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ORIGINAL ARTICLE

Physico-chemical properties, wax composition, aroma profiles, and antioxidant activity of granulated non-centrifugal sugars from sugarcane cultivars of Thailand

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Abstract Non-centrifugal cane sugar (NCS) is globally consumed and has various health benefits. It is mostly produced in hardened block form, which is less convenient than in granulated form for food applications. In terms of the traditional processing of NCS, preparation of granulated products is difficult due to the impurities found in the cane juice extracted from the whole stalk. Therefore, the aim of this study was to characterize and determine the physico-chemical properties, wax composition (policosanols and long-chain aldehydes), volatile aroma profiles, and antioxidant activity of traditional NCS in granular form made from four different cane cultivars of Thailand. The total soluble solid, pH, color, and mineral content varied among the sugarcane cultivars, whereas there was no significant difference in the total sugar, phenolic and flavonoid content. The total policosanol, a cholesterol-lowering nutraceutical wax component, and long-chain aldehyde contents were similar in the NCS products amongst three cultivars, and ranged from 2.63 to 3.69 mg/100 g. The granulated NCS products, in which acetaldehyde and dimethyl sulfide were the main volatile compounds, gave less aroma components than traditional NCS. The use of different sugarcane cultivars thus influenced the quality attributes of granulated non-centrifugal sugar products.

Keywords Non-centrifugal cane sugar · Physico-chemical properties · Policosanol · Volatile aroma components · Antioxidant activity

Introduction

Sugarcane is one of the essential sources for sweetener manufacturing, including non-centrifugal cane sugar (NCS), also called "cane brown sugar". NCS has its own characteristic taste, aroma and nutritional value. It is consumed in most sugarcane growing regions and countries of the world, and known under many different names, such as Gur (India), Panela (Mexico and South America), Jaggery (Africa), Htanyet (Myanmar), Panocha (Philippines), Rapadura (Brazil), Kokuto or Kurozato (Japan), and Naam Taan Oi (Thailand) (Jaffé 2012; Thakur 1999). By a non-centrifugal method, NCS is traditionally obtained through the dehydration of sugarcane juice using open pans without removing molasses and, therefore, it is packed with natural minerals, vitamins, and phytochemicals. The sugar making process also involves the production of Maillard reaction compounds, which are strongly involved in the generation of the color and aroma of the NCS products (Asikin et al. 2016). The volatile aroma composition, as well as other components, is an important food property for acceptance and preference by consumer (Payet et al. 2005).

The interest in the functional components in NCS, such as polyphenols and policosanols (PCs), has considerably increased in recent years due to their promising biological

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properties (Jaffé 2012). The phenolic and flavonoid constituents from NCS and other sugarcane products have been reported to possess potent antioxidant, antimutation, and tyrosinase inhibitory effects (Duarte-Almeida et al. 2007, 2011; Jaffé 2012; Takara et al. 2007). PC is a mixture of primary long chain alcohols with chain lengths varying from 22 to 34 carbon atoms, including docosanol (C22), tetracosanol (C24), hexacosanol (C26), octacosanol (C28), and triacontanol (C30), which possess cholesterol and lipid-reducing properties (Chen et al. 2008). They are naturally found in the wax of plant surfaces, leaves, seeds, and fruits of many food crops, but their therapeutic effectiveness has been demonstrated primarily on PCs derived from sugarcane wax (Chen et al. 2009; Irmak and Dunford 2005; Irmak et al. 2006; Wang et al. 2007).

Thailand is one of the world's top five sugarcane producers, and is one of the world's leading refined sugar exporting countries after Brazil, India, and China (FAO 2016). In Thailand, traditional NCS is commonly known as Naam Taan Oi, which is produced mostly by an open pan boiling method in an artisanal sugarcane process. There are more than 41 cane cultivars in Thailand, including the famous varieties Suphanburi 50 (SP50), Uthong 12 (UT12), and Khon Kaen 3 (KK3). Thai NCS is produced mostly as bricks of different size. This is because it is the easiest to do so, and the traditional technique to fabricate NCS in the form of bricks has been passed from generation to generation. Although this shape is useful in terms of storage, it is not convenient for application both in household and industry. To reduce this limitation, granulated NCS is the best option. NCS granules can be produced by removing the peel of the cane stalk prior to juice extraction in order to reduce the impurities in the cane juice, and thus achieve optimum sugar crystallization and degree of saturation.

Therefore, the aim of this study was to characterize and determine the physico-chemical properties, wax composition (PCs and long-chain aldehydes), volatile aroma profiles, and antioxidant activity of traditional NCS in granular form made from four different cane cultivars of Thailand, including SP50, UT12, and KK3, and the new hybrid cultivar, SRS2000-5-14 (SRS2), developed by the Sukhothai Agricultural Research and Development Center, Thailand.

Materials and methods

Standards and reagents

Standard minerals (calcium, sodium, potassium, magnesium, iron, copper, and zinc), sugars (sucrose, fructose, and glucose), PC standards (docosanol, tetracosanol, hexacosanol, octacosanol, and triacontanol), gallic acid, rutin, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were obtained from Sigma-Aldrich (St. Louis, MO, USA). The chemicals used as standards to identify the volatile aroma components were obtained from Sigma-Aldrich, Tokyo Chemical Industry (Tokyo, Japan), and Wako Pure Chemical Industries (Osaka, Japan). The derivatization reagent *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA) was obtained from GL Science (Tokyo, Japan), and pyridinium chlorochromate was purchased from Sigma-Aldrich. All reagents and chemicals were of analytical grade unless stated otherwise.

Granulated NCS preparation

Whole stalks of four different sugarcane cultivars, including Suphanburi 50 (SP50), Uthong 12 (UT12), Khon Kaen 3 (KK3), and SRS2000-5-14 (SRS2), were used as source materials. SP50, UT12, KK3, and SRS2 cane cultivars were grown in the same field at the Sukhothai Agricultural Research and Development Center, Sukhothai, Thailand, and the samples were collected at the stage of maturity (12 month cultivation) during the sugarcane harvesting season in February 2014. The outer rinds covered with waxy materials were separated from the fresh sugarcane stalks by manual peeling, followed by juice extraction with a two-roller power crusher. The total soluble solid (TSS) of the fresh juice was measured with a hand held refractometer. In order to make traditional granulated brown sugar, the juice (300 mL) was then filtered using a muslin cloth and transferred to a household open pan to be heated at a temperature of 110–120 °C. The sugarcane juice was concentrated by boiling without adding any lime or other chemicals. The progression of the boiling-concentration process was monitored until granulated NCS was formed. Additionally, a sample of Thai traditional NCS (TP), the commercial product of the One Tumbon One Product (OTOP) project, was obtained from a community enterprise at Payao Province, Thailand. Without the rind peeling process, TP was prepared by evaporation of cane juice of SP50 cultivar in an open pan with the addition of a food-leavening agent to obtain the granulated NCS product. All NCS samples were ground using a dry blender (Iwatani Corporation, Tokyo, Japan), passed through a 30-mesh size sieve and kept dry at −20 °C until use.

Physico-chemical properties

The moisture content of the NCS samples was measured based on the weight loss of the samples after drying at 105 °C for 1 h until the constant weight obtained. The color of the NCS products was determined following the International Commission for Uniform Methods of Sugar

Analysis (ICUMSA) GS1/3–7 protocol (ICUMSA 2003), and was expressed as the international unit of color for sugar products. The minerals (calcium, sodium, potassium, magnesium, iron, copper, zinc) were quantified with an atomic absorption spectrophotometer (Model 2100, Perkin Elmer, Massachusetts, USA) (AOAC 2005O). The sugar (sucrose, fructose, glucose) content was analyzed using high-performance liquid chromatography equipped with a refractive index detector (Shimadzu HPLC class VP series, Shimadzu Corporation, Kyoto, Japan). The chromatography was conducted using an Inertsil NH_2 column (4.6 mm × 250 mm; GL Sciences Inc., Tokyo, Japan) with a mixture of acetonitrile and water (4:1, v/v) as the eluent at 1 mL/min (Weerawatanakorn 2013). All analyses were carried out in triplicate.

Wax extraction and policosanol and long-chain aldehyde analysis

Sample extraction and preparation

Soxhlet extraction was carried out following the reported method by Asikin et al. (2008). Briefly, 9 g of NCS was placed in a thimble filter (Advantec No. 84, Tokyo, Japan) and extracted with 150 mL of a mixture of hexane and methanol (20:1 v/v) with an extraction time of 10 h for both PC and long-chain aldehydes. The solvent was removed using a rotary-evaporator under vacuum at 40 °C, and the dry residue wax extracts were diluted either with toluene or with chloroform to obtain 1-mL extract samples for further analysis.

The aldehyde standards were synthesized by oxidation of their alcohol forms using pyridinium chlorochromate as described by Pérez-Camino et al. (2003). Briefly, each form of the 1 mM alcohol standards (tetracosanol, hexacosanol, octacosanol, and triacontanol) and 9 mM pyridinium chlorochromate were stirred in 50 mL of dichloromethane for 1.5 h at room temperature. The oxidized mixture was then eluted with dichloromethane through a short silica gel-60 column (6 × 2 cm i.d.), and the separated standard products were then dried under a nitrogen stream and diluted in toluene.

Policosanol (PC) and long-chain aldehyde determination

For the quantification of PC and long-chain aldehyde compounds by gas chromatography-flame ionization detection (GC-FID), a mixture of PC or long-chain aldehyde standards was prepared in toluene (0.01–0.5 mg/mL). An Agilent 6890 N GC system equipped with a fused capillary column (DB 5, 0.25 mm i.d. × 30 m; Agilent J&W, Santa Clara, CA, USA) and an FID system were used for the quantitative analysis of PCs and long-chain aldehydes. The GC injector and FID detector were both set at 350 °C, and helium was used as carrier gas. Aliquots (1 μL) were injected with a split ratio of 1:10. The oven temperature was initially set to 150 °C, then increased at the rate of 4 °C/min to 320 °C, and kept at 320 °C for 15 min. The amounts of both alcohol and aldehyde forms were calculated based on calibration curves obtained by injection of different concentrations of standard mixtures of PCs and aldehydes. PC and long-chain aldehyde contents have been expressed as mg per 100 g of sample on a wet basis.

For mass spectrometry (MS) analysis, the PC standards (or wax extracts) were also dissolved in chloroform for derivatization with MSTFA (2:1, v/v). The mixed derivatization solutions, including 0.5 mL of wax extracts in chloroform and 250 μL of MSTFA, were heated at 50 °C for 15 min, followed by the addition of chloroform to obtain 1-mL aliquots for analysis. Trimethylsilyl derivatives of the alcohol forms were analyzed by a Shimadzu GC-MS QP-2010 Plus (Shimadzu Corporation) equipped with a fused capillary column DB-5 MS (0.25 mm i.d. × 30 m, Agilent J&W) under the same GC conditions described above. However, the MS profile of the aldehydes was analyzed without silylation. The injection volume was 0.3 μL and the split ratio was 1:10. For MS detection, the electron impact ion source and interface were maintained at 200 and 280 °C, respectively. The MS acquisition scan range and rate were set at (m/z) 30–500 amu and 2 scans/s, respectively, and the ionization voltage was 70 eV.

Volatile aroma component analysis

Three grams of each sample were placed in a 20-mL glass headspace vial and sealed with an aluminum crimp cap. Samples were equilibrated at 40 °C for 30 min using an Agilent G1888 headspace autosampler (Agilent J&W). Vaporized volatiles were injected using a split ratio of 1:10 to an Agilent 7890A GC system equipped with a fused silica capillary column (DB-Wax column, 60 m × 0.25 mm i.d., 0.25 μm film thickness, Agilent J&W). The GC injector and FID were set at 250 °C, and the oven was initially programed at 40 °C (held for 5 min), then increased to 200 °C at a rate of 5 °C/min and kept at 200 °C for 3 min. Helium was used as the carrier gas at a flow rate of 23 cm/s. The peak area response of the volatile compounds was monitored in order to evaluate the relative amount (%) of the volatile aroma components in the NCS samples.

The MS data of the volatile compounds were obtained using an Agilent 7890A GC coupled with an Agilent 5975C mass spectrometer (Agilent J&W). The column and oven program specifications for GC–MS analysis were as described above. For MS detection, both ion source and

interface were maintained at 230 °C. The MS acquisition scan range and rate were set at (m/z) 29–300 amu and 1.77 scans/s, respectively, and the ionization voltage was 70 eV. The volatile components were identified based on the linear retention indices (RIs) to a homologous series of *n*-alkanes (C5–C20), as well as comparisons of MS fragmentation patterns of the volatile compounds to corresponding compounds from the National Institute of Standards and Technology (NIST) MS Library, Version 2008, and to co-injected authentic standards. All analyses were carried out in triplicate (Asikin et al. 2014).

Phenolic content and antioxidant determination

Total phenolic content

The total phenolic content was determined with the Folin–Ciocalteu assay (Medini et al. 2014). An aliquot of diluted extract (2 mL) was added to 5 mL of Folin–Ciocalteu reagent. The mixture was shaken and allowed to stand for 6 min, before adding 4 mL of 7% Na_2CO_3 aqueous solution. The solution was then mixed thoroughly. After incubation at 45 °C for 15 min, the absorbance at 760 nm was recorded. The total phenol content had been expressed as milligrams of gallic acid equivalents (GAE) per gram of sample from a calibration curve of gallic acid.

Total flavonoid content

The total flavonoid content was measured by a colorimetric assay according to Meda et al. (2005). Briefly, a 2 g sample was extracted with methanol (10 mL). One milliliter of 2% aluminium chloride ($AlCl_3$) in methanol was mixed with the same volume of the methanol extract of the sample or a standard solution of rutin (5–80 mg/L). The absorbance at 415 nm was recorded after 10 min against a blank sample without $AlCl_3$. The mean value of three readings was calculated and expressed as mg rutin equivalent (RAE) per gram of sample.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity

The DPPH method was applied following the report by Medini et al. (2014). The sample (1 g) was extracted with methanol (50 mL). Extracts (1 mL) of the samples were added to 2 mL of a 0.2 mM DPPH–methanol solution. After incubation in the dark at room temperature for 60 min, the absorbance was determined at 517 nm. The percentage inhibition of free radical DPPH was calculated from $(A_{blank} - A_{sample}/A_{blank}) \times 100$, where A_{blank} is the absorbance of the control reaction and A_{sample} is the absorbance in the presence of the plant extract.

Statistical analysis

All the data have been expressed as the mean ± standard deviation (SD) of three replicas, and one-factor ANOVA followed by the Tukey test was used for the statistical analysis. Trends were considered significant when the means of compared sets differed at $p < 0.05$.

Results and discussion

Physico-chemical properties of NCS products from different cultivars

The filtered cane juice was concentrated in an open pan at a temperature of 110–120 °C to obtain the granulated NCS products. The average time for this process was 20 min, with a maximum yield of 28.8% and a minimum yield of 17.7% for the products made from SP50 and SRS2, respectively (Table 1). The total soluble solid (TSS) of the cane juices ranged from 17.31° to 18.10° Brix and significantly varied among the different sugarcane cultivars. The TSS of traditional cane brown sugar (TP), 18.46° Brix, was also recorded for comparison purposes. The moisture content of the granulated NCS products ranged from 0.98 to 2.98%, following the Thai standard guidelines for NCS products, which require values between 0.7 and 3%. However, the moisture content of TP in this study was higher than the standard requirement. The brown color of the NCS products from KK3 and SRS2 cultivars was much darker than that of the SP50 and UT12. The color of NCS is an important quality attribute that has an impact on the consumer acceptances and their preferences. Regardless of the original pigment of raw cane juice, the color of NCS products was mostly regulated by chemical reactions including the Maillard browning reaction. The NCS products made from Thai cane cultivars in this study have a lighter color value (IU 2292–5285) than Japanese NCS products (IU 12,410–29,529) (Asikin et al. 2014, 2016). The high pH (6.96) of TP resulted from the application of food additives with alkaline properties.

There were differences in the fructose and glucose content between the products made from SP50 and UT12 cultivars, while there was no statistically significant difference in the total sugar and sucrose levels of the products (Table 2). In addition, the total sugar content of the product made from the new cultivar, SRS2, was the highest among all cultivars. The total sugar levels were similar to those of traditional cane sugar (processed without peeling the outer layer rinds), indicating that the peeling process has no effect on the sugar content of the NCS products.

One of the quality characteristics of NCS compared to refined sugar was its nutritional value and, in

Table 1 Yield and physico-chemical properties of NCS products from different sugarcane cultivars

Property	SP50	UT12	KK3	SRS2	TP
Yield (%, w/v)[a]	22.8 ± 2.8	19.6 ± 0.4	19.9 ± 1.2	17.7 ± 0.5	n/a
Total soluble solid (°Brix)[b]	17.31 ± 0.01e	17.83 ± 0.01d	18.10 ± 0.01b	18.01 ± 0.01c	18.46 ± 0.01a
Moisture content (%)	0.98 ± 0.03d	1.70 ± 0.05c	2.98 ± 0.03b	1.17 ± 0.08d	4.28 ± 0.08a
ICUMSA color unit (IU)	2641.7 ± 40.7c	2291.7 ± 28.9d	5116.7 ± 84.4a	5285.4 ± 25.3a	4277.1 ± 40.2b
pH	5.75 ± 0.01c	5.76 ± 0.01c	5.83 ± 0.00b	5.82 ± 0.01b	6.96 ± 0.01a

[a] Yield value was defined as percentage of weight of the obtained sugar product on the cane juice

[b] Total soluble solid value was taken from sugarcane juice prior to NCS preparation

Values are expressed as the mean ± SD ($n = 3$) and values with different letter within the same row are significantly different ($p < 0.05$)

Table 2 Sugar (g/100 g) and mineral (mg/100 g) content of NCS products from different sugarcane cultivars

Component	SP50	UT12	KK3	SRS2	TP
Sucrose	83.80 ± 4.57a	80.15 ± 2.95a	78.80 ± 6.15a	83.79 ± 0.78a	75.37 ± 0.13a
Fructose	2.68 ± 0.08c	3.33 ± 0.15b	5.30 ± 0.20a	4.93 ± 0.06a	5.08 ± 0.08a
Glucose	3.35 ± 0.10c	4.05 ± 0.09b	6.93 ± 0.20a	6.70 ± 0.09a	6.22 ± 0.20a
Total sugars	89.83 ± 4.53a	87.53 ± 3.14a	91.03 ± 5.83a	95.43 ± 0.74a	86.67 ± 0.25a
K	19.80 ± 21.66d	675.55 ± 14.45a	253.10 ± 11.03b	138.29 ± 1.71c	273.53 ± 0.48b
Na	2.43 ± 0.04c	2.33 ± 0.11c	1.94 ± 0.09d	3.13 ± 0.07b	5.60 ± 0.15a
Ca	1.01 ± 0.08c	1.15 ± 0.06c	1.63 ± 0.12b	0.90 ± 0.11c	10.35 ± 0.13a
Mg	6.88 ± 0.11b	5.67 ± 0.30c	1.70 ± 0.11d	0.61 ± 0.04e	9.68 ± 0.54a
Fe	0.79 ± 0.02c	1.77 ± 0.04a	1.41 ± 0.78b	0.80 ± 0.04c	0.28 ± 0.01d
Cu	0.71 ± 0.01a	0.74 ± 0.01a	0.64 ± 0.02a	0.34 ± 0.02b	0.07 ± 0.06c
Zn	0.60 ± 0.01a	0.61 ± 0.01a	0.45 ± 0.02b	0.36 ± 0.01c	0.42 ± 0.15b
Total minerals	32.25 ± 1.62e	687.81 ± 14.37a	260.88 ± 10.89c	144.42 ± 1.85d	299.94 ± 0.84b

Values are expressed as the mean ± SD ($n = 3$) and values with different letter within the same row are significantly different ($p < 0.05$)

particular, its mineral content. As shown in Table 2, the different cane cultivars presented difference of mineral content values. K levels (19.80–675.55 mg/100 g) were the highest among all the minerals. K is an essential mineral necessary to balance fluids and other minerals in the body, and maintain a normal blood pressure (Aburto et al. 2013). K concentration in the product made from UT12 was the highest one (675.55 mg/100 g), followed by Mg, Na, and Ca. TP was prepared from sugarcane cultivar SP50, but there was a huge difference in K levels of TP and the developed NCS products. This might be due to variations in the post-harvest period and the geographic origin of the sugarcane cultivar (Qudsieh et al. 2001). The current daily recommendation for sugar intake is 4–8 teaspoons (16–32 g) depending on one's energetic needs (1600–2400 kcal/day). From our data, the amount of K obtained from NCS ranged between 3 and 27 mg per 4 teaspoons. Trace amounts of Cu and Zn were also found in the developed products.

Wax composition of NCS products from different cultivars

Derived from sugarcane wax, long-chain aliphatic alcohols (PCs) are of great interest in nutraceuticals owing to their beneficial health effects such as reducing platelet aggregation, reducing plasma low-density lipoprotein levels, and inhibiting cholesterol synthesis (Chen et al. 2008). Besides PCs, long-chain aldehydes were also found in plant natural waxes, and there was limited information in the literature concerning on the quantities of long-chain aldehydes in plants, particularly sugarcane and its by-products. Thus, both PC and long-chain aldehyde contents in the developed NCS products was investigated. This is the first report on PC and long chain aldehyde content in NCS products developed from various cultivars of Thai sugarcane.

The amount of PC and long-chain aldehydes in the NCS products from different cane cultivars is shown in Table 3. Excluding the product made from SP50, the predominant PC of the NCS products was octacosanol (C28), followed

Table 3 Wax composition (PCs and long chain aldehydes, mg/100 g) of NCS products from different sugarcane cultivars

Component	SP50	UT12	KK3	SRS2	TP
Docosanol	0.45 ± 0.07a	0.23 ± 0.03ab	0.24 ± 0.01ab	0.41 ± 0.13a	0.15 ± 0.02b
Tetracosanol	0.39 ± 0.01a	0.18 ± 0.03bc	0.15 ± 0.02c	0.24 ± 0.02b	0.17 ± 0.03bc
Hexacosanol	0.14 ± 0.00ab	0.10 ± 0.01b	0.12 ± 0.01b	0.14 ± 0.01b	0.19 ± 0.03a
Octacosanol	0.16 ± 0.01b	1.36 ± 0.25a	1.34 ± 0.02a	1.52 ± 0.15a	1.38 ± 0.27a
Triacontanol	0.86 ± 0.12a	0.70 ± 0.12ab	0.78 ± 0.01ab	0.84 ± 0.07a	0.51 ± 0.07b
Total PCs	2.00 ± 0.20b	2.58 ± 0.43ab	2.64 ± 0.06ab	3.14 ± 0.20a	2.41 ± 0.42ab
Tetracosanal	0.23 ± 0.12a	0.14 ± 0.01a	0.19 ± 0.02a	0.10 ± 0.02a	0.11 ± 0.02a
Hexacosanal	0.19 ± 0.04ab	0.15 ± 0.03bc	0.11 ± 0.02bc	0.27 ± 0.02a	0.08 ± 0.01c
Octacosanal	0.21 ± 0.02b	0.24 ± 0.04b	0.14 ± 0.01b	0.17 ± 0.03b	0.56 ± 0.14a
Triacontanal	ND	ND	ND	ND	0.27 ± 0.05
Total aldehydes	0.63 ± 0.18a	0.54 ± 0.08a	0.45 ± 0.04a	0.55 ± 0.05a	0.85 ± 0.20a

Values are expressed as the mean ± SD ($n = 3$) and values with different letter within the same row are significantly different ($p < 0.05$)

ND not detected

by triacontanol (C30). The current trend was in accordance with previous studies on sugarcane PC content, in which octacosanol was found to be the main component of sugarcane wax (Asikin et al. 2008; Irmak et al. 2006; Menendez et al. 2005). The product made from SP50 had the lowest PC level (2.00 mg/100 g), while that made from SRS2, the recent bred sugarcane cultivar, had the highest PC content (3.14 mg/100 g). The PC distribution of the developed product made from SRS2 was comprised of 13.1% docosanol (C22), 7.6% tetracosanol (C24), 4.5% hexacosanol (C26), 48.4% octacosanol (C28) and 26.8% triacontanol (C30). Meanwhile, that of SP50 was comprised of 22.5% docosanol (C22), 19.5% tetracosanol (C24), 7.0% hexacosanol (C26), 8.0% octacosanol (C28) and 43% triacontanol (C30). Asikin et al. (2008) found that the highest and the lowest total PC values of 7 different types of Japanese NCS products were 85.69 and 6.96 mg/100 g, respectively. The PC contents found in this study were somewhat lower than those in Japanese NCS products, which may be due to differences in the cane varieties and the manual peeling process. TP represented the traditional NCS produced by artisan farmers in a northern province of Thailand, which contained about 2.41 mg/100 g of PC (57.3% octacosanol), while UT12 and KK3 products contained 2.58 and 2.64 mg/100 g, respectively, of which about 52.7 and 50.8% was octacosanol, respectively.

The long-chain aldehyde content was lower than that of PC in all NCS products, and no triacontanal (C30) was detected in any of the brown sugars with the exception of TP (Table 3). In this study, although with no statistical difference, the highest long-chain aldehyde content was found in TP with 0.85 mg/100 g, comprised of 12.9% tetracosanal (C24), 9.4% hexacosanal (C26), 65.9% octacosanal (C28) and 31.8% triacontanal (C30). The GC data also revealed that the product made from KK3 has the lowest level of total long chain aldehydes (0.45 mg/100 g). In a previous study, very low levels of long-chain aldehydes in Japanese NCS products were also reported (Asikin et al. 2008). On the other hand, the developed NCS products in this study, regardless of which cane cultivar, contained a certain amount of long chain aldehydes. Compared to the traditional product made from SP50 (TP), there was no statistically significant difference in the total PC and long-chain aldehyde content between the developed NCS products, indicating that peeling the cane prior to NCS preparation has no significant effect on both PC and long chain aldehyde content. However, the previous results confirmed the high content of PCs and long-chain aldehydes in the rind rather than the pith of the sugarcane, and that the wax composition varies depending on the sugarcane cultivar, and on sugarcane maturity level (Asikin et al. 2012).

The total PC and long chain aldehyde contents (Table 3) were 2.63, 3.12, 3.09, 3.69, and 3.26 mg/100 g for SP50, UT12, KK3, SRS2, and TP, respectively. The data obtained here on the policosanol and long chain aldehyde contents of NCS products from various cultivars could be used, together with other indicators, for screening purposes in sugarcane breeding. The developed granulated NCS contains not only nutrients, but also cholesterol reducing bioactive compounds that provide specific health benefits beyond those of basic sweeteners. These functional sweeteners are a promising ingredient in functional food and beverage manufacturing.

Volatile aroma components of NCS products from different cultivars

Identified by their RIs and GC–MS spectra, a total of 24 volatile components were found in the NCS products

Table 4 Volatile aroma components (relative concentration, %) of NCS products from different sugarcane cultivars

No	RI[a]	Component	SP50	UT12	KK3	SRS2	TP	Identification[b]
1	697	Acetaldehyde	3.94 ± 0.08c	3.40 ± 0.22c	8.83 ± 0.38b	4.51 ± 0.97c	23.98 ± 0.72a	RI, MS, Std
2	738	Dimethyl sulfide	14.96 ± 2.42c	75.64 ± 0.98a	46.35 ± 1.74b	69.16 ± 2.16a	ND	RI, MS, Std
3	781	Propanal	ND	ND	1.02 ± 0.07	ND	ND	RI, MS, Std
4	807	2-Methyl propanal	ND	3.42 ± 0.21c	9.62 ± 3.06b	1.39 ± 0.14c	16.49 ± 0.34a	RI, MS, Std
5	894	Methanol	3.74 ± 0.39b	4.37 ± 0.21b	2.99 ± 0.58b	2.65 ± 0.43b	11.28 ± 0.72a	RI, MS, Std
6	907	2-Methyl butanal	0.56 ± 0.19d	2.67 ± 0.26c	3.89 ± 0.21b	1.26 ± 0.06d	6.35 ± 0.21a	RI, MS, Std
7	911	3-Methyl butanal	0.72 ± 0.32d	1.84 ± 0.12c	3.46 ± 0.28b	1.70 ± 0.14c	7.35 ± 0.23a	RI, MS, Std
8	925	Isopropyl alcohol	34.70 ± 4.74a	ND	7.59 ± 2.61b	ND	ND	RI, MS, Std
9	932	Ethanol	22.57 ± 5.61a	1.46 ± 0.19b	3.78 ± 0.49b	3.12 ± 0.68b	6.28 ± 0.23b	RI, MS, Std
10	970	2,3-Butanedione	ND	ND	ND	ND	1.65 ± 0.07	RI, MS, Std
11	973	Pentanal	2.11 ± 0.66a	ND	1.34 ± 0.03a	1.03 ± 0.35a	0.74 ± 0.15a	RI, MS, Std
12	998	Decane	3.41 ± 0.94a	0.67 ± 0.13b	2.61 ± 0.68a	1.21 ± 0.09b	1.05 ± 0.04b	RI, MS, Std
13	1077	Hexanal	0.96 ± 0.11b	0.44 ± 0.03c	2.04 ± 0.21a	0.98 ± 0.13b	0.62 ± 0.08bc	RI, MS, Std
14	1091	Undecane	1.66 ± 0.10a	0.32 ± 0.05b	ND	0.99 ± 0.34ab	ND	RI, MS, Std
15	1192	Dodecane	2.36 ± 0.23a	0.86 ± 0.14b	0.90 ± 0.30b	1.82 ± 0.45ab	ND	RI, MS, Std
16	1263	Dihydro-2-methyl-3(2H)-furanone	ND	ND	ND	ND	2.85 ± 0.19	RI, MS, Std
17	1293	Tridecane	1.73 ± 0.23a	0.84 ± 0.12b	1.28 ± 0.23ab	1.52 ± 0.25ab	ND	RI, MS, Std
18	1301	1-Hydroxy-2-propanone	ND	ND	ND	ND	1.07 ± 0.07	RI, MS, Std
19	1456	Acetic acid	1.13 ± 0.42b	ND	ND	ND	10.90 ± 1.33a	RI, MS, Std
20	1467	2-Furaldehyde	ND	0.76 ± 0.12b	ND	ND	5.06 ± 0.08a	RI, MS, Std
21	1512	2-Acetyl-furan	ND	ND	ND	ND	0.63 ± 0.10	RI, MS
22	1635	Butanoic acid	1.03 ± 0.41	ND	ND	ND	ND	RI, MS, Std
23	1658	2-Propenoic acid	0.47 ± 0.10b	1.96 ± 0.35ab	ND	5.30 ± 0.85a	ND	RI, MS
24	1679	3-Methyl butanoic acid	ND	ND	ND	ND	0.98 ± 0.09	RI, MS, Std
Total relative percentage			96.03 ± 0.27	96.68 ± 0.35	95.72 ± 0.67	96.65 ± 0.37	97.28 ± 0.11	
Total identified (peak area 1.E + 06)			3.25 ± 0.39	8.00 ± 1.16	3.24 ± 1.16	3.90 ± 0.57	4.70 ± 0.39	

Values are expressed as the mean ± SD ($n = 3$) and values with different letter within the same row are significantly different ($p < 0.05$)

ND not detected

[a] Retention indices relative to *n*-alkanes on a polar DB-Wax column

[b] Identification based on retention index (RI), NIST MS library (MS), and authentic standards analyzed by mass spectrometry (Std)

produced from different cane cultivars. These volatile components comprised 7 aldehydes, 5 alcohols, 4 acids, 4 hydrocarbons, 3 Maillard reaction products (MRPs), and 1 sulfur component (Table 4), which represent 96.03–96.72% of the total volatile components. The MRPs included dihydro-2-methyl-3(2H)-furanone, 2-furaldehyde, and 2-acetyl-furan. Whereas 16 volatile molecules were identified for the NCS from SP50, 14 volatile compounds were detected for the NCS products from UT12, KK3 and SRS2. Although 16 constituents were present in the samples made from SP50, including TP, the NCS made from UT12 had the highest flavor intensity (8.0 E + 0.6), represented by the total peak area response, indicative of a more potent volatile flavor. The main volatile aroma component of the NCS products made from UT12, KK3, and SRS2 was dimethyl sulfide, while that of the SP50 sugar was isopropyl alcohol. Remarkably, both dimethyl sulfide and isopropyl alcohol were not found in the TP sample.

It appears that, among the developed NCS products, only UT12 contained an MRP component, 2-furaldehyde (0.76%), while the traditional product TP contained a significant amount of MRPs (8.54%). These sugary flavor components were formed during NCS production via non-enzymatic Maillard reactions (Asikin et al. 2014; Cho et al. 2010). Regarding its relative percentage to the total peak area of the aroma compounds, the SP50 cultivar presented more than half its content (63.5%) as alcohol components, followed by sulfur compounds (15.6%). The major volatile compound in the developed NCS products was found to be a sulfur compound (48.8, 78.2, and 69.2% for KK3, UT12, and SRS2, respectively), followed by aldehyde compounds

(31.6, 11.5, and 11.2%, respectively). Although it was made from the same cultivar (SP50), the main components of NCS TP were aldehydes rather than alcohols (which were the second main component). Regarding the alcohols, isopropyl alcohol was the major volatile compound of the developed NCS product from SP50, while methanol was found to be the major compound in TP. In terms of aldehyde aroma compounds, acetaldehyde was the major flavor in TP and the SP50, UT12, and SRS2 products, while 2-methyl propanal was the major volatile in the NCS product made from KK3. Each volatile compound in the NCS samples presents the overall aroma character and quality. The published literature indicates that predominant acetaldehyde gives an ethereal, fresh, green, pungent-like smell, whereas dimethyl sulfide provides an intense, lactone-like, sulfurous, cabbage aroma (Cheng 2010). A combination of other volatile constituents gives complex odor characteristics to the developed NCS products.

The volatile profiles of the developed NCS products and TP in this study were different from the flavor profiles of previously reported raw cane and NCS products (Asikin et al. 2014, 2016; Payet et al. 2005). This might be due to the shorter heating duration employed (15–20 min) this study, compared to that of the TP manufacturing process. The chemical reaction process of flavor generation, therefore, differs from those in previous studies. Flavor is one of the most important properties in food products, and is an important factor in the determination of its acceptance and preference by the consumers. Consequently, the different aroma profiles of NCS—including acidic aroma or roasted flavor—, which are closely related to the different cane cultivars, play an important role in the manufacture of brown sugar-based food and beverage products, such as bakery, confectionary, and fruit juice products.

Antioxidant activity of NCS products from different cultivars

The overall results of the phenolic content and antioxidant activity of granulated NCS products from different cultivars are shown in Table 5. Compared to the developed NCS products, statistical analysis showed that the traditional sugar product (TP) has the highest total phenolic (13.95 mg GAE/g) and flavonoid (2.09 mg RAE/g) contents, and that there were statistically significant differences ($p < 0.05$). The results here also indicate that peeling the outer layer rind before cane juice extraction significantly affected the total phenolic content, total flavonoid content, and the antioxidant activity of the final NCS products. Sugarcane juice contained pigments made from phenolic, phenylpropanoid and flavonoid compounds such as naringenin, tricin, apigenin and luteolin. The whole cane stalks consisted of phenolic compounds mainly as phenylpropanoids, including caffeic, chlorogenic and coumaric acids, and the slight level of flavones such as apigenin, tricin and luteolin derivatives (Duarte-Almeida et al. 2011). Feng et al. (2014) reported that sugarcane rind contained higher total phenolic and total flavonoid content than the other parts of the sugarcane stalk.

NCS product made from SP50 had the lowest total phenolic and flavonoid content, and antioxidant value. Consequently, these factors significantly influenced the antioxidant activity of the NCS products as determined from the DPPH radical-scavenging activity assay ($p < 0.05$). Positive correlations have been found between antioxidant activity against DPPH radicals and the total phenolic and flavonoid content in NCS products (Pearson's coefficients $R^2 = 0.830$ and 0.774, respectively). The phenolic compounds in the NCS products were thus strongly related to their antioxidant activity. The results suggested that different sugarcane cultivars resulted in different polyphenol content and antioxidant activity in brown sugar products. Duarte-Almeida et al. (2011) reported the variation of phenolic profiles and antioxidant capacity of culms and sugar cane products from three varieties of sugarcane. Moreover, the results by Payet et al. (2005) suggested that the difference between commercial sugar products from various cane cultivars influenced the polyphenol content and scavenging properties of sugar products.

Conclusion

The use of different sugarcane cultivars had an impact on the physical and chemical properties of the final sugar products, including the polyphenol content and antioxidant activity. The PC contents of the developed granulated NCS products, produced with an additional rind peeling process, was slightly different from that of the traditionally

Table 5 Antioxidant activity of NCS products from different sugarcane cultivars

Property	SP50	UT12	KK3	SRS2	TP
Total phenolic content (mg GAE/g)	2.33 ± 0.05c	3.71 ± 0.07b	3.42 ± 0.08b	3.00 ± 0.03bc	13.95 ± 0.44a
Total flavonoid content (mg RAE/g)	0.03 ± 0.01b	0.05 ± 0.01b	0.04 ± 0.01b	0.03 ± 0.01b	2.09 ± 0.03a
DPPH radical-scavenging activity (% inhibition)	38.04 ± 0.51d	59.65 ± 0.60b	52.40 ± 0.63c	50.62 ± 0.46c	71.08 ± 0.52a

Values are expressed as the mean ± SD ($n = 3$) and values with different letter within the same row are significantly different ($p < 0.05$)

processed NCS (TP). The major volatile components of the developed NCS products were acetaldehyde and dimethyl sulfide. Aldehydes, alcohols, sulfur, and hydrocarbon aroma compounds contributed to the overall aroma quality of the granulated NCS products. Understanding the variation in volatile composition and characteristics, as well as other chemical properties of NCS products, plays a key role in the formulation of brown sugar-based food and beverage products, as well as in cane cultivar breeding.

Author contributions Both MW and YA initiated the study conception and design and the analysis and interpretation of data, and drafted the manuscript; MK performed the wax composition analysis; HT, KW, and C-TH participated in the design of the study and revised the manuscript; RC performed the physico-chemical properties analyses.

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Compliance with ethical standards

Conflicts of interest The authors declare that there is no conflict of interest.

References

Aburto NJ, Hanson S, Gutierrez H, Hooper L, Elliott P, Cappuccio FP (2013) Effect of increased potassium intake on cardiovascular risk factors and disease: systematic review and meta-analyses. BMJ 346:f1378

Asikin Y, Chinen T, Takara K, Wada K (2008) Determination of long-chain alcohol and aldehyde contents in the non-centrifuged cane sugar Kokuto. Food Sci Technol Res 14:583–588

Asikin Y, Takahashi M, Hirose N, Hou DX, Takara K, Wada K (2012) Wax, policosanol, and long-chain aldehydes of different sugarcane (*Saccharum officinarum* L.) cultivars. Eur J Lipid Sci Technol 114:583–591

Asikin Y, Kamiya A, Mizu M, Takara K, Tamaki H, Wada K (2014) Changes in the physicochemical characteristics, including flavor components and Maillard reaction products, of non-centrifugal cane brown sugar during storage. Food Chem 149:170–177

Asikin Y, Hirose N, Tamaki H, Ito S, Oku H, Wada K (2016) Effects of different drying–solidification processes on physical properties, volatile fraction, and antioxidant activity of non-centrifugal cane brown sugar. LWT-Food Sci Technol 66:340–347

Association of Official Analytical Chemists (AOAC) (2005) Official methods of analysis of the AOAC, 18th edn. AOAC, Gaithersburg

Chen ZY, Jiao R, Ma KY (2008) Cholesterol-lowering nutraceuticals and functional foods. J Agric Food Chem 56:8761–8773

Chen Y, Dunford NT, Edward J, Carver B, Goad C (2009) Policosanol contents and composition of wheat varieties as affected by environment. J Sci Food Agric 89:310–314

Cheng H (2010) Volatile flavor compounds in yogurt: a review. Crit Rev Food Sci Nutr 50:938–950

Cho I, Lee S, Jun H, Roh H, Kim Y (2010) Comparison of volatile Maillard reaction products from tagatose and other reducing sugars with amino acids. Food Sci Biotechnol 19:431–438

Duarte-Almeida JM, Negri G, Salatino A, de Carvalho JE, Lajolo FM (2007) Antiproliferative and antioxidant activities of a tricin acylated glycoside from sugarcane (*Saccharum officinarum*) juice. Phytochem 68:1165–1171

Duarte-Almeida JM, Salatino A, Genovese MI, Lajolo FM (2011) Phenolic composition and antioxidant activity of culms and sugarcane (*Saccharum officinarum* L.) products. Food Chem 125:660–664

Feng S, Luo Z, Zhang Y, Zhong Z, Lu B (2014) Phytochemical contents and antioxidant capacities of different parts of two sugarcane (*Saccharum officinarum* L.) cultivars. Food Chem 151:452–458

Food and Agriculture Organization of the United Nations (FAO) (2016) Sugarcane production quantities by country (1993–2014). http://faostat3.fao.org/browse/Q/QC/E. Accessed 16 Feb 2016

ICUMSA (2003) ICUMSA methods book supplement 2002. Verlag Dr. Albert Bartens KG, Berlin

Irmak S, Dunford NT (2005) Policosanol contents and compositions of wheat varieties. J Agric Food Chem 53:5583–5586

Irmak S, Dunford NT, Milligan J (2006) Policosanol contents of beewax, sugar cane and wheat extracts. Food Chem 95:312–318

Jaffé WR (2012) Health effects of non-centrifugal sugar (NCS): a review. Sugar Technol 14:87–94

Meda A, Lamien CE, Romito M (2005) Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chem 91:571–577

Medini F, Fellah H, Ksouri R, Abdelly C (2014) Total phenolic, flavonoid and tannin contents and antioxidant andantimicrobial activities of organic extracts of shoots of the plant *Limonium delicatulum*. J Taibah Univ Sci 8:216–224

Menendez R, Marrero D, Mas R, Fernandez I, Gonzalez L, Gonzalez R (2005) In vitro and in vivo study of octacosanol metabolism. Arch Med Res 36:113–119

Payet B, Cheong AS, Smadja J (2005) Assessment of antioxidant activity of cane brown sugars by ABTS and DPPH radical scavenging assays: determination of their polyphenolic and volatile constituents. J Agric Food Chem 53:10074–10079

Pérez-Camino MC, Moreda W, Mateos R, Cert A (2003) Simultaneous determination of long-chain aliphatic aldehydes and waxes in olive oils. J Chromatogr A 983:283–288

Qudsieh HYM, Yusof S, Osman A, Rahman RA (2001) Physicochemical changes in sugarcane (*Saccharum officinarum* var yellow cane) and the extracted juice at different portions of the stem during development and maturation. Food Chem 75:131–137

Takara K, Otsuka K, Wada K, Iwasaki H, Yamashita M (2007) 1,1-Diphenyl-2-picrylhydrazyl radical scavenging activity and tyrosinase inhibitory effects of constituents of sugarcane molasses. Biosci Biotechnol Biochem 71:183–191

Thakur AK (1999) Potential of jaggery (gur) manufacturing in Punjab state. In: Proceedings of the National Seminar on Status, problems and prospects of jaggery and Khandsari Industry in India. Indian Institute of Sugarcane Research, Lucknow, pp 70–76

Wang MF, Lian HZ, Mao L, Zhou JP, Gong HJ, Qian BY, Fang Y, Li J (2007) Comparison of various extraction methods for policosanol from rice bran wax and establishment of chromatographic fingerprint of policosanol. J Agric Food Chem 55:5552–5558

Weerawatanakorn M (2013) Dicarbonyl compounds and sugar contents of Thai commercial beverages. Songklanakarin J Sci Technol 35:631–639