UNIVERSITÀ DEGLI STUDI DEL MOLISE

Department of Agricultural, Environmental and Food Sciences

International PhD Course in:

AGRICULTURE, TECHNOLOGY AND BIOTECHNOLOGY

(CURRICULUM: FOOD SCIENCE, TECHNOLOGY AND BIOTECHNOLOGY)

(CYCLE XXXII)

Related disciplinary scientific sector: AGR/15 (Scienze e Tecnologie Alimentari)

PhD Thesis

Green extraction of polyphenols from cocoa shells and microencapsulation to produce a functional chocolate bar

Coordinator of PhD Course: Prof. Giuseppe Maiorano

Supervisor: Prof. Luciano Cinquanta

Co-Supervisor: Prof. Emanuele Marconi

PhD Student: Melania Grassia

159267

Academic Year 2018/2019

ABSTRACT

Il presente progetto di dottorato ha coinvolto l'Università del Molise, l'azienda Dolceamaro srl e l'Università di Ankara (Turchia), come partner estero. Lo scopo del progetto è stato quello di realizzare una tavoletta di cioccolato funzionale arricchita di polifenoli, estratti dal pericarpo delle fave di cacao. Le materie prime migliori, a disposizione dell'azienda, sono state lavorate per ottenere la massa di cacao. Dagli scarti di lavorazione, invece, sono stati estratti i polifenoli con un metodo "green". Tali composti sono stati incapsulati con spray dryer per preservarli dalle avverse condizioni di lavorazione (luce, ossigeno e temperatura) e mascherarne il sapore amaro e astringente. Il processo di microincapsulazione è stato ottimizzato scegliendo la combinazione migliore dei seguenti parametri: velocità del flusso in entrata, rapporto core:coating agent e temperatura di atomizzazione. Le capsule sono state successivamente aggiunte alla massa di cacao per realizzare una tavoletta funzionale di cioccolato fondente.

Il prodotto è stato sottoposto a un ciclo d'invecchiamento accelerato per valutarne la stabilità e le proprietà chimico-fisiche nel tempo e rispetto a una tavoletta standard. Infine, sono state analizzate le caratteristiche organolettiche. Il prodotto ottenuto ha mostrato dei difetti nell'aspetto ma non nel gusto. Inoltre i polifenoli, in numero maggiore rispetto allo standard, si sono conservati più a lungo.

Il progetto di dottorato rappresenta uno studio preliminare per la valorizzazione di un sottoprodotto dell'industria del cacao nella stessa filiera produttiva (economia circolare).

The PhD project involved the University of Molise, Dolceamaro srl Company and the University of Ankara (Turkey) such as foreign partner. The aim was to produce a functional chocolate bar enriched with polyphenols extracted by cocoa shells. At the beginning the better raw materials of the Company were selected in order to produce cocoa mass. Moreover, the manufacturing waste were used like a source of polyphenols, extracted by green technique. They were encapsulated using spray dryer technique for preserving from the adverse process conditions (light, oxygen, high temperature) and masking their bitter taste. The process was optimized choosing the best combination of feed flow rate, core: coating ratio and inlet temperature. Then capsules were added to cocoa mass to produce dark chocolate bar.

The product was submitted to accelerated test in order to evaluate its stability during time, compared with a standard sample. Finally, the organoleptic characteristic was evaluated by sensorial analysis. The functional chocolate bar presented some defects in the appearance but not in the taste. Furthermore polyphenols, that are more than standard sample, were preserved better over time.

The PhD project was a preliminary study for the technology development of a waste recovery process in chocolate industry, according with circular economy.

TABLE OF CONTENTS

INTRODUCTION

Project partner

Dolceamaro srl is an important confectionery company placed in Molise (Italy). The company was founded in 1975 by Pietro and Rosa Papa, who started manufacturing Jordan almonds. It has been continued due to the action of their sons Claudio and Silvano. After few years, they added chocolate products line, including chocolate moulds and dragee. At the same time, they develop a new single wrapped production line, which includes cookies. In 2012, Dolceamaro made a new product, in order to meet the increasing market demands: the macaroon.

Today, there are 5 production lines: the production and packaging of chocolate moulds, dragee, sugar coated products, macaroon and the packaging of cookies. The main target markets of the Company are Department Store Retail, Large Retailing, Travel & On board Retail, Food Service, Normal Trade and Event and Ceremonies Market. Its turnover is 7.800 K€. The company size is 4000 m^2, organized in 3 structures and it consists in about 60 employees. At present, the company is increasing its sizes, building new production facility, buying new machineries, investing in technological innovation. Dolceamaro wants to differentiate itself in the marketplace, improving the quality of products and the effectiveness of its processes. Nowadays, the mission of the company is the supply chain integration with research and development sector in order to satisfy consumer needs, enhancing the culture and the human value of the territory. Dolceamaro collaborates with various institutes and departments in order to carry on several projects for the design of innovative products. The research actives are based on the values of environmental sustainability, the healthy nutrition education and the high quality of raw materials such as cocoa beans and almonds. Furthermore, the growing demand for healthy products consumers drives the company to the production of functional foods. Currently the traditional confectionery market slows in Europe and North America. The market strategy to overcome this decreasing could be the developing of functional products targeting the healthy food.

Project main objective

The current food system is considered “linear” because the manufactures use finite resources to produce food with a consequence wasteful and pollution. However, this model involves huge costs and it can’t support the fast growing population, the economical developments and urbanization. For this reason, the new economy approach is based on the reducing or the reusing of industrial food waste with benefits such as efficiency and profitability of the processes. The circular economy is a model to preserve and valorize the resources as long as possible and it is included in the UN Sustainable Developments Goals for 2030 (https://sustainabledevelopment.un.org/).

The confectionery industry produces approximately up to 625 tones waste/year. They include un-used raw materials, wastes from the pre-processing phases (e.g. shelling, forming chocolate…) and production wastes (e.g. dough, chocolate mass…)
(https://www.wasteroadmap.co.za/download/6_UCT_CSIR_Final_Report.pdf).

Cocoa shells are the external part of the cocoa beans and they constitute about 11% of the annual cocoa production. Shells are usually considered a waste of chocolate manufacturing because they are indigestible for human diet and dangerous for animal feed due the high theobromine content. Therefore, they are left on the cocoa plantation and used like a constituent of fertilizers (Minifie, 1999). The composition of shells suggests using them like a source of high value extracts: polyphenols. Cocoa polyphenols are bioactive compounds with potential benefits for human health due their antioxidant activity.

The majority of published papers are related to classic extraction of cocoa shells that require large amounts of organic solvents, long-time of extraction and high energy consumption (Balentić et al., 2018). For example, Hernández-Hernández et al. (2018) compared different traditional extraction methods of cocoa shells, showing that the methanol/water extraction leads the highest yield of total polyphenols. However, this system is not adapted to extract compounds that will be added in food. The present research aims to develop an efficient alternative technique that promotes sustainable processes using no toxic solvent and renewable plant material to extract a safe and high quality product.

However, during the chocolate processes, about 90% of the initial content will be lost because of the working conditions (high temperature, oxygen contact…). In this study, a part of the lost amount of polyphenols have been recovered from shells and added in the final product.

Several studies have already described the incorporation of these biocompounds in a food matrix in order to improve its healthy and organoleptic properties. For instance, Pasrija et al. (2015) added green tea polyphenols into bread, observing an improvement of its quality characteristics and functionality. In its study, Pešić et al. (2019) suggested that the enrichment of meat- and cereal-based products with grape polyphenol extracts is a good strategy to formulate a healthier diet.

However, this project is more ambitious, because the extracted polyphenols will enrich chocolate to produce a functional bar. Therefore, it represents an example of circular economy due the recovery of a waste of chocolate manufacturing in the same production line in order to valorize the final product.

CHAPTER 1 GREEN EXTRACTION OF POLYPHENOLS FROM COCOA SHELL

An introduction to cocoa

Cocoa is the crop of *Theobroma cacao* (C. Linneo, "food of God") and belongs to *Sterculiaceae* spp. The tree grows in equatorial area, between tropics of Cancer and Capricorn, because requires high temperature (18-35°C) and humidity (80%) and annual rainfall (up to 2000 mm) (Afoakwa, 2011). Another important aspect for its development is the indirect exposition to the light of sun, so it grows protected by taller plants (banano, coconut…). Cocoa tree also needs of deep well-drained soil that is porous and rich in nutritive substances (https://www.britannica.com).

Generally, the young plants are cultivated in a nursery until they will be transferred in the plantation. They are placed at suitable distance from each other (3 meters) and can reach 15 m of height. In the plantation cocoa trees are maintained at 5 m to facilitate the harvest (Fig.1a). The flowers are produced in clusters directly on the trunk of the tree and only a few of them will develop a fruit. The cocoa pod is egg-shaped, 20-30 cm large and 20 cm long, with a different color depending on its variety and the maturation state. Inside the fruit there are a great number of seeds, called beans, covered with a sweet white pulp (Fig.1b).

Fig.1- Cocoa plantation (a) and cocoa pods (b) (https://www.123rf.com)

The origin of cocoa is dated back to 12000 b.C but around 10000 b.C two main genetic groups, ''Criollo'' (*Theobroma cacao cacao*) and ''Forastero'' (*Theobroma cacao spherocarpa*) have been originated on the both sides of the Amazonian river: the Ande and Brazil sides respectively. A third group, called Trinitario, derived from the cross-breeding between Criollo and Forastero.

In 2008, Motamayor et al. suggested 10 genetic clusters from which more than 12000 cocoa varieties were originated, but commercially the previous classification remain the most commonly used (Fig.2).

Criollo's variety constitutes only 1% of total cocoa production, because its survival is greatly affected from the adverse environmental conditions. The taste and flavor of cocoa are fine and delicate, rich in secondary notes of long duration. It represents an esteemed raw material and its price is a result of an agreement between the producers and the buyer. Usually cocoa Criollo is used for the production of high quality chocolate. At the opposite, Forastero is commonly used for the production of commercially chocolate and cocoa derivates; it represents the 90% of the total production because of its great resistance to diseases. Despite of its high yield, the quality isn't much appreciated: Forastero results to have a bitter taste and a few secondary aromas.

The price of this cocoa (for mass consumption) is established every day on the international market and listed on the London and New York stock market.

Finally, Trinitario, being a hybrid between the previous varieties, represents a good compromise between the yield and the quality of the cocoa

Criollo and Trinitario varieties for their valuable features are also identified like "flavoring" or "fine" cocoa (Caraceni, 2020). At the opposite "bulk" cocoa beans come from Forastero trees.

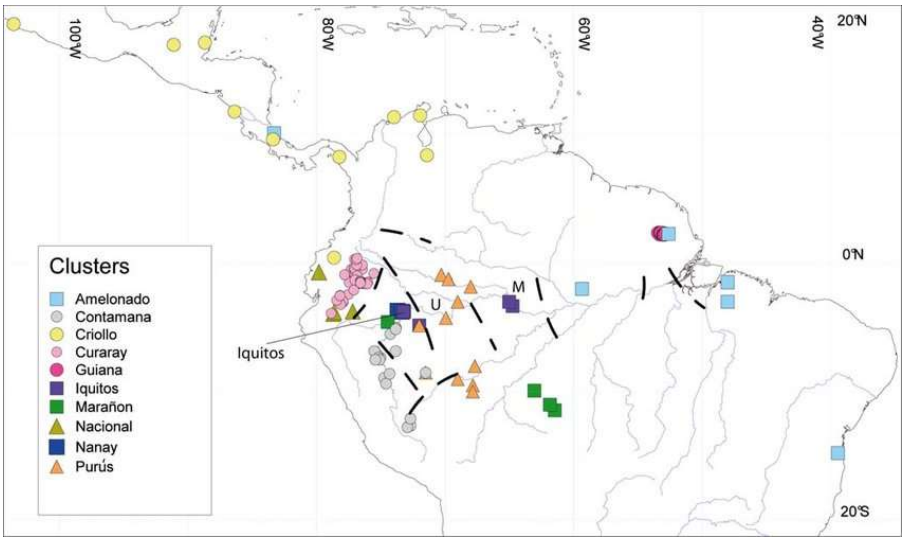


Fig.2- Geographic and genetic population differentiation of the original cocoa variety (Motamayor et al., 2008)

Cocoa manufacturing and byproduct

Cocoa harvest is spread twice a year, generally before and later rain season. The harvest time depends on the climate and variety of cocoa and it changes from country to country. The ripe pods are removed by cultivators manually by using a sharpened blade, called machete. Within ten days after the harvest, pods are opened to extract the beans (https://www.icco.org). Expert cultivators can open until 500 pods in an hour, paying attention to don't damage seeds. During this operation, there is a first beans selection, removing the defective ones. Cortex is left in the field and used as fertilizer, while beans are submitted to the next process: fermentation. The freshly extracted beans are placed on banana leaves inside baskets or in wooden boxes to fermentate. This process leads the germination and the

formation of aromas precursors of the cocoa. Fermentation lasts between 3 and 10 days, depending on the variety and specific features of the beans. It can be divided in two different steps: the anaerobic and aerobic phases. During the first 24-48h, yeasts liquefy the cocoa pulp, producing ethanol from carbohydrates; after this passage, alcohol diffuses into cotyledons and it will be oxidized into lactic acid by LAB (lactic acid bacteria) and then in acetic acid by AAB (acetic acid bacteria). The fermentation loads an increasing of temperature (from 25-30°C to 35-40°C) and the production of organic acids (succinic and acetic acids) and secondary metabolites such as higher alcohols, aldehydes, ketones and fatty acid esters, that contribute to the final cocoa flavors (De Vuyst & Weckx, 2016).

After fermentation, beans will be dried to reduce the moisture from 60% to 7.5%. This operation avoids the growth of spontaneous bacteria and moulds and prevents the development of off-flavors. Drying can be done naturally, thought sun energy, or artificially in greenhouses. Another consequence of this process is the development of the chocolate color of the beans, which depends on the reactions that form new compounds. Deus et al. (2018) showed that during cocoa drying there is a reduction of polyphenols due their oxidation at high temperature and moisture content, but this phenomenon is less relevant using traditional method (by direct sun light). After drying cocoa beans are stored in jute sacks and placed in a ventilated warehouse in order to prevent the moisture reabsorbing. Finally, with a few exceptions, cocoa beans reach the port of export, where they are loaded onto cargo vessels (https://www.icco.org). The standard method to evaluate the quality of beans is called “cut test”: they are longitudinally cut and submitted to visual inspection in order to identify the number of unfermented, moldy, split, insect damaged and flat beans. The first process of cocoa manufacture is the raw beans cleaning to remove stones and grit, metals and bean cluster (Minifie, 1999). After this phase, beans are roasted in order to sterile the product. The relationship between time and temperature affects the degree of cocoa roast: the thermal processing of cocoa beans varies in the range of temperature between 130 and 150 °C and for time between 15 and 45 min (Belitz et al., 2009; Krysiak, 2002, 2006). The main consequences of roasting are: the loss of bean moisture; the nibs became more friable, making shell cracking; the loss of volatile acids, that are produced during fermentation, reducing the bitterness and acidity; the development of aromas due the Maillard reaction, that involves amino acids, proteins partly denatured and reducing sugars. In some industry, instead of roasting the whole bean, members roast only nibs (Minifie, 1999).

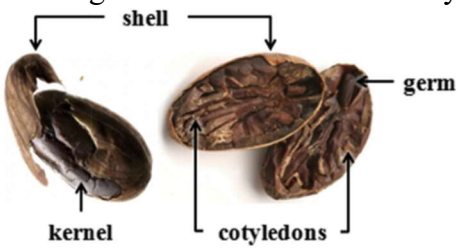


Fig.3- Cocoa bean composition (Okiyama et al., 2017)

Cocoa beans are composed by two cotyledons (the nibs) and a small embryo plant, all covered with a skin (Okiyama et al.,

2017) (Fig.3). Winnowing consists in the separation of the valuable part, nibs, from the shells, that are usually considered a byproduct of cocoa manufacturing.

After winnowing, nibs are reduced in liquor through grinding process that breaks cell walls, containing until 55% of fat. Into machine, frictional force generates heat that melts fats, making the paste more fluid. The viscosity of liquor depends on the degree of roasting and moisture content of the nib. The refined cocoa liquor is stored in tanks at about 90-100°C. Fat can be partially removed from liquor by using hydraulic presses. Depending on the pressure and the time of the treatments two kinds of cocoa cakes can be obtained:

1) High fat cake (22-24% of residual fat)
2) Low fat cake (10-12% of residual fat).

Cocoa butter extracted is then pumped in a different tank. After pressing, the remaining cake is grinded to produce cocoa powder (Afoakwa, 2011).

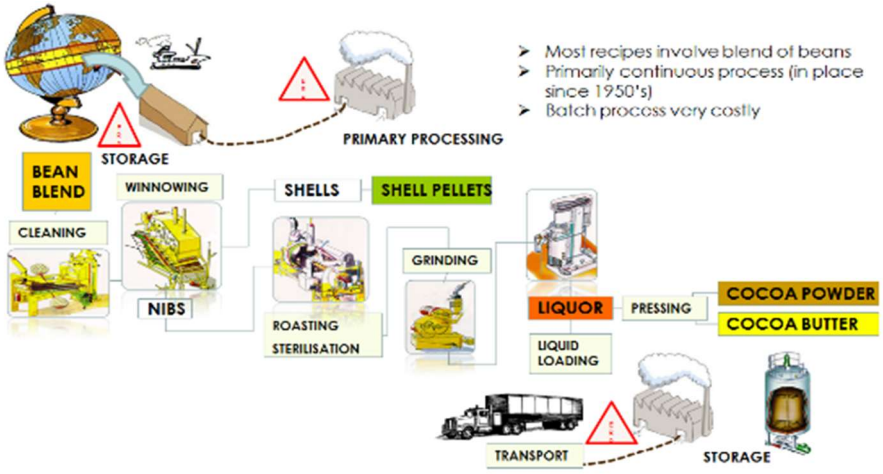


Fig.4- Cocoa manufacturing (https://www.eurococoa.com)

Cocoa chemical composition

Cocoa is usually consumed as hedonistic food and for its healthy properties in the western world (Zimmermann & Ellinger, 2020). A general chemical composition is reported in Tab.1.

Tab.1- Chemical composition of cocoa beans (Lima et al., 2011)

Constitutents	Roasted nibs	
Water[a]	3.0	3.7
Fat	54.0	54.0
Protein	12.5	
Starch	6.0	6.0
Fiber	2.5	2.5
Ash	3.0	2.8
Theobromine	1.3	1.3
Caffeine	0.2	0.1
Others	—	
References	(Valiente et al., 1994)	(Minifie, 1980)

[a]Varies according to the degree of drying and roasting.
*Nibs consist of shelled and ground cotyledons of commercial cocoa beans.

Lipids

Cocoa is composed by more than 50% of fat. In cocoa butter, fatty acids are organized as triacylglycerol (TAG), which included POP, POS, SOS. These TAG structures contain palmitic (C16), stearic (C18) and oleic (C18:1) acid. Its composition depends on the growing conditions of cocoa beans. Cocoa butter is a light yellow fat, with a melting point of 35°C. The manufacturing process and the characteristics of the final product (texture, viscosity, melting behavior, flavor and taste) are affected by fat properties (Afoakwa, 2010). Cocoa butter can crystallize in several different forms, according to the condition of cooling (Tab.2). The stability of these different forms affects the color, fat blooming and shelf life of the chocolate.

Tab.2- Polymorphic forms of cocoa butter (https://www.chemistryviews.org)

Crystal form	Formation conditions	m. p. [°C]
I	rapid cooling of the melt	17.3
II	rapid cooling of the melt at 2 °C/min	23.3
III	crystallization of the melt at 5–10 °C, converts into II at 5–10 °C	25.5
IV	crystallization at 16–21 °C	27.3
V	slow crystallization of the melt	33.8
VI	from form V after several months at RT	36.3

For chocolate production there are some vegetable fats that can be used as alternative to cocoa butter (max 5% of total fat). They can replace cocoa butter completely or in parts. Based on their composition and the consequently properties, fats are distinguished in:

(a) Cocoa butter equivalent (CBE): non-lauric (not containing lauric acid) plant fats, which are similar in their physical and chemical properties to cocoa butter and mixable with it in every amount without altering the properties of cocoa butter;

(b) Cocoa butter extender (CBEX): similar to CBE, but not mixable in every ratio with cocoa butter;

(c) Cocoa butter improvers (CBIs): with higher content in solid triglycerides than CBE, used for improving soft cocoa butters;

(d) Cocoa butter replacer (CBR): non-lauric fats with a distribution of fatty acid similar to cocoa butter, but with a completely different structure of triglycerides; they can't completely replace cocoa butter;

(e) Cocoa butter substitutes (CBSs): lauric plant fats (containing lauric acid), chemically totally different to cocoa butter, with some physical similarities; they can substitute cocoa butter to 100% (Lipp & Anklam, 1997).

Carbohydrates

Cocoa contains carbohydrates such as sugars and starch. Sugars are contained in the cotyledons of bean and included galactose, raffinose, stachyose, melibiose, sorbose, mannitol, inositol. Their amount depends on the origin and variety of cocoa and it is strongly reduced during fermentation due the absorption of the pulp. Sucrose decreases until near zero, whereas fructose and glucose increase correspondingly. During roasting, most of the reducing sugars disappear and non-reducing sugars decrease. The mean value of starch in cocoa bean is about 5.30% with 36% amylase. Its content is affected by harvesting and fermentation process. Finally, fiber constitutes about 17.8% and 16.1% in raw and roasted cocoa bean, respectively (Knight, 1999).

Proteins

Proteins are present only in small amounts in cocoa. They include: albumin, globulin, prolamine and glutenin. Their proportion changes with the bean variety and origin and due their reactions with polyphenols. A certain amount of protein nitrogen is present as free amino acids that are partly destroyed through thermolysis and Maillard reaction.

Minerals and vitamins

Cocoa is an excellent source of essential minerals such as calcium, copper, iron, manganese, magnesium, phosphorus, potassium, and zinc. Moreover, cocoa is not high in vitamins except for the shells that are rich of vitamins D2 (Rucker, 2008).

Alkaloids

Cocoa beans contain methylxanthines (about 4%) such as theobromine, caffeine and theophylline as traces (Franco et al., 2013). Researchers show that the ratio theobromine/caffeine depends on cocoa genotype. They are stored with polyphenols in a single large vacuole (Afoakwa, 2010) and their content decrease during fermentation due the migration from cotyledons to shells. During roasting, theobromine and caffeine form adducts with diketopiperazines that provide the specific bitterness of roasted beans and confer the astringency of chocolate. Methylxanthines are usually known owing to their bioactive potential and synergistic effects with flavanols (Langer et al., 2011).

Polyphenols

Cocoa is a rich source of polyphenols, which represent about 12-18% of dry weight of unfermented beans. They are stored in the pigment cells of the cotyledons (Wollgast & Anklam, 2000) and include flavan-3-ols (37%), anthocyanins (4%) and proanthocyanidins (58%). Catechins include monomeric forms, such as (-)-epicatechin, (+)-catechin, (+)-gallocatechin, (-)-epigallocatechin and the polymeric compounds called procyanidins (A or B forms), obtained by the link between the monomeric subunits. Procyanidins are represented by dimers (B1-B5), trimers (C1), and oligomers (until 10 subunits, D) (Barišic et al., 2019 & Andùjar et al., 2012) (Fig.5).

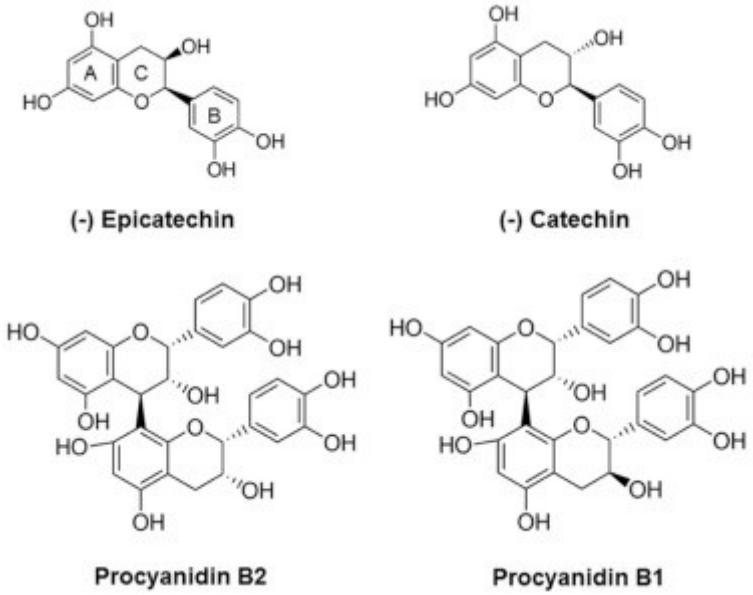


Fig.5- Structure of main cocoa polyphenols (Martìn & Ramos, 2016)

The composition of polyphenol content depends on the different types of cocoa, different countries of origin, and manufacturing condition (Maleyki & Ismail, 2010).

In the production of cocoa, the amount of flavonoids decreases of about 90% (Rusconi & Conti, 2010) due the oxidation and thermal effects of the fermentation, drying, roasting, alkalinization, and storage process (Langer et al., 2011). During fermentation polyphenols moved from cotyledons to the external parts of the seeds. Their content decreases due the hydrolysis and oxidative phenomena occurring during the first 24-48 h of fermentation (Sabahannur et al., 2018). This effect is significantly influenced by the pH and temperature achieved by seed (pH=6 and T=35.5°C), because these are the optimal conditions for the oxidase enzyme activity (Misnawi, 2003). Roasting also affects the destruction of polyphenol cells due their exposition to high temperature and oxygen (Wollgast & Anklam, 2000). Generally, the polyphenols content of cocoa products is very low by the higher temperature and longer processing time. Żyżelewicz et al. (2016) showed the great degradation of polyphenols, such as epicatechin and procyanidin B2, during roasting process at different operation conditions.

Cocoa polyphenols have many implications on human health due their antioxidant and antiradicals activities. Grassia et al. (2019) reports some of the main positive effects of polyphenols: the increasing of HDL-cholesterol in the human serum; the improving of blood pressure, inulin resistance, blood lipids and cognitive reactions; the reduction of coronary heart disease and diabetes.

Cocoa polyphenols have a different bioavailability in the human body: while the monomeric and dimer forms are well absorbed in a small intestine (Williamson, 2009 and Andùjar et al., 2012), oligomers larger than trimers improve the digestive process because they contribute to the formation of microflora into the colon (Gonthier et al., 2003).

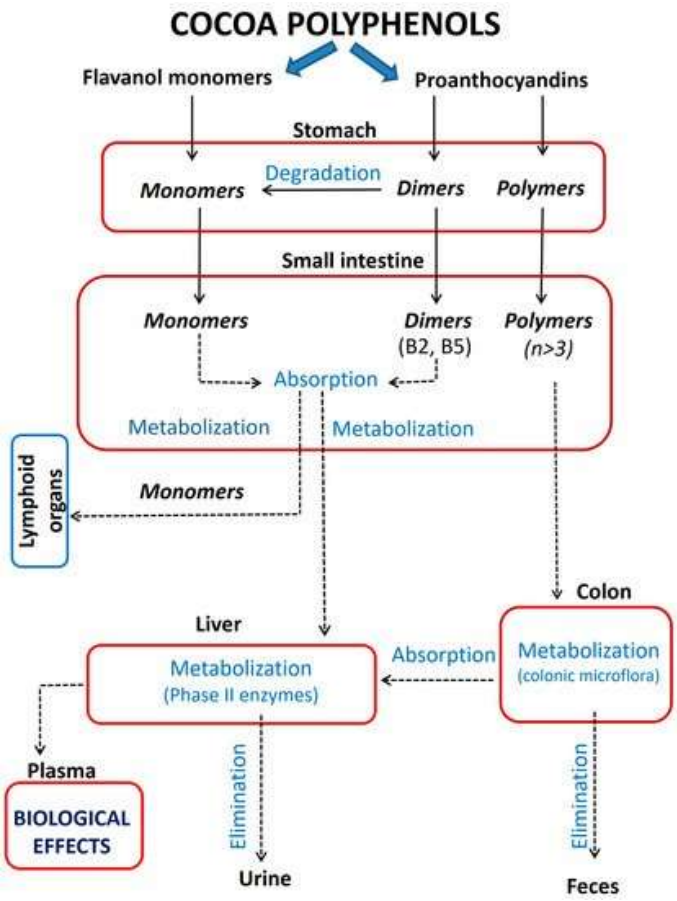


Fig.6- Absorption of polyphenols (Aprotosoaie et al., 2016)

Green extraction from cocoa shell

Food industry waste is a growing economically and ecologically problem of the modern society. Nowadays the recovery and re-usage of byproduct is a necessity considering the growing world population and disappearing of raw materials.

Cocoa shells constitute the 12% of whole bean and they are usually considered a waste of cocoa manufacturing. On the basis of annual production of cocoa beans (1200000 tons), about 140000 tons of shells are produced. The recent studies show that this raw material can be used like a source of natural substances, for instance polyphenols (epicatechin, catechin, and procyanidins).

Usually, traditional extraction techniques are slow and require high amounts of solvents and heating (Balentić et al., 2018). According with the sustainable aim of this research a green extraction method has been used to extract polyphenols from cocoa shell.

A general definition is that "Green extraction is based on the discovery and design of extraction processes, which will reduce energy consumption, allows use of alternative solvents and renewable

natural products, and ensure a safe and high quality of extract/product" (Rombaut et al. 2014).
Generally, the six principles of green extraction are:

1) Use of renewable plant resources
2) Use of alternative solvents (water or agro-solvents)
3) Reduce energy consumption
4) Produce co-products instead of waste
5) Reduce unit operation
6) Generate a biodegradable extract without contaminants.

Aim

The company Dolceamaro SRL usually buys for its productions four types of cocoa beans of different origins (Ivory Coast, Cameroon and Peru), varieties (Forastero and Trinitario) and type of production (conventional and organic). At the beginning of the thesis it was established which raw material was more interesting from a nutritional point of view. Since it was identified, the company has asked its supplier to produce that cocoa mass. The by-products of the production, the cocoa shells, were thus sent to the University of Molise where they were analyzed. Cocoa shells, rich in a large amount of phenolic compounds, are therefore a good and inexpensive source of these biocompounds with a significant antioxidant effect.
According to the sustainable objective of this research, a green extraction method was used to extract polyphenols from cocoa shells, assessing the main conditions for improving their yield.

Materials and methods

Nutritional Analysis

Cocoa beans and cocoa shells were analysed in duplicate for moisture, ash, protein, total fat, fiber and total polyphenols. Total carbohydrate content was estimated by difference. Moisture contents of the samples was determined by the gravimetric method by drying 5 g of ground sample at 101 ± 2 °C to constant weight in an air oven. Ash contents was determined by using a muffle furnace at 550–600 °C for 4 h. Fat content of chocolate samples were measured by Soxhlet Extraction Method according to AOAC (1990) Official Method 963.15. The protein content has been determined based on total nitrogen content measured with Kjeldhal procedure, multipled by a factor of 6.25 (Protein = Nitrogen * 6.25). The chemical composition of the cocoa fiber was determined by the AOAC method 991.43 (AOAC, 1995).

Sample preparation

The cocoa beans were grounded for 30 s in a mixer. To remove the fat, 5 g of these grounded beans were mixed with 25 mL of n-hexane for 3 min. The mixture was centrifuged at 3000 rpm during 25 min at 4°C using J2-21 Beckman Centrifuge. This process was repeated four times and the defatted sample was left overnight at room temperature to remove the solvent.

Phenolic extraction procedure (traditional method)

To extract phenolic compounds, 2 g of sample was mixed with 50 mL of an 80% methanol solution for 2 h at 50°C using a thermostatic bath. The extract was filtered through a Whatman No. 1 filter paper.

Phenolic extraction procedure (green extraction)

The equipment used for extraction of polyphenols was ultrasonic bath 2210 Bransonic (125 kW- 47 kHz ± 6%).

2 g of dried and ground samples were placed in a thermostatic water bath shaker with 10 ml of deionized water at room temperature of 19°C ± 1 °C for 15 min. The liquid extract was separated from solids by centrifugation at 5000 rpm for 10 min. The supernatant was transferred to a 10 ml flask and deionized water was added to make the final volume 10 ml. All samples were extracted in duplicates.

Total phenolic content

The total phenolic content in the extract was determined by Folin Ciocoltau method. 250 µl of the extract was diluted with distilled water to 10 ml. Aliquots of 100 µl of samples were mixed with 500 µl of Folin-Ciocalteau reagent. After 3 min, 1 ml of 7.5% sodium carbonate was added. The mixtures were allowed to stand for 2 h at room temperature. The total polyphenol content in the extract was calculated and expressed as gallic acid equivalents (GAE; g/100 g dry mass) using a gallic acid (0-120 mg/l) standard curve. All samples were prepared in duplicates.

Effect of extraction procedures and different parameters

Accordingly, the objective of this research was to find the most suitable and nontoxic solid-solvent extraction of polyphenols from cocoa shells which enables the use of the extract in food industry. Further, to elucidate how different solvents, time and particle size affect the quantitative extraction of polyphenols.

Solvents

Three different solvents and their mixtures were used to identify the most suitable one for the recovery of polyphenols. The solvents used in this experiment were: deionized (DI) water, ethanol, aqueous

ethanol 75%, aqueous ethanol 50%, aqueous ethanol 25%, and methanol (using in the traditional method).

Extraction Time

To study the effect of extraction time, samples were extracted with water at room temperature for (15, 30, 45, 60, 90,120) min.

Particle size

Six solutions with different particle size (μm) of ground shells were studied (φ>1000; >850; >710; >500; >250; <250). For physical separation, shells were passed through five different sieves.

Statistical analysis

All experiments were performed in triplicate. Data are reported as the mean and standard deviation (SD) of three replicates. Statistical significance of treatment effects was determined with ANOVA. Least significant differences were obtained with a Scheffè's test ($p < 0.05$). The statistical analyses were performed with SPSS version 13.0 for Windows (SPSS, Inc., Chicago, IL).

Results and discussion

LEGEND

C1= Camerun Cocoa bean (var. Forastero) conventional

C2= Ivory Coast Cocoa bean (var. Forastero) conventional

C3= Ivory Coast Cocoa bean (var. Forastero) organic

C4= Venezuela Cocoa bean (vat. Trinitario) conventional

Tab.3 – Chemical composition of different cocoa beans and cocoa shells. Different letters within same column indicate significant difference (P < 0.05).

SAMPLE	MOISTURE	DRIED MATTER	TOTAL FAT	CARBOHYDRATES	PROTEINS	FIBERS	ASH	TOTAL POLYPHENOLS
	g/100g	g/100g	g/100g	g/100g	g/100g	g/100g	g/100g	g/100g
C1	5.09 ± 0.57a	94.91 ± 0.57	52.81 ± 0.22a	14.06	10.90 ± 1.95	16.9 ± 0.3a	3.57± 0.75a	1.65 ± 0.07a
C2	6.56 ± 1.21a	93.44 ±1.21	54.30 ± 0.50a	10.73	13.09 ± 3.32	16.5 ± 0.5a	3.40 ± 0.90a	2.29± 0.05b
C3	5.98 ± 1.29a	94.02 ± 1.29	52.32 ± 0.30a	7.91	12.47 ±1.06	20.2 ± 0.6b	4.38 ± 0.82b	2.25 ± 0.07b
C4	6.08 ± 1.42a	93.11 ± 1.42	50.93 ± 0.71b	9.8	13.09 ± 1.67	20.4 ± 0.2b	3.83 ± 0.68a	2.38 ± 0.01c
COCOA SHELLS	3.18 ± 0.03	96.82 ±0.03	13.56 ± 0.61	20.11	13.87 ± 0.25	46.9 ± 0.4	5.56 ± 0.35	1.23 ±0.01

Food quality was determined by three main features: external quality (e.g. commercial variety), the consumption value (manufacture and manufacturing) and biological quality expressed by the balance of nutrients. For the interest of cocoa industry total fats and total polyphenols are two chemical parameters with more importance than others. The former gives the organoleptic and rheological

characteristics of final product; the latter confers astringent and bitter sensations and contributes significantly to the green and fruity flavors of cocoa liquors (Aprotosoaie et al., 2016). Further, some studies demonstrated that flavan-3-ols may be efficacy in the prevention and treatment of human *Non-alcoholic fatty liver disease* (NAFLD) (Vauzour et al., 2018), which is related to total fats. Tab.3 shows the different nutritional compounds of several cocoa beans and cocoa shells, that were studied. Data show that the concentration of flavan-3-ols in cocoa depend on their variety (genotype) and origin. According with Wollgast'study (Wollgast & Anklam, 2000) cocoa beans from Venezuela showed the highest amount of total polyphenols in the fermented bean but the lowest content of fats. Forastero seeds contain few flavan-3-ols than Trinitario sample, probably because they were undergone a longer fermentation to reduce the bitterness. Further, it was evident that the type of production didn't influence the value of these chemical compounds. For this reason, sample n. 2 contained the best compromise between fatty acids (54.3 g/100 g) and total polyphenols (2.29 g/100 g).

According with Dolceamaro SRL, cocoa beans chosen were processed to produce cocoa mass. In the processing of the product, the first step is cleaning, to remove most of the impurities (Okiyama et al., 2017). After cleaning the whole beans are roasted along with the shells, being subjected to subsequent shelling. A review of the literature indicates that changes in polyphenol content in cocoa bean during convective roasting were investigated in terms of temperature. It has been shown that with increasing heat treatment, especially above 130°C level of polyphenols in cocoa beans is significantly reduced (Żyżelewicz et al., 2016). For this reason, data shown that shell contained less total polyphenols (1.23 g/100 g) than whole cocoa beans. At the opposite, the content of dietary fiber (46.9 g/100 g) and ash (5.6 g/100 g) were very higher.

According with the eco-sustainable principle of this research, it was studied the efficient methods for extraction polyphenols from cocoa shell using green procedure. The most common equipment used for extraction purposes are ultrasonic baths. Extraction mechanism involves two types of physical phenomena: diffusion through the cell walls and washing out the cell's content once the walls are broken. Ultrasound waves interact with the plant material to alter its physical and chemical properties. The cavitation effects of these waves facilitate the release of extractable compounds and enhance mass transport by disrupting the plant cell walls. Further a shorter extraction duration generally means a reduction of oxidation and degradation of natural compounds (Rombaut et al. 2014).

It is generally known that the yield of chemical extraction depends on the type of solvents with varying polarities, extraction time and temperature, as well as on the chemical composition and

physical characteristics of the samples. Therefore, it was studied the effect of extraction solvent such as water, ethanol and their combination on the total polyphenols.

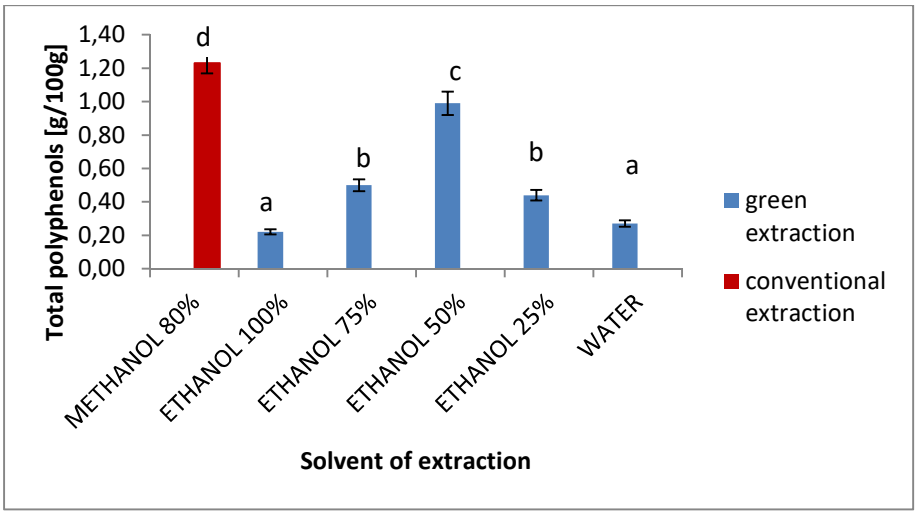


Fig.7- Effect of extraction time on total polyphenol content. Different letters indicate significant difference (P< 0.05).

As shown in (Fig.7), considering only green solvents, 50% ethanol shows the highest total polyphenols extraction and there isn't significant difference between 75% and 25% ethanol concentration. While ethanol 100% extracts show the lowest total polyphenols yield. The low solubility of the polyphenols in absolute organic solvents may be due to strengthening of the hydrogen bonds between polyphenols and protein in these solvents. On the other hand, the increase in solubility upon the addition of water to organic solvents could be due to weakening of the hydrogen bonds in aqueous solutions. Another hypothesis regards the increase of ionization of the polyphenols in such solutions.

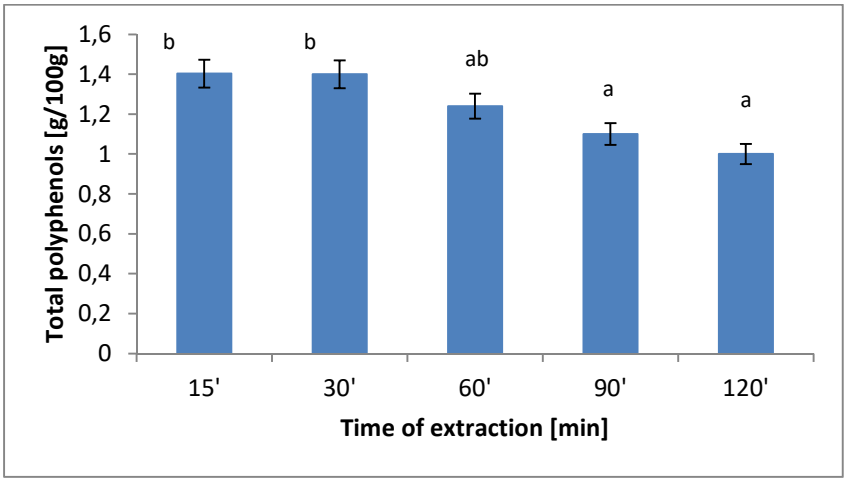


Fig.8- Effect of extraction time on total polyphenol content. Different letters indicate significant difference (P< 0.05)

The effect of extraction time was also studied. As shown in (Fig.8), as the extraction time increased (15-30 min) the yield of polyphenols didn't change. But times longer than 30 min showed to cause rapid polyphenols degradation.

Finally, it was studied the influence of particle size of ground cocoa beans on the yield of extraction. In this case, as particle size increased, the amount of total polyphenols extracted decreased (Fig.9). It was found that extracting with smaller particle sizes could produce a higher yield. Smaller particle sizes offer greater surface area for mass transfer. Finer particles, however, are more prone to agglomeration (Baldosano et al. 2015).

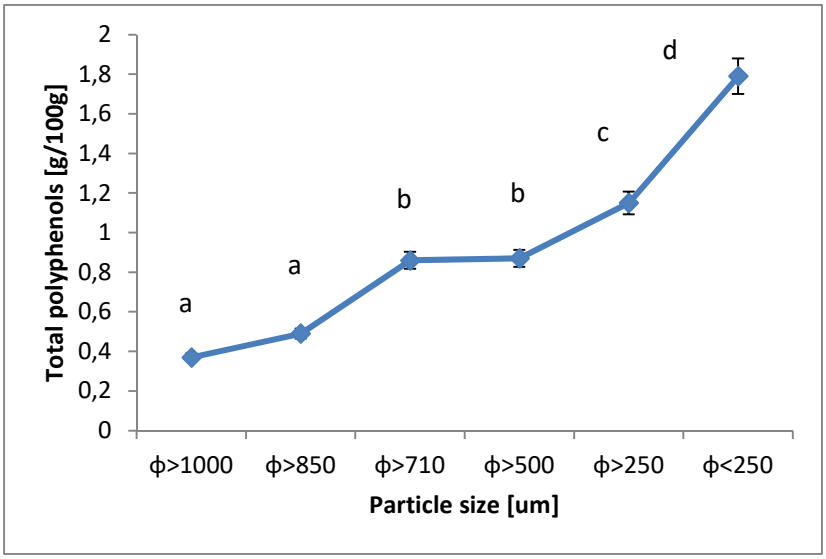


Fig.9- Effect of particle size of cocoa on total polyphenol content. Different letters indicate significant difference (P< 0.05)

The recovery of phenolic compounds from cocoa shell is influenced by the extraction time (15 min), particle size of raw material (<250 µm) and extraction solvent (50% ethanol). The yield of flavan-3ols extraction in this condition (1.79 g/100g) is more than the value obtained with the traditional procedure (1.23 g/100g). The effects caused by ultrasound in the extraction media are attributed to the cavitation phenomenous. The ultrasound waves generate bubbles in the media. When they reach a critical diameter, bubbles collapse causing the release of micro-jets and shock waves directed towards the solid surface. The structure of cocoa cells will be destroyed with the consequence release of their content in the solvent (Rombault et al., 2014). For this reason, small particles were broken faster. On the other hand, traditional method consists in the maceration procedure, where the extraction is carried out according to the principle of polyphenols diffusion into extraction solvent.

The study showed the effectiveness of ultrasound in the polyphenol extraction in a shorter time than conventional extraction process (15 min vs 2 hours), that means a less reduction of oxidation and degradation of natural compounds. Therefore, in addition to the higher yields in polyphenol (>30%), also the kinetic of extraction was improved due a reduction of extraction duration.

Bucić-Kojić et al. (2008) showed that temperature of extraction (50°C) had a positive influence on conventional maceration of total polyphenols, especially for flavan-3ols. At the opposite, ultrasonic and extraction trials were conducted at room temperature over time with low energy consumption, because the devices used hadn't got the temperature regulation settings.

In conclusion, ultrasound extraction has proven to be an efficient green technology when compared to conventional maceration.

In this way all features of green extraction were been fulfilled: use of cultivate plant as raw material choosing the varieties with much higher concentrations of active ingredients (principle 1); use of safety, environmental and economical alternative solvent (principle 2); the reduction of energy consumption and wasted produced using ultrasound technique that improve the efficiency of extraction (principle 3); re-use of cocoa shell, the production of co-products instead of waste (principle 4); the reduction of number of unit operations as cycle of extraction to optimize the yield (principle 5); the re-cycling of the byproducts and biodegradability of the products obtained (principle 6).

Green extraction makes use of physical and chemical phenomena that are fundamentally different compared with those applied in conventional extraction techniques. It is also a new concept to meet the challenges of the twenty-first century, to protect both the environment and consumers, and in the meantime to enhance competition of industries to be more ecological, economical, and innovative (Rombaut et al., 2014).

Bibliography

- Afoakwa E. O., (2011) Industrial chocolate manufacture- processes and factors influencing quality, Chocolate Science and Technology
- Afoakwa E. O., (2010) Cocoa cultivation, bean composition and chocolate flavor precursor formation and character, Chocolate Science and Technology
- Andùjar I., Recio M.C., Giner R.M., Rìos J.L. (2012) Cocoa Polyphenols and Their Potential Benefits for Human Health, Oxidative Medicine and Cellular Longevity, https://doi.org/10.1155/2012/906252
- AOAC (1990) Official method of analysis of AOAC international, 15th ed., Association of Official Analytical Chemistry, Gaithersburg
- AOAC (1995) Official method of analysis of AOAC international, 16th ed., Association of Official Analytical Chemistry, Gaithersburg
- Aprotosoaie A.C., Miron A., Trifan A., Luca V.S., Costache I.I. (2016) The Cardiovascular Effects of Cocoa Polyphenols- An Overview, Diseases, Vol. 4, No. 4
- Baldosano, Hilary Y et al. (2015) Effect of Particle Size, Solvent and Extraction Time on Tannin Extract from Spondias Purpurea Bark through Soxhlet Extraction, Proceedings of the DLSU Research Congress, Vol. 3, 4-9.
- Balentić J., Ačkar Đ., Jokić S., Jozinović A., Babić J., Miličević B., Šubarić D., Pavlović N. (2018) Cocoa Shell: A By-Product with Great Potential for Wide Application, Molecules, Vol. 23
- Barišic V., Kopjar M., Jozinovic A, Flanjak I., et al. (2019) The Chemistry behind Chocolate Production, Molecules, Vol. 24, No. 17
  https://doi.org/10.3390/molecules24173163
- Belitz H.D., Grosch W., Schieberle P. (2009) Food chemistry, 4th ed., Berlin, Germany, Springer
- Bucić-Kojić A., Planinić M., Tomas S., Jakobek L., Šeruga M. (2008) Influence of solvent and temperature on extraction of phenolic compounds from grape seed, antioxidant activity and colour of extract, International Journal of Food Science and Technology, Vol. 44, 2394–2401
- Caraceni R. (2020) La degustazione del cioccolato, Hoepli, 2nd ed., Milano, Italy
- Deus V.L., De Cerqueira e Silva M.B., Maciel L.F., Miranda L.C.R, Hirooka E.Y., Soares S.E., Ferreira E.S., Bispo E.S. (2018) Influence of drying methods on cocoa (Theobroma

cacao L.): antioxidant activity and presence of ochratoxin A, Food Science and Technology, Vol. 38, No. 1, 278-285
https://doi.org/10.1590/fst.09917

- De Vuyst L., Weckx S. (2016) The cocoa bean fermentation process: from ecosystem analysis to starter culture development, Journal of Applied Microbiology, Vol. 121, No. 1, 5-17 https://doi.org/10.1111/jam.13045
- Franco R., Oñatibia-Astibia A., Martínez-Pinilla E. (2013) Health Benefits of Methylxanthines in Cacao and Chocolate, Nutrients, Vol. 5, 4159-4173
- Gonthier M.P., Donovan J.L., Texier O., Felgines C., Remesy C., and Scalbert A. (2003) Metabolism of dietary procyanidins in rats, Free Radical Biology and Medicine, Vol. 35, No. 8, 837–844
- Grassia M., Salvatori G., Roberti M., Planeta D., Cinquanta L. (2019) Polyphenols, methylxanthines, fatty acids and minerals in cocoa beans and cocoa products, Journal of Food Measurement and Characterization, Vol. 13, No. 3, 1721–1728 https://doi.org/10.1007/s11694-019-00089-5
- Hernández-Hernández C., Viera-Alcaide I., Sillero A.M.M., Fernández-Bolaños J., Rodríguez-Gutiérrez G. (2018) Bioactive compounds in Mexican genotypes of cocoa cotyledon and husk, Food Chemistry, Vol. 240, 831-839.
- Knight I. (1999) Chocolate and Cocoa: Health and Nutrition, Knight International
- Krysiak W. (2002) Roasting conditions and cocoa bean quality, Acta Agrophysica, Vol. 77, 51–60
- Krysiak W. (2006) Influence of roasting conditions on coloration of roasted cocoa beans, Journal of Food Engineering, Vol. 77, 449–453
- Langer S., Marshall L.J., Day S.J., Morgan M.R.A. (2011) Flavanols and Methylxanthines In Commercially Available Dark Chocolate: A Study Of The Correlation With Nonfat Cocoa Solids, Journal of Agricultural And Food Chemistry, Vol. 59, 8435–8441 http://www.researchgate.net/publication/51244078
- Lima L.J.R., Almeida M.H., Nout M.J.R., Zwietering M.H. (2011) Quality Determinants of Commercial Cocoa Beans, with Particular Reference to the Impact of Fermentation, Critical Reviews in Food Science and Nutrition
http://dx.doi.org/10.1080/10408391003799913
- Lipp M., Anklam E. (1997) Review of cocoa butter and alternative fats for use in chocolate-Part A. Compositional data, Food Chemistry, Vol. 62, No. 1, 73-97

- Maleyki M.J.A., Ismail A. (2010) Antioxidant properties of cocoa powder, Journal of Food Biochemistry, Vol. 34, No. 1, 111-128 https://doi.org/10.1111/j.1745-4514.2009.00268.x
- Martín M.A., Ramos S. (2016) Cocoa polyphenols in oxidative stress: Potential health implication, Journal of Functional Foods, Vol. 27, 570-588 https://doi.org/10.1016/j.jff.2016.10.008
- Minifie W.B. (1999) Chocolate, cocoa, and confectionery: Science and technology third edition, Aspen Publishers, Inc. Gaithersburg, Maryland, 135-164
- Misnawi D.S. (2003) Effect of Cocoa Bean Polyphenols on Sensory Properties and Their Changes during Fermentation, Pelita Perkebunan, Vol. 19, No. 2, 90-103.
- Motamayor J.C., Lachenaud P., Mota J.W.S., Loor R. (2008) Geographic and genetic population differentiation of the Amazonian chocolate tree (Theobroma cacao L), PLoS ONE Vol. 3, No. 10
- Okiyama D.C.G., Navarro S.B.L., Rodrigues C.E.C. (2017) Cocoa shell and its compounds: Applications in the food industry, Trends in Food Science & Technology, Vol. 63, 103-112 https://doi.org/10.1016/j.tifs.2017.03.007
- Pasrija D., Ezhilarasi P.N., Indrani D., Anandharamakrishnan C. (2015) Microencapsulation of green tea polyphenols and its effect on incorporated bread quality, Food Science and Technology, Vol. 64, 289-296
- Pešić M.B., Milinčić D.D., Kostić A.Z., Stanisavljević N.S., Vukotić, G.N., Kojić, M.O., Gašić, U.M. et al. (2019) In vitro digestion of meatand cereal-based food matrix enriched with grape extracts: How are polyphenol composition, bioaccessibility and antioxidant activity affected?, Food Chemistry, Vol. 284, 28-44
- Rombaut N., Tixier A.S., Bily A., Chemat F. (2014) Green extraction processes of natural products as tools for biorefinery, Biotechnology & Applied Microbiology, Vol. 8, No. 4 1932-1031
- Rucker R. (2008) Nutrition Properties of Cocoa, Chocolate: History, Culture, and Heritage https://doi.org/10.1002/9780470411315.app10
- Rusconi M., Conti A. (2010) Theobroma cacao L., the food of the Gods: a scientific approach beyond myths and claims, Pharmacological Research, Vol. 61, No. 1, 5–13
- Sabahannur S., Alimuddin S., Rahmawati A. (2018) Changes in Phenol Level and Antioxidant Activity of Cocoa Beans During Fermentation and Roasting Journal of Food Research, Vol. 7, No. 4, 23-29

- Vauzour D., Rodriguez-Ramiro I., Rushbrook S., Minihane A.M., et al., (2018) n-3 Fatty acids combined with flavan-3-ols prevent steatosis and liver injury in a murine model of NAFLD, Molecular Basis of Disease, Vol. 1864, 69–78
- Williamson G. (2009) Bioavailability and health effects of cocoa polyphenols, Inflammopharmacology, Vol. 17, No. 2
  https://doi.org/10.5539/jfr.v7n4p23
- Wollgast J., Anklam E. (2000) Polyphenols in chocolate: Is there a contribution to human health, Food Research International, Vol. 33, 449-459
- Zimmermann B.F., Ellinger S. (2020) Cocoa, Chocolate, and Human Health, Nutrients, Vol. 12, No. 3, 698-701
- Żyżelewicz D., Krysiak W., Oracz J., Sosnowska D., Budryn G., Nebesny E. (2016) The influence of the roasting process conditions on the polyphenol content in cocoa beans, nibs and chocolates, Food Research International
  http://dx.doi.org/10.1016/j.foodres.2016.03.026
- https://it.123rf.com/archivio-fotografico/cocoa.html?sti=mxto3ucbafexpbyepo|
- https://www.britannica.com/topic/cocoa-food/Fermentation
- https://www.chemistryviews.org/details/ezine/808827/Chocolate__The_Noblest_Polymorphism_II.html
- https://www.eurococoa.com/en/cocoa-story/cocoa-story-the-production-process-from-cocoa-beans-to-semi-finished-products/
- https://www.icco.org/about-cocoa/trading-a-shipping.html
- https://www.icco.org/faq/58-cocoa-harvesting/131-what-time-of-year-is-cocoa-harvested.html
- https://sustainabledevelopment.un.org/?menu=1300
- (https://www.wasteroadmap.co.za/download/6_UCT_CSIR_Final_Report.pdf)

CHAPTER 2 MICROENCAPSULATION OF POLYPHENOLS

Microencapsulation and state of the art

Microencapsulation process was developed in 1970 and consists in the covering of a liquid, solid or gas compound (core) with a coating material (shell) (Gibbs et al., 2009).

In food industry this new technology is used to assure the protection of sensitive nutrients and their following controlled release (Onwulata, 2012).

The microcapsules have a size range between 1 micron and 1 mm. They could be distinguished in two groups, according with their structure: microcapsule and microsphere. The former is composed by a central core of active substance covered by a coating material; the latter consists in a uniform matrix in which the core is dispersed or dissolved (Teixeira da Silva et al., 2014). Furthermore, there are other different structures depending on their shape and their composition (Fig.10).

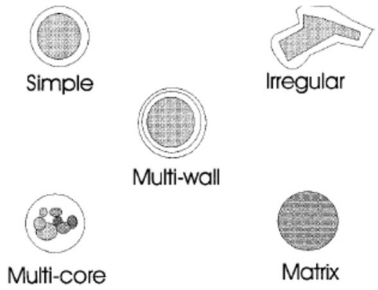


Fig.10- Structures of different capsules (Gibbs et al., 2009)

Nowadays the interest in this particular technique is increasing because of its many advantages. First, capsule protects the nutrients from the adverse external conditions, increasing their preservation. Then, the wall masks the off-taste of internal biocompounds like as polyphenols or ω−3 fatty acids (Onwulata, 2012). In other cases, capsules capture and maintain the aromas of some substances (e.g. chewingum). Encapsulation could be used also to change the density of a liquid or transforming it in a solid. In some applications it needs to dilute an active material in a small amount. This technique allows separating two or more substances placed in the same matrix. Finally using capsules, it is possible to control the release of the core material in a specific place of the human body (Munin & Edwards-Lévy, 2011). For all of these reasons, microencapsulation is widely used in the food and pharmaceutical industries.

Today functional foods are an important segment of food's market. A common definition of functional food is a product with a positive effect on human health beyond basic nutrition, instead of the prevention or the reduction of some diseases (Martirosyan & Singh, 2015). These products could be natural or processed, for example adding some biocompounds. In the finished foods microcapsules

allow to protect the active substance from the technological conditions to which is submitted. Furthermore, the active compound should act in a specific part of the human body to explicate its particular function, maintaining its bioactive loading during the transport through the digestive tract. This phenomenon is influenced by a wide range of factors such as the nature of the bioactive substance, the ratio between the core and wall, the chemical and physical properties of the covering layer. The diffusion or degradation of the wall and the internal conditions of the organism (pH, temperature and pressure) are the main mechanisms involved in the release (Teixeira da Silva et al., 2014).

In the food industries microencapsulation is adapted to a wide range of active compounds. For instance, probiotics, live microorganisms that confer a beneficial effect to human healthy, are often submitted to this technology in order to preserve their vitality. Among non microbial products, substances such as vitamins, fatty acids, enzymes or polyphenols have been encapsulated (Champagne & Fustier, 2007).

About vitamins and minerals, their bioavailability could be reduced by interactions with other food ingredients. Moreover, some of these are sensitive to high temperature or they aren't water solubility (Estevinho et al., 2016). Fatty acids, like, omega-3 and omega-6, bring a lot of health benefits but they are undesirable by consumers due their smell. They are also susceptible to oxidation (Kaushik et al., 2014). Finally, polyphenols are wide used in food and pharmaceutical industries due their antioxidant property. Like previous compounds, they oxidize very fast, change their color, and are more sensitive to light and heat. Lastly, many of these molecules confer astringent and bitter taste to food (Munin & Edwards-Lévy, 2011). To overcome the above mentioned problems, microencapsulation techniques are developed.

Spray drying

Spray-drying is a technique used to produce a dry powder from a liquid phase in pharmaceutics and food technology. This invention was developed by Samuel Percy for production of milk powder in 1920 (Keshani et al., 2015).

The principle of spray drying is the atomization of the liquid product in a gas current, such as air or nitrogen, to obtain a powder.

Every spray dryer is composed by: a source of hot air, that includes an air supply fan, an air heater and an air distribution system, an atomizing system, a drying chamber and a powder recovery system (Fig.11a).

Initially, a mixture of coating agent and core substance is introduced in the atomizer where gas transforms feed into droplets. When the medium evaporates, leading the evaporation of the solvent,

the powder entraps the core within the material. After, the dry food particles are separated from air in a cyclone separator (Fig.11b) (Singh & Heldman, 2009).

The main advantage of spray drying technique is the production of stable and high quality particles (Munin & Edwards-Lévy, 2011) with a specific size and moisture content (Keshani et al., 2015). This unit operation is cheap, continue and easy to use, with a quick response time. It could be used to encapsulate heat sensitive and heat-resistant products, such as flavors, lipids, and carotenoids. However, this technique isn't adapted to encapsulate probiotics, because the high temperature gas could kill bacteria or reducing their viability (Gharsallaoui et al., 2007). The best inconvenience is the limited number of wall materials available and soluble in water (Desai & Park, 2005).

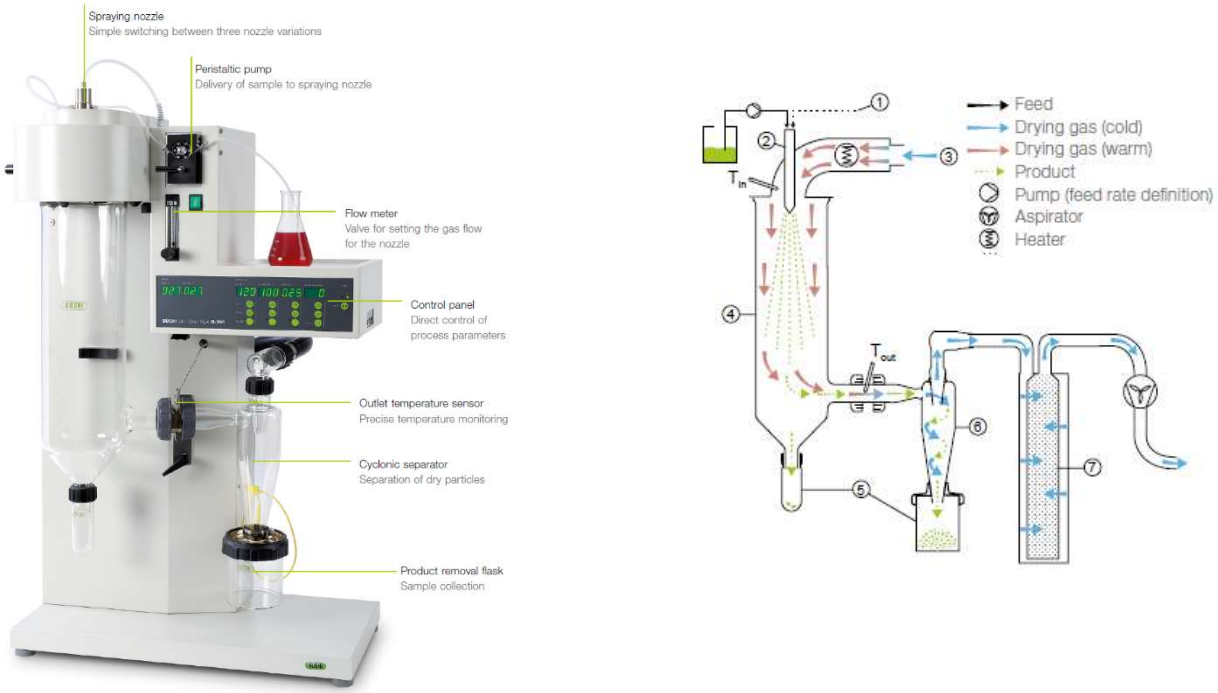


Fig.11- Diagram of the equipment of conventional spray-drying (a) and its mechanism (b) (https://www.buchi.com)

Spray drying theory and thermodynamic approach

The spray drying encapsulation technique is based on several phases that influence the yield and the quality of formed capsules. The first step is the emulsion preparation of the core material in the wall solution (usually water is used like solvent), which with is often immiscible. Usually this emulsion is homogenized and stabilized for a certain time until it is fed into spray dryer. The wall and core material properties, their ratio and the emulsion characteristics are the primary factors that determine the effectiveness of encapsulation (De Vos et al., 2010). The parameters studied in this phase are solids content, density, surface tension and viscosity of the emulsion. In particular, a higher solid concentration leads less liquid to vaporize with a consequently increasing of the outlet temperature and decreasing of the product moisture. The capsules size will be big and easier to separate from the

solvent, causing the yield increase (https://www.buchi.com). About viscosity, high values affect the formation of elongated and large droplets during the atomization with adverse effect in drying. This parameter is influenced by the nature of the coating agent and the ratio between it and core substances. Emulsions are generally obtained by two types of emulsification techniques such as low energy emulsification (phase inversion temperature) and high energy/pressure emulsification like ultrasonication and microfluidization (Jafari et al., 2006).

Coating Agent

One of the most important step in the microcapsule developing is the choose of wall materials, that depends on the core substance and the desirable features of the final product. Usually selected materials should have the following characteristics:

1) good rheological properties in the solution in order to allow easy workability at high concentration;
2) good emulsification property with the core material assuring the stabilization of the final emulsion;
3) no-reactivity with core material during the encapsulation process;
4) the controlled release of the core substance in specific condition;
5) the protection of the bioactive compound against adverse conditions (e.g. oxygen, heat, light, humidity);
6) high solubility in solvents like water or ethanol;
7) low cost;
8) food grade status.

The selection of the coating agent affects the encapsulation efficiency and microcapsules stability during storage.

Coating materials belong to wide range of natural substances: carbohydrates (e.g. maltodextrins, cyclodextrins), proteins (e.g. gelatine, soy protein, whey protein…), lipids (wax, fats, oils), gums (gum acacia, agar, sodium alginate) or cellulose esters and ethers (Gharsallaoui et al., 2007).

Category	Coating materials	Widely used methods
Carbohydrate	Starch,maltodextrins, chitosan, corn syrup solids, dextran, modified starch, cyclodextrins	Spray- and freeze-drying, extrusion, coacervation, inclusion complexation
Cellulose	Carboxymethylcellulose, methyl cellulose, ethylcellulose, celluloseacetate-phthalate, celluloseacetate-butylate-phthalate	Coacervation, spray-drying, and edible films
Gum	Gum acacia, agar, sodium alginate, carrageenan	Spray-drying, syringe method (gel beads)
Lipids	Wax, paraffin, beeswax, diacylglyerols, oils, fats	Emulsion, liposomes, film formation
Protein	Gluten, casein, gelatin, albumin, peptides	Emulsion, spray-drying

Fig.12- Coating materials for microencapsulation (Desai & Park, 2005)

Maltodextrin

Maltodextrin is a polysaccharide that consists of D-glucose units linked with α-(1-4) bonds and with branched segments linked by α-(1-4) bonds. It has a dextrose equivalent (DE), an inverse measure of the number of anhydro α -D-glucose units, less than 20. Maltodextrin is obtained from the partial hydrolysis of a starch of several botanical sources (e.g. wheat, corn, rice) by acids and/or enzymes. Maltodextrins were introduced between 1967 and 1973 by Fred Armbruster and co-workers of the Corn Products Refining (Bemiller & Whistler, 2009).

This raw material is generally recognized as safe (GRAS) so it is widely used in foods formulation (Rowe et al., 2006). It has an energy value of about 4 kcal/g and could be digestible in small intestine (Lovegrove, 2017).

The physicochemical properties of maltodextrins depend on several factors. The nature of starch determines the ratio between amylase and amylopectin. Usually, maize, wheat and rice have the same properties but some variety of them, like as waxy maize, contain more amylopectin than amylase. The number of DE influences the hygroscopicity, solubility, osmolality, viscosity, cohesiveness of the solution in which maltodextrin is dessolved. Moreover, the stability and solubility of the solution, the viscosity, crystallization, and sweetness are affected by the molecular-weight of saccharides. Maltodextrin has gelling property that improves the stability of the emulsion.

Maltodextrins with different dextrose equivalents are commonly used like coating agent in encapsulation process because of its high water solubility, low viscosity, low sugar content (Balasubramani et al., 2015). Tonon et al. (2011) studied the effect of maltodextrin with different

degree of polymerization (10 DE and 20 DE) on the hygroscopicity of acai juice powder showing the lowest moisture in the powder made with maltodextrin 10DE. This phenomenon depends on the links between the hydrogen of the water molecules and the hydrophilic groups available in the maltodextrin, that are more in the molecule with a great number of ramifications. The chemical structure influences also the particle size, that have a higher mean diameter in maltodextrin 10DE than maltodextrin 20DE, relating to the molecular size of each agent.

Maltodextrin is also important to reduce the deposition of particles on the the wall of drying chamber, improving the yield of final product (Quek et al., 2007).

Generally, a high amount of maltodextrin can reduce the moisture of the powder due the increasing of total solid content.

During the spray drying process an increased of maltodextrin concentration form a skin that trap air bubbles in the particles, reducing the bulk density.

At the opposite, this raw material raises the feed viscosity with the resulting development of the size capsules without changing their solubility.

Furthermore, maltodextrin provides a less hygroscopicity of the final product (Tonon et al., 2008).

Finally, it has neutral taste and aroma so it is really adapted to preserve flavours (Fernandes et al., 2014).

The atomization

The atomization consists in the breaking of the liquid feed in small droplets. The consequence of this phenomenon is the moisture transfer from the droplets to the surrounding medium. This step influences the structure, the velocity and the size distribution of the droplets and the characteristics of the final product. In fact, the increasing of surface-volume ratio leads a faster drying rate with a less lost of thermo-sensitive product (Anandharamakrishnan & Ishwarya, 2015).

Ohnesorge (1936) explained the atomization mechanism like a combination of several factors, which are viscosity, density, surface tension and jet size. Mathematically it is espressed by the dimensionless Ohnesorge number (Oh):

$$Oh = \frac{\sqrt{We}}{Re} = \frac{\mu}{\rho \sigma L} = \frac{viscous\ forces}{\sqrt{(inertia\ x\ surface\ tension)}}$$

where:

We= Weber number;

Re= Reynolds number;

μ, ρ and σ are the viscosity, density and surface tension of the feed droplet, respectively;

L is the characteristic dimension of the feed droplet.

The atomization is carry on by an atomizer that could be classified in three main types: rotary atomizer, pressure nozzle atomizer, two-fluid nozzle atomizer (https://www.gea.com). Their characteristics are described in Tab.4.

The choose of the atomizer configuration depends on the nature and the viscosity of raw materials and the desirable characteristics of the finished product. To optimize heat and mass transfer is important to create a maximum heat-transferring surface between the dry air and the liquid. If the energy that push the liquid to the contact with the gas stream is high, the formed droplets will be fine; if the energy is kept costant, the size of particles increases when feed flow rate rises. (Gharsallaoui et al., 2007).

Tab. 4- Atomizator configurations and their characteristics (Anandharamakrishnan & Ishwarya, 2015)

	Rotary atomizer	**Pressure nozzle (or hydraulic) atomizer**	**Two-fluid nozzle atomizer**
Figure	 Fig.13a- (www.indiamart.com)	 Fig.13b- (www.indiamart.com)	 Fig.13c- (www.buchi.com)
Principle	It is composed by a wheel that rotates at high velocity. Since the feed enters in the wheel, it is thrown out like a spray by the centrifugal force.	The liquid feed fall through an orefice by pressure energy leading the its breaking in small droplets.	It breaks the liquid through the impact with high-velocity air. Compressed air shears field producing wide range of droplet sizes.
Mean droplet size:	30–120 µm.	120–250 µm.	30–150 µm.
Relationship between atomization parameters:	The particle diameter is directly proportional to feed rate and feed viscosity, and inversely proportional to wheel speed and wheel diameter.	The particle diameter is directly proportional to feed rate and viscosity, and inversely proportional to atomization pressure.	The particle diameter is directly proportional to feed rate and viscosity and inversely proportional to atomization pressure.
Advantages	• Production of uniformly size droplets. • Low pressure required.	• Production of great particle size • Production of particle with less occluded air	• Capability to hand highly viscous feed • Production of finer particles • Easier control of the droplet size.
Disadvantages	• Less capability to hand higly viscous feed • Great environmental pollution • Not usable in horizontal spray dryer	• Sprays is less homogeneous at high feed rates	• Hight cost for compressed air required • Production of particle with more occluded air • More temperature gradient between the droplets and the drying medium • Overspray: fine particles tend to occlude the nozzle

The drying

The drying process could be divided in two main steps. In the first stage, droplets keep in contact with heat air, leading the evaporation of the solvent and the consequence contraction of droplets. In the second step of drying process, solid fraction precipitates, covering the droplets surface and forming a layer called croast (Mezhericher et al., 2010).

At the beginning, the atomization particles evaporate from the surface with the consequence loosing of water, reducing diameter and solids formation near the external part.

The internal temperature droplets increase until a costant value (drying of the air at wet bulb temperature) because of the contact between hot air and liquid feed; the evaporation of droplet water is carried on at costant temperature, affecting by the water vapor partial pressure. In this case the rate of water diffusion from the droplet core to its surface is costant and has the same value of the evaporation water from the surface. During this phase, the evaporation of a liquid droplet of diameter (d) is proportional to its surface area. Mathematically, the Peclet Number (Pe) describes the main parameters that hand the drying process (Huang, 2011):

$$Pe.C = \frac{\delta C}{\delta r}$$

where:

C is the concentration of the solute relating on its weight

R is the droplet radius

$$Pe = \frac{k}{D}$$

κ is the evaporation rate;

D is the diffusion rate.

The spray-air contact between droplets and hot air can take place through two different systems: co-current process and counter-current ones.

In the first arrangement, feed flow and hot air have the same direction, so the products dried instantaneously, with a low thermal degradation. The temperature of the droplets is kept low due to the high rate of evaporation at the wet-bulb temperature. Wet-bulb temperature is the thermal energy of hot air used for evaporation: when a part of thermal energy, called latent heat, is used for vaporization, the remaining cold air (evaporative cooling) keeps the temperature of the droplets below the outlet temperature of the drying air. It also drives the particles in the system.

In counter-current process, the liquid is sprayed in the opposite direction of the flow of hot air so product is exposed to high temperatures for more time. The particles outlet temperature reaches the value of air temperature. It isn't adapted to the encapsulation of thermo-sensitive products.

During the second stage the precipitation of solid fraction leads the covering of the droplet surface with a layer of solid component. The croast can be rigid, assuring the mantaince particles size or flexible, reducing their size.

During this event the evaporation rate depends on the rate of water vapor diffusion through the dried surface. This phase, called “the falling rate period”, leads the heating of the particles (about 20°C lower than the air outlet temperature) (Gohel et al., 2002).

The second step continues until the reaching of balances of temperature and vapor partial pressure between liquid (droplets) and gas phases vapour (drying medium). So, heat transfer is carried out from air to product (because of different temperature) while water is transferred in the opposite direction (because of different vapor pressure) (Fig.14)

The drying process depends on the product nature and air inlet temperature. Usually the time for the passage of the sprayed particle through the drying zone is about 15-30 s (Fogler & Kleinschmidt, 1938).

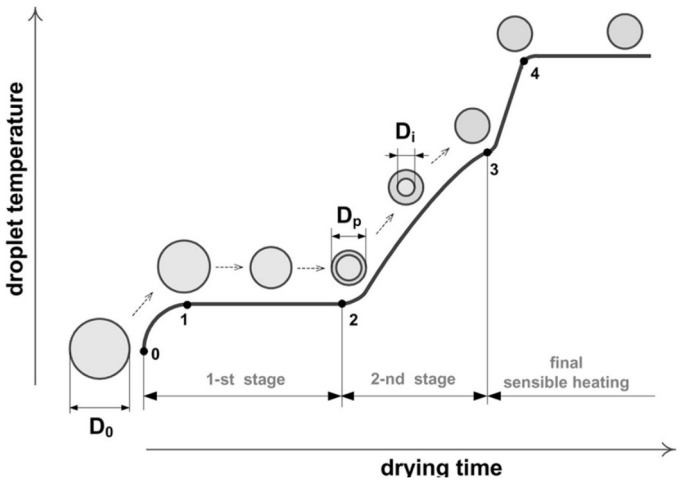


Fig.14- Drying phase: D0 is the initial diameter; Dp is the particle diameter; Di is the internal diameter (Mezhericher, 2010).

Particle separation

After the evaporation, the temperature of the particle rises to the general temperature of drying chamber and the product is separated from air in a cyclone separator. The aim of the cyclone is the reducing of product losses in the atmosphere: the densest particles are recovered at the base of the drying chamber while the finest ones pass through the cyclone to be separated from the humid air (Gharsallaoui et al., 2007).

One of the main consequence of cyclon is the particles wall deposition that reduces the yield of the process. Many factors influence this phenomenon. First, the characteristics of the drying chamber such as the wall materials (e.g. nylon is better than stainless because of its less adhesive) and the

geometries (conical, lantern and hour-glass, horizontal configuration, parabolic geometry). Some researchers studied that the chamber conformation changes the air flow, and consequently, the particle deposition. However, this aspect has to be considered with the time during particles stay in the drying chamber and the volume of the site. A large drying chamber reduces particle deposit. Moreover, the nature of capsules (coating agent) affects the deposition: when particles are above the glass transition temperature, T_g, they became sticky due to heating and humidity absorbtion. A more stickness of the products means a more adhesivity on the wall.

This aspect is also influenced by process parameters, such as inlet temperature and flow feed. The former, increasing of T_g, leads more flux deposition. About flow feed, if it is high, droplets size increases with a lost of moisture content and a corresponding increase in deposits on the wall.

In this condition the particles aggregate each other, affecting the increase of deposition rate and the decrease of drying yield (Keshani et al., 2015).

Parameters

The characteristics of the formed capsules depend on several factors that could be distinguished in: the operating conditions of the drying process, the properties of the feed and the structural features of the spray dryer. (Handscomb et al., 2009; Zbicinski, 2017). These parameters affect the particle size and morphology, their storage stability, process yield, crystalline state, moisture content, the density (Kemp et al., 2016) and the bioavailability of the encapsulated substances.

The main operation conditions regard the atomization pressure, the spray angle, the inlet and outlet temperature and the flow feed.

About the atomization pressure, if raw material and type of nozzle don't change, the diameter of the final particles increases at low pressure following the equation reported by Masters (1991):

$$\frac{D_2}{D_1} = \left(\frac{P_2}{P_1}\right)^{-0.3}$$

where

D_1 and D_2 are the diameter particles at the biginning and at the end of the atomization process respectively;

P_1 and P_2 are different pressure ($P_1 < P_2$). This is because high pressure allows a more division of the liquid feed in particles.

The rate feed is related to the drying of the formed droplets in the atomizer stage (Zbicinski et al., 2002): if the flow rate rises, the size of the particles increase because more liquid passes through the nozzle.

Inlet temperature is the temperature of the drying air. Usually when it is high, the thermal efficacy increases but it also involves a greater loss of heat-sensitive products. The air inlet temperature is

directly proportional to the moisture content and the drying rate of the capsules. Low inlet temperature leads a low drying rate with the consequently formation of capsules rich of water with the agglomeration tendency. On the other hand, a high temperature causes more water evaporation with the breaking of the coating membrane.
Outlet temperature is the temperature of the particles before to enter into cyclon. This temperature cannot be regulated because it is the results of the heat and the mass transfert in the drying chamber. This parameter influences the final moisture content and the particles agglomeration (Maas et al., 2011).
During the drying phase, the spray angle is an important parameter correlated with the particles size. It is measured at the nozzle orifice and depend on the nozzle's liquid tangential velocity, that is the speed at which the feed liquid enters in the nozzle before it is divided into fine droplets.
The choice of spray angle changes this rate and it is related to the type of air flow (co-current or counter-current). Usually in co-current process, the angle is wider then counter current ones, with a consequently speed tangential velocity.

Feed properties like viscosity, density and surface tension, are related to the solid content and the ratio between coating agent and core substances.
About the feed viscosity, higher values involve more atomization energy in order to fight the viscous force during the process. The correlation between viscosity and diameter particle (D) is the following:

$$\frac{D_2}{D_1} = \left(\frac{\mu_2}{\mu_1}\right)^{-0.2}$$

where μ_1 and μ_2 ($\mu_1 < \mu_2$) are two different feed viscosities.
Finally the glass transition temperature, also related to the feed viscosity, is associated with product stickiness and the particles deposition. It depends on the constituent solutes present in the feed.
High tension surface of the liquid feed also can hinder its flow: for these reason, emulsion is usually submitted to homogenyzation before spray dryer process.

About equipment parameters, the atomizator geometry affectes the particle size and the flow feed: usually stable nozzles are adapted for rough solution and rotary nuzzles for finer particles production. At the costant value of the other parameters, if the rotary rate (in rotary nuzzle), pressure (in pressure nozzle) and ratio air/liquid (in biphases nozzle) rise, the particle diameter decreases.

Aim

According with the propose of this research, the encapsulation should allow to increase the antioxidant property of a functional chocolate bar, masking the bitter and astringent flavour of

enriched polyphenols. Therefore, the aim of this study is to find the best conditions to encapsulate polyphenols from cocoa shells using spray dried technique. The choose of the coating material, the best ratio between maltodextrin and core and the optimal process parameters (flow feed and inlet temperature) are investigated. The encapsulation is evaluated in term of yield, efficiency and stability of the capsules.

Matherials and methods

Raw material and chemicals

Cocoa shells are collected by the Dolceamaro SRL (Monteroduni (IS), Italy). The materials are then submitted to extraction procedure, illustrated in the previous paragraph.

Maltodextrin (dextrose equivalent (DE) 17.0 - 19.9) is obtained from the hydrolysis of starch of wheat by enzymes. It is a white powder with a soft sweet taste. The composition of maltodextrin is reported as follows:

Dextrose %/ds 2 HPLC 210

Maltose %/ds 7 HPLC 210

Maltotriose %/ds 9 HPLC 210

Polysaccharides%/ds 82 HPLC 210

The moisture content is less than 5% and its physiscal properties include the conducibility <= 150 µS/cm at 25 °Brix and density of 450-600 g/dm3. Furthermore, this polysaccharide is free soluble in water while it slightly soluble in anhydrous alcohol. Maltodextrin is purchased from CHIMPEX INDUSTRIALE SPA (Caivano (NA), Italy).

Ultrapure water from a Milli-Q System (Millipore Inc., USA) is used. Ethanol (96% v/v), Methanol (99.8% v/v), Acetic Acid (99.8% v/v), Gallic acid, Calcium Carbonate, DPPH e Folin-Ciocalteau's phenol reagents are purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA)

Encapsulation process

Polyphenols extract are encapsulated, using maltodextrin as coating materials. Coating solutions (10% w/v) are prepared 1 day before the encapsulation process to obtain full dissolution in the solvent (water). For encapsulation, 1g of lyophilized polyphenols are mixed with 1:5, 1:10 and 1:15 core to coating ratio and the mixture are homogenized at 7000 rpm for 10 min in a high speed homogenizer (DH. WHG02118, DAIHAN Scientific CO., Ltd. Korea) until obtaining a good dispersion.

Spray-drying is carried out in an equipment Buchi B-290 spray dryer (Buchi Labortechnic AG, Switzerland) using a liquid feed volumetric flow rate of 6 mil/min and 9 mil/min.

The drying air inlet temperature of 120°C and 150°C nozzle air flow-rate, 600 NL (litters at normal conditions)/h and aspiration 75% (28 m^3/h).

Capsules are stored at room temperature and protected from the light until following analyses.

Studied parameters

Coating agent:

MD	10%

Ratio:

Coating agent:core	1:5
Coating agent:core	1:10
Coating agent:core	1:15

Inlet Temperature

Temperature	120°C
Temperature	150°C

Flow Feed

Flow Feed	20%
Flow Feed	30%

Yield

The dried yield was calculated using Eq. (1)

$EE\ (\%) = (W_C / (W_M + W_P)) * 100$

Where:

W_C is the weight of capsules;

W_M is the weight of dry maltodextrin;

W_P the weight of dry polyphenols.

Moisture content

1 g of capsules is kept in a hot-air oven at 105°C they reached a constant weight, and the moisture content is calculated in terms of the weight loss (AOAC, 2000).

Total phenolic content (TPC) and surface phenolic content (SPC)

The total phenolic (TPC) were determined following the methods of Saénz et al. (2009). One hundred milligrams of the prepared microcapsules were dispersed in 1 ml ethanol, acetic acid, and water

(50:8:42). This dispersion was agitated using a Vortex (1 min) and then an ultrasonicator twice for 20 min. The supernatant was centrifuged at 6000 rpm for 10 min and then filtered. The amounts of phenolic compounds were quantified by the Folin–Ciocalteau method (Singleton & Rossi, 1965). For the determination of surface phenolic compounds (SPC), 100 mg of microcapsules were treated with 1 mL of a mixture of ethanol and methanol (1:1). These dispersions were agitated in a Vortex at room temperature for 1 min and then filtered (0.45 lm Millipore filter) (Robert, 2010). The amounts of phenolic compounds were quantified by the Folin–Ciocalteau method (Singleton & Rossi, 1965).

The encapsulation efficiency (EE) was calculated using Eq. (2).

EE (%) = [(TPC−SPC) /TPC] x 100

Antioxidant activity

Antioxidant activity (AA) was determined using DPPH method (Tolun et al., 2016) with some modifications.

50 milligrams of microcapsules were dispersed in 1 ml ethanol:acetic acid:water mixture (50:8:42 v/v/). The emulsion was agitated using a Vortex (1 min) and then an ultrasonicator twice for 20 min. The supernatant was centrifuged at 6000 rpm for 10 min and then filtered through a filter 0.45 µm. The samples were diluted with ethanol:acetic acid:water mixture (50:8:42 v/v) after filtration.

100 mL of diluted samples were mixed with 3.9 mL of 25 ppm DPPH˙radical solution (2.5 mg DPPH in 100 mL MetOH) and left in the dark at 25◦C. For control, 100 mL of ethanol:acetic acid:water mixture (50:8:42 v/v) were used instead of samples. Finally, for each solution the absorption was measured at 517 nm by using a UV/VIS spectrophotometer.

The percentage inhibition (%I) of the free radical by the sample was calculated using the equation (3):

Percentage inhibition (%I) = [($Abs517_{control}$ − $Abs517_{sample}$)/ $Abs517_{control}$] × 100

Particle size analysis

The particle size distributions of microparticles were measured via a Malvern Mastersizer 3000E Hydro, (Malvern Instruments, Worcestershire, U.K.). Each individual particle size measurement was determined from the average of three readings made per sample. Mastersizer was used with a laser obscuration greater than 10%, experimental tests were repeated three times for each run, and micro-beads were characterized as milky particles with particle adsorption index equal to 1.582 and particle refraction index equal to 1.330. Small variation of optical indices did not show significant variation of results. Although the non spherical option was used, a certain amount of size distribution spread is related to the relative orientation of the particles passing by in the detection area respect to laser light

scattering detection sensors. Results were averaged using at least 20 independent measures. The particle size was expressed as volume weighted mean diameter D.

Statistical analysis

All experiments were performed in triplicate. Data are reported as the mean and standard deviation (SD) of three replicates. Statistical significance of treatment effects was determined with ANOVA. Least significant differences were obtained with a Scheffè's test ($p < 0.05$). The statistical analyses were performed with SPSS version 13.0 for Windows (SPSS, Inc., Chicago, IL).

Results and discussion

Yield

The yield of the powders under different process conditions were investigated (Tab.5). Generally, powder's yield showed an increase with the rising temperature at different core: coating material ratios. An opposite tendency was observed with the increase of flow feed from 6 ml/min to 9 ml/min. usually high temperature and low flow feed leads a greater efficiency in heat and mass transfer, improving the final yield (Tonon et al., 2008). According to Tolun et al. (2016), the yield also rises increasing the ratio core: coating agent because of maltodextrin reduces the deposition of particles on the wall of drying chamber (Quek et al., 2007) and allows a greater trap of the bioactive substance during the drying process. The best results were obtained for S3 (78.05%), S5 (73.32%) and S11 (80.68%) using 1:5, 1:10, 1:15 core: coating ratio, respectively.

Tab.5- Yield of microcapsules at different core:coating agent ratios, inlet temperature, outlet temperature and flow feed conditions

SAMPLE	RATIO CORE: COATING AGENT	INLET TEMPERATURE [°C]	OUTLET TEMPERATURE [°C]	FLOW RATE [ml/min]	YIELD [%]
S1	1:5	120	67	6	71.94
S2	1:5	120	51	9	73.98
S3	1:5	150	87	6	78.05
S4	1:5	150	71	9	59.44
S5	1:10	120	62	6	73.32
S6	1:10	120	50	9	61.15
S7	1:10	150	87	6	68.69
S8	1:10	150	72	9	68.77
S9	1:15	120	62	6	77.96
S10	1:15	120	57	9	70.93
S11	1:15	150	73	6	80.68
S12	1:15	150	60	9	76.30

Moisture

The moisture is one of the most important parameter evaluated to have useful information about the stability of the capsules. In 1:10 and 1:15 core: coating ratio, the moisture decreases with an increase

of the drying temperature. This phenomenon is influenced by the greater drying rate of water evaporation at high temperature. On the other hand, increasing the feed flow rate the difference between inlet and outlet temperatures rises (Tab.5), loading higher residual moisture content in the particles. About the core: coating agent ratio, increasing from 1:5 to 1:15 the moisture content decreases. This result confirms the data proposed by Singh & Heldman (2009). Maltodextrin with high degree of polymerization enhances the drying particles reducing the residual humidity in the final product. Furthermore, additives, like maltodextrin, reduce the glass transition temperature (T_g) of the product that provides less resistance to mass transfer. T_g is the temperature above the structure of feed pass from glassy state to rubbery state and it depends on the coating agent nature. According with the previous discussion the lowest value of moisture was found in S3 (3.92%), S7 (3.57%) and S11 (2.44%) for 1:5, 1:10 and 1:15 core: coating agent respectively (Fig.15)

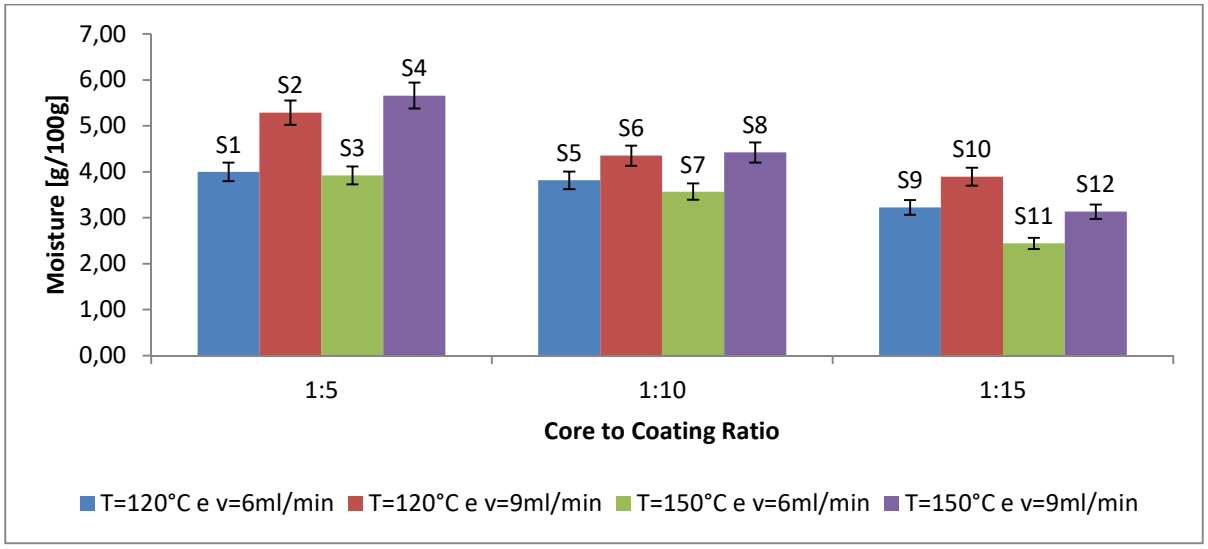


Fig.15– Moisture of microcapsules at different core: coating agent ratios, inlet temperature and flow feed conditions

The total polyphenol content (TPC), surface polyphenol content (SPC) and encapsulation efficiency

The total polyphenol content aren't significantly influenced by the inlet temperature and feed flow rate (Fig.16). Usually polyphenols are sensitive to high temperature but probably the range 120-150°C isn't sufficient to reduce their amount. These results are according with those obtained by Tolun et al. (2016), who studied grape polyphenols at different inlet temperatures.

On the other hand the total polyphenol content is affected by the core: coating agent ratio. The amount of the latter decreases as a result of the increasing concentration of maltodextrin in the mixture. In 1:5 ratio, the highest result (26.19) is obtained at 150°C and 6 ml/min (S3) (Fig.16). The same tendency could be observed for surface polyphenols, whose values aren't very high. Therefore, the encapsulation efficiency is correlated only to the core: coating agent ratio, with the highest values for

S3 (94.63%), and S2 (91.30%) (Fig.17). Finally, according with Poomkokrak et al. (2015), data suggests that encapsulation efficiency is not influenced by moisture content of the powder.

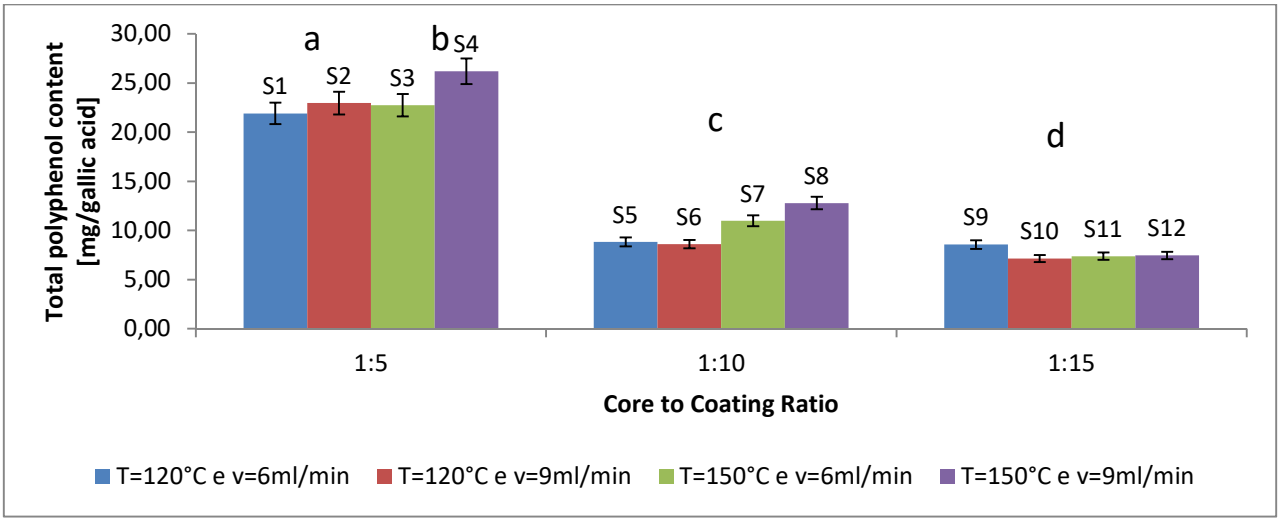


Fig.16- Total polyphenol content of microcapsules at different core: coating agent ratios, inlet temperature and flow feed conditions. Different letters indicate significant difference ($P < 0.05$)

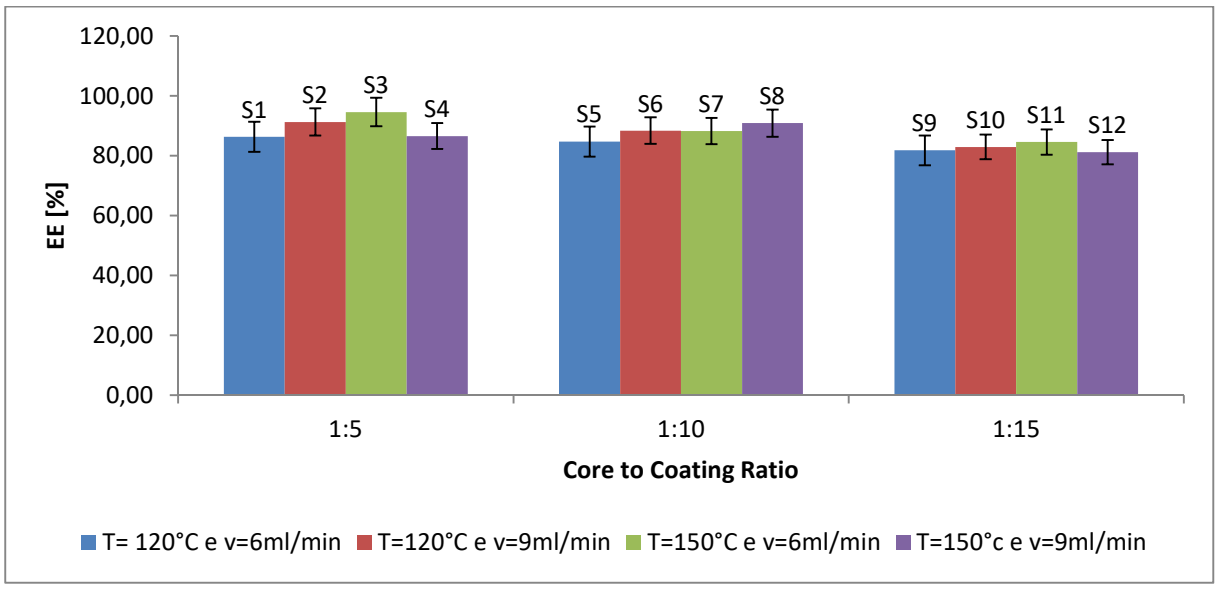


Fig.17- Encapsulation efficiency at different core: coating agent ratios, inlet temperature and flow feed conditions.

Antioxidant Activity

There is a positive correlation between polyphenols and antioxidant activity. For this reason, the AA% depends only with the core: coating ratio parameter. As shown in Fig.18 the averages AA% values are about 26.90% for 1:15; 52.35% for 1:10 and 63.75% for 1:5. Antioxidant activity decreases when coating material concentration increases due the dilution effect of maltodextrin that doesn't contain any active radicals.

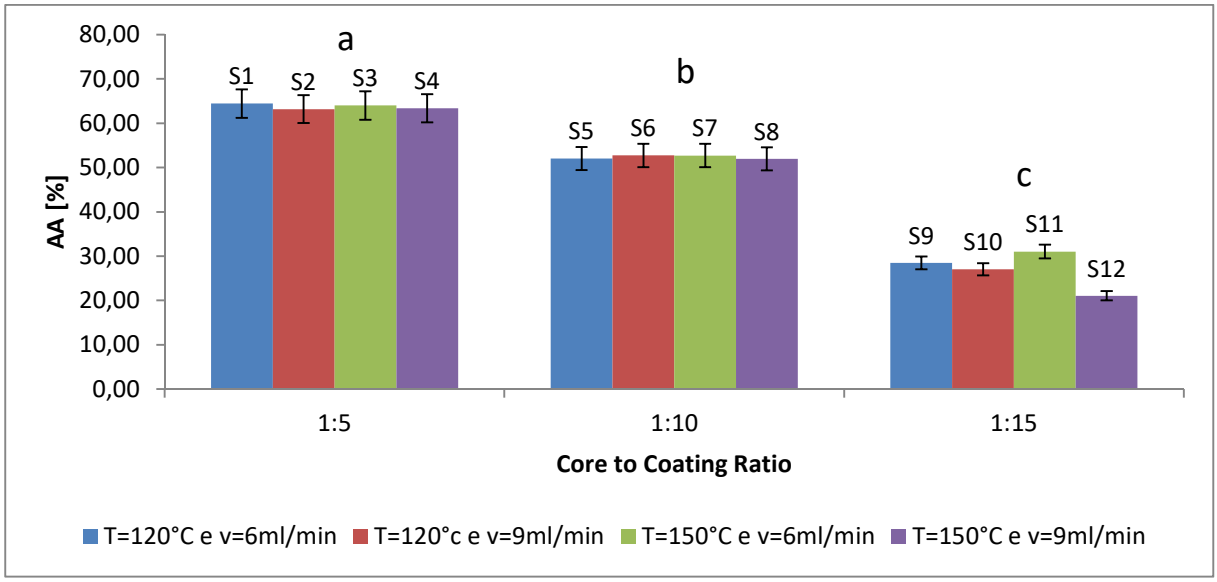


Fig.18- Antioxidant activity at different core: coating agent ratios, inlet temperature and flow feed conditions. Different letters indicate significative difference (P < 0.05)

Finally, considering the Total Polyphenol Content, the Encapsulation Efficiency and the Antioxidant Activity, the best results were related with the 1:5 core: coating ratio. Among these samples, S3 showed the highest yield and the lowest moisture content, resulting an optimum compromise of the studied conditions.

Particle Size

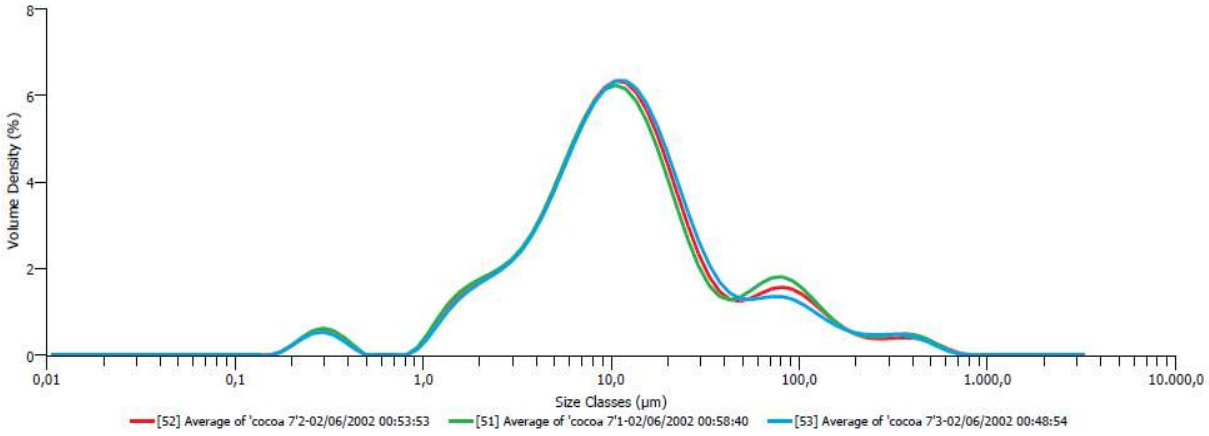


Fig.19- Particle size distribution of cocoa powder

The mean particle size of encapsulated particles was $D_{[4,3]}$ =30 µm (Fig.19). The encapsulated powder produced from maltodextrin showed a trimodal distribution with three sizes: approximately at 0.5 µm, 10 µm and 80 µm respectively. These wide range of size is affected by spray dried technique that cause changes in the emulsion structure with fragmentation of droplets during atomization and their following aggregation due recirculation which bring into contact particles of different drying stages.

Bibliography

- Anandharamakrishnan C., Ishwarya S.P. (2015) Spray Drying Techniques for Food Ingredient Encapsulation, John Wiley and Sons, Ltd., Chichester, UK
- AOAC (2000) Official method of analysis of AOAC international, 17th ed., Association of Official Analytical Chemistry, Gaithersburg
- Balasubramani P., Palaniswamy P.T., Visvanathan R., Thirupathi V., Subbarayan A., Maran Prakash J. (2015) Microencapsulation of garlic oleoresin using maltodextrin as wall material by spray drying technology, International Journal of Biological Macromolecules, Vol. 72, 210-217
  https://doi.org/10.1016/j.ijbiomac.2014.08.011
- Bemiller J., Whistler R. (2009) Starch: Chemistry and Technology, 3rd ed., 310-315, Academy press USA
- Champagne C.P., Fustier P. (2007) Microencapsulation for the improved delivery of bioactive compounds into foods, Current Opinion in Biotechnology, Vol. 18, No. 2, 184-190 https://doi.org/10.1016/j.copbio.2007.03.001
- Desai K.G.H., Park H.J. (2005) Recent Developments in Microencapsulation of Food Ingredients, Drying Technology, Vol. 23, No. 7, 1361-1394
  https://doi.org/10.1081/DRT-200063478
- De Vos P., Faas M.M., Spasojevic M., Sikkema J. (2010) Encapsulation for preservation of functionality and targeted delivery of bioactive food components, International Dairy Journal, Vol. 20, No. 4, 292-302
  https://doi.org/10.1016/j.idairyj.2009.11.008
- Estevinho B.N., Carlan I., Blaga A., Rocha F. (2016) Soluble vitamins (vitamin B12 and vitamin C) microencapsulated with different biopolymers by a spray drying process, Powder Technology, Vol. 289, 71-78
  https://doi.org/10.1016/j.powtec.2015.11.019
- Fernandes R.V.B., Borges S.V., Botrel D.A. (2014) Gum arabic/starch/maltodextrin/inulin as wall materials on the microencapsulation of rosemary essential oil, Carbohydrate Polymers, Vol. 101, 524-532
- Fogler B.B., Kleninschmidt R.V. (1938) Spray drying, Industrial and Engineering Chemistry, Vol. 30, No. 12, 1372-1384
  https://pubs.acs.org/doi/abs/10.1021/ie50348a007

- Gharsallaoui A., Roudaut G., Chambin O., Voilley A., Saurel R. (2007) Applications of spray-drying in microencapsulation of food ingredients: An overview, Food Research International, Vol. 40, 1107-1121
  https://doi.org/10.1016/j.foodres.2007.07.004
- Gibbs B.F., Kermasha S., Alli I., Mulligan C.N. (1999) Encapsulation in the food industry: a review, International Journal of Food Sciences and Nutrition, Vol. 50, No. 3, 213-224 https://doi.org/10.1080/096374899101256
- Gohel M.C., Amin A.F., Patel K.V., Panchal M. K. (2002) Studies in release behavior of diltiazem HCL from matrix tablets containing (hydroxypropyl) methyl cellulose and xanthan gum, Chemical and Pharmaceutical Bulletin, Vol. 141, 21-28
- Handscomb C.S., Kraft M., Bayly A.E. (2009) A new model for the drying of droplets containing suspended solids, Chemical Enginerring Science, Vol. 64, 628-637 http://dx.doi.org/10.1016/j.ces.2008.04.051
- Huang D. (2011) Modeling of particle formation during spray drying, European Drying Conference - EuroDrying' 2011, Palma, Balearic Island, Spain, 26-28
- Jafari S.M., He Y., Bhandari B. (2006) Nano-emulsion production by sonication and microfluidization - a comparison, International Journal of Food Properties, Vol. 9, 475-485
- Kaushik P., Dowling K., Colin B.J., Adhikari B. (2014) Microencapsulation of omega-3 fatty acids: A review of microencapsulation and characterization methods, Journal of Functional Foods, Vol. 19, Part B, 868-881
  https://doi.org/10.1016/j.jff.2014.06.029
- Kemp I.C., Hartwig T., Hamilton P., Wadley R., Bisten A. (2016) Production of fine lactose particles from organic solvent inlaboratory and commercial-scale spray dryers, Dry Technology, Vol. 34, 830-842
  http://dx.doi.org/10.1080/07373937.2015.1084314
- Keshani S., Daud W.R.W., Nourouzi M.M., Namvar F., Ghasemi M. (2015) Spray drying: An overview on wall deposition, process and modeling, Journal of Food Engineering, Vol. 146, 152-162
  http://dx.doi.org/10.1016/j.jfoodeng.2014.09.004
- Lovegrove C.H., De Noni E.I., Patel H., El S.N., Grassby T., Zielke C., Ulmius M., Nilsson L., Butterworth P.J., Ellis P.R., Shewry P.R. (2017) Role of polysaccharides in food, digestion, and health, Critical Reviews in Food Science and Nutrition, Vol. 57, No. 2 https://doi.org/10.1080/10408398.2014.939263

- Maas G.S., Schaldach G., Littringer E.M., et al. (2011) The impact of spray drying outlet temperature on the particle morphology of mannitol, Powder Technology, Vol. 213, 27–35
- Martirosyan D.M., Singh J. (2015) A new definition of functional food by FFC: what makes a new definition unique?, Functional Foods in Health and Disease, Vol. 5, No. 6, 209-223 https://doi.org/10.31989/ffhd.v5i6.183
- Masters K. (1991) Spray Drying Handbook, 5th ed., Longman Scientific and Technical, Harlow
- Mezhericher M., Levy A., Borde I. (2010) Theoretical Models of Single Droplet Drying Kinetics: A Review, Drying Technology, Vol. 28, No. 2, 278-293 https://doi.org/10.1080/07373930903530337
- Munin A., Edwards-Lévy F. (2011) Encapsulation of Natural Polyphenolic Compounds; a Review, Pharmaceutics, Vol. 3, No. 4, 793-829 https://doi.org/10.3390/pharmaceutics3040793
- Ohnesorge W.V. (1936) Die Bildung von Tropfen an Düsen und die Auflösung flüssiger Strahlen, Zeitschrift fürAngewandte Mathematik und Mechanik, Vol. 16, 355-358
- Onwulata C.I. (2012) Microencapsulation and functional bioactive foods, Journal of Food Processing and Preservation, Vol. 37, No. 5, 510-532 https://doi.org/10.1111/j.1745-4549.2012.00680.x
- Poomkokrak J., Niamnuy C., Choicharoen K., Devahastin S. (2015) Encapsulation of soybean extract using spray drying, Journal of Food Science and Agricultural Technology, Vol. 1, No. 1, 105-110
- Quek Y.S., Chok N.K., Swedlund P. (2007) The physicochemical properties of spray-dried watermelon powders, Chemical Engineering and Processing, Vol. 46, 386-392
- Robert P., Gorena T., Romero N., Sepulveda E., Chavez J., Saenz C. (2010) Encapsulation of polyphenols and anthocyanins from pomegranate (Punica granatum) by spray drying, International Journal of Food Science and Technology, Vol. 45, 1386-1394
- Rowe R.C., Sheskey P.J., Owen S.C. (2006) Handbook of pharmaceutical excipients, 5th ed., 442-444
- Singh R.P., Heldman D.R. (2009) Introduction to Food Engineering, Elsevier's Science & Technology
- Singleton V.L., Rossi J.A. (1965) Colorimetric of total phenolics with phosphomolybdic-phosphotungstic acid reagents, American Journal of Enology and Viticulture, Vol. 16, 144-158

- Teixeira da Silva P., Fries L.L.M., Ragagnin de Menezes C., Holkeml A.T., SchwanI C.L., WigmannI É.F., De Oliveira BastosI J., De Bona da Silva C. (2014) Microencapsulation: concepts, mechanisms, methods and some applications in food technology, Food Technology, Vol. 44, No. 7, 1304-1311
  http://dx.doi.org/10.1590/0103-8478cr20130971
- Tolun A., Altintas Z., Artik N. (2016) Microencapsulation of grape polyphenols using maltodextrin and gum arabic as two alternative coating materials: development and characterization, Journal of Biotechnology, Vol. 239, 23-33
  https://doi.org/10.1016/j.jbiotec.2016.10.001
- Tonon R.V., Brabet C., Hubinger M.D. (2008) Influence of process conditions on the physicochemical properties of acai (Euterpeoleraceae Mart.) powder produced by spray drying, Journal of Food Engineering, Vol. 88, 411-418
- Tonon, R.V., Brabet C., Hubinger MD. (2011) Spray drying of acai juice: Effect of inlet temperature and type of carrier agent. Journal of Food Processing and Preservation, Vol. 5, 691-700
- Zbicinski I. (2017) Modeling and scaling up of industrial spraydryers: a review, Journal of Chemical Engineering, Vol. 50, 757-767
  http://dx.doi.org/10.1252/jcej.16we350
- Zbicinski I., Delag A., Strumillo C., Adamiec J. (2002) Advanced Experimental Analysis of Drying Kinetics in Spray Drying, The Chemical Engineering Journal, Vol. 86, No. 1, 207-216
- https://www.buchi.com/en/products/spray-drying-and-encapsulation/mini-spray-dryer-b-290
- https://www.gea.com/en/expert-knowledge/milk-powder-manufacture/components-spray-drying-installation.jsp
- https://www.indiamart.com/pmindustries/spray-dryer.html

CHAPTER 3 FUNCTIONAL CHOCOLATE BAR PRODUCTION

Chocolate manufacturing

Chocolate is an emulsion of cocoa particles in a fat continuous phase. The production is composed by several phases, starting with the mixing of ingredients.

The basic ingredients for every type of chocolates are cocoa mass, sugar, cocoa butter, emulsifier and flavouring (for dark chocolate). For milk chocolate, producers also add powdered milk, while for white chocolate, they remove cocoa mass and use milk. Then, depending on type of chocolates (flavouring, gianduja…) other raw materials (tree nuts, flavours, dehydrated fruits), can be added.

Usually ingredients are mixed in a whole batch for 12-15 min. After this step, chocolate paste is refined up to maximum particle sizes of 20 μm using roll refiners. The right dimension of the particles affects whether the palatability of the final product or its workability. If the particles are very fine, more cocoa butter will be added in the recipe to improve the consistency of chocolate; on the other hand, greater particles lead to a sensation of roughness on the palate (Minifie, 1999). Five rolls mounted vertically, through which paste pass compose today's refiners, reducing its thickness gradually. Ball mill is another system used to reduce particle size, especially for chocolate high of fat. Chocolate mass is pumped through the ball mill, which is filled with steel balls rotated by a high-speed stirring gear. The operating rates, the pumping speed and the dimension of the balls affect the texture of the final chocolate (https://www.cacaochocolade.nl).

After the refining, some producers do the conching of chocolate to promote the development of its final texture and flavour. This step consists in the moving and warming of the mass for long time at high temperature (until 82°C). During this process the remaining moisture of the paste and the undesirable volatiles compounds (e.g. acetic acid) are removed. Furthermore, it helps the whole dispersion of solids in liquid fat. The prolonged mixing also causes the development of flavour, like caramel because of Maillard sugar reaction. Conching also affects the reduction of the astringency because of the oxidation of polyphenols and the formation of complexes between tannins and proteins (Barišic et al., 2019). Conching was invented by Rudolph Lindt in 1879. The conche was a machine, with the shape of a shell, in which large stone rollers mixed and aerated the liquid chocolate.

After conching, the liquid chocolate could be mould and sell to the manufactures (covered chocolate) or submitted to tempering for chocolate bar production. Currently there are only 273 known bean-to-bar chocolate makers (http://bean.bar) in the word, while everyone else work the cocoa mass or covered chocolate.

The following process operation is tempering that confers a good texture and colour to chocolate and prevent the bloom fat development. These properties depend on the nature of the crystalline form of cocoa butter, relating to temperature condition. Cocoa butter can exist in six polymorphs forms (from I to VI) with different physical characters (e.g. melting point) that influence the stability of the final product (Wille & Lutton, 1966). Usually the V form is associated to a shelf stable chocolate, the IV form is related to under-tempered chocolate and VI is often responsible of fat bloom defect, common for old chocolate bar (Bresson et al., 2011). A general machine tempering consists in a steel tank wrapped by a thermostatically controlled water jacket. The chocolate is continuously submitted to a decrease of temperature from 46-49° to about 28-29°C (for dark chocolate) in order to obtain the stable forms of cocoa butter. Finally, the tempered liquid chocolate is put in moulds and solidified by using cooling tunnel (Barišic et al., 2019). After cooling, moulds are removed and the chocolate bars packed.

Organoleptic property and sensorial analysis

The sensory analysis of food is an interdisciplinary scientific discipline based on the description, measurement and interpretation of product characteristics that can be perceived by human senses.

For chocolate evaluation all senses are involved in the analysis: sight for the appearance of food; smell, that is detected both before and during eating for the odours and flavours perception respectively; touch, that refers to the mouth feel (texture, consistency…); hearing because the sounds offers indication about food quality; taste, that involves only five sensations (salt, sweet, sour, bitter and umam). To improve the sensorial capability of chocolate is very important know the features of product (ingredients, production techniques…) and the training of panels with reference samples and the use of the scale.

During sensorial analysis, each judge has to work individually to avoid the influence from the group. He has good health, physical comfort and mental poise to not alter the perceptions. Judges shouldn't wear perfume, aftershave, deodorant, hand cream. Furthermore, before tasting they can't eat heavy. Ideally testing takes place in a quiet area, with adequate light and ventilation.

For chocolate analysis, tester can judge until 8 samples of 15-20 g each.

Actually, there aren't any official recognized methods to evaluate chocolate.

Compagnia del Cioccolato, has drowned a sheet in order to judge different types of dark chocolate bars and it is currently used for the "golden chocolate bar" award. The sheet is divided in six sections: five are related with the different senses and the last one regards the final sensation. For each parameter, that describes a feature of chocolate, is assigned a score. The final score (0-100) is obtained by the sum of the scores of each section and describes the quality of product:

86-100 is an excellent chocolate bar;

76-85 is a good quality chocolate bar;
63-75 is a mediocre chocolate bar (for GDO);
50-62 is a low quality chocolate bar;
<50 low quality of cocoa and bad chocolate manufacturing.

The first sense involved in the chocolate sensorial analysis is the sight in order to evaluate the appearance of the product. The colour represents an indicator of the product quality: often, reddish chocolate is originated from fine variety cocoa beans. But it also depends on the manufacturing, (alkalization and long roasting confers dark colour to chocolate) (Caraceni, 2020). During storage, chocolate can be subjected to fat bloom that is visible as gray stains on its surface and a loss of brilliance. This defect is related with errors during production process, such as tempering, forming and cooling or a wrong ratio between the ingredients. Another problem is the presence of holes on the product surface because of the introduction of air during manufacturing (Popov-Raljić et al., 2009). After a preliminary visual inspection, chocolate has to be broken with the hands: if the product has been well tempered, it will make a few crumbs.

In sensorial analysis also hearing constitutes an instrument to evaluate the quality of chocolate: snap is a term commonly used to describe the sound of chocolate braking. It depends on the conformation of cocoa butter due of tempering. When the sound is different, the chocolate results to be old or stored: at high temperature or humidity. Sometimes, a dull sound is related to the use of replicate vegetable fats or to a low refining (Caraceni, 2020).

Some of food properties are perceived through oral receptors during eating. The touch sense is tiled by the central part and the tip of the tongue: when you put a piece of chocolate in the mouth it blends, leading to the release of endorphins that offer a strong sense of well-being. Several parameters are evaluated with the touch. First is melting, estimated like the speed of melting and the sensation of filling in the mouth. It depends on the quantity of cocoa butter and the working processes (refining, conching and tempering). The fat also gives a refreshing and lubricating sensation after chocolate taste (Zamora, 2007).

A negative parameter is the astringency, a sensation perceived as a dry feeling in the mouth due the interaction between polyphenols and protein of saliva, that form precipitates or aggregates (Misnawi et al., 2005). This feature is correlated with the variety of cocoa beans (Forastero type contains more polyphenols than Criollo) and the use of mild conditions process that preserve polyphenols. Astringency is usually perceived after taste and it is linked with the acidity sensation.

The texture of chocolate involves mechanical properties such as hardness, cohesiveness, viscosity, elasticity, chewiness, brittleness, gumminess and adhesiveness. A good product must to be perceived

as silky, slippery, velvety and smooth. It is affected by the raw material and the manufacturing operations.

Fining of chocolate is a textural attribute influenced by the size of the particles and their distribution in the chocolate matrix, but also by the total fat and lecithin content (Beckett, 2009; Afoakwa et al., 2008). This parameter is linked with the aroma perceived during taste, because flavouring molecules are entrapped in ground particles.

Finally, rounding describes chocolates that are complex and have been crafted well to display balanced notes of flavour, acidity, aroma and polyphenols. Also this feature depends on the process and the quality of raw material.

One of the most important quality attribute of chocolate is flavour, that depends on bean genotype (primary flavour), manufacturing process (secondary flavour), and the type of doil and age of cocoa tree. The bean genotype affects the chemical composition of the bean, such as proteins, polysaccharides, and polyphenols. These components are precursors of flavour, that are formed during fermentation and drying processes. Cocoa bean fermentation and drying cause the break of proteins into amino acids and short chain oligopeptides and the degradation of polysaccharides into glucose and fructose. These chemical compounds react with each other to produce the cocoa flavour volatiles during roasting. Finally during fermentation and drying polyphenols are oxidized leading the reduction of the astringency and bitterness and increasing the flavour of cocoa beans (Kongor et al., 2016). In the (Fig.20) the most important flavours of chocolate are reported. They include positive flavours because confer a good taste to the product (e.g. fruity, floral, caramel/malt, nutty, green…). There are also off flavours such as over fermented, smoky, mouldy, unfermented, oily, caused by a wrong manufacturing or storage. Finally, the score of smell is related to the quality of flavours (positive/negative), their quantity (number of flavours perceived) and intensity (the length of time they last).

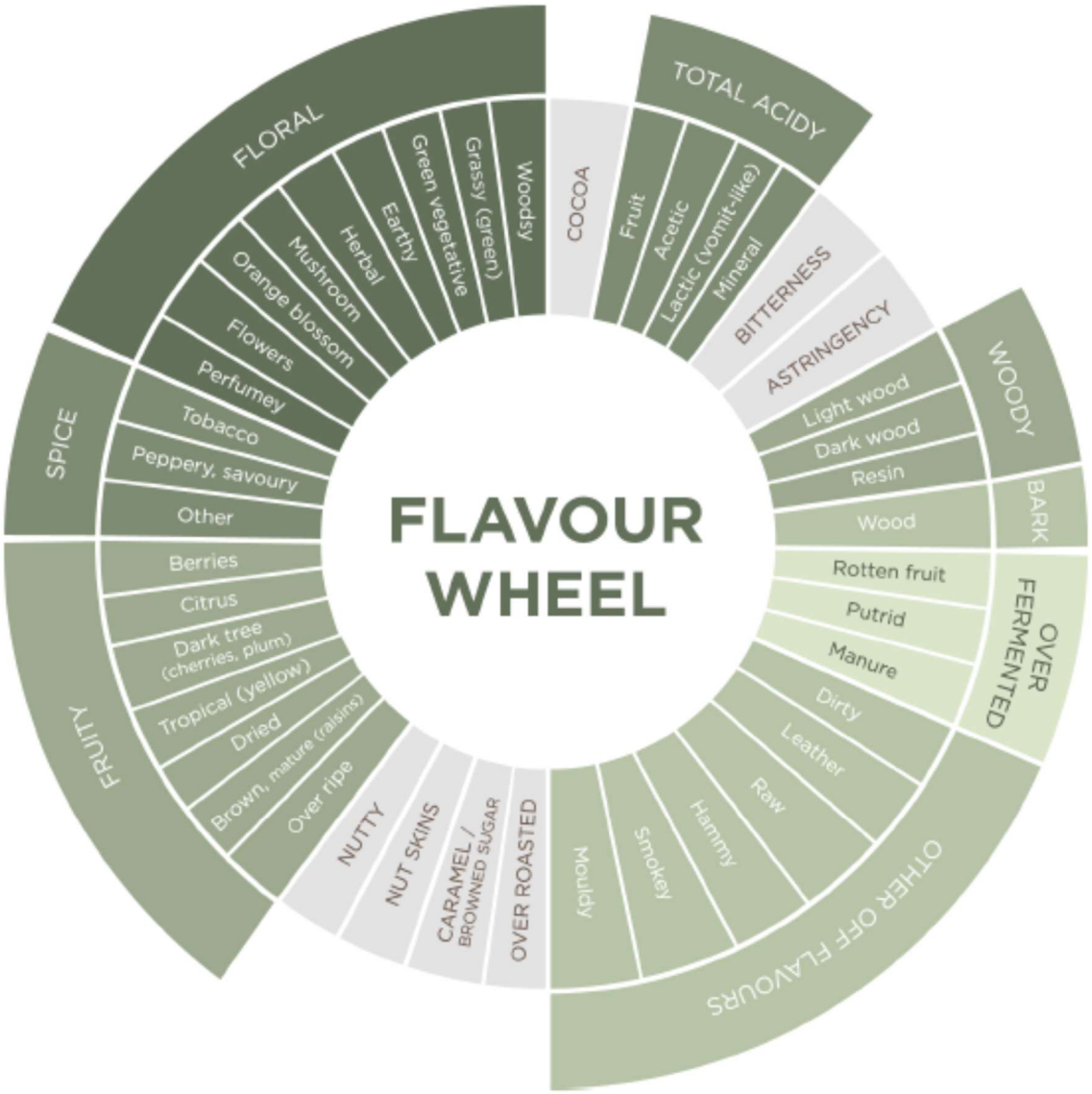


Fig.20- Flavour characterisation of cocoa (https://it.scribd.com)

The last sense, used for chocolate evaluation is taste. Taster can recognize only three attributes: acid, bitterness and sweetness. Acid is usually correlated with the quality of the bean and the manufacturing process such as drying, roasting and conching that can remove the volatile acids. Sweetness and bitterness depend on the amount of sugar and cocoa mass respectively but also to the bean genotype: Criollo and Trinitario are generally finer than Forastero variety.

Tab.6- Influence of several factors on the quality evaluation parameter (Caraceni, 2020)

	Ingredient ratio	Cocoa quality	Coltivation	Fermentation	Drying	Cleaning	Roasting	Shelling	Refining	Mixing	Conching	Tempering	Cooling	Packaging	Storage
Appareance	*								*		*	***	*	*	***
Sound	*								*		*	***	*	*	***
Finess									***		**				
Texture		**	*	**	*		*	*	**		**	**			
Astringency	*	***	*	**	*						*				
Rounding	**	***	*	**	*		**	*	**		**	*			*
Melting	***								**		*	*			*
Primary Flavour	***	***	*	**			**	*	*		*	*			*
Secondary Flavours	**	***	*	***	*	*	***	*	*		*	*			*
Off flavors	*	**	***	***	***	*	**	*			*				***
Flavor quality	*	***	*	**	*	*	**	*			**				*
Persistence	**	***	*	*			*		*		*				
Sweetness	***	**	*	*			*								
Bitterness	**	***	*	*			*								
Acidity	*	***	*	***	***		**		*		**				
Taste pleasure	**	***	*	**	*		*				*				

Shelf life

The shelf life of a food product is the period of time during which it remains safety and preserves its desired sensory, chemical, physical and microbiological characteristics. Furthermore, during this time, the nutritional compounds have to be compliance with the data reported in the label, if the product is stored in the recommended conditions (Kilcast and Subramaniam, 2000). Chocolate is considered a shelf stable product due to the properties of cocoa: the presence of antioxidant compounds such as polyphenols and tocopherols and the low moisture. It has a shelf life of about 12-24 months (Mishra et al., 2017) depending of the category of products, its formulation and packaging and the manufacturing and storage conditions.

The composition of chocolate influences the flavour and the texture of the bar. If incompatible fats (palm kernel oil and cocoa butter) are mixed together, they will lead to the bloom of the product. Furthermore, some ingredients, such as nuts, can accelerate the oxidative rancidity of the chocolate. Regarding manufacturing process, for moulded products tempering and moulding are the main steps to assure the good quality and stability of the chocolate. Time of cooling influences the texture of the product: if cooling is too fast, the chocolate could break; but if it is cooled too slowly the bar will be discoloured and soft. The problem is that these defects will result during the storage of the product, after the seal to the final consumer. The use of properly designed moulds and right cooling conditions could prevent soft texture, the appearance of boom or fat crystals.

Finally, the storage conditions are very critical to maintain the original characteristics of the chocolate: usually in the label, the manufacturer advises consumers to keep the product in a cool and dry environment (temperature between 12-21°C and 60% of humidity), away from off-odours and light, that could cause the degradation of antioxidant compounds (Stauffer, 2007).

Generally, the main defects that can occur during chocolate shelf life are changes in its sensory attributes, fat bloom development, water and fat migration and rancidity of the fat.

Fat bloom (Fig.20a) depends on the fat structures of cocoa butter with a consequence loss of gloss and snap. Usually this defect is related with incorrect temperature storage or wrong tempering condition that cause a melting and re-crystallization of the fat in unstable polymorphic forms. Generally, sensory changes precede bloom development. They include the reducing of smoothness and caramel flavour and the development of stale aromas. These off-notes are related with the development of fat's rancidity. Antioxidants compounds, as polyphenols, can slow down this "aging" effect.

Another consequence of a long storage is the sugar bloom (Fig.21) that is caused by the moisture absorption of chocolate surface from high humidity environment. This defect could also occur during cooling because of the difference temperature between cold product and warm ambient air at the end of cooling tunnel. In order to predict the shelf life of chocolate products, accelerated storage test are used. This "forced aging" can be conducted using two different methods: temperature cycling and isothermal storage at high temperature (below cocoa butter melting point). Both procedures allow understanding the changes that can occur during storage (Kilcast and Subramaniam, 2000).

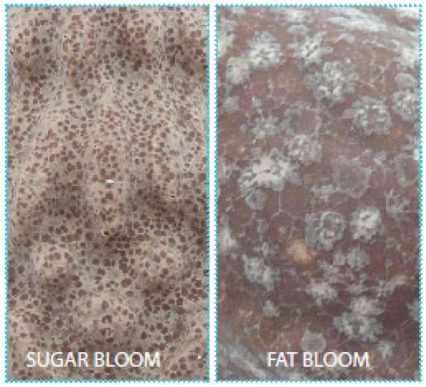


Fig.21- Sugar and fat bloom (www.anodscocoa.com)

Claim

The purpose of food labelling is to ensure the protection of consumers and the awareness of what they buy. In order to regulate the correct use of labelling by producers, Europe Parliament has issued the REGULATION (EU) No 1169/2011 on the provision of food information to consumers. In particular, the article 49 regards the voluntary informations like nutrition and health claims, for which it refers to REGULATION (EU) No 1924/2006. This law allows regulating the use of claims promoting equal conditions of competition between producers and protecting consumers from

misleading practices. In this way the voluntary indication has the only aim to promote the development of a healthier food diet (Peter, 2005).

Every claim have to be clear, accurate, based on scientific evidence and approved by Europe Commission. In the law, the nutritional and functional claims allowed are reported. Moreover, the research implies changes in the current knowledge and it leads to the request of new claims.

Currently, there is only a recognized claim about polyphenols, but it refers to those included in olives. In 2012, Barry Callebaut Belgium nv. requested to EFSA (European Food Safety Authority) the evaluation of the scientific data, in order to submit the claim related to cocoa flavanols and their healthy effect on the cardiovascular system. In the study, that supported the scientific evidence of claim, the Panel consumed at least 200 mg cocoa flavonols that are contained in 10 g of high flavanol dark chocolate, for 12 weeks. Research put in evidence the positive correlation between the consumption of chocolate and the maintaining of endothelium-dependent vasodilatation of the patients.

This declaration was restricted only to the use of Barry Callebaut for a period of five years from the date of publication of the law (31th March 2015). Therefore, starting from 21 April 2020 it will be extended to every product that satisfied the conditions reported in that regulation. On the other hand, claim doesn't constitute an authorization for the selling of cocoa polyphenol extract.

In conclusion, the REGULATION (EU) No 539/2015 introduced the authorization to update the list of permitted health claims made on foods, contained in the REGULATION (EU) No 432/2012, with the following declaration:

"Cocoa flavanols help maintain the elasticity of blood vessels, which contributes to normal blood flow".

This claim refers to the consumption of 200 mg/die of cocoa flavonols, contained in 10 g of dark chocolate, in the context of a balance diet for general population.

Aim

The chocolate production line of Dolceamaro Company consists in the tempering of cocoa liquor in order to produce bars. The aim of this project was to produce a functional chocolate bar enriched with encapsulated polyphenols. Generally, chocolate bar with 70% of cocoa solids contains about 500 mg/100g of total polyphenols. During manufacturing, some capsules of polyphenols were added to increase their amount and try to reach the recent claim "Cocoa flavanols help maintain the elasticity of blood vessels, which contributes to normal blood flow". This claim refers to the consumption of 200 mg/die of cocoa flavonols, contained in 10 g of dark chocolate. Furthermore, it was evaluated the sensorial characters of the functional chocolate bar and its stability during storage respect of a control sample.

Materials and Methods

Determination of shelf life

Accelerated storage test was conducted in order to predict the shelf life of the formed chocolate bars. After production, chocolate bars enriched with polyphenols (P) and the control samples (S), packed with PP film, were stored in incubator at isothermal condition of 25°C (Danzl & Rothkopf, 2016) at U=70% for 56 days. According with Stauffer (2007), one week of cycling corresponds to one month of shelf life.

Colour analysis

Colour parameters (L*, a*, b*) were measured with a CR-300 Chroma Meter (Minolta, Osaka) equipment. According to Caliskan & Nur Dirim (2013), the Hue angle H (°) and the Chroma C* values were calculated:

$$H (\circ) = \arctan (b^*/ a^*) \quad (1)$$

$$C^* = (a^{*2} + b^{*2})^{1/2} \quad (2)$$

Moisture

The chemical composition of the moisture was determined by the AOAC method (AOAC, 2000).

Water activity

Water activity was measured using an Aqua Lab Model Series CX2 water activity meter (Decagon Devices, Inc., Pullman, Washington 99163, USA)

Texture analysis

Sample texture was analyzed with a TA-XT2 Texture Analyzer (Texture Technologies Corp., Scardsale, NY) and Texture Expert Software. A 6 mm cylinder probe was used for simulate the bite compression. The conditions for the test were:

pretest speed of 2 mm/s, test speed of 2 mm/s, posttest speed of 5 mm/s, distance of 90%, relaxtion time of 5 s and force of 50g. Dimensions of chocolate pieces were 20mm X 20 mm X 20 mm (l x w x h). The parameters evaluated were hardness and adhesiveness.

Total polyphenol content

5g of ground chocolate was weighed accurately in a Falcon tube (50 mL). Fat was extracted three times with n-hexane (25 mL) by vortexing (3 min) and the tubes were centrifuged (15 min, 17°C, 5000 rpm). Phenolic compounds were extracted from the defatted, dried samples in Falcon tubes (50 mL) with 50 mL of ethanol/water (50:50, v/v) by vortexing (1 min) and subsequent sonication (20 min, T=20°C). Then, samples were centrifuged (15 min, 17°C, at 5000 rpm) and carefully decanted.

The supernatants were pooled and filtered with 0.45 µm PTFE filters (Chromacol, U.K.). The amounts of phenolic compounds were quantified by the Folin–Ciocalteau method (Singleton & Rossi, 1965).

The extracts of total polyphenols after filtration (0.20 µm polyester membrane, Sartorius Stedim Biotech GmbH, Goettingen, Germany) were injected in Inertsil® ODS C18 column (150 x 4,6 mm; 3,5 µm particle size; 100 A; Alltech Italia Srl). Epicatechin and catechin were determined using fluorescence detection with an excitation wavelength of 230 nm and emission at 321 nm. Theobromine and caffeine were detected using a photodiode array detector at 280 nm.
The binary system phases were solvent A (water: 85% phosphoric acid – 99.7: 0.3 (v/v)) and solvent B (water: acetonitrile: 85% phosphoric acid - 57.7: 40.0: 0.3(v/v/v)) with a constant flow rate of 2 ml $min^{-1}$and stop time after 35 min. The starting mobile phase condition was 0% B, so it was increased to 100% in 30 min to return to 100% A (30 min). The conditions were held at 100% B for the last four minutes. All flavonoids were determined using selected reaction monitoring (SRM), showed in the article of Ortega et al. (2010).

MS/MS conditions

HPLC was coupled to a triple quadrupole detector (TQD) mass spectrometer (Waters, Milford, MA, USA) using an electrospray ionization (ESI) source Z-sprayTM. For samples, the MS was operated in negative mode to analyze the procyanidins. The working conditions for the ionization source were as follows: capillary voltage, 3 kV; source temperature, 150 °C; cone gas flow rate, 80 L/h and desolvation gas flow rate, 800 L/h; desolvation temperature, 400 °C. Nitrogen (>99% purity) and argon (99% purity) were used as nebulizing and collision gases, respectively. The dwell time established for each transition was 100 ms for HPLC. Data acquisition was carried out by MassLynx v 4.1 software.

Sensory analysis

Twelve panellists (three males and nine females, aged 25–50 years) were trained in a specific course by Compagnia del Cioccolato, to become Chocolate Taster. Panellists were trained to assess the qualitative and quantitative differences between the products, including appearance, texture, flavour, hearing and taste. Sensory properties of chocolates were evaluated using all known relevant ISO standards (ISO 8589, ISO 8586-1, ISO 8586-2, ISO 5492, ISO 11036- 1988, 1984, 1993, 1994, 1992, 1994), that are compliance with IFST Guidelines for Ethical and Professional Practices for the Sensory Analysis of Foods (https://www.ifst.org/). Quality category was determined using scores spans: products, which were evaluated with less than 50 points, were considered as very low quality; scores within limits 50-62 characterized low quality; 63-75 medium quality; 76-85 good quality, 86-

100 excellent products. This result derived from the sum of the individual score related to each sensorial attribute (Fig.22).

Organoleptic evaluation sheet of DARK CHOCOLATE

Chocolate: _____

	Parameter	Value	Score
SIGHT	Appearence	From 0 (if porous, streaked, stained, uneven, grainy, opaque, etc.) to 4 (if homogeneous, glossy, uniform)	
HEARING	Snap (breaking sound)	From 0 (if deaf, soft) to 1 (if clear)	
TOUCH	Finess	From 0 (if grainy, sandy, floury, dusty, etc.) to 5 (if no particle is perceived)	
	Texture	From 0 to 2 (if very greasy, oily, sticky, chewy, etc.) from 3 to 5 (if pleasant) from 6 to 8 (if silky, creamy, sliding)	
	Astringency	From 0 (if unpleasant) to 5 (if absent or less perceived)	
	Rounding	From 0 to 2 (if flat) from 3 to 5 (if easily perceived) from 6 to 7 (if high)	
	Melting	From 0 (if slow) to 5 (if fast)	
		Tot.Touch	
FLAVOUR AND AROMA	Cocoa primary flavour	From 0 to 3 (if very weak) 4 to 7 (if slightly intense) from 8 to 10 (if intense, robust, easily perceived)	
	Secondary flavours	From 0 to 3 (if absent or less perceived) from 4 to 7 (if perceived but not easy to identify) from 8 to 10 (if easly perceived, intensive) from 11 to 12 (if finess and numerous) Flavours perceived:	
	Off-Flavours (mouldy, rancid, smooky, juta, carton, etc.)	From -5 (if clear perceived, unpleasant) to 0 (is absent) Off-flavours perceived:	
	General flavour	From 0 (if there are few flavours) to 5 (if it is rich of flavours)	
	Persistence of flavour	From 0 (if short) to 5 (if prolonged)	
		Tot. Flavour	

TASTE	Sweetness	From 0 (if unpleasant) to 6 (if less)	
	Bitterness	From 0 (if unpleasant) to 6 (if less)	
	Sourness	From 0 (if unpleasant) to 6 (if less)	
	Taste pleasure	From 0 to 3 (if less) from 4 to 7 (if good) from 8 to 10 (if perfect, balanced)	
		Tot.Taste	

Final sensation	From 0 (if unpleasant) to 5 (if very pleasant)	

50-62 **63-75** **76-85** **86-100** **TOTAL**

Date: _____

Notes:

Fig.22- Sensorial analysis sheet for dark chocolate

Statistical Analysis

Analyses were carried out in triplicate for both samples: standard chocolate (S), and chocolate enriched with polyphenol (P) at different times. Data obtained were analyzed by descriptive and analytical statistics. The aim of study is to identify if there are some differences between the samples during storage. For this purpose, repeated measures analysis of variance was performed, using mixed model approach (Bates et al., 2015). The effect of time and its interaction with both groups (S and P) was evaluated using R software (R Core Team 2019) at the significance level of $p \leq 0.05$.

Results and descussion

Color analysis

Tab.7- Color attributes: data are presented as mean±SD.

days	sample	L*	a	b	Hue Angle (°)	Chroma
$t_0 = 0$	S	29.56 ± 0.25	6.21 ± 0.09	4.56 ± 0.05	1.11 ± 0.03	7.71 ± 0.07
	P	28.62 ± 0.12	5.66 ± 0.17	3.53 ± 0.08	1.39 ± 0.06	6.67 ± 0.16
$t_1 = 14$	S	29.75 ± 0.09	6.25 ± 0.03	4.96 ± 0.04	0.98 ± 0.01	7.98 ± 0.04
	P	28.87 ± 0.40	5.47 ± 0.14	3.36 ± 0.14	1.42 ± 0.07	6.42 ± 0.17
$t_2 = 28$	S	29.57 ± 1.40	6.18 ± 0.19	4.43 ± 0.62	1.17 ± 0.26	7.61 ± 0.35
	P	33.91 ± 0.14	5.48 ± 0.11	5.40 ± 0.21	0.66 ± 0.03	7.69 ± 0.22
$t_3 = 42$	S	29.67 ± 0.58	5.88 ± 0.16	4.12 ± 0.31	1.19 ± 0.11	7.18 ± 0.27
	P	30.26 ± 0.67	6.33 ± 0.50	4.88 ± 0.29	1.03 ± 0.08	7.99 ± 0.54
$t_4 = 56$	S	29.23 ± 0.08	6.31 ± 0.10	4.65 ± 0.10	1.10 ± 0.01	7.84 ± 0.15
	P	29.25 ± 0.67	6.41 ± 0.42	4.46 ± 0.34	1.20 ± 0.08	7.81 ± 0.51

Commission Internationale de l'Eclairage (CIE) defined three parameters to identify colour: L* indicates lightness (+ = lighter, - = darker), a* is the red/green coordinate (+ = redder, - = greener), and b* is the yellow/blue coordinate (+ = yellower, - = bluer). The L*C*H colour space is similar to L*a*b*, but it describes colour differently using cylindrical coordinates instead of rectangular ones. In this colour space, L* indicates lightness, C* represents chroma (+ = brighter, - = duller), and H is the hue angle (https://sensing.konicaminolta.us). At the beginning samples have lightness difference: polyphenol enriched chocolate (29.6 ± 0.2) is darker than standard one (28.6 ± 0.1). The first bar showed a constant trend during storage instead of P that is very irregular. This trend depends on the wrong tempering and cooling of the chocolate that promoted the sugar bloom with consequence white spots on the surface (Fig.23a,b). The adding of capsules, containing maltodextrins, changed the rheology of chocolate (Fig.23c) making it more sensitive to storage conditions. Also values of "a" and "b" showed a significantly difference in time (from t_0 to t_5) and in the interaction (S vs. P). These results are also reflected by the Hue Angle and Chroma values showing a great fickleness. In conclusion, observing only data at the beginning and at the end of storage time, both samples, show a colour saturation: they passed from 7.71 ± 0.51 to 7.84 ± 0.15± 0.51 (S) and from 6.67 ± 0.16 to 7.81 (P) respectively (Tab.7).

These results are consistent with the literature data (Popov-Raljić, 2009), indicating to the appearance of gray that depends on the microstructure of chocolate and colour changes, that are specific for each kind of chocolate (Aguilera & Maior, 2004).

Fig.23a,b- Appearance of chocolate samples: standard on the right and P on the left; Fig.23c- Chocolate liqueur

Water activity and moisture

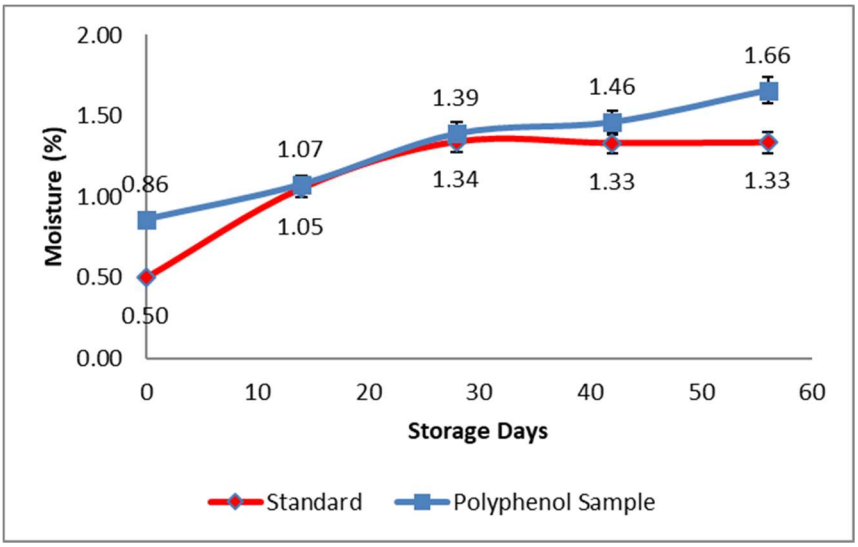


Fig.24- Moisture content during the accelerated storage test of chocolate bars (packed with PP film, T=25°C and RH=70%). (S) = control, (P) = sample with added phenols.

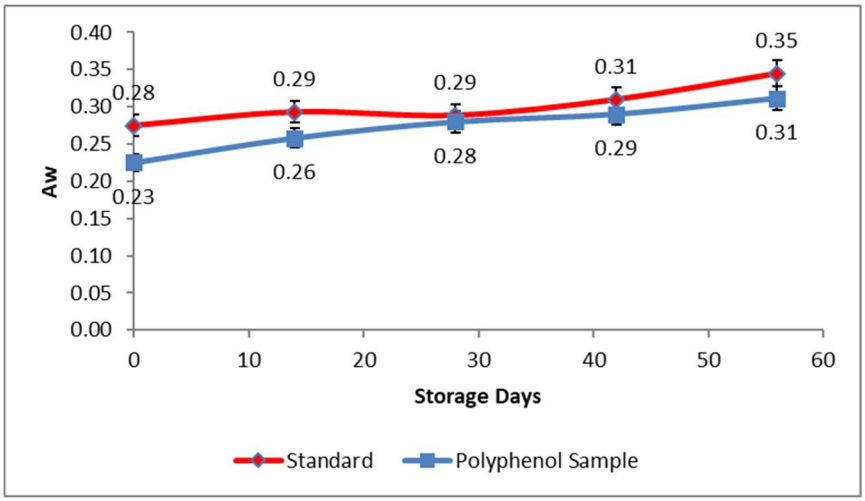


Fig.25- Aw during the accelerated storage test of chocolate bars (packed with PP film, T=25°C and RH=70%). (S) = control, (P) = sample with added phenols.

During storage, samples P and S show the same trends both for water activity and moisture (Fig.24, 25). Standard sample is characterized by an increasing of moisture from 0.50 ± 0.06% at the beginning to 134 ± 0.02% at time t_3; then, moisture keep constant during the remaining time. Instead of S, P sample shows water absorption during whole storage period. Chocolate bars are subjected to a significant increasing of moisture values due the high humidity and the warm temperature in time. These conditions affect the condensation of water into products (Kilcast and Subramaniam, 2000) leading the sugar bloom on the surface of chocolate (Fig.23a,b). The absorption is greater the more the structure is weaker.

However, the average moisture for both samples are considerate acceptable for Kirk et al. (2008), because they are included in the range of 0.8-2.3%. Regarding water activity, standard (S) and polyphenol enriched sample (P) show an increase during whole aging period. For both bars the final values are low (Aw= 0.3), that usually identifies a crisp and brittle products. During all times, the water activity of standard results higher than P sample, in which the migration of water is stabilized by the maltodextrin presence.

Total polyphenol content

The total polyphenol content into S and P samples are studied over time. The encapsulated powder incorporated chocolate had significantly higher polyphenols content than control. At time t_0 the total amount of P is 1231 mg/100g instead of S that contained 854 mg/100g. Furthermore Fig.26 shows a slight degradation of polyphenols for P sample during storage. At the end of storage treatment polyphenols of standard were reduced of about 50% while P, only 22%. This is a consequence of encapsulation technique that preserves biocompounds from the adverse storage conditions such as high humidity and temperature.

Comparing these results with those obtained for general chocolate bars 70% of cocoa solids, (Grassia et al., 2019), the amount of total polyphenols is very high, but not enough to reach the recognized claim related to them. Furthermore, this experiment needs a more in-depth analysis in order to evaluate the quality and the amount of polyphenols encapsulated in chocolate. The MS-MS HPLC, based on the method descript, allows only to recognize some flavonol species such as procyanidins at high molecular weight, that are contained in both samples at different concentration (Fig.27a,b,c,d). As shown in the images, despite it wasn't possible to quantify the several species, sample P contained less amount of polymerized forms because the encapsulation prevents the polymerization reactions.

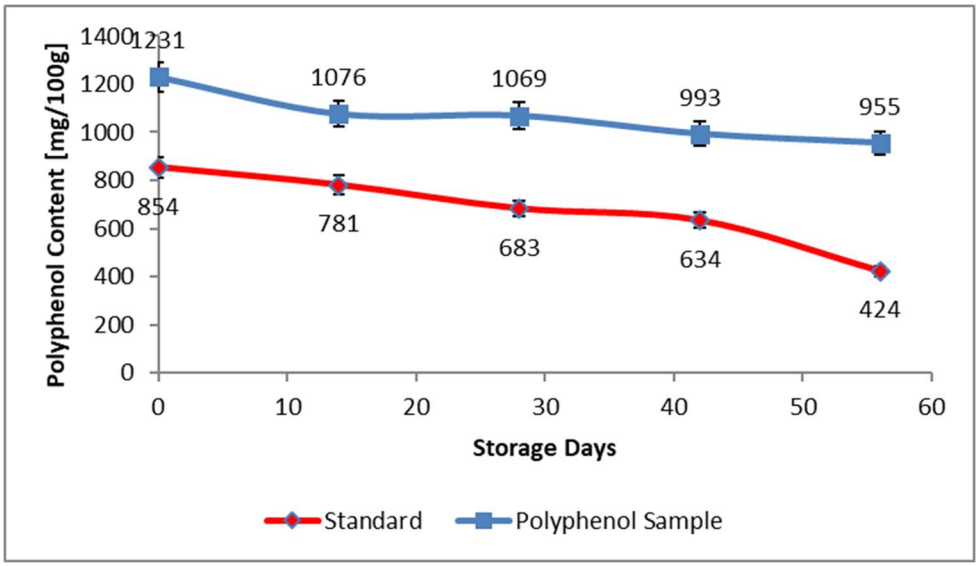
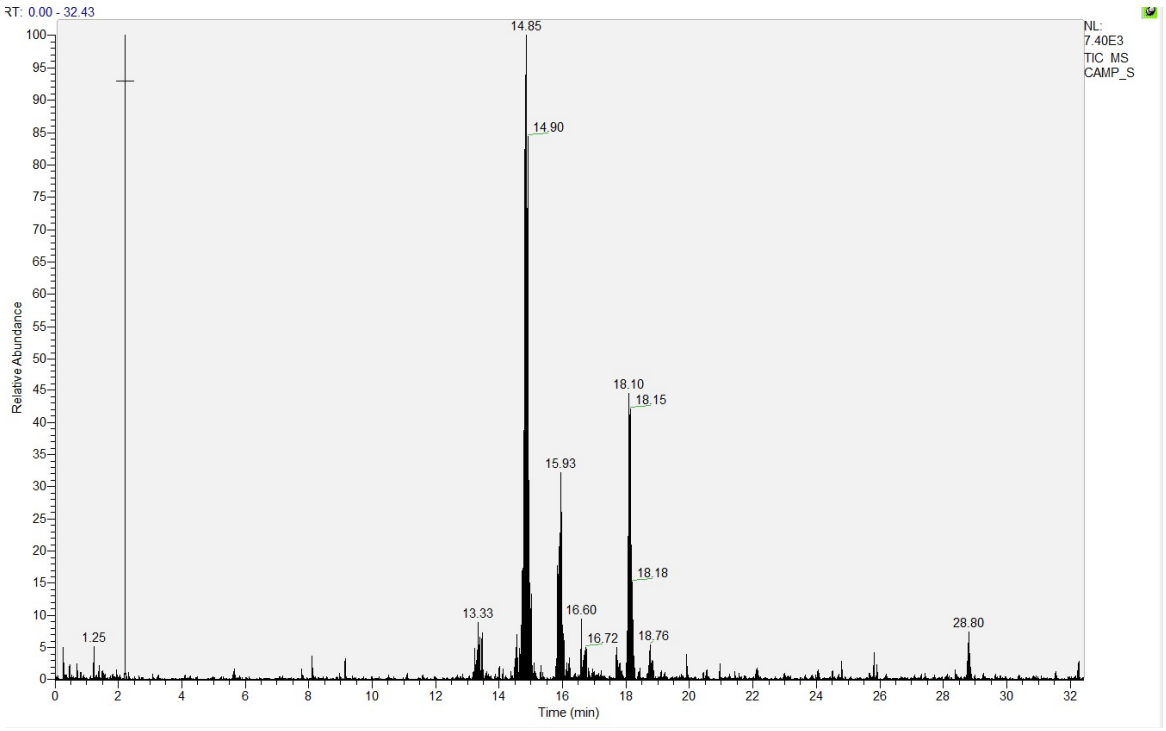
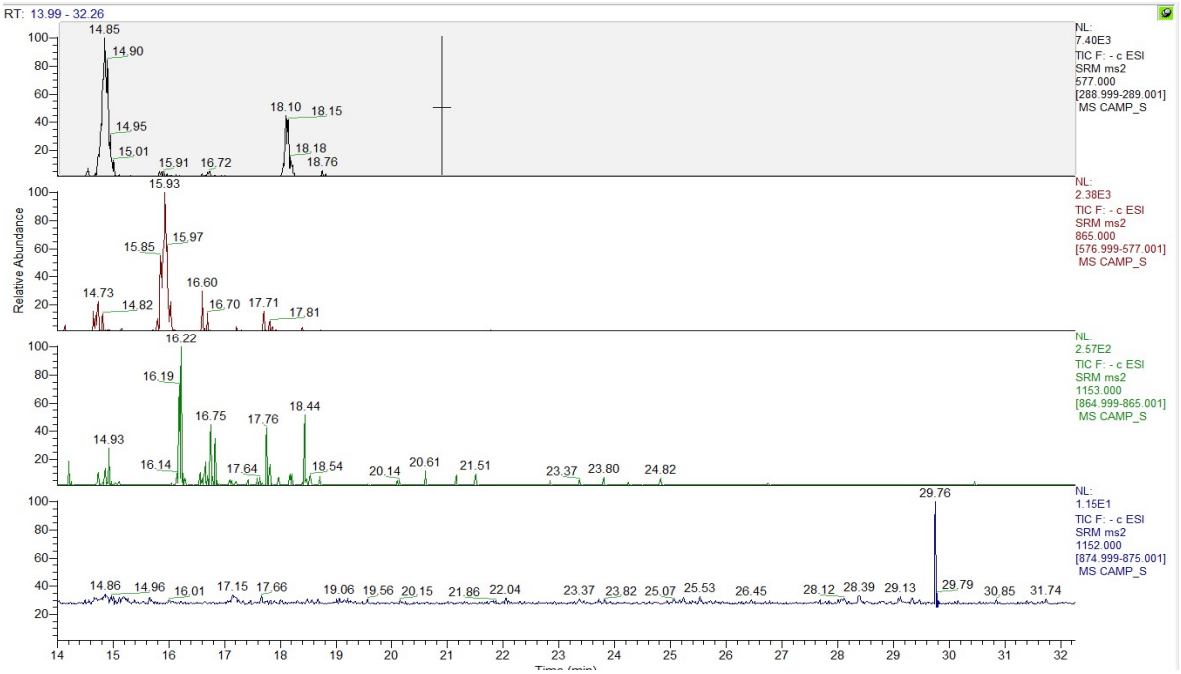


Fig.26- Total polyphenol content during the accelerated storage test of chocolate bars (packed with PP film, T=25°C and RH=70%). (S) = control, (P) = sample with added phenols.


RT: 0.00 - 32.43
NL:
7.40E3
TIC MS
CAMP_S
Relative Abundance
Time (min)
14.85
14.90
18.10
18.15
15.93
18.18
13.33
16.60
16.72
18.76
1.25
28.80


RT: 13.99 - 32.26
NL:
7.40E3
TIC F: - c ESI
SRM ms2
577.000
[288.999-289.001]
MS CAMP_S
NL:
2.38E3
TIC F: - c ESI
SRM ms2
865.000
[576.999-577.001]
MS CAMP_S
NL:
2.57E2
TIC F: - c ESI
SRM ms2
1153.000
[864.999-865.001]
MS CAMP_S
NL:
1.15E1
TIC F: - c ESI
SRM ms2
1152.000
[874.999-875.001]
MS CAMP_S
Relative Abundance
14.85
14.90
14.95
15.01
15.91
16.72
18.10
18.15
18.18
18.76
15.93
15.85
15.97
16.60
14.73
14.82
16.70
17.71
17.81
16.22
16.19
16.75
17.76
18.44
14.93
16.14
17.64
18.54
20.14
20.61
21.51
23.37
23.80
24.82
29.76
14.86
14.96
16.01
17.15
17.66
19.06
19.56
20.15
21.86
22.04
23.37
23.82
25.07
25.53
26.45
28.12
28.39
29.13
29.79
30.85
31.74

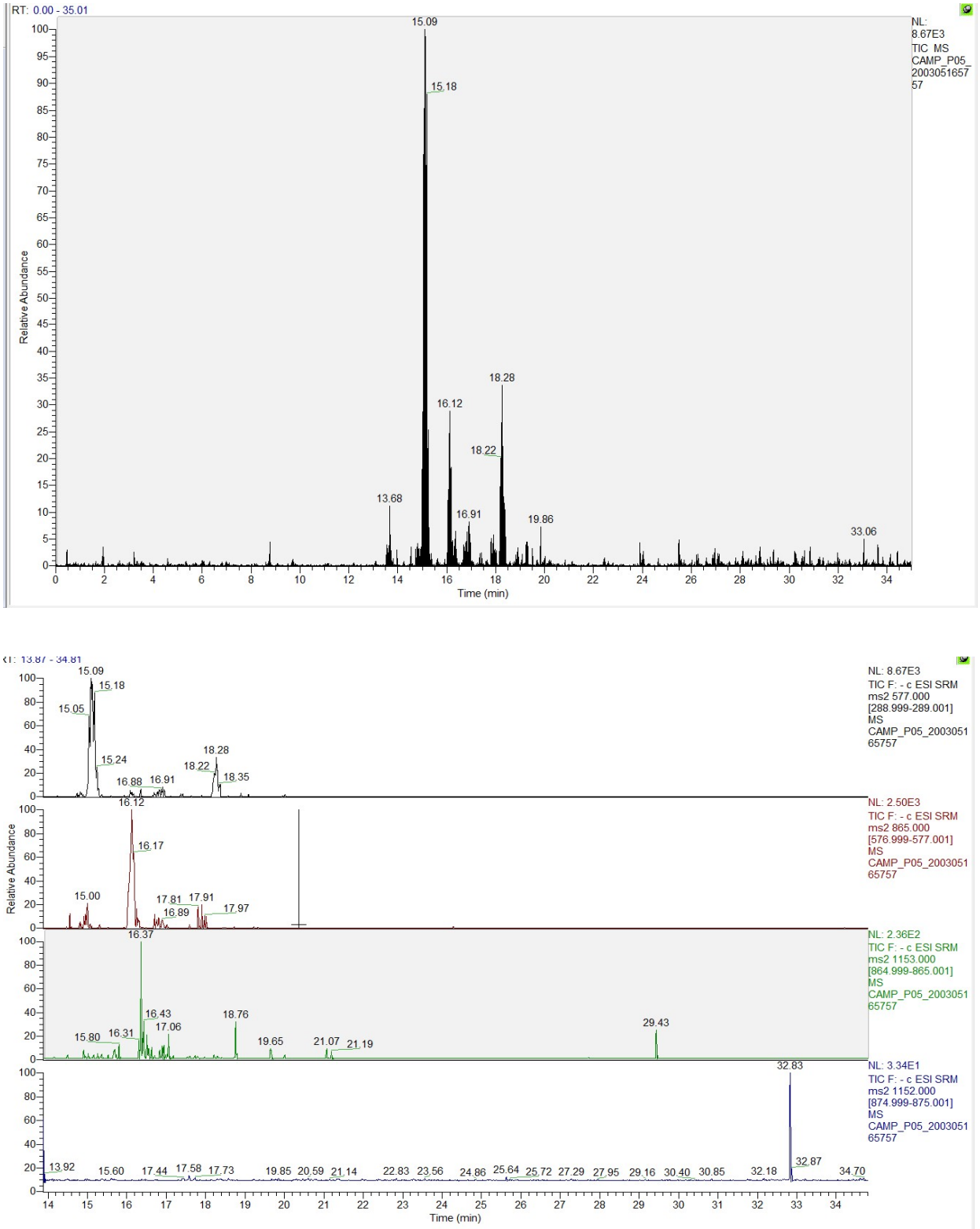


Fig.27a,b,c,d- Chromatogram of total procyanidins for (S) = control (a,b) and (P) = sample with added phenols (c,d)

Texture Analysis

Texture analysis included the study of two parameters: hardness and adhesiveness that are evaluated for both samples at different storage time. For P, hardness decreased rapidly in the first 42 days from

a value of 30798 g to 12843 g while standard sample showed a slightly reduction from 11269 g to 6479 g. Similar results are reported by Mishra et al. (2017) who investigated the changes of chocolate characters at different temperature storage. Hardness is correlated with the crystalline microstructure and particle size distribution of the product: smaller particle size leads an increase of hardness (Afoakwa et al., 2008). Therefore, P sample, that contain microcapsules of several sizes, with a medium diameter of 60 µm, show a greater reduction during time.

Adhesiveness, at the beginning of the observation, is the same for both samples but their trends vary during storage: bar enriched with polyphenols keeps this attribute constant during whole period; the standard shows a great reduction of adhesiveness probably due the less moisture absorption. Generally, storage of dark chocolate at high temperature caused decreases in hardness, cohesiveness, gumminess and chewiness (Nightingale, 2011)

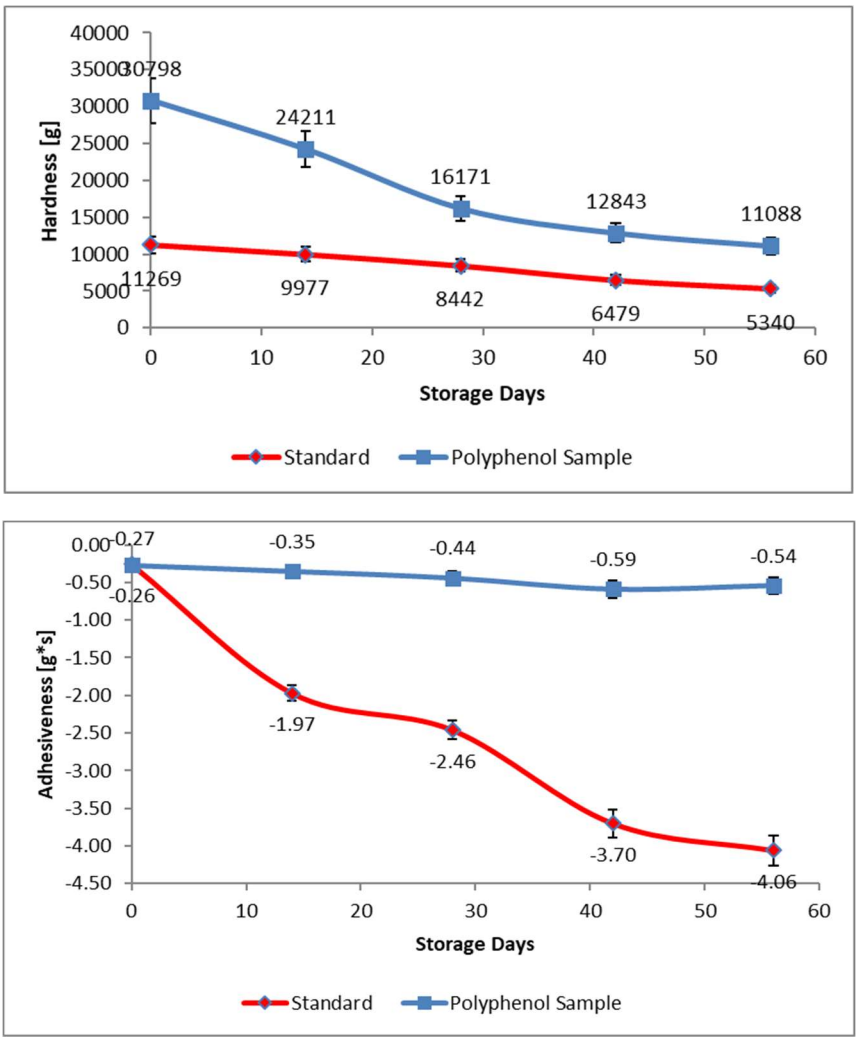


Fig.28a,b- Hardness (a) and adhesiveness (b) during the accelerated storage test of chocolate bars (packed with PP film, T=25°C and RH=70%). (S) = control, (P) = sample with added phenols.

Sensorial Analysis

The appearance

Appearance is the first characteristic evaluated in consuming a product. Based on the statistical test, samples show difference value of preference. Indeed, P shape looks irregular due the high viscosity of the chocolate with consequence not homogeneous distribution in the mould during cooling (Fig.23c). This problem affects also the fat blooming during storage with the formation of white spots on the surface (Fakhmi et al., 2016). Furthermore, the brown colour derives from anthocyanins which were red, purple and blue pigments in cocoa beans (Sampebarra, 2018). These results were confirmed also in the measurement of colour, reported in the previous section.

Hearing

Snap is a function of the amount and quality of the cocoa butter, the particles size and their distribution in chocolate and correct tempering. Despite of the difference in the structure, panellists didn't detect significantly difference between samples giving them the highest value (1/1).

Aroma

Aroma is the main parameter to determine level of preference in chocolate sensorial analysis.

For both samples, tasters didn't found any off flavours: it means that they are manufactured correctly. The only significant difference perceived by judges regards the primary aroma that depends on the genetic of cocoa beans, the main ingredient in the manufacture of chocolate. Standard's primary flavour (7/10) was evaluated better than P (5/19), maybe because maltodextrin, that cover polyphenol capsules, mask the strong aroma of cocoa. On the other hand, secondary flavours (6/12) were perceived with the same intensity ($p<0.5$).

Regarding persistence and general aroma's quality, testers didn't express any significantly difference between samples that reached medium high values of (3/4).

Texture

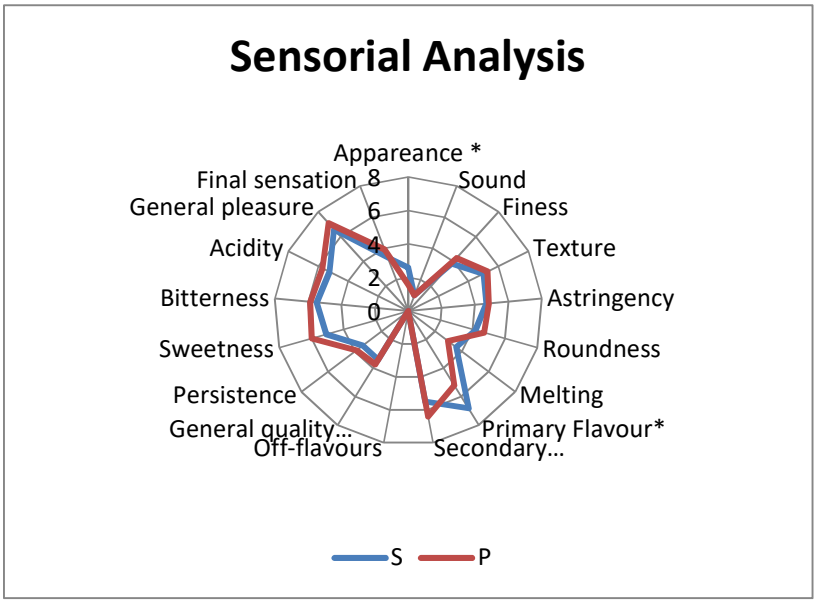
The texture can be detected by the sense of touch by eating chocolate directly (Listiyana, 2014). The results of statistical calculations didn't show any difference in quality attributes. Both chocolate bars were evaluated like good products with a soft texture that melt gently in the mouth. The difference of texture detected by instrument didn't find the same feedback during tasting because judges didn't perceived granularity.

Taste

The taste is a very important characteristic of the organoleptic properties of chocolate. It derived from alkaloid (theobromine, caffeine), phenolic components, pyrazine, some peptides and free amino acids that provide a balanced combination of different aromas on chocolate (Fakhmi et al., 2016). Also in this case no significantly differences were detected between two samples that are characterized by a

good compromise of sweetness (5/6), bitterness (5/6) and acidity (5/6). This result show that the adding of capsules, covered with maltodextrin, didn't modify the taste of the final product, hiding also the negative aspect of polyphenols such as bitterness and astringency.

Finally, based on the results of the study it concluded that both samples, S and P, could be considered like medium quality chocolate bars with a final score of 70/100 and 72/100.



Quality Attributes	Score
Appeareance	0-4
Sound	0-1
Finess	0-5
Texture	0-8
Astringency	0-5
Roundness	0-7
Melting	0-5
Primary Flavour	0-10
Secondary Flavours	0-12
Off-flavours	-5-0
General Flavour quality	0-5
Persistency	0-5
Bitterness	0-6
Sweetness	0-6
Acidity	0-6
General pleasure	0-10
Final sensation	0-5

Fig.29– Sensorial Analysis for (S) = control and (P) = sample with added phenols.

Bibliography

- Afoakwa E.O., Paterson A., Fowler M., Ryan A. (2008) Flavor Formation and Character in Cocoa and Chocolate: Critical Review, Critical Reviews in Food Science and Nutrition, Vol. 4, 840-857
- Aguilera M.M., Mayor G. (2004) Fat Migration in Chocolate: Diffusion or Capillary Flow in a Particulate Solid- A Hypothesis, Paper Journal of Food Science, Vol. 69, 167-174
- Barišic V., Kopjar M., Jozinovic A, Flanjak I. (2019) The Chemistry behind Chocolate Production, Molecules, Vol. 24, No. 17
  https://doi.org/10.3390/molecules24173163
- Bates D., Mächler M., Bolker B., Walker S. (2015) Fitting Linear Mixed-Effects Models Using lme4, Journal of Statistical Software, Vol. 67, No. 1, 1–48
  https://doi.org/10.18637/jss.v067.i01.
- Beckett S.T. (2009) Industrial Chocolate Manufacture and Use, Blackwell Publishing. Fourth Edition, Formerly Nestlé, York, UK. 732
- Bresson S., Rousseau D, Ghosh S., Marssi E.M., Faivre V. (2011) Raman spectroscopy of the polymorphic forms and liquid state of cocoa butter, European Journal Lipid Science & Technology, Vol. 113, 992–1004
  https://doi.org/10.1002/ejlt.201100088
- Caliskan G., Nur Dirim S. (2013) The effects of the different drying conditions and theamounts of maltodextrin addition during spray drying ofsumac extract, Food and Bioproducts Processing, Vol. 9, No. 1, 539-548
- Caraceni R. (2020) La degustazione del cioccolato, Hoepli, 2nd ed., Milano, Italy
- Danzl W., Rothkopft I. (2016) Comparing Storage Tests for Chocolate Shelf Life Prediction, 14th Euro Fed Lipid Congress, At: Ghent, Belgium
- Fakhmi M., Ikrawan Y., Cahyadi W. (2016) Differences in tempering time and temperature against the characteristics of peanut butter chocolate filling, Food technology, Food University, Bandung
- Grassia M., Salvatori G., Roberti M., Planeta D., Cinquanta L. (2019) Polyphenols, methylxanthines, fatty acids and minerals in cocoa beans and cocoa products, Journal of Food Measurement and Characterization, Vol. 13, No. 3, 1721-1728
  https://doi.org/10.1007/s11694-019-00089-5
- Kilcast D., Subramaniam P., (2000) The stability and shelf-life of food, Woodhead Publishing Limited, 224-231

- Kirk R., Sawyer R., Egan H. (2008) Composición y análisis de alimentos de Pearson, Novena reimpresión, Grupo Editorial Patria (Eds.) México D.F., México 773
- Kongorb J.E., Hinneh M., Vande Walle D., Afoakwa E.O., Boeckx P., Dewettinck K. (2016) Factors influencing quality variation in cocoa (Theobroma cacao) bean flavour profile - A review, Food Research International, Vol. 82, 44-52 https://doi.org/10.1016/j.foodres.2016.01.012
- Lipp M., Anklam E. (1997) Review of cocoa butter and alternative fats for use in chocolate-Part A, Compositional data, Food Chemistry, Vol. 62, No. 1, 73-97
- Listiyana D. (2014) Substitution of seaweed flour (Eucheuma cottonii) in making ekado as an alternative to high iodine foods in school children, Thesis, Faculty of Sport Science, Semarang State University
- ISO 8589 (1988) Sensory analysis- General guidance for the design of test rooms
- ISO 8586-1 (1993) Sensory analysis- General guidance for the selection, training and monitoring of assesors- Part 1: Selected assessors
- ISO 8586-2 (1994) Sensory analysis- General guidance for the selection, training and monitoring of assesors- Part 2: Experts
- ISO 5492 (1992) Sensory analysis- Vocabulary
- ISO 11036 (1994) Sensory analysis- Methodology- Texture profile
- Minifie W.B. (1999) Chocolate, cocoa, and confectionery: Science and technology, 3th edition, Aspen Publishers, Inc. Gaithersburg, Maryland, 135-164
- Mishral D., Chauhan, A.K., Bhardwaj R., Deshmukh G.P. (2017) Shelf Life Study of Guava Milk Chocolate, International Journal of Pure & Applied Bioscience (IJPAB), Vol. 5, No. 3, 1011-1017 https://doi.org/10.1016/j.idairyj.2009.11.008
- Misnawi S., Jamilah B., Nazamid S., (2005) Changes in polyphenol ability to produceastringency during roasting of cocoa liquor, Journal of the Science of Food and Agriculture, Vol. 85, 917-924
- Nightingale L.M., Lee S.Y., Engeseth N.J. (2011) Impact of Storage on Dark Chocolate: Texture and Polymorphic Changes, Journal of Food Science, Vol. 76, No. 1, 142-153
- Ortega N., Romero M.P., Macia A., Reguant J., et al. (2010) Comparative study of UPLC–MS/MS and HPLC–MS/MS to determine procyanidins and alkaloids in cocoa samples, Journal of Food Composition and Analysis, Vol. 23, No. 3, 298-305

- Peter W. (2005) Consumer Understanding and Use of Health Claims for Foods, Nutrition Science and Policy, Vol. 63, No. 7, 256-264
- Popov-Raljić J.V., Laličić-Petronijević J.G. (2009) Sensory Properties and Color Measurements of Dietary Chocolates with Different Compositions During Storage for Up to 360 Days, Sensors, Vol. 9
- R Core Team (2019) A Language and Environment for Statistical Computing, Vienna, Austria: R Foundation for Statistical Computing
  https://www.R-project.org/
- Sampebarra A.L. (2018) Characteristics of anthocyanin dyes from non fermented cocoa beans as a source of natural color substances, Journal of Agricultural Products Industry, Vol. 13, No. 1, 63-70
- Stauffer M. (2007) Conquering Shelf-life Issues of Chocolate, The Manufacturing Confectioner, 47-51
- Wille R.I., Lutton E.S. (1966) Polymorphism of cocoa butter, Journal of the American Oil Chemist's Society, Vol. 43, 491-496
- Zamora, E. (2007) Evaluación Objetiva de la Calidad Sensorial de Alimentos procesados. Instituto de Investigaciones para la Industria Alimenticia, MINAL, Cuba, Editorial Universitaria
- http://anodscocoa.com/resource-details/34/Fat-bloom-and-Sugar-Bloom-.html
- http://bean.bar/makers
- https://www.cacaochocolade.nl/main.php?lng=1&p=inhoud&h=5&g=1&s=4&z=0&sp=
- https://www.ifst.org/our-resources/ifst-guidelines-ethical-and-professional-practices-sensory-analysis-foods
- https://it.scribd.com/document/335175879/2-Cocoa-Quality-and-Flavour-Evaluation-Dr-Darin-Sukha
- https://sensing.konicaminolta.us/blog/identifying-color-differences-using-l-a-b-or-l-c-h-coordinates/

CONCLUSIONS

The aim of this research was to create a functional product: chocolate bars enriched with polyphenols from bean shells, through the collaboration between the University of Molise, a confectionery company (Dolceamaro) and a foreign partner (University of Ankara), which supported some innovation techniques in the realization of the project. According to today's widely shared European industrial objectives, the project was conducted using green technology to extract polyphenols, thus enhancing a by-product of cocoa production: the cocoa shell. This by-product has proven to be a rich source of important biocompounds such as polyphenols. The study has shown that the use of ultrasonic pretreatment, combined with the optimization of certain parameters such as low particle size, reduced energy consumption (ambient temperature and short time) and ecological solvents (aqueous ethanol), has improved the extraction efficiency. However, polyphenols are very sensitive to production conditions such as light, temperature and oxygen. For this reason, the spray drying technique has been experimented to encapsulate the polyphenols extracted from bean shells.

The spray drying process has been designed to obtain the best compromise between the core: coating agent ratio, inlet temperature and feed rate. Once the microcapsules were produced, they were added to the cocoa mass to obtain a functional chocolate bar. Although the amount in polyphenols was higher than that of a standard bar, both products were very similar. However, some defects were found, related to the appearance of the chocolate because the addition of microcapsules changed its rheology. In conclusion, this study represents a preliminary step for the recovery of biocompounds from cocoa shells and their inclusion in a food matrix. To improve the stability of the chocolate bar during storage, it is necessary to improve the balance of the ingredients by adding capsules before tempering in chocolate production. Further developments of this project are to extend the extraction technique also to other substances of technological interest (cocoa butter) and healthy (fibers).

ACKNOWLEDGEMENTS

This research was supported by “Programma Operativo Nazionale Ricerca e Innovazione 2014-2020”. I thank the colleagues from Department of Food Engineering of the University of Ankara who provided insight that greatly assisted the research, and some collegues of The University of Napoli. Finally, I would like to thank the Company Dolceamaro for its assistance during the research.

La borsa di dottorato è stata cofinanziata con risorse del
Programma Operativo Nazionale Ricerca e Innovazione 2014-2020 (CCI 2014IT16M2OP005),
Fondo Sociale Europeo, Azione I.1 “Dottorati Innovativi con caratterizzazione Industriale”

UNIONE EUROPEA
Fondo Sociale Europeo