Decolorization of Stevia Syrups by Coagulation/Flocculation Techniques

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ABSTRACT: Stevia syrup, produced from the condensed aqueous stevia leaves extracts, is used as a zero caloric sweetener. The main sweetness compound in stevia, containing 4 to 10% in dried leaves, is a diterpene glycoside, namely stevioside. Color of stevia syrup was deep brownish-yellow, resulted in unacceptable by the consumers. The objectives of this research were to extract stevia syrup with water by percolation techniques and to separate coloring components from stevia syrups by coagulation/flocculation techniques using ferrous sulfate ($FeSO_4$) combined with calcium oxide (CaO) compared with activated carbon. Results showed that $FeSO_4$ + CaO was the most effective technique for removing color of stevia extracts and stevia syrups. The color as lightness (L*), reddish/greenish (a*) and bluish/yellowish (b*) values increased from 0.63 ± 0.16, 1.57 ± 0.08 and 1.09 ± 0.02 to 18.69 ± 0.34, 30.80 ± 0.37 and 32.22 ± 0.58, respectively. Moreover, browning index of stevia syrup treated with $FeSO_4$ + CaO was higher (760.09 ± 7.49) than non-treat syrups (651.24 ± 7.98), and stevia syrup treated with activated carbon (575.16 ± 9.62). However, stevioside content of stevia syrup treated with $FeSO_4$ + CaO was lower than non-treated syrups and stevia syrups treated with activated carbon which their contents were 263.13, 411.94, and 307.40 mg/ 100 mL, respectively. This work has also demonstrated that coagulation/flocculation techniques by $FeSO_4$ + CaO are much effective and inexpensive technique for removing colorants from stevia syrups.

1. INTRODUCTION

Stevia rebaudiana Bertoni is herbaceous plant, indigenous to Paraguay and Brazil, which consists of main glycoside sweeteners namely stevioside and rebaudioside A (Sativa et al., 2004). Stevioside is main responsible for the sweetness of the products, which appears about 300 time more sweet than sucrose (Shi et al., 2002). The stevia extracts are used commercially as zero caloric natural sweetener, which can be substituted the synthetic sweetener.

Normally, there are several methods to extract stevia such as hot water (Teo et al., 2010) and solvent extractions including methanol or ethanol (Abou-Arab et al., 2010). However, there are many disadvantages for stevia extracted with solvents are bitter taste, consuming time, and product toxics (Teo et al., 2010; Rao et al., 2012 and Azmir et al., 2013).

Currently, the development of modern techniques such as, ultrasonic extraction, microwave-assisted extraction, and pressurized liquid extraction are modified with significant advantages over conventional methods (Rao et al., 2012; Charpe and Rathod, 2012; Jaitak et al., 2009). However, these methods are costly and giving the undesirable compounds such as pigment or bitter compounds. So, percolation technique is simple technique for sweetener extraction and applied to extract stevioside from stevia leaves (Persinos, 1973; Shi et al., 2002). Therefore, percolation techniques were selected for sweetener extraction.

Although purified crystal stevia sweetener is the form for commercial stevia sweetener, its crystal product is high costly and consume time. The stevia syrup is an alternative form with high yielding and easy-to-used products, which meet consumer demands (Al-Farsi et al., 2007).

One of the most important techniques for production of syrups is clarification and discoloration of extract in order to remove its undesirable color (Nasehi et al., 2012). The color of stevia syrups are from chlorophyll, other pigments such as carotenoid and also phenolic compounds such as tannins, alkaloids, and flavonoids (Savita et al., 2004; Edeoga et al., 2005). Coagulation/Flocculation technique is the one effective method for removing impurities and decolorization of water plant extracts especially stevia extracts (Zhou et al., 1984). Various chemical compounds can be used for coagulation/flocculation such as ferrous sulfate ($FeSO_4$), ferric sulfate ($Fe_2(SO_4)_3$), ferric chloride ($FeCl_3$) calcium oxide (CaO), and activated carbon (Parmar et al., 2011; Rybicki and Kurbiel, 1989; Zhou et al., 1984). In this study, $FeSO_4$ combined with CaO and activated carbon were used for decolorization of stevia syrup.

Therefore, the objectives of this research were to extract stevia syrup with water by percolation techniques and to separate coloring components from stevia syrups by coagulation/flocculation techniques using ferrous sulfate ($FeSO_4$) combined with calcium oxide (CaO), compared with activated carbon.

2. EXPERIMENT

2.1 Material

Dried stevia leaves were purchased from local market, Bangkok, Thailand. After removing twigs and branches, leaves were pulverized and used for further extraction.

2.2 Stevia extraction by percolation techniques

One kilograms of dried stevia leaves powders were percolated by water (20 L). Briefly, dried stevia leaves powders were packed onto Büchner funnel equipped with suction flask, and then the water was rinsed, passing through the stevia powders on the funnel, which gave the dark greenish stevia extract (E1).

2.3 Decolorization of stevia extracts by coagulation/flocculation techniques

2.3.1 Adding ferrous sulfate and calcium oxide

The stevia extract was flocculated with 1% of ferrous sulfate ($FeSO_4$) and 0.5% of calcium oxide (CaO), and pH of stevia extracts were adjusted to pH 9–10 by adding the small amount of calcium oxide (Shi et al., 2002).

2.3.2 Adding activated carbon

The stevia extract was mixed with 0.3% activated carbon from the total volume of stevia extract (Markosyan, 2015).

The mixed solution from 2.3.1 and 2.3.2 were stand for 24 hr. in room temperature for sedimentation, and then they were filtrated, which gave the transparent stevia extracts, abbreviated name E2 and E3, respectively.

2.4 Preparation the stevia syrups

The stevia syrups were prepared by evaporation (Buchi, Germany) of E1, E2 and E3 solutions, which their degree Brix were around 50. The stevia syrups were named S1, S2 and S3, respectively.

2.5 Color measurement

All samples were measured their color by a Miniscan EZ color reader with the CIELAB and CIELCH color system. L* represents the lightness, a* represents reddish/greenish, b* represents bluish/yellowish, C represents Chroma and h represents hue angles (the correlate of chromaticity or tone, The units are in the form of degrees° (or angles), ranging from 0° (red) through 90° (yellow), 180° (green), 270° (blue) and back to 0°).

The browning index (BI) represents the purity of brown color or brown pigment concentration (Guerrero et al., 1995; Palou et al., 1999). The browning index (BI) were calculated as the follows formula:

$$\mathrm{BI} = [100(\mathrm{x} - 0.31)]/0.172$$

Where:

$$X = \frac{a^* + 1.75L^*}{5.645L^* + a^* - 3.012b^*}$$

2.6 Stevioside content by HPLC-DAD

All samples were analyzed stevioside which they were carried out in isocratic mode at ambient temperature with High Performance Liquid Chromatography-Diode Array Detector (HPLC-DAD) using an Eclipse XDB-C18 column (4.6 mm ID × 250 mm, 5 µm). The injection volume was 20 µL, and flow rate was 0.5 mL/min for 10 min. The mobile phase included of water: acetronitrile (20:80 v/v). The wavelength of DAD was set at 210 nm. Stevioside standard and all samples were prepared the concentration at 1 mg/mL.

2.7 Statistical analysis

All treatments were carried out in triplicate. The results were expressed as mean±S.D. (standard deviation). The SAS statistical computer package was used to analyze the experimental data (SAS Version 6, SAS Institute, Cary, NC, USA). Significant differences ($p \leq 0.05$) between means were determined by Duncan's Multiple Range Test.

3. ANALYSIS

3.1 Color of stevia extracts and syrups

Color coordinate values (L*, a*, b*, Chroma and hue angles) of stevia extracts and syrups with different decolorization methods were significantly different ($p \leq 0.05$), as shown in Table 1 and 2.

Highest lightness value (L*) of stevia extracts (E1, E2 and E3), 64.78±0.54, was obtained with E2 and E1 was the lowest value (55.10±0.11). Redness values (a*) of stevia extracts were ranged from -1.51±0.06 to 1.82±0.06, which E3 had the highest value and E2 had the lowest value. Bluish values (b*) of stevia extracts were ranged from 10.48±0.73 to 34.42±0.13, which was similar to redness values (E3 was the highest and E2 was the lowest). Although Chroma values (C) of stevia extracts (E1, E2 and E3) were ranged from 10.58±0.73 to 34.47±0.13, Chroma values of E3 and E2 were highest and lowest, respectively. Hue angle values (h) of stevia extracts (E1, E2 and E3) were ranged from 86.97±0.10 to 98.21±0.26 (yellow green to green color), which E2 had the highest and E3 had the lowest value.

The decolorized stevia extracts by $FeSO_4$ + CaO (E2) gave the greater lightness and hue angle values when compared with control (E1) and activated carbon (E3) because ferrous ion (Fe^{2+}) from $FeSO_4$ could react with tannins, flavonoids and also some pigments, and remove the impurities and undesirable color from the stevia extracts (Shi et al., 2002; Meuser and Bauer, 2012). The E3 stevia extracts (remove color by activated carbon had a higher lightness value than control (E1) but lower than E2, which color of activated carbon might contaminate into the E3 sample. Moreover, CaO used to adjust pH in the stevia extracts around 9–10 could effect for pigments precipitation and color removing (Georgiou et al., 2003).

Table 1 Color value of stevia extracts with different decolorization methods

Sample	Color Measurement					Color
	L*	a*	b*	C	H	
E1	55.10 ±0.11[b]	-1.02 ±0.14[b]	31.11 ±0.58[b]	31.13 ±0.58[b]	91.88 ±0.28[b]	Dark green
E2	64.78 ±0.54[a]	-1.51 ±0.06[c]	10.48 ±0.73[c]	10.58 ±0.73[c]	98.21 ±0.26[a]	Light brown
E3	56.15 ±0.11[b]	1.82 ±0.06[a]	34.42 ±0.13[a]	34.47 ±0.13[a]	86.97 ±0.10[c]	Dark brown

a, b, c,… Means with different letters are significantly different according to Duncan's Multiple Range Test ($p \leq 0.01$).

Highest lightness value (L*) of stevia syrups (S1, S2 and S3), 18.69±0.34, was obtained with S2. This value was higher 30 and 144 times than S1 (0.63±0.16) and S3 (0.13±0.01), respectively. Redness values (a*) of stevia syrups were ranged from 0.56± 0.02 to 30.80±0.37, which a* of S2 was higher 20-55 time than a* values of S1 and S3. Bluish values (b*) of stevia syrups were ranged from 0.22±0.01 to 32.22±0.58, which S2 had the highest and S3 had the lowest value. For chroma values (C) of stevia syrups, S2 had the highest values (44.57±0.30), followed by S1 (1.91±0.05) and S3 (0.60±0.03), respectively. Hue angle values (h) of stevia syrups were ranged from 21.79±0.72 to 46.29±0.81 (red brown to brown color), which S2 had the highest (46.29±0.81), followed by S2 (34.71±1.92) and S3 (21.79±0.72), respectively.

When the extracts were concentrated by evaporation process to produce syrups, the color coordinate values of stevia syrups were darker than the stevia extracts. The dark color in stevia syrups might be from the phenolic, tannin, flavonoid compounds and some pigments originally presenting in stevia leaves, which gave dark green, yellow and brown color (Savita et al., 2004; Edeoga et al., 2005). The lightness, a*, b*, C and hue angle values of S2 (brown color) are greater than control (S1) and decolorized with activated carbon (S3). The color of S3 was darker than control (S1) might be cause from the black color of activated carbon.

From these results, $FeSO_4$ combined with CaO was the good method for remove the impurities and dark color from the stevia extracts and syrups, which give the light brown color.

Table 2 Color value of stevia syrups with different decolorization methods

Sample	Color Measurement					Color
	L*	a*	b*	C	H	
S1	0.63 ±0.16[b]	1.57 ±0.08[b]	1.09 ±0.02[b]	1.91 ±0.05[b]	34.71 ±1.92[b]	Dark green
S2	18.69 ±0.34[a]	30.80 ±0.37[a]	32.22 ±0.58[a]	44.57 ±0.30[a]	46.29 ±0.81[a]	Brown
S3	0.13 ±0.01[c]	0.56 ±0.02[c]	0.22 ±0.01[c]	0.60 ±0.03[c]	21.79 ±0.72[c]	Dark brown

a, b, c,… Means with different letters are significantly different according to Duncan's Multiple Range Test ($p \leq 0.01$).

3.2 Browning index of stevia extracts and syrups

The browning index (BI) of stevia extracts and syrups are shown in Table 3. The browning index of stevia extracts (E1, E2 and E3) were ranged from 15.30±1.30 to 90.28±0.45 and stevia syrups (S1, S2 and S3) were ranged from 575.16±9.62 to 760.09±7.49.

The browning index represents the purity of brown color or brown pigment concentration (Guerrero et al., 1996; Palou et al., 1999), which E2 was the lowest BI (15.30±1.30). It was indicated that $FeSO_4$ and CaO could remove the dark pigment or impurities in the extracts.

The browning index of stevia syrups increasing from the stevia extracts might cause from the molecules of tannin in stevia extracts had oxidation during concentration process (syrup production) (Fulcrand et al., 2006). Moreover, the browning index of S2 had the highest (760.09±7.49) values, which means S2 show the good high purity of brown color when compared with the others.

Table 3 Browning index of stevioside extracts (E1, E2 and E3) and syrups (S1, S2 and S3)**.**

Sample	Browning Index
E1	76.20±2.39[e]
E2	15.30±1.30[f]
E3	90.28±0.45[d]
S1	651.24±7.98[b]
S2	760.09±7.49[a]
S3	575.16±9.62[c]

a, b, c,… Means with different letters are significantly different according to Duncan's Multiple Range Test ($p \leq 0.01$).

3.3 Stevioside content in stevia extracts and syrups before and after decolorization

The stevioside content of stevia extracts and syrups are shown in Fig. 1. The stevioside content of stevia extracts (E1, E2 and E3) were ranged from 6.06 to 14.18 mg/100 mL and stevia syrups (S1, S2 and S3) were ranged from 236.13 to 411.94 mg/ 100 mL.

The stevioside content of stevia extracts were slightly different in all conditions. In stevia syrups, S2 had the lower stevioside content (236.13 mg/ 100 mL) than S1 (411.94 mg/ 100 mL) and S3 (307.40 mg/100 mL). $FeSO_4$ and CaO can react with tannins, flavonoids, pigments and undesirable color from the stevia extracts and also might be react with stevioside in stevia syrups and precipitation. For activated carbon, the stevioside contents in S3 was decreased when compared with control (S1) because stevioside could be adsorbed by activated carbon (Zhou et al., 1984).

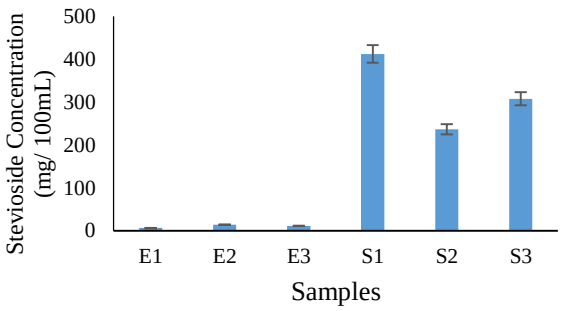

Fig. 1 Stevioside concentration of stevioside extracts (E1, E2 and E3) and syrups (S1, S2 and S3) analyzed by HPLC-DAD at 210 nm.

CONCLUSION

Results showed that stevia syrups decolorized by ferrous sulfate combined with calcium oxide were the most efficient method for removing the impurities and dark color, which gave the appreciable brown color than the control. However, the decolorized compounds resulted in the decrease of stevioside in stevia syrups.

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