Bleaching effects of massecuite on some properties of crystallized sugar beet and milk chocolate bars produced

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Abstract Four kinds of bleached, unbleached, second and third crystal sugars (BCS, UCS, SCS and TCS) were made from different massecuites in a sugar-beet factory, and their physiochemical (polarization, invert sugar, colorants, pH, ash and SO₂), microbiological and functional properties were measured. While the polarization of UCS, SCS and TCS were lower than BCS; their invert sucrose, colorants, pH and ash contents were significantly higher than BCS. The phenols and betaine of BCS, UCS, SCS, and TCS were 144, 401, 384 and 673 (mg/100 g); and 244, 791, 4662, and 6589 (mg/100 g); respectively. Whereas the phenol of milk chocolate bars (MCB) made with UCS, SCS, and TCS were only 10% higher than MCB completed with BCS; their betaine contents were substantially (up to 16 times) higher than the ones finished with BCS. Sensory evaluation showed that the MCB prepared with three sugars including UCS, SCS and TCS had significantly higher glossiness, brittleness, flavor and mouth feel than those made with BCS. The greater colorants, ash content and inverted sugars of UCS, SCS and TCS (in comparison with BCS)

made considerable improvements in the glossiness, flavor and brittleness of MCB, respectively. BCS had 8 ppm of toxic sulfur; whereas, UCS, SCS and TCS had no detectable sulfur and significantly higher beneficial copper content than BCS. No pathogenic microorganism were detected in UCS, SCS, TCS or their subsequent MCB. Our results highly recommend using UCS, SCS and TCS instead of BCS in food products (such as MCB) due to their higher health benefits.

Keywords Bleached and unbleached sugars · Phenols · Betaine · Sulfur · Milk chocolate bars · Sensory attributes

Introduction

Increasing the intake of antioxidant-rich foods in the daily diet is an important subject to protect public health against various diseases. Since the most common form of sweetening agent in the world is white (completely refined) sugar with very low antioxidants, it is essential to use (directly or indirectly) less-refined sugars with higher levels of antioxidants and without unsafe compounds. The production stages of different sugars show that it is possible to manufacture the proposed product with improved beneficial properties. The processing stages to obtain white (conventional) sugar from sugar beet are extraction, refining and concentration of sugar beet juice, crystallization of the massecuite (cooked mass), separation of crystals from heavy syrup in centrifugation and drying of sugar granules (Chen and Chou 1993; Van Der Poel et al. 1998; Henke et al. 2006). The manufacture of bleached (white) sugar from a suitable massecuite will not occur in a one-stage system when its crystal content is more than 55%. In fact, it is

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not an efficient process to separate sugar crystals from the heavy viscous liquid by centrifugation due to its very low mobility (Van Der Poel et al. 1998; Asadi 2007). The conventional white sugar is obtained from the centrifugation and bleaching of the first crystalized massecuite after separation of its attached syrup. The centrifugation runoff (attached syrup + washed water) of this stage is re-concentrated to make the second massecuite. Second-grade sugar is produced from the second crystalized massecuite through centrifugation but without bleaching. The procedure is repeated using the centrifugation runoff (from the second massecuite) to make third-grade sugar. The centrifugation runoff of this third massecuite (molasses) has high inverted sugars (mainly glucose and fructose), therefore, it is not feasible to re-concentrate and crystallize its sucrose.

Since sugar-beet juice has many colorants and bioactive compounds including phenolics, melanoidins, melanins, betaine and alkaline degradation products (Godshal et al. 1991), the conventional white (or bleached) sugar is produced from the first massecuite by removing the color from the surface of its crystal granules (Chen and Chou 1993). When the spray washing is eliminated at this stage, unbleached crystal sugar is created containing a color coating (mostly phenols and betaine) associated with some minerals.

There is a correlation between the phenol content and the antioxidant activity of sugar crystals and their sugary food products (Harish-Nayaka et al. 2009; Mohdaly et al. 2013). Antioxidant compounds of sugar beet molasses act as disinfectants against pathogens, anti-carcinogens, anti-mutagens, and heavy metal chelators in the human bloodstream (Godshal et al. 1991; Lameloise and Lewandowski 1994; Harish-Nayaka et al. 2009). Betaine, an anhydrous chemical form of $(\text{CH}_3)_3\text{NCH}_2\text{CO}_2$ (trimethylglycine) is a zwitterion (neutral) molecule combined with a nitrogenous water-soluble compound. It protects sugar beet cells against drought and the human body against osmotic stresses of kidney, fat accumulation in the liver, artherosclerosis, Alzheimers and stroke risk (Viertorinne et al. 1999; Craig 2004).

Unfortunately, the whitening process eliminates also betaine from sugar crystals and puts it (with possible degradation) primarily in the final dark brown molasses (Beckett 2011).

Although the phenolic and flavonoid contents of dark chocolate without sugar (based on gallic acid) is ~ 500 mg/100 g (Wollgast and Anklam 2000; Genovese and Lannes 2009; Bojňanská et al. 2011), the combination of milk chocolate with color-coated sugars containing betaine and phenols may enhance its functional properties substantially.

Liquid sulfur dioxide is used in sugar beet processing to reduce the dark color of the sugar-beet juice after purification and to prevent browning during juice concentration. Although the main portion of added sulfur (approximately 30 ppm) is eliminated from the refined juice during its vacuum evaporation, the residual sulfur remaining in the bleached sugar has harmful health effects. Sulfur compounds in sugar interact with biological molecules and can result in various medical disorders, such as neurologic abnormality, mental retardation, and dislocated ocular lenses (Izgüt-Uysal et al. 2005; Katz et al. 2011).

It was our purpose to study the effects of bleaching crystalized sugar-beet massecuite on its physio-chemical, functional (mainly phenolic and betaine) and microbiological properties in the resulting bleached, unbleached, second and third crystal sugars. Additionally, to compare the functional and sensory properties of milk chocolate bars (MCB) made with different crystal sugars under similar formulation and processing conditions.

Materials and methods

Materials

All chemicals (methanol, sodium carbonate, ethanol, lead acetate, sodium thiosulphate, rosaniline hydrochloride, copper sulphate pentahydrate, potassium chloride and sodium hydrogen phosphate) and reagents (gallic acid and Folin-ciocalteus) used in this study were analytical grade and obtained from Merck Chemical Co (Germany). Dowex resins (hydrogen form and hydroxide form) were obtained from Sigma Company (USA). Different equipment including WFX-210 Atomic Absorption Spectrophotometer (made by Beijing Rayleigh Analytical Instrument Corporation, Beijing, China), a Heidolph homogenizer (Germany), a Varian CARY 100 Conc. UV/VIS Spectrophotometers (USA), a Master-sizer (Model 2000, Malvern Instruments, UK, www.malvern.co.uk/ms2000), a Hettich Zentrifugen micro refrigerated separator (Germany), and Emerson air vacuum pump (USA) used for this study.

Methods

Processing methods

Different samples of bleached crystal sugar (BCS), unbleached crystal sugar (UCS), second crystal sugar (SCS) and third crystal sugar (TCS) were produced in Hegmatan Sugar-beet Factory located in Hamedan, Iran. After the extraction, refining, concentration and cooking of sugar beet juice (to prepare super saturated mother liquor or massecuite), it was conveyed to a crystallizer. At this

stage, the resulting 70 °C massecuite was cooled down and the sucrose crystallized at 40 °C until its crystal content reached to approximately 50–52%. According to Asadi (2007), the first prepared massecuite (~ 90% dry solid, ~ 90% sugar purity) was discharged by gravity to a perforated basket (pore size < 100 µm) in the batch centrifuge. The centrifugal force of the separator detached the crystallized granules of sugar from its group at 1200 RPM within 1.5–2 min. Sugar crystals could not pass through the perforated basket and made a layer of approximately 150 mm thickness on the internal wall of basket. A fine jet of pressurized saturated steam or hot water (100 °C) was sprayed on the sugar crystal layer by a series of nozzles moving up and down for 5–6 s during spinning to remove and wash away the color coating materials and produce BCS or white sugar. UCS was produced from the same massecuite under similar conditions but without the bleaching stage with hot water. The second massecuite (with ~ 45% crystals) and third massecuite (with ~ 35% crystals) were made by re-concentrating runoff obtained from centrifugation of the first and second crystallized massecuites. The SCS and TCS were produced in the same batch centrifuge but without the bleaching processes. Since the molasses or runoff of the third massecuite had considerable inverted sugars (very low sucrose) and high viscosity, it was not feasible to concentrate and crystallize its sucrose (Chen and Chou 1993; Kwok et al. 1999; Asadi 2007).

The MCB samples produced in the Pars Minoo Confectionary Company located in Iran. The product contains cocoa powder (16%), non-fat milk powder (14%), one of the prepared sugars (43%) and melted cocoa butter (27%) mixed to form a uniform primary paste of milk chocolate. The physiochemical analysis of cocoa powder, non-fat milk powder and cocoa butter (shown in Tables B, C and D of Appendix of electronic supplementary material) confirmed that their specifications matched with documented literatures. The resulting mixture was then conched in a low speed mixer for 3–6 h. In this process, a rotating surface scraper evenly distributed cocoa butter within the mixture and polished the chocolate particles. This process also enhances flavor development (due to the frictional heat of scraper), and releases volatiles, acids, and oxidative compounds of the resulting mixture. Lecithin and cocoa butter (~ 10% of the cocoa paste) were added to the mixture and the conching process was extended for ~ 1 h. The molten chocolate held in an incubator at 50 °C for one night and tempered sequentially at different times and temperatures conditions. Tempering is a process to produce chocolate with desirable glossy appearance, solid structure, and ‘snap’ texture when bitten. Chocolate made without tempering has a cloudy texture, dull appearance, can develop bloom or spots (even when kept at the proper

temperature), and can be soft to the taste. After tempering the liquid chocolate was cast into molds of milk chocolate bars and cooled down to 17–20 °C for solidification. A warm water bath was used to demold the solid milk chocolate bars. They were wrapped in aluminum foil and stored at 5 °C. The control sample of milk chocolate bars was prepared with BCS (white sugar) at similar conditions.

Methods of analysis

Physiochemical tests The polarization (apparent sucrose content), invert sugars (%), SO₂ content, ash, pH and color values (yellowness index) of all sugars were measured (ICUMSA 2009; Ieggli et al. 2011). In addition, Method 942.05 of AOAC (2000) was used to determine the copper content in different sugars and their resultant MCB.

Microbiological tests International Standards Organization methods of (2004), (2005), (2007), (1999) and (2008) were used to measure the total plate counts of *Enterobacteriaceae*, *E. coli*, *Salmonella*, *Staphylococcus*, yeasts and molds in the sugars and MCB, respectively.

Phenols determination The Folin-Ciocalteu Method (Wollgast and Anklam 2000) was used to measure and record the total phenol content of BCS, UCS, SCS, TCS, and their resulting MCB in mg GAE (gallic acid equivalent)/100 g of each product.

Betaine determination About 50–100 mg of each sugar or MCB was ground and mixed with 5 mL of methanol and kept for 24 h at 4 °C. After chloroform extraction, its supernatant was mixed with the hydroxide form of Dowex resin to transfer the complex betaine compounds of the extracted solution to the resin surface. The resulting solution was held for complete sedimentation and its decanted resin was washed with double distilled water and ammonium hydroxide to transfer the betaine compounds from the resin to aqueous solution. The solvent evaporated under vacuum (negative pressure of 0.1 MPa at 35 °C for 24 h) and was mixed with 0.6 mL of cold sulfuric acid (1 N) to dissolve its remaining materials followed by indirect cooling. This solution (0.5 mL) was mixed with 0.2 mL of KI-I₂, centrifuged at 4 °C for 5 min at 14,000 rpm, and cooled at 4 °C for 24 h to make iodine crystals of betaine. After adequate washing of iodine crystals with 1 N cold sulfuric acid, the betaine was dissolved in 0.8 mL of ethylene dichloride (containing 25 µl of iodide solution) and its absorbance was read at a wavelength of 365 nm. The standard linear graphs of pure betaine in methanol were prepared at different concentrations (from 50 to 550 µg/L with 100 unit intervals) and read at the same wavelength. By comparing the sample absorbance with the graph of standard solution, the betaine content was calculated in mg/g of original sample (Wall et al. 1960; Barak and Tuma 1979).

Sensory evaluation

After making MCB under similar conditions (formulations and processing methods) but with different sugars in each sample, they were cut into uniform rectangular pieces (4 cm × 4 cm × 1 cm) and inserted in clear and sealable plastic bags. A panel of 11 (5 female and 6 male) trained taste testers (aged 30–45 and 5 years taste assessing experience) was chosen to describe the levels of each descriptive attribute. After the panelists observed the surface glossiness of the MCB made with BCS, they tasted three quality attributes; brittleness, mouth feel and flavor) for “warm up or calibration” and discussed the results. Then the three-digit coded plastic bags of different MCB samples made with BCS, UCS, SCS and TCS were randomly presented to each panel member at room temperature. The evaluations of prepared samples were conducted in separate and individual booths with air ventilation under fluorescent light with even illumination (suggested by ISO Standards No. 8589 (2007)) 2 h after breakfast. Each sensory attribute was scored on a “9-point hedonic scale”, 1 (dislike very much or very poor) and 9 (like very much or very good) (Wollgast and Anklam 2000; Onwuka and Abasiokong 2006). Tap water was available for cleansing the palate between samples. The coded samples were offered 5 min apart to each panelist. Each replicate of the evaluation was divided into three sessions with a 30 min break in between to prevent taste fatigue.

Statistical analysis

A completely randomized experiment was designed to perform the planned tests and obtain the required data without bias. Then the Standard Statistical Package for Social and Natural Sciences, and Duncan method (for averaged data) were used to perform the necessary analysis of variances for the physiochemical and sensory data of the MCB containing different types of sugar. The Tukey method was used to compare the multiple means of the hedonic scores for glossiness, brittleness, mouth feel, and flavor of the MCB. Additionally, correlation coefficients were calculated to estimate the relationship between the properties of specific sugars and the sensory criteria of the resulting MCB samples.

Results and discussion

Physiochemical, microbiological and functional properties

Although the polarization and invert sugar of UCS, SCS and TCS were respectively lower and higher than BCS,

these sugars had high sweetness at similar physical state and temperature (Table 1). Since the concentration of refined sugar-beet juice took place at ~ 65 °C under vacuum pressure, some of its sucrose may have converted to glucose and fructose. The inverted sugars are dissolved by hot water or steam washing of crystalized massecuite during centrifugation. However, they remain with the crystals when the bleaching process is eliminated. Most probably, the higher ash content of the colored sugars (UCS, SCS and TCS) was the main reason that they had higher pH levels than BCS. Since a Maillard reaction may occur between sucrose and the remaining nitrogen compounds in concentrated sugar beet juice during its refining, concentration and crystal formation; it is necessary to control and maintain the pH of sugar crystals to neutral during its cooling temperature at 40 °C to minimize sucrose inversion (Finot et al. 1990).

The results of the mineral measurements showed that the ash and the copper content of UCS, SCS and TCS were higher than BCS (Table 1). The beneficial copper content of UCS, SCS and TCS were 4, 10 and 30 times higher than BCS, respectively. Similar differences were observed in the copper content of MCB made with the different sugars (Fig. 1). The copper contents of those made with UCS and TCS were 2.361 and 0.411 µg/g about 23 and 4 times higher, respectively, than the chocolate bar made with BCS. The copper content of white sugar (or BCS) obtained from the sugar beet in Turkey was in the range of 0.020–0.035 µg/g (Pohl and Stecka 2011) and not more than 0.104 (µg/g) in milk chocolate made with BCS (Ieggli et al. 2011). The beneficial and recommended copper intake with different foods is 1.3 mg or 1300 µg per day (Beckett 2011; Genovese and Lannes 2009). The copper content of food (while is less than the recommended level) prevents various health disorders and plays (as a cofactor) in many enzyme activities in the human organism. It is also a required element for iron transport, glucose metabolism, infant growth, and brain development (Katz et al. 2011).

In spite of the fact that all of the crystalized sugars had de-sulfurization process assisted by long vacuumed cooking process, the sulfur residue of the BCS was close to 8 ppm (Table 1). Although the refined sugar-beet juice was concentrated under vacuum pressure and some of sulfur compounds released, the resulting cooked mass had considerable sulfur residue. The remaining SO₂ in the massecuite penetrated inside the sugar granules to form sulfurous acid during the crystallization process (Izgüt-Uysal et al. 2005). When the bleaching process is omitted, the remaining molasses layer around the outer surfaces of crystals (with strong hygroscopic properties) pulls out the soluble SO₂ as moisture from the inside to the outside surfaces of particles without spending extra energy. While the sulfurous acid content of UCS evaporated (during

Table 1 Means of physico-chemical and microbiological properties found in BCS (bleached crystal sugar), UCS (unbleached crystal sugar), SCS (second crystal sugar) and TCS (third crystal sugar)¹

Samples	Pol (Z°) ²	Invert (%)	Color degree (IU) ³	PH	% Ash ⁴	SO ₂ (ppm)	Mesophilic CFU/g
BCS	99.86 ± 0.03 ^a	0.012 ± 0.01 ^a	50.67 ± 4.62 ^a	7.2 ± 0.05 ^a	0.01 ± 0.01 ^a	7.75 ± 0.17	ND
UCS	99.46 ± 0.58 ^a	0.23 ± 0.07 ^b	1778.33 ± 301.67 ^b	7.5 ± 0.09 ^b	1.19 ± 0.03 ^b	ND ⁵	19 ^a
SCS	96.93 ± 0.85 ^b	0.20 ± 0.02 ^b	1817.00 ± 158.49 ^c	7.5 ± 0.49 ^b	1.01 ± 0.06 ^b	ND	21 ^a
TCS	95.50 ± 0.56 ^c	0.27 ± 0.03 ^c	3533.00 ± 395.79 ^d	7.5 ± 0.32 ^b	1.38 ± 0.04 ^c	ND	24 ^b

¹The numbers with the different superscripts (a, b, c and d) in each column are significant ($p < 0.01$)

²Z° or Sucrose degree

³International units for color of sugar solution based on ICUMSA documents

⁴Mainly the mineral content of final sugar crystals

⁵Not detected

Fig. 1 The copper content of BCS, UCS, SCS, TCS and their resulting MCB (milk chocolate bars) with similar formulation and production conditions

drying process) and became almost zero ppm, this compound in BCS did not decrease because of its insufficient, outer surface molasses layer (Table 1). Since there was no bleaching process for production of SCS and TCS, a similar phenomenon happened and no sulfur compounds remained in the outer surface of these crystal sugars after centrifugation and low temperature drying.

The microbiological results showed that *E. coli*, *Salmonella* and *Staphylococcus* were not detected in the colored sugars (UCS, SCS and TCS) or the resulting chocolate samples. Their *Enterobacteriaceae*, yeasts and molds were less than 10 CFU (colony forming units)/g of each sugar or chocolate product (Table A in Appendix of electronic supplementary material). The mesophilic bacteria of UCS, SCS and TCS were higher than BCS and they belonged to the *Enterobacteriaceae* family (Table 1). Although this study did not measure the amount of pathogenic bacteria of

mesophilic microorganisms in the sugars, the cooking process at ~ 65 °C under vacuum pressure for at least 30 min, makes it unlikely that these pathogenic bacteria of the mesophilic group would survive. This is because the decimal reduction time (DRT) for the majority of bacterial cells heated at 65 °C is shorter than 1 min. The aerobic mesophilic bacteria of raw and refined cane sugar were reported as ~ 50 and 2 CFU/g of sugar, respectively (Wojtczak et al. 2012). Although sugar-beet roots have more microbial load than sugar cane (because of their direct root contacts with the soil and irrigation water) their numbers are comparable to sugar cane. It was possible to use supplementary processes (such as rapid cooling) of crystalized massecuite (before and after centrifugation) and maintain the mesophilic level of UCS, SCS and TCS at < 2 CFU/g.

The hot water sprayed inside the centrifuge bowl washes out the outer pigment layers of the sugar crystals with the result that BCS had much lower brown color than the other sugars. The centrifugation of massecuite without the washing process separates only the non-crystallized sugar syrup and produces granules of sugar crystals coated with layer of molasses and pigments. The average particle size diameter of crystallized sugar granules of BCS, UCS, SCS and TCS (measured with 3 replicates by laser diffraction system of Master-Sizer) with 50% cumulative mass (D50) were 707 ± 32 , 811 ± 25 , 584 ± 11 , and 689 ± 18 μm , respectively. Since most of the colorant compounds remained on the surface of unbleached sugars, these sugar granules were ~ 100 μm thicker than BCS.

The coating color of UCS, SCS and TCS originated from the natural pigments of raw sugar beet, which were not eliminated during the juice refining process. Additional coloring comes from the continuous browning reactions of glucose with amino acids (resulted respectively from the inverted sugars and nitrogen compounds) of sugar beet juice during the refining, concentration and crystallization. These processes transfer different browning colorants (such as melanoidines) to the final crystal sugar and molasses (Lameloise and Lewandowski 1994; Bojňanská et al. 2011). Figure A in Appendix of electronic supplementary material shows the colors of the four types of crystallized sugar obtained in this study. The UCS had a more brownish coating than SCS because it was produced directly from the first massecuite with high colorants and without hot water washing for color elimination. Since the second massecuite was made by mixing sugar syrup (runoff of the first centrifugation) with hot water (at least 3% of massecuite mass) for washing, it had less colorants than first massecuite, and the resulting SCS had less brownish color in comparison with UCS. However, the third massecuite made from the runoff of second massecuite but without hot water washing had more color compounds than second massecuite, and its resulting crystal sugar (TCS) had a darker color in comparison with SCS.

The three sugars (USC, SCS and TCS), and their resulting chocolate had higher phenols ($p < 0.01$) and betaine ($p < 0.01$) contents than those found in BCS and its related chocolate (Table 2). The phenolic contents of fresh sugar beet pulp and its juice are close to 180 mg and 90 mg GAE/100 g, respectively (Mohdaly et al. 2013). Since ~ 7 kg of sugar beet juice (with $\sim 14\%$ extractable sucrose) is needed to make 1 kg of crystal sugar, the phenolic content of crystallized massecuite before crystal separation with maximum 20% loss (during the refining process) was ~ 500 mg GAE/100 g. While BCS was not able to retain more than 30% of the phenolic content of the final sugar-beet massecuite, the phenolic content of UCS, SCS and TCS were 80, 76 and 134% of

the first massecuite (Table 2). Due to the differences between phenolic and betaine contents of BCS and colored sugars (UCS, SCS and TCS), noticeable color differences were observed between these crystal sugars (see Figure A in Appendix of electronic supplementary material).

According to Godshal et al. (1991), about 60 and 40% of the total pigments and colorant precursors (phenolics, betaine and amino acids) of light brown sugar are located in the outside and inside of their crystals, respectively. While the inside phenol content of UCS and SCS were very close to BCS, the granules of UCS and SCS had 240 and 231 mg of GAE/100 g more phenols (as a coating layer) respectively than BCS at similar conditions (Table 3). The difference in phenols of TCS and BCS on their outer surfaces was as much as 400 units (mg of GAE/100 g).

This process happened most probably due to the repeated re-concentration and cooking of the sugar beet runoff in production of SCS and TCS. These results show clearly that the bleaching process of crystallized sugar beet massecuite during centrifugation generates white sugar with very low phenolic compounds located mainly inside of crystals. Diet researchers studied the naturally occurring antioxidants such as flavonoids, phenolic acids, and polyphenols in various sweeteners. They found that the antioxidant capacity (as measured by ferric-reducing ability of plasma) of white sugar (BCS) and light brown sugar (with coating layer of phenolic compounds) were 0.009 and 0.385 mmol/100 g of sugar (> 40 times difference), respectively (Phillips et al. 2009).

While the quantity of phenolic compounds differed among BCS, UCS, SCS and TCS, there was no significant difference in phenols of MCB samples made with dissimilar sugars possibly because of the long conch-heating process of the milk chocolate bars. Almost 50% of bioactive compounds of the ingredients used in chocolate processing decompose during processing (Hoskin and Dimick 1984; Beckett 2011). In spite of this fact, the MCB made with UCS, SCS and TCS had $\sim 10\%$ more phenols than those made with BCS (Table 2). Higher contents of phenolic compounds is conceivable if necessary modifications in chocolate processing (mainly heating conditions) take place.

UCS, SCS and TCS with a residual molasses layer had 3, 19 and 27 times more betaine than BCS, respectively (Table 2). The betaine content of sugar beet molasses is ~ 100 mg/g (Wall et al. 1960; Viertorinne et al. 1999; Asadi 2007). Based on the Godshal et al. (1991) report for color compounds of sugar crystals, the outside betaine contents of BCS, UCS, SCS and TCS were 0, 500, 2800 and 4000 mg/100 g, respectively (Table 3). The betaine of MCB made with UCS was 300% more than the ones made with BCS (Table 2). This interesting result showed that betaine had more resistance to decomposition during the

Table 2 Phenols and betaine in BCS, UCS, SCS, TCS and resulting MCB (milk chocolate bars) plus their standard deviations

Sugar and MCB samples	Phenols in sugar or MCB in terms of GAE (mg/100 g)	Phenol ratio of MCB made with each sugar to phenol of MCB made with BCS	Betaine in sugar or MCB (mg/100 g)	Betaine ratio of MCB made with each sugar to Betaine of MCB made with BCS
BCS	144 ± 7.6 ^a	(BCS/BCS) = 1.00	244 ± 9 ^a	1.00
UCS	401 ± 3.1 ^b	(UCS/BCS) = 2.78	791 ± 19 ^b	3.24
SCS	384 ± 11.1 ^b	(SCS/BCS) = 2.67	4662 ± 94 ^c	19.11
TCS	673 ± 5.1 ^c	(TCS/BCS) = 4.67	6589 ± 80 ^c	27.00
MCB with BCS	1423 ± 28.0 ^a	(MCB _{BCS})/(MCB _{BCS}) = 1.00	169 ± 31 ^a	(MCB _{BCS})/(MCB _{BCS}) = 1.00
MCB with UCS	1527 ± 12.1 ^b	(MCB _{UCS})/(MCB _{BCS}) = 1.07	398 ± 18 ^b	(MCB _{UCS})/(MCB _{BCS}) = 2.36
MCB with SCS	1448 ± 5.8 ^a	(MCB _{SCS})/(MCB _{BCS}) = 1.02	2507 ± 31 ^c	(MCB _{SCS})/(MCB _{BCS}) = 14.83
MCB with TCS	1564 ± 7.1 ^b	(MCB _{TCS})/(MCB _{BCS}) = 1.10	2758 ± 63 ^c	(MCB _{TCS})/(MCB _{BCS}) = 16.32

Numbers with different superscripts (a, b and c) in each column are significantly different ($p < 0.01$) from each other

Table 3 The fractionation of total phenols and betaine in the inside and outside of BCS, UCS, SCS and TCS granules based on 40 and 60% distribution of color compounds in sugar crystals (Reproduced with permission from Godshal et al. 1991)^a

Sugar samples	Total phenols mg GAE/100 g	Outside phenols (mg GAE/100 g)	Inside phenols (mg GAE/100 g)	Total betaine (mg/100 g)	Outside betaine (mg/100 g)	Inside betaine (mg/100 g)
BCS	~ 144	~ Zero ^b (0%)	~ 144 (100%)	~ 200	~ Zero ^b (0%)	~ 200 (100%)
UCS	~ 401	~ 240 (60%)	~ 161 (40%)	~ 800	~ 500 (60%)	~ 300 (40%)
SCS	~ 384	~ 231 (60%)	~ 153 (40%)	~ 4700	~ 2800 (60%)	~ 1900 (40%)
TCS	~ 673	~ 404 (60%)	~ 269 (40%)	~ 6600	~ 4000 (60%)	~ 2700 (40%)

^aThe phenols and betaine of first massecuite of sugar beet are respectively about 600 and 100 mg/g

^bThere is no molasses layer in the outer surface of white or bleached sugar crystals (BCS) due to the hot water spraying or steaming process of crystalized massecuite during centrifugation, therefore its phenols and betaine are very low and negligible (almost zero)

processing (mainly heating) of MCB than their phenolic compounds.

Sensory properties and statistical analysis

The chocolate quality depends on its ingredients' specifications, formulation, processing conditions and shaping of the final product. The regulations of European Community (EC) permit the use of different sugars up to 55% of the total ingredients for making milk chocolates. This guideline enforces manufacturers to use sugar more than the legal minimum level of each non-sugar ingredient. Furthermore, EC permits to mix mostly (> 40%) sucrose and less than 5% of other saccharides (mainly glucose, fructose, lactose or maltose) in formulation of chocolate milk (Bolenz et al. 2006). This was the reason that 43% of each crystal sugar used in formulation of MCB to evaluate its effects on sensorial quality of finished product. Chocolate

is normally described by their external appearance (surface glossiness), internal appearance (brittleness), texture (mouth feel), taste (flavor) and aroma (Muñoz and Gutiérrez 2015). However, in this study aroma was not used as the milk powder masked cocoa aroma. The sensory evaluation data showed that the MCB made with colored sugars (UCS, SCS and TCS) had ($p < 0.05$) higher sensory scores than those made with BCS (Table 4). While the MCB made with BCS obtained less than 65% of its total possible sensory scores, those made with SCS, USC and TCS had respectively more than 80, 85 and 90% of the maximum scores. ANOVA results confirmed that the MCB made with colored sugars (UCS, SCS and TCS) had higher flavors, glossiness, brittleness, mouth feel and overall acceptance than those made with BCS (Table E in Appendix of electronic supplementary material). In other words, the means of sensory attributes in MCB made with colored sugars and BCS had significant differences; and the

Table 4 Mean and total scores of sensory evaluation of MCB (milk chocolate bars) made with four kinds of sugars (BCS*, UCS**, SCS*** and TCS****) with similar formulation and processing conditions

Kind of sugar	Mouth Feel ¹	Flavors ²	Brittleness ³	Glossiness ⁴	Total scores	Maximum possible score	Gaining scores out of maximum (%)	Average of scores
BCS	5.89 ± 1.25 ^a	6.44 ± 1.42 ^a	5.00 ± 2.02 ^a	5.11 ± 1.83 ^a	22.44	36.0	62.3	5.61
SCS	8.22 ± 2.24 ^b	8.89 ± 2.33 ^b	6.56 ± 2.01 ^b	6.44 ± 2.05 ^b	30.11	36.0	83.6	7.53
TCS	8.56 ± 1.84 ^b	8.56 ± 2.32 ^b	7.78 ± 2.11 ^c	7.89 ± 2.02 ^c	32.79	36.0	91.1	8.19
UCS	8.44 ± 1.92 ^b	8.78 ± 0.87 ^b	6.78 ± 1.29 ^b	7.44 ± 2.07 ^c	31.44	36.0	87.3	7.86

*MCB made with bleached crystal sugar (BCS)

** MCB made with unbleached crystal sugar (UCS),

and * MCB made with respectively second and third crystal sugars (SCS and TCS)

¹and ² significant at ($p < 0.01$), and

³and ⁴ significant at ($p < 0.05$)

probability to assume they are equal to each other were < 0.0003 (Table F in Appendix of electronic supplementary material).

Sugar properties and attribute descriptions of the resultant MCB

Effects of colorants on glossiness and flavor

When UCS, SCS or TCS with colorant compounds are used individually instead of BCS (as a sweetening agent) to make chocolate, they had considerable effects on mouth feel, flavor and glossiness of the resulting product. High correlations were obtained between the color degree of crystal sugars (specified in Table 1) with glossiness ($R^2 = 0.66$ and $r = 0.81$), flavor ($R^2 = 0.71$ and $r = 0.84$) and mouth feel ($R^2 = 0.81$ and $r = 0.90$) of the resulting MCB (Fig. 2 and Figure B in Appendix of electronic supplementary material). According to Afoakwa (2010), surface glossiness in the chocolate texture is tightly connected with crystalline structure of cocoa butter and its melting degree in the mouth. The cocoa butter improves not only the glossiness but also taste perception when hard materials of chocolate converts to liquid oil and provides a pleasant mouth feel. Since the UCS, SCS and TCS coated with molasses had greater tendency to absorb free moisture from the cocoa particles and crystals of cocoa butter, the resulting chocolate probably had less free moisture and more crystalline particles of cocoa butter and consequently more glossiness than those made with BCS.

The quantities of polyphenol, alkaloids (generated during conching), caramels, betaine, and to some extent the sweetness (associated with the presence of sugars) are key factors in the final flavor profile of cocoa liquor (Afoakwa et al. 2008). Since the UCS, SCS and TCS had much higher colorants than BCS, the resulting MCB had higher flavor

than those made with BCS (Tables 2, 4). Most chocolate consumers' prefer the milk chocolate to the dark chocolate because it releases their favored flavors of sweetness and caramel during chewing in the mouth (Guinard and Mazzucchelli 1999; Cooper et al. 2008). Most probably, the molasses layer of colored sugars (UCS, SCS and TCS) with the intensifying flavors of colorant (caramel, phenolic and betaine) compounds mixed with the cocoa liquor produced MCB with higher flavor.

Effects of ash content on flavor and brittleness

The higher ash content of individual UCS, SCS and TCS in comparison with BCS (Table 1) affected the flavor and to some extent the brittleness of MCB. The high correlations between the ash content of different crystal sugars and two combined sensory attributes of the MCB including flavor ($R^2 = 0.87$ and $r = 0.93$) and brittleness ($R^2 = 0.93$ and $r = 0.96$) confirmed these effects (Fig. 2 and Figure C in Appendix of electronic supplementary material). According to Sharif (1997), alkalization of cocoa liquor will reduce its acidity and improve the flavor of final chocolate considerably. Alkalization polymerizes the flavonoids (including phenols), reduces acidity and astringency of cocoa liquor and its resulting chocolate (Afoakwa et al. 2008). Possibly the ash contents of UCS, SCS and TCS (with at least 100 times more than BCS, see Table 1) enhanced the alkalization process during conching when they mixed with cocoa liquor and produced chocolate with stronger flavors in comparison with the chocolate made with BCS.

Effects of inverted sucrose on brittleness and glossiness

The higher inverted sugars of individual UCS, SCS and TCS in comparison with BCS (Table 1) affected the brittleness and the glossiness of MCB. The high correlations

Fig. 2 The effects of color degrees, ash content and invert sugar of BCS, UCS, SCS and TCS on sensory (flavor, brittleness and glossiness) attributes scores of their resultant MCB (milk chocolate bars)

between the inverted sucrose of different crystal sugars and two combined sensory attributes of brittleness ($R^2 = 0.94$ and $r = 0.97$) and glossiness ($R^2 = 0.92$ and $r = 0.96$) confirmed these effects (Fig. 2 and Figure D in Appendix of electronic supplementary material). According to Muñoz and Gutiérrez (2015), the force necessary to break the center of milk chocolate into two smaller pieces estimates its brittleness. The free moisture content in chocolate generally reduces brittleness and may make it more difficult to break or crush it (Afoakwa 2010). Since the invert sugars of colored sugars were 20–30 times higher than BCS, their hygroscopic nature lead to less free moisture and increased brittleness of MCB. Reducing the free moisture in chocolate (as discussed before) increases the glossiness of the final product.

Conclusion

When sugar-beet factories bleach massecuite to remove the color coating of crystalline sucrose and make white sugar, the bioactive soluble pigments are lost. The colored sugars (UCS, SCS and TCS) with molasses layers containing phenolic and betaine compounds around their crystals contain more bioactive compounds than white sugar (BCS). Furthermore, the elimination of washing stage (by hot water or wet steam) of crystalized massecuite during crystals separation had a potential to increase the copper content in their final sugar granules. Similarly the MCB

made with UCS, SCS and TCS had more copper and bioactive (phenols and betaine) compounds. Additionally, the flavors, brittleness and glossiness of resultant MCB improved significantly in comparison with those made with BCS (conventional white sugar). Since the amount of hot water used for washing of the sugar crystals during centrifugation is about 3–5% of the massecuite mass, the production of UCS (unbleached crystal sugar) instead of BCS (bleached crystal sugar) has potential to reduce the required energy and water needed in the processing of sugar remarkably.

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