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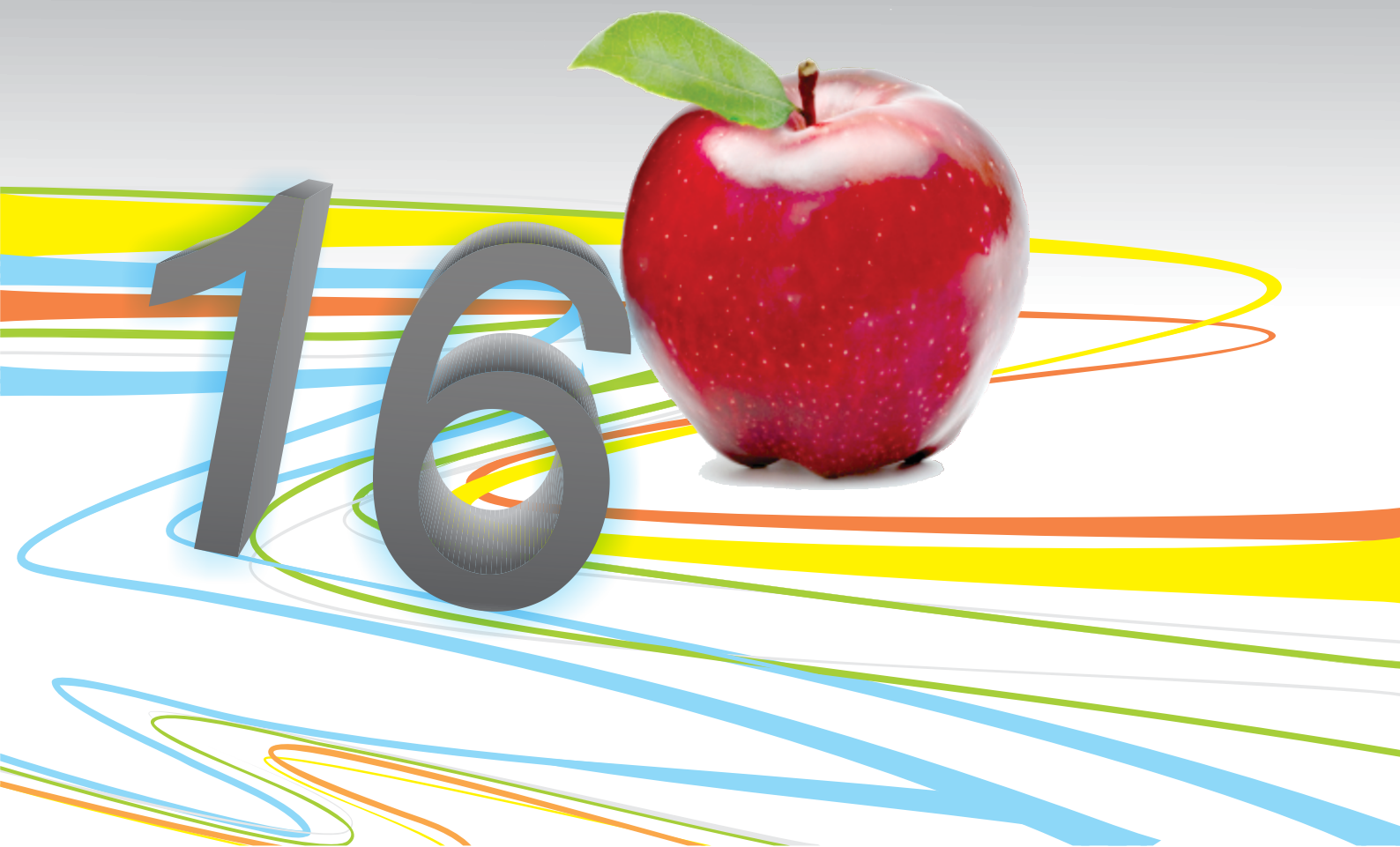

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Suizdavač:
Sveučilište Josipa Jurja Strossmayera u Osijeku, Prehrambeno-tehnološki fakultet Osijek,
Franje Kuhača 20, 31000 Osijek, Hrvatska

Tehnička priprema i dizajn:
Damir Alihodžić (Tuzla, BiH), Ivana Lauš (Osijek, Hrvatska), Kenan Biberkić (Tuzla, BiH)

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Editorial

Chocolate has gone through several evolutions from the Aztec period to present time, related to its significance and consumption.

To Aztecs and Mayas it represented food of gods, therefore it had ceremonial value, sometimes aphrodisiac, rather than hedonistic. When chocolate was introduced to Europe, sugar was added to it and its ceremonial characteristic is lost – it became purely hedonistic beverage, primarily for rich and influential parts of society. Nowadays, chocolate is becoming functional food, primarily due to being a rich source of polyphenols, but also as a matrix for different bioactive compounds which are added to it.

The second evolution could be attributed to the form of its consumption – for a very long period chocolate was consumed exclusively as a beverage because it was too hard in the solid state. Aztecs and Maya combined cocoa paste with cornmeal and spices and dissolved this mixture in water. The beverage was bitter and spicy. Aztecs drank it cold and called it Quetzacoatl, while Mayas preferred hot beverage. With introduction of chocolate to Europe, sugar was added to the beverage since Europeans drank it purely as an aphrodisiac and for pleasure and with time they began to dissolve cocoa paste in milk. With the industrial revolution and invention of cocoa press chocolate is becoming cheaper, it is produced and consumed in solid form and became available to all social niches.

The third form of evolution is the type. Along with dark chocolate, milk chocolate is produced in 19^{th} century, in 20^{th} century white chocolate is introduced and currently ruby chocolate is becoming new, 4^{th} chocolate.

From nutritional point of view, chocolate is complex and bitter-sweet. Due to high sugar content, chocolate is rich in energy and is often linked to obesity and diabetes, as well as dental issues. However, at the same time, dark chocolate is an excellent polyphenol source and is being recognized as a functional food. The efforts are constantly being made by academic and professional research to improve beneficial side of chocolate and reduce its negative side.

This issue is mostly dedicated to chocolate – from analytical aspect to its consumption, as well as by-product usage and new, ruby chocolate.

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EVALUATION OF FUNGICIDE RESIDUES IN APPLES AND THEIR IMPACT ON FOOD SAFETY

Vezirka Jankuloska[1]*, Ilija Karov[2], Gorica Pavlovska[1], Gjore Nakov[3], Midhat Jašić[4]

[1]Faculty of Technology and Technical Sciences Veles, "St. Kliment Ohridski" University -Bitola, Dimitar Vlahov 57, 1400 Veles, Republic of North Macedonia
[2]Agricultural Faculty, University "Goce Delcev" Shtip, Krste Misirkov 10-A, 2000 Shtip, Republic of North Macedonia
[3]Department of Biotechnology and Food Technologies, University of Ruse "Angel Kanchev", Branch Razgrad, Aprilsko vastanie Blvd. 47, 7200 Razgrad, Bulgaria
[4]University of Tuzla, Faculty of Technology, Univerzitetska 8, 75000 Tuzla, Bosnia and Herzegovina

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Summary

The apple is a fruit that has beneficial health effects due to the presence of biologically active components and is considered as a functional food. But today's production of apples cannot be imagined without chemical treatment. In addition to its favorable health effects, it may also have adverse effects on human health due to the presence of pesticides that are considered as contaminants. The purpose of this research is to evaluate the fungicide residues in fruit/apples. The fungicide residues were extracted from apple cultivar Idared with QuEChERS method and analyzed by ultra-performance liquid chromatography with a triple quadrupole mass spectrometer (UPLC-TQ/MS). The analyses have shown that the apples contain pesticides penconazole (10 μg/kg), boscalide (60 μg/kg), tebuconazole (11-40 μg/kg), myclobutanil (20-70 μg/kg), fenbuconazole (70 μg/kg), pyrimethanil (12-60 μg/kg) and carbendazime (azole) with range of 100-200 μg/kg. After comparing the concentration of detected pesticides with MRLs, it can be concluded that apples are safe for consumption.

Keywords: apples, food safety, pesticides, residues

Introduction

The apple *(Malus domestica)* is one of the three basic fruits produced in Republic of North Macedonia. Apple picking starts in late summer with the early varieties and continues in the autumnal months. Apples are highly represented in the national market, and a certain percentage are also exported (Picha, 2009). The total domestic consumption of apples is estimated at about 24.748 tons, which is about 20% of domestic production, while about 21.500 tons are purchased for industrial processing, 25% of the total apple production are sold on the national market, while 75% are processed or sold on foreign markets (Ministry of Agriculture, Forestry and Water management, 2010).

Various types of apples have different chemical composition. In accordance with the research conducted by Aziz et al. (2013) there is a difference in the chemical composition between *Malus sylvestris* and *Malus domestica*. The percentage of moisture, potassium and ascorbic acid is high in *Malus Sylvestris*. The ash, fat, fiber, protein and total sugar percentage are high in *Malus Domestica*. The factors which are affecting chemical composition of apples, as well as the content of some components are: cultivar, climate, harvesting period, soil, storage and others (Mujkanović et al., 2019). The apple is a source of vitamins and minerals, soluble and insoluble dietary fiber (Chen et al., 2014). Nearly half of the amount of vitamin C is found below the skin of the apple, and the skin contains insoluble fiber. Pectin, which plays a major role in the reduction of cholesterol and prevents atherosclerosis and cardiac diseases, is present in the apple. Because of these components that play an important role in the health of people, apples and their products are considered as functional foods. The apple jam from traditional apple cultivars can be used as functional food because it contains carbohydrates, potassium, zinc, iron and can be recommended to the sport players, little children, vegans and elderly (Mujkanović et al., 2019). The consumption of apples and their health benefits with regard to cancer, cardiovascular diseases, asthma and diabetes have been researched by Boyer and Liu (2004).

Apart from health benefits, the apples can also have adverse effects on health if they contain pesticides over the MRLs and are unsafe for consumption. Today's practice shows that apples in conventional production are treated with pesticides 10 to 15 times (Jankuloska et al., 2018). Many types of fruits are consumed raw, without any thermal processing and that is a potential health risk for the consumer. Thermal processing and treating the apples with steam (110 °C, 20-25 min) and removing the apples peel from the apples (65-70 °C, 3 min) was identified as the most effective step in reducing fosalone residues and the complete elimination of fenitrothion and tolifluanide (Štěpán et al., 2005). The presence of pesticide residues and their metabolites can be a potential hazard to human health that is

*Corresponding author: vezirka.jankuloska@gmail.com

manifested by acute or chronic toxicity. The priority is to detect them in the food in order to avoid the possible risks to human health.

Pesticides are used to protect the plants during cultivation in order to decrease the damage which can be caused by harmful organisms (fungi, insects, weeds, birds, snails, spiders, nematodes, bacteria and viruses). Pesticides permitted for use as means of protection in the Republic of North Macedonia are prescribed in the list of approved active substances (Official Gazette of Republic North Macedonia, 2013). It is mandatory to manage the appearance of pesticide resistance in the pest, to uphold the waiting period, to follow the recommendations of the advisory service, as well as the instructions of the pesticide manufacturer. The European Commission has adopted a list of maximum residue levels (MRLs) for pesticide used in the production of food and feed, regulated by Regulation EC 396/2005 (Official Journal of the European Union, 2005). In the Republic of North Macedonia, the maximum residue levels (MRLs) for pesticides are regulated with the Regulation on general food safety requirements regarding maximum tolerated levels of pesticide residues in or on food (Official Gazette of Republic of North Macedonia, 2013).

Difenoconazole, fenbuconazole and trifloxystrobin in apples (Golden Delicious and Idared) were extracted with QuEChERS method and analyzed with LC-MS/MS. The concentration content was: 0.01-0.41 mg/kg difenoconazole (Jankuloska et al., 2018), 0.01 to 0.07 mg/kg fenbuconazole (in Golden Delicious apples) (Jankuloska et al., 2017b) and 0.04-0.14 mg/kg trifloxystrobin (Jankuloska et al., 2017). According to the results obtained the authors concluded that the apple is safe for consumption. The chlorpyrifos content in apple from the Resen region is smaller than the MRLs and the use of chlorpyrifos doesn't depend on the variety of apple but rather of the locations where the apple is grown (Jankuloska et al., 2017a).

Fungicide residues (quintozene, pyrimethanil, captan, folpet, tebuconazole, carboxin, flutolanil, fludioxonil) were analyzed with GC and matrix solid phase dispersion (MSPD) in fruit and vegetables (apples, carrots, orange, artichoke, tomatoes and zucchini). The concentration content was smaller than the MRLs. LOQ and LOD were under 0.1 mg/kg (Navarro et al., 2002). Azoxystrobin and trifloxystrobin were analyzed in apple with GC, with electron capture detector (ECD) and nitrogen phosphor detector (NDP) and limit of detection (LOD) were found to be 0.02 mg/kg for azoxystrobin and 0.01 mg/kg for trifloxystrobin (Giza and Sztwiertnia, 2003).

From the above stated we can conclude that pesticides play an important role in the food safety and the purpose of this research is to evaluate the fungicide residues level in fruits/apples.

Materials and methods

The experimental part of the research was carried out in Resen at two different locations (Evla and Kriveni). Field research and trials took place in stages and lasted throughout 2016. The evaluation of fungicides is done during apple vegetation until harvest time. At the end of May, the first samples were taken for analysis (hazelnut-sized apples) and transported for the analysis of residues. The second sampling stage was when apples reached the size of a walnut. The third sampling stage took place in the ripening period. The fourth sampling stage for residue analysis took place at the time of harvest (September-October). To determine the exact maturity for apple harvesting, several objective measures and indicators were used, such as the color of the skin, the hardness, content of soluble hard materials, the development of ethylene and the content of starch. Apples were randomly sampled. The mass of samples for analysis was 1 kg (for sampling). Fresh apples with peel and mesocarp without treatment were analyzed.

Extraction of pesticide residues

Following the latest trends in the extraction of pesticide residues, the QuEChERS method was applied in this study according to the MKS EN 15662:2011 standard (Standardization Institute of Republic of North Macedonia, 2011). The homogenized sample of apple fruits with weight of 10 ± 0.1 g is transferred at 50 ml centrifuge tube. 10 ml of acetonitrile and a buffered mix of salts for extraction and separation (4 g ± 0.2 g $MgSO_4$, 1 g ± 0.05 g NaCl, 1 g ± 0.05 g trisodium citrate dihydrate, 0.5 g ± 0.03 g disodium hydrogen citrate sesquihydrate) are added. The tube was closed with a cap and the extract was vigorously mixed by hand (for 1 min). After the centrifugation (5 minutes at 3000 rpm), 1 ml of the acetonitrile extract was taken and transferred into a clean-up tubes containing 150 mg primary secondary amine (PSA) and 900 mg $MgSO_4$. The tubes were closed and the mixture was shaken 30 seconds, and then were centrifuged for 5 minutes at 4500 rpm. The cleaned and acidified extracts are transferred into auto sampler vials to be used for the multi-residue determination with chromatographic method (Anastassiades et al., 2003; Jankuloska et al., 2018).

Analysis with UPLC/TQ-MS

The analysis of pesticide residues in apples was conducted with the most sophisticated separation technique: ultra-performance liquid chromatography-triple quadruple mass spectrometry (UPLC-TQ/MS), Agilent UPLC 1290, detector DAD VL Agilent 1260 G1315D (Waldbronn, Germany), Agilent triple quadruple LC/MS detector 6420 (Agilent Technologies, Santa Clara, California, USA). UPLC BEH C-18 column with dimensions of 2.1 x 100 mm and pore size of 1.8 μm was used for separation (Table 1). The temperature of the column was 35 °C and the flow was 0.4 ml/min. The volume of injection is 0.7 μl. The solvents used in extraction and analysis were distilled and checked for any unwanted impurity prior to use. The mobile phase used consisted of solvent A: 0.1% (v/v) HCOOH and 5 mmol ammonium formate; solvent B: methanol with 0.1% (v/v) HCOOH and 5 mmol ammonium formate.
The accuracy of the method was checked with a standard addition method, showing good accuracy, repeatability, and reproducibility (RSD < 10%). The limit of detection (LOD) is in the range of 0.01 to 0.07 mg/kg and the limit of quantification (LOQ) is 0.01 mg/kg for all pesticides.

Table 1. Separation parameters on UPLC for pesticide residues

Time (min.)	*Flow rate (ml)*	*Solvent A[%]*	*Solvent B [%]*
0	0.4	95	5
0.5	0.4	95	5
3.50	0.4	50	50
17.00	0.4	0	100
20.00	0.4	0	100
20.10	0.4	95	5

Results and discussion

In this research we analyzed the fungicide residues in Idared apples in four development stages from cultivation.
Table 2 shows the present fungicides in apples from two locations (Evla and Kriveni).

Table 2. Concentration of fungicide residues in Idared from locations Evla and Kriveni in the first and second stage

Pesticides	**Concentration (μg/kg)/Evla, first stage**	**Concentration (μg/kg)/Kriveni, first stage**	**Concentration (μg/kg)/Evla, second stage**	**Concentration (μg/kg)/Kriveni, second stage**
Myclobutanil	20	70	n.d	20
Fenbuconazole	70	70	n.d	n.d
Penconazole	10	n.d	n.d	n.d
Tebuconazole	n.d*	11	n.d	40
Boscalide	60	n.d	n.d	n.d
Pyrimethanil	60	20	12	n.d

*n.d- not detected

At first stage, six fungicides were detected in apples (Table 2). From obtained results we can see that detected pesticides in apples from both locations are different. Respectively, in the Evla apples, penconazole, boscalide and tebuconazole were detected, while the same were not detected in the Kriveni apples.

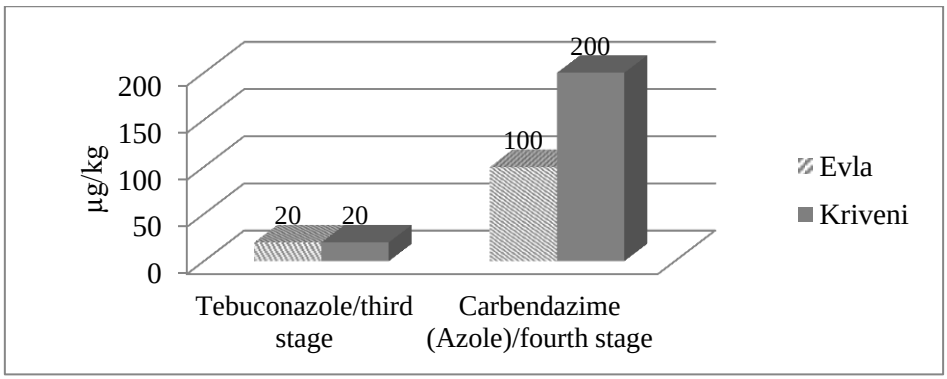

Fig. 1. Concentration of fungicide residues in Idared from locations Evla and Kriveni in the third and fourth stage of development of apples cultivation

As shown in Fig. 1 the tebuconazole fungicide is present in the apples from both locations in the same concentration of 20 μg/kg, while the remaining pesticide detected in the second stage is not present in the third stage apples. Navarro et al. (2002) used GC to analyze tebuconazole in fruits and vegetables and received a low pesticide concentration which is similar to our results. In this stage, 100 μg/kg carbendazime (azole) is detected in the Evla apples and 200 μg/kg is detected in the Kriveni apples. Similar results to these were obtained by Singh et al. (2009), during the analysis of apples grown under different conditions in which they detected carbendazime and chlorpyrifos but in low concentration.

In this study, there is a distinct presence of pesticides in all four stages, but it is also noticeable that the concentration of detected pesticides is different. The persistence of pesticides depends upon the dose of pesticides, mode of application, temperature and weather conditions at the time of application and on crop growth conditions (Tsochatzis et al., 2013; Trapp S. 2015), translocation of pesticides in nature, chemical properties, location of cultivation and type of formulations (Patel et al., 2016).

When assessing the importance of pesticides, what needs to be taken into consideration is whether their level is higher than the allowed maximum, and for that reason in this study we made a comparison of the concentration of pesticides represented in the fourth stage (time of harvest) in the apples from both locations.

The obtained results for the concentrations of the carbendazime (azole) in the Idared apples from the location of Evla and Kriveni and their maximum residue levels (MRLs) are presented in Fig. 2.

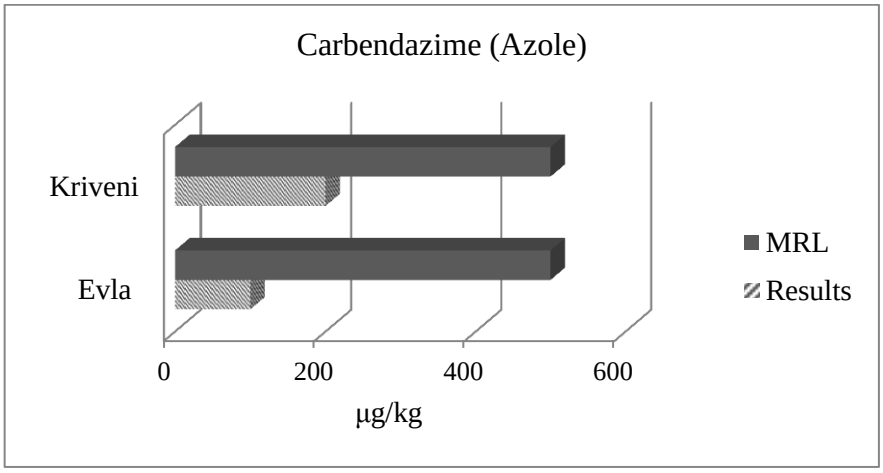

Fig. 2. Concentration of carbendazime (azole) in apples from different locations and maximum residue levels (MRLs)

Based on the results (Fig. 2) it can be seen that the carbendazime (azole) content (100 μg/kg) is at a legally tolerated level (500 μg/kg). Our results are similar to the ones obtained by Radišić et al. (2009), which analyzed the carbendazime level in fruit juices from apple, peach, orange and raspberry with LC-MS/MS. The carbendazime in apples from Kriveni is present at 200 μg/kg and this concentration is smaller than the MRLs. This result is similar to the result obtained by Morales et al. (2011), when they analyzed pesticides in peppers with LC-ESI-MS and extraction with QuEChERS. Carbendazime is systemic fungicide with protective and curative action used to protect plants against *Venturia* and *Podosphaera* in pome fruit and *Monilia* and *Sclerotinia* in stone fruit.

Conclusion

The aim of this research was to evaluate the represented fungicide residues in apples Idared from two different locations with QuEChERS and UPLC/TQ-MS. Different fungicides in different concentration content were detected in the four stages of apple cultivation. The different representation of pesticides depends on and is related to various factors and in our research, from the location of apple cultivation. But despite the different presence of fungicides in apples, they are safe for consumption.

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THE PHYSICAL PROPERTIES, POLYPHENOL CONTENT AND SENSORY CHARACTERISTICS OF WHITE CHOCOLATE ENRICHED WITH BLACK TEA EXTRACT

Ivana Lončarević[1*], Biljana Pajin[1], Jovana Petrović[1], Danica Zarić[2], Vesna Tumbas Šaponjac[1], Aleksandar Fišteš[1], Petar Jovanović[3]

[1]University of Novi Sad, Faculty of Technology Novi Sad, 21000 Novi Sad, Serbia
[2]IHIS Techno Experts d.o.o., Research Development Center, 11000 Belgrade, Serbia
[3]Eugen Chocolate d.o.o., 21412 Gložan, Serbia

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Summary

Chocolate contains a variety of different compounds such as fats, carbohydrates, proteins, vitamins, and minerals. Fat-free cocoa particles are also extremely rich in polyphenols. While dark chocolate is a most powerful source of antioxidants, the content of these components decreases with the reduction in the content of dark cocoa particles in chocolate. White chocolate differs from milk and dark through the absence of fat-free cocoa particles containing antioxidants.
Today's investigations are focused on finding new methods of creating functional confectionery products. Accordingly, in this study white chocolate was enriched with100 g kg^{-1} of encapsulated black tea extract in order to increase its polyphenol content. The addition of encapsulated black tea extract to white chocolate increased the viscosity of enriched chocolate mass to the extent that is acceptable for the production of this kind of product. Total polyphenol content in enriched chocolate was increased over 6 times compared to white chocolate. However, black tea encapsulate contributed to foreign taste of medications in enriched chocolate, making it unacceptable by consumers.

Keywords: white chocolate, encapsulated black tea extract, particle size distribution, rheology, polyphenol content, sensory characteristics

Introduction

Chocolate is a complex rheological system consisting of non-fat particles dispersed in a continuous fat phase primarily composed of cocoa butter (Afoakwa et al., 2009; Zarić et al., 2016). In addition to its own unique flavor and textural properties, chocolate can provide significant amounts of a number of essential nutrients with potential positive impact on human health (Steinberg et al., 2003). Nowadays, research is mostly focused to the polyphenolic fraction of cocoa mainly consisting of flavonoids (Lecumberri et al., 2007). Dark, milk, and white chocolates have different content of cocoa solids (fat free cocoa solids and cocoa butter) and milk fat (Rossini et al., 2011). Dark chocolate contains cocoa liquor, cocoa butter, powdered sugar, and emulsifier lecithin, while, in the case of milk chocolate, the milk powder is added and the content of cocoa solids is lower. White chocolate even doesn't contain fat-free cocoa solids and thus lacks polyphenols (Samsudin, 1996).
Nowadays, many confectionery products have been enriched with bioactive compounds since the market is increasingly searching for functional food that can improve life style (Glaberson, 2011). The bioactive compounds are very sensitive to heat, light, and water, so the encapsulation technique is commonly used in order to improve their stability and to change liquid solutions to powders for easier handling (Tumbas Šaponjac et al., 2016). The acceptability of new type of chocolate by consumers depends primarily on its taste, but also on mouth feeling, which mainly depends on the particle size and the viscosity of the molten chocolate mass. The size of the particles in chocolate is desirable to be 15–30 μm. If particles are bigger, the chocolate will cause a gritty mouth feeling and, on the other hand, smaller particles increase the specific surface area, more liquid phase is needed to cover it, and viscosity increases (Bolenz et al., 2014). Tea polyphenols have a wide range of pharmaceutical properties including antioxidative, antiarteriosclerotic, and anticarcinogenic (Turkmen et al., 2006). In the manufacturing of black tea, a large portion of the catechins are converted to theaflavins and thearubigens, which are responsible for the dark brown color and the taste of black tea. The remaining catechins account for 3–10% in brewed black tea (Hong et al., 2001).
Our previous work involved the production and quality analysis of white chocolate enriched with 60, 80, and 100 g $kg^{-1}$of encapsulated blackberry juice (Loncarevic et al., 2018[a]). This study was designed to examine the impact of encapsulated black tea extract

*Corresponding author: ivana.radujko@tf.uns.ac.rs

in amount of 100 g kg^{-1}on particle size distribution, rheological properties, total polyphenol content, and sensory properties of enriched white chocolate.

Materials and methods

Materials

The material used in this work included black tea extract encapsulated in maltodextrins by spray drying technique (Frutarom Etol d.o.o., Slovenia) in the form of black powder (in following text E). The composition of white chocolate is shown in our previous paper (Lončarević et al., 2018)[a], consisting cocoa butter (348.4 g kg^{-1}), powdered sugar (497.6 g kg^{-1}), whole milk powder (149.3 g kg^{-1}), sunflower lecithin (4.5 g kg^{-1}), and vanilla powder (0.2 g kg^{-1}).

Methods

Pre-crystallization of chocolate mass

Chocolate mass was tempered in modified Brabender farinograph where the original kneader is connected with two thermostats (Lauda Ecoline Staredtition E 215 T, Germany), which enables an immediate temperature change in the kneader and in half a minute in chocolate mass (Pajin et al., 2012). First, 120 g of white chocolate was melted in a farinograph kneader at 42 ºC for 30 minutes. Then, chocolate mass was gently stirred at 42 ºC for 60 min, followed by another 60 min at 29.5 ºC. The pre-crystallized chocolate mass was then poured into 50 g plastic mold and cooled in a refrigerator at 5 ºC for 90 minutes.

The same procedure was carried out in order to produce the control sample of white chocolate (in following text W) and chocolate sample enriched with 100 g $kg^{-1}$of encapsulated black tea extract (in following text WE), where the encapsulate was added to melted chocolate after 30 minutes at 42 ºC.

Particle size distribution

The particle size distribution (PSD) of E, W and WE was determined using a laser diffraction particle size analyzer (Mastersizer 2000, Malvern Instruments, UK). The Scirocco dispersion unit was used for dispersing E in the air while Hydro 2000 μP dispersion unit was used for dispersing chocolate in sunflower oil. PSD were described by the volume mean diameter d[4,3], and parameters d(0.1), d(0.5), d(0.9) representing the particle sizes where 10, 50 or 90% of the total particle volume was formed by particles smaller than this size, respectively.

Rheological properties of chocolate mass

Rheological properties of chocolate samples were determined using a rotational rheometer Rheo Stress 600 (Haake, Germany) at the temperature 40 ± 1 ºC (IOCCC, 2000). The shear rate was increased from 0 s^{-1} to 60 s^{-1} during a period of 180 s and then was kept constant for 60 s at max speed of 60 s^{-1} and after that was reduced from 60 s^{-1} to 0 s^{-1}, within 180 s. The obtained flow curves were fitted using Casson model, where the following parameters were obtained: Cason yield stress (Pa) and Casson viscosity (Pas).

Total polyphenol content (TPC)

The total polyphenol content in E was determined spectrophotometrically by a Folin-Ciocalteau method adapted to microscale (González-Molina et al., 2008), while the extraction of polyphenol compounds from W and WE and their determination was performed according to Belščak-Cvitanović et al. (2012) and Belščak-Cvitanović et al. (2015), as described in our previous work (Lončarević et al., 2018)[a].

Sensory analyses

The sensory analyses took place in the Sensory Laboratory of Faculty of Technology Novi Sad, University of Novi Sad, in individual booths, under white light, at room temperature (ISO 8589, 2007). It was performed by a trained sensory panel of 10 staff members from the Faculty of Technology Novi Sad, University of Novi Sad, who had experience in the assessment of chocolates (ISO 8586-1, 1993). During the training sessions, a list of definitions and reference for highest and lowest intensity were developed (ISO 5492, 2008). The sensory characteristics discussed are separated into four groups representing visual (color, glow), textural (hardness, melting rate, graininess), olfactory (cocoa butter aroma and GTE aroma), and gustatory (GTE flavor, cocoa butter flavor, GTE aftertaste) characteristics of chocolate. Panelists evaluated the intensity of each attribute using 15-cm unstructured scale marched on each end with anchors for lowest intensity and highest intensity and they placed a vertical mark on the scales according to their perception of each sensory attribute (ISO 4142, 2002). After that, the ratings were transferred into numbers (1–15) using a ruler. Evaluated attributes, their description, as well as their lowest and highest intensity are listed in Table 1.

Table 1. Description, lowest and highest intensity of evaluated attributes in chocolate samples

Attribute	Description	Lowest Intensity	Highest Intensity
Color	Intensity of black color of chocolate	light (color of white chocolate)	dark (E)
Glow	Intensity of gloss on chocolate surface	matte	Shine
Hardness	Force nedeed to break chocolate sample into two pieces with front teeth	extremly soft	extremly hard
Melting	Length of time to chocolate to melt	quickly	Slowly
Grainines	Sandy texture upon melting	smooth	Gritty
Black tea aroma	Intensity of smell corelated with encapsulate	none	Strong
Cocoa butter aroma	Intensity of smell corelated with coccoa butter	none	Strong
Cocoa butter flavor	The taste of white chocolate	mild	Strong
Black tea flavor	The taste of E	mild	Strong
Sweetness	The taste of table sugar	mild	Strong
Black tea aftertaste	The taste of E in the mouth after swallowing	none	Strong

Results and discussion

PSD od E and chocolate samples

PSD curves of W, E, and WE are presented in Fig. 1.

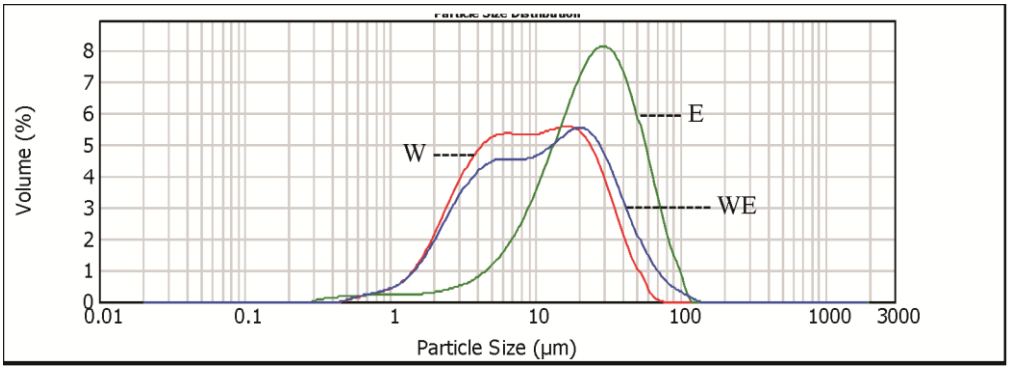

Fig. 1. Particle size distribution of white chocolate (W), encapsulated black tea extract (E) and enriched chocolate with 100 g $kg^{-1}$of E (WE)

White chocolate was milled using five roll mill as seen by multimodal particle size distribution curve. Observing the PSD curve of E, it can be noticed that this powder generally has larger particle sizes comparing to W, however, the largest volume of E consists of particles with diameters in interval 10-30 µm. This indicates that this encapsulate is suitable as supplement in chocolate production since consumers dislike sandy mouth feel, and prefer quickly melting without sticking (Bolenz & Manske, 2013).The PSD parameters are presented in Table 2.

Table 2. Particle size parameters of encapsulated black tea extract (E), white chocolate (W) and enriched chocolates with 100 g kg^{-1} of E (WE)

Particle size parameters (µm)	Sample		
	E	W	WE
d(0.1)	7.41 ± 0.11	2.59 ± 0.04	2.67± 0.06
d(0.5)	24.92 ± 0.07	9.18± 0.06	11.31± 0.13
d(0.9)	58.27 ± 0.14	29.35 ± 0.20	38.32± 0.04
d[4,3] (µm)	30.87 ± 0.09	13.06 ± 0.02	16.91 ± 0.10

Values represent the means (n = 3) ± standard deviation

The addition of 100 g kg^{-1} of E to white chocolate increased all PSD parameters in WE, where the volume mean diameter d[4,3] was increased from 13.06 μm in W to 16.91μm in WE. However, observing that chocolate particles have to be in a size below 30 μm, the addition of E did not increase the mean particle diameter above the acceptable limit.

Rheological properties of molten chocolate samples

The rheology of molten chocolate mass at defined temperature and processing conditions is mainly defined by its ingredient composition, fat content, choice of emulsifier, and particle size distribution (Pajin et al., 2013). Fig. 2 represents the impact of encapsulated black tea extract on rheological properties of enriched chocolate. The both chocolate samples exhibit a thixotropic flow where the addition of encapsulate increases viscosity of white chocolate mass due to a decrease in the free fat phase in the system when the particles of encapsulate were coated by cocoa butter and milk fat.

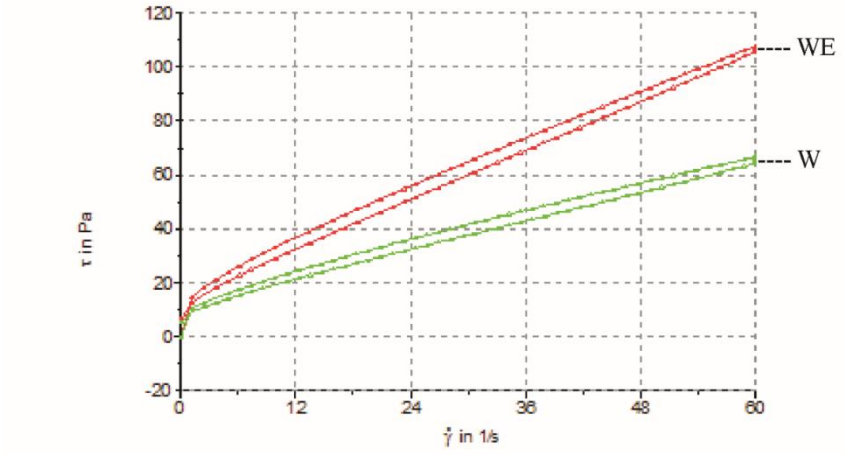

Fig. 2. Flow curves of white chocolate (W) and chocolate with 100 g kg^{-1} of encapsulated black tea extract (WE)

The addition of E to white chocolate increased the values of all rheological parameters in enriched chocolate sample, as shown in Table 3.

Table 3. Rheological parameters of white chocolate (W) and chocolate with 100 g kg^{-1} of encapsulated black tea extract (WE)

Rheological parameters	Sample	
	W	WE
Thixotropic curve area (Pa/s)	144.97 ± 5.00	244.2 ± 7.82
Casson yield stress (Pa)	4.11 ± 0.09	5.26 ± 0.07
Casson viscosity (Pas)	0.63 ± 0.05	1.83 ± 0.04

Values represent the means (n = 3) ± standard deviation

The Casson yield stress of chocolate mass at 40 ºC was increased from 4.11 Pa in white chocolate to 5.26 Pa in enriched chocolate. On the other hand, the addition of encapsulate increased Casson viscosity almost 3 times, from 0.63 Pas to 1.83 Pas. This also led to thixotropic changes within the system with increased value of thixotropic curve area in WE compared to W.

Total polyphenol content

The pure black tea encapsulate contains 1687.64 mg GAE/100g, as shown in Table 4.

Table 4. Total polyphenol contentof black tea encapsulate (E), white chocolate (W) and enriched chocolate (WE)

Sample	E	W	WE
Total polyphenol content (mg GAE/100 g)	1687.64 ± 133.96	40.75 ± 0.96	250.18 ± 4.99

Values represent the means (n = 3) ± standard deviation

The addition of 100 g kg^{-1} of E to white chocolate increased total polyphenol content in enriched chocolate for over 6 times, from 40.75 mg GAE/100 g in W to 250.18 mg GAE/100 g in WE. However, this value is lower compared to total polyphenol content in milk chocolates. Miller et al. (2006) reported that different milk chocolates contain 325-538 mg GAE/100 g. On the other hand, dark chocolate is the reachest source of total polyphenols, originating from dark solids of cocoa beans. Lončarević et al. (2018)[b] showed that dark chocolates made of 58%, 75%, and 88% of cocoa solids contain as much as 1741.98, 1852.72 and 1912.49 mg GAE/100 g, respectively.

Sensory analyses

The sensory characteristics of WC and enriched chocolate with 100 g kg^{-1} of E are shown in Table 5.

Table 5. Sensory characteristics of white chocolate (W) and enriched chocolate with 100 g $kg^{-1}$of encapsulated black tea extract (WE)

Sensory attribute	W	WE
Color	1.00 ± 0.00	11.26 ± 0.91
Glow	8.03 ± 0.16	7.32 ± 0.28
Hardness	6.28 ± 0.13	6.14 ± 0.23
Melting	13.78 ± 0.15	13.15 ± 0.20
Graininess	1.13 ± 0.08	1.29 ± 0.36
Cocoa butter aroma	13.93 ± 0.09	6.24 ± 0.31
Black tea aroma	1.00 ± 0.00	7.56 ± 1.64
Cocoa butter flavor	14.24 ± 0.09	2.10 ± 0.41
Black tea flavor	1.00 ± 0.00	14.12 ± 0.54
Sweetness	7.87 ± 0.07	2.86 ± 0.35
Black tea aftertaste	1.00 ± 0.00	10.54 ± 0.69

Values represent the means (n = 3) ± standard deviation

The addition of E to white chocolate changed the colour of white chocolate to black, where the gloss on the chocolate surface slightly decreased at the same time. While the hardness and melting of enriched chocolate was not affected, the addition of E caused sandy texture of enriched chocolate. Also, encapsulated black tea extract significantly reduced sweetness of enriched chocolate, but, at the same time, it masked the aroma and flavor of cocoa butter, and contributed to the foreign taste of medications.

Conclusions

The addition of 10 g kg^{-1} of encapsulated black tea extract to white chocolate increased its total polyphenol content over 6 times. The results also showed an increase in particle size distribution and viscosity of chocolate mass at the same time, which did not affect the textural characteristics of enriched chocolate, obtained by sensory analyses. While the addition of black tea encapsulate reduced the sweetness of white chocolate, it also contributed to the unpleasant flavor of enriched chocolate.

Acknowledgments

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RUBY CHOCOLATE – BIOACTIVE POTENTIAL AND SENSORY QUALITY CHARACTERISTICS COMPARED WITH DARK, MILK AND WHITE CHOCOLATE

Danijela Šeremet[1], Ana Mandura[1], Aleksandra Vojvodić Cebin[1], Marina Oskomić[1], Elodie Champion[2], Arijana Martinić[1], Draženka Komes[1]

[1]Department of Food Engineering, Faculty of Food Technology and Biotechnology, University of Zagreb, Pierottijeva 6, 10000 Zagreb, Croatia
[2]National Institute of Agronomy, Food Science and Environment, Agrosup Dijon, France

original scientific paper

Summary

Belgian-Swiss cocoa company Barry Callebaut has recently revealed the fourth type of chocolate - Ruby chocolate characterized by the fresh berry taste and reddish color. Since there is no published data about its bioactive content, the aim of this study was to compare Ruby chocolate with different, well-known types of chocolates (dark, milk, semisweet and white) according to bioactive content and sensory attributes. Dark chocolate exhibited the highest total phenolic content and antioxidant capacity followed by semisweet chocolate, while Ruby chocolate, regarding total phenolic content, was ranged between milk and white chocolate, but exhibited higher antioxidant capacity than milk chocolate, probably due to the higher content of flavan-3-ols and proanthocyanidins. Semisweet and dark chocolate obtained the highest score in chocolate distinctive odour, while for the same attribute, Ruby chocolate was estimated as least preferable chocolate. White chocolate with strawberry was used because of similar sensory characteristics as Ruby chocolate, regarding taste and fruity odour, and was rated with a higher score compared to Ruby. The highest intensity of acidity was determined in Ruby chocolate, which is its main characteristic. All estimated sensory attributes were scored the best for the semisweet chocolate, while Ruby chocolate was least acceptable chocolate.

Keywords: Ruby chocolate, bioactive potential, sensory evaluation

Introduction

Cocoa (*Theobroma cacao)*, known as the chocolate tree, belongs to the genus *Theobroma* and subfamily *Sterculioidea* of the mallow family *Malvaceae* (Afoakwa, 2016a), and its seeds, cocoa beans, contained in the tree fruit – the cocoa pod, are the main ingredient for chocolate production. Three morphogenetic groups of cocoa beans are Forastero, Criollo and their hybrid Trinitario (Qin et al., 2016). The estimated world total production of cocoa beans in 2017/2018 was 4 649 000 tones from which about 75% was located in Africa where the biggest producers are Ivory Coast, Ghana, Cameroon, and Nigeria (ICOO, 2019). Main nutritional ingredients of cocoa beans are fat, carbohydrates, proteins, fibers and minerals (K, P, Mg, Ca), but recently, more attention has been paid to the bioactive compounds - vitamins, sterols, phospholipids, alkaloids and polyphenols (Torres-Moreno et al., 2014; Todorovic et al., 2015). Unfermented cocoa beans are rich in polyphenols (12 – 18% of dry matter) (Kim and Keeney, 1984), natural antioxidants characterized by the aromatic feature and conjugated system with hydroxyl groups enabling them to neutralize reactive oxygen species and other free radicals which results in various health benefits (Zhang and Tsao, 2016). The most abundant polyphenols in cocoa beans are proanthocyanidins (58%), flavanols (37%), and anthocyanins (4%) (Di Mattia et al., 2017). From the group of alkaloids, the most represented is theobromine (2 – 3%), while caffeine and theophylline are found in low content (Aprotosoaie et al., 2015). Raw cocoa beans undergo different processes before including in chocolate formulation - fermentation, drying, roasting, grinding, conching and tempering, which all contribute to the chemical and bioactive content of the final product - chocolate (Di Mattia et al., 2017; Todorovic et al., 2015).

Chocolate can be defined as a semi-solid suspension of fine solid particles from sugar, cocoa and milk powder (depending on the type) in a continuous fat phase of cocoa butter (Afoakwa, 2016b) that melts at oral temperature and generates a smooth suspension (Ostrowska-Ligęza et al., 2019). Chocolate is consumed largely worldwide, and it is known as one of the most craved foods. Comparing to the highest chocolate consumption (9 kg/year) reported in Switzerland (Wickramasuriya and Dunwell, 2018), in Croatia it was noted to be 2.2 kg/year (GAIN, 2016). According to today´s scientific research, consumption of cocoa and cocoa-related products has

*Corresponding author: dkomes@pbf.hr

numerous health benefits, but cocoa is known as a medicine for thousands of years. Swedish scientist C. Linnaeus in 1753 named *Theobroma cacao* "food of the gods" (Lippi, 2015). Consumption of chocolate activates pleasure centers of the human brain and has stimulant, relaxing and antidepressive effects mostly due to the content combination of theobromine and caffeine deriving from cocoa beans, which results in unique psychopharmacological properties (Thamke et al 2008; Judelson et al., 2013; Tuenter et al., 2018). Meier et al. (2017) have reported that eating chocolate increases positive mood, particularly when it is eaten mindfully. Besides its psychological effect, chocolate consumption is related to other health benefits. According to Seem et al. (2019), among different types of chocolates and cocoa powders, dark chocolate, after unsweetened cocoa powder, has the biggest effect in supporting and preserving bone health. Preventive effects of cocoa polyphenols in cancer (Martin et al., 2013) and cardiovascular diseases (Kerimi and Williamson, 2015) has been extensively revised. In a scientific opinion stated by EFSA (2014), flavanols from cocoa beans contribute to the maintenance of normal endothelium-dependent vasodilation. Examples of commercial products that carry this claim are Acticoa™ cocoa powder and chocolate (Barry Callebaut, Switzerland).

Main categories of chocolate are dark, milk and white, corresponding to the content of cocoa solids, milk fat and cocoa butter, (Afoakwa, 2016b; Ostrowska-Ligęza et al., 2019), regulated by the EU Directive 2000/36/EC of the European Parliament and the Council relating to cocoa and chocolate products intended for human consumption. With the mentioned three types of chocolate, Barry Callebaut, Belgian-Swiss cocoa company, has recently released the fourth type of chocolate - Ruby chocolate. Ruby chocolate is characterized by fresh berry taste and reddish color. In a patent by Dumarche et al. as inventors and Barry Callebaut as assignee (US 9107430, 2015), it is claimed that red or purple cocoa-derived materials can be produced by treating cocoa nibs, obtained from raw cocoa beans which have higher polyphenol content than a fermented cocoa beans, with an acid with the suitable pKa value. It is preferred that cocoa beans are unfermented and dried in the sun. Acidic conditions (pH, water content, temperature and length of reaction) must be controlled in order to preserve polyphenols to a particular degree in nibs - at least 20 mg/g, but most preferably 40 to 60 mg/g (US 9107430, 2015). Ruby chocolate was presented at a launch event in Shanghai (China) in September 2017, but so far there is no literature data about its bioactive content.

Therefore, this study aimed to investigate the bioactive content and sensory characteristics of Ruby chocolate and compare them to the same parameters of already known types of chocolates.

Materials and methods

Chemicals and materials

In this study, six different types of chocolate were used - dark with 72% of cocoa parts (DC), semisweet with 38% of cocoa parts (SC), milk with 32% of cocoa parts (MC), Ruby (RC), white (WC) and white chocolate with strawberries (WSC), obtained in the local supermarket.

All chemicals used for experimental procedures were of analytical grade.

Sample preparation

Preparation of chocolate samples was carried out as described by Guyot et al. (1998) and Hammerstone et al. (1999), with some modifications. Firstly, chocolate samples were manually grated. In order to eliminate lipids, each sample was extracted with *n*-hexane. The phenolic compounds were extracted from defatted cocoa solids in the ultrasonic bath (Elma sonic S 60 Hz, Elma, Germany) with aqueous methanol (70%) (Adamson et al., 1999), and then centrifuged on SL8R centrifuge (Thermo Fisher Scientific). The supernatant was decanted and collected in a volumetric flask. Extracts were kept at +4 ˚C until use.

Total polyphenol content (TPC) and total flavonoid content

Total phenolic content in chocolate extracts was determined spectrophotometrically (Genesys 10S UV-VIS Spectrophotometer, Thermo Fisher Scientific, US) following a modified method of Lachman et al. (1998). Gallic acid was used for calibration and the results were expressed as gallic acid equivalents (GAE) per gram of original chocolate product (mg GAE/g) (Kramling and Singleton, 1969).

The determination of total flavonoid content was carried out according to the method of Ough and Amerine (1988). After precipitation and separation of flavonoid compounds with formaldehyde in acidic conditions, remaining non-flavonoid phenolics were measured using Folin-Ciocalteau reagent as described above (determination of total phenolic content). Flavonoid content was calculated as the difference between total phenolic and non-flavonoid content.

Since gallic acid was used as the standard in phenolics and-non-flavonoids determination, the content of flavonoids was also expressed as mg GAE/g of original chocolate product (Kramling and Singleton, 1969). All measurements were performed in triplicate.

Determination of antioxidant capacity

Antioxidant capacity of the chocolate extracts was determined using DPPH radical scavenging assay (Brand-Williams et al., 1995) and ABTS radical cation ($ABTS^{+}$) decolourization assay (Re et al., 1999). All measurements were performed in triplicate. For both assays, Trolox was used as the standard and the results were expressed as μmol Trolox equivalents per g of chocolate product (μM Trolox/g).

Determination of flavan-3-ols by vanillin and 4-dimethylaminocinnamaldehyde (p-DAC) assays

Chocolate extracts were analysed for their flavan-3-ols content by vanillin assay as described by Di Stefano et al. (1989) using 4% vanillin solution in methanol.
The content of flavan-3-ols was also determined by *p*-DAC assay, due to differences in used reagents and mechanisms of reactions. A standard procedure reported by Di Stefano et al. (1989) was used to estimate the flavan-3-ol content. Dissolved *p*-DAC in concentrated HCl and methanol was used as a reagent.
For calibration, (+)-catechin (CAT) standard was used. All measurements were performed in triplicate and the results were expressed as mg (+)-catechin per gram of chocolate product (mg (+)CAT/g).

Quantitative determination of proanthocyanidins

Proanthocyanidins (i.e. condensed tannins) were analysed by *n*-butanol/HCl assay of Bate-Smith (1973), with minor modifications. Solutions of cyanidin chloride were used for the construction of standard calibration curves and the results were expressed in mg of cyanidin chloride equivalents per g of chocolate product (mg CyE/g). All measurements were performed in triplicate.

Sensory evaluation

Chocolate samples were evaluated for sensory properties using quantitative descriptive analysis method, following ISO standards (International Standard ISO 8586/2012, 2012) and corresponding literature data on sensory evaluation, with some modifications (Camu et al., 2008; Luna et al., 2002). The sensory evaluation was conducted on six experimental samples using the internal sensory panel of researchers from the Faculty of Food Technology and Biotechnology with experience in sensory evaluations. The panel was formed of 20 trained personnel, 15 female and 5 male members, who had previous experience in the assessment of confectionery products. All panel members exhibited a good score in a taste sensitivity test and showed the ability to identify 5 of 7 commonly found food flavours. Firstly, they had undergone extensive training during two sessions to familiarize with similar samples and to reach a consensus of quantification of previously selected sensory attributes. During training sessions, a list of reference intensities ratings was developed in order to properly evaluate all sensory attributes of experimental samples. Proper conditions in the partitioned booth of sensory laboratory required for sensory evaluation were obtained, including equilibration of encoded samples served in Petri dishes at room temperature (22 °C) with white light illumination. For the experimental chocolate samples, three sessions in a period of one month were held. Followed attributes were evaluated for all six chocolates: milk, fruity and chocolate distinctive odour, mouthfeel, after taste, sweetness, acidity, milk taste, bitterness and astringency, with overall acceptability of each chocolate sample. Attention was especially focused on the sensory evaluation of Ruby chocolate, comparing its overall acceptable grade and individual parameters, such as acidity, fruity and milk odour, to other chocolate samples. The sensory attributes were assessed on a 1/9 point scale, defined as: 1; very weak, 5; moderate and 9; very strong. The average point number was calculated for each of the attributes.

Statistical analysis

All results expressed as the mean value ± standard deviation with Correlations between assays were performed using Microsoft Excel (MS Office 2010).

Results and discussion

Total phenolic content (TPC) of investigated chocolates - dark (DC), semisweet (SC), milk (MC), Ruby (RC), white (WC) and white chocolate with strawberry (WSC) is shown in Fig. 1. Among investigated samples, DC contained the highest TPC (8.11 mg GAE/g) with high correlation (0.98) with antioxidant capacity (DPPH: 40.75 μmol Trolox/g;

ABTS: 57.67 μmol Trolox/g) (Fig. 2), due to the highest content of cocoa solids, while the lowest values of TPC and antioxidant capacity were determined in WC (TPC: 0.36 mg GAE/g; DPPH: 2.85 μmol Trolox/g; ABTS: 0.64 μmol Trolox/g) and WSC (TPC: 0.04 mg GAE/g; DPPH: 0.51 μmol Trolox/g; ABTS: 1.75 μmol Trolox/g) (Fig. 1; Fig. 2). Todorovic et al. (2015) also reported higher TPC in dark (11.99 mg GAE/g) than in milk chocolates (2.70 mg GAE/g), as well as Laličić-Petronijević et al. (2016), who detected a higher TPC in dark (8.4 mg GAE/g) than in semisweet (6.4 GAE/g) and milk (1.6 mg GAE/g) chocolates. Similar results were also reported by da Silva Medeiros et al. (2015) and Belščak-Cvitanović et al. (2012). The lower values of TPC and antioxidant capacity of milk chocolate compared to dark and semisweet chocolates can be attributed to the formation of cocoa polyphenols-milk protein complexes through non-covalent hydrophobic interactions stabilized by hydrogen bonding (Jakobek, 2015), and to a smaller content of cocoa solids. For Ruby chocolate, as the most interesting chocolate due to the lack of data about its bioactive content, measured TPC was 1.35 GAE/g (Fig. 1), antioxidant activity 8.21 μmol Trolox/g determined by the DPPH method and 10.63 μmol Trolox/g determined by the ABTS method (Fig. 2). The values of antioxidant capacity of investigated samples determined by the ABTS method are in high correlation with TPC (0.98), and slightly higher compared to values obtained by the DPPH method which can be explained by the ability of ABTS radical to react with a broader range of antioxidative compounds (Mareček et al., 2017).

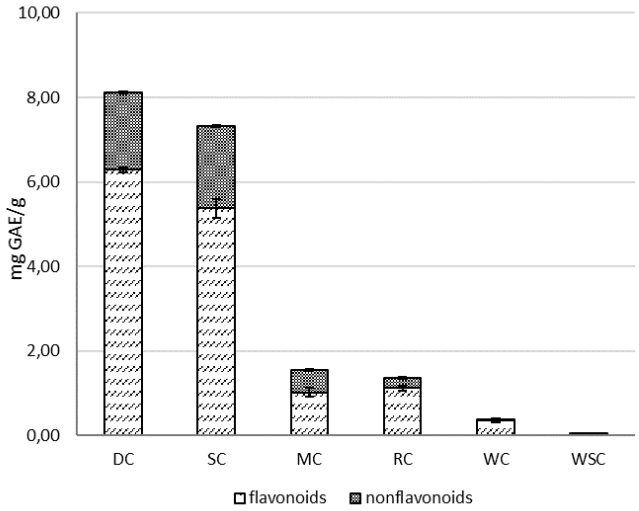

Fig. 1. Total flavonoids and non-flavonoids content (mg GAE/g chocolate ± SD) of analysed chocolates

In the investigated chocolates, as can be seen in Fig. 1, flavonoids are predominant among the polyphenolic compounds. The values of flavonoids ranged between 0.04 mg GAE/g for WSC and 6.28 mg GAE/g for DC. According to the literature, one of the most abundant subgroups of flavonoids in chocolate are flavan-3-ols, especially (-)-epicatechin and (+)-catechin, which can group together to form oligomeric and polymeric proanthocyanidins - polyphenolic compounds that contribute the most to the antioxidant capacity of chocolate (Di Mattia et al., 2017). The results for total flavan-3-ols and proanthocyanidins content of investigated chocolates are presented in Table 1. Flavan-3-ols content determined with vanillin assay exhibited up to 3.00 mg CAT(+)/g (DC) and, determined by *p*-DAC assay, up to 2.91 mg CAT(+)/g (DC). The highest content of proanthocyanidins was observed in DC (0.80 mg CyE/g), while their presence in WC and WSC was not observed. Similar results were reported by Belščak-Cvitanović et al. (2012; 2015), Todorovic et al. (2015) and Laličić-Petronijević et al. (2016). It is worth to highlight higher values of antioxidant capacity and higher content of flavan-3-ols (1.02 mg CAT(+)/g; 0.90 mg CAT(+)/g) and proanthocyanidins (0.10 mg CyE/g) of RC compared to MC (0.06 mg CAT(+)/g; 0.31 mg CAT(+)/g; 0.06 mg CyE/g) (Table 1), even though MC turned out to be richer in total polyphenols (Fig. 1). The high correlation between total proanthocyanidins and

total flavan-3-ols content using both vanillin and *p*-DAC assays (0.98) can be explained due to the chemical composition of proanthocyanidins since they are oligomeric and polymeric flavan-3-ols. The lower values measured by *p*-DAC assay may be the consequence of different structural requirements for obtaining a reaction and assay sensitivity. Thus, *p*-DAC reagent reacts only with a hydroxyl group at the C-6 position in the benzene ring, while vanillin reagent bonds on hydroxyl groups at C-6 and C-8 positions in molecules of flavan-3-ols (Porter et al., 1986).

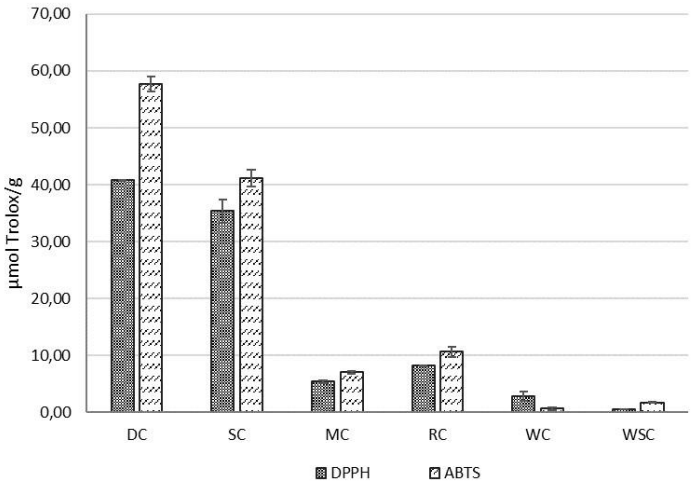

Fig. 2. Antioxidant capacity of analysed chocolates determined by ABTS (µmol Trolox/g chocolate ± SD) and DPPH assays (µmol Trolox/g chocolate ± SD)

Table 1. Total flavan-3-ols (mg CAT(+)/g ± SD) and proanthocyanidins (mg CyE/g ± SD content in analysed chocolates

Sample	Total flavan-3-ols[1](mg CAT(+)/g)	Total flavan-3-ols[2] (mg CAT(+)/g)	Total proanthocyanidins (mg CyE/g)
DC	3.71±0.03	2.91±0.72	0.80±0.02
SC	2.83±0.02	1.94±0.32	0.56±0.02
MC	0.06±0.01	0.31±0.06	0.06±0.00
RC	1.02±0.01	0.90±0.16	0.10±0.10
WC	n.d.	0.02±0.00	n.d.
WSC	0.03±0.00	n.d.	n.d.

n.d.= not detected
CAT(+)=catechine, CyE=cyanidin chloride equivalent
[1-2]Determined using vanillin and *p*-DAC assay
Results are expressed as the mean value ± standard deviation

Fig. 3a-b illustrate the score for evaluated sensory attributes in the analyzed chocolates. According to obtained results in odour attributes (Fig. 3a), WC showed the highest score in terms of milk odour, while WSC expressed the most dominant fruity odour among all samples. As expected, SC and DC obtained the highest score in chocolate distinctive odour, while for the same attribute, RC was estimated as at least preferable chocolate. In terms of fruity odour, WSC exhibited the highest score, while this attribute was less pronounced in RC. Also, various taste attributes were tested in order to rank the acceptability of RC among other, commonly consumed chocolates. As can be seen in Fig. 3b, SC and DC were the highest ranked samples in mouthfeel taste, described as one of the most significant sensory categories for chocolates (Dürrschmid et al., 2006). WC, WSC and RC were evaluated with lower scores than MC for the same attribute. Although it was expected for MC to exhibit the highest grade in milk taste, WC was evaluated with the highest score, while RC received an average score. Except for WSC, scores for aftertaste did not vary significantly between the samples, while sweetness and acidity were scored in a wider value range, as expected. WC and WSC showed the highest score in sweetness, while the lowest score was obtained for DC. The intensity of acidity was the highest in RC, followed by WSC and DC, which corresponds to data presented on Barry Callebaut

official web site (Anonymus, 2019) reporting the acidity as one of the prominent sensory attributes of Ruby chocolate, along with sweetness, sourness, creamy and red fruit flavour. The presence of bitterness and astringency was the most dominant in chocolates with the highest content of cocoa solids, SC and DC, since those sensory attributes are related with methylxanthines (caffeine and theobromine) and polyphenolic compounds, such as proanthocyanidins and flavan-3-ols in cocoa (Misnawi et al., 2003; Wollgast and Anklam, 2000; Luna et al., 2002; Belščak-Cvitanović et al., 2012).

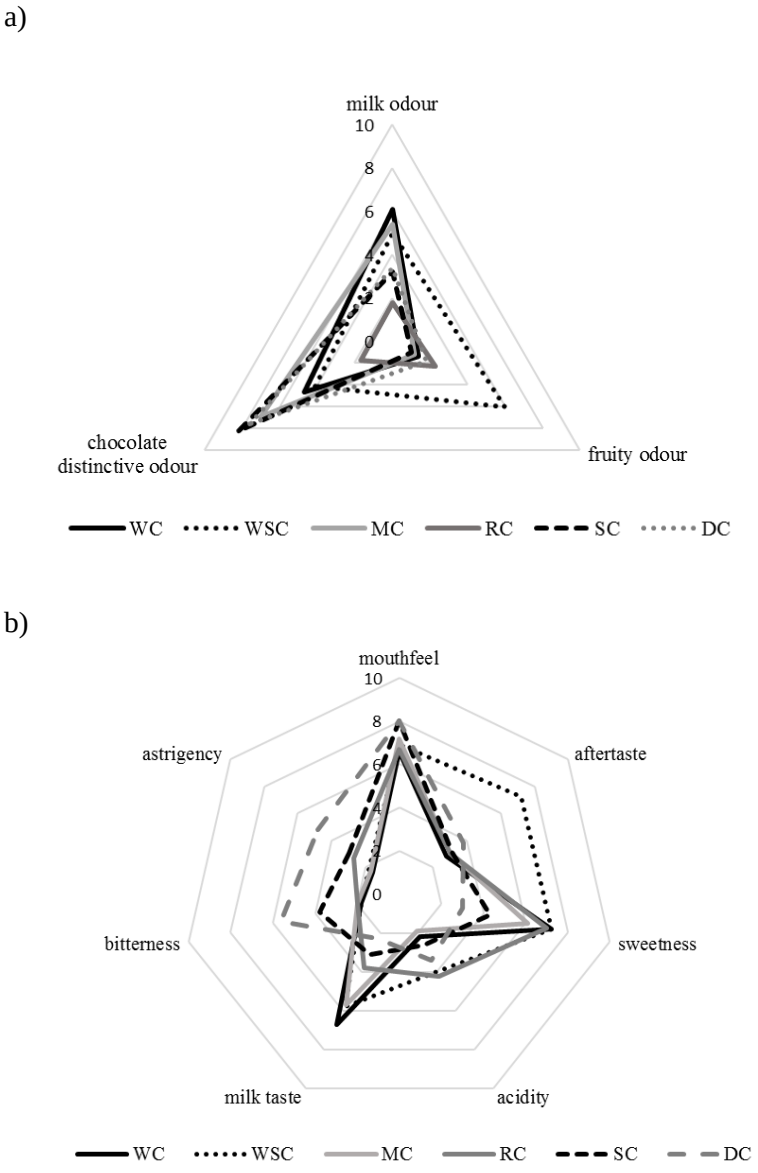

Fig. 3. Spider chart representing mean scores of the evaluated sensory attributes (a) odour attributes and (b) taste attributes for white chocolate (WC), white chocolate with strawberry (WSC), milk chocolate (MC), Ruby chocolate (RC), semisweet chocolate (SC) and dark chocolate (DC)

The overall acceptability, as a useful guideline in the final chocolate product assessment, was also evaluated and the best assessed was SC with the highest score (7.9), followed by DC (7.6) and MC (6.7) and further WC and WSC with average scores (5.4 and 6.1, respectively) while RC was at least acceptable chocolate (5.2).

Conclusions

According to results of bioactive potential of different chocolates, dark chocolate showed the highest value of total phenolic content correlated well with antioxidant capacity as expected, while Ruby chocolate exhibited moderate results of total phenolic

content ranging between milk and white chocolate, although showing higher antioxidant capacity compared to the milk chocolate. The highest content of flavan-3-ols and proanthocyanidins was determined in dark chocolate, while Ruby chocolate showed higher values of mentioned phenolic compounds than milk chocolate. A sensory evaluation assessed the semisweet chocolate with the highest score in terms of overall acceptability, while Ruby chocolate was least acceptable chocolate. Due to the lack of data about the bioactive composition and sensory assessment of Ruby chocolate, it is necessary to continue the examination in order to confirm the obtained results.

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EFFECT OF DIFFERENT STORAGE CONDITIONS ON FAT BLOOM FORMATION IN DIFFERENT TYPES OF CHOCOLATE

Svjetlana Škrabal[1], Đurđica Ačkar[2*], Jurislav Babić[2], Borislav Miličević[2], Antun Jozinović[2], Drago Šubarić[2]

[1]Polytechnic in Požega, Vukovarska ul. 17, 34000, Požega, Croatia
[2]Josip Juraj Strossmayer University of Osijek, Faculty of Food Technology Osijek, Franje Kuhača 20, 31000 Osijek, Croatia

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Summary

Chocolate is a product that is mostly composed of cocoa butter, cocoa mass and sugar. Cocoa butter is a sensitive component that is susceptible to changes when temperature fluctuations occur during storage. Moreover, chocolate is sensitive to changes in the humidity of the surrounding environment. At inappropriate temperatures, part of stable crystals of cocoa butter will melt, fat will come out on the surface of chocolate and fat bloom will appear. This phenomenon is one of the most common problems in the chocolate industry since it highly affects consumers' acceptability – grey layer on the surface will make consumers dislike the product and ones that are less familiar with the phenomenon will question the safety. In this research, chocolates with different contents of cocoa butter, milk fat and substitute fats were prepared. We examined the effects of different humidity (50, 65 and 75%) and temperatures (20 and 29 °C) during storage on the formation of fat bloom. Measurements of surface colour and thermophysical properties of the surface of chocolate were performed. Chocolates with higher content of milk fat proved to be the most stable. Chocolate containing only cocoa butter was also stable under different storage conditions. The substitute fat and high humidity additives have had a great impact on formation of fat bloom on surface of chocolate.

Keywords: fat bloom, chocolate, relative humidity, storage

Introduction

One of the major problems of the confectionery industry is the fat bloom of chocolate products. Fat bloom of the surface of chocolate is the result of complex processes that take place in chocolate over a period of time or as a consequence of a poorly managed production process, the use of inadequate raw materials or poor storage conditions (Kinta and Hatta, 2012; Silva et al., 2017).

Fat bloom results in changes in product properties, above all in appearance and texture, and thus in reducing the consumer's acceptance of the product. The grey look of the chocolate surface is caused by irregular shapes of cocoa butter crystals. Chocolate fat bloom does not appear only because of transition from the β (V) form of cacao butter to the β (VI) form but also because of transition of the unstable lower polymorphic forms to the β (V) form (Kinta and Hatta, 2007; Bahari and Akoh, 2018). Due to the partial mixing ability with the fat, the emulsifiers damage the crystalline structure of triglycerides and thereby increase the content of the liquid phase which leads to polymorphic transitions. However, they do not affect the transition of form V to form VI of cocoa butter, which slows down formation of fat bloom of the chocolate (Jovanović and Pajin, 2004). If temperature rises during storage of chocolate products, the lower melting triglycerides are transferred to the liquid state after which they are crystalized again by cooling. However, recrystallized fat will not be associated with the solid phase that has fats with higher melting point (Zhao and James, 2018).

Fat bloom of the surface of chocolate may also result from eutectic incompatibility of two fats (e.g., solid butter and cocoa butter or milk fat) (Ghosh et al., 2002; Timms, 2003). Fat compatibility depends on the thermal properties of fat (melting point and solid fat content), the size and shape of molecules (affected by fatty acid chain lengths and *cis* and *trans* structures of unsaturated acids) and polymorphism. As these properties are more similar, fats are more compatible. The formation of eutectic is achieved by mixing two incompatible fats (fat of a different percentage of solid triglycerides), the mixture of which has a lower melting point than each component. The final product is sensitive to fluctuations in temperature and appearance of fat bloom (Graef et al., 2005). If the fats of a similar fatty acid composition are mixed but have a different form or size of molecules, a stable crystal network is not formed, so the final product is also sensitive to fat bloom (Timms, 2003). Separation of certain fatty fractions in chocolate is often associated with migration of liquid fats to the surface by capillary transition that is supported by the difference in the concentration of triacylglycerols (TAG) (Smith et al., 2007).

*Corresponding author: dackar@ptfos.hr

Milk fat is present in milk chocolate. The triglyceride composition of milk fat is very complex because it contains more than 400 different triglycerides (Breitschuh-Apostolakis and Flöter, 2002). By studying the properties of chocolate after the addition of anhydrous milk fat and five dairy fractions obtained by dry fractionation of milk fat, Tietz and Hartel (2000) concluded that the lipids which are present in milk fat in much smaller proportions greatly affect the time of nucleation, rate of crystallization and the rate of fat bloom of chocolate surface. Milk fat affects the size of crystals, the way of crystallization and the transition of less stable polymorphic forms to more stable ones (Sonwai and Rousseau, 2010). The influence of milk fat depends on its type and on polar lipids in milk fat. It has been found that fat bloom of milk chocolates is directly related to the proportion of free fatty acids, diacylglycerol and monoacylglycerol (Ransom-Painter et al., 1997; Tietz and Hartel, 2000; Rousseau, 2006). Milk fat accelerates the appearance of fat bloom if laurel fat is present. Namely, milk fat fractions with higher melting point form a mixture with lauric acid which destabilizes β (V) crystals and accelerates the formation of fat bloom (Ransom-Painter et al., 1997). Fat bloom occurring during storage is characterized by the growth of small crystals on the surface and within the product after a certain period (Kinta and Hatta, 2012; Timms, 2003). Due to the oscillation of temperature during storage, there is a change in fat crystals, which creates new polymorphic forms (Sonwai and Rousseau, 2006). The chocolate surface is more sensitive to temperature changes than its interior. Increasing the temperature increases the proportion of liquid fat that is suppressed to the surface of the chocolate. Late temperature drop does not crystallize all liquid fat, but one part remains liquid inside of the chocolate. In addition, the surface of the chocolate becomes porous and the liquid can undisturbedly travel to the surface of the chocolate where it crystallizes under certain conditions. Properly packed chocolates are more resistant to fat bloom than those who are not packed properly (Torbica et al., 2013; Lonchampt and Hartel, 2004). Even when production meets all the requirements necessary to prevent fat bloom, the fat bloom may occur due to inadequate storage conditions. When the temperature is high enough (above 32 °C), cocoa butter is partially melted. Cooling cocoa butter that was melted uncontrollably crystallizes into unstable polymorphic forms due to lack of stable centres. Even the slightest temperature oscillations accelerate the appearance of fat bloom (Torbica et al., 2013). Sahari et al. (2013) have found in their research that it is possible to use *Camellia sinensis* tea as Cocoa Butter Replacer in the production of dark chocolates and that there is a possibility of inhibiting fat bloom.

Materials and methods

Materials

The materials used in this study were:

- whole milk powder, roller dried (Zvečevo, d.d., Požega, Croatia);
- whole milk powder, spray dried (Zvečevo, d.d., Požega, Croatia);
- whole milk powder, spray dried ("Laktopol" Sp.z. o.o. Warszawie, Poland);
- caramelized whole milk powder, roller dried (Zvečevo, d.d., Požega, Croatia);
- caramelized whole milk powder, roller dried (Hochdorf Swiss Milk AG, Hochdorf, Switzerland);
- skim milk powder ("Laktopol" Sp.z. o.o. Warszawie, Poland);
- hazelnut paste (Zvečevo, d.d., Požega, Croatia);
- cocoa butter equivalent (IllexaoTM CB 40, AarhusKarlshamn, Sweden).

Preparation of chocolate samples

The chocolates were produced by the standard process of production of chocolate masses in the "Zvečevo" confectionery factory. Technological parameters in the preparation of chocolate masses: mixing time 360 seconds, refining on a two-roll refiner, refining on a five-roll refiner, dry conching for 4 hours at a temperature of 52 to 56 °C and liquid conching for 20 hours at a temperature of 55 to 59 °C.
Percentages of different fats used in production of samples are given in Table 1.

Table 1. Fats used in formulations of chocolates

Sample	Cocoa butter (%)	Milk fat (%)	Hazelnut fat (%)	Vegetable fat (%)
MC-1	23.5	3.7 (roller dried and skim milk powder)	2.1	-
MC-2	23.5	3.7 (spray dried and skim milk powder)	2.1	-
MC-3	23.0	4.4 (spray dried and skim milk powder)	2.1	-
MC-4	19.48	3.9 (spray dried milk and powdered whey)	-	4.0
MC-5	23.8	6.0 (condensed milk)	-	-
MC-6	27.0	4.9 (spray dried and skim milk powder)	-	-
CO-1	23.8	-	-	4.0
CO-2	40.8	-	-	-

Moulding

Chocolate mass temperature (before pouring into moulds) was 30 - 30.5 °C, mould temperature was 29 °C, refrigerator temperature was 5 °C, the temperature of chocolate was 18 - 20 °C and the working room temperature for packing of chocolate was 21 - 24 °C.

Storage of samples

Produced chocolate was kept open for 55 days in a cooled incubator with humidity control (Climacell, Medical Intertrade) under controlled conditions:

- 12 hours at 20 °C and 12 hours at 29 °C with relative humidity below 50%,
- 12 hours at 20 °C and 12 hours at 29 °C with a relative humidity of 65% and
- 12 hours at 20 °C and 12 hours at 29 °C with 75% relative humidity.

Colour of the chocolate

Measurement of the colour of the upper surface of the samples was carried out immediately after the preparation of the samples and every 10 days. Last measurement was done on the 55th day. Chocolate colour measurement was performed using a Conica Minolta CR-600, and colour parameters L*, a* and b*. L* values range from 0 to 100 and give a rating of whether it is dark or light. If L*=0, the object is black, and if L*=100 then it is white. a* value can be positive or negative. Positive values point to red and negative to green. The b* value can also be positive or negative. If the value is positive, the result is yellow and, if negative, blue (Bricknell and Hartel, 1998).

Based on the measured values (10 for each sample), the whitening index (WI), whitening index change (ΔWI) and total colour change (ΔE) were calculated according to the following expressions:

$$WI = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{0,5} \quad (1)$$

$$\Delta WI = WI_n - WI_1 \quad (2)$$

$$\Delta E = [(\Delta L^*)^2 - (\Delta a^*)^2 + (\Delta b^*)^2]^{0,5} \quad (3)$$

Determination of thermophysical properties

To measure of thermophysical properties of chocolate samples were taken after 55 days of storage. The measurement was conducted on samples previously used to measure colour change and samples stored at room temperature (control samples). Samples were retrieved with scratching the surface layer of chocolate that changed colour during storage. 10 to 20 mg of samples were used, and the samples were weighed in a standard aluminium container (40 μL). The weighing vessel was hermetically sealed, and the measurement of thermophysical properties was conducted. The samples were subjected to the following temperature program:

- isothermal at 50 °C, 1 min;
- cooling from 50 °C to 0 °C (cooling rate was 10 °C/min);
- isothermal at 0 °C, 1 min;
- heating from 0 °C to 200 °C (heating rate was 10 °C/min).

To determine the thermophysical properties of the samples, Mettler-Toledo DSC model 822e was used, and the measurements were carried out under a nitrogen purity 5.0 (Linde). Enthalpy change (ΔH) was obtained from the DSC exothermic curve using the software STAR^e (Fig. 1).

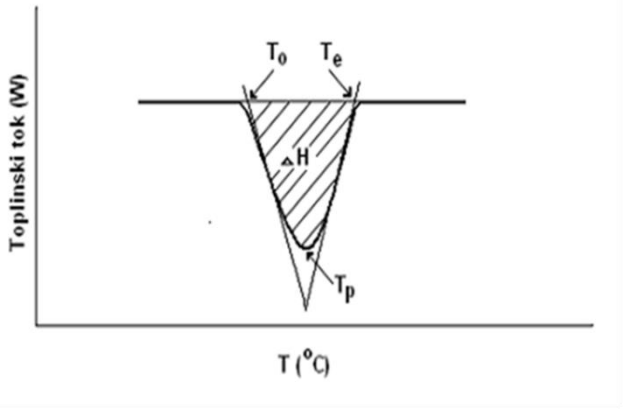

Fig. 1. Exothermal DSC curve and parameters determined by STAR^e software

Statistical analysis

Pearson correlation coefficient was performed using Statistica®, Version 13.4.0.14 (1984-2018 TIBCO Software Inc). Total colour change, whitening index, enthalpy of melting of sucrose and enthalpy of melting of cocoa butter were used as a variables. The data used for determination of correlation between variables were measured after 55 days at different relative humidities. Significant p-value was set at 0.05.

Results and discussion

Total colour change and whiteness index

Some of the ingredients and air humidity of the area in which samples were kept had a different effect on the fat bloom of the examined chocolates. Changes in some samples, especially those stored in the 75% air humidity, are so advanced that fat bloom appeared on the surface of the product along with changes in texture. Chocolates became brittle and "dry". Liang and Hartel (2004) stated that milk fat positively affects the stability of chocolate colour. This claim was confirmed in this study if samples MC-1 and MC-2 (containing 3.7% milk fat) were taken into account, especially at air humidity of 65% (Table 2). From previous research by Jovanović and Pajin (2004) it can be concluded that milk fat acts as a fat bloom inhibitor. Timms (2003) stated that at temperatures above 18 °C and relative humidity greater than 60%, polymorphic transition of cocoa butter increases and fat blooming process is accelerated. In accordance with this study, all samples had the most significant colour change at 75% relative humidity. The sample MC-1 contained roller dried milk powder, which resulted in a higher proportion of free milk fat that inhibits fat bloom (Metin and Hartel, 2012). As a result, the value of ΔWI in sample MC-1, after 55 days and at 65% humidity, was 3.17 (Table 2), and in sample MC-2, containing spray dried milk powder, 5.99. Sonwai and Ruosseau (2010) concluded that free dairy fat interacts with other fats and that its content affects the stability of the product. At air humidity of 75% both samples behaved similarly (intense fat bloom covered the entire surface of the product). This phenomenon is caused probably because of bounding of water to the protein and carbohydrates (sugar). This leads to the dissolution of sugar and the creation of pathways to release fat to the surface of chocolate (Rousseau, 2006). Changes observed at air humidity of 50% in both samples were of such intensity that the average consumer would not characterize it as negative, also those changes did not lead to changes in other product properties. For these two samples, the fact that they contain hazelnut paste should be also taken into account. Hazelnut paste contains oil fractions, which are relatively liquid at low temperatures and can easily migrate to the surface. Cocoa butter is easily dissolved in it, thereby tendency to migrate to the surface of chocolate is increased and because of that it creates a space for migration of other fats to the surface of the product. Hazelnut oil contains triolein as the main component and this TAG plays an important role in migration of fats (Smith et al., 2007). The MC-3 sample contained the same ingredients as the MC-2 sample, but the proportion of those ingredients was different. MC-3 sample contained about 0.7% more milk fat, which ultimately had a strong effect on product stability. At 75% moisture, colour change of the surface occurred on the 20th day. Changes in the MC-2 sample were significantly more pronounced at lower humidity. The MC-4 sample contained 19.48% cocoa butter, 3.9% milk fat, powdered whey and 4% vegetable fat. From the results shown in the Table 2 it is evident that the chocolate made from these raw materials was unstable even at lower humidity. At 65% humidity there was a significant change in the colour of the surface (the most intense of all tested samples), most likely due to the high hygroscopicity of whey and low milk fat content in the product (Keogh et al., 2006). The MC-5 sample contained 6% of milk fat (condensed milk) and was relatively stable during storage. Certain variations were recorded when measuring the surface colour of this sample, probably due to the raw material composition and the "non-homogeneous" colour of the surface. Stability of this sample can be attributed to a high proportion of milk fat. The same stability was observed in MC-6 sample which contained 4.90% of milk fat.

Table 2. Influence of relative humidity on whitening index (ΔWI) and total color change (ΔE) of chocolate samples

Sample	**MC - 1**		**MC - 2**		**MC - 3**		**MC - 4**		**MC - 5**		**MC - 6**		**CO - 1**		**CO - 2**	
	Relative humidity < 50%															
Days	**ΔWI**	**ΔE**	**ΔWI**	**ΔE**	**ΔWI**	**ΔE**	**ΔWI**	**ΔE**	**ΔWI**	**ΔE**	**ΔWI**	**ΔE**	**ΔWI**	**ΔE**	**ΔWI**	**ΔE**
10	1.8	1.31	1.2	1.35	-0.46	1.15	1.01	1.1	1.76	1.84	0.38	0.58	1.1	1.13	1.24	1.26
20	2.4	2.64	1.83	2.08	0.63	1.01	2.18	2.5	2.25	2.57	1.21	1.67	2.34	2.58	1.88	1.95
30	2.01	2.08	2.28	2.39	0.38	1.25	1.99	2.2	2.21	2.44	0.56	0.94	1.91	2.1	1.64	1.68

Table 2. Cont.

40	2.63	2.92	2.37	2.61	0.86	1.16	1.75	2	2.73	3.17	0.51	0.82	2.25	2.53	1.93	1.97
50	1.85	1.95	2.11	2.17	0.48	1.41	7.17	7.9	3.93	5.47	1.54	2.47	5.25	6.08	1.88	2.17
55	2.56	2.72	2.38	2.53	0.72	1.53	2.11	2.3	2.3	2.58	0.86	1.26	3.15	3.28	2.06	2.11
	Relative humidity 65%															
10	2.05	2.14	6.74	6.9	0.72	1.72	4.22	4.4	2.81	3.15	2.71	2.92	3.52	3.72	2.19	2.23
20	2.3	2.55	4.73	5.01	1.29	1.38	3.1	3.4	4.86	5.76	1.68	2.37	2.69	3.3	2.48	2.77
30	2.25	2.55	5.33	5.67	1	1.4	2.83	3.4	5.87	6.84	1.25	1.94	2.08	2.64	1.36	1.72
40	2.22	3.33	6.7	7.22	1.05	1.2	6.15	6.8	4.31	5.36	1.94	3.22	4.45	5.23	1.42	1.66
50	3.26	4.01	6.57	6.98	1.06	1.86	1.88	2.1	2.11	2.45	0.62	0.82	2.69	2.83	2.11	2.16
55	3.17	4.08	5.99	6.42	2.81	3.16	7.19	8.1	3.85	5.21	0.69	1.42	5.8	6.65	1.5	1.85
	Relative humidity 75%															
10	0.77	0.89	2.34	2.41	0.5	2.43	0.56	0.9	0.8	1.03	0.96	1.13	0.96	1.22	2.19	2.23
20	9.91	10.8	9.71	10.4	3.04	3.56	7.87	8.6	3.2	3.14	8.36	10.7	11.9	15.88	2.48	2.77
30	12.1	13.3	10.9	11.7	4.29	4.71	8.89	9.8	3.25	3.18	8.03	10.1	11.43	17.08	1.36	1.72
40	12.5	14	11.1	12.2	4.24	4.76	11.4	13	1.01	1	8.09	11	9.93	14.77	1.42	1.66
50	14.1	17	11.4	12.6	5.82	6.5	11.3	13	3.83	3.97	9.85	12.8	11.79	20.08	2.11	2.16
55	14.2	16.8	13.5	15.4	4.63	5.38	14.9	18	4.19	4.41	11.58	15.5	13.99	23.63	1.5	1.85

Chocolate production is increasingly using various CBE's to improve some of the quality parameters such as gloss, stability, solubility, etc. Also, CBE's can significantly affect the cost of the product. In chocolate production, CBE's replaces no more than 5% of cocoa butter. Although the triglyceride composition of CBE's fat is similar to the triglyceride composition of cocoa butter, it is not identical, and hence the formation of fat bloom may result from differences in composition (Yates, 2003). The presence of stable crystals of cocoa butter will allow proper nucleation and thus slow down the fat bloom (Kinta and Hartel, 2010). If the results for similar chocolates are compared, it is apparent that the changes at all storage conditions are very similar. The CO-1 sample contained 4% of CBE's, same as MC-4 sample. However, the MC-4 sample contained 3.9% milk fat, which resulted in a significantly lower colour change in the surface (inhibiting effect of milk fat) (Tietz and Hartel, 2000). The CO-1 had a change in colour at humidity below 50% and at 75% it was of a high intensity (ΔE was 23.63). The reason for this is probably the incompatibility of mixing CBE's with cocoa butter (Ghosh et al., 2002; Timms, 2003). In addition to this statement, the results obtained for the CO-2 sample, which was the only sample containing only cocoa butter, showed it was very resistant to the fat bloom under all conditions under which the research was carried out. Sample CO-2 which had a low sugar content and a high content of cocoa butter (from cocoa butter and cocoa mass), proved to be extremely stable during the 55 day storage at all conditions under which the research was conducted. The stability of such chocolate can be explained by the fact that there is one type of fat in the chocolate (cocoa butter) so there is no risk of fat bloom of the chocolate due to the incompatibility of mixing of fats (Kinta and Hatta, 2007).

On the other hand, low sugar content as well as other non-fat particles that have tendency to adsorb water did not affect the increase of the whitening index at higher relative humidity (the value of ΔWI at the beginning of storage was 1.24, while at the end of storage at 65% humidity was 1.50, and at 75% 2.59). Adsorption of moisture to hydrophilic particles that are the ingredients of chocolate can cause melting of sugar on the surface of chocolate (Kinta and Hatta, 2012). The results given in Table 2 confirm this conclusion. CO-1 sample contained 58% of sugar and it is apparent from the results that the value of whitening index significantly increased at 75% humidity after only 20 days of storage (ΔWI 11.9), which manifested itself through total colour change (ΔE 23.63 at the end of storage).

From the data obtained by the statistical analysis (Table 3), it is evident that there is a significant correlation between the whitening index and total colour change. This proves that in fact the color change that occurs on chocolates is patterned by mostly fat bloom, that is, an increase in the whitening index.

Table 3. Coefficient of correlation between analysed parameters

Variable	Total colour change	Whitening index	Enthalpy of melting of sucrose	Enthalpy of melting of cocoa butter
Total colour change	1.000000			
Whitening index	**0.957685**	1.000000		
Enthalpy of melting of sucrose	-0.219245	-0.226829	1.000000	
Enthalpy of melting of cocoa butter	**0.440923**	**0.412273**	**-0.637463**	1.000000

Bold values were considered significant at $p<0.05$

Thermophysical properties

Differential scanning calorimetry (DSC) was conducted prior to storage (control sample) and after storing chocolates under the following conditions: 50, 65 and 75% relative humidity, at 20 °C for 12 hours followed by 12 hours at 29 °C for 55 days. DSC analysis gave the results of relative content and proportion of fat (cocoa butter and other fat) and sucrose on the surface of the chocolate. The relative content and fat to sucrose ratio on the surface of the examined samples of chocolate is proportional to the enthalpy of melting of the above mentioned ingredients. From the results, it is apparent that in almost all samples (except MC-1 at 65% relative humidity) during storage at a higher relative humidity (65 and 75%), a change in the composition of the surface layer manifested by decreasing the sucrose content and increasing the proportion of fat. Svanberg et al. (2013) also concluded that during storage of incorrectly processed chocolate comes to increase of fat content on surface of chocolate. On the other hand, the temperature fluctuations during storage did not cause significant changes in composition of the chocolate surface at relative humidity of 50%. Figs. 2 and 3 show enthalpy of melting of sucrose and fat of chocolate samples. As mentioned, in most samples the temperature fluctuation during storage with higher relative humidity has reduced the sucrose content and increased the fat content on the surface. In the sample MC-6 (27.0% cocoa butter, 4.9% milk fat, spray dried and skimmed milk), the smallest changes in sucrose content on the surface of chocolate were observed, while in other samples there was a significant decrease in sucrose content during storage under the specified conditions. MC-1, MC-2, MC-4 had a reduction of sucrose content on the surface by about 40% during storage at a relative humidity of 65 and 75%. Also, in these samples and in MC-6 sample, the surface fat content raised at about 85% under the same conditions (except MC-1 at 65% relative humidity). Thereby, after storage at 75% moisture, the MC-6 sample had the highest enthalpy (38.22 J/g), probably due to the higher content of fat in the formulation (about 5% higher). CO-1 sample, which contained 4% of CBE's, stands out with a significant increase in fat content on the surface after storage at higher relative humidity. Therefore, when stored at 75% relative humidity the CO-1 sample showed an increase of fat slightly more than 100% (ΔH was 17.8 J/g for the control sample and after storage at 75% ΔH was 39.7 J/g). The increase of fat on the chocolate surface is also evident from the results for the whitening index. This correlation between whiteness index and enthalpy of melting of cocoa butter is confirmed by statistical analysis. Correlation coefficient also shows that there is statistically significant negative correlation between enthalpy of melting of sucrose and cocoa butter. The MC-4 sample also contained 4% of CBE's and there was also a significant increase in the fat content on the surface during storage (ΔH was 17.4 J/g for the control sample and after storage at 65% ΔH was 33.2 J/g). Relatively lower fat migration is likely the result of presence of milk fat (3.9%), which, as mentioned before, slows the appearance of fat bloom. MC-5 sample has higher content of cocoa butter and higher content of milk fat than MC-6. When these samples are compared it can be seen that sample MC-5 has higher enthalpy of melting of cocoa butter.

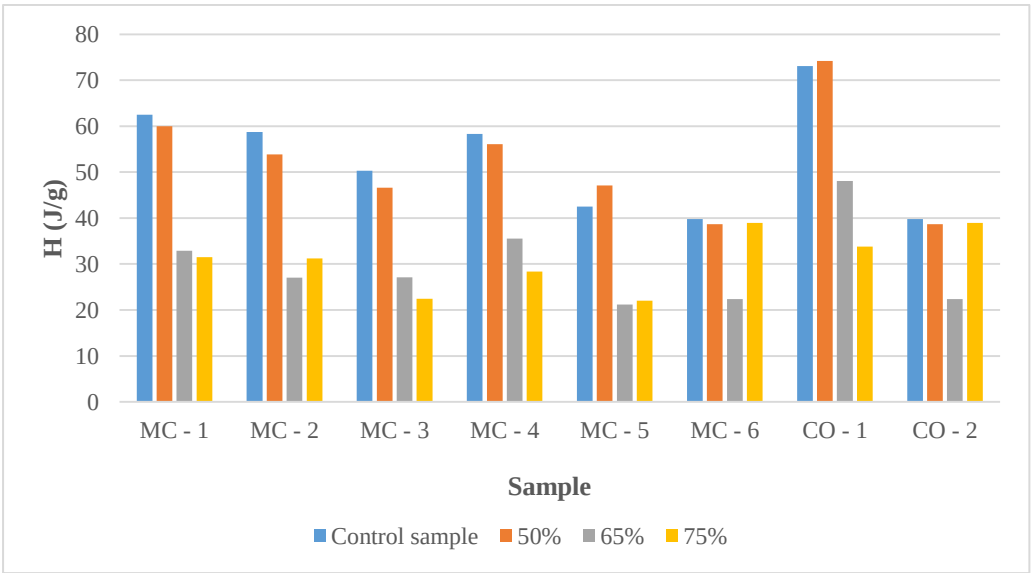

Fig. 2. Enthalpy of melting (H) of the sucrose obtained by DSC by analysing the surface layer of chocolates at different relative humidity

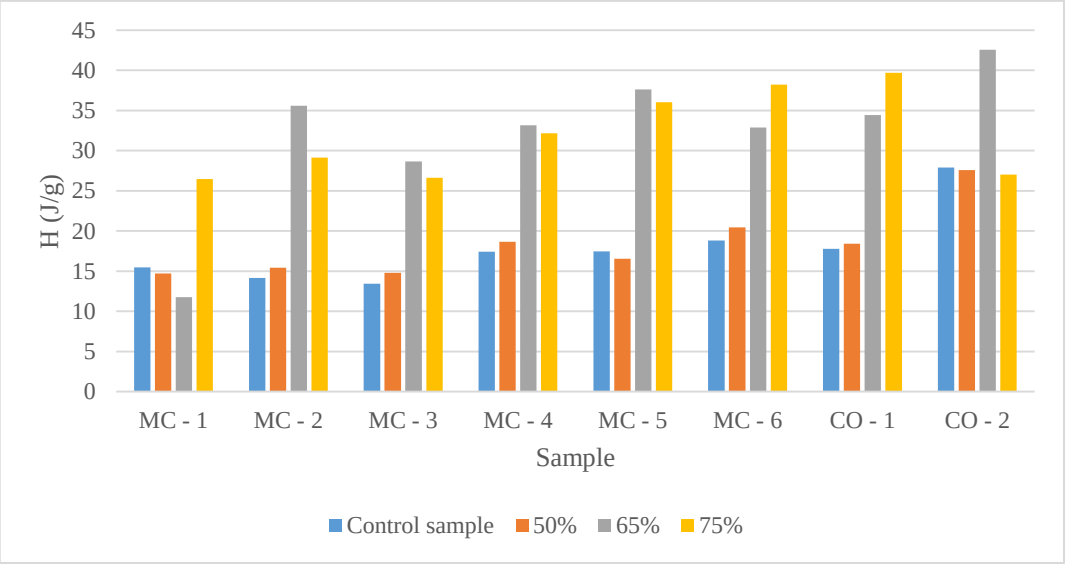

Fig. 3. Enthalpy of melting (H) of the cacao butter obtained by DSC by analysing the surface layer of chocolates at different relative humidity

Conclusions

Based on the research carried out it can be concluded that certain fat-containing ingredients and relative humidity during storage of samples had a significant effect on fat bloom formation on the surface of the examined chocolates. Changes in some samples, especially those kept at 75% relative humidity, were so advanced that along with appearance of fat bloom on the surface of the product, the change in the texture of chocolate also occurred. Chocolates containing milk fat, which originated from powdered roller dried milk, were more resistant to fat bloom due to the fact that the roller dried milk contains a higher content of free milk fat which is a fat bloom inhibitor. Similar behaviour was also observed in samples that contained more milk fat. Chocolates containing fat with lower melting point (hazelnut paste) were more prone to fat bloom at lower humidity. The addition of powdered whey in the manufacture of chocolate has led to the most intense formation of fat bloom due to the higher hygroscopicity of this ingredient. Similarly happened in samples containing spray-dried milk powder, which were more hygroscopic than the roller dried milk powder or condensed milk. Samples containing CBE's had more pronounced fat bloom than those with the same content of total fat but other types of fat. The reason for this occurrence is probably incompatibility of cocoa butter and added CBE's. A chocolate sample that did not contain any other fat except cocoa butter showed extremely good stability at all storage conditions and temperature fluctuations. Based on the results obtained by DSC, important conclusions were made, which explained the mechanism of fat migration. Based on analysis of the surface of bloomed chocolate it was found that over time (55 days), depending on the humidity of the air in the area where the samples were kept, changes of chocolate surface occurred. The slightest changes occurred when samples were stored at 50% humidity. At higher air humidity (65 and 75%) there was a significant change in surface composition. There was an increase in the fat content and decrease of the sugar content. From the obtained results it can be concluded that due to the increase in moisture content of the air, sugar decomposition occurs in the surface layers of chocolate, creating "pore" that allow fat to enter the surface (increased by temperature fluctuations). An increasing amount of fat bloom on the surface of chocolate was dependent on the composition of chocolate. Milk fat influenced the slowing of fat migration. Samples with a higher milk fat content, as well as samples containing rolled dried milk powder had a lower fat content on the surface of the product. Samples containing fat with lower melting point (hazelnut fat) and samples with CBE's had a high fat content on the surface of chocolate.

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CHOCOLATE, SNACKING AND SELECTED DIETARY HABITS IN PUPILS: BMI-FOR AGE APPROACH

Dragana Šoronja Simović[1], Biljana Pajin[1], Szabolcs Halasi[2], Aleksandar Fišteš[1], Ferenc Kiš[1], Miljana Djordjević[1], Andrej Ovca[3], Žana Šaranović[4], Zita Šereš[1*]

[1]University of Novi Sad, Faculty of Technology Novi Sad, Bul cara Lazara 1, 21000 Novi Sad, Novi Sad, Serbia
[2]University of Novi Sad, Hungarian language teacher training faculty, Štrosmajerova 11, 24000 Subotica, Serbia
[3]University of Ljubljana, Faculty of Health Sciences, Zdravstvena pot 5, 1000 Ljubljana, Slovenia
[4]Economics Institute, Kralja Milana 16, 11000 Belgrade, Serbia

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Summary

Besides pleasant taste, the potential health benefits of cocoa and chocolate products have been known for many years. Formerly accepted as a medicine, chocolate nowadays among consumers represents everyday delicacy primarily associated with caries, obesity, high blood pressure, and diabetes. However, recent studies indicate the potential role of chocolate in cardiovascular diseases prevention and benefits linked to lower body mass index (BMI). The presented study aims to examine adolescents' habits regarding chocolate, sweets, salty snacks and fast food consumption as well as corresponding products intake frequency and relate them with BMI. The study population encompassed 525 participants attending elementary and high schools in the province of Vojvodina, Serbia, from which 42.5% were boys and 57.5% girls. The risk for overweight and participants' nutritional status were determined through BMI assessment. The majority of participants had normal range nutritional status (70.5%) followed by overweight (19.8%) and obese (7.6%). Increased number of overweight and obese nutritional statuses was recorded among boys compared to girls. Results revealed that fruits as a snack are most frequently consumed between meals, followed by salty snacks and sweets, regardless of the participants' nutritional status. The highest share of sweets consumption between meals was noticed among participants with obese nutritional status (15%). Furthermore, more than 40% of participants with overweight and normal range nutritional status do not consume chocolate at all, while 2.5% of participants with obese nutritional status stated that they consume chocolate on a daily basis.

Keywords: adolescents, snack, consumption frequency, chocolate, body mass index

Introduction

Leading factor in the prevention of non-communicable diseases (type 2 diabetes and cardiovascular disease) and obesity increment worldwide is associated with a healthy diet maintaining. As recommended by the World Health Organization (WHO), a healthy diet should include high levels of fruits, vegetables, and whole grains consumption alongside with low intake of refined carbohydrates, saturated fats, and salt (WHO, 2018). Considering long life term implications, the corresponding healthy diet adherence should be promoted across all age groups. Despite the fact that parents commonly shape children's dietary habits, changes in the diet are possible during growing up as a consequence of diverse factors influence. In particular, the transition period between adolescence and young adulthood is recognized as a suitable period for the introduction and adoption of healthy dietary habits (Hilger et al., 2017). However, different factors such as school environment (polices regarding diverse food accessibility in schools, the proximity of food stores) socioeconomic status and sociological aspects (peer's body image perception) interfere with the healthy dietary habits embracing in the corresponding life period. All mentioned factors are related with the prolonged time of being outdoors upon beginning of school education (Krusinska et al., 2017; Stevenson et al., 2007). As a consequence of being outdoors, readily accessible food rich in fats and sugars such as fast food and sweets more often becomes a part of the daily food intake, most frequently in a form of snack or even as meal replacement. Another factor which contributes to the corresponding group's food preference is food taste which triggers the generation of related psychological effects such as pleasant fillings and positive mood. In this respect, chocolate is widely consumed although the corresponding mood benefits have been designated as ephemeral (Parker et al., 2006). Conversely, it has been demonstrated that chocolate consumption also induces negative fillings such as guilt, related to the cognition of its nutritional value, lack of control over eating behavior, as well as influence on slenderness and weight (Macht & Dettmer, 2006; Macht & Simons, 2000; Rodgers et al., 2011). Conventional chocolate, as a form of sweets, contains a high share of lipids and carbohydrates and represents a product rich in

*Corresponding author: zitas@tf.uns.ac.rs

calories while significant nutrients are absent (Cuenca-García et al., 2014). As a consequence, chocolate is considered as unhealthy food associated with caries, obesity, high blood pressure, and diabetes (Corti et al., 2009). However, an interesting fact is that Europe's historical documents associate chocolate with medicine indicating its use in treating numerous disorders such as angina and heart pain. The corresponding concept of potential health benefits provided by chocolate and cocoa beverages consumption was widely accepted until the early 1990s (Keen, 2001). In the past half of the century a noticeable increase in the manufacture of chocolate confectionery was observed (Kleinert-Zollinger and Beckett, 2017). Alongside with chocolate and related products production increase, the perception of chocolate among consumers changed. Previously considered as health beneficial, chocolate gained unfavorable attributes and has been associated with possible negative effects on consumers' health (Keen, 2001). The corresponding negative effects are primarily related to high fat and carbohydrate content in chocolate. Nevertheless, chocolate as well as cocoa also contain unique flavonoid types, primarily catechin and epicatechin, in a form of long polymers (pentamers, hexamers, etc.) (Natsume et al., 2000; Keen, 2001). The presence of such antioxidant compounds is associated with potential health benefits as a consequence of their antioxidant, antihypertensive, anti-atherogenic, antithrombotic and anti-inflammatory effects. Furthermore, the corresponding compounds exhibit influence on blood pressure (Golomb et al., 2012), insulin sensitivity, vascular endothelial function, and nitric oxide activation (Corti et al., 2009). Beneficial long-term effect on weight maintenance was also reported for the intake of catechins which are predominant in chocolate, fruit, vegetables, and tea (Hughes et al., 2008). Weight maintenance alongside with overweight and obesity development nowadays represent emerging problem worldwide. Factors such as high-density energy intake, sedentary lifestyle and lack of physical activity also contribute to the obesity development among young population (Meşe, et al., 2017). Body mass index (BMI) is applied for nutritional status assessment of an individual regarding obesity. According to the WHO, a 4 group classification considering BMI is presented: underweight (BMI<18.5 kg/m^2), normal range (BMI 19−24.99 kg/m^2), overweight (pre-obesity) (BMI≥ 25 kg/m^2) and obese (BMI≥30 kg/m^2) valid for adults of both genders (WHO, 2016). Children's nutritional status is estimated with special Growth Charts for school-aged children and adolescents as well as BMI cut-offs with respect to BMI for adults (WHO, 2007). As reported by Golomb et al. (2012), higher chocolate intake frequency has been related to lower BMI in adults.

Considering inconsistent opinions regarding chocolate consumption and possible health effects, the objective of the present study was to get an insight in young population habits towards chocolate, confectionary products and salty snacks consumption with respect to their BMI.

Materials and methods

Elementary and high schools in the province of Vojvodina, Serbia participated in the study. Cross sectional study involved 525 participants of Hungarian nationality from which 223 were boys (42.5%) and 302 were girls (57.5%) aged from 11 to 18 years. Participants were selected by random sampling among pupils attending 5th (10–12 years) and 7th (13–14 years) grade of elementary school and 3rd (17–18 years) grade of high school. The majority of the participants were from rural area (80%). The basic characteristics of the studied population are presented in Table 1. Participation in the survey was anonymous and on a voluntary basis. Before filling in the questionnaire, participants were informed regarding study's aims as well as that provided data would be handled as confidential.

Table 1. Basic characteristic of the studied population

Education level	Boys		Girls		Total	
	n	%	n	%	n	%
Elementary school 5th grade	66	48.9	69	51.1	135	25.7
Elementary school 7th grade	86	46.2	100	53.8	186	35.4
High school 3rd grade	71	34.8	133	65.2	204	38.9
Total	223	42.5	302	57.5	525	100

The first section of the questionnaire covered demographic data (gender and age) along with social and cultural data focused on degree course and place of residence. The second section was focused on questions

addressing snacking habits (which product is the most preferable snack) and frequency of chocolate consumption as well as accessible high-calorie foods consumption as snacks on daily basis during one week. Provided time for completing the questionnaire was 15 minutes during first class in the morning.

A database in Microsoft Excel 2010 was formed according to the answers provided by the surveyed participants. The survey results were analyzed by using pivot table function in the Microsoft Excel 2010 and expressed as the percentage of participants based on BMI. The assessment of the nutritional status was carried out according to the reference values of BMI-for-age (5 to 19 years) z-scores for boys and girls provided by the WHO (2007). Chi-square test was applied in order to determine the potential relationship between two categorical variables (BMI and frequency of selected products consumption) by testing the deviations of the obtained (empirical) frequencies from some of the expected (theoretical) frequencies. The null hypothesis assumes that there is no relationship between the variables. In cases where more than 20% of the cells had less than 5 expected frequencies, the p values were estimated with Monte Carlo simulation based on 10000 sampled tables (Barceló, 2019). The contingency coefficient (C) is used to estimate the strength of the relationship between the corresponding variables. If the contingency coefficient is less than 0.1, then there is no or negligible relationship between the variables, if C is between 0.1−0.3, then there is a weak relationship, whereas values between 0.3–0.5 or above 0.5 indicate a moderate or a strong relationship, respectively (Baguley, 2012). All statistical analyses were performed using IBM SPSS Statistics software (IBM Corporation, Armonk, USA) with the pre-defined 95% confidence interval ($p < 0.05$). Graphical representation of the analyzed results was performed using Statistica 13.3 (TIBCO Software Inc., USA).

Results and discussion

The majority of the surveyed population were elementary school pupils (35.4% and 25.7% from 7th and 5th grade, respectively) followed by high school pupils (38.9%). Considering gender, girls were more numerous regardless of the education level (Table 1). Nutritional status of the studied population according to the BMI-for-age addressing gender is reported in Table 2.

Regardless of gender, participants with normal range nutritional status were the most numerous (~70%, Table 2) indicating awareness and developed healthy eating habits among the studied young population. Participants with overweight nutritional status were the second largest group in the studied population with ~20% (Table 2).

Table 2. Distribution of participants by gender and (n) in the studied population according to the BMI-for-age

BMI classification*	Boys		Girls		Total	
	n	%	n	%	n	%
Underweight	2	0.9	9	3	11	2.1
Normal range	142	63.7	228	75.5	370	70.5
Overweight	53	23.8	51	16.9	104	19.8
Obese	26	11.6	14	4.6	40	7.6
Total	223	100	302	100	525	100

*according to the reference values of BMI-for-age (5 to 19 years) z-scores for boys and girls provided by the WHO (2007)

Underweight nutritional status in the studied population was recorded in a small share of 2.1% while obese nutritional status was represented with 7.6% share (Table 2). According to the results, more overweight and obese boys (23.8% and 11.6%, respectively) participated in the study compared to the girls (16.9% and 4.6%, respectively) (Table 2). Furthermore, the number of girls with normal range nutritional status was about 11% higher with respect to boys (Table 2). Considering underweight nutritional status, higher share was noticed among girls (3%) compared to boys (0.9%) (Table 2).

The presented results regarding sweets, including chocolate, fruit and salty snacks consumption habits and weekly consumption frequency of such products are expressed as a percentage based on the corresponding BMI classification. The obtained results from the statistical analysis were summarized in Table 3.

Results addressing the question about eating habits between meals are presented in Fig. 1. As noticeable on Fig. 1, a high share of participants declared that they consume fruits between meals (30−53%) especially in groups with overweight (53.9%), normal (43.8%) and obese (30%) nutritional statuses. The obtained results regarding fruit consumption are

in agreement with results from a study conducted among university students in Germany where 26.9% of students consumed fresh fruit several times on daily basis (Hilger et al., 2017). Following fruits, the second most consumed product are salty snacks with a share of 36.4−14.4% regardless of the nutritional status group. Salty snacks are the most consumed products between meals for underweight nutritional status group (36.4%) followed by normal (26.5%) and obese (25%) nutritional status groups. Sweets (chocolate and other confectionary products) consumption in the highest share was noticed for the obese nutritional status group (15%) and subsequently normal, underweight and overweight nutritional status groups (10.5%, 9.1%, and 5.8%, respectively). The consumption of cereal bars between meals is more frequent among obese (10%) and underweight (9.1%) nutritional status groups, while share of the corresponding product consumption in normal and overweight nutritional status groups is quite similar (5% and 6.6%, respectively).

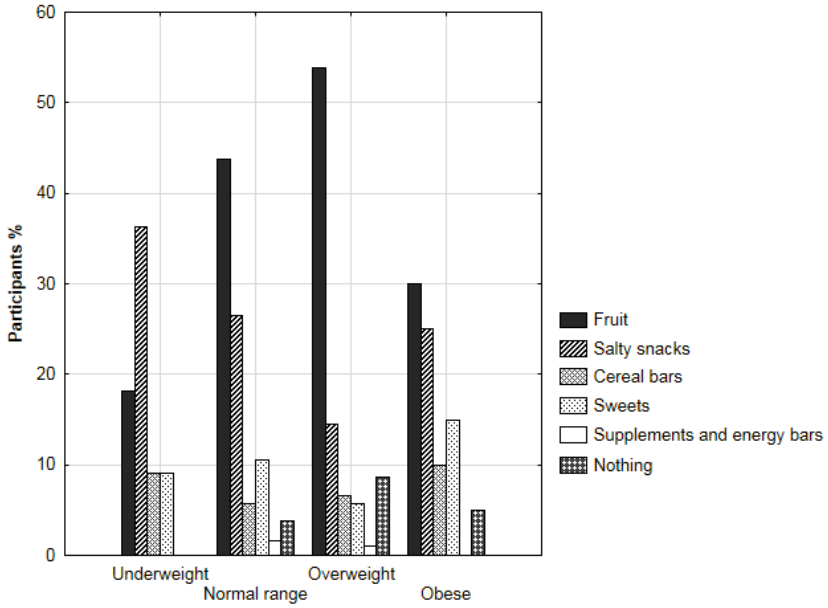

Fig. 1. Studied population eating habits between meals according to the BMI-for-age

Supplements and energy bars are not recognized as preferable snack between meals as noticeable on Fig. 1. Only participants from the normal range and overweight nutritional status groups reported consumption of the corresponding products in a small share of 0.9−1.6%. Snack skipping was most commonly among overweight participants (8.6%) followed by obese nutritional status participants probably due to the widespread opinion that a reduced number of daily meals will contribute to weight control.

The weekly consumption frequency of chocolate, fast food, sweets and salty snacks in the studied population considering classification according to BMI-for-age is illustrated in Fig. 2. As regards to chocolate consumption, 46.8% of participants with overweight nutritional status and 40.8% of participants with normal range nutritional status stated that they do not consume chocolate at all. However, chocolate consumption once a week was similar for all nutritional status groups ranging from 27% for normal range to 30% for obese participants (Fig. 2). Hilger et al. (2017) reported that 4.5% of university students ate chocolate several times on daily basis and also related the frequency of chocolate consumption with gender. When considering chocolate consumption two or three times per week, participants with underweight nutritional status were the most numerous (36.4 and 18.2%, respectively) followed by participants with normal range nutritional status (13.8 and 4%, respectively) (Fig. 2). The pattern of chocolate consumption from one to seven times within a week with a decreasing tendency, from day one to day seven, was observed only within participants with overweight nutritional status. Nevertheless, 2.5% of participants with obese nutritional status also stated that they consume chocolate every day in a week (Fig. 2). The obtained significance value is 0.044, indicating a statistically significant relationship. However, the value of contingency coefficient is under 0.3, indicating a weak relationship between BMI and chocolate consumption (Table 3).

Table 3. p-values and contingency coefficients from the statistical analysis of relationship between BMI and selected products consumption

Variables	Pearson Chi-Square value (χ2)	Degrees of freedom (df)	Asymp. Sig. (2-sided)[b]	Monte Carlo Sig. (2-sided)[c]			Contingency coefficient (C)
				Sig.	Lower bound	Upper bound	
Eating habits between meals	21.998[a]	15	0.108	0.115	0.107	0.123	0.210
Consumption frequency of:							
Chocolate	36.605[a]	21	0.019	0.044*	0.039	0.049	0.265
Sweets	32.930[a]	21	0.047	0.079	0.072	0.086	0.253
Fast food	22.134[a]	21	0.392	0.375	0.362	0.387	0.208
Salty snacks	16.248[a]	18	0.575	0.553	0.541	0.566	0.180

[a]More than 20% of the cells had less than 5 expected frequencies.
[b]Asymp. Sig. is the p-value based on chi-square approximation.
[c]Based on 10000 sampled tables.
*significant at $p < 0.05$.

With respect to sweets consumption, obtained results for all nutritional status groups were very similar to the chocolate consumption frequency (Fig. 2). The explanation of such similar results could be found in the fact that for most of the participants' term "sweets" relates with chocolate in the first place.

When addressing the fast food consumption, 55.8% of participants with overweight nutritional status do not eat fast food at all, likewise 53.5% of participants with normal and 50% of participants with obese nutritional status (Fig. 2). The corresponding results are consistent with the results from the study of Hilger et al. (2017) who found that 52.5% of university students consumed fast food less than once a week, while 1.9% of participants ate fast food frequently (4−7 times weekly). 21.9%, 25%, and 15% of participants with normal, overweight and obese nutritional status confirmed that they consume fast food once a week which was approximately two times higher compared to fast food consumption twice a week within these nutritional status groups (Fig. 2).

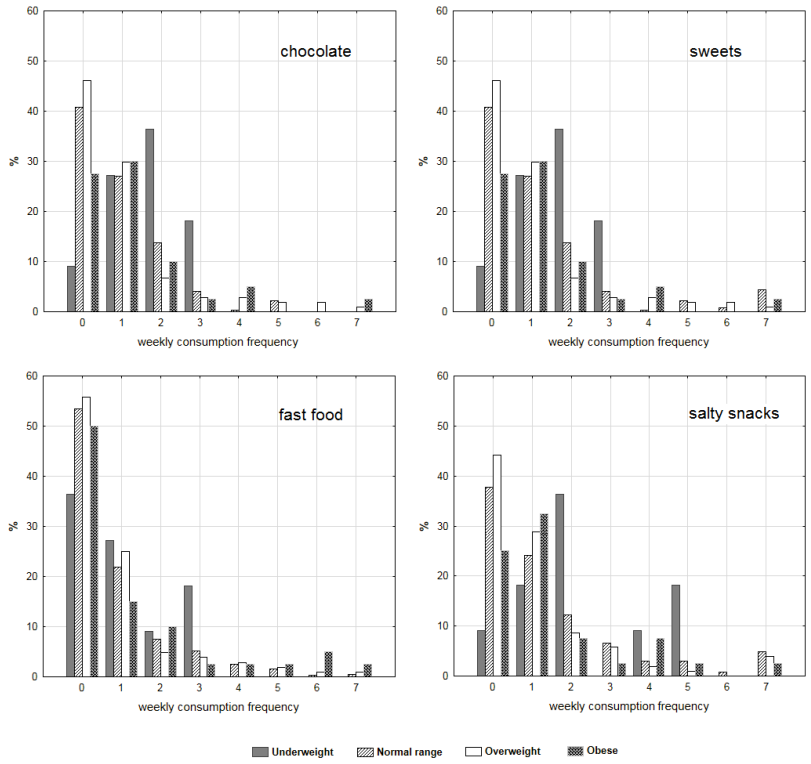

Fig. 2. Weekly consumption frequency of targeted products in the studied population according to the BMI-for-age

The observed fast food consumption frequency within participants with underweight nutritional status was one, two or three times per week with the highest percentage (27.3%) for fast food eaten once a week. Furthermore, the habit of fast food consumption, in higher extent, six or seven times per week was observed within participants with obese nutritional status (5 and 2.5%, respectively), while large differences in fast food consumption for four or five times per week were not observed within participants with normal, overweight and obese nutritional statuses (Fig. 2). Participants with obese nutritional status consume fast food more often that other participants in the sample, however, the chi-square test has showed no statistically significant dependence between BMI and fast food consumption ($\chi^2 = 22.13$, df = 21, p = 0.375).

As regard to salty snacks consumption, comparing participants with normal range and overweight nutritional status, large differences were not observed since 37% stated that they do not consume salty snacks at all (Fig. 2). Furthermore, similarly to fast food, a higher percentage of participants with obese nutritional status (25%) stated that they do not eat salty snacks when compared to participants with underweight nutritional status (9.1%). Nevertheless, the habit of salty snacks consumption once per week was the most notable among participants with obese nutritional status (32.5%) followed by participants with overweight, normal range and underweight nutritional status (Fig. 2). The snacks consumption frequency two, four or five times per week was higher among participants with underweight nutritional status (36.4, 9.1, and 18.2%, respectively) compared to other nutritional status groups (Fig. 2). However, the habit of salty snacks consumption every day in a week was observed within participants with normal range as well as overweight and obese nutritional status (2.5−4.8%) (Fig. 2). Nevertheless, statistical significance between BMI and salty snack consumption was not observed (Table 3).

Conclusions

The presented study relates adopted habits and consumption frequency in terms of chocolate, salty snacks, and fruit intake with the body mass index. As regards to habits related to food and products consumption between meals, surveyed participants' preference was directed towards fruits especially in overweight and normal range nutritional status groups. The highest share of sweets consumption including chocolate was observed within obese nutritional status group as well as tendency in snack exclusion from daily meals. Besides the fact that statistical analysis showed a statistically significant relationship between BMI and chocolate consumption the strength of the corresponding relationship was week according to the contingency coefficient. Although the most of participants fall within normal range nutritional status, increasing number of overweight and obese participants in elementary school imply that further education of young population should be conducted in order to adopt and practice balanced diet.

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THE INFLUENCE OF COCOA ON THE CARDIOVASCULAR SYSTEM

Inesa Jahić[1], Amra Bašić[1], Marizela Šabanović[2*]

[1]PZU „OS PHARM“, Prnjavor bb, 75 260 Kalesija, Bosnia and Herzegovina
[2]Faculty of Technology, University of Tuzla, Univerzitetska 8, 75 000 Tuzla, Bosnia and Herzegovina

review

Summary

Cocoa contains a range of chemicals that can interact with cells and tissue components, providing protection against the development and improvement of pathological conditions. The most important effects of cocoa and chocolate are related to cardiovascular disease. Due to the high content of flavonoids, it has numerous positive effects on cardiovascular diseases, including: antioxidant, anti-inflammatory, antithrombotic effects, and possibly increased HDL, lowering blood pressure and improving endothelial function. The beneficial effects of cocoa are most likely a consequence of reducing oxidative stress and increasing the bioavailability of nitric oxide. Although many positive effects of chocolate on the cardiovascular system have been proven, precautions in its use are mandatory.

Keywords: cocoa, flavanols, polyphenols, cardiovascular diseases, blood pressure

Introduction

Cocoa is the dried and fully fermented seed from the fruits of cocoa tree, lat. *Theobroma cacao* (McShea et al., 2009). The first data on the use of cocoa dates back to 1600 BC. In Honduras, archeologists have discovered specially designed bowls, thousands of years old, believed to be used by Aztecs to drink liquid cocoa (Henderson et al, 2007). It is known that consuming large amounts of fruits and vegetables, i.e. foods rich in natural polyphenols, are associated with a lower risk of coronary heart disease and stroke. Epidemiologicaly, a similar relationship was observed with cocoa.

Polyphenols

Cocoa contains about 380 known chemicals, of which 10 are psychoactive. Unprocessed cocoa beans are inseparable due to the high content of polyphenols that give them a bitter taste. In finished products such as chocolate, the content of cocoa can be reduced from 100% to 10% due to different production processes (Rusconi and Conti, 2010). Cocoa contains a large number of polyphenolic compounds, but is particularly rich in flavanoids, specifically flavanols. The flavanols are responsible for the bitterness of cocoa because they build complexes with saliva proteins (Manach et al., 2004). Cocoa contains the highest concentration of antioxidants in comparison with any other food. It has been shown to have the highest content of polyphenols (611 mg per serving) and flavonoids (564 mg per serving), higher than tea and wine together (Lee et al., 2003). Dark chocolate has a higher content of phenol compared to milk chocolate. Dark chocolate, in addition to the greater content of flavonoids, has better biological effects because milk in milk chocolate can inhibit intestinal absorption of flavanoids (Serafini et al., 2003). The main flavanols that cocoa contanis are catechin, epicatechin and procyanidins, which are mostly responsible for antioxidant activity in cocoa products (Ramiro-Puig and Castell, 2009). Thanks to their properties, flavonoids positively affect the cardiovascular system, including antioxidant and antithrombotic activity, immune regulatory properties and the endothelium (Corti et al., 2009).

Theobromine

In addition to polyphenols, cocoa also contains methylxanthine compounds, predominantly theobromine, and, in lower amounts, caffeine (Katz et al., 2011). The content of methylxantine depends on the genotype of cocoa tree. Theobromine stimulates the heart muscle, relaxes the bronchial smooth musculature in the lungs and plays a significant role in the transmission of intracellular signals (Shively and Tarka, 1984). In addition, theobromine has an antioxidant effect, and some antioxidant substances are believed to be effective in the treatment of depressive disorders (Scapagnini et al., 2014). Although new studies have shown that theobromine does not affect mood, there is a literature that states that theobromine and cocoa flavanols alone or in combination may have significant neurocognitive effects (Scholey and Owen, 2013).

*Corresponding author: marizela_sabanovic@yahoo.com

Minerals

Cocoa bean is a source of many essential minerals, including magnesium, copper, potassium and iron. These minerals can affect the health and function of blood vessels, influencing the nutritional value of cocoa. In its composition, there is mostly magnesium, which catalyzes a large number of biological reactions, including protein synthesis and energy production (Steinberg et al., 2003). The lack of magnesium in the body is associated with some diseases, among others with metabolic syndrome, insulin resistance and diabetes (Gums, 2004). Dark chocolate is an important source of copper that is essential in the body for processes such as iron transport, glucose metabolism, infant growth and brain development (Olivares et al., 1996).

Chocolate and cardiovascular diseases

According to the World Health Organization, by 2030, 23.6 million people will die of cardiovascular disease. The lifestyle, with a great emphasis on healthy nutrition, is one of the most important factors for the emergence, prevention and control of cardiovascular disorders. In general, chocolate is one of the most famous foods in the world, and there is more and more attention to its potential benefits in cardio metabolic health. It has been proven that cocoa products contain flavanol and as such have great potential in preventing cardiometabolic disorders (Corti et al., 2009).

Some studies indicate that chocolate consumption has a positive impact on human health, with various effects such as antioxidant, antihypertensive, anti-inflammatory, anti-atherogenic and antithrombotic effects, also affecting the function of vascular endothelium and activation of nitric oxide. These positive effects have been confirmed in meta-analysis supporting the positive role of cocoa and cocoa products on cardiovascular risk factors such as blood pressure, cholesterol levels, atherosclerosis, and insulin resistance. However, generally the effect of chocolate consumption on vascular functions is much more pronounced in healthy subjects compared to the impact on cardiovascular disease, where the evidence is much weaker. There are some limited studies that focus on the association of cholesterol with severe cardio metabolic outcomes (heart attack, diabetes, cardiovascular disease) (Buitrago-Lopez et al., 2011). Cardiovascular risk factors and diseases are associated with endothelial dysfunction or damage. According to meta-analysis published in 2008, consumption of food rich in polyphenols is mainly associated with an improvement in the endothelial function in the short-term and long-term form. This was established for tea consumption (Ras et al., 2011), red wine, grape juice and orange juice consumption (Morand et al., 2011). As cocoa is particularly rich in polyphenols, it doesn´t surprise that it induces NO (nitric oxide) dependent vasodilatation in rabbits (Karim et al., 2000) and improves endothelium in healthy people as well as in patients with cardiovascular risk factors (Sudano et al., 2012).

The influence of cocoa on blood pressure

The relationship between cocoa and blood pressure was observed in the island population in Central America, the Kuna Indians, who had a very low rate of hypertension and a constant healthy low pressure without age influence (Hollenberg, 2006; Kean, 1944). These effects are lost upon migration to urban Panama city and are likely linked to lower intake of natural cocoa drinks rich in flavanols. The tradition of this island population is consumption of three to four cups of cocoa-drinks a day, and it is assumed that they consume 1880 mg procyanidins per day (McCullough, 2006).

In a study in Iowa (USA) on the health of postmenopausal women (34,489 women) free of cardiovascular disease with a 16-year follow up, it was found that a regular intake of foods rich in flavonoids and reduced risk of death caused by cardiovascular disease was associated (Mink et al., 2007). A Zutphen study of the elderly, which included 470 elderly males without chronic disease, also suggests that the usual cocoa intake can reduce cardiovascular risk and is reversly linked to cardiovascular disease and comprehensive mortality (Bujisse et al., 2006).

In accordance with the relationship between consumption of cocoa and low incidence of hypertension, results of several short-term clinical studies show that the intake of certain chocolates can reduce blood pressure in humans. Grassi et al. (2005) studied 20 healthy young adults with a typical Italian diet with daily consumption of 100 g of dark chocolate or 90 g of white chocolate (assuming 500 mg and 0 mg of polyphenols) daily for 15 days, in random sequence with a 7-day washout period between treatments. They noticed that the addition of dark chocolate was associated with decreased systolic blood pressure, while white chocolate did not have these effects. The results have been extended to essential hypertensive patients and then to hypertensive patients with glucose intolerance (Grassi et al., 2005). Taubert et al. (2007) studied the effects of low-dose dark chocolate rich polyphenols in humans for 18 weeks. The intake of dark chocolate reduced systolic (-4.5 ± 1.35 mmHg) and

diastolic blood pressure (-2.5 ± 1.36 mmHg), as well as oxidative stress. A blood pressure decrease was followed by a constant increase in S-nitrosoglutation, indicating an improved NO formation. The above studies provide support for the inclusion of oxidative stress with the regulation of vascular tonus without the availability of NO formation (Taubert et al., 2007).

Commercially available dark chocolate (74% cocoa), but not white chocolate, improves the flow of vasodilatation by 80% in young healthy smokers. This effect was observed 2 hours after taking chocolate and lasted up to 8 hours. Since the plasma antioxidant status is significantly improved 2 hours after intake, it is likely that not only the induction of nitric oxide synthesis and increased NO levels but also reduction of oxidative stress and reduced degradation of nitric oxide to reactive species of oxidant, thus contributing to an improved function endothelium, especially in conditions of high loading with oxidative stress, as in smokers (Zhu et al., 2002). Indeed, antioxidants can prevent the transformation of NO into peroxynitrite and in turn protect against vasoconstriction and vascular damage (Wever, 1998). Oxygenate stress and reduced antioxidant defenses play a key role in the pathogenesis of atherosclerosis (Flammer, 2007).

Due to the importance of the cocoa effect in maintaining blood pressure, improving nitric oxide and endothelial function, its role in the antihypertensive effect can be explained. There is evidence that flavanols and foods rich in flavanol (Table 1), including cocoa, can inhibit the activity of angiotensin converting enzyme (ACE) *in vitro* (Actis-Goretta et al., 2006). ACE regulates the renin-angiotensin system, degrades angiotensin 1 to angiotensin 2, which stimulates the release of vasopressin or aldosterone and antidiuretic hormone, increasing sodium and water retention. It also inactivates vasodilators bradykinin and calidin. Whether ACE inhibits or mediates antihypertensive activity of cocoa flavanol in humans has not yet been fully clarified (Lavoie, 2003).

Table 1. Flavanol content in different foods

SOURCE	FLAVANOL CONTENT per mg/kg or mg/l
CHOCOLATE	460-610
BEANS	350-550
CHERRY	50-220
PEACH	50-14
BLUEBERRY	130
APPLE	20-120
GREEN TEA	100-800
BLACK TEA	60-500
RED WINE	80-300
CIDER	40

Precautions

Although many positive effects of chocolate on the cardiovascular system have been proven, precautions in its use are mandatory. Unfortunately, in the process of chocolate production, cocoa is heated and loses its nutritional and healing properties. So, because of the high caloric value (about 500 kcal/100g) and high sugar content, it is necessary to limit the intake of chocolate. It is believed that the intake of large amounts of sugar is associated with an increase in body weight, caries, diabetes, and is one of the risk factors for hypertension and dyslipidemia (Corti et al, 2009).

Conclusion

For centuries, people have been consuming and enjoying cocoa products and chocolate for good taste and for its beneficial effects on health. Although excessive consumption can have harmful effects, existing studies generally agree on a potentially useful association of chocolate consumption with a lower risk of cardio metabolic disorders. Over the past 10 years, many studies have confirmed that cocoa really improves vascular function, probably mediated by its high content of polyphenols. Reduction of oxidative stress and increased bioavailability of nitric oxide are most likely a consequence of beneficial effects of cocoa.

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COCOA BEAN SHELL – A PROMISING BY-PRODUCT RICH IN BIOACTIVE COMPOUNDS

Nika Pavlović[1*], Maja Miškulin[1], Krunoslav Aladić[2], Stela Jokić[3]

[1]Josip Juraj Strossmayer University of Osijek, Faculty of Medicine Osijek, Josipa Huttlera 4, 31000 Osijek, Croatia
[2]Croatian Veterinary Institute, Branch - Veterinary Institute Vinkovci, Josipa Kozarca 24, 32100 Vinkovci, Croatia
[3]Josip Juraj Strossmayer University of Osijek, Faculty of Food Technology Osijek, Franje Kuhača 20, 31000 Osijek, Croatia

review

Summary

Nowadays, when we are increasingly becoming a generation of large quantities of waste materials from various industries, there is an emerged need for certain solutions to suppress waste or make it more economical in some other way. Some by-products from the different food industries are rich in various bioactive compounds which could be utilized in other production processes. Finding the purpose and use of these compounds could be valuable for future generations. One of those by-products is cocoa bean shell (CBS), by-product in the processing of cocoa and its products, that has already proven to contain large amount of different bioactive compounds like theobromine, caffeine, specific phenolic compounds as well as dietary fibres and other valuable compounds which will be reviewed in this paper. CBS could be used in the production of functional products or even in food industry, cosmetic or pharmaceutics due to its high nutritional value what also makes it an economically acceptable raw material.

Keywords: by-product, cocoa bean shell, bioactive compounds, utilization

Introduction

During the production of different products in the food industry, large quantities of waste are also produced (Nguyen, 2014) that pollute the environment and cause major economic problems around the world. Consequently, people begin to think and explore in the direction of utilization and application of by-products from the food industry (Manzano et al., 2017; Okiyama et al., 2017; Pavlović et al., 2019; Viganó et al., 2015).

The fact of growing world population and disappearing raw materials, with a real threat of reduced food sources, does not surprise that the awareness is raising about the needs of storage and re-usage of materials that once were just a waste (Panak Balentić, 2018). Different types of waste are thrown away near the factories polluting the soil, nearby lakes and rivers, creating the major ecological problem while actually, those waste has the potential to be reused (Hamzat and Adeola, 2011).

By-products of the cocoa industry

Cocoa beans, as fermented and dried seeds of the *Theobroma cacao* plant are used as a main ingredient in the production of chocolate and its products. In Ghana, this raw material is called "golden pod" and has a "premium status" on the market because the cultivation of this plant is a tradition of private farms across the country, with the unique culture in the production of high quality dried cocoa beans. The main process in the cocoa industry includes a wide range of intermediate products that include cocoa liquor, cocoa butter, cocoa cake and raw cocoa powder. Cocoa pulp juice is a by-product used in the production of industrial alcohol and alcoholic beverages while the pod husks and shells are used as animal feed or fertilizer. After harvest, crucial chocolate precursors are formed in the fermentation process due to diverse biochemical transformation inside the cocoa beans. The fermentation step depends mostly on type of the seed, climate, season, diseases, turning, quantity of beans and pre-conditioning of the pulp (Afoakwa et al., 2013). It is known how during the process of fermentation significant amounts of certain phenolic compounds migrate from cocoa bean to the shell making this by-product rich in these compounds. So enriched cocoa bean shell (CBS) could be a potential source of bioactive compounds in different industries like food, cosmetic or pharmaceutics (Hernández-Hernández et al., 2018). Research on waste disposal in the way of finding new solutions for the use of different by-products would certainly reduce waste accumulation in a world that has become serious ecological and economical problem today.

CBS is a part of cocoa bean, which is separated from the cotyledon together with the nib before or after roasting (Arlorio et al., 2005). Since CBS is a waste in cocoa processing with certain bioactive compounds (Awarikabey et al., 2014), it is necessary

*Corresponding author: nika.felicita@gmail.com

to further explore and find suitable applications for its usage.

Bioactive compounds of CBS

While cocoa bean is only about 33% of the cocoa plant, 67% is considered as waste (Campos-Vega et al., 2018). It is already known how cocoa is included in the promotion of health due to the valuable bioactive compounds (Scapagnini et al., 2014) that can be extracted or even eliminated by various extraction methods. In this way, its further use in the food industry is enabled, leading again to the creation of new technologies (Awarikabey et al., 2014). Besides bioactive compounds (1.4%), CBS contains proteins (18.2%), carbohydrates (68.9%), fats (2.4%) and some minerals (9.1%) (Jurić and Nutrizio, 2014). CBS has been used also as fertilizer and as feed in animal nutrition (Afoakwa et al., 2013).

Methylxantines

From valuable bioactive compounds, CBS primarily contains methylxanthines theobromine and caffeine while theophylline concentrations are negligible (Okiyama et al., 2017; Pavlović et al., 2019). All three methylxanthines have similar psychoactive effects but different effects on the human organism (Okiyama et al., 2017). In cocoa fruit, theobromine is synthesized both in pericarp and in cotyledons of cocoa seeds. It accumulates firstly in young leaves, and during the ripening phase, its concentration decrease in the pericarp while in the cotyledons its concentration increases probably due to its migration from the pericarp. Theobromine is synthesized from AMP (Adenosine MonoPhosphate) and metabolised by demethylation via xanthine as well as in the seed and leaves of the cocoa plant. The rippening phase, growing as well as soil composition and climatic conditions significantly affect the methylxanthine content in cocoa plant (Smit, 2011). In the fermentation stage, the proportion of theobromine in the bean is decreased by cca 25% due to the migration of the methylxanthines from the beans to the CBS (Okiyama et al., 2017; Smit, 2011; Timbie et al., 1978). Theobromine, as naturally present in the plant, serves as its defense mechanism (Hartati, 2010). In addition to being toxic in larger quantities, some authors pointed how theobromine possess many pharmacological properties such as anticancer, diuretic and stimulative. The use of CBS in animal nutrition was found questionable primarily because of larger theobromine amounts which can have adverse effects such as damage to the reproductive and developmental system of animals (Adamafio, 2013). The higher quantities of caffeine limit the use of CBS in animal feed depending on the species and year of the animal (Hamzat and Adeola, 2011). The European Union restricted the theobromine quantity in feed to 300 mg/kg what means 13.7% w/w of CBS (Oduro-Mensah et al., 2018). Conclusions lead to the development of some of its removal methods so that such by-products can get even wider, better purpose and usage. Some methods of detheobromination are hot water extraction, alkaline treatment as well as treatment by microorganisms (Adamafio, 2013).

Phenolic compounds

Phenolic compounds are secondary metabolites found in pigment cells of cotyledons which perform different functions in the plant (Hernández – Hernández et al., 2017). Polyphenols are involved in plants growth and reproduction as well as in its protection from various pests. The polyphenol profile of every plant differs within the variety of each species (Bravo, 2009). Strong antioxidant activity of certain phenolic compounds reduces oxidative stress (Santos et al., 2017) and improves cardiovascular function (Aprotosoaie et al., 2016). Certain phenolic compounds migrate from cocoa beans into CBS during various stages in the production process such as fermentation, roasting and alkalization, thus reducing the polyphenol content in cocoa beans and increasing their content in CBS. That makes this by-product rich in these compounds. Strong antioxidant activity of CBS could also be associated with procyanidines (Ismail and Lye Yee, 2006; Okiyama et al., 2017). The most common phenolic compounds in cocoa are epicatechin (even about 35% of total phenolic content), catechin and procyanidine. Other cocoa bean polyphenols are galocatechin, epigallocatehin, epicatechin-3-*O*-gallate, quercetin, quercetin 3-glucoside, quercetin-3-arabinoside, clovamide and deoxycyclovamide (Hernández-Hernández et al., 2017). During the fermentation process, which may last from 5 to 10 days, the combination of endogenous and microbial enzymatic activities, with a temperature increase of about 50 °C, and diffusion of the metabolites inside and outside the cotyledon, results in polymerization of polyphenols as well as their ability to react with other compounds and thus the creation of certain complexes. For this reason, fermentation is a very important and most responsible step in reducing the content of flavan-3-ols in cocoa beans, specifically (-) – epicatechin. The amount of reduced polyphenols definitely depends on the degree of fermentation. The process of drying, after fermentation step, due to the decrease in water content also contributes to the

reduction of polyphenols in the beans, depending mostly on climatic conditions (Di Mattia et al., 2017). Hernández – Hernández et al. (2017) mentions different authors who refer to positive properties of phenolic compounds like antitumour, anti-inflammatory, antineurodegenerative, antibacterial and anticancer properties.

Other compounds of CBS

Dietary fibres

When consumed, dietary fibres are already known to be beneficial for the human health as well as for body function. CBS contains about 60% dietary fibres according to dry weight. 80% of those total fibres are insoluble dietary fibres while 17% are soluble ones and are mainly pectic substances (11.78% of the total dietary fibres). Their amount mostly depends on external factors such as climate and soil quality. High day and night temperatures, with a sufficient moisture, help to accumulate these compounds in CBS. Low amount of water soluble pectins in CBS was reported in non-fertile and non-volcanic soils. Also, the difference in the content of the water soluble pectins and the polyphenol content has been reported previously to be in relation to the heterogeneity of the seed coming from neighbor plantations, making the cocoa origin as a significant quality parameter (Bruna et al., 2009).
CBS were also characterized to have the high pectin ability in gel formation and is widely used in the production of jams, jellies, frozen foods and foods with reduced caloric value (Arlorio et al., 2001). The proportion of fibre in CBS depends on whether they are roasted or not. It is also concluded that in roasted seeds and shells, formation of Maillard compounds increases the fibre content (Redgwell et al., 2003). A positive constituent is that CBS contains fibres, but some monogastrics are unable to digest them, and because of that, some ways of improving their digestibility have been demonstrated by fermentation with *Plerotus* spp., while theobromine degradation was achieved by fermentation with *Aspergillus niger*. Certainly, prior using those methods, it is necessary to conduct an analysis on mycotoxicity (Bentil, 2012).

Lipid profile

Fatty acids are involved in transport and storage of energy in the body as they are essential compounds of all membranes and are also gene regulators (Rustan and Devon, 2005). Essential fatty acids are those that are needed for the biological functioning of the body and which the body cannot synthesize itself. Human body needs two such essential fatty acids: linoleic (C18: 2n-6) and α-linolenic (C18: 3n-3) acid (Glick and Fischer, 2013).
In addition to interesting bioactive profile, the CBS has also an interesting fatty acid composition, similar to that of cocoa butter. The main fatty acids of CBS are palmitic and oleic, while linoleic acid is even twice as much in CBS than in cocoa butter (Okiyama et al., 2017; El-Saied et al., 1981). Following the comparison of the chemical composition of CBS fat and cocoa butter fat, it can be noticed the similarity and higher nutritional value of CBS fat (El-Saied et al., 1981). Also, it was found how during the fermentation process some microorganisms affect the changes in lipid profile because the content of saturated fatty acids decreases in CBS extracts while the content of some unsaturated fatty acids (oleic, linoleic and gamma-linolenic fatty acids) which are important for our health, increases (Lessa et al., 2018).

Vitamin D

The CBS from fresh unfermented cocoa beans dried in the sun, may contain small amounts of vitamin D while the fermented ones can contain a much larger amounts. This is probably due to a specific precursor that is converted to vitamin D during exposure to sunlight. Knapp and Coward (1935) decided to make an experiment and determine how the fermentation step, as well as type of drying, affect the vitamin D content in the CBS. The fermented beans are usually put on tables and dried by turning in the sun during 6 days at least. Some beans can be also artificially dried. Yeasts, that contain ergosterol, develop during the fermentation process of the cocoa beans, and during the drying process in the tropical sun, ergosterol is converted into vitamin D. It was also noticed how this was only the case when CBS had been exposed to sunlight. During the process of artificially drying, vitamin D was absent in the CBS (Knapp and Coward, 1935). By adding a CBS into animal food, it is proven the increase of vitamin D content also in animal milk and butter (Knapp and Churchman, 1937).

Biogenic amines

Potentially toxic compounds such as biogenic amines like tyramine and the non-nutritive compounds such as phytic acid and trypsin inhibitors, do not reduce the possibility of further usage of CBS as a source of pectin. The pectin gel obtained from the CBS can be purified with ethanol and 2-propanol. Some studies pointed how biogenic amines can cause heart palpitations, hyper and hypotension, dizziness, headache and facial redness. The amount of these

compounds in CBS is relatively small and has no toxic effect (Arlorio et al., 2001).

HMF

In the food industry, because of different operation conditions and due to the chemical composition of raw materials, the synthesis of hydroxymethylfurfural (HMF), also 5-(hydroxymethyl)furfural, is a common occurrence in food. The reasons are mainly accumulation of simple sugars as well as some polysaccharides, proteins and amino acids, low pH and high temperatures (Kowalski et al., 2013). Jokić et al. (2018) investigated bioactive compounds in CBS extracts obtained after subcritical water extraction (SWE) and demonstrated how at higher temperatures HMF was detected in extracts. This is one of the reasons why it is always important to optimize the extraction procedure to obtained desired compounds in the final extract.

Thermal treatment such as roasting (especially foods rich in carbohydrates) also greatly contributes to the formation of HMF. Also, many polysaccharides are susceptible to hydrolysis in the acidic medium and can be transformed into simple sugars, which are starting material for the formation of HMF. HMF is a heterocyclic aldehyde with six carbon atoms, aldehyde and alcohol functional group. The basic substrates of HMF formation are monosaccharides fructose and glucose, and disaccharide sucrose which is easily transformed to both of them. Fructose dehydration is more effective and faster than glucose dehydration and occurs in three stages. The last step of dehydration forms the final structure of HMF. Due to possible adverse effects of HMF on human health, it is necessary to take into account the reduction of this compound in food by alteration in its possible production (Kowalski et al., 2013).

Some potential usage of CBS

CBS as a by-product in the cocoa industry has been still discarded regardless of its high nutritional value. Apart from being a source of fibre, it is a potential source of antioxidants, due to its high phenolic content (explained in text above), which makes it a good source as a raw material for further use. This high content of dietary fibre makes it interesting as an ingredient in food preparation (Martín-Cabrejas et al., 1994; Martínez et al., 2012; Okiyama et al., 2017). Relatively high values of dietary fibres together with phenolic compounds imply that this by-product is interesting to the food industry, in the manufacture of confectionery products, bakery or in the preparation of low calorie dietetic and fibre-rich products (Nsor-Atindana et al., 2012). The another innovative approach would be adding isolated polyphenolic compounds and water-soluble pectins from CBS further in chocolate, making it richer in antioxidant composition (Bruna et al., 2009).

Some studies have proven that the phenolic compounds of CBS that are added to some types of meat can reduce lipid oxidation with their antioxidant properties. Therefore, they can be recommended for oils that are intended for frying as a replacement for synthetic antioxidants (Okiyama et al., 2017). This can improve their quality and their stability without affecting the color of the oil. When added to oils, the formation of free fatty acids and peroxide levels is slowed down while antioxidant activity increased (Manzano et al., 2017).

Looking the situation today, when economic gains and profits are most important to the producers, the usage of antibiotics is increasing in livestock treatment and have preventive maintenance on their health. Resistance to pathogenic bacteria is becoming more frequent, and excessive use of antibiotics leads to their residues in the environment, which becomes serious environmental problem. Evidence is also made by various confirmatory studies where traces of antibiotics are found in some local and waste waters. It is the reason because some authors mention the use of by-products of tropical plants such as CBS as possible substitutions for antibiotics in animal feed due to substantial antimicrobial potential, improving the health of domestic animals on farms. Bioactive compounds of those by-products can influence pathogenic bacteria, avoiding the damage of the cell itself. Some bioactive compounds may act synergistic and increase antimicrobial activity against pathogenic bacteria. Certainly, these assumptions and research require extra caution because some bioactive compounds may be toxic (Guil-Guerrero et al., 2016).

As an adsorbent, CBS is mostly effective in removing heavy metals such as lead, chrome, cadmium, etc. There is also an increase in pH value and release of calcium, magnesium, potassium and sodium from the CBS as well (Meunier et al., 2003). Among all other natural materials, CBS with its low porosity is widely used in metal decontamination of soil (Meunier et al., 2004). The CBS based activated carbons show the potential for usage as an adsorbent in various water or wastewater treatments, as some studies confirm (Ahmad et al., 2012). It is also recommended usage of CBS in the biofiltration of wastewaters in the food production as well as biogas production. Some studies mention also the usage of phenolic extracts in prevention of dental cavities (Okiyama et al., 2017). Other authors concluded that CBS is a promising precursor for activated carbon for usage in adsorption of whey proteins (Pereira et al., 2014).

The CBS can be chemically modified based on diazonium chemistry. Because of the fast, simple preparation and the wide range of reactive functional groups of aryl diazonium salts, they are ideal for modifying surface properties of various materials and so as the CBS. These compounds grafted on the CBS surface changing its properties and making it a suitable adsorbent to remove pollutants such as heavy metals, gases or industrial dyes (Fioresi et al., 2017).
The CBS can be used as a fertilizer because it forms a humus substrate, but does not decompose easily, which can lead to its accumulation during the season (Nair, 2010).
The possibility of water bounding and polysaccharide composition makes the CBS suitable as a raw material for preparing fibre-based formulations. The various composition of CBS can be changed by various extraction methods and techniques, given the extracts interesting not only because of its aroma but also for its protein and saccharide content (Dongowski et al., 1991). An example is the addition of this compound (soluble cocoa dietary fibre) into the muffins as a substitute for fat, thus altering the dough properties. Except pleasant colors, it increases the density of dough, making its texture softer and smoother and reducing its later hardening. Disadvantages were difficulty to chewing and swallowing, stickiness and bitter taste (Okiyama et al., 2017).
Important facts are also expensive sources of protein concentrates that make the basis of animal feed. CBS is recommended for the ruminant diet but with the addition of certain compounds to improve the taste or dilute the toxic effects of CBS substances that could affect the used diet (Aregheore, 2002). In addition to being used as fuel for boilers, livestock feed and preparation of fertilizers, this by-product could be used as a food, cosmetic or pharmaceutical additive or food supplement with high nutritional value and due to that become an economically acceptable raw material (Okiyama et al., 2017).

Conclusion

The CBS, which is a food industry by-products produced in large quantities in the world, contains valuable bioactive compounds. Given its nutritional value, this by-product could become a raw material in newer productions, thereby reducing the amount of waste in the world. As a rich source of some bioactive compounds including theobromine, caffeine, flavonoids and dietary fibres which can be isolated by different extraction techniques, CBS can also be used in preparation of large spectrum of functional products.

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Conflicts of Interest

The authors declare no conflict of interest.

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FUNCTION OF FOOD ADDITIVES IN CHOCOLATE PRODUCTION

Veronika Barišić[1*], Drago Šubarić[1], Midhat Jašić[2], Jurislav Babić[1]

[1]Josip Juraj Strossmayer University of Osijek, Faculty of Food Technology Osijek, Franje Kuhača 20, 31000 Osijek, Croatia
[2]Faculty of Technology Tuzla, Univerzitetska 8, 75000 Tuzla, Bosnia and Herzegovina

professional paper

Summary

Chocolate is a complex product that has a specific texture. This complexity is due to the interactions between the ingredients used in production: cocoa butter, cocoa mass and sugar. Sugar gives bulk to chocolate and any change in the recipe changes the textural properties of the chocolate. Recently, there has been considerable production of low-sugar chocolates using other bulking agents and sweeteners. Some of the most common are isomalt, maltitol, lactitol, polydextrose etc. Emulsifiers that have been used in chocolate production almost from the beginning are also responsible for its texture and rheological properties. They reduce the interaction between the solid particles and increase the lipophilicity of the sugar particles. Lecithin and polyglycerol polyricinoleate are most commonly used, but some other emulsifiers have also been reported in production.

Keywords: food additives, chocolate, emulsifiers, sweeteners

Introduction

Food additives are by Regulation 1333/2008 "any substance not normally consumed as a food in itself and not normally used a characteristic ingredient of a food, whether or not it has nutritive value, the intentional addition of which to a food for a technological purpose in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food results, or may reasonably be expected to result, in it or its by-products becoming directly or indirectly a component of such food".

Two main reasons for using food additives are: making the product safer and making it look and taste better (Saltmarsh, 2013). Since chocolate is a very stable product that has a low content of water (Beckett et al., 2017), food additives that extend the shelf life of food are not necessary in this case.

Emulsifiers are kind of additives that are used in chocolate production for a very long time. They are used for modifying flow properties of chocolate mass for easier manipulation, moulding and acceptable sensory characteristics (Norn, 2015).

Other group of food additives commonly used in chocolate are bulk sweeteners. They are increasingly used in the last few years because of increased popularity of sugar-free products. Chocolate has up to 50% of sucrose in its formulation, so it is an ideal product for replacement of part or whole amount of sugar. Sucrose is responsible for chocolate texture and flavour and with its removal some other bulk sweeteners are used to maintain proper properties of chocolate (Beckett et al., 2017).

Bulk sweeteners

Chocolate production was for a long time unbelievable without sucrose, although lately, sugar-free chocolates have been increasingly studied and produced. In such chocolates different bulk sweeteners are used. They give a sucrose-like texture and taste, most commonly used are polysaccharides and sugar alcohols (polyols).

Sorbitol E420

Sorbitol is monosaccharide alcohol (Fig. 1) which has approximately 50% sweetness of sucrose. It is hygroscopic and gives a cooling effect during consumption and dissolving in the mouth (Beckett et al., 2017). Used in the production of sugar-free chocolate, it gives a suitable texture and mouthfeel (bulking agent) to sugar-free products (Smith and Hong-Shum, 2011). Also, it can be used as a sequestering and emulsifying agent in baked goods, mayonnaise, confectionery products, creams, etc. It has reduced calorie content (2.4 kcal/g) and can be used in products for diabetics. As for other polyols, its permitted addition is at *quantum satis* but content must be labelled if product contains more than 10% (laxative effect) (Saltmarsh, 2013).

When part of sucrose was replaced with sorbitol in milk chocolates, higher quality attributes occurred in this kind of chocolate. Also, using only sorbitol in milk chocolate results in lower viscosity, which needs to be corrected by using starch or guar gum (Rathnavati and Chavan, 2016).

*Corresponding author: veronika.barisic@ptfos.hr

Mannitol E 421

Mannitol is naturally present monosaccharide alcohol (Fig. 1) which adds mouthfeel and texture of sucrose to sugar-free chocolates. It has the greatest laxative effect in comparison with other sugar alcohols, so adults can take only 10 g of mannitol daily (Beckett et al., 2017). It is used in chewing gums and hard-boiled candies. Since it has low solubility in water it extends the shelf life of products and does not participate in Maillard reactions (Saltmarsh, 2013; Smith and Hong-Shum, 2011).

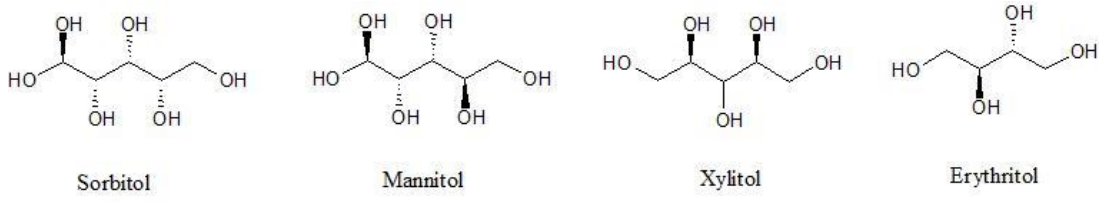

Fig. 1. Monosaccharide alcohols used in chocolate production

Isomalt E953

Isomalt is a polyol derived from a mixture of two disaccharide alcohols (Fig. 2) which has 40% sweetness of sucrose. It is non-hygroscopic, has prebiotic properties and cannot be used in chocolates that are conched at temperatures higher than 45 °C (Beckett et al., 2017). It is used as a bulking agent which gives sweetness and texture in the production of free sugar chocolates. It has an energy value of 2.4 kcal/g and is allowed at a *quantum satis* but if it contains more than 10% it has to be labelled that may have a laxative effect (Smith and Hong-Shum, 2011; Saltmarsh, 2013).

Belščak-Cvitanović et al. (2014) concluded in their study that chocolates with isomalt have larger particle size which directly affects textural properties of chocolate. Also, viscosity of chocolate with isomalt is higher when comparing to maltitol, xylitol and sucrose (Olinger, 1994). Isomalt's increase of chocolate viscosity can be associated with higher solid volume fraction in chocolate. This is because of isomalt's density which is slightly lower than of other sugar alcohols (Aidoo et al., 2013).

Maltitol E965

Maltitol is disaccharide alcohol (Fig. 2), bulk and anhydrous sweetener which can be used at elevated temperatures. It is present in the liquid and crystalline forms. Maltitol syrup has a high content of water so it cannnot be used in chocolates. The crystalline form has 90% sweetness of sucrose. It can be used as a sugar replacer in some other products, such as chewing gum, hard-boiled candies, jams, ice-creams, etc (Beckett et al., 2017; Smith and Hong-Shum, 2011; Saltmarsh, 2013).

Maltitol based chocolates produced and studied by Konar et al. (2018) showed lower sugar melting enthalpies than sucrose containing chocolates. Reason for this could be higher content of moisture in maltitol. This study also examined maltitol's influence on viscosity of chocolate and concluded that maltitol increases viscosity mostly because of different shape of particles and higher moisture content.

Son et al. (2017) examined effect of maltitol on bloom phenomenon in chocolate. This additive showed anti-blooming properties when compared with chocolate made only with sucrose.

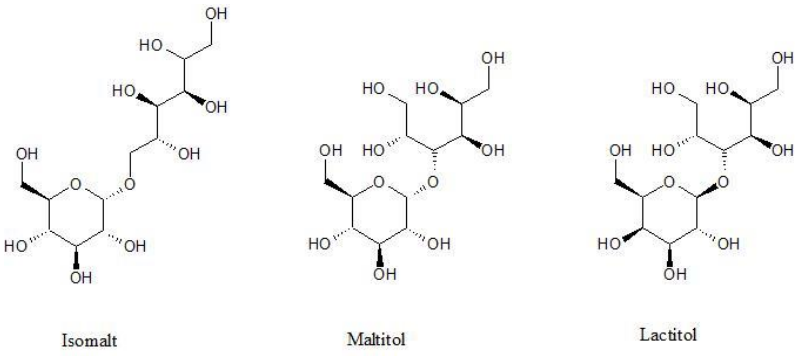

Fig. 2. Disaccharide alcohols used in chocolate production

Lactitol E 966

Lactitol is disaccharide alcohol (Fig. 2) that is hygroscopic and has a sweetness of 40% of sucrose. It is used in the production of sugar-free chocolates and has prebiotic properties. Because of its texture and bulking properties, it can replace sucrose in the chocolate formulation. Its energy value is 2 kcal/g and can be used in diabetic products (Beckett et al., 2017; Smith and Hong-Shum, 2011).
Belščak-Cvitanović et al. (2015) in their study examined the influence of different sweeteners on chocolate properties. The use of lactitol in chocolate formulation resulted in an increase of the hardness but decrease of elasticity of chocolate when compared to conventional chocolate. This is probably caused by the larger particles also caused by lactitol.
Although every change in chocolate affects its properties, using combinations of different sweeteners can produce a product that is not significantly different from the traditional one. Lactitol, maltitol and isomaltulose combinations are most commonly used. This produces a less calorific product with very satisfactory sensory properties (Martínez-Monteagudo et al., 2018; Mentink et al., 1994).

Xylitol E967

Xylitol is natural monosaccharide alcohol (Fig. 1) which can be found in fruits, vegetables and mushrooms. It is obtained by enzymatic conversion of glucose and has a cooling effect during digestion in the mouth (Beckett et al., 2017). It acts as a bulking agent and has the same sweetness as sucrose without any off-flavours. In addition, it can serve as a stabiliser, thickener, emulsifier, humectant, etc. (Smith and Hong-Shum, 2011). As other polyols, it has a laxative effect, cooling effect in the mouth, 2.4 kcal/g energy content and resists fermentation by oral bacteria (Saltmarsh, 2013).
Xylitol has high hydrophilicity because of its structure which has more active and free –OH groups which can react with water. Water in chocolate has big effect on chocolate viscosity. This was proven by Homayouni Rad et al. (2019) where formulation with only xylitol had the highest viscosity values in comparison with formulation with other polyols and theirs combination.

Erythritol E968

Erythritol is monosaccharide alcohol (Fig. 1) which occurs in different fruits and vegetables. This bulk sweetener has a very low caloric value (0.2 kcal/g), can be manipulated under elevated temperatures. Except as a sugar replacer, it can be used as humectant, sequestrant and flavour enhancer. It is possible to have a laxative effect if it's consumed excessively (Beckett et al., 2017; Saltmarsh, 2013).

Polydextrose E 1200

Polydextrose is a bulking sweetener used in sugar-free chocolates. Except for sugar replacement, it can be used also as a fat replacer. It has a caloric value of 1 kcal/g and because of the way of digestion, it is considered dietary fibre (Saltmarsh, 2013). Polydextrose is mostly composed of randomly bonded glucose units and small amounts of sorbitol. During consumption, polydextrose leads to a heating effect in the mouth (Murphy, 2001; Beckett et al., 2017).
In study conducted by Aidoo et al. (2014) polydextrose showed effect on increase of Casson yield stress, but Casson plastic viscosity remained similar to control. Also, particles of chocolate with polydextrose were smaller. This could be the reason for increased Casson yield stress.

Emulsifiers

Emulsifiers have been used in chocolate production for a very long time, mainly because they reduce the tension between the dispersed and continuous phase. They also affect chocolate's sensitivity to moisture and temperature changes, tempering behaviour, and fat migration (Garti and Aserin, 2012).

Lecithin E 322

Lecithin is the most widely used emulsifier in chocolate production, mainly because of its low cost and unique characteristics. Theodore Nicolas Gobley discovered it between 1845 and 1847 by extraction from egg yolk. It got named by the Greek word for egg yolk. Lecithin is of natural origin and can be found in soybeans, egg yolks and whole grains. The human body also contains lecithin, most of it in the vital organs (Garti and Aserin, 2012).
Commercial lecithin used in chocolate production consists of approximately 50-60 wt% phospholipids. The two most common forms used are liquid and powdered (de-oiled) lecithin. In addition to being used as an emulsifier for reducing viscosity in chocolate, it can also serve as an antioxidant (margarine, edible oils and fats), anti-spattering agent (margarine), release agent (baked goods), etc. (Saltmarsh, 2013; Smith and Hong-Shum, 2011).

The phospholipids present in lecithin are active components that contain a two-part molecular structure. One end of the molecule is lipophilic (fatty acid end) and the other is hydrophilic (phosphorus-containing end). Due to their nature, they are located on the border between oil and water (Saltmarsh, 2013). In chocolate, lecithin covers sucrose particles to develop acceptable flow of cocoa butter. This gives a uniform particle distribution in cocoa butter and prevents agglomeration of sugar particles (Schantz and Rhom, 2005). Powdered sucrose used in chocolate production has a larger surface area than granulated sucrose. Absorption of lecithin on the surface of sucrose increases the lipophilicity of sucrose by reducing the acceptor character of the sucrose surface by chains of phospholipids in lecithin. In this way, the interaction between sucrose particles is reduced (Garti and Aserin, 2012).
Ashkezary et al. (2018) and Tisoncik (2010) examined the influence of emulsifiers on chocolate hardness. They concluded that increasing the lecithin content results in softer chocolate texture. Also, Rousset et al. (2002) concluded that the addition of lecithin in the 0.1 to 0.3% content significantly changes the Casson yield value and plastic viscosity, but the addition above 0.5% increases the yield value because the higher lecithin content causes the formation of multiple layers on the surface of sucrose particles (due to reverse micelle formation).
Modified lecithins are also used in chocolate production, and Miyasaki et al. (2015) studied five different ones (acetylated, enzymatically hydrolyzed, hydroxylated, standard and defatted lecithin) and determined their impact on chocolate. Enzymatically hydrolyzed and hydroxylated lecithins had the most pronounced effect on the crystallization of cocoa butter. It was concluded that they had increased the onset temperature of crystallization so it may be said that they act as accelerators of the nucleation of cocoa butter.

Ammonium phosphatides E 442

Ammonium phosphatide is a substitute for synthetic lecithin used as an emulsifier in chocolate production to reduce viscosity. It has no taste and odour that are characteristic of lecithin obtained from soy. It has a similar effect to natural lecithin, settles on the surface of the sugar and reduces the agglomeration of sucrose particles. Unlike natural lecithin, it has a restriction on its use in chocolate. Under Regulation 1129/2011, it is allowed to add 10000 mg/kg of ammonium phosphatide to chocolate (Saltmarsh, 2013).
Unlike lecithin, it gives greater stability to the chocolate against oxidation, and it is possible to add it up to 1%, without affecting the taste and aroma of the product. Also, high doses will not adversely affect the viscosity of the chocolate as is the case with natural lecithin. It can also be added into ice cream coatings and various other confectionery products (Smith and Hong-Shum, 2011; Wood et al., 2004).

Citric acid esters of mono- and diglycerides of fatty acids E 472c

Citrems are used as emulsifiers in sausages and margarine and as a liquid controller in chocolate. They can be present as liquids, pastes and solids (Saltmarsh, 2013). Citrems are sensitive to hydrolysis, they contain water, have a low pH and cannot be stored at elevated temperatures. They are known to be good for reducing plastic viscosity and yield value of chocolates (Norn, 2015).
Beckett et al. (2017) reported that citric acid esters have an effect similar to a combination of PGPR and lecithin on chocolate viscosity. Also, Ashkezary et al. (2018) examined the influence of different emulsifiers on chocolate hardness. They concluded that citrem added in the 1% share had the greatest effect on softening of chocolates when compared with lecithin and PGPR.

Polyglycerol polyricinoleate E 476

Polyglycerol polyricinoleate (PGPR) is complex mixture of polycondensed esters of vegetable polyglycerol polyricinoleic acid. It is edible in oils and it is used as a viscous light brown liquid. PGPR is most commonly used in combination with lecithin because it does not significantly affect plastic viscosity but may therefore reduce yield value by 50%. It is usually added to chocolate in the proportion of 0.1 to 0.5%. Because it significantly influences the yield value, it facilitates the manipulation of chocolate during production and promotes the emergence of bubbles from finished chocolate (Smith and Hong-Sham, 2011; Garti and Aserin, 2012).
PGPR works by increasing the lipophilicity of the sugar surface by reducing the acidic character of the surface of the sugar particles. This reduces the interactions between the particles and increases the fluidity of the fat base. Middendorf et al. (2015) examined the effect of PGPR in cocoa butter suspension and concluded that this emulsifier and cocoa butter actually interact strongly with each other. Schantz and Rohm (2005) reported that best ratio of lecithin to PGPR for minimisation of yield stress is 30:70. Also, hardness of chocolate can be affected by PGPR content. The higher the content of emulsifier, the lower are the values for hardness. This

is because PGPR effects interaction between particles which is mostly responsible for texture of chocolates (Tisoncik, 2010).

Sorbitan tristearate E491

Sorbitan esters are obtained by the reaction of fatty acids with hexitol anhydride. They are non-ionic emulsifiers and are most commonly used for water-in-oil emulsions. They can be used in ice creams, baked goods, but also for modifying the crystal structure in chocolates. The most important role in chocolate is actually the inhibition of fat bloom that occurs during storage (Saltmarsh, 2013).
Cocoa butter, which is a major component in chocolate production, is known to exist in six polymorphic forms. During chocolate storage, the most common transition is from form V to form VI. This is also the reason for the fat bloom which happens during storage. Sorbitan tristearate, by its physical structure, has the effect of slowing this transition. Mostly by producing a rigid structure that will hinder and slow the transition (Garti and Aserin, 2012).

Conclusions

Bulk sweeteners used in the production of sugar-free chocolate have different sweetness intensities, calorific values and influence on the structure of chocolate. There is no universal bulk sweetener when producing chocolate. It is necessary to determine the final effect that is to be accomplish and based on that choose the right sweetener. The emulsifiers used in the production of all kinds of chocolate are already very well known. Most commonly used is soy lecithin, which can be combined with PGPR, but there are other emulsifiers that have benefits and limitations.

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