pH on caramelization and Maillard reaction kinetics in fructose-lysine model systems. *Journal of Food Science*, *66*(7), 926-931. https://doi.org/10.1111/j.1365-2621.2001.tb08213.x

Andres-Lacueva, C., Monagas, M., Khan, N., Izquierdo-Pulido, M., Urpi-Sarda, M., Permanyer, J., & Lamuela-Raventós, R. M. (2008). Flavanol and flavonol contents of cocoa powder products: influence of the manufacturing process. *Journal of Agricultural and Food Chemistry*, *56*, 3111-3117. https://doi.org/10.1021/jf0728754

Andruszkiewicz, P. J., D'Souza, R. N., Altun, I., Corno, M., & Kuhnert, N. (2019). Thermally-induced formation of taste-active 2,5-diketopiperazines from short-chain peptide precursors in cocoa. *Food Research International*, *121*, 217-228. https://doi.org/10.1016/j.foodres.2019.03.015

Annual report 2014/2015 (2017). International Cocoa Organization. https://www.icco.org/about-us/international-cocoa-agreements/cat_view/1-annual-report.html Accessed 03 January 2020.

Aprotosoaie, A. C., Luca, S. V., & Miron, A. (2016). Flavor chemistry of cocoa and cocoa products: an overview. *Comprehensive Reviews in Food Science and Food Safety*, *15* (1), 73-91. https://doi.org/10.1111/1541-4337.12180

Aremu, C. Y., Agiang, M. A., & Ayatse, J. O. I. (1995). Nutrient and antinutrient profiles of raw and fermented cocoa beans. *Plant Foods for Human Nutrition*, *48*, 217-223. https://doi.org/10.1007/bf01088443

Ariza, B. T. S., Suswati, E., Mufida, D. C., Setiawan, A. R., Isnaini, A., Setiawan, A. R., Rizal, M., Wahyudi, T., & Misnawi, J. (2016). Study on activity of cocoa ethanolic extract against Shigella dysenteriae. *Pelita Perkebunan*, *32*, 130-137. https://doi.org/10.22302/iccri.jur.pelitaperkebunan.v32i2.222

Astley, S., & Finglas, P. (2016). Nutrition and health. Reference Module in Food Sciences. https://doi.org/10.1016/B978-0-08-100596-5.03425-9

Bandi, J. P., Kubicek, K., & Raboud. P. B. (1984). Installation for solubilizing cocoa. US4438681A.

Baigrie B.D. (1994) Cocoa flavour. In: Piggott J.R., Paterson A. (eds) Understanding Natural Flavors. Springer, Boston, MA

Bartella, L., Donna, L. D., Napoli, A., Siciliano, C., Sindona, G., & Mazzotti, F. (2019). A rapid method of the assay of methylxanthines alkaloids: theobromine, theophylline and caffeine, in cocoa products and drugs by paper spray tandem mass spectrometry. *Food Chemistry*, *278*, 261-266. https://doi.org/10.1016/j.foodchem.2018.11.072

Bauermeister, J. (1989). Process for making cacao powder by disagglomeration and cacao powder granulate by subsequent agglomeration. EP0310790A2.

Beg, M. S., Ahmad, S., Jan, K., & Bashir, K. (2017). Status, supply chain and processing of cocoa – A review. *Trends in Food Science & Technology*, *66*, 108-166. https://doi.org/10.1016/j.tifs.2017.06.007

Biehl, B. (1986). Cocoa fermentation and problem of acidity, over-fermentation and low cocoa flavour. Incorporated Society of Planters.

Bonvehí, J. S., & Coll, F. V. (2000). Evaluation of purine alkaloids and diketopiperazines contents in processed cocoa powder. *European Food Research and Technology*, *210*(3), 189-195. https://doi.org/10.1007/PL00005510

Borthwick, A. D., & Da Costa, N. C. (2017). 2, 5-diketopiperazines in food and beverages: Taste and bioactivity. *Critical reviews in food science and nutrition*, *57*(4), 718-742. https://doi.org/10.1080/10408398.2014.911142

Chalin, M. L. (1972). Method of dutching cocoa. US3868469A.

Codex Alimentarius. Codex standard for cocoa powders (cocoas) and dry mixtures of cocoa and sugars. Codex Stan 105 (1981), pp. 1-4

Cremer, D. R., & Eichner, K. (2000). The reaction kinetics for the formation of Strecker aldehydes in low moisture model systems and in plant powders. *Food Chemistry*, *71*(1), 37-43. https://doi.org/10.1016/S0308-8146(00)00122-9

De Vuyst, L., & Weckx, S. (2016). The cocoa bean fermentation process: from ecosystem analysis to starter culture development. *Journal of Applied Microbiology*, *121*(1), 5-17. https://doi.org/10.1111/jam.13045

De Zaan cocoa, 2006. Cocoa & Chocolate manual. ADM Cocoa, Suiza.

Del Rio, D., Costa, L. G., Lean, M. E. J., & Crozier, A. (2010). Polyphenols and health: What compounds are involved? *Nutrition, metabolism and cardiovascular diseases*, *20*(1), 1-6. https://doi.org/10.1016/j.numecd.2009.05.015

Domínguez-Rodríguez, G., Marina, M. L., Plaza, M. (2017). Strategies for the extraction and analysis of non-extractable polyphenols from plants. *Journal of Chromatography A*, *1514*, 1-15.

El Gharras, H. (2009). Polyphenols: food sources, properties and applications – a review. *International Journal of Food Science & Technology*, *44* (12), 2512-2518. https://doi.org/10.1111/j.1365-2621.2009.02077.x

Ellis, L. D. (1990). Process for making dark cocoa. US5114730A.

Emire, S. A., Jha, Y. K., Mekam, F. (2013). Role of anti-nutritional factors in food industry. *Beverage & Food World*, 23-28.

Fei, L., Rodriguez-Garcia, J., Damme, I. V., Westwood, N. J., Shaw, L., Robinson, J. S., Warren, G., Chatzifragkou, A., Mason, S. M., Gomez, L., Faas, L., Balcombe, K., Srinivasan, C., Picchioni, F., Hadley, P., & Charalampopoulos, D. (2018). Valorisation strategies for cocoa pod husk and its fractions. *Current Opinion in Green and Sustainable Chemistry*, *14*, 80-88. https://doi.org/10.1016/j.cogsc.2018.07.007

Franco, R., Oñatibia-Astibia, A., & Martínez-Pinilla, E. (2013). Health benefits of methylxanthines in cacao and chocolate. *Nutrients*, *5* (10), 4159-4173. https://dx.doi.org/10.3390%2Fnu5104159

Gauda, E. B., & Martin, R. J. (2012). Chapter 43. Control of Breathing. Avery's diseases of the newborn. 9th edition. Edited by Gleason, C. A., and Devaskar, S. U.

Germann, D., Stark, T. D., & Hofmann, T. (2019a). Formation and characterization of polyphenol-derived red chromophores. Enhancing the color of processed cocoa powders: part 1. *Journal of Agricultural and Food Chemistry*, *67*, 4632-4642. https://doi.org/10.1021/acs.jafc.9b01049

Germann, D., Stark, T. D., & Hofmann, T. (2019b). Formation and characterization of polyphenol-derived red chromophores. Enhancing the color of processed cocoa powders: part

2. *Journal of Agricultural and Food Chemistry*, *67*, 4643-4651. https://doi.org/10.1021/acs.jafc.9b01050

Gobert, J., & Glomb, M. A. (2009). Degradation of glucose: reinvestigation or reactive α-dicarbonyl compounds. *Journal of Agricultural and Food Chemistry*, *57*, 8591-8597. https://doi.org/10.1021/jf9019085

Gu, L., House, S. E., Wu, X., Ou, B., & Prior, R. L. (2006). Procyanidin and catechin contents and antioxidant capacity of cocoa and chocolate products. *Journal of Agricultural and Food Chemistry*, *54* (11), 4057-4061. https://doi.org/10.1021/jf060360r

Gültekin-Özgüven, M., Berktaş, I., & ÖzÇelik, B. (2016). Change in stability of procyanidins, antioxidant capacity and in-vitro bioaccessibility during processing of cocoa powder from cocoa beans. *LWT - Food Science and Technology*, *72*, 559-565. https://doi.org/10.1016/j.lwt.2016.04.065

Hagerman, A. E. (1992). Tannin-protein interactions. Phenolic compounds in food and their effects on health (pp 236-247). ACD Symposium Series.

Holkar, C. R., Jadhav, A. J., & Pinjari, D. V. (2019). A critical review on the possible remediation of sediment in cocoa/coffee flavored milk. *Trends in Food Science & Technology*, *86*, 199-208. https://doi.org/10.1016/j.tifs.2019.02.035

Huang, Y., & Barringer, S. A. (2010). Alkylpyrazines and other volatiles in cocoa liquors at pH 5 to 8, by Selected Ion Flow Tube-Mass Spectrometry (SIFT-MS). *Journal of Food Science*, *75*(1), 121–127. https://doi.org/10.1111/j.1750-3841.2009.01455.x

Hurst, W. J., Krake, S. H., Bergmeier, S. C., Payne, M. J., Miller, K. B., & Stuart, D. A. (2011). Impact of fermentation, drying, roasting and Dutch processing on flavan-3-ol stereochemistry in cacao beans and cocoa ingredients. *Chemistry Central Journal*, *5* (53). https://doi.org/10.1186/1752-153X-5-53

Jolić, M. A., Redovniković, I. R., Marković, k., Šipušić, D. I., & Delonga, K. (2011). Changes of phenolic compounds and antioxidant capacity in cocoa beans processing. *International Journal of Food Science & Technology*, *46* (9), 1793-1800. https://doi.org/10.1111/j.1365-2621.2011.02670.x

Kofink, M., Papagiannopoulos, M., & Galensa, R. (2007). (-)-Catechin in cocoa and chocolate: occurrence and analysis of an atypical flavan-3-ol enantiomer. *Molecules*, *12*, 1274-1288. https://doi.org/10.3390/12071274

Kongor, J. E., Hinneh, M., de Walle, D. V., Afoakwa, E. O., Boeckx, P., & Dewettinck, K. (2016). Factors influencing quality variation in cocoa (Theobroma cacao) bean flavor profile – A review. *Food Research International*, *82*, 44-52. https://doi.org/10.1016/j.foodres.2016.01.012

Kopp, G. M., Hennen, J. C., Seyller, M., & Brandstetter, B. (2010). Process for producing high flavour cocoa. EP2241190A1.

Kruszewski, B., & Obiedziński, M. W. (2020). Impact of raw materials and production processes of furan and acrylamide contents in dark chocolate. Journal of Agricultural and *Food Chemistry*, *68* (8), 2562-2569. https://doi.org/10.1021/acs.jafc.0c00412

Lan, X., Liu, P., Xia, S., Jia, C., Mukunzi, D., Zhang, X., Xia, W., Tian, H., & Xiao, Z. (2010). Temperature effect on the non-volatile compounds of Maillard reaction products derived from xylose–soybean peptide system: Further insights into thermal degradation and cross-linking. *Food Chemistry*, *120*(4), 967-972. https://doi.org/10.1016/j.foodchem.2009.11.033

Li, Y., Feng, Y., Zhu, S., Luo, C., Ma, J., & Zhong, F. (2012). The effect of alkalization on the bioactive and flavor related components in commercial cocoa powder. *Journal of Food Composition and Analysis*, *25*(1), 17-23. https://doi.org/10.1016/j.jfca.2011.04.010

Lima, L. J. R., Kamphuis, H. J., Nout, M. J. R., & Zwietering, M .H. (2011). Microbiota of cocoa powder with particular reference to aerobic thermoresistant spore-formers. *Food Microbiology*, *28*(3), 573-582. https://doi.org/10.1016/j.fm.2010.11.011

Maleyki, M. J. A., Ismail, A. (2010). Antioxidant properties of cocoa powder. *Journal of Food Biochemistry*, *34*, 111-128. https://doi.org/10.1111/j.1745-4514.2009.00268.x

Martín, M. A., & Ramos, S. (2017). Health beneficial effects of cocoa phenolic compounds: a mini-review. *Current Opinion in Food Science*, *14*, 20-25. https://doi.org/10.1016/j.cofs.2016.12.002

Martín, M. A., Goya, L., & Ramos, S. (2013). Potential for preventive effects of cocoa and cocoa polyphenols in cancer. *Food and Chemical Toxicology*, *56*, 336-351. https://doi.org/10.1016/j.fct.2013.02.020

Méndez-Albores, A., Campos-Aguilar, A. Z., Moreno-Martínez, E., & Vázquez-Durán, A. (2013). Physical and chemical degradation of B-aflatoxins during the roasting and dutching of cocoa liquor. *Journal of Agricultural Science and Technology*, *15* (3), 557-567.

Méndez-Albores, A., De Jesús-Flores, F., Castañeda-Roldan, E., Arámbula-Villa, G., & Moreno-Martínez, E. (2004). The effect of toasting and boiling on the fate of B-Aflatoxins during pinole preparation. *Journal of Food Engineering*, *65* (4), 585-589. https://doi.org/10.1016/j.jfoodeng.2004.02.024

Miller, K. B., Hurst, W. J., Payne, M. J., Stuart, D. A., Apgar, J., Sweigart, D. S., & Ou, B. (2008). Impact of alkalization on the antioxidant and flavanol content of commercial cocoa powders. *Journal of Agricultural and Food Chemistry*, *56* (18), 8527-8533. https://doi.org/10.1021/jf801670p

Misnawi, S. J., Jamilah, B., & Nazamid, S. (2003). Effects of incubation and polyphenol oxidase enrichment on colour, fermentation index, procyanidins and astringency of unfermented and partly fermented cocoa beans. *International Journal of Food Science and Technology*, *38* (3), 285-295. https://doi.org/10.1046/j.1365-2621.2003.00674.x

Oduns, A. A., & Longe, O. G. (1998). Nutritive value of hot water- or cocoa-pod ash solution-treated cocoa bean cake for broiler chicks. *British Poultry Science*, *39* (4), 519-525. https://doi.org/10.1080/00071669888700

Ofosu, I. W., Ankar-Brewoo, G. M., Lutterodt, H. E., Benefo, E. O., & Menyah, C. A. (2019). Estimated daily intake and risk of prevailing acrylamide content of alkalized roasted cocoa beans. *Scientific African*, 6. https://doi.org/10.1016/j.sciaf.2019.e00176

Okiyama, D. C. G., Navarro, S. L. B., & Rodrigues, C. E. C. (2017). Cocoa shell and its compounds: applications in the food industry. *Trends in Food Science & Technology*, *63*, 103-112. https://doi.org/10.1016/j.tifs.2017.03.007

Ortega, N., Romero, M. P., Macià, A., Reguant, J., Anglès, N., Morelló, J. R., & Motilva, M. J. (2008). Obtention and characterization of phenolic extracts from different cocoa sources. *Journal of Agricultural and Food Chemistry*, *56* (20), 9621-9627. https://doi.org/10.1021/jf8014415

Pia, A. K. R., Pereira, A. P. M., Costa, R. A., Alvarenga, V. O., Freire, L., Carlin, F., & Sant'Ana, A. S. (2019). The fate of Bacillus cereus and Geobacillus stearothermophilus during alkalization of cocoa as affected by alkali concentration and use of pre-roasted nibs. *Food Microbiology*, *82*, 99-106. https://doi.org/10.1016/j.fm.2019.01.009

Quelal-Vásconez, M. A., Lerma-García, M. J., Pérez-Esteve, É., Arnau-Bonachera, A., Barat, J. M., & Talens, P. (2020). Changes in methylxanthines and flavanols during cocoa powder processing and their quantification by near-infrared spectroscopy. *LWT*, *117*, 108598. https://doi.org/10.1016/j.lwt.2019.108598

Quelal-Vásconez, M. A., Lerma-García, M. J., Pérez-Esteve, É., Talens, P., & Barat, J. M. (2020) Roadmap of cocoa quality and authenticity control in the industry: A review of conventional and alternative methods. *Comprehensive Reviews in Food Science and Food Safety*, *19*, 448-478. https://doi.org/10.1111/1541-4337.12522

Razzaque, M. A., Saud, Z. A., Absar, N., Karin, M. R., & Hashinaga, F. (2000). Purification and characterization of polyphenoloxidase from guava infected with fruit-rot disease. *Pakistan Journal of Biological Sciences*, *3*, 407-410. https://doi.org/10.3923/pjbs.2000.407.410

Rimbach, G., Melchin, M., Moehring, J., Wagner, A. E. (2009). Polyphenols from cocoa and vascular health –A critical review. *International Journal of Molecular Sciences, 10* (10), 4290-4309. https://dx.doi.org/10.3390%2Fijms10104290

Rodríguez, P., Pérez, E., & Guzmán, R. (2009). Effect of types and concentrations of alkali on the color of cocoa liquor. *Journal of the Science of Food and Agriculture*, *89* (7), 1186-1194. https://doi.org/10.1002/jsfa.3573

Saltini, R., Akkerman, R., & Frosch, S. (2013). Optimizing chocolate production through traceability: a review of the influence of farming practices on cocoa bean quality. *Food Control*, *29* (1), 167-187. https://doi.org/10.1016/j.foodcont.2012.05.054

Sarmadi, B., Aminuddin, F., Hamid, M., Saari, N., Abdul-Hamid, A., & Ismail, A. (2012). Hypoglycemic effects of cocoa (Theobroma cacao L.) autolysates. *Food Chemistry*, *134* (2), 905-911. https://doi.org/10.1016/j.foodchem.2012.02.202

Sarmadi, B., Ismail, A., & Hamid, M. (2011). Antioxidant and angiotensin converting enzyme (ACE) inhibitory activities of cocoa (Theobroma cacao L.) autolysates. *Food Research International*, *44* (1), 290-296. https://doi.org/10.1016/j.foodres.2010.10.017

Scalone, G. L. L., Textoris-Taube, K., Meulenaer, B. D., Kimpe, N. D., Wöstemeyer, J., & Voigt, J. (2019). Cocoa-specific flavor components and their peptide precursors. *Food Research International*, *123*, 503-515. https://doi.org/10.1016/j.foodres.2019.05.019

Schroder, T., Vanhanen, L., & Savage, G. P. (2011). Oxalate content in commercially, produced cocoa and dark chocolate. *Journal of Food Composition and Analysis*, *24* (7), 916-922. https://doi.org/10.1016/j.jfca.2011.03.008

Shankar, M. U., Levitan, C. A., Prescott, J., & Spence, C. (2009). The influence of color and label information on flavor perception. *Chemosensory Perception*, *2*(2), 53-58. https://doi.org/10.1007/s12078-009-9046-4

Singh, P., Kesharwani, R. K., & Keservani, R. K. (2017). Chapter 24. Antioxidants and vitamins: roles in cellular function and metabolism. Sustained energy for enhanced human functions and activity. Edited by Debasis Bagchi.

Stark, T., & Hofmann, T. (2006). Application of a molecular sensory science approach to alkalized cocoa (Theobroma cacao): structure determination and sensory activity of nonenzymatically C-glycosylated flavan-3-ols. *Journal of Agricultural and Food Chemistry*, *54* (25), 9510-9521. https://doi.org/10.1021/jf062403

Tanaka, M., & Terauchi, M. (1999). Cocoa powder rich in polyphenols, process for producing the same and modified cocoa containing the same. US6485772B1.

Taş, N. G., & Gökmen, V. (2016). Effect of alkalization on the Maillard reaction products formed in cocoa during roasting. *Food Research International*, *89*, 930-936. https://doi.org/10.1016/j.foodres.2015.12.021

Terink, J., & Brandon, M. J. (1981). Alkalized cocoa powders and foodstuffs containing such powders. US4435436A.

Todorovic, V., Milenkovic, M., Vidovic, B., Todorovic, Z., & Sobajic, S. (2017). Correlation between antimicrobial, antioxidant activity, and polyphenols of alkalized/nonalkalized cocoa powders. *Journal of Food Science, 82* (4), 1020-1027. https://doi.org/10.1111/1750-3841.13672

Tomas-Barberán, F. A., Cienfuegos-Jovellanos, E., Marín, A., Muguerza, B., Gil-Izquierdo, A., Cerdá, B., Zafrilla, P., Morillas, J., Mulero, J., Ibarra, A., Pasamar, M. A., Ramón, D., & Espín, J. C. (2007). A new process to develop a cocoa poder with higher flavonoid monomer content and enhanced bioavailability in healthy humans. *Journal of Agricultural and Food Chemistry, 55* (10), 3926-3935. https://doi.org/10.1021/jf070121j

Totlani, V. M., & Peterson, D. G. (2005). Reactivity of epicatechin in aqueous glycine and glucose Maillard reaction models: quenching of C2, C3, and C4 sugar fragments. *Journal of Agricultural and Food Chemistry*, *53*, 4130-4135. https://doi.org/10.1021/jf050044x

Totlani, V. M., & Peterson, D. G. (2007). Influence of epicatechin reactions on the mechanisms of Maillard product formation in low moisture model systems. *Journal of Agricultural and Food Chemistry, 55* (2), 414-420. https://doi.org/10.1021/jf0617521

Trout, R. B. (2001). Method for making dutched cocoa. EP1278428B1.

Turcotte, A. M., Scott, P. M., & Tague, B. (2013). Analysis of cocoa products for ochratoxin A and aflatoxins. *Mycotoxin Research*, *29* (3), 193-201. https://doi.org/10.1007/s12550-013-0167-x

Wiant, M. J., William R., Lynch, W.R., & LeFreniere, R. C. (1989). Method for producing deep red and black cocoa. US5009917A.

Wissgott, U. (1988). Process of alkalization of cocoa in aqueous phase. US4784866A.

Wollgast, J., & Anklam, E. (2000). Review on polyphenols in Theobroma cacao: changes in composition during the manufacture of chocolate and methodology for identification and quantification. *Food Research International*, *33* (6), 423-447. https://doi.org/10.1016/S0963-9969(00)00068-5

Zhang, L., Xia, Y., & Peterson, D. G. (2014). Identification of bitter modulating Maillard-Catechin reaction products. *Journal of Agricultural and Food Chemistry*, *62* (33), 8470-8477. https://doi.org/10.1021/jf502040e

Zhu, Q. Y., Holt, R. R., Lazarus, S. A., Ensunsa, J .L., Hammerstone, J. F., Schmitz, H. H., & Keen, C. L. (2002). Stability of the flvan-3-ols epicatechin and catechin and related dimeric procyanidins derived from cocoa. *Journal of Agricultural and Food Chemistry*, *50* (6), 1700-1705. https://doi.org/10.1021/jf011228o