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

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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

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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).

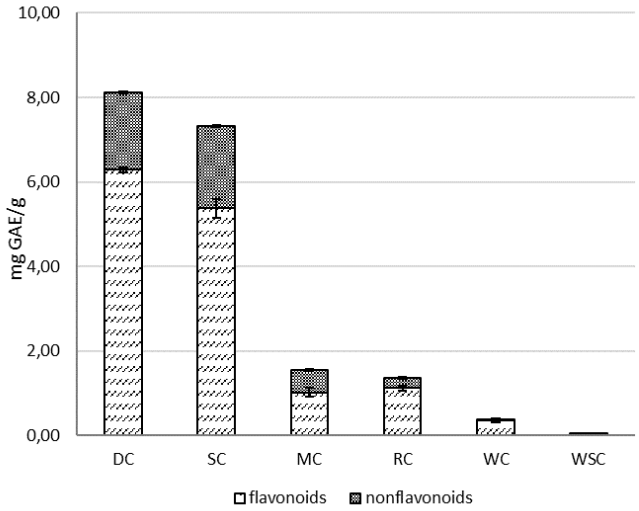

Fig. 1. Total flavonoids and non-flavonoids content (mg GAE/g chocolate ± SD) of analysed chocolates

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).

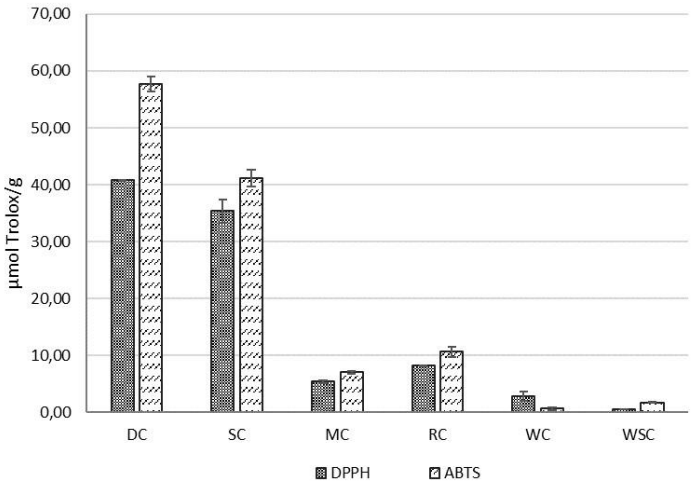

Fig. 2. Antioxidant capacity of analysed chocolates determined by ABTS (µmol Trolox/g chocolate ± SD) and DPPH assays (µmol Trolox/g chocolate ± SD)

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).

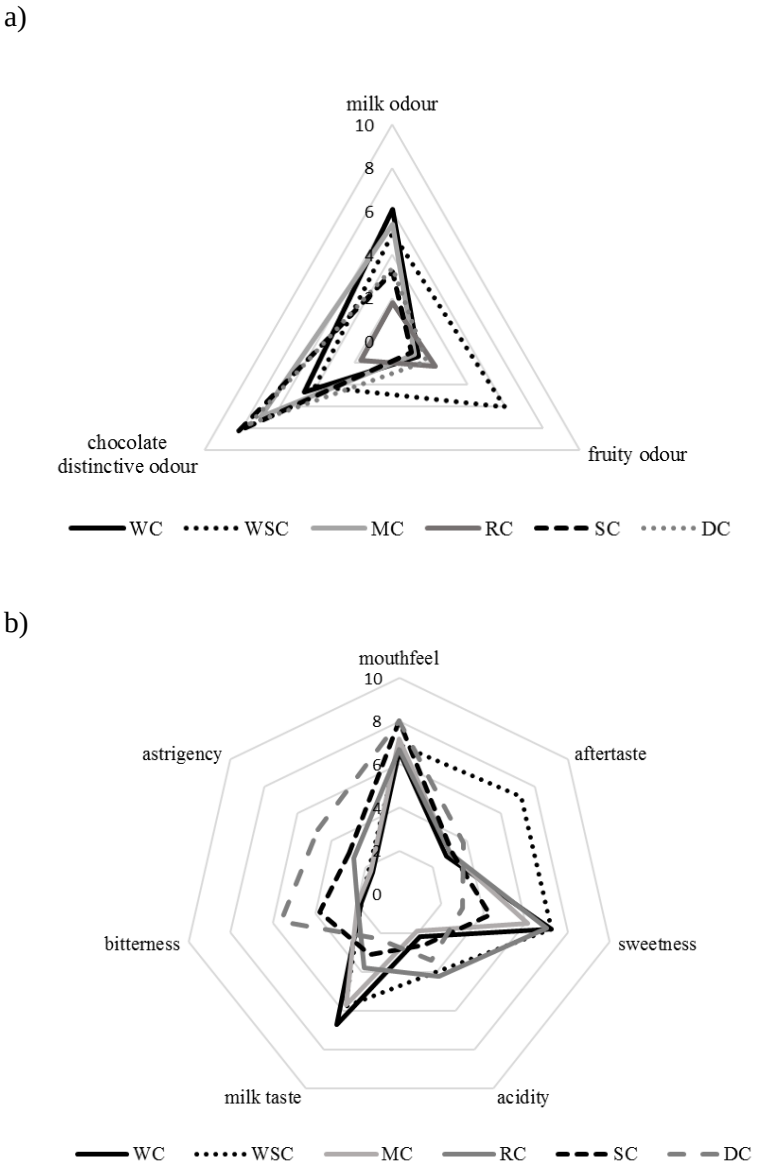

Fig. 3. Spider chart representing mean scores of the evaluated sensory attributes (a) odour attributes and (b) taste attributes for white chocolate (WC), white chocolate with strawberry (WSC), milk chocolate (MC), Ruby chocolate (RC), semisweet chocolate (SC) and dark chocolate (DC)

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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