Australian Journal of Crop Science AJCS

AJCS 15(02):187-195 (2021) ISSN:1835-2707
doi: 10.21475/ajcs.21.15.02.p2270

The relationship between soil chemical properties and phytochemical contents of non-centrifugal cane brown sugar in Thailand

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Abstract

In Thailand, NCS products from sugarcane are traditionally produced by the evaporation of raw cane juice obtained from a pressing machine. NCS products enhance flavor and supply both energy and nutritional value through the vitamins, minerals and bioactive compounds which occur naturally in sugarcane produced without molasses removal. Soil quality and choice of cane cultivar affect the nutraceutical components of brown cane sugar products grown in diverse areas. Differences in nutraceutical components of NCS products obtained by traditional processes were investigated form sugarcane planted in two different areas of Sukhothai Province, with varied soil qualities based on the same cane variety (LK-92-11). The soil classification was carried out according to the Staff Soil Survey (2014) and classified as Aquic Haplustalfs and Aeric Endoaquepts soil types. NCS products from sugarcane planted in Aquic Haplustalfs contained high nutraceuticals and antioxidant activities compared with sugarcane planted in Aeric Endoaquepts. EC and potassium content of the soils showed significant negative correlation with policosanols, total phenolics, total flavonoid contents, and antioxidant activities of the NCS products. Pearson's correlation indicated that higher electrical conductivity and potassium content of the soils were attributable to lower amounts of policosanols, total phenolics, and total flavonoid contents as well as antioxidant activities. We suggested that electrical conductivity and potassium content of soil should be concerned and monitored to obtain high level of nutraceutical of NCS product made from sugarcane.

Keywords: Sugarcane; nutraceuticals; soil quality; brown sugar products; antioxidant.
Abbreviations: NCS_non-centrifugal sugar; EC_Electrical conductivity; PC_Policosanols.

Introduction

Sugarcane (*Saccharum officinarum* L.) is the most important feedstock of sugar production since it provides nearly 70% of the sugar consumed worldwide (Feng et al., 2014). Increased health risks from excessive intake of refined sugar have sparked global interest for healthier sugar substitutes (García et al., 2017). Brown cane sugar is the most appealing commercial substitute because of its various biological functions with beneficial activities on human health (Jaffe, 2012; Asikin et al., 2014). NCS product or brown cane sugar is available in many countries under different names as Gur (India), Panela (Mexico and South America), Jaggery (Africa), Rapadura (Brazil), Kokuto or Kurozato (Japan), and Naam Taan Oi (Thailand) (Weerawatanakorn et al., 2016). According to the FAO (1994), non-centrifugal sugar product is the technical term for brown cane sugar. NCS is traditionally produced by dehydrating the sugarcane juice without molasses removal. This significantly reduces the loss of natural minerals, vitamins, and phytochemicals (Weerawatanakorn et al., 2016).

The unique brown color of NCS is generated by chemical reactions involving non-enzymatic browning (Maillard reaction) and caramellization (Takahashi et al., 2016), while the presence of phenolic compounds, mainly phenylpropanoids and flavonoids, also plays an important role in brown color generation (Duarte-Almeida et al., 2007; Iqbal et al., 2017). The NCS contains nutrients including amino acids, minerals, vitamins, and bioactive compounds such as phenolic acids (chlorogenic, caffeic and coumaric acids), flavonoids (derivatives of tricin, apigenin and luteolin) (Takara et al., 2002), and long-chain alcohols, known as policosanols (PC). The various beneficial biological functions regarding human health have been reported for NCS products including anti-cariogenic, antitoxic-cytoprotective, anti-carcinogenic, and antioxidant effects (Payet et al., 2005; Jaffe, 2012). Among several flavonoid derivatives isolated from NCS extracts, tricin showed *in vitro* antiproliferative activity against several human cancer cell lines (Duarte-Almeida et al., 2007) and may be considered safe enough for clinical development as a cancer chemopreventive agent (Verschoyle et al., 2006). Other interesting components in NCS including PC and long-chain aldehydes (policosanals) have also been reported. PC comprise a group of long-chain (C22-C34) aliphatic primary alcohols that possess cholesterol and lipid-reducing properties (Weerawatanakorn et al., 2016). They are mainly found in sugarcane wax, especially in the rind of the stalk (Nuissier et al., 2002; Purcell et al., 2005) and remain in brown cane sugar if production lacks the refining step (Asikin et al., 2008). The

PC content of Japanese NCS products, known there as "Kokuto", and Thai NCS, known as Nam Tann Oi ranged from 7.0-85.7 and 2.6-3.7 mg/100g, respectively (Asikin et al., 2008; Weerawatanakorn et al., 2016).
Thailand is among the world's leading sugarcane producers and refined sugar exporters (FAO 2017). Sugarcane is a significant Thai economic plant (Prasara et al., 2016) and crop sustainability must be maintained. Soil quality is an important criterion to realize yield and quality of sugarcane and cane products (Cherubin et al., 2016). Many factors including genotype, ontogeny, and environment affect amounts and types of phytochemicals in a plant. Few information have been made to link soil properties to phytochemical contents in crops and final food products (Zhao et al., 2006). Therefore, the effect of soil quality variation in two different sub-districts was assessed regarding the nutraceutical contents including PC, tricin, phenolic compounds and antioxidant activity of sugar products produced by traditional methods of evaporation in an open pan. Soil characteristics, physicochemical properties, antioxidant activity, policosanol and tricin contents of NCS products (Nam Tann Oi) were analyzed to determine any significant relationships.

Results and Discussion

Chemical properties of soil in sugarcane field

Two sugarcane field areas in Sukhothai Province were selected to analyze soil qualities. The first area was Koh Ta Liang Sub-district, Srisomrong District (SR) and the second was Yanyaow Sub-district, Sawankhalok District (SK). Following the Soil Survey Division Staff, (2014), soil from the first area was classified as Aeric Endoaquepts. The soils were composed of very deep clay that formed in flood basin deposits on alluvial plains. The study site was a flat plain with waterlogged soil condition during the rainy season. It had a clay texture with black or gray surface color. Subsoil color was light brown to brown with mottles of yellowish brown, reddish brown and red with limestone, iron, and manganese (Fig 1b.). Based on chemical properties (Table 1.), the top soil (0-20 cm depth) had an organic matter content of 1.7%, a total N content of 0.05%, an available P (Bray II) content of 3.97 mg kg^{-1} and an exchangeable K content of 68.4 mg kg^{-1}. Therefore, sugarcane grown in Aeric Endoaquepts soil was unsuitable with limitations of waterlogging based on Agricultural Economics Crop Zoning data that prepared by the Land Development Department (Ministry of Agriculture and Cooperatives, 2018). Waterlogged condition has an impact on agro-morphological traits leading to decrease of sugarcane productivity (Islam et al., 2011; Anitha et al., 2016; Khaiyam et al., 2018;).
The soil from the second area (Yanyaow Sub-district, Sawankhalok District) was classified as Aquic Haplustalfs soil derived from alluvial fans. Data (Table 1) shows that the soil had a moderate permeability and vegetation with land application currently as sugarcane. Soil characteristics and properties indicated that topsoil and subsoil textures were silty loam to silty clay. Soil color was brownish brown and dark brown (Fig 1a.). Based on chemical properties, the topsoil (0-20 cm depth) had relatively high content of organic matter (2.5%), while nitrogen (0.05%) and exchangeable potassium content (26.2 mg kg^{-1}) were very low. Available phosphorus content (10.61 mg kg^{-1}) was at a moderate level. The Aquic Haplustalfs soil was more suitable for sugarcane planting because of silty loam to silty clay texture compared to Aeric Endoaquepts soil (Jongskul, 2016).
Moreover, Aquic Haplustalfs soil of Yanyaow Sub-district had higher soil organic matter content compared to soil of Koh Ta Liang Sub-district. Bokhtiar (2015) mentioned that 2.5 to 3.0% soil organic matter was necessary for sustainable sugarcane production.

Physicochemical properties of NCS products

Following the traditional evaporation process, phytochemical compounds of NCS products made from sugarcane cultivar LK-92-11 grown under the two different soil qualities, were evaluated. NCS products were made every week for three weeks during February 2016. Sugarcane was harvested from Koh Ta Liang Sub-district (SR1-3) and Yanyaow Sub-district (SK1-3). The obtained NCS products (Fig 2.) were processed to analyze physicochemical properties and phytochemical contents. Total soluble solids (Brix) and pH of raw cane juice from LK-92-11 cane cultivar grown in the two different areas were assessed as 21.8-22.0 ^{o}B and pH 4.6-5.0, respectively. Juice analysis also indicated that the pH of raw cane juice form sugarcane planted in Aquic Haplustalfs ranged from 4.6 to 5.2 and in Aeric Endoaquepts was 5.0 (data not shown). Total soluble solid contents indicating the sweetness of NCS products obtained from two areas are slightly different since the major constituent of non-centrifugal brown sugar is sucrose (80-87%) following monosaccharide including fructose and glucose (Simko and Polovkov, 2017; Asikin et al., 2017).
Physicochemical properties of NCS products (Table 2.) indicated that color value of sugarcane product from Aquic Haplustalfs soil (40,200-36,566 IU) was significantly ($p \leq 0.05$) higher than sugarcane from Aeric Endoaquepts soil (14,700-20,533 IU). Color formation of NCS products is caused by four different mechanisms including: (1) melanoidin from Maillard reaction; (2) caramelization from thermal degradation of sugar; (3) degradation of reducing sugar; (4) oxidative reaction of phenolic compound (Carolyn & Bucheli, 1994). Phenolic and flavonoid pigments therefore retained in final products play an important role on color of NCS products (Payet et al., 2005). With the same process condition, total phenolic and flavonoid contents of NCS products produced by cane from Aquic Haplustalfs (Yanyaow Sub-district) were higher than sugarcane from Aeric Endoaquepts soil (Koh Ta Liang Sub-district) (Table 3).
Wax content is an indicator of policosanol contents of NCS products from sugarcane. The wax content of NCS product from the Aquic Haplustalfs soil (2.61-3.00%) showed higher than one from the Aeric Endoaquepts (1.59-2.04%). The amount of sugarcane surface wax is cultivar dependent and affected by the maturity stage, extract juice process, nutrients in the soil and time of harvesting (Colombo et al., 2005). Total acidity of NCS products in sugarcane from the Aquic Haplustalfs soil (0.89-1.10%) was also higher than products from the Aeric Endoaquepts (0.30-0.35%). This was in agreement with pH of raw cane juice found in the study but it was contradict to the pH of soil in the two areas. The result might imply that the more acidic of raw juice and products is not contributed to the more acidic property of soil. Zambrosi (2014) found that soil pH affects phosphorus availability for plant uptake. Low soil pH (acidic condition) severely limits phosphorus availability to plants, which may cause deficiency symptoms and link to plant growth (Zambrosi et al, 2014). The result showed that the Aeric Endoaquepts soil (pH of 5.1) is more acidic than Aquic Haplustalfs soil (pH of 6.2) and the phosphorus content of Aeric Endoaquepts soil was lower than one of Aquic Haplustalfs soil (Table 1).

Effect of soil properties in the two areas on policosanol and tricin components of NCS products

Policosanols comprise a group of long-chain aliphatic primary alcohols (C22-C30) as characteristic nutraceuticals in sugarcane. They exhibit health promotion by reducing platelet aggregation as well as serum low-density lipoprotein levels, and inhibiting cholesterol synthesis, leading to the risk reduction of atherosclerosis (Noa & Mas, 2005; Singh et al., 2006). An overview of the beneficial health effects of C-28 has also been reported (Taylor et al., 2003). The flavonoid tricin is a nutraceutical not normally found in the most consumed plant food, which has been reported in rice, cereal, and sugarcane (Irmak et al., 2006). Tricin exhibits chemopreventive properties as growth inhibition of human malignant breast tumor cells and protection of murine gastrointestinal carcinogenesis (Cai et al., 2005; Verschoyle et al., 2006). Compositions of PC contents of all NCS products (Table 3.) showed oxtacosanol (C28-OH) and triacosanol (C30-OH) as the main components. PC contents of NCS products from the Aquic Haplustalfs soil (8.80-9.38 mg 100 g^{-1}) were higher than from the Aeric Endoaquepts soil (7.80-8.17 mg $100g^{-1}$). The two different soil types gave sugar products with significant differences ($p \leq 0.05$) in PC contents. This result was in accordance with wax contents in Table 2. Tricin contents of NCS products from the Aeric Endoaquepts soil (113.39-124.83 µg $100g^{-1}$) were higher than from the Aquic Haplustalfs soil (96.58-111.43 µg $100g^{-1}$) (Table 3.). This phenomenon suggested that soil properties affected PC and tricin contents in NCS products.

Total phenolic contents (TPC), total flavonoid contents (TFC) and antioxidant properties of NCS

Other indicators of nutraceutical food product compounds include total phenolic contents (TPC), total flavonoid contents (TFC) and antioxidant activities. TPC (35.76-38.37 mg GAE $100g^{-1}$) and TFC (27.35-31.46 mg RUE 100 g^{-1}) of NCS products from the Aquic Haplustalfs soil were higher than from the Aeric Endoaquepts soil (17.69-23.28 mg GAE $100g^{-1}$ and 12.28-17.01 mg RUE 100 g^{-1}, respectively) (Table 3). TPC of NCS products found here were much lower than values reported by Payet et al. (2005) for commercial brown sugars produced in different countries (117.4 to 418.1 mg GAE $100g^{-1}$) (Nayaka et al., 2009), for Jaggery, an Indian NCS, (383 mg GEA $100g^{-1}$) and for Kokuto, a Japanese NCS (92.88 to 154.92 mg GAE $100g^{-1}$) (Takahashi et al., 2016). This might be due to differences in phenolic and flavonoid extraction methods such as duration, solvent, time and sugarcane varieties. Radical scavenging activities of the two different NCS products were determined by DPPH, FRAP and ORAC assay. The NCS products from the Aquic Haplustalfs soil showed high antioxidant activity (DPPH, FRAP and ORAC radical scavenging activity) compared with product from the Aeric Endoaquepts soil. Antioxidant activities concurred with results of TPC and TFC contents. Higher bioactivities as antioxidant activities were attributed to large amounts of bioactive compounds. The results suggested that NCS product from Aquic Haplustalfs soil contain high value of nutraceutucal contents and bioactivity as antioxidant activity compared with product from the Aeric Endoaquepts soil. In addition, the high value of phytochemicals and antioxidant activities might be from the higher soil organic matter content of Aquic Haplustalfs soil compared with the Aeric Endoaquepts soil.

Pearson's correlation between soil properties and phytochemical components of NCS from the two different soil types

Pearson's correlation was used to evaluate the relationship between soil properties and nutraceutical contents of the NCS products. Results are shown in Table 4. Except for tricin, PC contents and other nutraceutical indicators including TFC, TFC, and antioxidant activities by, DPPH, FRAP and ORAC assay showed negative correlation with soil electrical conductivity (EC) and exchangeable potassium content (K). This suggested that soil with higher EC and exchangeable K contents produced sugarcane and NCS products with lower nutraceutical and antioxidant activities. The relationship of soil component and nutraceutical contents is shown in Fig.3. The EC and exchangeable K values of the Aquic Haplustalfs soil were 0.02 ds m^{-1} and 26.2-17.1 mg kg^{-1}, respectively compared with the Aeric Endoaquepts soil at 0.03-0.05 ds m^{-1} and 68.4-16.3 mg kg^{-1}, respectively. The recent result was disagreement with the report of Dumas (2003) who reported that potassium deficiency might result in a decrease of lycopene level in tomato (Dumas et al., 2003).

On the contrary, the pH value, available phosphorus content (P), and organic matter (OM) had a strong positive correlation with PC content and other nutraceutical indicators including TFC, TFC, and antioxidant activities by, DPPH, FRAP and ORAC assay, except for tricin contents. The pH value, available phosphorus content, and organic matter of the Aquic Haplustalfs soil were 6.2-6.1 (60 meter depth), 10.61-1.77 mg kg^{-1}, and 2.1-2.5%, respectively whereas those of the Aeric Endoaquepts soil were 5.1-5.7, 3.9-2.6 mg kg^{-1}, and 1.3-1.7%, respectively. To date, little information in the literature has been given to link soil conditions to phytochemical, the secondary metabolites with potential health-promoting effect of crop. Oliveira (2003) concluded that amount of carotenoid in grapes (*Vitis vinifera*) were more affected by soil characteristics than irrigation. There was a 60% reduction of carotenoids in irrigated grapes grown on a lower-water-retention soil compared to non-irrigated grapes (Oliveira et al., 2003). Mozafar (1993) reported that carotenoid content in fruit and vegetables tended to increase with higher nitrogen value (Mozafar, 1993). The result that Aeric Endoaquepts soil was characterized as the limitations of waterlogging and lower nitrogen value might explain that NCS product from Aeric Endoaquepts soil contain low value of phytochemical contents and bioactivity as antioxidant activity compared with product from the Aquic Haplustalfs soil. Given the limitations of most published studies, the future researchs are required.

Materials and Methods

Plant materials and Study area

The experimental field was located in Sukhothai Province, Thailand. Two areas of the natural cane field environment were planted with the same sugarcane cultivar of LK-92-11 under similar crop management to assess differences in soil quality. The farmers were asked to apply the same crop management. Fertilizer of NPK (15-15-15) at a rate of 21 kg N ha^{-1}, together with urea (46-0-0) at a rate of 65 kg N ha^{-1} were applied only one time. The first area was Koh Ta Liang Sub-district (17°13'36.7"N and 99°86'09.3"E), Srisomrong

Table 1. Soil physicochemical properties of Aeric Endoaquepts and Aquic Haplustalfs.

Physicochemical properties	Depth (cm)	Organic matter (%)	Nitrogen (%)	Available phosphorus (mg kg^{-1})	Exchangeable potassium (mg kg^{-1})
Aeric Endoaquepts (Kautaliang Sub-district, Srisomrong District (SR)) (x = 0591578, y = 1894546)					
	0-20	1.7 (medium)	0.05 (very low)	3.97 (low)	68.4 (medium)
	20-40	1.3 (rather low)	0.03 (very low)	2.64 (very low)	16.3 (very low)
		1.1 (rather low)	0.05 (very low)	3.84 (very low)	9.7 (very low)
	60-80	1.0 (rather low)	0.05 (very low)	4.54 (low)	10.0 (very low)
	80-100	1.2 (rather low)	0.03 (very low)	2.83 (low)	11.1 (very low)
	100-120	1.0 (rather low)	0.03 (very low)	2.61 (very low)	11.1 (very low)
	120-140	0.8 (low)	0.03 (very low)	2.83 (very low)	9.6 (very low)
Aquic Haplustalfs (Yanyaow Sub-district, Sawankhalok District (SK)) (x = 0587717, y = 1910831)					
	0-20	2.5 (relatively high)	0.05 (very low)	10.61 (medium)	26.2 (very low)
	20-40	2.1 (medium)	0.05 (very low)	13.62 (medium)	17.1 (very low)
	40-60	1.1 (rather low)	0.03 (very low)	1.77 (very low)	1.1 (very low)
	60-80	0.7 (low)	0.03 (very low)	4.26 (low)	0.7 (very low)
	80-100	1.0 (rather low	0.03 (very low)	1.38 (very low)	1.0 (very low)
	100-120	0.9 (low)	0.03 (very low)	2.41 (very low)	0.9 (very low)

Fig 1. Soil types from a: Yanyaow Sub-district, Sawankhalok District as Si Satchanalai (Aquic Haplustalfs) (x = 0587717,y = 1910831), and b: Kautaliang Sub-district, Srisomrong District as Chai Nat (Aeric Endoaquepts) (x = 0591578,y =1894546).

Table 2. Physicochemical properties of non-centrifugal sugarcane products from the two different soil qualities.

NCS product	ICUMSA color (IU)	Moisture content (%)	Water activity ^{ns}	Total acidity (%)^{ns}	Wax content (%)
SK1	40200± 0.07d	8.99±0.09b	0.52±0.20	0.89±0.01	3.00±1.19d
SK2	36566±0.05c	9.59±0.03a	0.56±0.10	1.07±0.00	3.87±2.37e
SK3	39500±0.01d	9.06±0.15ab	0.57±0.10	1.10±0.01	2.61±0.82c
SR1	14700±0.01a	7.08±0.30c	0.52±0.01	0.37±0.01	1.59±0.26a
SR2	15900±0.02a	6.00±0.38d	0.48±0.01	0.30±0.01	2.04±0.12b
SR3	20533± 0.02b	8.68±0.11b	0.52±0.02	0.35±0.01	1.64±0.08a

Values are the mean ± SD, n=3. Means in the same column with same superscript letter are not significant at p<0.05.
SK = sugar products from cane planting in Si Satchanalai (Aquic Haplustalfs); SR = sugar products from cane planting in Chai Nat (Aeric Endoaquepts); NS = non-significant difference; NCS products were produced and corrected for three consecutive analyses.

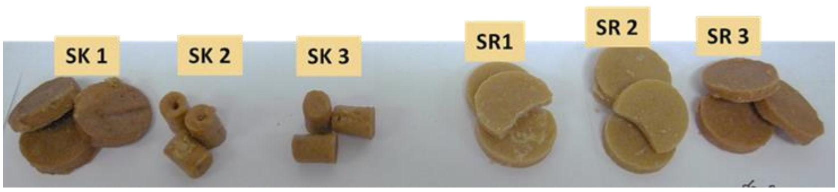

Fig 2. Non-centrifugal sugar products produced by sugarcane cultivar of LK-92-11 harvested from different soil types (SK1-SK3 from Si Satchanalai (Aquic Haplustalfs) and SR1-SR3 from Chai Nat (Aeric Endoaquepts).

Table 3. Nutraceutical contents and antioxidant activity of NCS products from the two different soil qualities.

Nutraceutical	NCS products					
	SK1	SK2	SK3	SR1	SR2	SR3
Policosanol						
Docosanol (C-22)	1.34±0.12	1.35±0.02	1.47±0.02	1.42±0. 08	1.08±0.19	0.18±0.06
Tetracosanol (C -24)	0.46±0.01	0.51±0.06	0.52±0.02	0.66±0.30	0.52±0.01	0.14±0.02
Hexacosanol (C-26)	0.58±0.09	0.61±0.05	0.58±0.03	0.62±0.13	0.58±0.02	0.29±0.04
Octacosanol (C -28)	4.20±0.25	4.14±0.08	3.90±0.06	3. 04±0.10	3.00±0.22	2.73±0.26
Triacontanol (C-30)	1.97±0.08	2.07±0.14	1.54±0.23	1.86±0.52	1.74±0.04	1.29±0.34
Total policosanol (mg $100g^{-1}$ wb)	8.55±0.13a	8.68±0.19a	8.00±0.21a	7.59±0.87b	6.88±0.45c	4.63±0.54d
Total policosanol (mg/$100g^{-1}$ db)	9.38±0.14b	9.60±0.21a	8.80±0.23c	8.17±0.94d	7.32±0.48f	7.80±0.26e
Tricin contents (µg $100g^{-1}$)	111.43±11.36ab	106.48±3.29ab	96.58±3.90b	128.04±2.44ab	113.39±7.56a	128.43±4.89a
TPC (mg GAE$100g^{-1}$)	35.76±0.06c	38.95±0.05a	38.37±0.14b	17.69±0.17f	19.14±0.10e	23.83±0.16d
TFC (mg RUE$100g^{-1}$)	27.35±0.84b	31.46±0.07a	31.24±0.37a	12.28±0.56e	15.36±0.42d	17.01±0.24c
% DPPH radical Inhibition	96.72±0.01a	95.91±0.07a	95.26±0.12a	66.63±1.25b	73.67±0.07c	87.97±0.76d
FRAP (mg $FeSO_4$ g^{-1})	1,391.2±9.2b	1,455.7±7.7a	1,468.0±33.6a	791.2±7.5e	883.6±11.4d	974.0±9.2c
ORAC (mgTE g^{-1})	31.07±2.47ab	39.43±5.63a	38.16±8.22a	13.95±2.68b	13.98±1.87b	21.97±1.26ab

Values are the mean ± SD, n=3. Means in the same horizontal with same superscript letter are not significant at p<0.05.
SK = sugar products made from cane planting in Si Satchanalai (Aquic Haplustalfs); SR = sugar products made from cane planting in Chai Nat (Aeric Endoaquepts); NS = non-significant difference; NCS products were produced and corrected for three consecutive analyses.

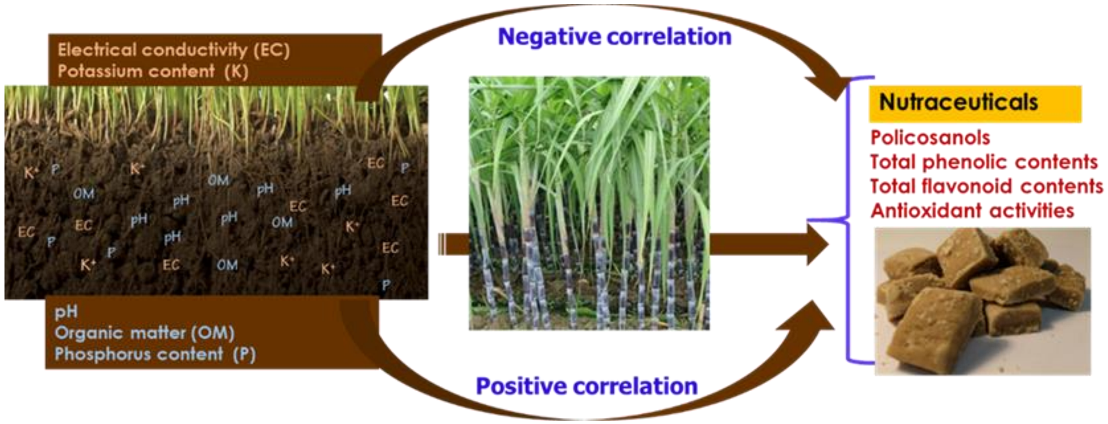

Fig 3. The relationship of soil properties and nutraceutical of non-centrifugal sugar products from pearson's correlation.

Table 4. Pearson's correlation between soil properties and chemical properties of NCS products from the two different soil types.

Properties	%OM	pH	EC (dSm^{-1})	%N	Available phosphorus ($mg\ kg^{-1}$)	Exchangeable potassium ($mg\ kg^{-1}$)
PC ($mg\ 100\ g^{-1}$)	0.07	0.81*	-0.70	-0.12	0.33	-0.51
Tricin ($mg\ 100\ g^{-1}$)	-0.08	-0.72	0.70	0.35	-0.23	0.51
Flavonoid ($mg\ 1\ g^{-1}$)	0.56	0.85*	-0.85*	0.25	0.64	-0.23
Phenolic contents ($mg\ g^{-1}$)	0.51	0.86*	-0.85*	0.23	-0.61	-0.27
% DPPH	0. 49	0.89*	-0.81*	0.22	0.61	-0.32
FRAP ($mg\ FeSO_4\ g^{-1}$)	0.52	0.87*	-0.84*	0.23	0.63	-0.27
ORAC ($mg\ TE\ 100g^{-1}$)	0. 30	0. 89*	-0. 70	-0.20	0.59	-0.50

** Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed). PC: Policosanol contents, FRAP: Ferric reducing antioxidant power, ORAC: Oxygen radical absorbance capacity. OM: Organic matter, EC: Electrical conductivity

District (SR), and the second site was Yanyaow Sub-district (17°28'10.2"N and 99°82'52.8"E), Sawankhalok District (SK). Following the Köppen classification, climate at the site was assessed to be Aw as tropical, savannah, wet with three seasons; (I) rainy, (II) dry and cool, and (III) dry and hot. Mean annual temperature was 28 ^{O}C and average annual precipitation 1,208 mm. In the rainy season (May to October), most rain fell between July and September (Meteorological Department, Ministry of Information and Communication Technology, 2017). Sugarcane samples were collected from each sampling area at the same time and sample place with soil sample for making non-centrifugal cane brown sugar and analysis of phytochemical contents.

Soil at Koh Ta Liang Sub-district was classified according to the Soil Survey Manual compiled by Soil Science Division Staff (2014) as Aeric Endoaquepts soil (Chai Nat soil series; (Cn) the National Soil Classification System of Thailand). This soil is formed from alluvium and occurs on river levees and alluvium fans. The relief varies from nearly level to gently undulating with slopes ranging from 0 to 2%. For Yanyaow Sub-district, soil was classified as Aquic Haplustalfs soil (Soil Survey Division Staff, 2014) (Si Satchanalai series (Sir); the National Soil Classification System of Thailand).

Standards and reagents

2, 2-diphenyl-1-picrylhydrazyl (DPPH), 2, 2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), and Folin-Ciocalteu's reagent were purchased from Sigma-Aldrich, Germany. 6-Hydroxy-2, 5, 7, 8-tetramethylchromane-2-carboxylic acid (Trolox), and rutin hydrate were obtained from Sigma-Aldrich (China). Gallic acid was obtained from Merck KGaA (Germany). Standard policosanols (C20-C30) were purchased from Sigma-Aldrich (Switzerland and Germany). Standard tricin was obtained from Dalton Pharma Services (Canada).

Soil sampling

Soil samples were collected in November 2016 as disturbed soil from seven plots at each site. Each plot was divided into 3 parts and 5 samples were randomly collected from each part between row and inter-row and then combined into one sample. Samples were collected at two depths of 0-20 and 20-40 cm. Fifteen soil sub-samples were collected from each sampling area (0.6 ha). 1 kg of the composite soil sample was taken for soil chemical analysis.

Soil laboratory analysis

Electrical conductivity (EC)

Electrical conductivity of saturated soil-paste extracts was measured according to Rhoades et al. (1996). A 1 g soil sample was dissolved in distilled water (5 mL) with continuous stirring for 30 min. After settling, measurement using an electrical conductivity meter was expressed as $dS\ m^{-1}$.

Determination of organic matter (OM) in soil

Determination of soil organic matter was based on the method used by Walkley and Black (1996). A 0.5 g sample was mixed with 1 N potassium dichromate ($K_2Cr_2O_7$) solution (5 ml) and sulfuric acid (H_2SO_4) (10 mL). The mixture was then left to stand for 30 min before adding distilled water (15 mL) and titrating with 5 N ferrous sulfate ($FeSO_4$) with *O*-phenolphthalein as the pH indicator. The value of organic matter was calculated from the equation below:

% Organic carbon = $\underline{(N\ K_2Cr_2O_7 \times V\ K_2Cr_2O_7 - N\ FeSO_4 \times V\ FeSO_4)}$ $\underline{\times 0.003 \times 100 \times 1.33}$ Weight of sample

Where, N = concentration of chemical, V = volume

% organic matter = % organic carbon x 1.724.

Determination of total nitrogen (N)

Determination of soil nitrogen was based on the Kjeldahl method (Bremner, 1996) and used to determine total nitrogen (N).

Determination of available phosphorus

Available phosphorus was determined according to the Bray II method (Bray and Kurtz, 1996). Soil sample of 2 g was mixed with the extractor Bray II (20 mL) and shaken for 40 sec before filtration (filter paper No. 42) andmeasured by a spectrophotometer (Milton Roy, Spectronic Genesys 5) at 882 nm. Available phosphorus was calculated from the obtained linear calibration curve of phosphorus and expressed as $mg\ L^{-1}$.

Determination of exchangeable potassium

Exchangeable potassium (K) was determined according to Pratt (2017). A soil sample of 5 g was shaken with 50 mL of ammonium acetate (NH_4OAc) (1 N). The mixture was then filtered (filter paper No. 42) and atomic absorption spectrophotometry was determined at 766 nm. Exchangeable potassium was calculated from the linear calibration curve of potassium and expressed as mg L^{-1}.

Preparation of non-centrifugal sugar product (NCS)

Non-centrifugal sugar product (NCS) was produced from the LK-92-11 sugarcane cultivar in both areas as Yanyaow Sub-district (Si Satchanalai soil series) and Koh Ta Liang Sub-district (Chai Nat soil series) in Sukhothai Province, Thailand. After manually harvesting mature sugarcane (February 2016), whole stalks were immediately pressed to obtain raw cane juice. Following the traditional process, the raw cane juice was evaporated in an open pan (60 L) for 1.30 hr to produce NCS. This process was repeated every week for 3 weeks in February 2016. The obtained NCS products (Fig 2.) were stored at -20 ^{O}C for further physicochemical and phytochemical analyses.

Analyses of the physicochemical and phytochemical properties of NCS products

Moisture content and water activity analyses

Moisture content was evaluated based on weight loss of a 2 g sample during oven drying at 105 ^{O}C for 3 hr, while water activity (a_w) was measured using an IC-5000 AW-LAB Water Activity Analyzer (Novasina AG, Lachen, Switzerland).

Color value analysis

Color was determined according to the International Commission for Uniform Methods of Sugar Analysis (ICUMSA, 2003). Color value was calculated following ICUMSA and expressed as a color unit (Asikin et al., 2014). The sample (1 g) dissolved in distilled water (100 mL) was adjusted to pH 7.0 with 0.1 N sodium hydroxide (NaOH) solution. The mixture was then filtered (filter paper No. 42) and absorbance was measured at 420 nm by a spectrophotometer (Thermo Spectronic, USA). Color value was calculated using the equation:

ICUMSA color unit or IU = (absorbance x 1,000)/ (b x C)

Where b is the cell path length used (cm)

C is the concentration of sugar solution (g mL^{-1}).

Phenolic compounds and antioxidant activities determination

Sugar samples (20 g) were extracted with 70 mL of MeOH solution (1:1v/v) with stirring for 60 min followed by filtration (filter paper No. 4). The solvent was removed using a rotary evaporator and the extract was adjusted with MeOH solution to a final volume of 50 mL (Weerawatanakorn et al., 2016). The extract was used to analyze total phenolic contents, total flavonoid contents (TFC), DPPH radical scavenging activity, and oxygen radical absorbance capacity (ORAC).

Total phenolic contents (TPC)

TPC of sugar extract was determined based on the Folin-Ciocalteu method of Weerawatanakorn et al. (2016), calculated from a linear calibration curve of gallic acid and expressed as mg GAE 100 g^{-1}.

Total flavonoid contents (TFC)

TFC was performed following the method of Weerawatanakorn et al. (2016). Results were expressed as mg rutin equivalent 100 g^{-1}.

DPPH radical scavenging activity

The ability of antioxidants in the sugar sample to inhibit DPPH radicals was determined following Weerawatanakorn et al. (2016). The antioxidant activity was calculated as a percentage of DPPH radical inhibition.

Ferric reducing antioxidant power assay (FRAP)

FRAP is based on the reduction of $FeIII^{+}$ to $FeII^{+}$ due to the action of antioxidants present in the sample. FRAP was determined according to the method described by Manohar et al. (2017) with activity expressed as mg $FeSO_4$ g^{-1}.

Oxygen radical absorbance capacity (ORAC)

ORAC was determined following the method described by Asikin et al. (2014), with slight modifications. Fluorescence intensity was kinetically recorded every minute for 60 min with fluorescent filters set at wavelengths of 485 nm excitation and 530 nm emission. Area under the curve (AUC) was calculated for relative fluorescence value. The ORAC values of the samples were compared with Trolox using a linear calibration curve and expressed as micromoles of Trolox equivalents (TE) per g of sugar samples.

Policosanol analysis

Policosanol (PC) extraction was modified from the method of Weerawatanakorn et al. (2017) and Meerod et al. (2018). GC-FID was used to quantify policosanol contents. For qualification of PC compounds, PC standards or samples were prepared in chloroform (0.5 mL) and derivatized with MSTFA (2:1 v/v) (250 µL) at 50℃ for 15 min followed by addition of chloroform to obtain a 1 mL for mass spectrum identification.

GC-FID analysis was performed by a Shimadzu GC-2010 equipped with a fused capillary column (DB 5, 0.25 mm i.d. × 30 m; J&W Scientific, Folsom, CA, USA) and a flame ionization detector set at 350℃. Helium was used as a carrier gas and sample solution (1 µL) were injected with a split ratio of 1:10. Oven temperature was initially set to 150℃, increased to 320 ℃ at the rate of 4℃ min^{-1}, and then kept at 320 ℃ for 15 min. The PC content of sample was obtained by peak area calibrated by injecting mixture standards of PC at different concentrations.

Tricin content analysis

Tricin extraction was modified from the method of Colombo et al. (2005) and Meerod et al. (2018). Purified tricin (40 µL) of NCS products was quantified by the liquid chromatography-mass spectrometry (LC-MS). An Agilent Technologies 1100 (Germany) with C-18 Column LiChroCART RP-18e (150 x 4.6 mm, 5 µm) (Purospher STAR Merck, USA) was used. Mobile phase was a linear gradient of acetonitrile (solvent A) and 7% MeOH (0.1% formic acid) (solvent B) at a flow rate of 1.0 mL min^{-1}. The gradient elution program was set at a constant ratio between solvent A and B (30:70). Temperature of the oven was set at 40 ℃. For mass detection, Nebulizer gas (N_2) pressure was set at 60 psi with dry gas flow at 13 L min^{-1} and dry temperature of 320 ℃.

Statistical analyses

The experiments were conducted in triplicate and the results were expressed as mean ± S.D. Research data were analyzed by Completely Randomized Design (CRD) and statistically significant differences were compared using Duncan's new multiple range test at a probability of $p < 0.05$.

Conclusions

Non-centrifugal sugar products from sugarcane are sweeteners containing nutrients and phytochemical compounds which are beneficial to human health. Sugarcane planted in two different types of soil series provided variation of nutraceutical compounds in final NCS products obtained by traditional processes. NCS products from sugarcane planted in soil classified as Aquic Haplustalfs soil (Si Satchanalai) contained high amounts of nutraceuticals compared with NCS products from sugarcane planted in soil of Aeric Endoaquepts soil(the Chai Nat series). Higher nutraceutical contents of NCS products were found in Aquic Haplustalfs soil, with lower EC and exchangeable K values of 0.02 ds m^{-1} and 26.2-17.1 mg kg^{-1}, respectively, while lower values were recorded in Aeric Endoaquepts soil with higher EC and exchangeable K values of 0.03-0.05 ds m^{-1} and 68.4-16.3 mg kg^{-1}, respectively. The high phytochemical and antioxidant activity of NCS products is associated with the high organic matter of soil. This result is of benefit to sugarcane breeding research and can be used to improve sugarcane cultivars to meet increased quality demands in terms of nutraceutical components as well as in similar research involving the development of food products from sugarcane.

Acknowledgements

This research was financially supported by the National Research Council of Thailand, Research Project Numbers R2560B031 and RDG5950040

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