UNIVERSITAT POLITÈCNICA DE VALÈNCIA

DEPARTAMENTO DE TECNOLOGÍA DE ALIMENTOS

Application of new technologies to cocoa alkalization

Submitted by

Damián Valverde García

PhD Supervisors:

Prof. José Manuel Barat Baviera

Dr. Édgar Pérez Esteve

Valencia, January 2020

JOSÉ MANUEL BARAT BAVIERA, PhD in Food Technology and Professor at the *Universitat Politècnica de València*, and ÉDGAR PÉREZ ESTEVE, PhD in Food Technology

CERTIFY:

That the work *"***Application of new technologies to cocoa alkalization"** has been developed by Damián Valverde García under their supervision in the Departamento de Tecnología de Alimentos of the *Universitat Politècnica de València*, as a Thesis Project in order to obtain the degree of PhD in Food Science, Technology and Management at the *Universitat Politècnica de València.*

Valencia, 28th of January 2020.

Prof. José Manuel Barat Baviera   Dr. Édgar Pérez Esteve

Acknowledgements

Agradecimientos

Cuando una etapa de la vida termina, antes de levar anclas y aventurarse en la siguiente, uno debe honrar a todos los que han estado a su lado a lo largo de la pasada travesía. Y la travesía que ha supuesto la presente tesis doctoral ha sido larga, tres años nada más ni nada menos, con todos los momentos y personas que ello conlleva. Esta sección es para ellos, para las personas y sus momentos, para todos sin excepción, pues igual de agradecer es el haber contribuido con un pequeño gesto que el haber estado ahí apoyando durante toda la travesía.

Comenzaré dando las gracias a una persona que ya no está, a mi abuela. Ella siempre creyó en mí y considero que el primer agradecimiento debe ser para ella.

Continuaré con José Manuel Barat y con Édgar Pérez Esteve, personas que han sido esenciales para que este proyecto llegase a buen puerto. A ambos quiero agradecerles el haberme acogido, tanto como doctorando y como miembro más del grupo, y también el haber estado siempre ahí, empujando y sumando, confiando y aconsejando, para que la embarcación llegase a buen puerto.

También quiero dar las gracias a todos los integrantes del grupo, tanto a los que siguen como a los que cambiaron de rumbo y ahora van hacia otros puertos: Amira, Ana, Chiara, Cristina Cabrera, Cristina Fuentes, Héctor, Isabel, María, María Jesús, Nataly, Samu, Susana, Vicky… A todos ellos, gracias por haber estado ahí y por todos vuestros apoyos, sonrisas, debates, comidas y todo cualquier pequeño momento que me hayáis dado. Sois una segunda familia para mí.

Quiero aprovechar también esta página para dar las gracias a mis tres mejores amigas, Cristina Sala, Lorena Martínez y Miriam Gómez, por haber estado ahí con sus consejos, opiniones o simplemente escuchando. También a mi familia, que lleva apoyándome desde mucho antes de que el galeón zarpase rumbo a la tesis doctoral.

A todas estas personas y a todas las que no haya mencionado, gracias de corazón. Este pequeño logro también es vuestro y por eso os dedico esta sección.

Abstract

"Application of new technologies to cocoa alkalization" is a PhD thesis that aims to study the effects of two alternative techniques on the physicochemical and functional features of alkalized cocoa, and to evaluate their suitability to replace the traditional treatment.

In chapter I, the conventional system for alkalization in closed and pressurized vessels has been replaced by extrusion. This technique has been proved to be a fast, continuous and less energy-consuming method, characteristics that the traditional system lacks and that have promoted its application to cocoa alkalization. On the one hand, the effects produced by temperature, water content and alkali type and concentration were evaluated on the physicochemical and functional features of cocoa. In general, alkali type and concentration were the main variables increasing the pH, darkening the sample, but also reducing the functional content, while water content and temperature exerted minor effects. On the other hand, the temperature and the water content were the variables exhibiting the greatest effect on the moisture content, leading to reductions of almost 50%. Moreover, the extrusion alkalization products were compared to traditionally produced cocoa powders based on physicochemical, functional and sensory criteria. The results revealed that extrusion was faster and able to dark cocoa in a greater extent than the conventional treatment and to produce sensory acceptable powders. These results confirm that extrusion is a feasible and promising alternative method able to alkalize cocoa in a fast, continuous and less energy-consuming manner.

In chapter II, microwave heating has been studied as a substitute of the traditional alkalization treatment. This heating method has proven to be fast, versatile and able to preserve functional and sensory features of the treated products, which has made interesting its application to cocoa alkalization. The effects of the microwave heating technique on water content, power, duration, pressure application and alkali type and concentration were analyzed. In general, alkali type and concentration were the main variables inducing the darkening of cocoa, increasing its pH and reducing the concentrations of catechin and epicatechin.

However, these variables did not affect the antioxidant activity and the total phenol content of the treated cocoas in comparison to the untreated one. Treatment pressure, power and duration exerted positive effects on the antioxidant activity, the total phenol content or both. These changes were related to the capacity of these variables, in combination with the alkali agent, to release non-extractable polyphenols from the matrix and to the induction of different chemical reactions. Additionally, the effects of the different variables were studied on the moisture content. Power and duration of the microwave heating treatment were decisive in the reduction of the water content, which was significantly reduced up to 70%. In terms of the evaluation of the suitability of the developed microwave heating alkalization method, the produced cocoas were compared to the traditionally alkalized ones in their physicochemical, functional and sensory characteristics. The developed fast microwave heating treatment showed to be able to dark cocoa as much as the traditional method, to produce sensory equivalent powders and to lead to the obtaining of cocoas with higher antioxidant activity and phenol content.

In conclusion, this PhD thesis has applied extrusion and microwave heating as promising alternatives to cocoa alkalization. These technologies have demonstrated to dark cocoa in a significantly faster fashion than the traditional alkalization treatment, while keeping similar sensory profile and functional features, and substantially drying the product.

Resumen

«Aplicación de nuevas tecnologías a la alcalinización de cacao» es una tesis doctoral centrada, por un lado, en el estudio de los cambios fisicoquímicos y funcionales causados por dos técnicas de alcalinización alternativas a la convencional y, por otro, en la comparación de dichas propiedades con las de muestras comerciales.

En el capítulo I, se describe un sistema de alcalinización basado en extrusión. Esta tecnología se ha aplicado a la alcalinización de cacao dada su capacidad de tratar la materia prima de forma rápida y continúa, y su bajo consumo energético. En primer lugar, se estudiaron los efectos de diversas variables de proceso sobre las propiedades fisicoquímicas y funcionales de cacaos en polvo. En general, de todas las variables, el tipo y la concentración de álcali fueron las principales responsables del incremento de pH, del oscurecimiento de las muestras y de la reducción en el contenido de compuestos funcionales. En cuanto a la humedad, fueron la temperatura y el contenido en agua las que mostraron causar los mayores efectos, llegando a producir reducciones de casi un 50%. Además de evaluarse el efecto de las diferentes variables, en este capítulo también se compararon los cacaos producidos mediante extrusión con los alcalinizados por el método convencional. Los resultados mostraron que la extrusión, en menos de cinco minutos, fue capaz de oscurecer y de producir cacaos en polvo con un perfil sensorial, una capacidad antioxidante y un contenido en polifenoles totales relativamente similar al de los productos comerciales.

En cuanto al capítulo II, en este se ha estudiado la técnica de calentamiento por microondas como una alternativa al tratamiento de alcalinización tradicional. La tecnología microondas ha demostrado ser rápida, versátil y capaz de preservar las características funcionales y sensoriales, lo que ha hecho interesante su aplicación a la alcalinización de cacao. En los trabajos que forman este segundo capítulo, se estudiaron los efectos de diversas variables de proceso sobre el producto. En general, el tipo y la concentración de álcali fueron las variables principalmente responsables del oscurecimiento del cacao, del incremento del pH y de la reducción de las concentraciones de catequina y epicatequina. Sin embargo, el álcali empleado no redujo la actividad antioxidante ni al contenido en fenoles totales, propiedades que se vieron incrementadas por la presión, la potencia y la duración del tratamiento. Estos incrementos se relacionaron con la capacidad de estas variables para liberar a los polifenoles no extractables y para promover determinadas reacciones químicas. Además, también se estudió el efecto de las diferentes variables sobre la humedad. La potencia y la duración del tratamiento fueron las que se mostraron esenciales para lograr el secado del producto, llegando a producir reducciones de hasta el 70%. Por otro lado, además de estudiarse el impacto de las diferentes variables, también se compararon cacaos producidos por microondas con muestras producidas por el método convencional para evaluar su similitud con los cacaos comerciales. Los resultados mostraron que el microondas, en solo cuatro minutos, fue capaz de oscurecer el cacao y de mantener un perfil sensorial similar al producido por el método tradicional, a la par que conducía a una mejora en sus propiedades funcionales.

En resumen, los métodos para la alcalinización de cacao desarrollados en el marco de la presente tesis doctoral han demostrado ser unas alternativas muy prometedoras a la tecnología convencional. Ambas técnicas no solo han sido capaces de oscurecer el cacao tanto como el método comercial en un tiempo mucho menor, sino que también han conseguido el secado parcial de la muestra y unas características sensoriales y funcionales comparables o incluso mejores que las del método convencional.

Resum

«Aplicació de noves tecnologies a l'alcalinització de cacau» és una tesi doctoral centrada, d'una banda, en l'estudi dels canvis fisicoquímics i funcionals causats per dos tècniques d'alcalinització alternatives a la convencional i, d'altra banda, en la comparació d'aquestes propietats amb les de mostres comercials.

En el capítol I, es descriu un sistema d'alcalinització similar al convencional però basat en extrusió. Aquesta tecnologia s'ha aplicat a l'alcalinització de cacau per la seua capacitat de tractar la matèria primera de forma ràpida i continua, i pel seu baix consum energètic. En primer lloc, es van estudiar els efectes de diverses variables de procés sobre les propietats fisicoquímiques i funcionals de cacaus en pols. En general, de totes les variables, el tipus i la concentració d'àlcali van ser les principals responsables de l'increment de pH, de l'enfosquiment de les mostres i de la reducció en el contingut de compostos funcionals. Pel que fa a la humitat, van ser la temperatura i el contingut en aigua les que van causar els majors efectes, arribant a produir reduccions d'un 50%. A més d'avaluar l'efecte de les diferents variables, en aquest capítol també es van comparar els cacaus produïts mitjançant extrusió amb els alcalinizats pel mètode convencional. Els resultats van mostrar que l'extrusió, en menys de cinc minuts, va a ser capaç d'enfosquir i de produir cacaus en pols amb un perfil sensorial, una capacitat antioxidant i un contingut en polifenols totals relativament similar al dels productes comercials.

Pel que fa al capítol II, en aquest s'ha estudiat la tècnica de calfament per microones com una alternativa a l'alcalinització tradicional. La tecnologia microones ha demostrat ser ràpida, versàtil i capaç de preservar les característiques funcionals i sensorials, el que ha fet interessant la seua aplicació a l'alcalinització de cacau. En els treballs que formen aquest segon capítol, es van estudiar els efectes de diverses variables de procés sobre el producte. En general, el tipus i la concentració d'àlcali van ser les variables principalment responsables de l'enfosquiment del cacau, de l'increment del pH i de la reducció de les concentracions de catequina i epicatequina. No obstant això, l'àlcali empleat no va reduir l'activitat antioxidant ni al contingut en fenols totals, propietats que es van incrementar per la pressió, la potència i la duració del tractament. Aquests increments es van relacionar amb la capacitat d'aquestes variables per alliberar els polifenols no extractables i per promoure determinades reaccions químiques. A més, també es va estudiar l'efecte de les diferents variables sobre la humitat. La potència i la duració del tractament van ser les que es van mostrar essencials per aconseguir la deshidratació del producte, arribant a produir reduccions de fins al 70%. D'altra banda, a més d’estudiar-se l’impacte de les diferents variables, també es van comparar els cacaus produïts per microones amb mostres produïdes pel mètode convencional per tal d’avaluar la seua similitud amb els cacaus comercials. Els resultats van mostrar que el microones, en només quatre minuts, va ser capaç d'enfosquir el cacau i de mantenir un perfil sensorial similar al produït pel mètode tradicional, al mateix temps que conduïa a una millora en les seues propietats funcionals.

En resum, els mètodes per a l’alcalinització de cacau desenvolupats en el marc de la present tesi doctoral han demostrat ser unes alternatives molt prometedores per a substituir la tecnologia convencional. Les dos tècniques no només han estat capaces d'enfosquir el cacau tant com el mètode comercial en un temps molt menor, sinó que també han aconseguit la deshidratació parcial de la mostra i unes característiques sensorials i funcionals comparables a les del mètode convencional.

TABLE OF CONTENTS

1. PREAMBLE

1. PREAMBLE

This PhD thesis called "Application of new technologies to cocoa alkalization" forms part of the project "Study of the relationship between the processing variables and the nutritional and functional changes of cocoa powder. Development of a predicting methodology to processing" (Project RTC-2016-5241-2) funded by 2016-2019 European Region Development Fund.

This project has two aims: to understand the changes in cocoa induced by processing and to guarantee a precise prediction of the final characteristics of cocoa. In the present, it is difficult to make reliable predictions because there is not so much available information about the effects of the different treatments on cocoa.

For reaching the previous objectives, this project has been divided into three working lines: (1) study of the influence of the raw material and of the processing conditions on the physicochemical properties of cocoa powder, (2) development of fast methods for the analysis of cocoa and (3) design and application of alternative methods oriented to preserve the nutritional, functional and sensory characteristics of cocoa powder.

This doctoral thesis is part of the third working line of the previously described project. It aims to design new alkalizing techniques, which are faster and that cause lesser functional losses in comparison to the traditional treatment, while producing the desired changes in cocoa.

In this thesis, two different alternative treatments for alkalization have been developed: an extrusion and a microwave heating one. Both methods have interesting advantages that have made their application to cocoa alkalization a matter of interest. Extrusion is continuous, low energy consumption and fast technique widely employed by the industry for transforming powdered materials with the application of temperature and pressure. On the other hand, microwave heating, although being discontinuous, is a fast technique that uses electromagnetic waves as a heating source. This technique has a high penetration power and has been reported for preserving the nutritional, functional and sensory properties of the treated materials.

The application of the above mentioned technologies to cocoa, the study of their effects on the material and the evaluation of their suitability are what issued the present thesis.

2. OBJECTIVES

2. OBJECTIVES

The main objectives of the present thesis were to develop fast, more sustainable and less damaging methods for cocoa alkalization and to study their effects on the physicochemical and functional features, while evaluating the suitability of these new alkalization technologies for replacing the traditional method. In order to fulfill the main objectives, two different techniques were tested:

A. Extrusion. The specific objectives were:
    a. To design an extrusion alkalization treatment able to produce powders with physicochemical and sensory characteristics similar to commercial cocoas
    b. To determine the effect of the variables of the extrusion alkalization method on the physicochemical and functional features of cocoa
    c. To analyze if the developed extrusion alkalization treatment was able to improve the functional characteristics of commercial cocoas
B. Microwave heating. The specific objectives were:
    a. To design an alkalization treatment based on microwave heating capable of producing similar physicochemical changes than those exhibited by the commercial cocoas
    b. To determine the effect of the variables of the microwave thermal alkalization method on the physicochemical and functional features of cocoa
    c. To analyze if the developed alkalization treatment based on microwave was able to improve the functional characteristics of commercial cocoas
    d. To produce cocoas sensory similar to the traditionally alkalized ones

3. GENERAL INTRODUCTION

Based on the review "Changes in cocoa properties induced by alkalization process: a review" submitted to Comprehensive Reviews in Food Science and Food Safety

1. Cocoa production chain

Cocoa is one of the most distributed and well-known products around the world. The color and flavor 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 food appreciated by consumers, a relevant ingredient for industry and an exceptional source of research 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, but also in confectionery fillings and different skin products. The solid part separated from butter, known as cake, is milled to produce a fine powder. This product is then introduced into the formulations of an assortment of food products, such as frozen desserts, bakery products, confectionery coatings, dairy products and instant premixes (Beg et al., 2017; De Zaan, 2006).

In essence, the described cocoa production chain has not changed in the last 150 years, although the used equipment has been renovated and automated, which has not only improved the efficiency, but has also cut the time, of the process (Beg et al., 2017).

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

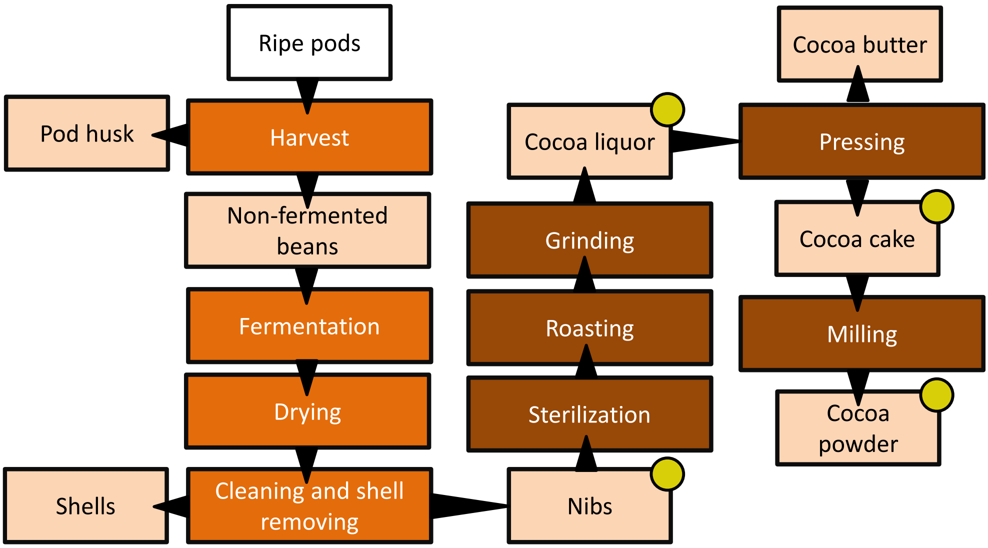

Figure 1. General scheme of natural cocoa powder production. The color of the primary process is grey, the secondary one is dark grey, while the different obtained products are depicted in light gray. Balck circles indicate the products that can be alkalized.

Alkalization, also known as "Dutching", was a treatment conceived first by Coenraad Johannes van Houten in the 19th century to enhance cocoa powder solubility. However after its implementation, industry showed it had the capacity to modify color and flavor, 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 alkalized, it is no longer considered natural and, 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 alkalized cocoa (pH above 7.6) (Miller et al., 2008). As expected, strong alkalized products exhibit darker colors, lower astringent, bitter and acidic notes, and more solubility.

Cocoa is so complex, but important, for the food industry that different scientific works have focused on improving and revealing the effects of the 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 (2015) described the cocoa production chain, the health effects of cocoa, and how flavor compounds and precursors are affected by the different production chain steps. Saltini, Akkerman and Frosch (2013) reported how farming practices affect cocoa bean quality. Wollgast & Anklam (2000) conducted their work about the alterations of polyphenols during general chocolate processing, and the methods used for the identification, isolation, purification and quantification of polyphenols. Kongor et al (2016) reviewed the factors influencing the bean flavor 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. Fei et al (2018) used their review to show strategies for valorizing cocoa pod husks and their fractions. Finally, Okiyama, Navarro and Rodrigues et al (2017) centered their work on cocoa shell applications in the food industry.

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. Additionally by using this knowledge, it aims to describe the most commonly used technology and parameters by industry for cocoa alkalization, as well as the physico-chemical, nutritional, functional, microbiological and sensory changes that Dutching produces to cocoa.

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 pressurized reactors with a continuous kneading system (Wissgott, 1988; Trout, 2001; Wiant, Lynch and LeFreniere., 1989), although some authors have described unpressurized versions of this process (Tanaka and Terauchi, 1999; Ellis, 1990; and Terink and Brandon, 1984), and have even designed novel systems by applying other types of technologies, such as extrusion (Chalin, 1972; Bandi, Kubicek and Raboud, 1984; and Bauermeister, 1989).

In an attempt to describe all the variables that can be combined during an alkalization process to develop a cocoa product with specific properties (color, flavor, etc.), patents related to alkalization processes have been reviewed. After analyzing 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 commonest and most recommended conditions are presented to gain detailed insight into the alkalization processing conditions and its effects.

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

Table 1. Continuation.

Author	Procedure	T (°C)	Alkali (%)	Water content (%)	P (atm)	t (min)	Developed product
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 will 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 the different cocoa components lead to the desired color, flavor and solubility changes, which are the modifications that alkalization aims to make.

Several kinds of alkalis have been reported to be employed during alkalization, such as NaOH, Na_2CO_3, $NaHCO_3$, KOH, K_2C0_3, $KHCO_3$, $(NaH_4)_2CO_3$, $Ca(OH)_2$ and $CaCO_3$. All these salts are included in the Codex Alimentarius as authorized 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 color. Its combinations and concentrations depend on their basicity, the final desired color to be obtained and the alkali off-flavor that they confer. In line with this, with their patent Kopp et al (2010) showed two examples of obtaining dark black cocoas using different combinations of alkali. In one, these authors used 2.4% of NaOH and 12% of $(NaH_4)_2CO_3$, while they replaced half of NaOH with 2% of K_2CO_3 in the other This replacement did not affect the final color, but significantly reduced the alkali off-flavor of cocoa. This example stresses the importance of combining alkali agents to avoid this negative perception, but to make the desired color modification.

After analyzing different patents, it can be generally established that K_2CO_3 and NaOH are the most widely employed alkali agents during alkalization, although NaOH has been reported as being that which is best able to darken cocoa (Rodríguez, Pérez and Guzmán, 2009).

In addition to 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 in taste.

2.2. Water content

In general, water within the 10% and 50% range is added to the alkalization mixture. The importance of this variable lies in its ability to transport the alkali agent to the color precursors that will be oxidized during alkalization. To ensure that the alkali is well distributed, the amount of added water is necessary to wet the cocoa material, but it is important to bear in mind that adding more water than necessary will entail longer drying (Wiant, Lynch and LeFreniere, 1989).

2.3. Temperature

Temperature is another important variable for determining color and treatment duration. Generally increasing temperature leads to darker colors and faster reactions. The most frequently used range of temperatures goes from 60°C to 130°C. For dark black cocoas, the maximum recommended temperature is 150°C because higher ones not only negatively affect cocoa flavor, but also produce darker colors (Wiant, Lynch and LeFreniere, 1989).

In relation to the color of the final product, temperature plays a crucial role. Lower temperatures are needed to produce red cocoas compared to obtaining black products (Wissgott, 1988; Wiant, Lynch and LeFreniere, 1989). Indeed Ellis (1992) reported two methods for producing red and dark red cocoas by treating the sample at 74-79°C and 82°C, respectively. This inventor also reported two other processes for producing brown and black cocoa, in which the material was treated at 85-87°C and 88°C, respectively. As we can see, an increasing temperature allows the sample to darken, but increasing it too much results in losses of red chromophores and the formation of other color compounds. Regarding this change in colored compounds’ composition profile, another section explains how alkalization leads to the formation of red chromophores, but also to its agglomeration, which leads brown high-molecular-weight compounds to appear (Germann, Stark and Hofmann, 2019a).

In another work, Wiant, Lynch and LeFreniere (1989) patented two processes for producing dark red and black cocoa using a pressurized reactor, where the difference lay in aeration, duration and temperature. These authors treated cocoa at 65-100°C to produce dark red powders and at 120-135°C to obtain dark black ones. Once again, this emphasizes the need to apply milder temperatures to produce red products.

2.4. Pressure

Applying pressure is another parameter that can be used to shorten treatment duration. By way of example, when comparing the different works describing a process for producing black cocoa (Terink and Brandon, 1981; Kopp et al., 2010; Wiant, Lynch and LeFreniere, 1991; Ellis, 1990; Chalin, 1972), it can be seen that the traditional system (pressure between 1 and 12 atm) requires a reaction time of between 30 and 120 minutes. However during extrusion, which applies a pressure between 47 and 75 atm, duration is less than 5 minutes. In addition to speeding up the process, pressurization has been associated with cocoa red coloration intensity (Wissgott, 1988).

2.5. Treatment duration

Regarding treatment duration, Wiant, Lynch and LeFreniere (1991) recommended general exposure to last between 5 and 180 minutes, and specified a duration ranging from 60 to 180 minutes for black products. So it is worth underlining the importance of treatment duration in relation to color generation and the appearance of off-flavors. As stated in Section 3.2.3 about temperature, temperatures higher than those recommended can lead to loss of red chromophores and to the appearance of undesired off-flavors. These same defects can happen if exposure is longer than recommended.

2.6. Aeration

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

One example of the importance of aeration is that described by Ellis (1992) with a method for producing 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 exhibits 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 an air injection for 30 minutes to produce dark back cocoa, while applying the same injection for 60 minutes to obtain a bright red one. Other inventors (Ellis, 1992) have applied hydrogen peroxide as oxidizer to enhance the change in cocoa color from light brown to red, which consequently reduced treatment duration.

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

2.7. Summary

It can be generally established that the most employed and recommended conditions are: temperatures between 60°C and 130°C; alkali concentrations between 1% and 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 colors. Aeration and temperature also play a key role in cocoa color 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. However, a general recommendation for traditional treatment is that exposure time lasts between 5 and 180 minutes.

3. Desired changes induced by alkalization in cocoa products

Up until this section, the most widespread technology and treatment conditions to alkalize cocoa have been reviewed. They all have three main missions: improving the solubility of powder, darkening cocoa color and modifying the product's flavor 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 the 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, such as using different stabilizers and emulsifiers. Another approach is to reduce the proportion of cocoa powder included in formulation. To do so however, a material richer in soluble components must be produced in advance (Holkar, Jadhav and Pinjari, 2019).

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

Regarding cocoa solubility, cell structures of this material are characterized 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, Jadhav and Pinjari, 2019).

Some authors have reported NaOH, one of the most widely employed alkalis in alkalization, in other matrices to break ester links and to hydrolyze cell walls. These effects, combined with other alkalization conditions (temperature, pressure, etc.), along with reducing fat content, can be responsible for the increase in solubility caused by Dutching (Domínguez-Rodríguez, Marina and Plaza, 2017).

3.2 Color

During alkalization, alkali agents, combined with aeration, temperature and pressure, are applied to change cocoa color from light brown to dark, and even to red, by forming brown and red compounds (Rodríguez, Pérez and Guzmán, 2009; Oduns and Longue, 1998; Adeyeye, 2016; Stark and Hofmann., 2006; Totlani and Peterson, 2005, 2007; Germann, Stark and Hofmann, 2019a, 2019b).

Color changes, as explained in detail in a further section, are produced by several chemical reactions, which are enhanced by the alkaline media generated by alkali agents, fed by the oxygen from injected air, accelerated by temperature and facilitated by pressure. The formation of Maillard reaction products, the oxidation and polymerization 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 taking place during alkalization that lead to visual color changes.

3.3. Flavor

Cocoa has four main characteristic markers in flavor terms: cocoa, acidity, bitterness and astringency. All these perceptions are conferred by different groups of compounds and may be negatively affected by alkalization. Other kinds of flavors are studied in cocoa, such as alkalinity (introduced by the alkali), cocoa characteristic notes (aromatic, nutty, sweet, fruity notes, etc.) and different off-flavors (burnt, metallic, raw, muddy, etc.) (De Zaan, 2006).

As mentioned in following sections, fats, sugars, diketopiperazines, bioactive peptides, different polyphenols, methylxanthines and volatile compounds have been related to modifications in bitterness, astringency and other cocoa flavor notes. Some compounds have even been reported as modulators of these sensory perceptions (Zhang, Xia and Peterson, 2014). In particular, it has been documented that alkalization induces the chemical modification of polyphenols and their modifications are translated into changes in their flavor properties.

Through the degradation and modification of the aforementioned compounds, alkalization has been reported to positively reduce the bitter, acidic and astringent tastes of cocoa. 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, the general perception is a reduction in the aforementioned tastes.

4. Composition and chemical changes produced in cocoa by alkalization

Cocoa powder is known for being a nutritive ingredient with a high and diverse functional content. Although composition depends not only on the bean's origin, but also on the way cocoa is treated, the natural cocoa powder composition can be decomposed into complex carbohydrates (58%), proteins (20%) and fats (11%) as 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, such as flavonols (quercetin, isoquercetin), flavones (luteolin, apigenin), flavanones (naringenin), anthocyanins and phenolic acids, methylxanthines (theobromine, theofilline and caffeine), as well as a wide variety 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, Jadhav and Pinjari, 2019).

During natural powder alkalization, numerous complicated chemical reactions take place and are responsible not only for color and flavor development, but also improved solubility. The same chemical reactions can produce desirable or undesirable changes in the nutritional, functional and microbiological characteristics of cocoa. Table 2 briefly presents the reported modifications to the physico-chemical, nutritional, functional, microbiological and sensory features that may occur during cocoa alkalization.

Table 2. Reported changes in the physico-chemical, nutritional, functional, microbiological and sensory properties induced in cocoa by alkalization.

Attribute	Reported changes	Mechanisms
Carbohydrates	• No change of total carbohydrates • Decreasing of reducing sugars	• Maillard reactions
	References: 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
	References: Li et al., 2012; Taş and Gökmen, 2016	
Maillard reaction products	• Degradation of 3-deoxyglucosone • Increase of glucosone and diacetyl • Glyoxal, HMF and methylglyoxal unchanged	• Oxidation of Amadori compounds and glucose
	References: Taş and Gökmen, 2016	
Fat	• Reduction of total fat content	• Hydrolysis and saponification of triglycerides
	References: 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 in one mineral	• Alkali contribution
	References: Oduns and Longe, 1998; Adeyeye, 2016	

Table 2. Continuation.

Attribute	Reported changes	Mechanisms
Polyphenols	• Total phenol content is reduced • Antioxidant activity is reduced • Flavanols content is reduced • Relation epicatechin/catechin is inversed • 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	• Oxidation of polyphenols and their interaction with amino acids, proteins, peptides, other flavonoids and Maillard products • Epimerization of (+)-catechin to (-)-catechin • Monomerization of procyanidins • Isomerization of (-)-epicatechin to (-)-catechin • Glycosilation of flavanols • Modifications induced by Maillard reaction products
	References: 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	

Table 2. Continuation.

Attribute	Reported changes	Mechanisms
Methylxanthines	• Theobromine and caffeine are reduced	Theobromine interacts with bases and forms salts
		References: Oduns and Longe, 1998; Li et al., 2012
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
		References: Li et al., 2012
Anti-nutritional factors	• Reduction in tannins and increase in phytins and oxalates	• Degradation • Releasing from cells due to the treatment
		References: 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

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-alkalized and alkalized 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 this sugar can be the main one that interacts with amino acids during Maillard reactions. Taş and Gökmen (2016) analyzed the concentration of reducing sugars (sucrose, glucose and fructose). They did not observe any significant reduction in glucose and fructose, but noted a significant reduction in sucrose, which they associated with dipping cocoa beans in alkaline solution.

4.2. Proteins, peptides, amino acids and Maillard reaction products

During alkalization, due to temperature, enzymes and the media generated by alkali, proteins are degraded by deamination and oxidation reactions (Rodríguez, Pérez and Guzmán, 2009; Oduns and Longe, 1998; Méndez-Albores et al., 2013). Rodríguez, Pérez and Guzmán (2009) observed a reduction in crude protein due to thermal processing, which they increased by adding alkali. Of all the tested alkalis, NaOH was the most aggressive for proteins and led to a 12% loss.

Other authors have studied the degradation of proteins and observed a 45.5% reduction after alkalizing with an equivalent cocoa pod ash concentration to 50 g/kg of NaOH (Oduns and Longe, 1998). In agreement with Oduns et al, 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 relation to protein content, the protein estimation methodology, alkalization treatments and the conditions employed by the different authors were compared. The main differences found among these works seemed to be based on the conditions employed. Adeyeye (2016) did not define the alkalization process, while Oduns and Longe (1998) alkalized 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 alkalized 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%).

On 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 in its associated functional activities have not being studied in relation to cocoa alkalization, the reduction reported in the protein content produced 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 that have been documented to form during cocoa processing are diketopiperazines. These compounds are cyclic dipeptides that have been associated with part of the bitter taste of cocoa and also with different functional effects (Andruszkiewicz et al., 2019). Although these molecules have not been studied during alkalization, they might form as a result of treatment, and may contribute to the flavor 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) analyzed lysine concentration during the Dutching of beans and did not notice any reduction. Li et al (2012) studied the 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 obtained contradictory results as regards losses of amino acids and sugars. This contradiction can be associated with the different employed raw materials and alkalizing methods. Li et al observed a significant degradation for lysine and fructose, while Taş and Gökmen did not notice any change. Li’s group alkalized 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 alkalized 160 g of cocoa beans with 7.5% Na2CO3 for 30 minutes. The differences in raw material, alkalization agent and alkalizing 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) analyzed content in amino acids using HPLC equipment and measuring at 338 nm. Taş and Gökmen (2016) carried out acid hydrolysis with the sample before analyzing lysine content in a UPLC system coupled to a TQ detector. Acidification, degradation of proteins and the consequent release of amino acids, in this case lysine, could explain why this compound did not reduce in the work 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. flavor, taste and color. 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 alkalization on the generation of these compounds by alkalizing unroasted samples. For this purpose, they monitored changes in the concentration of α-Dicarbonyls (3-deoxyglucosone, glucosone, glyoxal, methylglyoxal, diacetyl and 5-hydroxymethylfurfural). They found that alkalization negatively affected 3-deoxyglucosone content, had no effect on 5-hydroxymethylfurfural, methylglyoxal and glyoxal, and significantly increased the concentration of both glucosone and diacetyl. The increase in the previous Maillard reaction products is 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 flavor through its interaction with fatty acids, which leads to their hydrolization and saponification.

Rodríguez, Pérez and Guzmán (2009) observed a 16% decrease in total fat, but did not detect any soapy flavor in their samples. In line with them, Méndez-Albores et al (2013) also detected a 16% reduction in the crude fat content of cocoa liquors alkalized 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 in crude fat and one of 65.9% for fatty acid content. These losses were significantly more marked than those reported by Rodríguez, Pérez and Guzmán (2009) and Méndez-Albores et al (2014).

The differences in fat content found by the previous researchers can be explained by the employed raw material. Rodríguez and Méndez-Albores' teams alkalized cocoa liquor (fat content ≈50%) and Adeyeye (2016) treated cocoa cake (fat content ≈10%). Cocoa cake has less fat content than liquor because it is obtained after pressing and partly removing fat. This means that the same fat degradation is seen, expressed as a percentage, as greater degradation in cocoa cake than in liquor, but they can be considered similar in absolute values. This can explain the greater degradation observed by Adeyeye (2016) in cake compared to that reported in liquor by other authors.

In general, all researchers agree that alkalization induces the hydrolization 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 flavor developing in cocoa, which is certainly undesirable.

4.4. Minerals

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

Accordingly, Adeyeye (2016) analyzed the mineral content of cocoa powders alkalized in a factory, and did not found any significant increase in mineral content. Sodium slightly increased in the alkalized samples versus the non-alkalized 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 alkalized 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 more marked increase with only 30 g of NaOH/kg. The difference in ash content between both these works might be related to the alkalizing 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 defense against pathogenic diseases and infections, and also in their different maturation processes. More than 8000 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 places cocoa as the food with the highest flavanol content based on its dry weight (Martín, Goya and Ramos, 2013; Martín and Ramos, 2017; Aprotosoaie, Luca and Miron, 2015).

Of 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, Luca and Miron, 2015).

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 flavor, 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 et al., 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) analyzed total polyphenol, flavanol content and antioxidant activity of alkalized 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 alkalized cocoa, by Gu et al (2006) in commercial cocoa powders, which were 51% for the antioxidant activity and 78% for procyanidins, and by Jolić et al (2011) in commercially alkalized cocoa nibs, which were 64% for total phenolic content, 59% for total procyanidins and 39% for the 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, as well as a 100% loss at pH 9 after 4 h. 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, the increase in 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 increased polyphenol oxidase activity (Rodríguez, Pérez and Guzmán, 2009; Misnawi, Jamilah and Nazamid , 2003), effects that reduce the amount of polyphenols (Li et al., 2012).

The two compounds, epicatechin and catechin, are major polyphenols in cocoa. Their common stereoisomers are (-)-epicatechin and (+)-catechin, with (+)-epicatechin and (-)-catechin being rare conformations lacking in natural cocoa. In general, (-)-epicatechin content is higher than (+)-catechin content (Gültekin-Özgüven, Berktaş and ÖzÇelik, 2016; Andres-Lacueva et al., 2008).

When cocoa is alkalized, the epicatechin/catechin ratio is inversed by the generation of (-)-catechin (Gültekin-Özgüven, Berktaş and ÖzÇelik, 2016; Hurst et al., 2011; Kofink, Papagiannopoulos and Galensa, 2007; Ortega et al, 2008). The appearance of this phenol is due to the isomerization of (-)-epicatechin to (-)-catechin, the epimerization of (+)-catechin to (-)-catechin, and the monomerization of procyanidins (Gültekin-Özgüven, Berktaş and ÖzÇelik, 2016;

Hurst et al., 2011; Andres-Lacueva et al., 2008; Jolić et al., 2011). Unlike the findings reported by the previous authors, other groups of researchers have found that after alkalization, epicatechin continued to be the major flavanol (Todorovic et al., 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, it will be seen that they simply quantified (+)-catechin content, and did not bear in 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, Berktaş and ÖzÇelik, 2016; Rimbach et al., 2009). As alkalization increases (-)-catechin concentration, it can be stated that this cocoa production chain step reduces the bioaccessibility of cocoa polyphenols. Gültekin-Özgüven, Berktaş and ÖzÇelik (2016) defines this term as the percentage of procyanidins solubilized in chyme (water phase) after each digestion step (gastric and duodenal). They also found that alkalization significantly reduced this characteristic.

As well as the degradation, there are reports that epimerization and isomerization and non- enzymatic glycosylation modifications modify catechin and epicatechin, and affect cocoa sensory characteristics (Stark and Hofmann, 2006). These authors identified different glycosylated flavanols generated by alkalization, and also discovered that this modification eliminated the associated bitterness, 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 1000 µmol/L, respectively, while their flavan-3-ol-3-glycosides have one that ranges 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, Xia and Peterson (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, which stresses that bitterness is not only related to bitter compounds, but also to compounds capable of modulating it.

In addition to taste, other authors have associated alkalization and the chemical reactions induced by an alkaline pH with the formation of chromophores in cocoa (Figure 2). Germann, Stark and Hofmann (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 can be responsible for part of cocoa's color (Stark and Hofmann, 2006; Totlani and Peterson, 2005, 2007; Germann, Stark and Hofmann, 2019a, 2019b). In their work, Germann, Stark and Hofmann (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. In addition, these authors reported an increase in the high-molecular-weight products that exhibited a reddish-brown color, which they assumed to be the major contributors of cocoa darkening. None of the mentioned molecules was found in non- alkalized cocoas.

In a second work about the characterization of the unpolar chromophores deriving from catechin and epicatechin, Germann, Stark and Hofmann (2019b) found that xanthenocatechins and xantheno-derived chromophores, a newly detected group of compounds, contributed to the red color of alkalized cocoa.

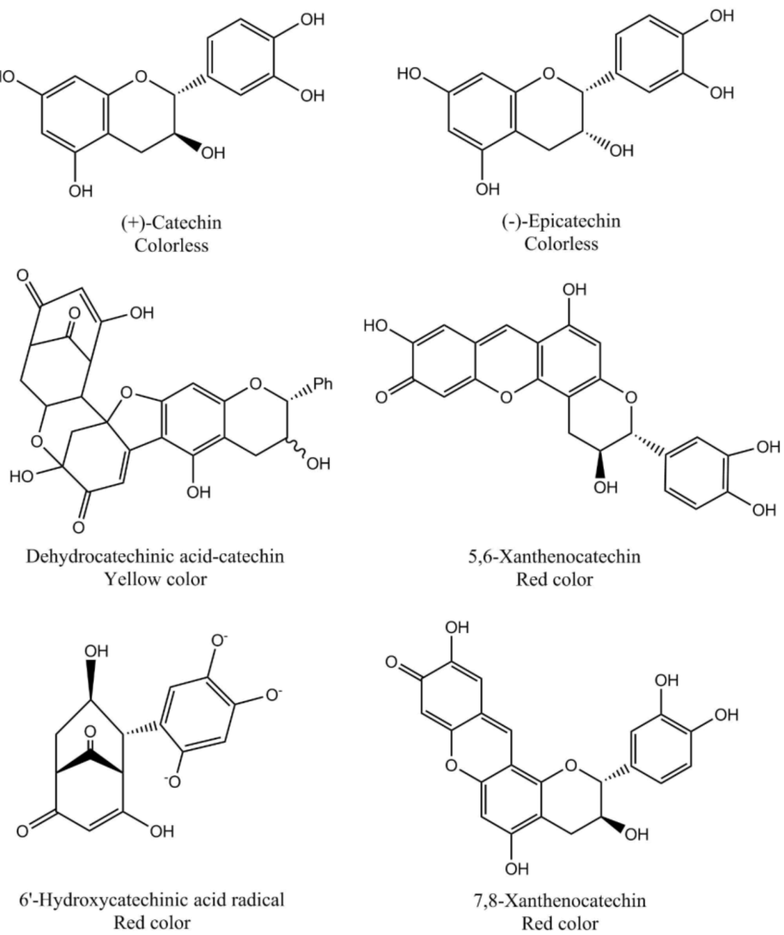

Figure 2. Examples of catechin and epicatechin-derived chromophores generated during alkalization.

Finally, 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 are able to 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 alkalized) 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 the different cocoa extracts, regardless of their degree of alkalization, had a similar antimicrobial effect to those found in some herbal extracts like oregano, rosemary and celery. The authors also tested the effect of alkalization of different cocoa powders on antimicrobial activity and found that Dutching did not significantly affect activity against *Gram-Positive* bacteria, but significantly enhanced action against *Gram-negative* bacteria compared to the non-alkalized 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.

As this section reveals, cocoa polyphenols are generally involved in all desired changes in the product. They are responsible, at least in part, for color and change in flavor, and are also connected to cocoa solubility because their interaction with other compounds may be responsible for insoluble complexes appearing. In the end, given the relevant content and variety of polyphenols, it could be stated that cocoa is actually what it is mainly thanks 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, and cardiovascular and metabolic effects, among others (Aprotosoaie, Luca and Miron, 2015; Franco, Oñatibia-Astibia and Martínez-Pinilla, 2013). Apart from positive effects, methylxanthines have been reported to have several acute adverse effects, such as tachycardia, feeding intolerance, seizures and cardiac dysrhythmias, which are effects commonly found with caffeine at usual therapeutic levels (Gauda and Martin, 2012).

In cocoa, theobromine is the methylxanthine with the highest concentration, followed by caffeine and lastly by theophylline. All these compounds have been reported to degrade during alkalization. Oduns and Longue (1998) observed that 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 came about 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. 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 the interaction of these compounds with the employed alkali agent, and also with their conversion into salts. In addition, 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, Luca and Miron, 2015).

4.7. Volatile flavor compounds

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

Cocoa flavor is composed of non-volatile flavors (mentioned in previous sections) and by the volatile flavor compounds studied by Li et al (2012). These researchers identified more than 80 volatile compounds belonging to different groups related to flavor (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 flavor 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 citric flavor 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 flavoring 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 alkalizing conditions. Polyphenols have hydroxyl groups that are able to 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 alkalized some samples by adding reducing sugar (glucose), which ended up with the formation of more flavor compounds during alkalization via different chemical processes, such as the production of Schiff's bases, Amadori rearrangements and Strecker degradations.

4.8. 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, able to combine with proteins, cellulose and other molecules to form insoluble structures; hydrocyanate, 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 polymerization of catechins, were reduced by 38.6%. This agrees with the observations made in previous sections because polyphenols and their polymers are degraded by alkalization. The above 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 several alkalization levels. These authors concluded that they did not observe any relation between oxalates content and Dutching because the level among different powders was generally similar. Although these results apparently go against the increment in oxalated observed by Adeyeye (2016), it can be stated that Schroder, Vanhanen and Savage (2011) used a group of natural and alkalized cocoas of different brands whose origins and producers were not related, which makes it difficult to draw conclusions about the effect of Dutching.

4.9. Spores

Another important aspect related to the bacterial quality of cocoa is the effect that alkalization has on spores. During fermentation, different bacteria and yeasts are allowed to grow to create the different precursors required to produce the desired cocoa flavor and color. After fermentation, bacterial content is significantly reduced by a sterilization 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 products containing this powder (Lima et al., 2011).

The effects of alkalization on spores have been studied by several authors. Lima et al (2011) analyzed microorganism content in a set of commercial cocoa powders. These researchers only detected thermoresistant spores in alkalized cocoa powders, but not in non-alkalized 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. In another work, 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.

4.10. 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 be contaminated, 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. Mycotoxin-producer species like *Aspergillus* and *Penicillium* were detected (Méndez-Albores et al., 2004).

In general, heat treatment over 250°C can effectively lower the concentration of mycotoxins. For example, aflatoxins content in maize lowered by 81% after being roasted at 285°C for 7 minutes (Méndez-Albores et al., 2013). In cocoa, Méndez-Albores et al (2013) roasted cocoa beans at 250°C 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 set a maximum of 20 ng/g given their hepatotoxic, teratogenic, mutagenic and carcinogenic properties. As shown, that limit is not reached after cocoa roasting.

After roasting, the authors alkalized samples using three different alkalis (NaOH, KOH, $Ca(OH)_2$) at three concentrations (10, 20 and 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. 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 the Mexican law specifications.

Other authors have studied the concentrations of aflatoxins (B1, B2, G1, G2) and ochratoxin in cocoa products (Turcotte, Scott and Tague, 2013). These researchers observed that when natural cocoas were alkalized, the concentration of aflatoxins B1, B2, G1 and G2, and ochratoxin lowered by 67.7%, 70.5%, 44%, 100% and 68.2%, respectively. The concentrations of all the analyzed mycotoxins were lower than the maximum level set out by law.

5. Conclusion

On the whole, this review offers a complete 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 developed new and alternatives techniques, traditional alkalization continues to be the most widely method followed by industry. The data collected and shown in this review can be employed as a guide to design new alkalization methods, and to even optimize existing ones.

This review also provides insight 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 the induced changes are negative because the safety of cocoa products is enhanced.

After analyzing all the published information, it can be concluded that polyphenols are responsible, at least in part, for the color, flavor 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.

6. 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

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. (2015). 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.

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

Bispo, E. S., Ferreira, V. L. P., Santana, L. R. R., & Yotsuyanagi, K. (2005). Sensory profile and acceptance of alkalinized cocoa (Theombroma cacao L.) powder. Food Science and Technology, 25 (2), 375-381. http://dx.doi.org/10.1590/S0101-20612005000200033

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

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

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.

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 indez, 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

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

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

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

4. CHAPTER I

Extrusion

4.1. Introduction to extrusion

1. Extrusion: a general view

Extrusion is a well-known process widely employed by the food industry for the production of different kinds of products such as pasta, snacks, biscuits, surimi and chocolate. In general, extrusion can be seen as a system that leads to the transformation of granular and powdered foods into larger pieces.

The extruder is composed by a horizontal and cylindrical barrel with two openings: the hopper or feeder, which is the entrance of the material into the extruder, and a final gate limited by a piece called "die", which reduces the size of the exit and also shapes the material that leaves the machine (Figure 3). Inside the barrel, there are one or even two screws with different sizes and thickness that move, mix and press the material. At the same time that it is moved through the extruder, the friction produced when the material is sheared and the effects of several heater bands lead to the increase of the temperature that melt the treated sample. When the mass reaches the die, it gets expanded, partially dried and cooled by the drastic change in pressure and temperature, and by the vapor that leaves the material (Kristiawan et al., 2018).

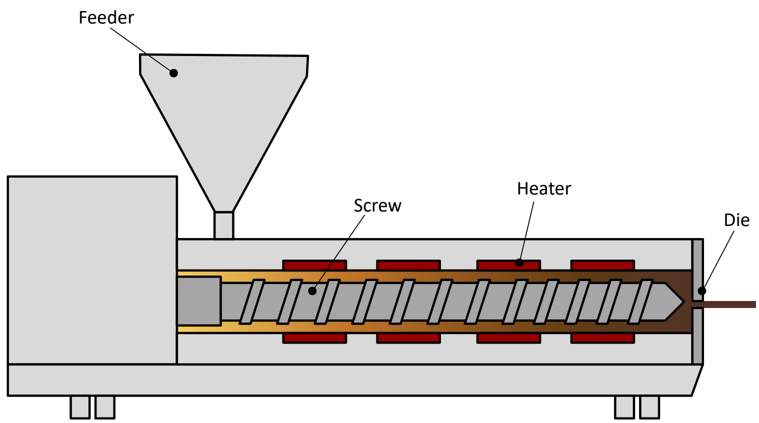

Figure 3. Extruder illustration.

According to the temperature reached in the extruder, the process is classified into cold extrusion or extrusion cooking. Cold extrusion is mainly used for mixing and shaping foods without significantly affecting the characteristics of the final product. During the process, the material does not reach more than 100°C and it is moved in a slow motion inside a smooth tube for having a reduced friction. On the other hand, in extrusion cooking, the food is heated above 100°C and compressed, which produces sensory, composition and microbiological changes in the material.

As a technique, extrusion has the advantages of being a versatile and continuous process that combines mixing, cooking, kneading, shearing, shaping and forming, and that can be employed with different kind of ingredients and operating conditions. In addition, extrusion does not produce any effluents, has higher productivity and lower processing cost than other methods (Fellows, 2000). Despite the initial inversion, the fast speed, the lesser energy consumption in comparison to other heating techniques and the partial drying of the sample reduce further costs and make its implementation a worthy investment. Moreover, extrusion has shown to be a suitable system for many extruded foods that cannot be easily produced by other technics. The disadvantage is that its use is limited to granular or powdered materials and that there is limited information about the effects of extrusion on different materials like, for example, about cocoa (Fellows, 2000; Chokshi and Zia, 2004; Alam et al., 2016).

2. Types of extruded food products

As a versatile technique, extrusion can be used not just for treating different kind of powdered materials, also for obtaining different shapes and textures according to the extrusion temperature, generated pressure, extrusion speed, type and number of screws, and type of die. Table 3 shows a collection of the different food products obtained in the industry by the application of extrusion.

In the case of pasta, for example, with a single composition, several dies and a cutter blade, all known shapes, from spaghetti and macaroon to letters and animal forms can be produced.

Table 3. List of products obtained through extrusion (Fellow, 2000).

Category	Examples
Confectionery products	Fruit gums, liquorice, toffee, fudge, boiled sweets, creams and chocolate, chewing gum, caramel and peanut brittle
Cereal products	Crispbread, breakfast cereals, expanded snackfoods, pasta products, pre-cooked flours, croutons, weaning food, soup, beverage bases, instant drinks, biscuits and baby food
Protein-based products	Texturized vegetable protein (soy protein), petfood, sausage products, frankfurters, hot dogs, surimi and processed cheese

3. Effect of extrusion on food composition and sensory characteristics

Several authors have studied the effects of extrusion on the color, texture, flavor, the different macromolecules and polyphenols of different food matrices (pineapple leather, green banana flour, purple potato and pea flour, corn meal, legume seeds, oats…). This section focuses on giving an overview of the compositional and sensory changes of the different products during extrusion.

3.1 Effect of extrusion on the composition

From a compositional point of view, extrusion has been reported to increase the digestibility of proteins by means of inactivating anti-nutritional factors and by the exposition of new sites to an enzymatic digestion though denaturalization. This effect is especially interesting to increase the low digestibility of plant proteins (Zhang et al., 2017; Fellows, 2000).

In addition to proteins, starches are also affected by extrusion. Depending on the processing conditions, the starch granules can be expanded and broken, and the macromolecules such as amylose and amylopectin reduced to lower molecular weight structures. This effect was associated to the increased sugar and insulin blood levels after the consumption of those products (Moisio et al., 2015; Fellows, 2000). Other authors observed in a study with green banana flour that extrusion can decrease some texture properties associated to starches, such as pasting properties (Sarawong et al (2014)). This was caused by the degradation and partly gelatinization of the starches present in the food matrix. In addition, extrusion induced the destruction of oligosaccharides related to flatulence such as raffinose and stachyose (Singh, Gamlath and Wakeling, 2007).

In relation to amino acids and sugars, both have been reported to decrease due to their association to Maillard reaction products and acrylamide formation, compounds whose generation increased after extrusion. Acrylamide is a carcinogenic molecule while Maillard reaction products, in addition to be defined as color compounds, have been reported as carcinogenic, cytotoxic and mutagenic (Singh, Gamlath and Wakeling, 2007). These increases are a nutritional disadvantage of extrusion, although the formation of Maillard products is desired and searched from a sensory point of view.

Lipid content has been also reported to be slightly affected by extrusion. Although this technique indirectly promotes fat oxidation by creating air bubbles in the matrix, it also reduces the oxidation by inactivating lipolytic enzymes. As in high fat products, rancidity is also a concern in the storage of extruded materials due to the expansion and aeration of the treated mass. For that reason, the packaging in a nitrogen atmosphere is a good way to slow down the oxidation processes of extruded products (Singh, Gamlath and Wakeling, 2007).

In the case of fibers, these molecules have been reported to be significantly affected by extrusion in its content and solubility. In their review, Alam et al (2016) showed that several authors studied the fiber content and solubility in different materials (wheat bran, pea hulls, rice…) and found an increase in the solubility of dietary fiber, which other authors related to an increase in concentration of soluble fibers.

In the case of vitamins, they differ in structure and properties, which makes them to be affected in a different way. While vitamins D and K are stable, vitamins C, A and E are significantly lost during extrusion, because of their high sensibility to oxygen and heat. In terms of mineral, none of them are degraded by extrusion and iron has been reported to increase due to the contamination from the metallic pieces of the extruder (Alonso et al., 2001). Another effect of the extrusion treatment is the inactivation of anti-nutritional factors that reduce the mineral absorption and the consequent increase in their bioavailability. In addition to anti-nutritional compounds, the degradation of polyphenols and the solubilization and modification of the chelating properties of some fibers also enhance mineral absorption (Singh, Gamlath and Wakeling, 2007).

In the case of functional components like polyphenols, they are negatively affected by the thermomechanical effects of extrusion, especially at long residence times and low moisture contents. Sharma et al (2016) studied the total phenol content and antioxidant activity profiles in pineapple leather after extrusion. They found an important decrease in phenolic content with high temperatures and screw speeds, and an increase in the antioxidant activity with temperature, which they associated with the lysis of cells and the releasing of their components to the media, and with the formation of Maillard pigments that enhanced the antioxidant activity in comparison with the raw sample. The decreases of different anti-nutritional factors and of the total phenol content have been also observed with the increases in temperature and water content by El-Hady and Habiba (2003). In the case of the antioxidant activity, its increase or maintenance after an extrusion treatment in comparison to the untreated material has been also reported by other authors such as Shih, Kou and Chiang (2009) and Nayak et al (2011).

From a microbiological point of view, low water content and the heat applied during extrusion make most of the extruded-cooked products sterile after the treatment. In the case of spores, there has not been any important research in this area, although some studies pointed out that spores of *Bacillus stearothermophilus* are importantly reduced by the application of high temperatures during extrusion (Nikmaram, 2015).

In addition to microorganisms, the effects of extrusion on the mycotoxin content have been also studied. Several authors have analyzed the content of different mycotoxins (fumonisins B1 and B2, total aflatoxins (B1, B2, G1, G2, M1), ochratoxin, deoxynivalenol, moniliformin and zearalenone) and reported different levels of reductions according to the treatment conditions (temperature, extrusion speed, moisture content, type of die…) (Castells et al., 2004; Kabak, 2009). For example, Scudamore, Banks and Guy (2004) evaluated the degradation of ochratoxin A in contaminated whole wheat grain and found that increasing the temperature from 116 to 133°C produced an increment of 20%. As it is shown in this work and in agreement with other researchers, the temperature is the main variable negatively affecting mycotoxins, although the other ones are also important. For example, Cortez-Rocha et al (2002) found that replacing the tapered-angular die by a tapered-circular one changed the reduction of fumonisin B1 from 63-99% to undetectable-90%.

3.2. Effects of extrusion on the sensory features

Apart from modifications of the composition of the material, extrusion has been associated to changes in texture, color and flavor, which will be commented in this section.

In terms of texture, there are two key factors determining this characteristic: the composition of the material and the treatment conditions (pre-conditioning, die temperature, screw speed, energy input, residence time and pressure). During extrusion, different physical and chemical changes take place (hydration, gelation and shearing of starches, denaturalization or reorientation of proteins, and melting of fats) and lead to the obtaining of the final and desired physical properties (Singh, Gamlath and Wakeling, 2007; Kristiawan et al., 2018).

As an example of the importance of the composition of texture, Brennan et al (2008) studied the expansion of two extruded breakfast cereals, one with the addition of wheat bran (rich in insoluble fibers) and one product without, and found a reduction of the expansion and an increase in hardness due to the decrease in the water solubility and to the increase bulk density of the material. As an example of the treatment conditions, Liu et al (2011) studied the modifications of the texture properties of extruded rice and showed the effects of the different extrusion conditions (water content, screw speed and temperature) on the hardness, adhesive force, springiness, gumminess and cohesiveness of the product. They found that all the properties were affected by extrusion. As examples, they observed that increases in screw speed leaded to higher springiness and to lower adhesive force and gumminess, and that increases in water content produced a higher adhesive force and a lower springiness. These examples illustrate how the textural properties depend on the composition and the treatment conditions, which mean that each material will behave in a different manner under the extrusion treatment.

In addition to the effects on the texture, extrusion has shown to degrade and volatilize the flavor compounds present before the process. On the one hand, extrusion induces thermal degradation, polymerization, oxidation and interactions of the flavor compounds with other ingredients. On the other hand, when the material leaves the machine and gets expanded, the flavor molecules are volatilized together with the steam (Yuliani et al., 2004). Due to these effects, it is usual in the industry to spray-add the desired flavor after the treatment, although it has several problems such as their non-uniform distribution on the matrix and their oxidation (Yuliani et al., 2004).

During extrusion, in addition to the color fading produced by the expansion of the product after its releasing from the extruder, color can be changed by the degradation of pigments, formation of Maillard reaction compounds, caramelization of carbohydrates and by the oxidative decomposition of fats and proteins (Chen et al., 1991). As an example of this darkening, Nayak et al (2011) made and extruded different proportions of potato with dry peas and evaluated the changes in color. The authors found a higher reduction in the luminosity, the redness and in saturation of the extruded samples in comparison to the non-treated ones. The researchers related these reductions to the formation of Maillard compounds, to the increase of amylose content and to the degradation of purple anthocyanins.

4. Extrusion and cocoa

In terms of studying the effects of extrusion on cocoa, any scientific work has been found and only some of the patents found describe the role of the extruder with different aims. For example, extrusion has been reported for the production of cocoa powder agglomerates employing the mentioned technique as a tool for reducing the energy consumption, the dust explosions and the bacterial counts of the product after fermentation (Rapp, 1977). In other patent, it has been employed for producing granular cocoa, which has higher fluidity, solubility and avoids the formation of dust clouds (Hara, Takeuchi and Morishima, 1979). Moreover, the extruder has been used for the production of chocolate and some confectionary products (Jury and Walker, 1997, Crook, Jury and Mackey, 1997, Mackey, 1994; Chaveron et al., 1983; Nappen and Marotta, 1979).

Regarding the employment of extrusion for the alkalization of cocoa, just one patent describing an alkalization method based on extrusion has been found (Chalin, 1972). This author described a process in which cocoa cake and nibs are pulverized and mixed with an alkali solution and later treated in the extruder at known conditions. This patent does not show information about the concrete physicochemical and sensory changes induced by extrusion, and does not evaluate the evolution of the functional properties of the product (antioxidant activity, total phenol content and the concentrations of any polyphenol).

Having in mind this lack of knowledge, a new extrusion alkalization method has been designed and applied for studying its effects on cocoa. The chapter 1 of this thesis describes the impact of this alternative alkalization method on the physicochemical and functional features as well as its suitability for producing acceptable alkalized cocoas.

5. References

Alam, M. S., Kaur, J., Khaira, H., & Gupta, K. (2016). Extrusion and extruded products: changes in quality attributes as affected by extrusion process parameters: a review. Critical Reviews in Food Science and Nutrition, 56, 445-473. https://doi.org/10.1080/10408398.2013.779568

Alonso, R., Rubio, L. A., Muzquiz, M., Marzo, F. (2001). The effect of extrusion cooking on mineral bioavailability in pie and kidney bean seed meals. Animal Feed Science and Technology, 94 (1-2), 1-13. https://doi.org/10.1016/S0377-8401(01)00302-9

Brennan, M. A., Monro, J. A., & Brennan, C. S. (2008). Effect of inclusion of soluble and insoluble fibers into extruded breakfast cereal products made with reserve screw configuration. International Journal of Food Science & Technology, 43 (12), 2278-2288. https://doi.org/10.1111/j.1365-2621.2008.01867.x

Castells, M., Marín, S., Sanchis, V., & Ramos, A. J. (2004). Fate of mycotoxins in cereals during extrusion cooking: a review. Food Additives & Contaminants, 22 (2), 150-157. https://doi.org/10.1080/02652030500037969

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

Chaveron, H., Pontillon, J., Billon, M., Adenier, H., & Kamoun, A. (1983). Process for preparing a chocolate paste. US4713256A.

Chen, J., Serafin, F. L., Pandya, R. N., Daun, H. (1991). Effects of extrusion conditions on sensory properties of corn meal extrudates. Journal of Food Science, 56 (1), 84-89. https://doi.org/10.1111/j.1365-2621.1991.tb07981.x

Chokshi, R., & Zia, H. (2004). Hot-melt extrusion technique: a review. Iranian Journal of Pharmaceutical Research, 3 (1), 2-16.

Cortez-Rocha, M.O., Trigo-Stockli, D.M., Wetzel, D.L. & Reed, C.R. (2002). Effect of extrusion processing on fumonisin B1 and hydrolysed fumonisin B1 in contaminated alkali-cooked corn. Bulletin of Environmental Contamination and Toxicology, 69 (4), 471-478. https://doi.org/10.1007/s00128-002-0086-6

Crook, S. J., Jury, M., & Mackley, M. R. (1997). Extrusion of chocolate. EP0820701A2.

Rapp, R. D. (1977). Process for producing cocoa powder agglomerates. DE3716227A1.

El-Hady, E. A. A., & Habiba, R. A. (2003). Effect of soaking and extrusion conditions on antinutriens and protein digestibility of legume seeds. LWT-Food Science and Technology, 36 (3), 285-293. https://doi.org/10.1016/S0023-6438(02)00217-7

Fellows, P. J. (2000). Chapter 14: extrusion cooking. *Food Processing Technology* (pp. 294-308). Cambridge and Boca Raton: Woodhead Publishing Limited and CRC Press LLC.

Hara, J., Takeuchi, M., & Morishima, T. (1979). Method of producing granular cocoa. US4308288A.

Jury, M., & Walker, J. H. (1997). Continuous extrusion of chocolate. EP0797922B1.

Kabak, B. (2009). The fate of mycotoxins during termal food processing. Journal of the Science of Food Agriculture, 89 (4), 549-554. https://doi.org/10.1002/jsfa.3491

Kristiawan, M., Micard, V., Maladira, P., Alchamieh, C., Maigret, J. E., Réguerre, A. L., Emin, M. A., & Della Valle, G. (2018). Multi-scale structural changes of starch and proteins during pea flour extrusion. Food Research International, 108, 203-215. https://doi.org/10.1016/j.foodres.2018.03.027

Liu, C., Zhang, Y., Liu, W., Wan, J., Wang, W., Wu, L. Zuo, N., Zhou, Y., & Yin, Z. (2011). Preparation, physicochemical and texture properties of texturized rice produce by improved extrusion cooking technology. Journal of Cereal Science, 54 (3), 473-480. https://doi.org/10.1016/j.jcs.2011.09.001

Mackey, M. R. (1994). Isothermal preparation of chocolate products. US5439695A.

Moisio, T., Forssell, P., Partanen, R., Damerau, A., & Hill, S. E. (2015). Reorganization of starch, proteins and lipids in extrusion of oats. Journal of Cereal Science, 64, 48-55. https://doi.org/10.1016/j.jcs.2015.04.001

Nappen, B. H., & Marotta, N. G. (1978). Preparation of dispersible chocolate liquor by extrusion. US4191786A.

Nayak, B., Berrios, J. J., Powers, J. R., & Tang, J. (2011). Effect of extrusion on the antioxidant capacity and color attributes of expanded extrudates prepared from purple potato and yellow pea flow mixes. Journal of Food Science, 76 (6), 874-883. https://doi.org/10.1111/j.1750-3841.2011.02279.x

Nikmaram, N., Kamani, M. H., & Ghalavand, R. (2015). The effects of extrusion cooking on antinutritional factors, chemical properties and contaminating microorganism of food. International Journal of Farming and Allied Sciences, 4 (4), 352-354.

Sarawong, C., Schoenlechner, R., Sekiguchi, K., Berghofer, E., & Ng, P. K. W. (2014). Effect of extrusion cooking on the physicochemical properties, resistant starch, phenolic content and antioxidant capacities of green banana flour. Food Chemistry, 143, 33-39. https://doi.org/10.1016/j.foodchem.2013.07.081

Scudamore, K. A., Banks, J. N., & Guy, R. C. E. (2004). Fate of ochratoxin A in the processing of whole wheat grain during extrusion. Food Additives and Contaminants, 21, 488-497. https://doi.org/10.1080/02652030410001670166

Sharma, P., Ramchiary, M., Samyor, D., & Das, A. B. (2016). Study of the phytochemical properties of pineapple fruit leather processed by extrusion cooking. Food Science and Technology, 72, 534-543. https://doi.org/10.1016/j.lwt.2016.05.001

Shih, M. C., Kou, C. C., & Chiang, W. (2009). Effects of drying and extrusion on colour, chemical composition, antioxidant activities and mitogenic response of spleen lymphocytes of sweet potatoes. Food Chemistry, 117 (1), 114-121. https://doi.org/10.1016/j.foodchem.2009.03.084

Singh, S., Gamlath, S., & Wakeling, L. (2007). Nutritional aspects of food extrusion: a review. International Journal of Food Science & Technology, 42 (8), 916-919. https://doi.org/10.1111/j.1365-2621.2006.01309.x

Yuliani, S., Bhandari, B., Rutgers, R., & D'Arcy, B. (2004). Application of microencapsulated flavor to extrusion products. Food Reviews International, 20 (2), 163-185. https://doi.org/10.1081/FRI-120037159

Zhang, B., Liu, G., Ying, D., Sanguansri, L., & Augustin, M. A. (2017). Effect of extrusion conditions on the physico-chemical properties and in vitro protein digestibility of canola meal. Food Research International, 100 (1), 658-664. https://doi.org/10.1016/j.foodres.2017.07.060

4.2. Development of a sustainable, fast and continuous cocoa alkalization method based on extrusion

Valverde, D., Sanchez-Jimenez, V., Pérez-Esteve, É., Barat, J.M.

Departamento de Tecnología de Alimentos. Universitat Politècnica de València, Camino de Vera, s/n, 46022, Valencia, Spain

Submitted to Innovative Food Science and Emerging Technologies

Abstract

Traditional alkalization, essential for darkening color, modifying flavor and increasing cocoa powder solubility, is a discontinuous time-consuming technique that employs considerable energy. We herein propose extrusion as a promising alternative to improve and increase the sustainability of the traditional process. The aims of this work were twofold: on the one hand, to characterise the effects of extrusion on alkalised cocoa physico-chemical features; on the other hand, to determine if alkalized powders possess similar characteristics to those obtained by conventional treatment. The results showed that alkali was the main variable to increase pH and to diminish color. Compared to commercial samples, the developed cocoas had darker colors than, and similar sensory properties to, their reference commercial cocoas. These findings confirm that extrusion is suitable for producing high sensory acceptable alkalized products quickly, sustainably and continuously.

Keywords: extrusion, alkalization, cocoa, Dutching, color.

1. Introduction

In the last years, climate change has become the challenge of the 21st century and is pushing all kinds of industries to responsibly use energy and reduce their pollution. Using renewable energy sources, looking for interesting applications of what is usually called “waste” and replacing traditional production techniques with more sustainable alternatives have become musts for industry.

Cocoa is a well-known product found worldwide. It is harvested in countries 20º north and south of the Ecuador, although it is processed practically in all continents. The following figures indicate the magnitude of this industry: the world bean production for 2014/15 was 4,236 million tons, of which 73% was produced in Africa and 37% processed in Europe to obtain derived products, such as cocoa liquor, cocoa powder or chocolate (International Cocoa Organization, 2017).

Given its importance, a minor change in this industry in sustainability terms can have an enormous impact on the world. This is the reason why we focused on applying and evaluating alternative techniques to enhance the whole cocoa production chain’s sustainability. In this work, we focused on alkalization from all possible steps because it has been the least studied.

Alkalization, also called “Dutching”, was a treatment initially conceived by van Houten in the 19th century to increase cocoa powder solubility. However, its effects on cocoa color and taste made its application also interesting for darkening and reducing the bitterness and astringency of powders. Technically, alkalization consists of treating cocoa in combination with an alkali solution in a closed, heated and pressurized vessel (De Zaan cocoa, 2006).

Alkalization as a process has three main industrial drawbacks: it is performed in batches; it implies considerable energy and time; it takes up 2 h or more (Terink and Brandon, 1981; Ellis, 1990; Wissgott, 1985). For these reasons, looking for a fast, continuous and less energy-consuming alternative method is a key point for the cocoa industry to improve and make alkalization a more sustainable step, and it is here where extrusion appears as a promising alternative.

Extrusion has been widely employed by the food industry to transform granular and powdered foods into larger pieces. Its applications include a considerable number of food products like pasta, snacks, biscuits, pet food, etc. (Fellows, 2000).

The advantages of extrusion are that it is a versatile and continuous technique, it combines mixing, cooking, kneading, shearing, shaping and forming, and can be employed with different kinds of ingredients and under diverse operating conditions. Extrusion also enables higher productivity and lower processing costs than other methods, and is an exceptional system for many extruded foods that cannot be easily produced by other techniques. One disadvantage of extrusion is that its use is limited to granular or powdered materials, and it can volatilize or even degrade flavor compounds (Yuliani et al, 2004).

Extrusion has been reported to modify the texture, color, flavor and microbiological content of different materials due to several kinds of modifications, such as: hydration, gelation and shearing of starches; denaturalization or reorientation of proteins; melting of fats (Singh, Gamlath and Wakeling, 2007). Of these modifications, changes in texture are the most relevant for the cocoa industry.

Extrusion also increases protein digestibility by inactivating anti-nutritional factors and by exposing new sites to enzymatic digestion though denaturalization (Zhang et al., 2017; Fellows, 2000). Finally from a microbiological point of view, low water content and heat applied during extrusion make most extruded-cooked products bacteria-free. Moreover, some studies point out that Bacillus stearothermophilus spores are significantly reduce in number after applying high temperatures during extrusion (Nikmaram, 2015).

Bearing in mind the general technological advantages of extrusion, and the fact that cocoa is a granular or powdered material, extrusion could serve as an extraordinary continuous, sustainable and fast alternative for modifying natural cocoa properties through alkalization. We were unable to find any scientific works that have studied the effects of extrusion on cocoa. The only reference in the literature is a patent published in 1974, which mentions the possibility of using extrusion for cocoa alkalization, but does not describe the effects of this treatment on cocoa physico-chemical characteristics (Chalin, 1974).

As information about the effects of extrusion variables on cocoa is lacking, the objectives of this work were to evaluate the effect of different processing variables (water content, alkali type and concentration, temperature) on the physico-chemical properties of alkalized powders. Moreover, as increased sustainability should not impede the production of similar properties, another study objective was to study if the physico-chemical and sensory properties of the produced cocoas were comparable to those of traditional alkalized samples.

2. Materials and methods

2.1. Material and methods

The cocoa employed for the extrusion experiments was a natural powder from the Ivory Coast. The commercial samples used as the control standard cocoas were all provided by Olam Food Ingredients SL (Cheste, Spain), and were: three natural, one dark natural, three light, two medium and two strongly alkalized cocoas. Sodium hydroxide, potassium carbonate, sodium carbonate, gallic acid, Folin-Ciocalteu reagent, methanol and acetone were supplied by Scharlau (Sentmenat, Spain). Trolox was provided by Across Organics (Geel, Belgium). (-)-Epicatechin and (+)-Catechin were acquired from Sigma-Aldrich (Darmstadt, Germany).

2.2. Cocoa extrusion

Before extrusion, each cocoa powder was properly mixed with the corresponding amounts of water and alkali in a Blixer (Robot Coupe, Mataró, Spain).

Mixtures were then placed inside a single-screw extruder 19/25 from Brabender (Duisburg, Germany). Extruder conditions were selected according to the results obtained in previous works. To determine the influence of the type and concentration of alkali, water content and temperature, the operational conditions were set as follows: die size (4 mm), extrusion speed (120 rpm), screw dimensions (1:1) and feeding speed (10 rpm). The temperatures in the different extruder modules were 37°C in module 1, 65°C in module 2, 60ºC or 100°C in module 3, depending on the assay temperature and the corresponding temperature in module 4. Treatment took less than 5 minutes.

Once extruded, samples were dried overnight at 100°C and turned into a powder in a coffee milling machine.

2.3. Experimental design

A response surface methodology was followed to define the combination of conditions to be applied, and to evaluate the relations between the process variables (water content (X_1), temperature (X_2) and alkali concentration (X_3)) and the response parameters (pH, color and moisture). Statistical modelling and analyses were carried out by the design assistant of the experiments of Statgraphics Centurion (Manugistics Inc., Rockville, MD, USA). The selected experimental ranges of the three independent parameters were those found to be the most widely used ones in different alkalization patents (Chalin, 1974; Ellis, 1992; Wiant et al., 1989; Wissgott, 1985; Terink and Brandon, 1981; Kopp et al., 2009). The design selected for the surface response modelling was an orthogonal and central composed design 2^3+star. The experimental conditions for the analysis are shown in Table 4.

After the data analysis, the behavior of each response variable (pH, color and moisture) in relation to the evaluated independent parameters was fitted in a quadratic polynomial model as shown in Eq. 1.

$$y = a_0 + \sum_{i=1}^{3} a_i X_i + \sum_{i=1}^{3} a_{ii} X_{ii} + \sum_{i \neq j=1}^{3} a_{ij} X_i X_j \quad (Eq. 1)$$

where "y" represents the response variable (pH, color or moisture), "a_o" is the constant, "a_i", "a_{ii}" and "a_{ij}" are the linear coefficients and their interactions, and "X_i" and "X_j" are the experimental data for each variable.

The previous surface response methodology was carried out separately for the two alkali agents herein employed: NaOH and K_2CO_3. For all the models, the R^2 statistical values were obtained to evaluate their suitability.

Table 4. Water content, temperature and alkali concentration for constructing the surface response.

Point	X_1 (%)	X_2 (°C)	X_3 (%)
1	20	150	6
2	31.4	105	3.5
3	25	105	6.7
4	30	75	1
5	25	105	3.5
6	25	105	3.5
7	30	75	6
8	25	105	0.3
9	20	75	1
10	30	150	1
11	25	162.9	3.5
12	20	75	6
13	18.6	105	3.5
14	25	75	3.5
15	20	150	1
16	30	150	6

2.4. Physicochemical and sensory analyses

2.4.1. Moisture

The moisture content of samples was calculated according to the recommendations described in the De Zaan cocoa manual (De Zaan, 2006). Moisture content was determined by Eq. 2.

$$Moisture\ (\%) = \frac{\big((W_1 - A) - (W_2 - A)\big) \times 100}{(W_1 - A)} \quad (\text{Eq. }2)$$

where, “W1” is the weight of the sample plus the cup before drying, “W2” is the weight of the sample and cup after drying, and “A” is the weight of the dried and empty aluminium cup.

2.4.2. pH and color determinations

The color and pH of the produced and commercial samples were determined following the methods described by De Zaan (2006).

pH measurements were taken using a penetration electrode and a pH-Meter BASIC 20+ from Crison (Barcelona, Spain). pH was used to classify samples into different categories according to Miller’s scale (Miller et al., 2008). Those cocoas with a pH between 5 and 6 were considered natural cocoas, those with a pH between 6 and 7.2 were taken as lightly alkalized, between 7.2 and 7.6 were moderately alkalized, and those over 7.6 were strongly alkalized.

The color parameters of samples were analyzed by a Minolta spectrophotometer CM 3600-d (Ramsey, USA). The intrinsic color of samples was measured in the reflectance mode with the specular component exclude (SCE). Data were converted in the CIE-L*a*b* color space. All the samples were read in triplicate.

To calculate the total color difference (ΔE), the formula reported by Rodríguez et al (2009) was used (Eq. 3).

$$\Delta E = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad (\text{Eq. }3)$$

where ΔL* is the difference in luminosity between an untreated sample and a treated one, Δa* is the difference in red/green, and Δb* is the difference in yellow/blue coordinates. The ΔE for the extruded samples was calculated using the values of the untreated cocoas, while the ΔE for the commercial ones was calculated with the mean value of the natural references.

2.4.3. Sensory analyses

Sensory analyses were performed according to UNE-ISO 6658:2019 by a trained panel made up of nine people. Seven different markers were analyzed: cocoa and chocolate taste, acidity, astringency, alkalinity, body and bitterness. They were scored from 1 to 5, where 0 represented no taste and 5 denoted a very intense taste. Of the 32 samples, a selection of cocoas comprising 2-4 samples from each category was made for this study. For this selection, only those samples with pH values and color coordinates that fell within the range described by standard commercial samples were included.

3. Results and discussion

This work presents extrusion as a fast, sustainable and continuous technique to replace traditional alkalization. However, this is rendered useless if the proposed alternative does not produce similar products to commercial ones. To verify if the method was able to produce the necessary changes and how they were made, this work was divided into two parts: the first one focused on analysing the impact of the different extrusion conditions on the physico-chemical properties of cocoa; the second one centred on comparing the produced products with traditionally obtained ones.

3.1. Effect of extrusion variables on cocoa physico-chemical characteristics

3.1.1. Model fitting

In this work, two independent experiments were run according to the alkali used in the treatment (K_2CO_3 and NaOH) to model changes in pH, color and moisture in relation to water content, temperature and alkali concentration. Table 4 shows the coefficients for each response variable that fitted the experimental data in the corresponding quadratic equation. It also indicates their statistical significance.

Table 4. Regression coefficients of the quadratic equations for pH, color and moisture in the samples treated with K_2CO_3 and NaOH.

Regression coefficients	L*				C*				h*				pH				Moisture loss			
	K_2CO_3		NaOH		K_2CO_3		NaOH		K_2CO_3		NaOH		K_2CO_3		NaOH		K_2CO_3		NaOH	
	Coef.	F-value	Coef.	F-value	Coef.	F-value	Coef.	F-value	Coef.	F-value	Coef.	F-value	Coef.	F-value	Coef.	F-value	Coef.	F-value	Coef.	F-value
X_0	45.22		41.69		36.81		34.11		97.02		112.31		-0.68		1.15		-0.85		-25.90	
X_1	-1.71	0.52	-1.14	0.83	-1.99	0.15	-0.24	0.08	-2.95	1.15	-1.41	0.45	0.48	1.96	0.13	0.57	0.22*	12.84	3.43**	25.26
X_2	-0.08	0.81	-0.09	3.78	0.05	2.38	-0.12	0.87	-0.14	0.21	-0.44	1.93	0.02	2.41	0.05	0.35	0.11**	20.93	-0.28***	76.83
X_3	-1.52**	20.48	-4.42**	66.61	-0.47*	13.65	-5.00**	57.31	-3.63*	17.07	-12.44*	65.43	0.65*	19.81	1.31***	73.53	-3.27	0.82	3.41	0.24
X_1^2	0.02	0.65	0.01	0.35	0.03	1.66	-0.01	0.12	0.03	0.46	-0.02	0.1	-0.01	0.64	0.01	0.01	0.02	0.07	-0.04	1.28
X_1X_2	0.01*	6.63	0.01	1.74	0.01	4.29	0.01	1.08	0.01	4.74	0.02	3.54	-0.01*	6.24	-0.01	2.03	-0.01	0.89	-0.01	2.11
X_1X_3	0.03	0.46	0.06	2.84	0.03	0.87	0.11	4.29	0.07	0.83	0.33*	7.04	-0.01	0.59	-0.02	2.81	0.17	1.68	-0.10	3.19
X_2^2	-0.01	1.35	-0.01	0.35	-0.01	2.89	-0.01	0.05	-0.01	0.7	-0.01	0.06	0.02	0.61	0.01	0.08	0.01	0.97	0.01**	20.78
X_2X_3	0.01	0.43	0.01	5.12	0.01	0.01	0.01	3.04	0.01	1.14	0.02	1.5	-0.01	1.64	-0.01*	7.09	-0.01	0	-0.01	3.27
X_3^2	-0.04	0.17	0.07	0.54	-0.14	2.59	-0.10	0.57	-0.12	0.35	-0.30	1.02	0.01	0	-0.02	0.35	-0.21	0.42	0.09	0.43
R^2	0.86		0.93		0.82		0.92		0.82		0.93		0.85		0.93		0.92		0.98	

Significance: * (0.01<p-value<0.05), ** (0.001<p-value<0.01), *** (p-value<0.001).

An analysis of variance (ANOVA) of the models showed that the resulting equations exhibited regression coefficients (R^2) above 0.8 in all the response parameters, which meant that the proposed models explained more than 80% of the variability of the different response parameters.

In addition, the significance of the coefficients and the impact of the different variables were evaluated to identify which affected the various response parameters. Among the significant coefficients, the highest F-value indicated which variable most affected a given parameter. Bearing in mind this criterion, and as seen in Table 4, alkali concentration was the main parameter that affected the pH and color of samples (p-value<0.05), while it was temperature, followed by the water content added to cocoa, for moisture.

3.1.2. Effect of extrusion variables on pH

pH is an important characteristic in the cocoa industry because it is used to classify cocoa into different categories (Miller et al., 2008). pH also conditions sample color because its increases the enzyme activity of polyphenol oxidase (Rodríguez et al., 2009; Misnawi et al., 2003) and enhances several chemical reactions that darken cocoa (Germann et al., 2019a, 2019b).

Figure 4 shows the effects of temperature and K_2CO_3 and NaOH concentrations on pH. As seen in it, the pH of the produced cocoas ranged from 5 to 10, which meant that powders of different alkalization degrees can be produced with both alkali types. Indeed the type and concentration of alkali proved to be determining factors for this characteristic. As seen, the higher the alkali concentration, the higher the final pH of the alkalized powder. Regarding type, as expected pH increased more with NaOH than with K_2CO_3 due to the higher alkalinity of NaOH.

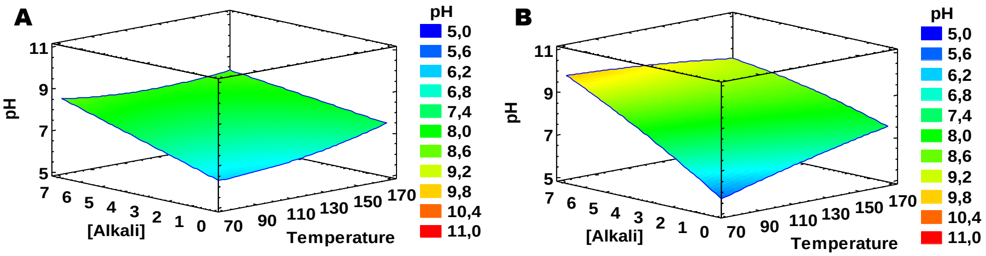

Figure 4. Surface responses of the effect of temperature and alkali concentration on pH. (A) Surface responses of the samples treated with K_2CO_3; (B) surface responses of the samples treated with NaOH, with an initial 20% water content.

In addition to alkali type and concentration, the combinations of water content and temperature for K_2CO_3, and alkali concentration and temperature for NaOH, had a negative impact on increases in pH (p-value<0.05). For example, when the K_2CO_3 concentration was 6% and temperature was 160°C, pH changed from 8 to 6.5 because water content rose from 20% to 30%. With NaOH, when the alkali concentration was 6% and water content was 30%, pH changed from 8.9 to 7.9 as temperature increased from 75°C to 160°C.

3.1.3. Effect of extrusion treatment variables on color

In this section, the effects of the extrusion variables on the color of the extruded cocoa powders were evaluated. Figure 5 shows the effects of K_2CO_3 and NaOH concentrations and temperature on color.

In color terms, the extrusion treatment without alkali generally increased the darkening (L*), saturation (C*) and redness (h*) of the untreated cocoa. For example, in the samples treated with 20% water content, 0% alkali and at 70°C, L*, C* and h* were 20, 20 and 52, and were 25, 25 and 54 in the untreated cocoa, both respectively. This meant that the extrusion treatment was able to darken cocoa when alkali was lacking.

When an alkali was included, samples were darker, redder and less saturated as the alkali concentration increased, which made cocoa color change from the light brown of the untreated powder to dark brown. In addition to alkali concentration, which in statistical terms was one of the variables with the strongest impact on color, alkali type was also relevant. According to the obtained results (Fig. 4), and in agreement with other authors like Rodríguez et al (2009), NaOH was the alkali that darkened the samples the most.

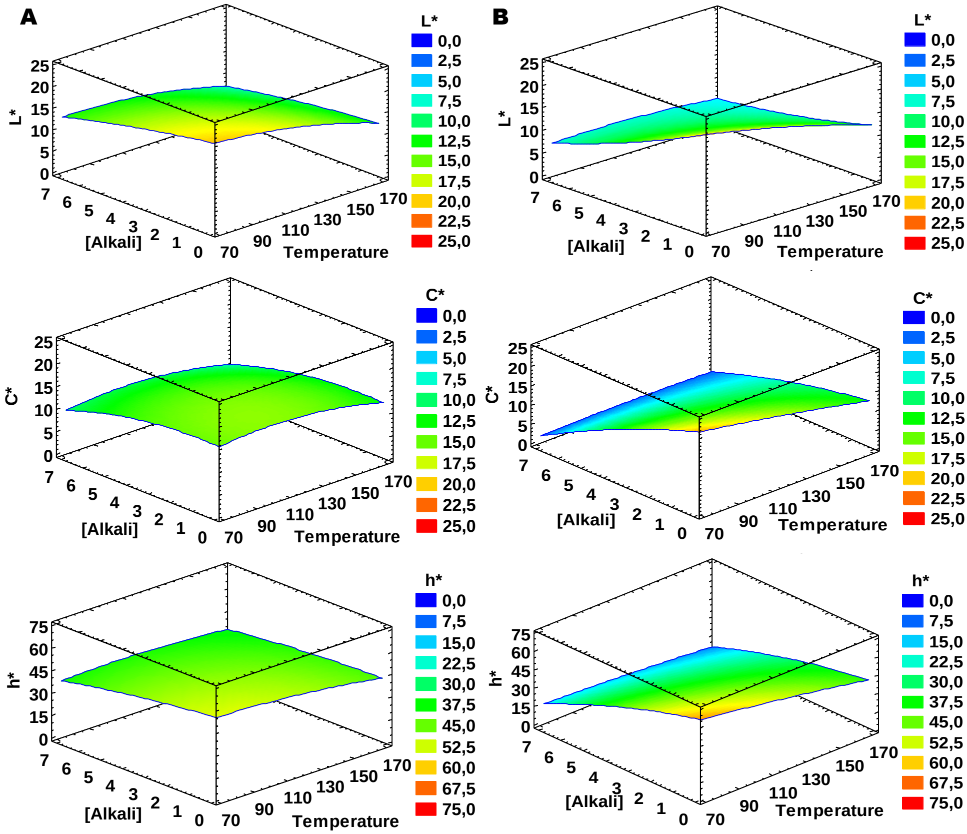

Figure 5. Surface responses of the effect of temperature, water content and alkali concentration on color components. (A) Surface responses of the samples treated with K_2CO_3 and (B) surface responses of the samples treated with NaOH, with an initial water content of 20%.

In addition to alkali, L* in the samples treated with K_2CO_3 was affected by the water content and temperature combination. Raising water content at high temperatures increased L*, while raising water content led to lower L* values at low temperatures. By way of example, with the cocoas treated with 6% of K_2CO_3 at 160°C, L* increased from 9.6 to 14.6 when water content rose from 20% to 30%, while at 75°C and with 6% alkali, L* lowered from 14.2 to 13.4 as water content increased.

With NaOH, apart from the effect of alkali, h* was affected by the water content and alkali concentration combination. Increasing the water content in the cocoas with high NaOH concentrations reduced h* less, while the same increase at low alkali concentrations more markedly reduced this parameter. For example, in the samples treated with 6% NaOH at 80°C, h* went from 24.9 to 31.1 when water content increased from 20% to 30%. In the cocoas treated with 1% NaOH at 80°C, h* went from 56.5 to 46.4 as water content increased by the same order.

Furthermore when comparing Figures 4 and 5, we observed an inverse relation between pH and color. When pH rose with treatment, color consequently reduced, and vice versa. Between the two employed alkali agents, lower pH and color values were obtained when NaOH was used compared to K_2CO_3. This highlights the importance of alkali selection during cocoa color development.

3.1.4. Effect of extrusion treatment variables on moisture loss

Moisture content in products like cocoa powders, which are nutritionally rich and need to be stored for medium and long time periods, is a negative feature that must be reduced to maintain the product's nutritional and microbiological qualities. It is generally considered that cocoa powder moisture must be lower than 5% to assure good conservation during its shelf life. To reach this content, in the traditional alkalization system performed in a pressurized vessel, the material is first treated, and then separately dried, to reduce moisture content to less than 5% (De Zaan, 2006).

Due to the aforementioned advantages and to evaluate changes in moisture in the extruded cocoas, the effects of temperature, water content, type and alkali concentration were studied in relation to moisture loss at the end of the extrusion process (Fig. 6).

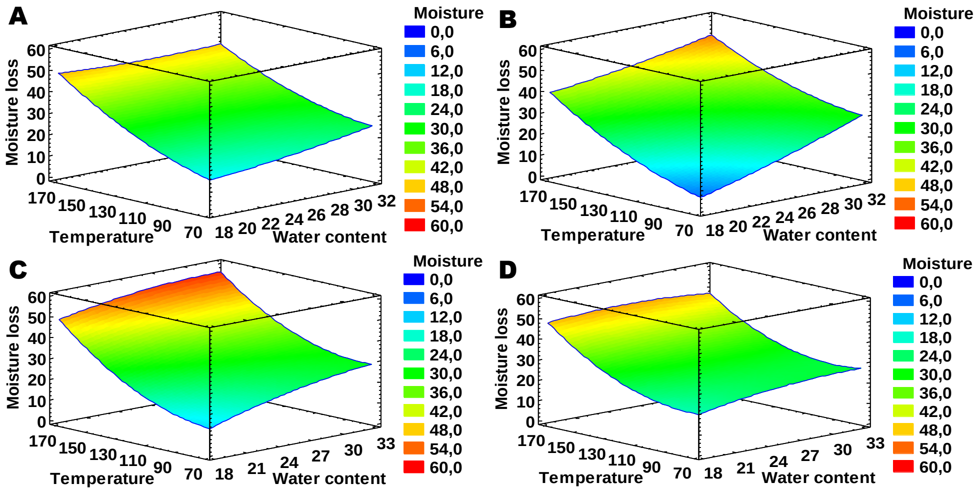

Figure 6. Surface responses showing the effect of water content, temperature, type and concentration of alkali on moisture loss (%) during the extrusion treatment. (A) The samples treated with 0.28% K_2CO_3, (B) the samples treated with 6% K_2CO_3, (C) the samples treated with 0.28% NaOH and (D) the samples treated with 6% NaOH.

Water content and temperature, but more specially temperature, increased moisture loss up to 50%. For example, moisture loss was approximately 45% for the 160ºC, 30% water content and 6% alkali conditions. This revealed that, although further drying was still necessary, the proposed method was able to significantly lower water content, which consequently reduced the costs and duration of the drying step compared to the commercial alkalization treatment.

3.2. Comparison to commercial samples

3.2.1. Color comparison

An essential part of designing and applying a new method as an alternative to another is to evaluate its suitability to be that replacement. In this work, apart from studying the effects that the different extrusion variables had on cocoa physico-chemical features, the powders produced by extrusion were compared to traditionally prepared commercial standards.

Of the 32 extruded cocoas, 19 samples were selected because their pH, L*, C* and h* values met the commercial color specifications or had even lower color values than the commercially specified ones. Figure 4 shows the total color differences of all the selected extruded cocoas, classified by their pH values.

ΔE gives a general idea of how similar two samples are, but does not indicate if samples are darker or lighter. In our case, ΔE was used to obtain an overview of the similarity of the produced and commercial samples.

As observed in Figure 7, all the selected extruded cocoas belonging to the natural, light and moderate alkalized groups had a ΔE above 2. This meant that the color of these samples could be detected by the human eye as being different from that of commercial cocoas (Lee and Coater, 1999). Some of the strong alkalized cocoas had ΔE values below 2, which meant that they were not differentiable from their commercial reference. Others had ΔE values of 10, which implies that cocoas clearly differed from their commercial references.

When the color components (L*, C* and h*) of all the selected extruded samples were analyzed, all the cocoas, except for the strong alkalised ones, had lower L* and C* values. This meant that they were darker and less saturated than commercial cocoas, with h* meeting commercial color specifications.

With the strong alkalized cocoas, most samples obtained L*, C* and h* values that met commercial specifications, although two of the samples alkalized with NaOH had lower L*, C* and h* values, which meant that they were darker in color, redder and less saturated. By way of example, L* of 6, C* of 3.4 and h* of 14.4 were obtained for cocoa S_6_20_150. The specifications of the strong alkalized cocoas were 8-13 (L*), 6-15 (C*) and 28-45 (h*).

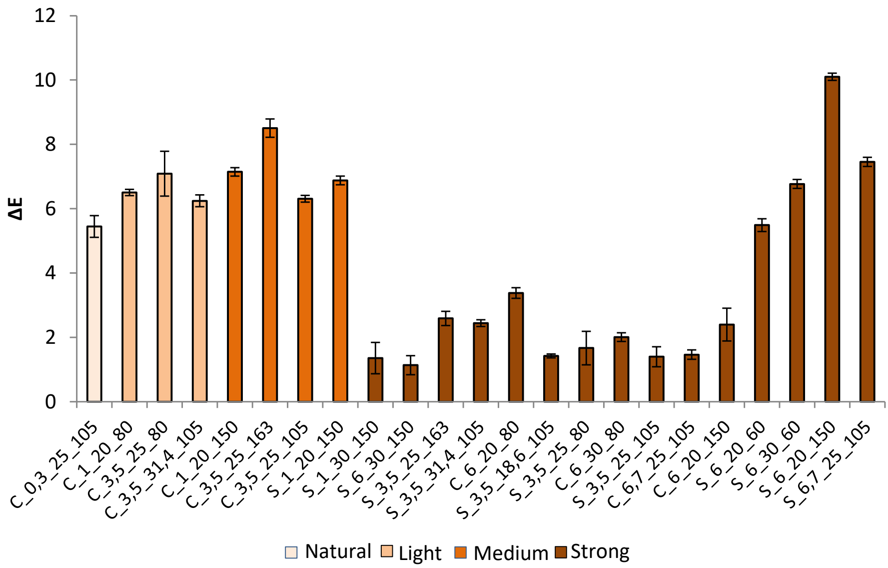

Figure 7. Total color difference (ΔE) of the extruded selected samples compared to their reference cocoas. Samples are divided to four groups: light orange (natural), orange (light), dark orange (medium) and brown (strong). The developed samples are coded in this order: the alkali employed (K_2CO_3 (C) or NaOH (S)), the proportion of alkali (%), the proportion of water (%) and temperature (°C).

Most cocoas had lower L*, C* or h* values which, if necessary, could be adjusted by modifying the treatment conditions for having similar colors to commercial cocoas. Indeed the built response surfaces are a useful tool for adjusting alkali concentration, water content and temperature according to, for example, a desired final color.

In addition to being able to darken cocoa, extrusion proved effective in cutting the time and energy use of traditional alkalization. Extrusion needs 92% less time than traditional alkalization and does not involve heating the tank's content before treating it, which speeds up the process and cuts the treatment's energy use.

Extrusion also reduces the time and energy use of the cocoa production chain by significantly lowering the moisture content of samples. Therefore, extrusion was generally shown as a more sustainable technique than conventional treatments.

3.2.2. Sensory evaluation of the selected samples

After the physico-chemical characterization, different samples were selected and sensory-evaluated by a trained panel formed by nine people. Figure 8 shows the spider graphs used to compare the extruded and commercial cocoas.

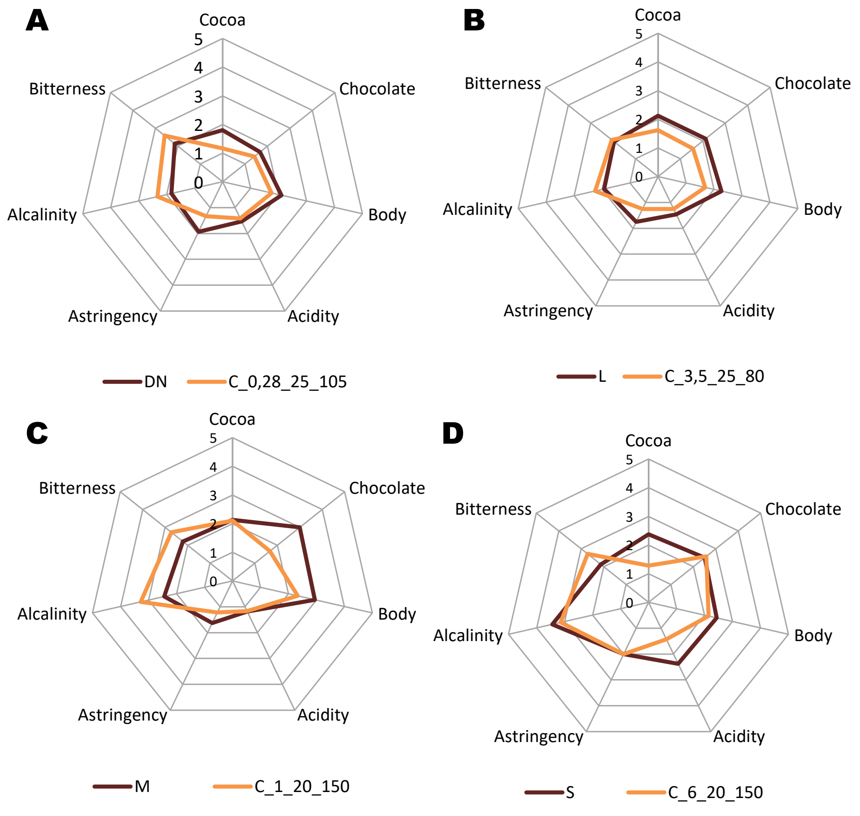

Figure 8. Comparison made between the extruded and commercial powders for seven sensory-evaluated characteristics. Samples are grouped according to their alkalization level as: (A) natural, (B) lightly, (C) moderately, (D) strongly alkalized.

As we can see, the commercial and extruder cocoas were similar. For dark natural cocoas (Figure 8A), which were heat-treated without alkali, the sensory patterns only differed in terms of cocoa taste intensity and astringency.

When cocoas were alkalized (Figure 8B, C and D), their behavior was also similar. At the lightly alkalized levels, the commercial and extruded samples did not differ for any evaluated marker. At the medium alkalized level, chocolate taste was less intense. At the strongly alkalized level, cocoa taste and acidity were lower in the extruded samples than in the commercial ones. Despite these differences, the obtained cocoas had similar sensory characteristics to the alkalized powders.

The similar sensory patterns stress the suitability of extrusion to replace the traditional alkalization method. In a 92% shorter time and continuously, extrusion obtained cocoas with similar color and sensory characteristics, and reduced samples' associated energy and moisture content.

4. Conclusions

The effects of alkalization on cocoa physico-chemical properties are dominated by the alkali agent and its concentration, which makes the substitution of the traditionally employed technique possible. As we show here, extrusion has the capacity to speed up, reduce energy use and turn traditional alkalization into a continuous system, while making similar color and sensory changes to those of traditionally alkalized products.

Further analyses have to be done to reveal the effects of extrusion on functional content, and to study the significance of the conditions employed in this work (screw and feeding speed, screw type and die size).

Acknowledgements

Damián Valverde would like to thank the Regional Ministry of Education, Culture and Sports of Generalitat Valenciana for his PhD grant and also to Marta Puchol Miquel for her contributions during the sensory evaluations.

Funding

The authors would like to acknowledge the financial support of the Spanish Government and European Regional Development Fund (Project RTC-2016-5241-2).

References

Chalin, M. L. (1974). Method of dutching cocoa. US3868469A.

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

Ellis, L. D. (1992). Process for making dark cocoa. US5114730A.

Fellows, P. J. (2000). Chapter 14: extrusion cooking. Food Processing Technology (Second Edition). Woodhead Publishing Limited.

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 (16), 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 (16), 4643-4651. https://doi.org/10.1021/acs.jafc.9b01050

Kopp, G. M., Hennen, J. C., Seyller, M., & Brandstetter, B. (2009). Process for producing high flavour cocoa. EP2241190B1.

Kristiawan, M., Micard, V., Maladira, P., Alchamieh, C., Maigret, J. E., Réguerre, A. L., Emin, M. A., & Della Valle, G. (2018). Multi-scale structural changes of starch and proteins during pea flour extrusion. Food Research International, 108, 203-215. https://doi.org/10.1016/j.foodres.2018.03.027

Lee, H. S., & Coates, G. A. (1999). Thermal pasteurization effects on color of red grapefruit juices. Journal of Food Science, 64 (4), 663-666. https://doi.org/10.1111/j.1365-2621.1999.tb15106.x

Miller, K. B., Hurst, W. F., 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 indez, 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

Nikmaram, N., Kamani, M. H., & Ghalavand, R. (2015). The effects of extrusion cooking on antinutritional factors, chemical properties and contaminating microorganism of food. International Journal of Farming and Allied Sciences, 4 (4), 352-354.

Rodríguez, P., Pérez, E., & Guzmán, R. (2009). Effect of the 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

Singh, S., Gamlath, S., & Wakeling, L. (2007). Nutritional aspects of food extrusion: a review. International Journal of Food Science & Technology, 42 (8), 916-919. https://doi.org/10.1111/j.1365-2621.2006.01309.x

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

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

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

Wissgott, U. (1985). Method of alkalization of cocoa in the aqueous phase. CH663324A5

Yuliani, S., Bhandari, B., Rutgers, R., & D'Arcy, B. (2004). Application of microencapsulated flavor to extrusion products. Food Reviews International, 20 (2), 163-185. https://doi.org/10.1081/FRI-120037159

Zhang, B., Liu, G., Ying, D., Sanguansri, L., & Augustin, M. A. (2017). Effect of extrusion conditions on the physico-chemical properties and *in vitro* protein digestibility of canola meal. Food Research International, 100, 658-664. https://doi.org/10.1016/j.foodres.2017.07.060

4.3. Functional changes induced by extrusion during cocoa alkalization

Valverde, D.[1], Behrends, B.[2], Pérez-Esteve, É.[1], Kuhnert, N.[2], Barat, J.M.[1]

[1]Departamento de Tecnología de Alimentos. Universitat Politècnica de València, Camino de Vera, s/n, 46022, Valencia, Spain

[2]Department of Life Sciences & Chemistry, Jacobs University, Campus ring 1, 28759, Bremen, Germany

Submitted to Food Research International

Abstract

Polyphenols are a group of secondary metabolites with different roles in the development and defense of plants that have shown to exhibit relevant effects on human health. In dry cocoa beans, polyphenols represent 10-15% of the weight, which has been reported to be highly degraded by traditional alkalization. Designing a new technique able to maintain the functional features of cocoa will be a great innovation for the industry. In this work, we study the effects of a developed extrusion alkalization method on the functional characteristics of cocoa. Results showed that extrusion negatively affected the phenolic profile. Of all the studied variables, type of alkali and concentration were the most significant in the reduction of the evaluated polyphenols. Furthermore, the comparison between extruded and traditionally produced powders revealed that extrusion produces similar reductions in the functional features than the conventional technique, but in a faster, less energy-consuming and continuous fashion.

Keywords: extrusion, alkalization, cocoa, Dutching, polyphenols, technology, flavanols.

1. Introduction

Polyphenols are secondary metabolites of plants that, in dry cocoa beans, represent the 10-15% of the dry weight (Martín et al., 2013 and 2017; Aprotosoaie, Luca and Miron, 2015). The importance of this group of compounds is related to their sensory and functional properties. In the case of sensory features, there are polyphenols that have been identified as pigments, as astringent and bitter compounds or as molecules able to modulate the flavor (El Gharras, 2009). In the case of the functional activity, several polyphenols have shown to have different in vitro beneficial health effects such as protection of neurons, stimulation of vasodilation, improvement of insulin secretion and inhibition of cancer cell proliferation (Del Rio et al., 2009).

Cocoa alkalization is an additional step of the cocoa production chain, in which the material is treated with an alkali solution, pressure and temperature inside closed and pressurized vessels. This treatment aims to dark the color of cocoa, increase the solubility of the powder and reduce the astringency and bitterness of natural cocoa (De Zaan cocoa, 2006).

In addition to the desired modifications in the physicochemical and sensory features of cocoa, alkalization has been reported to reduce the presence of polyphenols, methylxanthines, vitamins, amino acids and sugars, among other compounds (Brandon and Terink, 1981; Ellis, 1990; Wissgott, 1985; Li et al., 2012; Huang and Barringer, 2010). Specifically, conventional alkalization has been associated with the production of important reductions in the polyphenolic content of cocoa. For example, Gültekin-Özgüven et al (2016) analyzed the total polyphenol, flavanol content and antioxidant activity of alkalized cocoa liquors and found that the previous features were reduced an 87%, 83% and 50%, respectively. In other work, Gu et al (2006) found a reduction of 51% in the antioxidant activity and of 78% in the content of procyanidins in commercial cocoas, and Jolić et al (2011) a loss of 64% of total polyphenols, 59% of total procyanidins and of 39% of the antioxidant activity when cocoa nibs were alkalized.

One technique that has been applied as an alternative to cocoa alkalization, but whose effects have not been studied yet, is extrusion (Chalin, 1974). This technology has been widely applied by the food industry for the production of different kind of products as pasta, chocolate, chewy gums, breakfast cereals and baby food, among others (Fellow, 2000). Extrusion is based in the introduction of a powdered material in an extruder and in its continuous shearing, heating and pressurization that lead to the obtaining of a compacted product.

Extrusion has been reported to negatively affect the content of polyphenols of different food matrices, although it has been also documented an increase in the antioxidant activity, which has been related to the lysis of cells and to the formation of Maillard reaction products with an enhanced antioxidant activity (Sharma et al., 2016; El-Hady and Habiba, 2003; Shih, Kou and Chiang, 2009; Nayak et al., 2011).

In this work, due to the lack of information about the effects of extrusion alkalization on the functional characteristics of cocoa, we have developed and applied a cocoa alkalization method based in extrusion. One of our objectives is to study the effects of the different processing variables (water content, alkali type and concentration, and temperature) of the developed alkalization method on the functional features of cocoa. In addition, the different extruded powders are compared to the traditionally produced ones to determine if extrusion is less aggressive than the conventional alkalization method.

2. Materials and methods

2.1. Materials

The natural cocoa employed for the extrusion experiments was a natural powder from Ivory Coast. The commercial samples used as control were: three natural, one dark natural, three light, two medium and two strongly alkalized cocoas. All were provided by Olam Food Ingredients SL (Cheste, Spain). Trolox was provided by Across Organics (Geel, Belgium). (-)-Epicatechin, (+)-Catechin, avicularin, peptides B1 and B2, trimer C1, tetramer A2, clovamide and hyperoxide were provided by Phytolab (Vestenbergsgreuth, Germany). Clovamide was provided by

Biozol (Eching, Germany) and vitexin from Merck (Darmstadt, Germany). Potassium carbonate, sodium carbonate, sodium hydroxide, Gallic acid, methanol, Folin-Ciocalteu reagent and acetone were provided by Scharlau (Sentmenat, Spain).

2.2. Cocoa extrusion

Before extrusion, each cocoa powder was properly mixed with the corresponding amounts of water and alkali in a Blixer (Robot Coupe, Mataró, Spain). The mixtures were then introduced in a single screw extruder 19/25 from Brabender (Duisburg, Germany). The extruder conditions were selected for being the most common in different alkalization patents (Chalin, 1974; Ellis, 1992; Wiant et al., 1989; Wissgott, 1985; Brandon and Terink, 1981; Kopp et al., 2009). To study the influence of the concentration and type of alkali, the temperature and the water content the operational conditions were fixed: dimensions of the screw (1:1), size of the die (4 mm), feeding speed (10 rpm) and extrusion speed (120 rpm). Temperature in the extruder was: 37°C in module 1, 65°C in module 2, 60°C or 100°C in module 3 depending of the temperature of the assay, and the corresponding temperature in module 4. The treatment lasts less than 5 minutes. Once extruded, the samples were dried overnight at 100°C and powdered by employing a coffee milling machine.

2.3. Experimental design

A response surface methodology was used to stablish the combination of conditions to be applied and to stablish the relationships between the process variables (water content (X_1), temperature (X_2) and alkali concentration (X_3)) and the response parameters (antioxidant activity, total phenol content and concentration of ten different polyphenols). The statistical modeling and analysis was performed by the design assistant of experiments of Statgraphics Centurion (Manugistics Inc., Rockville, MD, USA). The selected design for the surface response modeling was an orthogonal and central composed design 2^3+star. The experimental conditions for the analysis are shown in Table 5.

Table 5. Water content, temperature and concentration of alkali for the construction of the surface response.

Point	X_1 (%)	X_2 (°C)	X_3 (%)
1	20	150	6
2	31.4359	105	3.5
3	25	105	6.71797
4	30	75	1
5	25	105	3.5
6	25	105	3.5
7	30	75	6
8	25	105	0.282029
9	20	75	1
10	30	150	1
11	25	162.923	3.5
12	20	75	6
13	18.5641	105	3.5
14	25	75	3.5
15	20	150	1
16	30	150	6

After the data analysis, the behavior of each response variable in respect to the evaluated independent parameters was fitted in a quadratic polynomial model as shown in Eq. 4.

$$y = a_0 + \sum_{i=1}^{3} a_i X_i + \sum_{i=1}^{3} a_{ii} X_{ii} + \sum_{i \neq j=1}^{3} a_{ij} X_i X_j \quad (Eq. 4)$$

where “y” represents the response variable, “a_o” is the constant, “a_i”, “a_{ii}” and “a_{ij}” are the linear coefficients and their interactions, and “X_i” and “X_j” are the experimental data for each variable.

The previous surface response methodology was carried out separately for the two alkali agents employed in this work: NaOH and K_2CO_3. For all the models, the R^2 statistical values were obtained for evaluating their suitability.

2.4. General functional characterization

2.4.1. Obtaining of the polyphenolic extract

For extracting the polyphenols present in the samples, an extraction protocol based in the combination of the conditions described by Andres-Lacueva et al (2008), Arranz et al (2009) and Hellström and Mattila (2008) has been employed. In this method, 1 g of cocoa powder was subjected to three extraction cycles: in the two firsts ones, cocoa was dissolved in 20 mL of a mixture of methanol and hydrochloric acid 16 mM (50:50); and in the third and last one, cocoa was dissolved in 20 mL of a mixture of acetone and distillated water (70:30). During each cycle, cocoa was sonicated for 15 minutes at room temperature in an ultrasound bath model Elmasonic S 40H (Elma, Singen, Germany). After the treatment, the samples were centrifuged at 10.000 rpm, at 4°C during 15 minutes. The supernatants of each step were kept in dark and then combined and taken to a final volume of 60 mL. The polyphenolic extracts were kept at 4°C until their analyses.

2.4.2. Total phenolic content

Total polyphenolic content was quantified by using the method described by Todorovic et al. (2015) with some changes. For the assay, 50 µL of each polyphenolic extract was mixed with 0.45 mL of methanol/water (1:1) and 5mL of Folin-Ciocalteu solution. Then, 4 mL of Na_2CO_3 solution were added to the previous mixture and it was kept in darkness for 1 hour. The absorbance of the samples was measured at 750 nm. The results were expressed in g of Gallic acid/100g cocoa powder.

2.4.3. Antioxidant activity

The determination of the antioxidant activity of cocoa samples was performed by following the DPPH method described by Todorovic et al. (2015) with some changes. For the assay, 6 µL of each polyphenolic extract were mixed with 294 µL of methanol. Then, 2.7 mL of the DPPH solution were added. Next, the samples were shaken and kept for 1 h in darkness before being measured at 517 nm. The results were expressed in g of Trolox/100g of cocoa.

2.4.4. Determination of polyphenols

The quantification of catechin, epicatechin, their oligomers and the other four polyphenols was done by HPLC following the method described by D'Souza et al (2017) with some modifications. A ZORBAX Eclipse Plus C18 column (2.1x100 mm) (Agilent Technologies, Waldbronn, Germany) was used. The employed mobile phases were: 0.05% aqueous formic acid (phase A) and acetonitrile with 0.05% of formic acid (phase B). The gradient was: 0-1 min, 8% phase B; 1-2.5 min, 8-12% phase B; 2.5-8 min, 12-16.5% phase B; 8-9 min, 16.5-17% phase B; 9-10 min, 17-17.5% phase B; 10-11 min, 17.5% phase B; 11-12 min, 17.5-18.5% phase B; 12-13 min, 18.5% phase B; 13-23 min, 18.5-95% phase B; 23-33 min, 95% phase B; 33-40 min, 95-8% phase B. The other chromatographic conditions were: UV detection at 280 nm, column temperature of 40°C, injection volume of 2 µL and a flow rate of 0.4 mL/min. The HPLC equipment was an Agilent 1260 HPLC system (Agilent Technologies, Waldbronn, Germany).

3. Results

3.1. Model fitting

To study the evolution of the antioxidant activity, the total phenol content and the concentrations of ten different polyphenols, a response surface methodology has been employed. In this work, two groups of response surfaces have been built, one with K_2CO_3 and the other with NaOH, for modeling and analyzing the effects of alkali concentration, water content and temperature on the functional features of cocoa. Table 6 shows the coefficients for each response variable that fit the experimental data into the corresponding quadratic equation, showing also their statistical significance.

Analysis of variance (ANOVA) of the models showed that most of the resulted equations have regression coefficients (R^2) higher than 0.8. This means that the models are correctly fitting the difference responses.

In addition to R^2, the significance of the different coefficients was evaluated to identify which ones are affecting the different response parameters. In general, from all the variables, alkali concentration is the main one affecting the concentration of the evaluated polyphenols (p-value<0.05), alone or in combination with other variables. In the case of the antioxidant activity and the total phenol content, the models are affected by different variables according to the employed alkali. In samples treated with K_2CO_3, they were mainly affected by temperature (p-value<0.05), while in samples treated with NaOH, the antioxidant activity was not influenced by any of the studied variables, meaning that the operational features fixed in this work (screw and feeding speed, die type…) or type of alkali could be the ones affecting it. In the case of the total phenol content, it was slightly affected by water content.

Table 6. Regression coefficients of the quadratic equations for functional features of the samples treated with K_2CO_3 and NaOH.

Regression coefficients	Antiox. Activity		Total phenol content		Avicularin		Peptide B1		Peptide B2		Catechin		Epicatechin		Clovamide		Hyperoside		Trimer C1		Vitexin	
	K_2CO_3	NaOH	K_2CO_3	NaOH	K_2CO_3	NaOH	K_2CO_3	NaOH	K_2CO_3	NaOH	K_2CO_3	NaOH	K_2CO_3	NaOH	K_2CO_3	NaOH	K_2CO_3	NaOH	K_2CO_3	NaOH	K_2CO_3	NaOH
X0	7.85	3.13	5.06	4.68	6.20	3.59	23.26	23.11	151.37	79.41	125.66	70.78	239.20	108.51	44.21	23.77	2.05	-1.23	95.56	26.81	0.43	0.18
X1	-0.18	-0.08	-0.14	-0.1220*	-0.41	-0.07	-1.01	-0.89	-9.96	-1.21	-5.66	-2.14	-14.97	-1.65	-2.86	-0.23	0.25	0.18	-6.70	-0.11	0.01	0.02*
X2	-0.05*	0.01	-0.02**	-0.01	0.00	-0.01	-0.04	-0.03	0.05	-0.17	-0.26	0.00	0.06	-0.08	0.05*	-0.08	-0.08	0.01	-0.05	0.01	-0.01	0.00
X3	-0.31	0.03	-0.19	-0.13	-0.25*	-0.74***	-0.53**	-2.86**	-7.61**	-20.84***	-3.07**	-11.15**	-12.60**	-28.59***	-1.80**	-4.70***	0.42	-0.48	-2.58*	-9.91***	0.07	-0.09**
$X1^2$	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.01	0.14	0.01	0.06	0.01	0.21	0.01	0.04	-0.01	-0.01	0.00	0.10	0.00	0.00	0.00
X1X2	0.00	0.00	0.01*	0.00	0.00	0.00	0.00	0.00	0.03	-0.01	0.01	0.00	0.03	-0.01	0.01	0.01	0.00	0.00	0.02	0.00	0.00	0.00
X1X3	0.00	0.00	0.00	0.00	0.00	0.01**	0.05	0.08	0.20	0.27*	0.21	0.35	0.33	0.43	0.04	0.10*	0.00	-0.01	0.07	0.12*	0.00	0.00
$X2^2$	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
X2X3	0.00	0.00	0.00	0.00	0.00	0.01*	-0.01	0.00	0.00	0.04*	-0.01	0.02	0.00	0.05	0.00	0.01	0.00	0.01*	0.00	0.02*	0.00	0.01**
$X3^2$	0.03	0.01	0.02	0.02	0.01	0.01	-0.10	-0.06	-0.18	0.85**	-0.45	-0.30	-0.17	0.91	-0.01	0.07	-0.08	0.04	-0.16	0.49**	-0.01	0.01
R^2	0.84	0.66	0.88	0.79	0.73	0.98	0.87	0.84	0.83	0.96	0.86	0.80	0.87	0.93	0.84	0.99	0.44	0.80	0.80	0.96	0.41	0.91

Significance: * ($0.01<p\text{-value}<0.05$), ** ($0.001<p\text{-value}<0.01$); *** ($p\text{-value}<0.001$).

3.2 Effects of the variables of extrusion treatment on the antioxidant activity and the total phenolic content

In this section, the effect of water content, temperature, type and concentration of alkali were evaluated on the functional characteristics of the developed powders. The results are shown in Figure 9.

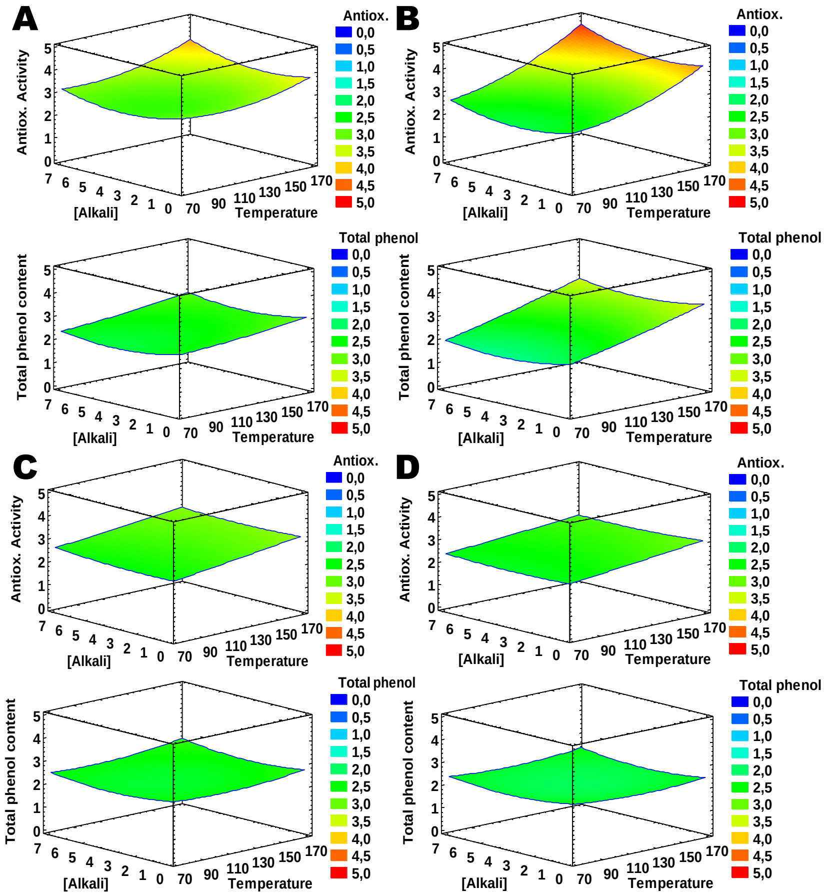

Figure 9. Surface responses of the effect of the temperature, the concentration and type of alkali, and the water content on the antioxidant activity and the total phenolic content. (A) Surface responses of the samples treated with K_2CO_3 and 20% of water content, (B) with K_2CO_3 and 30% of water content, (C) with NaOH and 20% of water content and (D) with NaOH and 30% of water content.

In general, the antioxidant activity and the total phenolic content are reduced by extrusion. In the non-treated cocoa, the antioxidant capacity and total phenolic content are 4.7±0.2 and 4.4±0.3 g/100g, while, in the extruded ones, they are under 3 at soft extrusion conditions without alkali (20% water content and 75°C). This points that extrusion, in the absence of alkali, reduces the previous features in comparison to non-treated cocoa.

In relation to K_2CO_3 (Figure 9A and B), the use of this alkali leads to increases in the antioxidant activity and the total phenolic content as the temperature is being raised. In addition, the increase in the water content combined with high temperatures leads to higher total phenolic contents. For example, when the samples are treated with 6% of K_2CO_3 at 160°C, the total phenolic content is increased from 2.7 to 3.3 g/100g as the water content is raised. This means that, when working with K_2CO_3, high water contents and high temperatures will be desirable for obtaining the best antioxidant activity and total phenol content.

In relation to NaOH (Figure 9C and D), the use of this alkali does not produce any effect on the antioxidant activity. In the case of the total phenol content, a slight significant reduction from 2.6 to 2.2 mg/100g is observed when the water content is increased in samples treated at 160°C with 6% of NaOH. This means that the use of high water contents might be avoided in terms of preserving the functional properties.

3.3. Effect of the variables of the extrusion treatment on catechin, epicatechin and their oligomers

In addition to the overview provided by the total phenolic content and the antioxidant activity, the concentrations of catechin and epicatechin, the two main cocoa polyphenols, and their oligomers were analyzed. The results are shown in Figure 10.

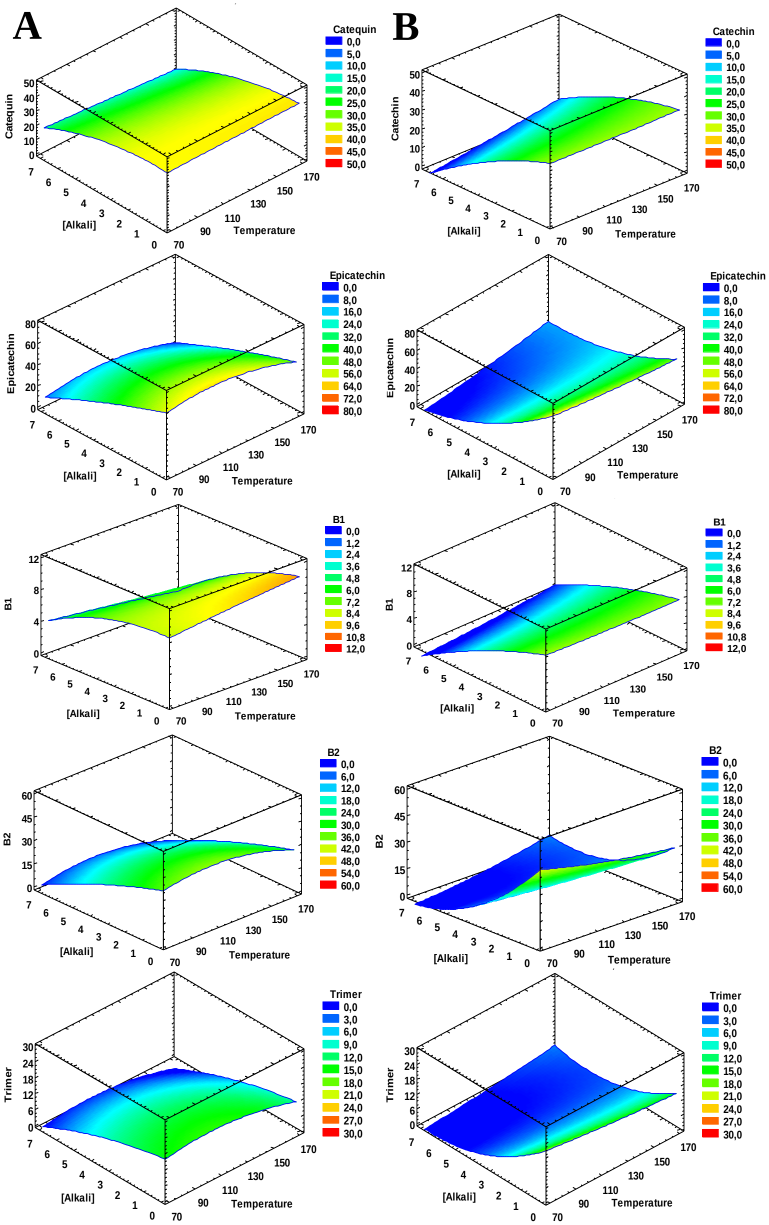

Figure 10. Effects of the concentration of alkali and temperature on catechin, epicatechin and their oligomers. (A) Samples treated with K_2CO_3 and 20% of water. (B) Samples treated with NaOH and 20% of water.

In order to stablish the effect of the processing variables, the raw material was characterized. In general, as other authors have previously reported, extrusion leads to losses of polyphenols (Sharma et al., 2016). For example, the concentrations of catechin, epicatechin, dimers B1 and B2, trimer C1 and the tetramer A2 in non-treated cocoa are 36±1, 70±3, 7.2±0.5, 41±2, 22±1 and 12.3±4 mg/100g, respectively, while, at soft conditions (0.28% of alkali, 20% of water content and 70°C), they are 37±2, 56±3, 8.4±0.4, 34±3 and 14.5±0.6 mg/100g with K_2CO_3, and 31±1, 57±5, 7.4±0.1, 38±3 and 17±1 with NaOH, respectively, not being the tetramer detected in any situation. As can be observed, the concentrations of epicatechin and trimer are slightly reduced at soft conditions and the tetramer reduced to undetected levels.

In terms of K_2CO_3, Figure 10A shows the effects of this alkali on catechin, epicatechin and its oligomers. In general, all of them are significantly reduced as the concentration of alkali is increased. In addition, the compounds share the same degradation patterns, which was expectable since all the compounds showed in Figure 10 are catechin, epicatechin or combinations of both.

As an example of the degradations induced by an increase in the K_2CO_3 concentration, catechin and epicatechin are seen to change from 37 and 56 to 23 and 22 mg/100g, respectively, when alkali was increased from 0.28 to 6%. In addition to their reductions, it can be seen that catechin is degraded a 37% and epicatechin a 60%. These results are in agreement with other authors in two facts: (1) cocoa alkalization leads to the general degradation of polyphenols (Gültekin-Özgüven et al., 2016; Miller et al., 2008; Gu et al., 2006; Jolić et al., 2011; Zhu et al., 2002) and (2) that (-)-epicatechin is more sensible to alkalization than (+)-catechin (Gültekin-Özgüven et al., 2016; Andres-LaCueva et al., 2008).

In addition to K_2CO_3, the effects of NaOH as an alkali agent were tested (Figures 10B). For example, when cocoa is treated with 6% of alkali and 25% water content at 105°C, the concentrations of catechin and epicatechin are 21 and 22 mg/100g for K_2CO_3, and 8 and 4 mg/100g for NaOH, respectively. In general, treating with NaOH produces a similar trend that the observed with K_2CO_3, although the losses were higher with NaOH for all the compounds.

Then, using NaOH will be unadvised in alkalization if the functional features of darkened cocoas want to be maximized.

The ability of NaOH to reach higher degradations in comparison to K_2CO_3 is based on its capacity for producing a higher increase of the pH. During alkalization, the generation of an alkaline media enhances several chemical processes as the monomerization of the polymers, the oxidation and chemical rearrangement of catechin and epicatechin (Gültekin-Özgüven et al., 2016; Hurst et al., 2011; Lacueva et al., 2008; Jolić et al., 2011), and other kind of reactions such as their non-enzymatic glycosylation and their interaction with Maillard reaction products (Stark and Hofmaan., 2006; Totlani and Peterson, 2005, 2007; Zhang et al., 2014). Reaching higher pH values can promote all these reactions and lead to higher reductions of the polyphenol content, which is what happens with NaOH in comparison to K_2CO_3.

Furthermore, apart from the concentration of alkali, other variables have exhibited an effect on the concentration of dimer B2 and trimer C1. Both compounds exhibited a similar behavior, being their concentrations reduced at low NaOH concentrations as the water content was increased. As an example, dimer B2 in samples treated at 75°C with 0.28% of NaOH is decreased from 38.3 to 28.7 mg/100g as the water content is increased from 20 to 30%. In addition, both compounds are increased by raising the temperature at high alkali concentrations. For example, in samples treated with 20% of water content and 6% of alkali, the content in dimer B2 changes from 0 to 3.9 mg/100g as the temperature increased from 75 to 160°C.

All the degradations observed in this section are in contradiction with the reported evolution of the antioxidant activity and total phenol content (Figure 9). These two features are increased or maintained at high temperatures and alkali concentrations, while catechin, epicatechin and their oligomers see their concentration significantly decreased at those strong conditions (Figure 10). At this respect, it is important to point that although the two main polyphenols in cocoa see their concentrations reduced, others could be released and formed as a consequence of the alkalization treatment. On the one hand, several researchers have reported that in food matrixes there are two fractions of polyphenols: the free and normally analyzed ones, and the non-extractable fraction, which is formed by polyphenols link to other molecules or cellular structures. The non-extractable group has been found to be higher than the extractable one in different matrices and also to be released by some treatments such as the use of NaOH during alkalization (Gonzales et al., 2015; Domínguez-Rodríguez et al., 2017). Taking that into account, it could be possible that extrusion, combined with the employed alkalis, could be able to release them. On the other hand, the formation of new compounds could be also responsible of the maintenance in the antioxidant activity and the total phenol content. As an example, several authors have reported that at the same time that (-)-epicatechin and (+)-catechin are degraded by cocoa alkalization, (-)-catechin is formed (Gültekin-Özgüven et al., 2016; Hurst et al., 2011; Kofink et al., 2007; Ortega et al, 2008). The increase in this compound, as well as of other ones, could be responsible of the observed maintenance of the mentioned features.

3.4. Effect of the variables of the extrusion treatment on other polyphenols

In addition to catechin, epicatechin and its oligomers, the effects of the treatment on other polyphenols (clovamide, hyperoxide, vitexin and avicularin) were evaluated. These compounds were selected for their different functional effects and have been divided into two groups according to their shared behaviors: one composed by avicularin and clovamide, and a second one by vitexin and hyperoside. Figure 11 shows the evolution of the previous compounds as an effect of the extrusion alkalization treatment.

With respect to the first group, it has to be said that avicularin (or quercetin-3-α-L-arabinofuranoside) is a plant flavonoid and quercetin derivative that has been reported to have anti-inflammatory, anti-allergic, antioxidant, anti-tumor and hepatoprotective effects (Vo et al., 2012), and that clovamide (or N-caffeoyl-L-dihydroxyphenyl-alanine) is a polyphenol-amino acid conjugate that has been reported to have anti-inflammatory, antioxidant, neuroprotective and anti-Alzheimer’s disease effects (Bouchez et al., 2019).

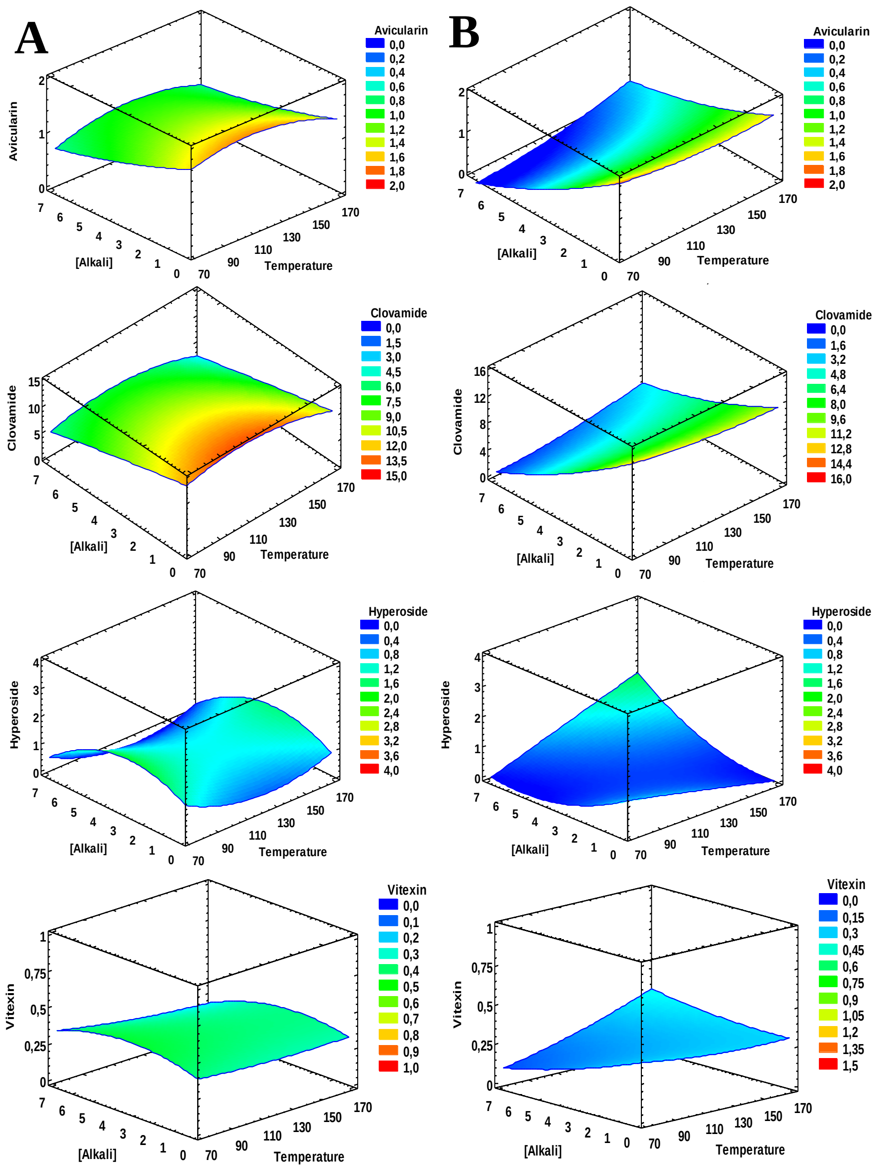

Figure 11. Effects of the concentration of alkali and temperature on vitexin, avicularin, hyperoside and clovamide. (A) Samples treated with K_2CO_3 and 20% of water. (B) Samples treated with NaOH and 20% of water.

Both compounds are, in general, in higher concentrations in extruded cocoas than in the untreated one. As an example, in non-treated cocoas, the concentrations of avicularin and clovamide are 1.32±0.06 and 10±1 mg/100g, while, in soft extruded cocoas (0.28% of alkali, 75°C, 20% of water content), the mean concentration values of these compounds are 1.6±0.1 and 12.3±0.9 mg/100g. This shows that extrusion, in the absence or with low amounts of alkali, can have a positive impact on cocoa functionality.

On the other hand, when the samples are alkalized (Figure 11A and B), the concentrations of avicularin and clovamide significantly decrease as the concentration of alkali is being raised. For example, in cocoas treated at 75°C and with a 20% of water content, avincularin and clovamide are reduced from 1.6 and 13.6 to 0.8 and 7.4 mg/100g as the concentration of K_2CO_3 is increased from 0.28 to 6%. This behavior is similar to the observed with catechin and its oligomers. In addition, the treatment with NaOH is more aggressive and leads to higher reductions than the one with K_2CO_3.

Apart from the alkali concentration, clovamide is also positively influenced by the temperature in samples treated with K_2CO_3. On the other hand, in cocoas treated with NaOH, avicularin is positively affected by the combinations of alkali-water content and temperature-alkali, and clovamide of alkali-water content.

With respect to the second group of compounds, vitexin (or apigenin-8-C-glucoside) is a c-glycosilated flavone that has a large number of pharmacological activities (anti-cancer, anti-Alzheimer disease, anti-hypertensive, anti-spasmodic, anti-depressant, antioxidant, anxiolytic effects, anti-inflammatory and anti-nociceptive activities, among others) (He et al., 2016) and hyperoside/hyperin (or quercetin-3-O-galactoside) is a type of flavonoid have been documented to have anti-inflammatory, anti-nociceptive, cardioprotective, hepatoprotective and gastrimucosal-protective effects (Verma et al., 2013).

Hyperoside and vitexin are both, generally, in lower concentrations in extruded cocoas than in the untreated one. For example, in non-treated cocoas, hyperoside and vitexin concentrations are 4.4±0.1 and 0.7±0.05 mg/100g, while in softly extruded samples (0.28% of alkali, 75°C, 20% of water content), they have a mean concentration of 0.95±0.07 and 0.35±0.08 mg/100g, respectively.

When cocoa is alkalized by extrusion (Figure 11A and B), vitexin and hyperoside exhibit a different behavior depending of the alkali that is employed. On the one hand, in samples treated with K_2CO_3, the concentrations of both compounds increase at medium alkali concentrations (3.5%). On the other hand, in cocoas treated with NaOH, both are increased as the temperature and the alkali concentration are risen. The observed shared behavior of these molecules suggests a common synthetic pathway, because their surface responses have a similar trend and, at the same time, one that totally differs from the exhibit by the other polyphenols studied in this work.

Furthermore, in respect to the modeling of these molecules, the statistical analyses showed that any variable have a significant effect on the hyperoside and vitexin concentrations when they are treated with K_2CO_3 and, also, that the R^2 of the models are low (Table 6). As was said in that section, it seems that the program could be unable to model the behavior of these parameters possibly due to their anomalous distributions. But, despite of that, if the response surfaces are analyzed (Figure 11A), it can be seen a clear increment at concentrations of 3.5% of K_2CO_3.

From the analysis of the effects of the treatment variables on cocoa, it can be concluded that the alkali concentration and type are the main variables negatively affecting the concentration of the different polyphenols. The water content and the temperature were also important, but in a lesser extent. From all the compounds, hyperoside and vitexin are the only ones increasing due to the alkalization treatment. This suggests that the release or formation of other polyphenols like these two could be after the observed maintenance and increase of the antioxidant activity and the total phenol content. Moreover, these results make necessary a deep analysis of the polyphenol profile for identifying the compounds whose concentration in being increased and for understanding the real functional importance of alkalized cocoa.

3.5. Comparison to commercial samples

3.5.1. Functional comparison

After the evaluation of how the different variables of extrusion alkalization affected the functional features of cocoa, the produced samples were compared to a set of commercial powders for studying the suitability of the new alkalizing method.

For the comparison study, cocoas with the darkest colors belonging to each alkalization level were selected. The results are shown in Figures 12, 13 and 14.

In the case of the antioxidant activity (Figure 12A), any difference is found at natural and slight alkalization levels between the extruded and traditionally produced cocoas, while, at the medium and strong ones, the extruded samples show to have a higher antioxidant activity.

With respect to the total polyphenol content (Figure 12B), any significant difference is found between the samples belonging to the different alkalization levels, with the exception of the slightly alkalized cocoas. This indicates that extrusion, despite of being reported to produce important losses in the total phenol content (Sharma et al., 2016), is generating similar losses than the conventional alkalization method.

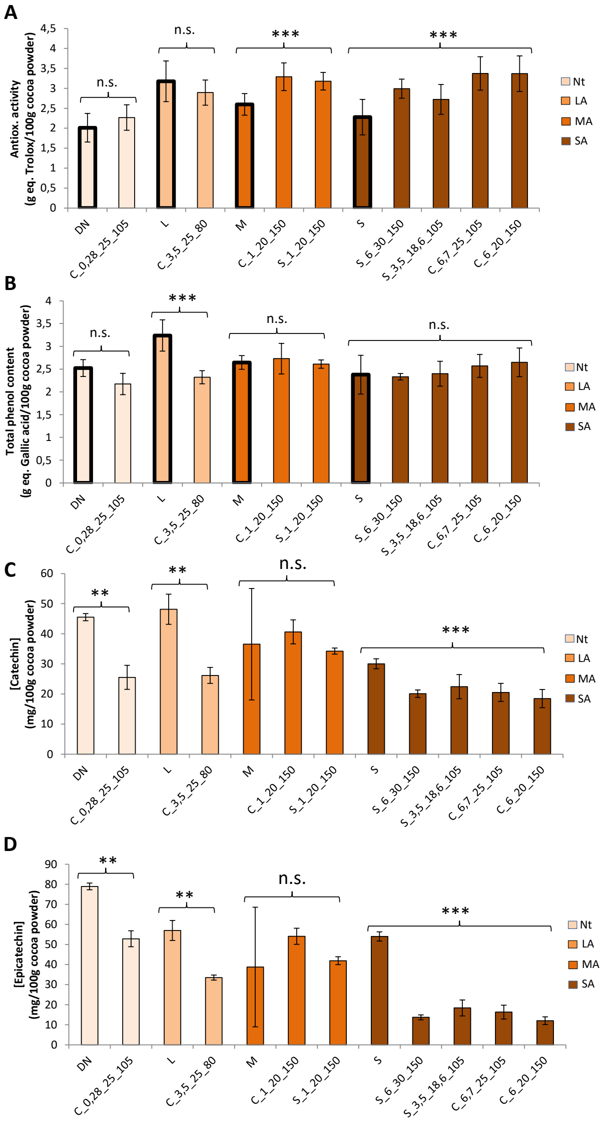

Figure 12. Comparison of the selected samples to the commercial cocoas in their functional characteristics. (A) Antioxidant activity, (B) total phenol content, (C) concentration of catechin and (D) epicatechin. Cocoas are colored according to their level of alkalization: natural (Nt), lightly (LA), moderately (MA) and strongly alkalized (SA). The commercial samples are surrounded by a black line and are: dark natural (DN), lightly (L), moderately (M) and strongly alkalized (S). Significance: non-significant (n.s.), * (0.01<p-value<0.05), ** (0.001<p-value<0.01) and *** (p-value<0.001).

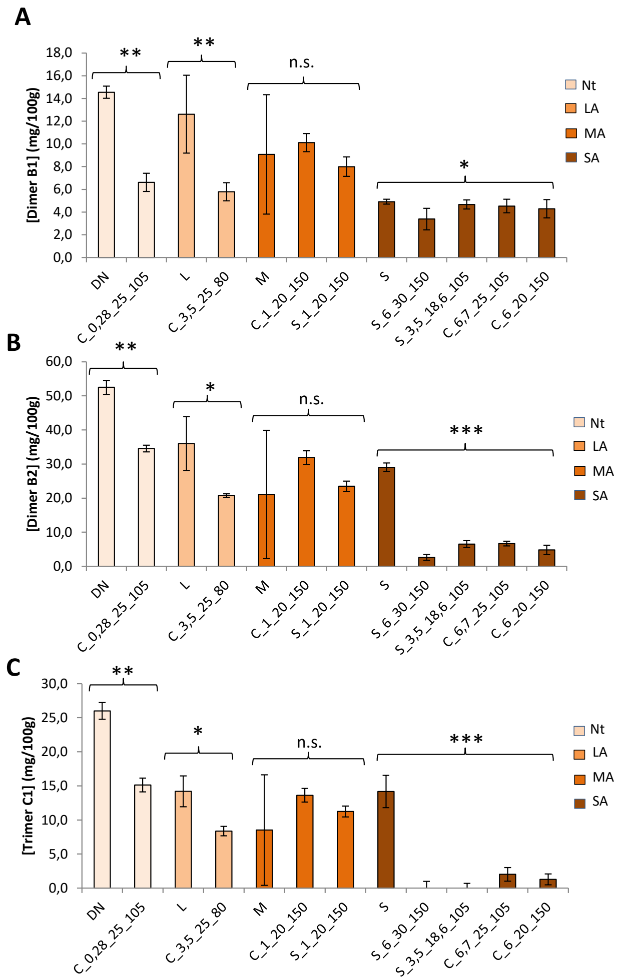

Figure 13. Comparison of the selected samples to the commercial cocoas in the other analyzed polyphenols. (A) Dimer B1, (B) dimer B2 and (C) trimer C1. Cocoas are colored according to their level of alkalization: natural (Nt), lightly (LA), moderately (MA) and strongly alkalized (SA). The commercial samples are surrounded by a black line and are: dark natural (DN), lightly (L), moderately (M) and strongly alkalized (S). Significance between commercial and extruded cocoas in each group: non-significant (n.s.), * (0.01<p-value<0.05), ** (0.001<p-value<0.01) and *** (p-value<0.001)

Regarding catechin and epicatechin content (Figure 12C and D), in almost all the alkalization levels, the extruded samples have lower catechin and epicatechin concentrations than the conventionally alkalized powders, which shows extrusion as a most aggressive technique. Furthermore, if the concentration of the oligomers of catechin and epicatechin are studied, it will be seen that they are reduced as the alkalization level increase and that their concentrations are generally lower in comparison to the exhibited by the commercial cocoas (Figure 13).

Finally, the evolution of the other four analyzed polyphenols (avicularin, clovamide, hyperoside and vitexin) was studied and its results are shown in Figure 14. An important variability is observed in some commercial samples, but it has to be remembered that this values are obtained by averaging different traditionally produced cocoas belonging to that group of alkalization.

In general, three of the polyphenols (avicularin, hyperoside and vitexin) have a lower (or similar) concentration in extruded samples than in traditionally alkalized ones. Clovamide (Figure 14B) is the only polyphenol whose concentration is higher in extruded cocoas than in the commercial ones. This molecule is an example of a polyphenol that increases through extrusion and that explains the higher antioxidant activity and similar total phenol content observed between the extruded and the commercial cocoas despite of the general reduction in concentration of catechin and its oligomers (Figure 12A and B).

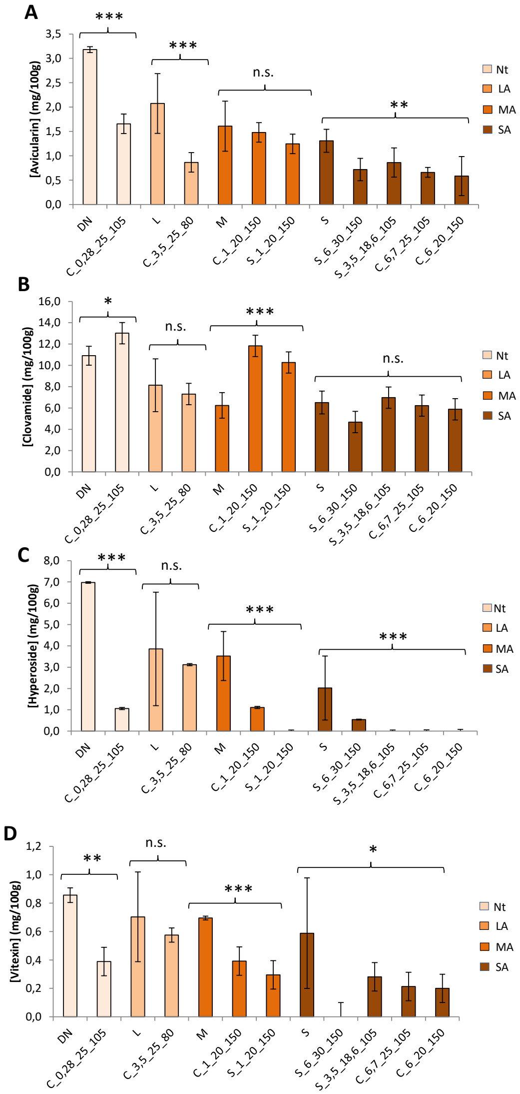

Figure 14. Comparison of the selected samples to the commercial cocoas in the other analyzed polyphenols. (A) Avicularin, (B) clovamide, (C) hyperoside and (D) vitexin. Cocoas are colored according to their level of alkalization: natural (Nt), lightly (LA), moderately (MA) and strongly alkalized (SA). The commercial samples are surrounded by a black line and are: dark natural (DN), lightly (L), moderately (M) and strongly alkalized (S). Significance between commercial and extruded cocoas in each group: non-significant (n.s.), * (0.01<p-value<0.05), ** (0.001<p-value<0.01) and *** (p-value<0.001).

4. Conclusions

The present work has analyzed and characterized the effects of extrusion alkalization on the functional features of cocoa.

From all the evaluated variables of the extrusion alkalization method, alkali type and concentration are the ones mainly reducing the concentration of all the studied polyphenols. In the case of the antioxidant activity and the total phenol content, both features remain mostly unchanged and even increased after alkalization, which could be related to the release and formation of new polyphenols such as hyperoside and vitexin.

With respect to the comparison between extrusion and the alkalization treatment, extrusion has shown to not improve the functional characteristics of cocoa, although its fast speed, continuous fashion treatment and less energy-consumption make this alkalization method an interesting replacement of the traditional one.

Acknowledgments

Damián Valverde would like to thank the Regional Ministry of Education, Culture and Sports of Generalitat Valenciana for his PhD grant.

Funding

This work was funded by the Spanish Government and European Regional Development Fund (Project RTC-2016-5241-2).

References

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

Aprotosoaie, A. C., Luca, S. V., & Miron, A. (2015). 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

Arranz, S., Saura-Calixto, F., Shaha, S., & Kroon, P. A. (2009). High Contents of Nonextractable Polyphenols in Fruits Suggest That Polyphenol Contents of Plant Foods Have Been Underestimated. Journal of Agricultural and Food Chemistry, 57 (16), 7298-7303. https://doi.org/10.1021/jf9016652

Bouchez, P., Benites, V. T., Baidoo, E. E. K., Mortimer, J. C. , Sullivan, M. L., Scheller, H. V., & Eudes, A. (2019). Production of clovamide and its analogues in Saccharomyces cerevisiae and Lactococcus lactis. Letters in Applied Microbiology, 69 (3), 181-189. https://doi.org/10.1111/lam.13190

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

Chalin, M. L. (1974). Method of dutching cocoa. US3868469A.

D'Souza, R. N., Grimbs, S., Behrends, B., Bernaert, H., Ullrich, M. S., & Kuhnert, N. (2017). Origin-based polyphenolic fingerprinting of *Theobroma cacao* in unfermented and fermented beans. Food Research International, 99, 550-559. https://doi.org/10.1016/j.foodres.2017.06.007

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

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. https://doi.org/10.1016/j.chroma.2017.07.066

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.

Gonzales, G. B., Raes, K., Vanhoutte, H., Coelus, S., Smagghe, G., & Camp J. V. (2015). Liquid chromatography–mass spectrometry coupled with multivariate analysis for the characterization and discrimination of extractable and nonextractable polyphenols and glucosinolates from red cabbage and Brussels sprout waste streams. Journal of Chromatography A, 1402, 60-70. https://doi.org/10.1016/j.chroma.2015.05.009

Gu, L., House, S. E., Wu, X., Ou, B., & Prior, R. L. (2006). Procyanidin and catechin contents and anxiotidant 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

He, M., Min, J. W., Kong, W. L., He, X. H., Li, J. X., Peng, B. W. (2016). A review on the pharmacological effects of vitexin and isovitexin. Fitoterapia, 115, 74-85. https://doi.org/10.1016/j.fitote.2016.09.011

Hellström, J. K., & Mattila, P. H. (2008). HPLC Determination of Extractable and Unextractable Proanthocyanidins in Plant Materials. Journal of Agricultural and Food Chemistry, 56 (17), 7617-7624. https://doi.org/10.1021/jf801336s

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 (7), 1274-1288. https://doi.org/10.3390/12071274

Kopp, G. M., Hennen, J. C., Seyller, M., & Brandstetter, B. (2009). Process for producing high flavour cocoa. EP2241190B1.

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

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

Miller, K. B., Hurst, W. F., 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

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

Sharma, P., Ramchiary, M., Samyor, D., & Das, A. B. (2016). Study of the phytochemical properties of pineapple fruit leather processed by extrusion cooking. Food Science and Technology, 72, 534-543. https://doi.org/10.1016/j.lwt.2016.05.001

Stark, T., & Hofmaan, 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

Todorovic, V., Redovnikovic, I. R., Todorovic, Z., Jankovic, G., Dodevska, M., & Sobajic, S. (2015). Polyphenols, methylxanthines, and antioxidant capacity of chocolates produced in Serbia. Journal of Food Composition and Analysis, 41, 137-143. https://doi.org/10.1016/j.jfca.2015.01.018

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

Verma, N., Amresh, G., Sahu, P. K., Mishra, N., Rao, C. V., & Singh, A. P. (2013). Pharmacological evaluation of hyperin for antihyperglycemic activity and effect on lipid profile in diabetic rats. Indian Journal of Experimental Biology, 51, 65-72.

Vo, V. A., Lee, J. W., Chang, J. E., Kim, J. Y., Kim, N. H., Lee, H. J., Kim, S. S., Chun, W. & Kwon, Y. S. (2012). Avicularin inhibits lipopolysaccharide-induced inflammatory response by suppressing ERK phosphorylation in RAW 264.7 macrophages. Biomolecules & Therapeutics, 20 (6), 532-537. https://dx.doi.org/10.4062%2Fbiomolther.2012.20.6.532

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

Wissgott, U. (1985). Method of alkalization of cocoa in the aqueous phase. CH663324A5

Zhang, B., Liu, G., Ying, D., Sanguansri, L., & Augustin, M. A. (2017). Effect of extrusion conditions on the physico-chemical properties and *in vitro* protein digestibility of canola meal. Food Research International, 100, 658-664. https://doi.org/10.1016/j.foodres.2017.07.060

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

5. CHAPTER II

Microwave heating

5.1. Introduction to microwave heating

1. Microwave heating: a general view

Microwaves are a type of electromagnetic radiation with a wavelength between 300 MHz and 300 GHz. Their ability for heating lies in the interaction of this energy with the ions and polar molecules present in the matrix. Specifically, microwaves lead to the continuous reorientation and collision of the ions and polar compounds, which cause the heating of the material. This heating is followed by the generation of a vapor gradient that goes from the inside to the outside of the treated mass, drying it (Khan et al., 2018; Chandrasekaran, Ramanathan and Basak, 2013).

Microwave heating as an alternative technique for cooking food has become popular in the last decades due to its several advantages: it has a higher penetration power that reduces the processing time, it is easier to use and safer than other heating techniques, it needs low maintenance and it is better than conventional heating techniques for preserving the nutritional, functional and sensory properties of the product (Vadivambal and Jayas, 2007; Khan et al., 2018; Chandrasekaran, Ramanathan and Basak, 2013).

In the food area, microwave heating has been applied to dry, pasteurize, sterilize, temper, thaw and bake foods, among other applications. Moreover, despite the different possible applications and its advantages, this technology is not frequently used in industrial processes due to technical and economic issues related to the non-uniform distribution of the temperature. As an example, in order to assure an optimal heat transfer of a concrete material, previous experimental information about the microwave heating profile of a concrete food product and the suitable knowledge to technically reduce the heating issues associated to microwave are needed (Vadivambal and Jayas, 2007; Khan et al., 2018; Chandrasekaran, Ramanathan and Basak, 2013).

Despite these disadvantages, microwave heating is an exceptional tool with a great versatility in its use and with the capacity of preserving the nutritional, functional and sensory characteristics of the material under treatment. These properties have promoted the research about its impact on different features of food, which will be overviewed in the next sections.

2. Effect of microwave heating treatment on the composition and sensory characteristics of food

Several researchers have analyzed the effects of microwave heating on the color, texture, flavor, the different macromolecules and polyphenols of different food matrices (grape seeds, herring, broccoli, silver catfish, hazelnuts, barley, banana chips, chocolate, green coconut water, roasted peanuts, apple juice…). This section focuses in giving an overview of the compositional and sensory changes of the different products during the microwave heating treatment.

2.1. Effect of microwave heating on the food composition

From a compositional point of view, microwave heating has been reported to increase starch and protein digestibility. Negi, Boora and Khetarpaul (2001) studied the effects of microwave cooking on the digestibility of these macromolecules in moth beans. The authors observed an important rise in protein and starch digestion. They related these increases to the ability of the heat treatment to gelatinize starches, to swell and rupture starch granules, to denaturalize proteins and to destroy anti-nutritional factors, effects that increase the activity of digestive enzymes and the availability and accessibility of proteins and starches.

In terms of the lipid content, Weber et al (2008), Türkkan, Cakli and Kilinc (2008) and Regulska-Ilow and Ilow (2002) have studied the changes of the lipid profile induced by several cooking methods in hearing, seabass and silver catfish. In their work, Weber et al (2008) observed that microwave did not change the total fatty acid and polyunsaturated fatty acid contents. These authors also study the evolution of different oxidation markers (conjugated dienes, thiobarbituric acid reactive substances (TBARS), free fatty acids and peroxides) and found a significant reduction of the free fatty acids and increment of the TBARS value in microwave heated samples, which indicate an increase of the lipid oxidation reactions due to this treatment. In the case of Regulska-Ilow and Ilow (2002), they came to the same conclusion that Weber's group. They observed that the polyunsaturated fat content and the oxidation quality indicators (peroxide and anisidine values) of hearing were not modify by the microwave thermal treatment. Finally, Türkkan, Cakli and Kilinc (2008) applied different cooking methods to seabass with the aim of studying the differences in proximate and fatty acid composition. They found general reductions of total and monounsaturated fatty acids, the maintenance of n-6 fatty acids and the increases of polysaturated and n-3 fatty acids in comparison to raw seabass. The differences observed among these authors and the previous ones in the changes in the lipid content could be related to the raw material and the different treatment conditions.

Other researchers (Uquiche, Jeréz and Ortíz, 2008) employed the microwave technique as a pretreatment for oil extraction from Chilean hazelnut. They discovered that microwave increased the extraction oil yield and quality. In addition, they observed a higher protection against oxidation when the microwave was applied, which the authors related to the inactivation of oxidative enzymes.

Microwave has been also documented to enhance the formation of advanced glycation end-products in comparison to conventional heating, although it has being also reported to reduce the formation of these compounds. The so-called advanced glycation end-products (name given by medical researchers) or Maillard reaction products (name given by food researchers) are molecules formed by the interaction of amino acids (especially lysine) and sugars, and contribute to important sensory characteristics (flavor, taste and color). Despite these desired effects, these compounds have been related to inflammation and several diseases (Wei, Liu and Sun, 2018). With respect to the formation of the Maillard reaction products, Fu, Li and Li (2012) made a comparison of the formation capacity between different thermal methods and found that microwave was the technique that achieved the highest losses of lysine out of water heating, drying oven heating and microwave heating. This points that microwave heating is decreasing the nutritional quality of the product by reducing the available content in amino acids and sugars.

In addition, as was mentioned previously in this section, microwave heating has been reported to inactivate anti-nutritional factors in food matrices. Most of the enzymes are heat sensible, which makes the different thermal treatments able to inactivate them. Polyphenol oxidase has been reported to be destroyed with temperatures of 50°C. Sharma and Gujral (2011) applied the microwave technology to cook barley and observed a reduction in the polyphenol oxidase ranging from 45% to 77%. Huang et al (2007) reported that the microwave treatment inactivated enzymes present in tea, leading to the maintenance of the functional molecules though storage. In this comparison study of the microwave and oven heating techniques, a control sample was not included, which limits the conclusions that can be drawn. Matsui et al (2007) studied the changes in activity of anti-nutritional factors such as peroxidase and polyphenol oxidase after the microwave treatment of green coconut water. They found the microwave to be an interesting approach for the inactivation of these enzymes.

Apart from enzymes, there are other types of anti-nutritional factors that have been reported to be affected by microwave heating. In this respect, Khattab and Arntfield (2009) and Hefnawy (2011) showed microwave cooking as a technique able to destroy tannins (compounds that inhibit enzymes that especially digest proteins and carbohydrates), phytic acid (an anti-nutrient that reduces the bioavailability of minerals, proteins and carbohydrates by binding to them), trypsin inhibitors (that reduce the protein digestibility) and flatulence-causing oligosaccharides such as raffinose, stachyose and verbascose. In their work, Khattab and Arntfield (2009) concluded that all thermal treatments are useful for decreasing the anti-nutritional compounds of different kinds of beans, although boiling, microwave cooking and autoclaving demonstrated to be the most effective ones.

In terms of polyphenol content, the microwave technology has shown to be less aggressive than other methods and to be an interesting tool for the extraction of polyphenols. An important number of publications can be found in literature employing microwaves for the extraction of polyphenols from different matrices (Pan, Niu and Liu, 2003; Dahmoune et al., 2015; Li et al., 2011; Bai et al., 2010; Du et al., 2009; Nkhili el al., 2009; Bouras et al., 2015). At this respect, Hayat et al (2010) studied the release of non-extractable polyphenols from citrus mandarin peels by microwave heating. They observed that microwave was able to release bound polyphenols in a simple and fast fashion, increasing total phenol content and antioxidant activity. López-Brenguer et al (2007) studied the effects of microwave cooking conditions on the bioactive compounds of broccoli. They cooked the broccoli immersed in water, detected a reduction in total phenol content after a microwave treatment and found polyphenols in the media. The authors concluded that their reported losses in phenolic compounds were due to the leaching of polyphenols into the cooking water, which could have been avoided by cooking without water.

As in other thermal treatments, the application of the microwave technology is able to effectively inactivate bacteria in food matrixes. Siguemoto et al (2018) compared the inactivation effect of microwave and conventional thermal pasteurizing methods over *Escherichia coli* O157:H7 and *Listeria monocytogenes*. The authors concluded that both treatments were able to reach the maximum allowed microbial concentration recommended by the FDA, which presents the microwave thermal heating as a promising alternative for pasteurization of apple juices.

In addition to the bacteria, the effects of microwave heating on the mycotoxins produced by molds have been studied. Herzallah, Alshawabkeh and Fataftah (2008) evaluated the effect of sunlight exposition, γ-radiation and microwave heating on artificially contaminated feeds. The authors observed that their microwave treatment was able to reduce the total aflatoxin and aflatoxin B1 contents, although they found that microwave was the least effective method. The researchers explained this lower effect by the low heating time that they employed in comparison to the other techniques and also to the geometrical dimension of the feed. In other work, Mobeen et al (2011) evaluated the effectiveness of microwave heating to destroy the aflatoxin present in peanuts and derived products. The authors found a 60% reduction of B1 and a 100% one in the case of aflatoxin B2, showing that the microwave technology is able to decontaminated food.

2.2. Effect of microwave heating on the sensory features of food

From the sensory characteristics, texture is one of the most affected ones. Microwave heating is known to generate a vapor gradient that goes from the inner parts of the material to the surface, drying it and leading to undesirable changes such as toughness, texture disruption and sogginess. These modifications of the product happen when it is taken to a boiling state. For example, the moisture loss generates a crispy feeling inside the product, which is a positive characteristic, but it also produces an undesirable sogginess due to the displacement of vapor from the core to the surface. In addition, in foods with low content of starches, the reduction in the moisture content has been associated with toughness. In general, in products with a minimum content of 37% of starches, it has been observed that the microwave treatment is responsible for the increased toughness of bakery products, due to changes in the solubility of amylose, its dispersion and crystallization during cooling (Mizrahi, 2012).

Volatile compounds related to flavor have been also reported to be affected by the microwave thermal treatment. In general, it is considered that microwave heating is able to degrade low molecular volatile compounds, to evaporate the high water solubility volatile ones and to enhance the lipid oxidation and the generation of its associated flavor molecules. In addition, microwave thermal treatment has been associated to the apparition of off-flavor compounds in avocado, peanuts and banana chips (Guzmán-Gerónimo, López and Dorantes-Alvarez, 2008; Mui, Durance and Scaman, 2002; Schirack et al., 2006).

In terms of color, microwave has shown to not be able to reach the superficial temperature needed for browning reactions. This happens because, although the food under treatment is being heated, the air surrounding it is cold, which induces water condensation and cooling of the surface. İçöz, Sumnu and Sahin (2004) compared the color changes in a bakery product treated by microwave, by microwave plus susceptors and by a conventional oven. The authors concluded that the microwave by itself was not able to dark the samples, while the microwave plus susceptors and the conventional oven were.

3. Microwave heating and cocoa

Different authors have focused their efforts in studying the effects of microwave treatment on the production and analysis of cocoa, although none of them have applied this technology on the alkalization of this food material.

Among the applications that have been described, microwave has been employed for digestions, polyphenol extractions, drying, roasting, increasing the moistening and the inactivation of enzymes.

Digestions are an important step to eliminate macromolecules from a matrix that will be analyzed in its content in heavy metals (cadmium, arsenic). Hartwig et al (2016) describes a procedure to combine the microwave technology with UV radiation for the digestion of cocoa and its further analysis by ICP-MS. Other authors have employed the microwave digestion as a preliminary step before the analysis of heavy metals in cocoa and chocolate (Kruszewski, Obiedziński and Kowalska, 2018; Lo Dico et al., 2018).

Some works have employed the microwave technology to polyphenol and fat extraction from cocoa samples. The microwave assisted extraction has different advantages that the conventional methods do not have: it depends on electric energy, which makes the process automatic; there is not a direct contact between the samples and the heat source, which makes the heating more effective and specific; it allows to have a safer working place; is does not need solvents; it reaches high potencies; and it is a relatively fast system (Irina and Mohamed, 2012; Vinatoru, Mason and Calinescu, 2017). These advantages have attracted the attention of several authors, which have developed quick and high efficiency methods for the extraction of theobromine, caffeine and fat (González-Nuñez and Cañizares-Macías, 2011; ElKhori et al., 2007). In addition, other researchers mention in their patents the microwave technology as a mean for extracting polyphenols from low fermented nibs (Kealey et al., 1996).

Other applications of microwave to the treatment or to the analysis of cocoa have been less studied. Among them, there are works about the employment of the microwave to drying (Guda, Gadhe and Jakkula, 2017; Firihu and Sudiana, 2016; Bernaert, Camu and Lohmueller, 2008), to roasting (Rother et al., 2009), to increase the moistening of a mixture of cocoa powder and sugar (Hofstaetter and Lee, 1995), and to inactivate the polyphenol oxidase (Pons-Andreu, Cienfuegos-Jovellanos and Ibarra, 2004).

Having in mind all the described advantages of microwave heating (higher preservation of nutritional, functional and sensory characteristics of the material under treatment, chapter 2 of this thesis is focused on the designing of a novel microwave-based cocoa alkalization process, in the study of how the different process variables affect the physicochemical and functional characteristics of cocoa, and in the evaluation of the suitability of the developed microwave heating alkalization treatment as an alternative to traditional cocoa alkalization.

4. References

Bai, X. L., Yue, T. L., Yuan, Y. H., & Zhang, H. W. (2010). Optimization of microwave-assisted extraction of polyphenols from Apple pomace using response Surface methodology and HPLC analysis. Journal of Separation Science, 33, 23-24. https://doi.org/10.1002/jssc.201000430

Bernaert, H., Camu, N., & Lohmueller, T. (2008). Method for processing cocoa beans. EP2299838B1.

Bouras, M., Chadni, M., Barba, F. J., Grimi, N., Bals, O., & Vorobiev, E. Optimization of microwave-assisted extraction of polyphenols from *Quercus bark*. Industrial Crops and Products, 77, 590-601. https://doi.org/10.1016/j.indcrop.2015.09.018

Chandrasekaran, S., Ramanathan, S., & Basak, T. (2013). Microwave food processing- a review. Food Research International, 52 (1), 243-261. https://doi.org/10.1016/j.foodres.2013.02.033

Dahmoune, F., Nayak, B., Moussi, K., Remini, H., & Madani, K. (2015). Optimization of microwave-assisted extraction of polyphenols from *Myrtus communis* L. leaves. Food Chemistry, 166, 585-595. https://doi.org/10.1016/j.foodchem.2014.06.066

Du, F. Y., Xiao, X. H., Luo, X. J., & Li, G. K. Application of ionic liquids in the microwave-assisted extraction of polyphenolic compounds from medicinal plants. Talanta, 78 (3), 1177-1184. https://doi.org/10.1016/j.talanta.2009.01.040

ElKhori, S., Jocelyn Paré, J.R., Bélanger, M. R., & Pérez, E. (2007). The microwave-assisted process (MAP^{TM1}): Extraction and determination of fat from cocoa powder and cocoa nibs. Journal of Food Engineering, 79 (3), 1110-1114. https://doi.org/10.1016/j.jfoodeng.2006.01.089

Firihu, M. Z., & Sudiana, N. (2016). 2.45 GHz microwave drying of cocoa bean. ARPN Journal of Engineering and Applied Sciences, 11 (19), 11595-11598.

Fu, Q., Li, L., & Li, B. (2012). Formation of Nε-(carboxymethyl)lysine in saccharide-lysine model systems by different heat treatments. International Journal of Food Engineering, 8 (3), 1-17. https://doi.org/10.1515/1556-3758.2724

González-Nuñez, L. N., & Cañizares-Macías, M. P. (2011). Focused microwaves-assisted extraction of theobromine and caffeine from cacao. Food Chemistry, 129 (4), 1819-1824. https://doi.org/10.1016/j.foodchem.2011.05.118

Guda, P., Gadhe, S., & Jakkula, S. (2017). Drying of cocoa beans by using different techniques. International Journal of Agriculture Innovations and Research, 5 (5), 859-865.

Guzmán-Gerónimo, R. I., López, M. G., & Dorantes-Alvarez, L. (2008). Microwave processing of avocado volatile flavor profiling and olfactometry. Innovative Food Science & Emerging Technologies, 9 (4), 501-506. https://doi.org/10.1016/j.ifset.2008.05.003

Hartwig, C. A., Pereira, R. M., Rondan, F. S., Cruz, S. M., Duarte, F. A., Flores, E. M. M., & Mesko, M. F. (2016). The synergic effect of microwave and ultraviolet radiation for chocolate digestion and further determination of As, Cd, Ni and Pb by ICP-MS. Journal of Analytical Atomic Spectrometry, 31 (2), 523-530. https://doi.org/10.1039/C5JA00388A

Hayat, K., Hussain, S., Abbas, S., Farooq, U., Ding, B., Xia, S., Jia, C., Zhang, X., & Xia, W. (2009). Optimized microwave-assisted extraction of phenolic acids from citrus mandarin peels and evaluation of antioxidant activity *in vitro*. Separation and Purification Technology, 70 (1), 63-70. https://doi.org/10.1016/j.seppur.2009.08.012

Hefnawy, T. H. (2011). Effect of processing methods on nutritional composition and anti-nutritional factors in lentils (*Lens culinaris*). Annals of Agricultural Sciences, 56 (2), 57-61. https://doi.org/10.1016/j.aoas.2011.07.001

Herzallah, S., Alshawabkeh, K., & Fataftah, A. A. (2008). Aflatoxin decontamination of artificially contaminated feeds by sunlight, γ-radiation and microwave heating. The Journal of Applied Poultry Research, 17 (4), 515-521. https://doi.org/10.3382/japr.2007-00107

Hofstaetter, D., & Le, V. T. (1995). Improving wettability of powder by microwave treatment. WO1996034536A1

Huang, Y., Sheng, J., Yang, F., & Hi, Q. (2007). Effect of enzyme inactivation by microwave and oven heating on preservation quality of green tea. Journal of Food Engineering, 78 (2), 687-692. https://doi.org/10.1016/j.jfoodeng.2005.11.007

İçöz, D., Sumnu, G., & Sahin, S. (2004). Color and texture development during microwave and conventional baking of breads. International Journal of Food Properties, 7 (2), 201-213. https://doi.org/10.1081/JFP-120025396

Irina, I., & Mohamed, G. Chapter 5: Biological Activities and Effects of Food Processing on Flavonoids as Phenolic Antioxidants. Advances in Applied Biotechnology, Prof. Marian Petre (Ed.), InTech.

Kealey, K., Snyder, R., Romanczyk, L., Geyer, H., Myers, M., Whitacre, E., Hammerstone, J., Schmitz, H. (1006). Dry drink mix and chocolate flavored drink made therefrom. US20020064584A1.

Khan, M. K., Ahmad, K., Hassan, S., Imran, M., Ahmad, N., & Xu, C. (2018). Effect of novel technologies on polyphenols during food processing. Innovative Food Science & Emerging Technologies, 45, 361-381. https://doi.org/10.1016/j.ifset.2017.12.006

Khattab, R. Y., & Arntfield, S. D. (2009). Nutritional quality of legume seeds as affected by some physical treatments 2. Antinutritional factors. Food Science and Technology, 42, 1113-1118.

Kruszewski, B., Obiedziński, M. W., & Kowalska, J. (2018). Nickel, cadmium and lead levels in raw cocoa and processed chocolate mass materials from three different manufacturers. Journal of Food Composition and Analysis, 66, 127-235. https://doi.org/10.1016/j.jfca.2017.12.012

Li, Y., Skouroumounis, G. K., Elsey, G. M., & Taylor, D. K. (2011). Microwave-assistance provides very rapid and efficient extraction of grape seed polyphenols. Food Chemistry, 129 (2), 570-576. https://doi.org/10.1016/j.foodchem.2011.04.068

Lo Dico, G. M., Galvano, D., D'ascenzi, C., Macaluso, A., Vella, A., Giangrosso, G., Cammilleri, G., & Ferrantelli, V (2018). Toxic metal levels in cocoa powder and chocolate by ICP-MS method after microwave-assisted digestion. Food Chemistry, 245, 1163-1168. https://doi.org/10.1016/j.foodchem.2017.11.052

López-Berenguer, C., Carvajal, M., Moreno, D. A., & García-Viguera, C. (2007). Effects of microwave cooking conditions on bioactive compounds present in broccoli inflorescences. Journal of Agricultural and Food Chemistry, 55 (24), 10001-10007. https://doi.org/10.1021/jf071680t

Matsui, K. N., Granado, L. M., Oliveira, P. V., & Tadini, C. C. (2007). Peroxidase and polyphenol oxidase thermal inactivation by microwaves in Green coconut water simulated solutions. LWT- Food Science and Technology, 40 (5), 852-859. https://doi.org/10.1016/j.lwt.2006.03.019

Mizrahi, S. (2012). Mechanisms of objectionable textural changes by microwave reheating of foods: a review. Journal of Food Science, 77, 57-62. https://doi.org/10.1111/j.1750-3841.2011.02515.x

Mobeen, A. K., Aftab, A., Asif, A., & Zuzzer, A. S. (2011). Aflatoxins B1 and B2 contamination of peanut and peanut products and subsequent microwave detoxification. Journal of Pharmacy and Nutrition Sciences, 1, 1-3.

Mui, W. W. Y., Durance, T. D., & Scaman, C.H. (2002). Flavor and texture of banana chips dried by combinations of hot air, vacuum, and microwave processing. Journal of Agricultural and Food Chemistry, 50 (7), 1883-1889. https://doi.org/10.1021/jf011218n

Negi, A., Boora, P., & Khetarpaul, N. (2001). Effect of microwave cooking on the starch and protein digestibility of some newly released moth bean (*Phaseolus aconitifolius* Jacq.) cultivars. Journal of Food Composition and Analysis, 14 (5), 541-546. https://doi.org/10.1006/jfca.2001.1013

Nkhili, E., Tomao, V., Hajji, H. E., Boustani, E. E., Chemat, F., & Dangles, O. (2009). Microwave-assisted water extraction of green tea polyphenols. Phytochemical Analysis, 20, 408-415. https://doi.org/10.1002/pca.1141

Pan, X., Niu, G., & Liu, H. (2003). Microwave-assisted extraction of tea polyphenols and tea caffeine from Green tea leaves. Chemical Engineering and Processing: Process Intensification, 42 (2), 129-133. https://doi.org/10.1016/S0255-2701(02)00037-5

Pons-Andreu, J. V., Cienfuegos-Jovellanos, E., & Ibarra, A. (2004). Process for producing cocoa polyphenol concentrate. WO2005115160A1.

Regulska-Ilow, B., & Ilow, R. (2002). Comparison of the effects of microwave cooking and conventional cooking methods on the composition of fatty acids and fat quality indicators in herring. Molecular Nutrition, 46 (6), 383-388. https://doi.org/10.1002/1521-3803(20021101)46:6%3C383::AID-FOOD383%3E3.0.CO;2-L

Rother, M., Himberg, M., Gaukel, V., & Schuchmann, H.P. (2009). Microwave roasting low loss factor microwave applications. 12th International Conference on Microwave and High Frequency Heating, AMPERE, 403-406.

Schirack, A. V., Drake, M. Sanders, T. H., & Sandeep, K. P. (2006). Impact of microwave blanching on the flavor of roasted peanuts. Journal of Sensory Studies, 21 (4), 428-440. https://doi.org/10.1111/j.1745-459X.2006.00075.x

Sharma, P., & Gujral, H. S. (2011). Effect of sand roasting and microwave cooking on antioxidant activity of barley. Food Research International, 44 (1), 235-240. https://doi.org/10.1016/j.foodres.2010.10.030

Siguemoto, E. S., Gut, J. A. W., Martinez, A., & Rodrigo, D. (2018). Inactivation kinetics of Escherichia coli O157:H7 and Listeria monocytogenes in apple juice by microwave and conventional thermal processing. Innovative Food Science & Emerging Technologies, 45, 84-91. https://doi.org/10.1016/j.ifset.2017.09.021

Türkkan, A. U., Cakli, S., & Kilinc, B. (2008). Effects of cooking methods on the proximate composition and fatty acid composition of seabass (*Dicentrarchus labrax*, Linnaeus, 1758). Food and Bioproducts Processing, 86 (3), 163-166. https://doi.org/10.1016/j.fbp.2007.10.004

Uquiche, E., Jeréz, M., & Ortíz, J. (2008). Effect of pretreatment with microwaves on mechanical extraction yield and quality of vegetable oil from Chilean hazelnuts (*Gevuina avellana* Mol). Innovative Food Science & Emerging Technologies, 9 (4), 495-500. https://doi.org/10.1016/j.ifset.2008.05.004

Vadivambal, R., & Jayas, D. S. (2007). Changes in quality of microwave-treated agricultural products –a review. Biosystems Engineering, 98 (1), 1-16. https://doi.org/10.1016/j.biosystemseng.2007.06.006

Vinatoru, M., Mason, T. J., & Calinescu, I. (2017). Ultrasonically assisted extraction (UAE) and microwave assisted extraction (MAE) of functional compounds from plant materials. TrAC Trends in Analytical Chemistry, 97, 159-178. https://doi.org/10.1016/j.trac.2017.09.002

Weber, J., Bochi, V. C., Ribeiro, C. P., Victório, A. M., & Emanuelli, T. (2008). Effect of different cooking methods on the oxidation, proximate and fatty acid composition of silver catfish (*Rhamdia quelen*) fillets. Food Chemistry, 106 (1), 140-146. https://doi.org/10.1016/j.foodchem.2007.05.052

Wei, Q., Liu, T., Sun, D. W. (2018). Advanced glycation end-products (AGEs) in foods and their detecting techniques and methods: a review. Trends in Food Science & Technology, 82, 32-45. https://doi.org/10.1016/j.tifs.2018.09.020

5.2. Improving the functional profile of alkalized cocoas through microwave heating processing

Valverde, D., Pérez-Esteve, É., Barat, J.M.

Departamento de Tecnología de Alimentos. Universitat Politècnica de València, Camino de Vera, s/n, 46022, Valencia, Spain

Submitted to Food Chemistry

Abstract

Alkalization is a cocoa production chain step that aims to modify the sensory and technological characteristics of natural cocoa. This process is very time-consuming, involves high energy use and significantly reduces the product's functional content. In this work, a novel alternative microwave heating (MwH) processing was applied to alkalization to improve cocoa's functional properties and to increasing the sustainability of this step. The results showed that MwH preserved unprocessed cocoa's antioxidant activity and total phenol content, which were maintained even at high alkalization levels. MwH also alkalized cocoa in a 93% shorter time than the traditional method by drying the product and obtaining similar color and sensory characteristics. This work proves that MwH alkalization is a fast, less damaging and sustainable technique to increase cocoa's functionality.

Keywords: microwave, alkalization, cocoa, Dutching, polyphenols, sustainability.

1. Introduction

Alkalization, also known as “Dutching”, is an optional cocoa production step chain. It is based on applying an alkaline solution, pressure and temperature to natural cocoa to darken color, reduce astringency and bitterness, and increase powder solubility (Afoakwa, 2010; De Zaan cocoa, 2006).

Although alkalization improves cocoa sensory properties, it has also been reported to lead to the degradation and loss of polyphenols, methylxantines, amino acids and sugars (Li et al., 2012, 2014; Gültekin-Özgüven et al., 2016). In terms of polyphenols, several researchers have observed significant losses due to alkalization. Gültekin-Özgüven et al (2016) studied the total polyphenol, flavanol content and the antioxidant activity of alkalized cocoa liquors. They reported reductions of 87%, 83% and 50% in the respective characteristics. Miller et al (2008) analyzed a set of commercial cocoa powders and found that 89% of total flavanols were degraded in highly alkalized cocoas. These works show that alkalization reduces one of the most interesting groups of compounds of cocoa because this food has been reported to have the highest flavanols content. Cocoa has even been documented to have a higher content in this class of compounds than wine and tea, which makes the effects of alkalization highly undesirable from a functional point of view (Martín, Goya and Ramos, 2017).

Another drawback of alkalization is that this process usually takes more than 30 minutes, with up to 4-5 h between processing and subsequent drying (Wiant, Lynch and LeFreniere, 1989; Trout, 2000; Wissgott, 1985). Exposure time depends on the applied conditions and the desired final product characteristics. In drying terms, it is a necessary step to reduce moisture content to below 5% and to avoid microbiological spoilage and quality decay (De Zaan, 2006; Ellis, 1990; Kopp et al., 2009; Wissgott, 1985). These findings evidence three operational disadvantages of alkalization: lengthy duration, drying necessity and associated high energy use.

In this work, we proposed using MwH technology to improve the industrial method and to respond to two of society’s demands; on the one hand, the population demands healthier food products, which makes the preservation of nutritional and functional contents a relevant step to be taken. In line with this, MwH has been reported to improve the food quality of processed products and to increase extracting polyphenols from several matrices (Khan et al., 2018; Mei et al., 2012), which has drawn attention to this technique being applied to alkalization; on the other hand, climate change has become an urgent global issue and society demands fast effective measures to fight it. Here industry plays a key role and its adaptation to overcome this global crisis is essential. Along with the different actions that can be implemented, replacing traditional techniques with more sustainable ones is an important step to be taken. MwH is known as a fast technique that takes less time than traditional methods, and applying this technique will speed up different processes and may also reduce the associated energy use.

Nowadays, MwH is applied to the cocoa production chain for extracting polyphenols (González-Nuñez and Cañizares-Macías, 2011), drying (Guda et al., 2017; Firihu and Sudiana, 2016), roasting (Zzaman and Yang, 2013), and inactivating enzymes (Pons-Andreu et al., 2004), but not for alkalizing cocoa.

Based on MwH's ability to extract polyphenols and to improve the quality of processed products quality, our objective was to apply MwH to cocoa alkalization to preserve the functionality of raw materials, while also cutting time and energy use.

2. Materials and Methods

2.1. Materials

Natural cocoa cake from the Ivory Coast and different traditionally produced cocoas used as commercial standards were provided by Olam Food Ingredients SL (Cheste, Valencia, Spain). Potassium carbonate, sodium hydroxide, gallic acid, Folin-Ciocalteu reagent, sodium carbonate, methanol and acetone were supplied by Scharlau (Sentmenat, Spain). (-)-Epicatechin and (+)-catechin came from Sigma-Aldrich (Darmstadt, Germany). Trolox was provided by Across Organics (Geel, Belgium).

2.2. Microwave alkalization

The developed microwave-assisted method for alkalizing cocoa cake included four steps: preparing the mix, processing it in a microwave, drying and grinding. In the first step, 100 g of cocoa cake were mixed with the corresponding proportion of water (20-30%) and alkalizing agent (0-6%). In the second step, the mix was transferred to a vessel and processed in a microwave oven at the corresponding power and for the established duration. Then the processed mix was dried at 100ºC, ground using a coffee milling machine and kept for future analyses.

Experiments were done in an open or in a hermetically closed pressure cooker OLL-SS-10775R (Sogo, Valencia, Spain), which enabled samples to be processed either without (WP) or with pressure (P). The microwave oven employed for processing was the Daewoo microwave oven KO6-8A6K (Sabadell, Spain) with a maximum rated power of 720 W. For the unpressurized samples, processing was stopped every minute and cocoa paste was manually mixed. Processing was not interrupted with the pressurized samples.

2.3. Experimental design

2.3.1. Determining processing limits

To set the maximum possible processing duration, different samples were made following the procedure described in Section 2.2. First, cocoa was processed at maximum rated power (720 W) for different durations. Second, samples were sensory-analyzed to determine up to what point processing was acceptable. Finally, the process was repeated by applying less power.

2.3.2. Studying the influence of the different parameters on alkalization

A full factorial design was used to study the impact of the different parameters (water content —20% and 30%—, pressure —absence or presence—, type of alkali —K_2CO_3 and NaOH—, and concentration of alkali —0, 1, 3.5 and 6 g/100g—). Other parameters, such as amount of cocoa cake (100 g), processing time (4 min), power (720 W) and power intensity (90%), remained constant in all the experiments.

2.4. Physico-chemical analysis

2.4.1. Color and pH

Color coordinates and pH were determined according to the De Zaan cocoa manual (De Zaan, 2006) with some modifications. pH measurements were taken using a pH-Meter BASIC 20+ (Crison, Barcelona, Spain). Samples' intrinsic color was analyzed by a Minolta spectrophotometer CM 3600-d (Ramsey, USA) and their intrinsic color was measured in the reflectance mode with the specular component exclude (SCE). Data were acquired using the CIE-Lab color space. All the samples were read in duplicate.

2.4.2. Moisture

Moisture content was determined by following the protocol provided in the De Zaan cocoa manual (De Zaan, 2006). Equation 5 was used for the calculations.

$$Moisture\ (\%) = \frac{\left((W_a - A) - (W_b - A)\right) \times 100}{(W_a - A)} \quad (\text{Eq. }5)$$

where: "W_a" is the weight of cocoa and the cup before drying, "W_b" is the weight of the sample and the cup after drying, while "A" is the weight of the dried and empty aluminum cup.

2.5. Functional characterization

2.5.1. Obtaining the cocoa polyphenolic extract

To extract the polyphenols present in cocoa powder, an extraction protocol optimized by combining the conditions described by Arranz et al. (2009), Andres-Lacueva et al. (2008) and Hellström and Mattila (2008) was used. In this method, 1 g of cocoa powder was subjected to three extraction cycles. In the first one, cocoa was suspended in 20 mL of methanol and hydrochloric acid 16 mM (50:50). The mixture was then sonicated for 15 minutes at room temperature in an ultrasound bath model Elmasonic S 40H (Elma, Singen, Germany). After processing, samples were centrifuged at 10000 rpm and 4°C for 15 minutes. The supernatant was collected and another cycle like the first one was run. Then a third extraction cycle was run by suspending the precipitate of the second cycle in 20 mL of acetone and distilled water (70:30). Finally, the supernatants of each step were combined and taken to a final volume of 60 mL. The final polyphenolic extracts were kept at 4°C until analyzed.

2.5.2. Antioxidant activity

The antioxidant activity of the polyphenolic extracts was spectrophotometrically determined according to the DPPH method described by Todorovic et al. (2015) with some modifications.

For the assay, 6 µL of each polyphenolic extract were dissolved in 294 µL of methanol. Then 2.7 mL of the DPPH solution were added to 300 µL of each calibration point and sample. After incorporating DPPH, samples were mixed and kept for 1 h in the dark before measuring absorbance at 517 nm. The results were expressed as g equivalent to Trolox/100 g of cocoa cake.

2.5.3. Total phenol content

This method was taken and adapted from that described by Todorovic et al. (2015) with some modifications. For the assay, 50 µL of each sample were mixed with 0.45 mL of methanol/water and 5 mL of Folin-Ciocalteu solution. Then the mixture was shaken, allowed to react for 2 minutes and was mixed with 4 mL of sodium carbonate. The final mixture was kept in the dark for 1 h and the absorbance was measured at 750 nm. The results were expressed in g equivalent to gallic acid/100 g cocoa powder.

2.5.4. Concentration of catechin and epicatechin

The determination of catechin and epicatechin was made according to the HPLC method described by Niemenak et al (2006) with some modifications. The filtered polyphenolic extracts were injected into a Liquid Purple C18 reverse phase column (250 x 4.4mm) from Análisis Vínicos (Tomelloso, Spain). The employed mobile phases were 2% aqueous acetic acid (phase A) and acetonitrile, water and acetic acid at a 40:9:1 v/v/v proportion (phase B). The gradient was 0-8 min, 10% phase B;8-20 min, 10-15% phase B; 20-35 min, 15-90% phase B; 35-37 min, 90-10% phase B; 37-45 min, 10% phase B. The other chromatographic conditions were UV detection at 280 nm, column temperature of 40°C, injection volume of 30 µL and a flow rate of 1.2 mL/min. The employed equipment was a 1260 Infinity II HPLC system from Agilent Technologies (Madrid, Spain).

2.6. Sensory analysis

Sensory analyses were done by a trained panel made up of nine people according to UNE-ISO 6658:2019. Seven different markers were analyzed: cocoa taste, chocolate taste, acidity, astringency, alkalinity, body and bitterness. They were scored from 1 to 5, where “1” was lack of taste and “5” was very strong taste. The best cocoas produced by the microwave (in terms of color and functional characteristics) were compared to a set of commercial cocoas commonly employed by Olam International for sensory analyses.

2.7. Statistical analysis

All the statically analysis were performed by employing the Statgraphics Centurion XVII software (Virginia, USA).

3. Results and Discussion

This work is divided into two sections: one to characterize the effects of the newly developed alkalization method on different cocoa features and the other to evaluate the suitability of the proposed MwH processing as a less damaging method for cocoa polyphenols and as a shorter technique that employs less energy.

3.1. Studying the influence of processing on physico-chemical properties

A full factorial design was used to know how to define the different variables affecting pH and color. For all these analysis, power intensity and the selected duration were set at 648 W and 4 minutes for all the conditions. These conditions were chosen according to a preliminary study, which determined tolerance to power and time exposure before off-flavors appeared. The effects produced by water content, alkali type and concentration and pressure application are shown in Figure 15.

pH is a relevant characteristic for the cocoa industry as it is employed to classify cocoa into different alkalization levels. When samples were alkalized without alkali, pH was similar to the unprocessed sample one (pH=5.5). When an alkali was included (Figure 15A), pH increased and reached maximum values of 9.4 when NaOH at 6% was used. These observed changes in pH depended on the concentration and type of alkali, which were not affected by other variables (water content and applied pressure).

In color terms, the MwH method, done without adding alkali, was able to slightly reduce the L* and C* of cocoas compared to the unprocessed sample by 13.8% and 10.5%, respectively. No change was observed in h*.

When cocoa was alkalized, all the variables of pressure, water content and alkali reduced the color coordinates (Figure 15B, 1C and 1D), although a multifactorial ANOVA analysis revealed that alkali type and concentration were those that mainly reduced them (Table 7). From the employed alkalis, both NaOH and K_2CO_3 decreased all the color coordinates, and NaOH led to the most marked reductions. This was expected because the mentioned alkali was the strongest base.

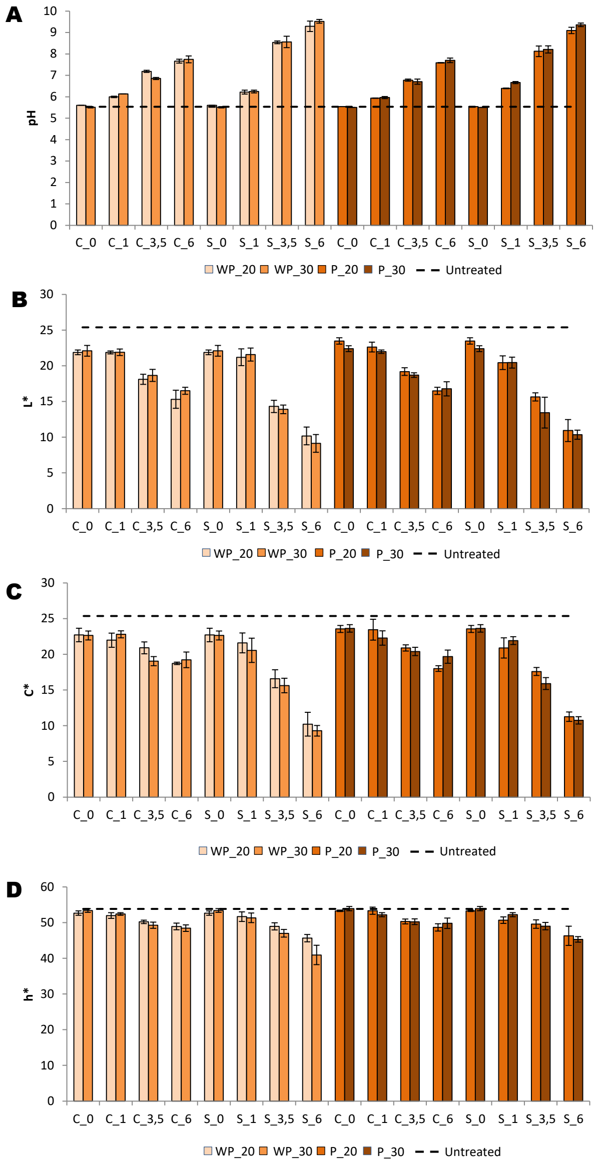

Figure 15. Effect of the different variables on color and pH. (A) pH, (B) L*, (C) C* and (D) h*. Each graphic is divided into two groups according to pressure (light orange columns (WP), dark orange columns (P)), and is subdivided by water content (20 or 30). Sample code: employed alkali (K_2CO_3 (C) or NaOH (S)) and alkali concentration (0, 1, 3.5, 6%). The dotted line represents the unprocessed sample.

Table 7. The F-values were obtained from a multifactorial ANOVA analysis for color, moisture and functional characteristics.. Components: alkali type (A), alkali concentration (C), pressure (P) and water content (H).

		L*	**C***	**h***	**Moisture**	**Antiox. activity**	**Total phenol content**	**[Catechin]**	**[Epicatechin]**
Main components	A	751.15***	908.91***	158.19***	0,04ns	19.86***	87.76***	9.41**	10.71**
	P	22.70***	30.59***	38.59***	970,87***	39.79***	0.27ns	0.39ns	7.49**
	C	1678.26***	1112.12***	467.98***	7,05***	8.69***	12.90***	64.06***	217.45***
	H	7.14**	6.31*	6.37*	388,42***	8.71**	1.72ns	3.78ns	10.82**
Interactions	AP	1.91ns	1.82ns	3.64ns	3,29ns	7.76**	0.31ns	1.85ns	12.40**
	AC	175.95***	265.84***	49.64***	3,64*	15.26***	17.06***	3.57*	9.26***
	AH	8.07**	3.79ns	7.63**	1,72ns	0.43ns	0.01ns	0.90ns	13.82***
	PC	6.77***	0.89ns	3.95**	9,06***	9.95***	1.56ns	1.42ns	1.87ns
	PH	16.72***	1.94ns	13.33***	0,04ns	20.12***	1.86ns	0.14ns	14.99***
	CH	2.00ns	8.33***	10.89***	0,28ns	5.55**	2.25ns	2.01ns	0.49ns
	APC	2.65*	2.50ns	4.30**	2,15ns	3.83*	0.96ns	1.06ns	12.31***
	APH	0.34ns	0.42ns	7.69**	2,01ns	0.92ns	2.23ns	0.90ns	0.33ns
	ACH	5.42**	3.92**	10.76***	0,99ns	2.92*	0.04ns	3.66*	11.31***
	PCH	1.84ns	0.54ns	5.39**	0,09ns	0.80ns	1.29ns	0.66ns	7.29***
	APCH	1.09ns	8.31***	1.97ns	0,23ns	2.08ns	0.46ns	2.10ns	0.70ns

Significance: ns (not significant), * (p-value>0.05), ** (0.01<p-value<0.05), ** (0.001<p-value<0.01) and *** (p-value<0.001).

3.1.2. Moisture content

The effect of the different variables was also studied on moisture content. The initial moisture of all the samples was 37.3% (moisture content of the dry powders (7.3±0.5%), plus 30% of added water). After the treatment, samples reached a final moisture content of ca. 6% (WP) and 10% (P). It means that processing was able to reduce moisture content up to 70%.

From the different variables, the statistical analysis (Table 7) showed that pressure mainly affected moisture, followed by water content and alkali concentration. Applying pressure generally led to higher moisture contents, but when NaOH concentrations went above 1%, the moisture of the pressurized samples reduced to similar values to the unpressurized cocoas. The reduction associated with an increased NaOH concentration could be related to the degradation of the compounds responsible for water retention.

3.1.3. Influence of processing on functional properties

After analyzing the impact of the different variables on color, pH and moisture, their effects on functional features were studied. The effect of each variable is shown in Figure 16.

The processed samples displayed similar antioxidant activity (Figure 16A) to the unprocessed cocoa samples (4.7±0.5 g/100 g). Antioxidant activity also significantly increased when NaOH concentrations over 3.5% were applied. This effect was not observed when K_2CO_3 was used.

When pressure was applied, the antioxidant activity of samples processed with NaOH increased, which did not take place in the unpressurized samples processed under the same conditions. This finding suggests that pressure enhanced the antioxidant activity of cocoa. Table 7 shows that, with all the variables, applying pressure had the strongest effect on antioxidant activity.

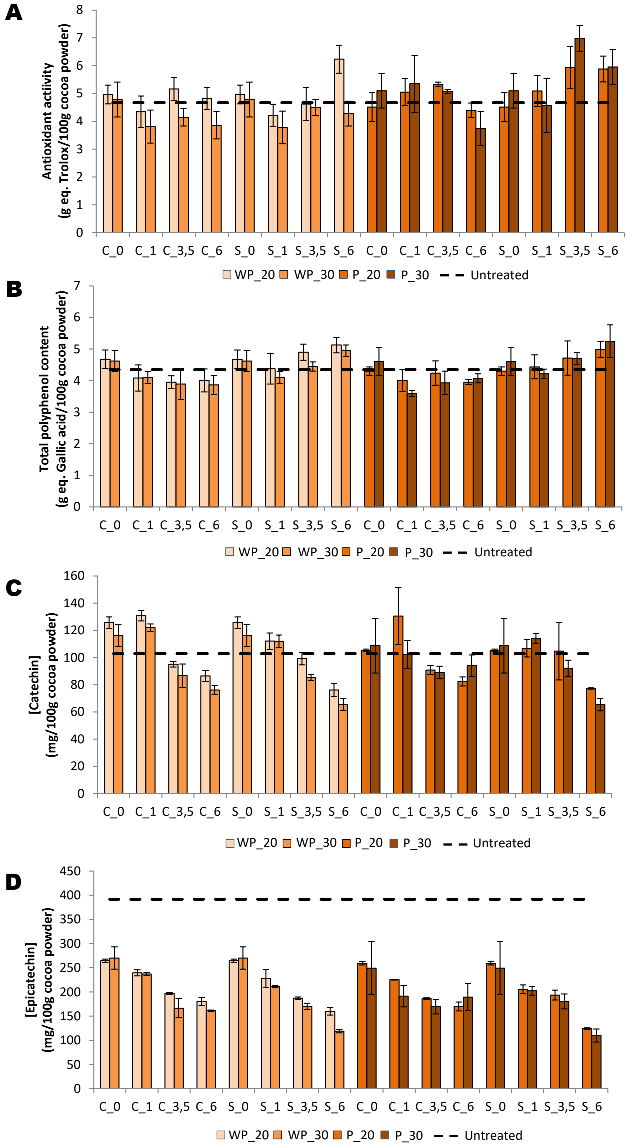

Figure 16. Effect of the different variables on functional characteristics. (A) Antioxidant activity, (B) total phenol content, (C) [Catechin] and (D) [Epicatechin]. Each graphic is divided into two groups according to pressure (light orange columns (WP), dark orange columns (P)), and is subdivided by water content (20 or 30). Sample code: employed alkali (K_2CO_3 (C) or NaOH (S)) and alkali concentration (0, 1, 3.5, 6). The dotted line represents the unprocessed sample.

The total phenol content did not generally change due to processing compared to the unprocessed cocoa (Figure 16B). Of all the variables (Table 7), only the type and concentration of alkali had a significant effect on total phenol content, and only high NaOH concentrations significantly increased total phenol content from 4.3±0.1 g/100 g (unprocessed cocoa) to 5.2±0.5 g/100 g.

The antioxidant activity preservation and total phenol content observed in the cocoas that underwent MwH could be related to both the inactivation of anti-nutritional factors and the extraction of non extractable polyphenols performed by the combination of MwH processing and the employed alkalis (Gonzales et al., 2015; Domínguez-Rodríguez et al., 2017).

A more in-depth polyphenolic content analysis was done to study the catechin and epicatechin concentrations (Figure 16C and D). The catechin concentration was negatively and significantly affected by the type and concentration of alkali, while the other variables had no negative effect on it (Table 7). MwH markedly reduced epicatechin compared to the unprocessed cocoa (Figure 16D). In addition, all the studied variables negatively impacted this characteristic, especially alkali concentration. Increments in this variable led to losses that exceeded 50%. This sensitivity in epicatechin, but not in catechin, indicated that the epicatechin form was more sensitive to microwave energy and to alkali than catechin. This could be due to the structure of epicatechin having been associated with different heat and light sensitivity (Liu et al., 2016). In addition, (+)-catechin and (-)-epicatechin have been reported to be transformed into (-)-catechin, a flavanol that is absent in non-alkalized cocoa (Gültekin-Özgüven et al., 2016; Hurst et al., 2011; Lacueva et al., 2008). The generation of this polyphenol, whose presence was not measured in this work, could be partly associated with the maintenance of antioxidant activity and total phenol content.

It is also worth mentioning that water content and pressure generally had a slight effect on functional characteristics (Table 7). From them, pressure was only relevant for antioxidant activity. For the other functional characteristics, the effect of pressure was slightly significant than for the other variables. The same was observed for water content, which was slightly significant in all cases.

3.2. Evaluating MwH suitability for cocoa alkalization

After demonstrating the MwH's capacity to quickly darken the color of natural powder without negatively affecting functional properties, the goal of this section was to verify that the physico-chemical, functional and sensory characteristics of the cocoas produced by MwH were comparable to those obtained by traditional processing.

3.2.1. Color comparison

As reducing color is one of the main cocoa alkalization goals, the powders with similar color and pH values to commercial ones were selected to be analyzed for their functional content.

To determine which samples were similar to traditionally produced ones, a set of commercial cocoas with different alkalization levels was characterized. The lowest and highest pH and color values obtained for the commercial samples were set as the limits (see Table 8), which were used to select the similar produced samples to the commercial ones.

Table 8. Minimum and maximum commercial limits for color (L*, C*, h*) and pH, and number of samples produced by the microwave, selected to be included among specifications.

Alkalization levels	Commercial limits				MwH cocoas in the specifications
	pH	L*	C*	h*	
Natural	5-6	21-27	19-24	49-56	4
Light	6-7.2	18-21	18-22	45-48	4
Medium	7.2-7.6	15-22	16-22	42-49	2
Strong	7.6-8.8	8-15	6-16	28-45	2

As seen in Table 8, microwave processing was able to produce cocoas with similar color and pH values to the natural, slight, medium and strong alkalinized commercial samples in only 4 minutes.

3.2.2. Functional comparison

All the samples with similar pH and color values to traditionally produced cocoas were compared to those for their functional features.

Figure 17 shows the graphical representation of the functional characteristics of both the commercial and MwH produced cocoas.

In antioxidant activity and total phenol content terms (Figure 17A and B), traditional processing without alkali (DN) reduced both antioxidant activity and the total phenol content of natural cocoa by almost 50%. Unexpectedly, MwH did not result in these reductions, and both features reduced when natural cocoa was alkalized by the traditional method. In the samples alkalized by the designed MwH method, the alkalized samples displayed more antioxidant activity and higher total phenol content than their references. The natural, slight, medium and strong MwH cocoas had 20±6%, 55±17%, 77±11% and 152±77% higher antioxidant activity and 11±4%, 24±4%, 50±1% and 92±8% higher total phenol content than their commercial references. These results showed that MwH alkalization preserved the antioxidant activity and total phenol content of the unprocessed cocoa at all the alkalization levels, even at the highest one.

An in-depth analysis based on catechin and epicatechin revealed that both compounds were negatively affected by MwH compared to the unprocessed cocoas (N) (Figure 17C and D). All the developed samples had lower catechin content than the natural and commercial cocoas. Only the strongly alkalized samples were similar to their references. Specifically, MwH reduced catechin content to 44±4%, 51±5%, 50±1% and 0±8% compared to the respective natural, light, medium and strong alkalized references.

With epicatechin (Figure 17D), all the MwH-alkalized cocoas had a lower concentration than the unprocessed material, but a higher concentration than traditionally alkalized cocoas. MwH increased the epicatechin concentrations in the light, medium and strong alkalized cocoas to 13±9%, 89±8% and 162±11%.

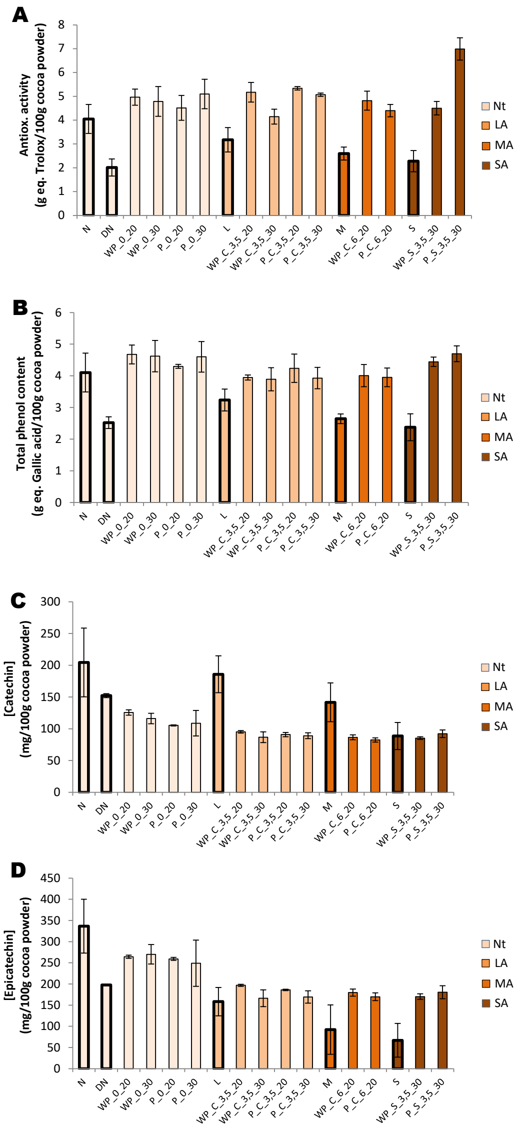

Figure 17. Functional properties of the commercial and selected cocoas. (A) antioxidant activity, (B) total phenol content, (C) [Catechin] and (D) [Epicatechin]. Samples were divided into pH and natural (Nt), light (LA), medium (MA) and strong (SA). The commercial cocoas surrounded by a thick black line had these codes: N (natural), DN, (dark natural), L (lightly alkalized), M (moderately alkalized) and S (strongly alkalized). The microwave-alkalized sample codes are unpressurized or pressurized (WP or P), NaOH or K_2CO_3 (S or C) and proportion of alkali and water.

In general, this section showed that the microwave-produced samples had improved functional characteristics than traditionally alkalized ones. This improvement seemed to result from the greater extractability of antioxidant molecules, their better preservation and the formation of new compounds.

3.2.3 Sensory evaluation

Finally, two cocoas at each level of alkalization, one unpressurized and the other pressurized, were chosen for being sensory characterized by a trained panel. The results are shown in Figure 18.

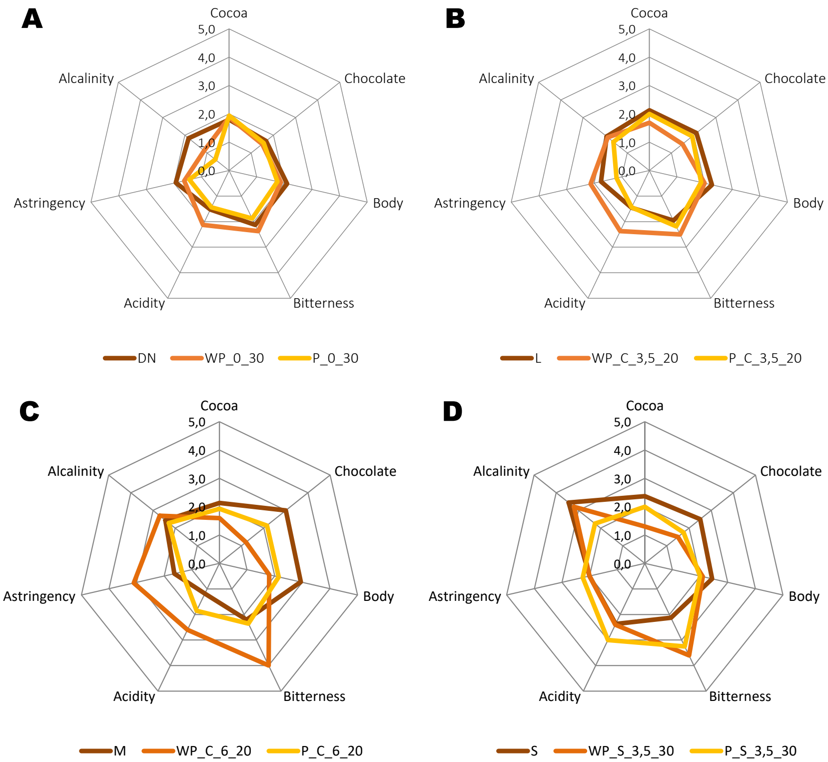

Figure 18. Evolution of the sensory profile of the commercial and MwH cocoas. Samples have been grouped as in: (A) dark natural, (B) light alkalized, (C) medium alkalized and (D) strong alkalized. Color code: unpressurized cocoa (dark orange), pressurized (light orange) and commercial (brown).

As we can see, MwH was able to produce cocoas' sensory characteristic similarly to commercial ones at all alkalization levels.

At the dark natural (Figure 18A) and light alkalization levels (Figure 18B), both the developed and commercial cocoas were almost similar in all the markers. At the medium level (Figure 18C), the pressurized sample was similar to its commercial reference, albeit with slightly less chocolate taste and body. The unpressurized sample was defined as being more astringent, acidic and bitter, and having less body, cocoa and a milder chocolate taste than the commercial cocoa. Finally, at the strong alkalization level (Figure 18D), the produced samples had less cocoa, a milder chocolate taste and more bitterness. Of the pressurized and unpressurized cocoas, the pressurized ones were perceived as being less alkaline, more acidic and had a stronger cocoa taste.

Figure 18 shows that the microwave and alkali combination led to increased bitterness (comparison of Figure 18A and D) and pressure had a positive effect on the sensory profile. The effect of the microwave and alkali combination could be related to the release of bitter polyphenols. Finally, several biochemical reactions induced by pressure could lie behind the sensory improvements produced by this variable.

In conclusion, in only 4 minutes microwave processing was able to produce cocoas with similar color and sensory features to commercial ones, but with higher functional content.

3.2.4. Time spent and energy use

As the previous sections describe, cocoa with different alkalization levels can be prepared by processing samples for 4 minutes. This period is shorter than those reported by other authors to reach the same alkalization level by traditional processing. For example, Wiant, Lynch and LeFreniere (1989) patented two conventional methods for alkalizing cocoa cake, one for obtaining dark red cocoa that needed 5-60 minutes and another for producing dark black cocoa that took 60-90 minutes.

With a mean duration of 60 minutes for cocoa alkalization it can be stated that the developed MwH method reduced the time needed to process cocoa by 93%. However, MwH not only accelerated the alkalization process, but it also reduced the energy used for the milling step for fracturing cocoa cake rocks and reduced the required drying by removing up to 70% of moisture content. All these accelerations consequently reduced the energy used in all these steps, which makes cocoa powder production a much more sustainable process.

4. Conclusions

Applying microwave heating reduced the duration of processing by 93%, moisture content by 70% and, consequently, the time spent and energy used in producing alkalized cocoa powder. Furthermore, the proposed technique was able to achieve similar colors and sensory features as powders produced by conventional processing in just 4 minutes. Yet the most interesting finding was that microwave heating preserved the functional properties of unprocessed cocoas and allowed all alkalized powders to significantly enhance their functional features than traditionally obtained ones.

From the studied variables, alkali type and concentration were very important factors for darkening cocoa and reducing its functional features. In terms of the sensory properties, pressurization, which generally had a slight effect on cocoa, was essential for obtaining the best products.

This work opens a way for the future implementation of microwave heating alkalization to industry, which will increase the sustainability of this process, cut the time needed and enhance the functional properties of products.

Acknowledgements

Damián Valverde would also like to thank the Regional Ministry of Education, Culture and Sports of Generalitat Valenciana for his PhD grant.

Funding

The authors would like to acknowledge the financial support of the Spanish Government and European Regional Development Fund (Project RTC-2016-5241-2).

References

Afoakwa, E. O. (2010). Chocolate science and technology. Wiley-Blackwell.

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

Arranz, S., Saura-Calixto, F., Shaha, S., & Kroon, P. A. (2009). High Contents of Nonextractable Polyphenols in Fruits Suggest That Polyphenol Contents of Plant Foods Have Been Underestimated. Journal of Agricultural and Food Chemistry, 57 (16), 7298-7303. https://doi.org/10.1021/jf9016652

De Zaan cocoa, (2006). Cocoa & Chocolate manual. ADM Cocoa, Switzerland.

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. https://doi.org/10.1016/j.chroma.2017.07.066

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

Firihu, M. Z., & Sudiana, N. (2016). 2.45 GHz microwave drying of cocoa vean. ARPN Journal of Engineering and Applied Sciences, 11 (19), 11595-11598.

Gonzales, G. B., Raes, K., Vanhoutte, H., Coelus, S., Smagghe, G., & Camp J. V. (2015). Liquid chromatography–mass spectrometry coupled with multivariate analysis for the characterization and discrimination of extractable and nonextractable polyphenols and glucosinolates from red cabbage and Brussels sprout waste streams. Journal of Chromatography A, 1402, 60-70. https://doi.org/10.1016/j.chroma.2015.05.009

González-Nuñez, L. N., & Cañizares-Macías, M. P. (2011). Focused microwaves-assisted extraction of theobromine and caffeine from cacao. Food Chemistry, 129 (4), 1819-1824. https://doi.org/10.1016/j.foodchem.2011.05.118

Guda, P., Gadhe, S., & Jakkula, S. (2017). Drying of cocoa beans by using different techniques. International Journal of Agriculture Innovations and Research, 5 (5), 859-865.

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

Hellström, J. K., & Mattila, P. H. (2008). HPLC Determination of Extractable and Unextractable Proanthocyanidins in Plant Materials. Journal of Agricultural and Food Chemistry, 56 (17), 7617-7624. https://doi.org/10.1021/jf801336s

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.

Khan, M. K., Ahmad, K., Hassan, S., Imran, M., Ahmad, N., & Xu, C. (2018). Effect of novel technologies on polyphenols during food processing. Innovative Food Science and Emerging Technologies, 45, 361-381. https://doi.org/10.1016/j.ifset.2017.12.006

Kopp, G. M., Hennen, J. C., Seyller, M., & Brandstetter, B. (2009). Process for producing high flavour cocoa. EP2241190B1.

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

Li, Y., Zhu, S., Feng, Y., Luo, C., Ma, J., & Zhong, F. (2014). Influence of Alkalization Treatment on the Color Quality and the Total Phenolic and Anthocyanin Contents in Cocoa Powder. Journal of Food Science Biotechnology, 23 (1), 59-63. https://doi.org/10.1007/s10068-014-0008-5

Liu, M., Zheng, Y., Wang, C., Xie, J., Wang, B., Wang, Z., Han, J., Sun, D., & Niu, M. (2016). Improved stability (+)-catechin and (-)-epicatechin by complexing with hydroxypropyl-β-cyclodextrin: effect of pH, temperature and configuration. Food Chemistry, 196, 148-154. https://doi.org/10.1016/j.foodchem.2015.09.016

Martín, A. M., Goya, L., Ramos, S. (2017). Protective effects of tea, red wine and cocoa in diabetes. Evidences from human studies. Food and Chemical Toxicology, 109, 302-314. https://doi.org/10.1016/j.fct.2017.09.015

Mei, C., Yong-Zhou, W., Guang, L., & Xiao-Ping, W. (2012). Effects of Different Drying Methods on the Microstructure and Thermal Oxidative Aging Resistance of Natural Rubber. Journal of Applied Polymer Science, 126 (6), 1808-1813. https://doi.org/10.1002/app.34300

Miller, K. B., Hurst, W. F., 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

Niemenak, N., Rohsius, C., Elwers, S., Ndoumou, D. O., & Lieberei, R. (2006). Comparative study of different cocoa (Theobroma cacao L.) clones in terms of their phenolics and anthocyanins contents. Journal of Food Composition and Analysis, 19 (6-7), 612-619. https://doi.org/10.1016/j.jfca.2005.02.006

Pons-Andreu, J. V., Cienfuegos-Jovellanos, E., & Ibarra, A. (2004). Process for producing cocoa polyphenol concentrate. WO2005115160A1.

Rodríguez, P., Pérez, E., & Guzmán, R. (2009). Effect of the 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

Todorovic, V., Redovnikovic, I. R., Todorovic, Z., Jankovic, G., Dodevska, M., & Sobajic, S. (2015). Polyphenols, methylxanthines, and antioxidant capacity of chocolates produced in Serbia. Journal of Food Composition and Analysis, 41, 137-143. https://doi.org/10.1016/j.jfca.2015.01.018

Tow, W. W., Premier, R., Jing, H., & Ajlouni, S. (2011). Antioxidant and Antiproliferation Effects of Extractable and Nonextractable Polyphenols Isolated from Apple Waste Using Different Extraction Methods. Journal of Food Science, 76 (7), 163-172. https://doi.org/10.1111/j.1750-3841.2011.02314.x

Trout, R. B. (2000). Method for making dutched cocoa. EP1278428B1.

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

Wissgott, U. (1985). Method of alkalization of cocoa in the aqueous phase. CH663324A5

Zzaman, W., & Yang, T. A. (2013). Effect of Superheated Steam and Convection Roasting on Changes in Physical Properties of Cocoa Bean (Theobroma cacao). Journal of Food Science and Technology Research, 19 (2), 181-186. https://doi.org/10.3136/fstr.19.181

5.3. Effects of power and duration of the microwave alkalization method on the properties of alkalized cocoa powders

Valverde. D., Pérez-Esteve, É., Barat, J. M.*

Departamento de Tecnología de Alimentos. Universitat Politècnica de València,
Camino de Vera, s/n, 46022, Valencia, Spain

Submitted to Food Chemistry

Abstract

Microwave technology has experienced a significant development in the last decades due to its interesting advantages in several fields. In the cocoa production chain, this technique has being applied for different purposes, but not for alkalization. Based on that, a microwave alkalization method has been developed in our laboratory. In this work, the effects of power and duration of the microwave treatment in combination with alkali type and concentration have been studied. Different surface responses were built to analyze the physicochemical and functional properties of the produced cocoas. The results pointed that microwave by itself slightly affected the evaluated characteristics and that in combination with an alkali, its effects were enhanced and leaded to higher functional features. In addition, high reductions in moisture content were reached by the microwave treatment, making this new alkalization process even a more interesting alternative than the traditional one.

Keywords: microwave, alkalization, cocoa, power, duration, Dutching, polyphenols.

1. Introduction

Cocoa powder is one of the widest employed cocoa products by the food industry. It is used in the formulation of different cocoa-based products due to its ability to provide the typical chocolate flavor and color (Afoakwa, 2011).

Apart from its sensory properties, cocoa have attracted attention in the last years due to its composition and protective health effects. Concretely, it is a rich source of fiber (26-40%), proteins (15-20%), carbohydrates (15%) and lipids (10-24%), in addition to minerals and vitamins. Furthermore, cocoa is known to be rich in polyphenols and methylxanthines, being exactly the food with the highest flavanol content based on its dry weight basis. Major polyphenols in cocoa are catechin, epicatechin and their dimers (procyanidins B1 and B2), although there are others present in less quantities such as quercetin, isoquercetin and luteolin (Martín et al., 2013; De Zaan cocoa, 2006; Maleyki et al., 2010). Due to its polyphenol content, cocoa has been associated with the prevention of different kind of cancers, cardiovascular diseases and diabetes (Martín et al., 2013; Martín et al., 2017; Santos and Macedo, 2018).

Polyphenols, in addition to other compounds such as peptides and sugars, are significantly reduced during cocoa processing, especially during alkalization (Miller et al., 2008; Andres-Lacueva et al., 2008; Gültekin-Özgüven et al., 2016; Payne et al., 2010; Hurst et al., 2011; Todorovic et al., 2017; Taş and Gökmen, 2016; Li et al., 2012). For example, Gültekin-Özgüven et al (2016) have reported the total phenol content, flavanols and antioxidant activity to be reduced an 87%, 83% and 50%, respectively, after alkalization, losses similar to the ones reported by Miller et al (2008) and also by Gu et al (2006).

The so-called alkalization, also known as “Dutch process”, is an optional step of the cocoa production chain applied for reducing the characteristic bitterness and astringency of cocoa, for darkening its color and also for increasing the powder solubility (Afoakwa, 2011; De Zaan cocoa, 2006).

During alkalization, cocoa is mixed with an alkali solution and submitted to a pressurized thermal treatment. From a technological point of view, different technologies have been described for alkalization. As an example, Wissgott (1988) described an alkalization process in a closed vessel that needed up to 4 h for producing cocoa with improved color, taste and dispersion. Other example is Wiant et al. (1989) that patented two methods for producing dark red and black cocoa in a closed vessel, needing for them 45 minutes and more than 2 h, respectively. But duration is dependent on the technology and although the conventional alkalization is a long process that is performed in a closed pressurized vessel, there are authors that have applied alternative technologies to alkalization such as Chalin (1972) and Bandi et al. (1984). Chalin performed his alkalization step inside an extruder and needed less than 5 minutes for producing a homogeneous, dark and sterile cocoa with a good suspension capacity in beverages. In the case of Bandi et al., they employed a vapor injection system that just needed 1 minute for obtaining alkalized cocoas similar to the marketed ones.

As can be seen, traditional alkalization is a long method that new technologies can replace due to its different advantages. Among the emerging technologies, microwave heating has become popular in the last decades due to its characteristics: short duration, versatility and improvement in quality of treated products (less losses and higher extraction yields) (Khan et al., 2018). In addition to those advantages, microwave has been reported to inactivate and destroy several anti-nutritional factors such as tannins (compounds that inhibit enzymes that especially digest proteins and carbohydrates), phytic acid (an anti-nutrient that reduces the bioavailability of minerals, proteins and carbohydrates by binding to them), trypsin inhibitors (that reduce the protein digestibility), flatulence-causing oligosaccharides such as raffinose, stachyose and verbascose, and enzymes such as the polyphenol oxidase (Sharma et al., 2011; Matsui et al., 2007; Hefnawy., 2011).

With relation to its application, microwave heating has been applied to the production of cocoa at several levels: to digest before the analysis in heavy metals (Hartwig et al., 2016), to extract fat, polyphenols and methylxanthines (González-Nuñez and Cañizares-Macías, 2011; Kealey et al., 1996), to dry (Guda, Gadhe and Jakkula, 2017; Firihu and Sudiana, 2016) and to roast (Rother et al., 2009), among other applications.

Based on these advantages and also in the novelty of the application of this technology to cocoa alkalization, our laboratory developed a microwave-based alkalization method quicker, more sustainable and less aggressive to the sample than the traditional one. In this work, we study the impact of power and duration of the treatment on the physicochemical and functional properties of cocoa with the objective of understanding how the different variables affect the characteristics of this material.

2. Materials and methods

2.1. Materials

Natural cocoa cake from Ivory Coast employed as raw material and the different cocoa standars were provided by Olam Food Ingredients SL (Cheste, Spain). Trolox was provided by Across Organics (Geel, Belgium). Sodium hydroxide, Folin-Ciocalteu reagent, methanol, potassium carbonate, sodium carbonate, gallic acid and acetone were provided by Scharlau (Sentmenat, Spain). (-)-Epicatechin and (+)-Catechin were provided by Sigma-Aldrich (Darmstadt, Germany).

2.2. Microwave alkalization

The developed procedure for alkalizing cocoa cake had four steps: preparing the mix, treating it by microwave, drying and grinding. In the first step, 100 g of cocoa cake are mixed with 30% of water and with the corresponding alkali agent proportion (from 0.28 to 6%). In the second step, the mix is transferred into a pressure cooker OLL-SS-10775R made by Sogo (Valencia, Spain) and treated in a Daewoo microwave oven KO6-8A6K (Sabadell, Spain) at corresponding power and duration. Then, the treated mix is dried at 100°C, grinded using a coffee milling machine and stored for future analyses. All the starting conditions for the assays were set according to a previous work.

2.3. Experimental design

To define the combination of conditions to be applied for evaluating the relationships between the process variables (alkali concentration (X1), time (X2) and power (X3)) and the response parameters (pH, color, moisture, antioxidant activity, total phenol content and concentrations of catechin and epicatechin), a response surface methodology was followed. The statistical modeling and analysis was performed by the design assistant of experiments of Statgraphics Centurion (Manugistics Inc., Rockville, MD, USA). Experimental ranges of the three independent parameters were stablished by previous tests in the laboratory. The selected design for the surface response modeling was an orthogonal and central composed design 2^3+star. Experimental conditions for the analysis are shown in Table 9 and 10.

Table 9. Tested concentration of alkali, time and power for building the response surfaces with alkali.

Point	X1 (%)	X2 (°C)	X3 (%)
1	1	1	90
2	3.5	3.5	50
3	1	6	90
4	3.5	3.5	0
5	0.3	3.5	50
6	3.5	0.3	50
7	6	6	90
8	3.5	3.5	50
9	1	6	10
10	3.5	3.5	100
11	6	1	90
12	1	1	10
13	3.5	6.7	50
14	6.7	3.5	50
15	6	1	10
16	6	6	10

Table 10. Tested durations and power intensities for building the response surface without alkali.

Point	X2 (°C)	X3 (%)
1	6	90
2	1	90
3	6	10
4	0.8	50
5	1	10
6	3.5	93.1
7	3.5	6.9
8	3.5	50
9	6.2	50
10	3.5	50

After data analysis, the behavior of each response variable (pH, color, moisture, antioxidant activity, total phenol content and concentrations of catechin and epicatechin) with respect to the evaluated independent parameters was fitted in a quadratic polynomial model as shown in Eq. 6.

$$y = a_0 + \sum_{i=1}^{3} a_i X_i + \sum_{i=1}^{3} a_{ii} X_{ii} + \sum_{i \neq j=1}^{3} a_{ij} X_i X_j \quad (Eq. 6)$$

where "y" represents the response variable (pH, color or moisture), "a_o" is the constant, "a_i", "a_{ii}" and "a_{ij}" are the linear coefficients and their interactions, and "X_i" and "X_j" are the experimental data for each variable.

The previous surface response methodology was carried out without any alkali and with the two different alkali agents: NaOH and K_2CO_3. For all the models, the R^2 statistical values were obtained for evaluating their suitability.

2.4. Physicochemical analysis

2.4.1. Moisture

Moisture content of the samples was determined by following the protocol described by De Zaan (De Zaan cocoa, 2006) and calculated by using the Eq. 7.

$$\text{Moisture (\%)} = \frac{\left((\mathrm{W}_1 - \mathrm{A}) - (\mathrm{W}_2 - \mathrm{A})\right) \times 100}{(\mathrm{W}_1 - \mathrm{A})} \qquad \text{(Eq. 7)}$$

where “W_1” is the weight of cocoa plus the cup before drying, “W_2” is the weight of cocoa and the cup after drying and “A” represents the weight of the dried and empty aluminum cup.

2.4.2. Color and pH

Color coordinates and pH were determined according to the De Zaan cocoa manual (De Zaan, 2006) with some modifications. pH measurements were taken using a pH-Meter BASIC 20+ (Crison, Barcelona, Spain). Samples’ intrinsic color was analyzed by a Minolta spectrophotometer CM 3600-d (Ramsey, USA) and their intrinsic color was measured in the reflectance mode with the specular component exclude (SCE). Data were acquired using the CIE-Lab color space. All the samples were read in duplicate.

2.4.3. Functional characterization

2.4.3.1. Cocoa polyphenol extraction

For extracting the polyphenols present in cocoa, an extraction protocol optimized by combining the procedures described by Andres-Lacueva et al. (2008), Arranz et al. (2009) and Hellström and Mattila (2008) was employed. In this method, 1 g of cocoa powder was subjected to three extraction cycles. In the first and second one, cocoa was suspended in 20 mL of methanol and hydrochloric acid 16 mM (50:50). Then, the mixture was sonicated for 15 minutes at room temperature in an ultrasound bath model Elmasonic S 40H (Elma, Singen, Germany). Then, the samples were centrifuged (10000rpm, at 4°C during 15 minutes) and supernatants collected. Finally, a third extraction cycle was performed by suspending the precipitate of the second cycle in 20mL of acetone and distillated water (70:30), the sample centrifuged and all the supernatants combined and taken to a final volume of 60 mL. The final polyphenolic extracts were kept at 4°C until their analyses.

2.4.3.2. Antioxidant activity

Quantification of the antioxidant activity was performed by the DPPH method described by Todorovic et al. (2015) with some modifications.

For the antioxidant determination, 6µL of each extract were diluted in 294 µL of methanol. Then, the diluted samples or 300µL of each calibration point were mixed with 2.7 mL of the DPPH solution. Once added the radical, samples were shaken and kept for 1 h in darkness before measuring at 517 nm. The results were expressed in g of Trolox/100 g of cocoa cake.

2.4.3.3. Total phenol content

This method is an adapted version of the described by Todorovic et al. (2015). For the total phenol determination, 50 µL of each extract were diluted in 0.45 mL of methanol/water (1:1) and 5 mL of Folin-Ciocalteu solution. Afterwards, 4 mL of Na_2CO_3 solution were added to the previous mix and it was kept in darkness for 1h. Absorbance of the samples was measured at 750 nm. The total phenol content was expressed in g of gallic acid/100 g cocoa powder.

2.4.3.4 Concentration of catechin and epicatechin

Quantification of epicatechin and catechin was performed according to an adapted HPLC method described by Niemenak et al (2006). The filtered polyphenolic extracts were injected in a reverse phase column Liquid Purple C18 (250 x 4.4 mm) from Análisis Vínicos (Tomelloso, Spain). Mobile phases were: acidic water with 2% of acetic acid (phase A) and acetonitrile, water and acetic acid (40:9:1 v/v/v) (phase B). Gradient was: 0-8 min, 90% phase A; 8-20 min, 90-85 phase A; 20-35 min, 85-10% phase A; 35-37, 10-90% phase A; 37-45 min, 90%

phase A. The other chromatographic conditions were: column temperature of 40°C, UV detection at 280 nm, injection volume of 30 µL and a flow rate of 1.2 mL/min. The equipment was a HPLC model 1260 Infinity II from Agilent Technologies (Madrid, Spain).

3. Results

3.1. Model description

Three different groups of surfaces were built according to the presence and type of alkali: without alkali, K_2CO_3 and NaOH. Table 11 shows the coefficients for each response variable that fit the experimental data into the corresponding quadratic equation, showing also their statistical significance.

Analysis of variance (ANOVA) reveals that in most of the samples treated with K_2CO_3 and NaOH, the regression coefficients (R^2) were higher than 0.9 in the different response parameters. This means that the proposed models explain more than 90% of the variability of the different response parameters. In the case of the samples treated without any alkali, almost all the models, excluding the one of the moisture content, had a noticeable low R^2, which means that the model is not able to precisely predict the behavior of those variables, even though all the experimental points slightly differs between them. For example, for the pH, all the samples had a value between 5.2 and 5.4, which does not explain the low R^2 of the model.

In addition, the significance of the different variables and its impact by a Pareto diagram were evaluated to identify which ones were affecting the different response parameters. For cocoas alkalized without an alkali agent, all the analyzed variables, with the exception of the moisture content, were slightly or even not affected by the modification of power and duration.

Table 11. Regression coefficients of the quadratic equations for the physico-chemical and functional properties in samples treated without alkali and with K_2CO_3 and NaOH. Codes: X1 is alkali concentration, X2 is duration and X3 is power.

Without alkali	**pH**	**L***	**C***	**h***	**Moisture**	**Antiox. activity**	**Total phenol content**	**[Catechin]**	**[Epicatechin]**
X0	5,206	21,813	20,594	51,795	28,965	3,864	3,437	72,909	327,351
X2	0,002	0,025	0,047*	0,151	0,403***	0,068	0,146	5,035*	-1,347
X3	0,000	0,001	0,015	0,001	-0,045***	0,012	0,005	0,163*	-2,913
$X2^2$	0,000	0,003	-0,032	-0,040	-0,043	-0,021	-0,023	-0,262	-2,193
X2X3	0,000	0,000	0,001	0,001	-0,037**	0,001	0,000	0,012	0,338**
$X3^2$	0,000	0,000	0,000	0,000	0,0004	0,000	0,000	0,000	0,014*
R^2	42,584	19,19	35,1	38,349	99,04	38,991	38,971	89,959	91,445

K_2CO_3	**pH**	**L***	**C***	**h***	**Moisture**	**Antiox. activity**	**Total phenol content**	**[Catechin]**	**[Epicatechin]**
X0	5.730	23.204	22.725	54.441	28.778	5.001	3.865	105.827	251.008
X1	0.335***	-1.645***	-0.505***	-1.511***	-0.521***	-0.317*	-0.273*	-15.000	-37.852*
X2	0.001	-0.804	-1.443	-1.456	-0.088***	-0.431**	-0.244**	-9.993	-26.277
X3	-0.001	0.003	-0.1*	-0.115**	0.001	0.001**	0.004*	-0.708	-0.243
$X1^2$	-0.001	0.090	-0.009	0.105*	0.080	0.043*	0.029*	2.110	5.833*
X1X2	0.003	0.000	0.028	0.025	-0.009	0.014	0.026*	0.086	-0.892
X1X3	0.000	-0.003	-0.100*	-0.187**	0.000	-0.002*	-0.002*	-0.059	-0.215
$X2^2$	-0.003	0.112	0.201**	0.210**	0.066	0.048*	0.025	0.808	3.477
X2X3	0.000	-0.002	0.003	0.002	-0.043***	0.003**	0.001	0.175	0.199
$X3^2$	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.006
R^2	98.512	96.520	96.658	98.601	99.711	94.423	92.817	67.400	85.209

NaOH	**pH**	**L***	**C***	**h***	**Moisture**	**Antiox. activity**	**Total phenol content**	**[Catechin]**	**[Epicatechin]**
X0	5.587	22.639	20.861	52.084	30.526	3.620	2.769	57.058	117.313
X1	1.182***	-3.671***	-2.168***	-1.997***	-1.035	0.106334*	0.139**	-0.203**	1.885***
X2	-0.213	0.626	0.508	0.144	-0.878***	0.016*	-0.042	1.485	30.343
X3	0.009	0.017	0.035	0.024	-0.082***	0.020*	0.018527*	0.347	1.623*
$X1^2$	-0.098**	0.280*	0.088	0.087	0.117**	-0.001	0.001	0.371	-0.233
X1X2	0.006	-0.041	0.032	0.077	0.027	0.018	0.014	-0.646	-1.589
X1X3	0.001	-0.001	-0.010*	-0.013*	0.002	0.001	-0.002	-0.095*	-0.239*
$X2^2$	0.036	-0.040	-0.109	-0.089	0.110**	-0.006	-0.003	0.101	-2.988
X2X3	0.000	-0.007	-0.002	0.001	-0.033***	0.003	0.002	0.021	-0.043
$X3^2$	0.000	0.000	0.000	0.000	0.001**	0.000	0.000	0.000	-0.002
R^2	97.907	97.001	97.544	96.111	99.823	82.232	87.351	85.425	93.268

Significance: * (0.01<p-value<0.05), ** (0.001<p-value<0.01); *** (p-value<0.001).

In the case of samples alkalized with K_2CO_3 or NaOH, concentration of alkali showed to be the main variable negatively affecting the physico-chemical and functional characteristics of cocoa (p-value<0.05), with the exception of the antioxidant activity and the total phenol content in samples treated with NaOH, which were positively correlated with the alkali concentration (p-value<0.05). In addition, duration and power of the treatment showed a positive effect on the antioxidant activity and total phenol content in alkalized cocoas (p-value<0.05). In the case of the moisture content, it was, for all the tested conditions, negatively correlated with power and duration.

3.2. Impact of duration and power, and of alkali type and concentration on the physico-chemical parameters

In this section, the effects of power and duration were analyzed independently and combined with the variables that seem to be the most relevant and basic variables of alkalization: type and concentration of alkali.

Three different treatments were assayed: without alkali, with K_2CO_3 and with NaOH. For each one of the conditions, different response surface graphics were developed, treating all the cocoas with 30% of water content and under pressurized conditions.

Figure 19 shows the effect of power combined with duration on the pH and the color components.

In the case of pH, the incorporation of an alkali agent leaded to an increase of the pH of natural cocoa. For example, adding 6% of K_2CO_3 produced an increase from 5.2 to 7.8, while treating with 6% of NaOH raised the pH to 9.8. Neither power nor duration modified the pH.

Other evaluated characteristic was the color of alkalized samples. Color components of natural cocoa have a value of 25.4±0.4 for L*, 25.4±0.6 for C* and 53.8±0.4 for h*. In general, cocoas treated by microwave heating without any alkali had lower values of luminosity. An an example, the mean values for microwave heated cocoas were 22.0±1.0, 20.6±1.2 and 52.1±0.8 for the L*, C* and h*, respectively. As power and duration did not show any effect on the color, the observed reductions in these features were related to the humidification and drying of cocoa.

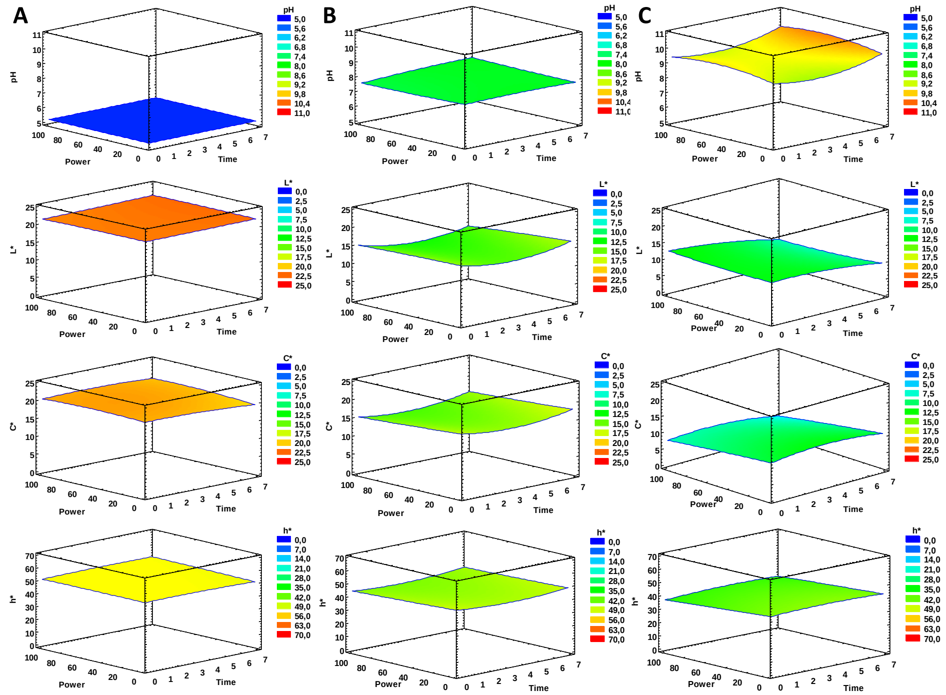

Figure 19. Response surface graphics of color and pH of different types of alkalis and power-time combinations. (A) Pressurized samples treated without alkali, (B) pressurized samples treated with K_2CO_3 and (C) pressurized samples treated with NaOH. Alkali concentration of the samples with alkali was 6% and water content of 30%.

When the samples were alkalized, the color components suffered a high reduction. From the tested alkalis, NaOH was the one leading to the lowest color values. For example, when the samples were treated for 6 minutes without any alkali and with a power of 90%, the L*, C* and h* were 20, 18 and 50, respectively, while they were 14, 15 and 45 when treated with K_2CO_3, and 9, 6 and 40 when treated with NaOH.

In terms of the effect of power and duration of the microwave treatment on the color of alkalized samples, both exerted a slight reduction effect on cocoas treated with K_2CO_3 and NaOH (Figure 19B and C), although the main variable significantly reducing the color was the alkali agent.

3.3. Impact of duration and power, and of alkali type and concentration on the functional characteristics

In this section, the effect of power and duration were evaluated on the antioxidant activity, total phenol content and concentrations of catechin and epicatechin.

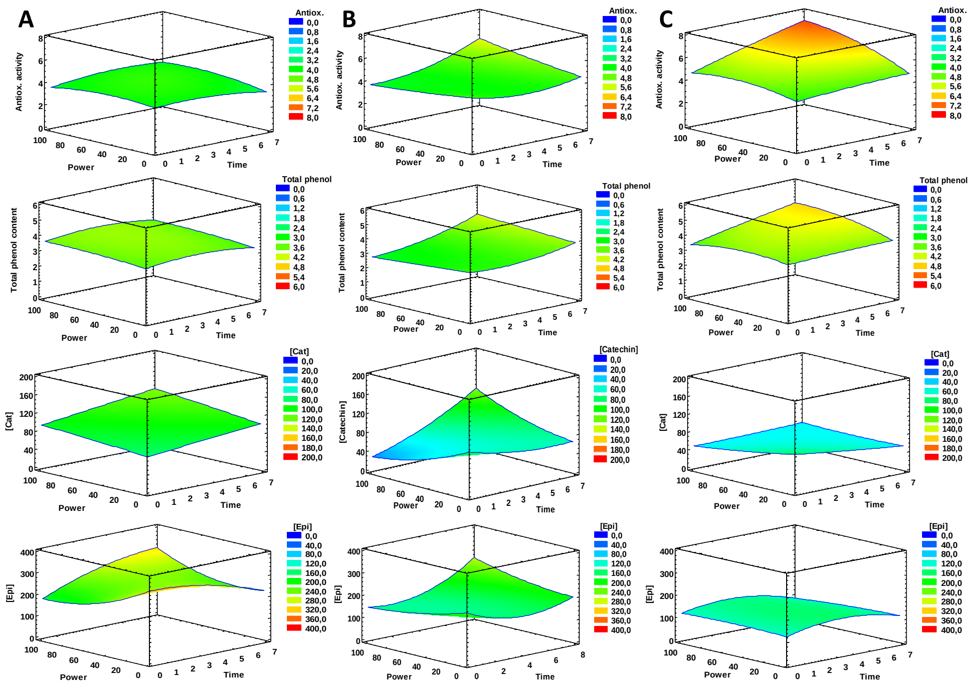

Figure 20. Response surface graphics of the functional attributes produced by different types of alkalis and power-time combinations. (A) Pressurized samples treated without alkali, (B) pressurized samples treated with K_2CO_3 and (C) pressurized samples treated with NaOH. Alkali concentration of the samples with alkali was 6% and water content of 30%. Data expressed in grams by 100 g of cocoa for the antioxidant activity and the total phenol content, and in milligrams by 100 g of cocoa for catechin and epicatechin.

Figure 20 shows the effect of power and duration combined with alkali on the functional characteristics of the alkalized cocoas.

In general, microwave heating alkalization produced slight reductions of almost all the evaluated characteristics (Figure 20A, B and C). Non-treated cocoa had an antioxidant activity of 4.7±0.5, a total phenol content of 4.4±0.3 and concentrations of catechin and epicatechin of 103±5 and 392±12, respectively. When that cocoa was treated by the developed microwave heating treatment without an alkali, its values were reduced to 4.5, 4, 90 and 200 at mild conditions (3.5 min and 50% of power). The previous losses were even higher when K_2CO_3 and NaOH were included. For example, application of 3.5% of these alkalis at mild conditions led to an antioxidant activity, total phenol content and concentrations of catechin and epicatechin of 3.7, 3.1, 84 and 168 with K_2CO_3, and of 5.7, 4.1, 66 and 216 with NaOH, respectively. As can be seen, all the features were lower than the ones of untreated cocoa with one exception: the antioxidant activity in samples treated with NaOH. In these samples, the antioxidant activity was significantly higher than in natural cocoa.

In terms of power and duration, any of this features had an impact on the antioxidant activity and the total phenol content in samples treated without an alkali (Figure 20A). In the case of the main polyphenols in cocoa, increases in power and duration led to slight increases of catechin and epicatechin.

When the samples were treated with K_2CO_3 (Figure 20B), the combination of high powers and long durations led to a slightly higher antioxidant activity, total phenol content and concentrations of the two main polyphenols in comparison to the non-alkalized cocoa. From soft conditions (10% of power and 1 min) to the harsh ones (90% of power and 6 min), the antioxidant activity, total phenol content and concentrations of catechin and epicatechin changed from 4.2, 3.2, 80 and 188 to 5.1, 3.9, 87 and 192 mg/100 g, respectively.

In the case of NaOH (Figure 20C), the combination of power and duration led to increased antioxidant activities and total phenol contents, and to reduced catechin and epicatechin concentrations. The lack of correlation between the increases of the antioxidant activity/total phenol content and the decreases of the two main polyphenols in cocoa indicates that the observed increases in the first two functional characteristics may be related to other compounds whose concentration is increased by the treatment. On the other hand, in samples treated with K_2CO_3, the increases in catechin and epicatechin matched with the increases in total phenol content and antioxidant activity, which meant that those polyphenols were partially responsible of the increases of these features.

All the previous observed increases could be related to the release of polyphenols and antioxidant compounds from cells due to the combined effect of microwave heating and the alkalis. Some authors have reported that there are two groups of polyphenols in food: the extractable ones, the normally measured; and the non-extractable ones, which are linked to other compounds or trapped in the matrix. NaOH has been reported to break ester links and to hydrolyze cell walls, effects that combined with the action of the microwave could be responsible of the increases in the functional features (Gonzales et al., 2015; Domínguez-Rodríguez et al., 2017; Hayat et al., 2010). In respect to K_2CO_3, the combined effects of alkali with microwave heating could also explain the observed increases, although its effects were lower than the ones of NaOH.

3.4. Effect of power and duration, and of alkali type and concentration on the moisture content

The last characteristic that was evaluated was moisture content in relation to power, duration and also alkali.

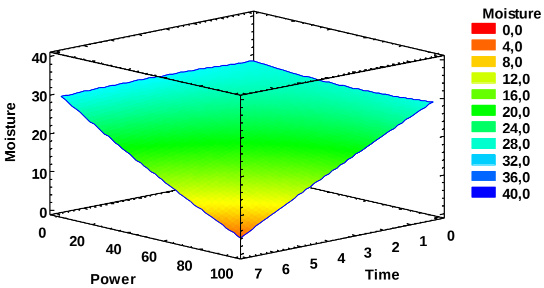

Figure 21. Surface response that shows the evolution of moisture content according to microwave heating power and duration. Initial water content was 30%, the samples were pressurized and alkali concentration was 6%.

Figure 21 shows a general surface response that illustrates the drying capacity of microwave heating. Only one surface is shown because the results for the samples treated without alkali, with K_2CO_3 and with NaOH were similar.

Microwave heating was able to reduce the added water content and natural cocoa moisture from the initial 37% (value obtained from summing the water content and the moisture of the powder) to values lower than 8% at high powers and durations. This high reduction of moisture content is a positive feature that leads to less drying durations and costs of the alkalized cocoa, which are important industrial advantages that increase the sustainability of the general process.

4. Conclusions

The influence of power and duration of a novel cocoa alkalization method based on microwave heating are shown in this work. In non-alkalized cocoas, power and duration has a slight, almost null, effect on pH, color and functional properties of the samples. When an alkali, especially NaOH, was included during the treatment, the effect of the microwave variables were enhanced and increases in the antioxidant activity and in the total phenol content were found. These increases were related to the release of non-extractable polyphenols from cocoa. In addition, a significant reduction of the moisture content was observed in the treated samples as power and duration were raised.

In general, this work has shown that the combination of the effects of alkali agent and microwave heating increases the functionality of cocoa at the same time that significantly dries the product, making interesting the application of this technique to cocoa alkalization.

Acknowledgements

The authors would like to acknowledge the financial support of the Spanish Government and European Regional Development Fund (Project RTC-2016-5241-2). Damián Valverde would also like to thank the Regional Ministry of Education, Culture and Sports of Generalitat Valenciana for his PhD grant.

References

Afoakwa, E. O. (2011). Chocolate science and technology. John Wiley & Sons.

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

Arranz, S., Saura-Calixto, F., Shaha, S., & Kroon, P. A. (2009). High Contents of Nonextractable Polyphenols in Fruits Suggest That Polyphenol Contents of Plant Foods Have Been Underestimated. Journal of Agricultural and Food Chemistry, 57 (16), 7298-7303. https://doi.org/10.1021/jf9016652

Bai, X. L., Yue, T. L., Yuan, Y. H., & Zhang, H. W. (2010). Optimization of microwave-assisted extraction of polyphenols from Apple pomace using response Surface methodology and HPLC analysis. Journal of Separation Science, 33, 23-24. https://doi.org/10.1002/jssc.201000430

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

Bouras, M., Chadni, M., Barba, F. J., Grimi, N., Bals, O., & Vorobiev, E. Optimization of microwave-assisted extraction of polyphenols from *Quercus bark*. Industrial Crops and Products, 77, 590-601. https://doi.org/10.1016/j.indcrop.2015.09.018

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

Dahmoune, F., Nayak, B., Moussi, K., Remini, H., & Madani, K. (2015). Optimization of microwave-assisted extraction of polyphenols from *Myrtus communis* L. leaves. Food Chemistry, 166, 585-595. https://doi.org/10.1016/j.foodchem.2014.06.066

De Zaan cocoa, (2006). Cocoa & Chocolate manual. ADM Cocoa, Switzerland.

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. https://doi.org/10.1016/j.chroma.2017.07.066

Firihu, M. Z., & Sudiana, N. (2016). 2.45 GHz microwave drying of cocoa bean. ARPN Journal of Engineering and Applied Sciences, 11 (19), 11595-11598.

Gonzales, G. B., Raes, K., Vanhoutte, H., Coelus, S., Smagghe, G., & Camp J. V. (2015). Liquid chromatography–mass spectrometry coupled with multivariate analysis for the characterization and discrimination of extractable and nonextractable polyphenols and glucosinolates from red cabbage and Brussels sprout waste streams. Journal of Chromatography A, 1402, 60-70. https://doi.org/10.1016/j.chroma.2015.05.009

González-Nuñez, L. N., & Cañizares-Macías, M. P. (2011). Focused microwaves-assisted extraction of theobromine and caffeine from cacao. Food Chemistry, 129 (4), 1819-1824. https://doi.org/10.1016/j.foodchem.2011.05.118

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

Guda, P., Gadhe, S., & Jakkula, S. (2017). Drying of cocoa beans by using different techniques. International Journal of Agriculture Innovations and Research, 5 (5), 859-865.

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

Hartwig, C. A., Pereira, R. M., Rondan, F. S., Cruz, S. M., Duarte, F. A., Flores, E. M. M., & Mesko, M. F. (2016). The synergic effect of microwave and ultraviolet radiation for chocolate digestion and further determination of As, Cd, Ni and Pb by ICP-MS. Journal of Analytical Atomic Spectrometry, 31 (2), 523-530. https://doi.org/10.1039/C5JA00388A

Hayat, K., Hussain, S., Abbas, S., Farooq, U., Ding, B., Xia, S., Jia, C., Zhang, X., & Xia, W. (2009). Optimized microwave-assisted extraction of phenolic acids from citrus mandarin peels and evaluation of antioxidant activity *in vitro*. Separation and Purification Technology, 70 (1), 63-70. https://doi.org/10.1016/j.seppur.2009.08.012

Hefnawy, T. H. (2011). Effect of processing methods on nutritional composition and anti-nutritional factors in lentils (*Lens culinaris*). Annals of Agricultural Sciences, 56 (2), 57-61. https://doi.org/10.1016/j.aoas.2011.07.001

Hellström, J. K., & Mattila, P. H. (2008). HPLC Determination of Extractable and Unextractable Proanthocyanidins in Plant Materials. Journal of Agricultural and Food Chemistry, 56 (17), 7617-7624. https://doi.org/10.1021/jf801336s

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://dx.doi.org/10.1186%2F1752-153X-5-53

Kealey, K., Snyder, R., Romanczyk, L., Geyer, H., Myers, M., Whitacre, E., Hammerstone, J., Schmitz, H. (1006). Dry drink mix and chocolate flavored drink made therefrom. US20020064584A1.

Khan, N., Monagas, M., Andres-Lacueva, C., Casas, R., Urpí-Sarda, M., Lamuela- Raventos, R. M. (2012). Regular consumption of cocoa powder with milk increases HDL cholesterol and reduces oxidized LDL levels in subjects at high-risk of cardiovascular disease. Nutrition, metabolism and cardiovascular diseases, 22 (12), 1046-1053. https://doi.org/10.1016/j.numecd.2011.02.001

LeFreniere, R. C., William R., Lynch, W.R., & Wiant, M. J. (1991). Method for producing deep red and black cocoa. US5009917A.

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

Li, Y., Skouroumounis, G. K., Elsey, G. M., & Taylor, D. K. (2011). Microwave-assistance provides very rapid and efficient extraction of grape seed polyphenols. Food Chemistry, 129 (2), 570-576. https://doi.org/10.1016/j.foodchem.2011.04.068

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., 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

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

Matsui, K. N., Granado, L. M., Oliveira, P. V., Tadini, C. C. (2007). Peroxidase and polyphenol oxidase thermal inactivation by microwaves in Green coconut water simulated solutions. LWT- Food Science and Technology, 40, 852-859. https://doi.org/10.1016/j.lwt.2006.03.019

Miller, K. B., Hurst, W. F., 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

Niemenak, N., Rohsius, C., Elwers, S., Ndoumou, D. O., & Lieberei, R. (2006). Comparative study of different cocoa (*Theobroma cacao L.*) clones in terms of their phenolics and anthocyanins contents. Journal of Food Composition and Analysis, 19 (6-7), 612-619. https://doi.org/10.1016/j.jfca.2005.02.006

Pan, X., Niu, G., & Liu, H. (2003). Microwave-assisted extraction of tea polyphenols and tea caffeine from Green tea leaves. Chemical Engineering and Processing: Process Intensification, 42 (2), 129-133. https://doi.org/10.1016/S0255-2701(02)00037-5

Payne, M. J., Hurst, W. J., Miller, K. B., Rank, C., Stuart, D. A. (2010). Impact of fermentation, drying, roasting, and Dutch processing on epicatechin and catechin content of cacao beans and cocoa ingredients. Journal of Agricultural and Food Chemistry, 58 (19), 10518-10527. https://doi.org/10.1021/jf102391q

Rother, M., Himberg, M., Gaukel, V., & Schuchmann, H.P. (2009). Microwave roasting low loss factor microwave applications. 12th International Conference on Microwave and High Frequency Heating, AMPERE, 403-406.

Santos, H. O., Macedo, R. C. O. (2018). Cocoa-induced (Theobroma cacao) effects on cardiovascular system: HDL modulation pathways. Clinical Nutrition ESPEN, 27, 10-15. https://doi.org/10.1016/j.clnesp.2018.06.001

Sharma, P., & Gujral, H. S. (2011). Effect of sand roasting and microwave cooking on antioxidant activity of barley. Food Research International, 44 (1), 235-240. https://doi.org/10.1016/j.foodres.2010.10.030

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

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

Todorovic, V., Redovnikovic, I. R., Todorovic, Z., Jankovic, G., Dodevska, M., & Sobajic, S. (2015). Polyphenols, methylxanthines, and antioxidant capacity of chocolates produced in Serbia. Journal of Food Composition and Analysis, 41, 137-143. https://doi.org/10.1016/j.jfca.2015.01.018

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

6. GENERAL DISCUSSION

In the present thesis, two alternative alkalization methods have been designed, applied and evaluated as substitutes of the traditional treatment employed by the cocoa industry. The conventional system is based on the use of pressurized reactors in which cocoa is mixed with an alkali solution and treated with a combination of temperature and pressure. In general, it is characterized for having high energy consumption, long duration (needing, according to the conditions, some hours) and for being aggressive with the functional and nutritional components. This thesis aims to develop and apply two alternative techniques for improving the traditional alkalization treatment, to study the effects of these new methods on cocoa and to evaluate their suitability for darkening the raw material while maintaining or improving the physicochemical, functional and sensory features in comparison to the commercial products. The two technologies assayed in this work were: extrusion and microwave heating.

In **Chapter I**, extrusion was assessed as a fast, less energy-consuming and continuous alkalization method in comparison to the conventionally employed one. In general, during extrusion, cocoa can be treated with temperature, pressure and with the addition of an alkali, emulating the traditional alkalization treatment, which made interesting its application to cocoa alkalization.

The extruder consists of a thermal horizontal tube with one or even two screws inside. When a powdered material is introduced, it is mixed by the action of the screw, cooked by the heating blocks and compressed until the mass is released through the die.

Extrusion as an alkalization treatment has been previously reported, although its effects on the features of cocoa have not been studied yet. In this chapter, it is aimed to describe the impact of a newly designed extrusion treatment on the physicochemical and functional characteristics of cocoa. The results obtained were divided in two articles: one evaluating the physicochemical changes of cocoa due to extrusion (article 1) and the other studying the evolution of the functional characteristics of the material (article 2). At the same time, both articles were divided into two sections: the first one aimed to analyze the effects of extrusion variables on the features of cocoa and the second one to compare the features of the extruded materials with those of commercial ones.

The first step of the work with the extruder, not included neither in article 1 nor 2, was to confirm if our extrusion method was able to dark cocoa. The preliminary tests showed that the color coordinates of the extruded samples were highly reduced down to values similar to the exhibited by the traditionally alkalized cocoas, which allowed to further study the effects of the technique.

Once the capacity of extrusion for darkening cocoa was validated, the effects of the different treatment variables (water content, temperature, alkali type and concentration) were evaluated on the color, pH and moisture of the material (article 1), and also on the antioxidant activity, the total phenol content and in the concentration of ten different polyphenols (article 2).

From all the variables, the alkali type (NaOH or K_2CO_3) and concentration were the main ones increasing the pH, reducing the color coordinates (L*, C* and h*) and decreasing the concentration of the different analyzed polyphenols, with the exception of vitexin and hyperoside.

The alkali is an important factor for its relation to the pH. In fact, pH is an essential characteristic during cocoa alkalization because the generation of an alkaline media enhances several chemical reactions that lead to the different changes observed after alkalization.

From the two alkalis employed in this work, NaOH was the agent reaching the lowest colors and concentrations of catechin, epicatechin, their oligomers and other polyphenols, and the highest pH values. With respect to the antioxidant activity and the total phenol content, the concentration of NaOH did not show to have any correlation with these characteristics, while K_2CO_3 increased both features at harsh conditions (high temperatures and concentrations of alkali). Although NaOH has been reported to release non-extractable polyphenols from other matrixes, the combination of this alkali with extrusion seems to lead to a degradation that counteracts its releasing effects.

In the case of the other studied variables, the increases of water content showed to produce a light reduction of the antioxidant activity and the total phenol content, and to reduce the dimer B2 and trimer C1 concentrations. In terms of the increases in temperature, it incremented the pH, slightly reduced the color of the material and increased the antioxidant activity and the concentrations of the two mentioned polyphenols.

When the extruded powders were compared to the traditionally produced cocoas, it was observed that the color of the extruded samples classified as natural, light and medium alkalized were clearly darker and less saturated than their commercial references. On the other hand, the strong alkalized and extruded cocoas had a similar color than their references, although some of them were darker, redder and less saturated. This showed that the extruder, in less than five minutes, was able to dark cocoa as much as the traditional method.

In terms of the functional characteristics of the extruded cocoas, the antioxidant activity and the total phenol content were maintained and even slightly increased in comparison to the commercial cocoas. On the other hand, although the previous general functional markers remained mostly unchanged, the two major polyphenols in cocoa, catechin and epicatechin, and their oligomers had a generally lower concentration in the alkalized extruded samples than in the commercial cocoas. In the case of the other studied polyphenols, clovamide was the only one whose concentration was increased by the treatment. The increase in concentration of this polyphenol is an example of a compound that explains the observed maintenance of the antioxidant activity and the total phenol content. In general, alkalization by extrusion, although being faster, continuous, more sustainable and capable of reaching darker colors than the traditional method, is not able to improve the functional characteristics of the conventionally alkalized cocoas.

Finally, the extruded cocoas were sensory evaluated in order to determine the effect of the alkalization by extrusion on the flavor of cocoa. The sensory profiles revealed that the extruded products were similar to their commercial references. This remarks the suitability of extrusion to replace the conventional treatment, because it has reached a similar color, pH, functional content and sensory perception, while being faster, less energy-consuming and continuous.

In **Chapter II**, a newly unreported microwave heating alkalization treatment for cocoa is described. It is based in the traditional alkalization method, but replacing the conventional heating source by a microwave one. Chapter II is formed by two different articles. Both works, due to the absence of information regarding the application of microwave heating to alkalization, were preceded by the stablishment of the treatment limits of the novel designed process for avoiding the burning and the consequent generation of off-flavors. Once they were stablished, a set of samples was produced by keeping the power and the duration of the treatment fixed at 648 W and 4 minutes, respectively. The effects of the treatment variables (water content, temperature and alkali type and concentration) were evaluated on the physicochemical and functional properties (article 3). In addition, the effects of the power and the duration of the technique were studied in article 4 by keeping the water content at 30% and applying pressure during it, because article 3 showed that these conditions were the most desirable ones.

With respect to the effects of the different variables, as was observed with extrusion, alkali type and concentration were responsible for almost all the desired physicochemical changes. They reduced the color values of cocoa and increased the pH. These variables were able to improve the antioxidant activity and the total phenol content of the alkalized samples. However, the concentrations of catechin and epicatechin were reduced.

From the employed alkalis, as was observed in Chapter I, NaOH was the one leading to the lowest color, catechin and epicatechin concentrations, and to the highest pH, antioxidant activity and total phenol content values. The ability of NaOH to reach the highest color reductions and antioxidant activities is, as previously said, related to the higher reached pH and to the biochemical reactions that are enhanced under basic conditions.

In absence of alkali, power and treatment duration only had light, or in most situations null, effect on the pH, color and functional features (article 4). A slight decrease of L*, C* and h* was found when comparing the microwave alkalized cocoas with the non-treated ones, although that reduction was associated with the humidification and post-treatment drying of the material.

In cocoas treated with K_2CO_3 or NaOH, the power and the duration of the treatment did not exert any effect on the color and the pH, although their combination leaded to general increases in the antioxidant activity and total phenol content. When the microwave treatment was combined with K_2CO_3, increases in catechin and epicatechin were observed and showed a well correlation with the increases of the antioxidant activity and the total phenol content. When the effects of NaOH were combined to the ones of the microwave thermal treatment, the increases in antioxidant activity and total phenol content were the highest, even overpassing the values of non-treated cocoa. However, the concentrations of catechin and epicatechin were the lowest ones among all the studied situations. The combined effects of NaOH and the microwave heating treatment could be behind these increases, in special, of the antioxidant activity. In the case of K_2CO_3, its combination with the microwave treatment could also explain the observed increases, although they were lower than those detected with NaOH.

In addition to the effects of the power and the duration on the previous characteristics, their impact on the moisture content was also studied. Increases of these two variables leaded to a moisture loss that reached values over 70%. This, besides of showing that the power and the duration were the main variables affecting this characteristic, also showed that microwave heating was, as the extruder, able to dry the samples, which may speed up the whole process and reduce its associated economic and energy consumption costs.

Other analyzed variables of the treatment were the pressure and the water content (article 3). In terms of pressure, its application had a significant effect on the antioxidant activity, which was increased with the application of pressure, and on the moisture content, which was also increased in pressurized samples. In the case of the water content, in general, it had a light effect on the different evaluated characteristics, being just relevant for the moisture content, which was lower as the added water content was lowered.

In addition to evaluate the impact of the different variables, the cocoa powders treated by the microwave were also compared to the traditionally produced ones in their physicochemical, functional and sensory characteristics. Microwave heating showed to be able to reach similar color and pH values than the commercial cocoas in four minutes. In comparison to extrusion, despite using the same alkali concentrations, the microwave-treated powders did not reach such dark colors.

The proposed microwave heating treatment, in addition to dark cocoa, was also able to preserve similar and even to increase the antioxidant activity and total phenol content in comparison to untreated powders. This was an unexpected result. Alkalization has been shown as a treatment that significantly reduces the functional features. The traditional processing and extrusion showed to produce significant losses, but, during the microwave heating treatment, any reduction was observed in the antioxidant activity and total phenol content in comparison to the untreated cocoas. The release of non-extractable polyphenols and the inactivation of anti-nutritional factors were associated to the observed maintenance of the functional characteristics.

In terms of catechin, the microwave treated samples had a lower content at low alkalization levels and a similar one at the strong degree of Dutching in comparison to their reference commercial cocoas. In the case of epicatechin, this polyphenol had higher concentrations in almost all of the levels of alkalization, especially at the strong ones, in respect to the traditionally produced cocoas.

In general, alkalization is considered to reduce the nutritional and functional content of cocoa, although the present thesis has shown that the microwave alkalization method lead to the obtaining of dark cocoas without losing and even increasing the functional properties of the final product.

Finally, in relation to the sensory properties of cocoa, the samples produced by the microwave were compared to a set of commercial powders in terms of the alkalinity, acidity, bitterness, body, astringency and cocoa and chocolate tastes. The obtained results pointed out that microwave alkalization was able to produce cocoas sensory similar to the commercial ones. Furthermore, pressure application showed to reduce the undesired astringency, bitterness and acidity, and increase the cocoa and chocolate tastes exhibited by the non-pressurized samples, which made desirable to apply pressure during the treatment. In addition, an increased bitterness was observed as the alkalization intensity was increased, which may be related to the release of bitter compounds. This explains the increases of this flavor and also of the functional features that were reported to be raised by the pressure application.

As a conclusion, this thesis describes the development and characterization of two alternative techniques suitable for being good replacements of the traditional alkalization treatment. Both are fast, able to dark cocoa as much as the conventional method, to provide sensory characteristics similar to those of traditionally produced cocoas and to reduce the moisture content, which will speed up even more the general process and reduce its costs and energy consumption. In terms of preserving or improving the functional features of the material, microwave heating is more successful than extrusion, although it has to be taken into account that extrusion, while not preserving the functional properties of natural cocoa, is continuous, ready to implement and able to reach darker colors than the discontinuous microwave heating treatment.

7. CONCLUSIONS AND PERSPECTIVES

Conclusions

- Extrusion and microwave heating have shown to be suitable alternative methods for replacing the traditional alkalization treatment. Both are fast, less energy consuming, able to dark cocoas as much as the conventional method and capable of producing cocoas sensory similar to the commercial powders.
- In terms of preserving or improving the functional features of the material, the microwave heating method is more successful than extrusion, although it has to be taken into account that extrusion is a continuous method able to reach darker colors than the ones reached by the discontinuous microwave heating treatment.
- Microwave heating is capable of maintaining the antioxidant activity and the total phenol content of the untreated cocoa even at strong levels of alkalization, although the content of catechin and epicatechin are negatively affected.
- From the variables of the extrusion and microwave treatments, the alkali type and concentration are the two main variables affecting the features of cocoa during extrusion and microwave alkalization. Specifically, during the microwave treatment, they enhanced the effects of other variables such as the power and duration on the functional characteristics.
- From the employed alkali agents, NaOH is the one producing the darkest colors and the highest losses of the studied polyphenols, while maintaining or even increasing the antioxidant activity and the total phenol content.
- Release of non-extractable polyphenols and the production of new ones could be the cause of the observed behavior of the antioxidant activity and the total phenol content.
- Extrusion and microwave heating have reduced the moisture content up to 50% and 70%, respectively, which consequently reduce the drying durations and costs.

Future perspectives

- Development of pilot plant experiments to study the industrial suitability of the designed extrusion and microwave heating alkalization methods.
- Employment of conveyor belts coupled to an industrial microwave heating equipment for converting the developed discontinuous method into a continuous one.
- Study of the polyphenol profiles in order to reveal the specific changes induced by microwave heating and extrusion alkalization conditions.
- Evaluation of the effects of extrusion and microwave heating alkalization on cocoa microbiology.

8. SCIENTIFIC CONTRIBUTIONS

List of scientific papers

Valverde, D., Pérez-Esteve, É., Barat, J.M. Changes in cocoa properties induced by alkalization process: a review. Submitted to Comprehensive Reviews in Food Science and Food Safety.

Valverde, D., Sanchez-Jimenez, V., Pérez-Esteve, É., Barat, J.M. Development of a sustainable, fast and continuous cocoa alkalization method based on extrusion. Submitted to Innovative Food Science and Emerging Technologies.

Valverde, D., Behrends, B., Pérez-Esteve, É., Kuhnert, N., Barat, J. M. Functional changes induced by extrusion during cocoa alkalization. Submitted to Food Research International.

Valverde, D., Pérez-Esteve, É., Barat, J.M. Improving the functional profile of alkalized cocoas through microwave heating processing. Submitted to Food Chemistry.

Valverde, D., Pérez-Esteve, É., Barat, J.M. Effects of power and duration of the microwave alkalization method on the properties of alkalized cocoa powders. Submitted to Food Chemistry.

Patents

Barat, J. M., Pérez-Esteve, É., **Valverde, D.** R-20228-2018 - Alcalinización rápida de cacao mediante tecnología microondas (Reference number: P201930746). Registration date: 19-08-2019

Oral communications

Valverde, D., Sánchez-Jiménez, V., Pérez-Esteve, É., Barat, J. M. (2019). Extrusion as an alternative method for cocoa alkalization. Fifth International Congress on Cocoa, Coffee and Tea (Bremen, Germany).

Poster communications

Valverde, D., Puchol-Miquel, M., Lerma-García, M. J., Pérez-Esteve, É., Barat, J. M. (2019). Effect of the origin and the alkalization on the cocoa polyphenol profile. VI International Student Congress of Food Science and Technology (Valencia, Spain).

Valverde, D., Lerma-García, M. J., Pérez-Esteve, É., Fuentes, A., Barat, J. M. (2018). Identification of polyphenols extracted from olive stones by HPLC-MS. 4th International & 5th National Student Congress of Food Science and Technology (Valencia, Spain).

Valverde, D., Iannello, M. C., Ruiz-Rico, M., Pérez-Esteve, É., Lerma-García, M. J., Barat, J. M. (2018). Obtention of an olive leaf extract rich in antioxidants and its encapsulation in silica supports. 4th International & 5th National Student Congress of Food Science and Technology (Valencia, Spain).

Valverde, D., Iannello, M. C., Bernardos, A., Lerma-García, M. J., Pérez-Esteve, É., Barat, J. M. (2017). Development of an enzyme-responsive nanocontainer for folic acid controlled release in the small intestine. XI International Workshop on Sensors and Molecular Recognition (Valencia, Spain).

Iannello, M. C., **Valverde, D.**, Ruiz-Rico, M., Lerma-García, M. J., Pérez-Esteve, É., Barat, J. M. (2017). Encapsulation of olive leaf extract in capped-mesoporous silica particles. XI International Workshop on Sensors and Molecular Recognition (Valencia, Spain).

Predoctoral stay in a foreign institution

Predoctoral stay in Kuhnert Group from Jacobs University (Bremen, Germany). From May to July of 2019. Learning to analyze samples employing HPLC-MS equipment and to interpret big data sets.