Total polyphenol content and antioxidant properties of cold brew coffee extracts as affected by ultrasound treatment and their application in low fat pork sausage

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ABSTRACT
The total phenolic content and antioxidant activities of the organic spent coffee ground obtained from different brewing methods were investigated. Among various brewing methods, the organic spent coffee ground showed the highest total phenolic content (TPC) and antioxidant activities and was selected as raw material for extracting phytochemicals using ultrasound assisted extraction (UAE) with different temperature, time, and solvent ratios. In terms of extraction time, the TPC and antioxidant activities were continuously increased until the extraction time reached to 40 min and then dramatically decreased indicating the appropriate extraction time. The extract with highest TPC (78.85 mg/g of antioxidant crude extracts) and antioxidant activities was obtained when extracted with UAE at 50 °C, solvent ratios of 1:20 g/ml and 40 min. The assays of 2,2-diphenyl-1-picyrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ATBS) and ferric reducing antioxidant power (FRAP) were detected at 717.11 µM Trolox/antioxidant crude extracts, 711.23 µM Trolox/antioxidant crude extracts, and 2401.90 µM Ammonium ferrous sulfate/antioxidant crude extracts, respectively. When applied the antioxidant crude extracts from organic cold brew spent coffee ground into low fat pork sausage at 1.00%, the improvement of TPC and antioxidant activities was obtained. After storage for 14 days, the TPC and DPPH scavenging activity was increased to 18.07 mg/g of antioxidant crude extracts/100 g pork sausage and 159.76 µM Trolox/100 g pork sausage, respectively, while the 2-thiobarbituric acid reactive substance values were reduced to 9.18 g malondialdehyde/100 g pork sausage. The rancidity of the sausage was ceased, and the shelf-life was prolonged when the extract was added.

KEYWORDS
Antioxidant activities; brewing methods; organic spent coffee ground; low fat pork sausage; ultrasound assisted extraction

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**Introduction**

Coffee was the second popular beverage among consumer around the world.\(^{[1]}\) The coffee bean cultivation was originated in Africa and then expanded to other continents except in Europe in the past year.\(^{[2]}\) Coffee can be classified into various species. One of the famous species is Arabica\(^{[3]}\) since it has a good taste and intense aroma.\(^{[4]}\)

Many brewing techniques had been introduced to provide customer-designed beverage characteristics. These techniques were adapted and developed by geographic, cultural, and customer personal preferences. The brewing techniques could be referred to as solid–liquid extraction. The brewing process started after dried coffee grounds absorbed water both inside and outside coffee particles, then the bioactive compounds were transferred out of the coffee ground into a coffee brew. The coffee brewing techniques could be separated by their extraction characteristic as pressure (moka and espresso), infusion (drip), and immersion (cold brew).\(^{[1,5-7]}\) Moka was the most well-known stovetop brewing technique, which was very popular in an Italian household. The moka coffee was generated through pressure and steam that passed through the coffee ground cake in the middle section of the moka pot.\(^{[8]}\) The espresso machine was a common and well known brewing technique. The principle of the espresso extraction process was the pressure, which forced the hot water through the compressed finely ground coffee.\(^{[5,7]}\) Instead of pressure, the filter or drip brewing principle depended on the water pressure and gravity force in the extraction chamber.\(^{[7,8]}\) In this past decade, cold brewing had caught the coffee market’s attention as the beverage provided a new sensory taste, less acidity, and less bitterness. Cold brew process started by steeping the extra-course coffee ground at 20–25°C for 8–24 h before collecting the coffee beverage.\(^{[9]}\) The lack of heat in this brewing process was difficult for several polar compounds to be extracted into the coffee compared to hot brewing process resulted in difference bioactive compounds and antioxidant capacity in the coffee and spent coffee ground (SCG) obtained from different brewing techniques.\(^{[7,10]}\) A coffee had been reported as the source of antioxidant activity\(^{[11]}\) as same as their SCG.\(^{[12]}\) The most abundant bioactive compound in SCG was caffeine followed by chlorogenic acids and phenolic acids, such as (caffeic, ferulic, gallic, p-coumaric, syringic, trans-cinnamic, and vanillic acid).\(^{[13]}\) The researcher found that the espresso SCG after solvent extraction contains TPC at 16 mg GAE/g with FRAP 0.10 mM FE (II)/g.\(^{[14]}\) As coffee becomes a popular beverage worldwide, the SCG was estimated to be increased each year.\(^{[15]}\) Despite, the large quantity of SCG produced from either coffee shop, coffee factory, or household, the SCG was stilled mostly underutilized.\(^{[16]}\) The important bioactivity composition in SCG still remained after the brewing process especially for cold brew method. The brewing could not extract all of the bioactive compounds from the roasted coffee ground.\(^{[7,17]}\) Hence, SCG is a prominent by-product that suited to be used as a raw material for antioxidant extraction.

Antioxidant compounds usually been applied to meat and meat product to prolong shelf life by retarding the lipid oxidation process, which causes major changes in the deterioration of meat. As lipid oxidation process was commonly promoted the negative changes of physico-chemical properties of meat, which eventually effect to meat quality. This process could be reduced by the use of synthetic antioxidant like butylated hydroxytoluene (BHT) or butylated hydroxyanisole (BHA), which had been restrictively applied, however they may cause potential human health risk and increase toxicity. To reduce the use of synthetic antioxidant, natural antioxidant could be used. Since the bioactive compounds or phenolic compounds in natural antioxidant have the potential to inhibit or delay oxidation through the reduction of free radical chain reaction and protection of low-density lipoprotein from oxidation.\(^{[18-20]}\) The lipid oxidation could be determined by TBARS assay. In fresh sausage study, after added green tea extract into the sausage, the TBARS was lowered after processing and during storage.\(^{[18]}\) In addition, the frankfurter sausage with 500 ppm of green tea extract could reduce the TBARS value during storage at 4°C.\(^{[19]}\)

The ultrasound assisted extraction (UAE) process was one of the green technologies in the field of phytochemical extraction. This process used low energy, low solvent, and less time compared to conventional extraction. The principle was based on the creation of cavitation bubble at high-intensity
frequency 20 kHz-10 MHz through compression and rarefaction. Many physical phenomena had been found during the mentioned frequency passes through the extracted solvent including high-speed microjet, shock wave, turbulent flow, sono-capillary, and liquid whirlpools after cavitation bubbles collapsed on the solute material. This high-speed microjet enhanced the erosion and fragmentation of the solute cell wall, then the sono-capillary channels were created, which then induced the penetration and mass transfer of the solvent. All of these phenomena could improve the UAE quality. Not only the physical phenomena but chemical phenomena also occurred during the UAE process such as hot spot. The hot spot occurred after the cavitation collapse could provide high energy, which was then ruptured the O-H bond and created the free radical species. These phenomena could reduce the quality of UAE process during the long extraction period. The extracted temperature, time, and solvent ratio had been reported as the factors that influenced the cavitation quality.

The UAE was widely used for extraction many kind of natural materials such as Annona muricata (DPPH 638.02 μM AE/g), lemon-scented tea tree (Leptospermum petersonii) leaves (TPC 98.91 mg GAE/g DW, DPPH 889.29 mM TE/g DW), guava (Psidium guajava L.) (4.07 mg/g gallic acid), and mango seed (TPC 121.66 mg GAE/gDM) but not for the extraction of organic spent coffee ground.

Organic spent coffee ground still contained important bioactive compound and still mostly unutilized. The green technology extraction as UAE process could be applied and utilized this valuable organic waste to obtain the antioxidant crude extract, which could be applied to meat product and prolong the shelf life. For these reasons, this research studied these three UAE parameters (solvent, temperature, and time) to investigate the effect of the UAE conditions on antioxidant capacity of cold brew organic SCG (CSCG). The application of organic CSCG extracted by UAE was studied using pork sausage as a meat model and the changes during storage were proposed.

Materials and methods

Raw material preparation

The light roasted organic Arabica ground coffee and CSCG were provided by a private company in Chiang Mai, Thailand. The ground coffee was stored in the sealed package at room temperature, 1000 g/package, and hot brewing to obtain hot brew organic SCG (HSCG) by drip, moka, and espresso brewing technique within 4 weeks. The CSCG was frozen before grounded and sieved through 50 meshes, then stored at −18 °C prior analyze and extract. The solvent used in this study was 95% food grade ethanol (Thailand’s distributor). All chemical reagents used for total phenolic content (TPC) and antioxidant measurements were analytically graded (Sigma, USA).

Preparation of HSCG

The light roasted organic Arabica ground coffee was grounded and sieved through 50 meshes before each brewing process. Three hot brewing process was used in this research as drip, moka, and espresso brewing technique. The drip organic SCG (DSCG) obtained by added hot water (92–96 °C) 250 ± 2 ml onto 25 g of ground coffee, which was already placed on the paper filter (daiso unbleached paper coffee filters, Japan), allowed the hot water to drip on to the bottom vessel until the total brewing time was 3.30 min. The moka organic SCG (MSCG) was produced according to Angeloni et al. The brewing started by adding 150 ml of hot water (92–96 °C) into the bottom chamber of moka pot (BUYZONE-moka pot, China), then placed 14 g of ground coffee at the medium section before placed onto the hot plate at 130 °C for 3 min. The espresso organic SCG (ESCG) was collected after brewing by the espresso machine (Sage Espresso Machine, model: BES875UK, Italia).
The SCG from all brewing processes was freeze dried until the moisture content was below 5% before grounded and sieved through 50 meshes, then stored at −18 °C prior analyze and extract. The HSCG (DSCG, MCG, and ESCG) which showed the highest TPC and antioxidant activity were further used as raw material to investigate the effect of UAE conditions compared to CSCG.

**Preparation of antioxidant crude extracts from HSCG and CSCG**

The HSCG and CSCG were mixed with 95% ethanol before extraction using ultrasound with heat (thermosonication at 40 kHz) (D6 series, GT SONIC, China). The parameters studied were solvent ratio, temperature, and time (Table 1). After the finished extraction, the crude extract was then filtered through Whatman No. 1 filter paper before evaporated the solvent by rotary evaporator (Rotavapor® R-100, Buchi, Germany) at 40°C. Both crude extracts were dissolved in 5 ml of 95% ethanol called antioxidant crude extracts from HSCG or antioxidant crude extracts from CSCG, then kept at −18°C (modified from Natnoi and Pirak[31]) prior to the analysis of total phenolic content (TPC), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), and ferric reducing antioxidant power (FRAP).

**Determination of TPC of antioxidant crude extracts from HSCG and CSCG**

The TPC was analyzed by the Folin–Ciocalteu method according to Abrahão et al.[32] with some modification. The sample (100 µl) was mixed with the same content of Folin–Ciocalteu reagent (10% v/v) before leaving the mixture to react for 2 min then added 10% w/v calcium carbonate (100 µl) and distilled water (1 ml). The mixed solution was left in a dark place at room temperature for 60 min. The development of reaction color was measured at 765 nm by a spectrophotometer. The gallic acid was used as a standard (0–100 µg/ml).

**Determination of antioxidant capacity by DPPH of antioxidant crude extracts from HSCG and CSCG**

DPPH assay was based on the reduction of the purple color of the DPPH solution occurred from target sample to scavenge the free radical. The assay was performed according to the method of Xu et al.[33] with slight modifications. The sample (250 µl) was mixed with 750 µl DPPH solution (0.1 mM methanolic solution). The absorbance was recorded at 517 nm after incubated in the dark place at room temperature for 30 min. The free radical scavenging ability was evaluated through Troloox standard curve (0–600 µM/ml).

**Determination of antioxidant capacity by ABTS of antioxidant crude extracts from HSCG and CSCG**

The ABTS assay was based on the generation of a blue to the green color of ABTS, which occurred from sample antioxidant capacity. The procedure was performed according to Wu et al.[34] with some modification. The equal ratio of ABTS solution (7 mM) and potassium peroxide sulfate (2.45 mM) was

| Table 1. The parameters of UAE with three factors including solvent ratio, temperature, and time and the condition of HSCG and CSCG extraction process. |
|-----------------------------------|
| UAE parameters | Condition                     |
| Solvent ratio | 1:10, 1:20 g/ml |
| Temperature   | 40°C and 50°C               |
| Time          | 20, 30, 40 and 50 min       |
mixed before leaving the solution in darkness at 4°C for 12–16 hr. The mixed solution (900 µl) was added to sample (100 µl). The solution was kept in a dark place at room temperature for 10 min then measure absorbance at 734 nm. The free radical scavenging ability was evaluated through Trolox standard curve (0–600 µM/ml).

**Determination of antioxidant capacity by FRAP of antioxidant crude extracts from HSCG and CSCG**

The FRAP assay was different from DPPH and ABTS assay as the assay monitors the redox-active compound generated from ferric iron (Fe³⁺) to ferrous iron (Fe²⁺) not the development of synthetic color reaction. The assay was followed by the method of Abrahão et al.⁴² with a slight modification. The FRAP solution was prepared by mixing the TPTZ solution (10 mM, dissolved in 40 mM HCl) with ferric chloride (20 mM) and acetate buffer (300 mM, pH 3.6) in ratio 1:1:10, respectively. The FRAP solution 2.7 ml was added into 270 µl of distilled water and 90 µl of sample. The solution was incubated at 37°C for 30 min and measured the absorbance at 595 nm. The ferrous sulfate (500–2000 µM/ml) was used as a standard.

**Effect of antioxidant crude extracts from CSCG on properties of low fat pork sausage**

The antioxidant crude extracts, which provided the best TPC and antioxidant activities, were selected to use in this part. Based on the preliminary research, the recipe for low fat pork sausage is shown in Table 2. The antioxidant crude extracts were added into the product at 0.75% w/w (recipe 1) and 1.00% w/w (recipe 2). The low fat pork sausages were stored at 4°C before measuring of TPC, DPPH, and 2-thiobarbituric acid reactive substances (TBARS) on days 1, 7 and 14.

**Determination of TPC and DPPH on low fat pork sausage with antioxidant crude extracts obtained from CSCG**

All low fat pork sausages were diced and grounded for 1 min before mixed with distilled water (1:5 g/ml) for 20 min by magnetic stirrer, then centrifuge at 5000 rpm for 5 min (modified from Isaza et al.⁴³) before determined by TPC and DPPH. The measurements were similar to the previous experiment of TPC and DPPH of antioxidant crude extracts from HSCG and CSCG.

**Determination of lipid oxidation by TBARS of low fat pork sausage with antioxidant crude extracts obtained from CSCG**

Low fat pork sausages were minced by grinder for 1 min before mixed with thiobarbituric acid (TCA) at a ratio of 1:2.5 g/ml, then mixed by Vortex mixer at the highest speed for 2 min. Added 2-thiobarbituric (0.02 M) at the same ratio as TCA then centrifuge at 3,000 g for 10 min before collecting the supernatant. The supernatant was boiled for 45 min then cooled in an ice bath immediately before measuring the solution absorbance at 532 nm. The 1,1,3,3-tetramethoxyxpropane was selected as a standard curve (0–12 nM/ml) (modified from Jayawardana et al.⁴⁶).

**Statistical analysis**

Each parameter was analyzed in triplicate. The full factorial in CRD design was applied for each parameter in the study. The statistical significance of the variable was determined at a 5% probability level (Duncan’s post hoc test using SPSS version 19). Pearson’s correlation test was used to analyze the relationship between TPC and three antioxidant capacity assays using SPSS version 19.
Results and discussion

**TPC and antioxidant activities of HSCG and CSCG**

The TPC and antioxidant capacity of all HSCG were significantly ($p \leq .05$) lower than CSCG (Table 3). Among the SCG from hot brewing process, the DSCG showed the highest TPC value compared to MSCG and ESCG, respectively. The use of pressure combined with hot water in the espresso brewing process could generate high kinetic energy and mass transfer leading to high brewing capacity. In terms of moka, the pressure used in the process was lower than the espresso process, which resulted in lower extracted quality.\(^{37}\) In addition, the mass transfer of the drip brewing process depends only on the gravitational force inside the filter chamber, then the mass transfer was even lower.\(^{44}\) This brewing capacity could be explained the difference in TPC and antioxidant activity of SCG between hot brewing technique. The TPC, DPPH, ABTS, and FRAP of CSCG were significantly ($p \leq .05$) higher than DSCG, MSCG, and ESCG, respectively. According to Belitz et al.\(^{38}\) and Rao and Fuller,\(^{39}\) hot water could extract more phenolic compounds during the brewing method compared to cold water. This could explain the lower TPC and antioxidant activity in HSCG compared to CSCG. The TPC of ESCG, MSCG, DSCG, and CSCG were different from some previous research due to the difference in plantation and coffee variety.\(^{40,41}\) Górecki and Hallmann\(^{42}\) reported the difference in phenolic content between the plantation with and without pesticide (organic). The researcher found that the phenolic content was mainly attributed to the pest defense system, which provided higher phenolic content in organic plantation. TPC and antioxidant activities of DSCG and CSCG were higher than other SCG samples, then these samples were selected as raw material to further investigate the effect of UAE conditions.

![Figure 1](image-url) The bioactive compound found in SCG (A) Caffeine and (B) Chlorogenic acid.
Table 3. The total phenolic content and antioxidant activity obtained from DPPH, ABTS, and FRAP assay of organic SCG from different brewing techniques including cold-brew technique and the three different how-brewing techniques.

| Brewing technique | Sample  | TPC (mg GAE/g organic SCG) | DPPH (µM Trolox/g organic SCG) | ABTS (µM Trolox/g organic SCG) | FRAP (mM ferrous sulfate/g organic SCG) |
|------------------|---------|-----------------------------|---------------------------------|---------------------------------|----------------------------------------|
| Hot brew         | ESCG    | 13.8 ± 0.2^a                | 157 ± 9^a                      | 161 ± 6^a                       | 0.14 ± 0.02^a                          |
|                  | MSCG    | 14.0 ± 0.4^b                | 163 ± 9^b                      | 168 ± 8^a                       | 0.21 ± 0.07^b                          |
|                  | DSCG    | 17.3 ± 3.5^c                | 190 ± 6^c                      | 200 ± 8^b                       | 0.36 ± 0.02^c                          |
| Cold brew        | CSGG    | 18.9 ± 0.9^d                | 211 ± 5^d                      | 255 ± 10^c                      | 0.49 ± 0.01^d                          |

^Values are expressed as means ± SD (n = 9). The number in each column marked with different letter were significantly different (p ≤ 0.05): ESCG = organic spent coffee ground from espresso technique, MCG = organic spent coffee ground from moka technique, DSCG = organic spent coffee ground from drip technique, and CSGG = organic spent coffee ground from cold brew technique.

**Effect of UAE condition (solvent ratio, temperature, and time) on antioxidant crude extracts from HSCG and CSGG**

Based on the statistical analysis, this research found a significant interaction between the two factors as time and temperature, while the solvent ratio was not significantly affected.

According to the previous result, the antioxidant crude extracts from HSCG (DSCG) and CSGG were selected as raw material for UAE process. The solvent, temperature, and time were important parameters for UAE quality. The solvent used in this experiment was ethanol 95% due to its high polarity, nontoxic to humans and the environment. In addition, ethanol could evaporate from the extracts at low temperature, which could minimize the energy. The effect of different ethanol ratios (1:10 and 1:20 g/ml) at fixed temperature and time (50°C for 40 min) on antioxidant crude extracts from HSCG and CSGG is shown in Figure 2. The antioxidant crude extracts from HSCG after extracted with ethanol ratio 1:20 g/ml showed significantly (p ≤ .05) higher in TPC and antioxidant capacity (DPPH, ABTS, and FRAP) compared to 1:10 g/ml. These results were similar to the antioxidant crude extracts from CSCG. During extraction with UAE, a high solvent ratio could enhance the interfacial area between cavitation bubble and solute surface, which accelerates the driving force and mass transfer of bioactive compound. In addition, after increasing the UAE time, the TPC also increased indicating that the plant cell wall was disrupted by the ultrasonic wave and the TPC could be released from the plant matrix. In addition, the longer UAE time could enhance the diffusivity of the solvent into plan matrix and increase disruption and solubility of bioactive compound. Similar results were also reported as the yield of TPC was increased when solvent ratio increased. However, the solvent ratio could enhance extraction quality up to one ratio, then the quality was decreased by excessive solvent could over consume the cavitation effect. If the solvent ratio was insufficient, the extraction quality would be decreased because of low infusion and mass transfer.

The two different extracted temperatures as 40°C and 50°C and four different extracted times as 20, 30, 40, and 50 min were selected to study the effect of temperature and time on antioxidant crude extracts from HSCG and CSGG (Figure 3). The TPC and antioxidant activities of antioxidant crude extracts from HSCG and CSGG after extracted at 40°C were lower than extracted at 50°C during 20–40 min of extraction with a solvent ratio of 1:20 g/ml. The TPC and antioxidant activity of both antioxidant crude extracts were increased along with extraction time. However, the values of both antioxidant crude extracts after extracted at 50°C were decreased after 40 min of extraction. The increase of extraction temperature could reduce solvent viscosity and increase mass transfer, which led to high extraction quality. Most of the previous studies reported an increase in a phytochemical compound after extraction at a temperature between 30 and 50°C and then decrease above 50°C. This could be attributed to UAE above a certain temperature that could reduce the intensity of cavitation bubble or promote free radical then the phenolic compound was retarded. During UAE, the contact time between cavitation bubble and solute cell wall had to be enough to enhance the penetration and mass transfer.
However, this research finds a decrease in the TPC and antioxidant activity after extract at 50 °C above 40 min. These results could be explained by two different theories. First, the overexpressed cavitation bubble could generate the free radical, which leads to the degradation of the bioactive compound through oxidation process. Second, the diffusion of solvent in and out the plant cell was at their final equilibrium, then the interpenetration could not be promoted.

**Correlation between TPC, DPPH, ABTS, and FRAP of antioxidant crude extracts from HSCG and CSCG**

The correlation between TPC and antioxidant capacity (DPPH, ABTS, FRAP) of antioxidant crude extracts from CSCG and HSCG were shown in Tables 4 and 5, respectively. This research observed a high correlation between TPC and all three antioxidant activities in antioxidant crude extracts from CSCG with the highest correlation between TPC and DPPH (0.895) as shown in Table 4. The high correlation could confirm that the result of antioxidant capacity in antioxidant crude extracts from CSCG was based on phenolic content.

In contrast, antioxidant crude extracts from HSCG exhibited a moderate correlation between TPC and antioxidant capacity with the lowest correlation between TPC and FRAP (0.430) (Table 5). These observations could be assumed that the antioxidant activity of antioxidant crude extracts from HSCG was not mainly contributed from phenolic content. Jiménez-Zamora et al. found that not only total polyphenols attributed to antioxidant activity but also melanoidin as well. As the TPC and antioxidant...
activity of antioxidant crude extracts from CSCG in this study were significantly \((p \leq .05)\) higher than antioxidant crude extracts from HSCG then this sample was selected as the source of antioxidants in the food model.
Effect of antioxidant crude extracts from CSCG on properties of pork sausage

According to TPC and antioxidant activity from the previous results, the extract from antioxidant crude extracts from CSCG was selected to apply in low fat pork sausage as a meat model in this study to enhance flavor and extend the product shelf life. The properties of low fat pork sausage were analyzed through TPC, DPPH, and TBARS. The antioxidant crude extract from CSCG was extracted at 50°C, 40 min, and solvent ratio 1:20 g/ml before added in low fat pork sausage (0.75% and 1.00%). The TPC, DPPH, and TBARS of low fat pork sausage with antioxidant crude extracts from CSCG during storage at 4°C for 1–14 days (Figure 4). The TPC and DPPH were gradually decreased, while TBARS was increased in all low fat pork sausage samples during storage. The TPC and DPPH of low fat pork sausage with antioxidant crude extracts from CSCG were significantly ($p \leq .05$) higher than the control low fat pork sausage, while the TBARS were significantly ($p \leq .05$) lower. The TPC, DPPH, and TBARS of both low fat pork sausage with antioxidant crude extracts from CSCG (0.75 and 1.00%) were significantly different ($p \leq .05$) at the storage time from days 7–14. The TPC and DPPH of low fat pork sausage with antioxidant crude extracts from CSCG were varied according to proportion of the antioxidant crude extracts from CSCG. The TPC and DPPH of low fat pork sausage with antioxidant crude extracts from CSCG 1.00% was approximately 3 and 11 times higher than the control low fat pork sausage. Furthermore, the TBARS was approximately 2 times lower in the low fat pork sausage with antioxidant crude extracts from CSCG 1.00% compared to control low fat pork sausage during the storage time. Jayawardana et al. suggest that high antioxidant capacity in the black and green tea extracted product could regrade the lipid oxidation process which was then reduce the TBARS value. From this
investigation, added antioxidant crude extracts from CSCG which contained high TPC and antioxidant activity in low fat pork sausage could reduce the lipid oxidation during the storage period. In summary, as the antioxidant crude extracts from CSCG showed high potential in terms of antioxidant capacity, these could be further applied in other food matrix to retard the product rancidity.

Conclusion
In this study, both HSCG and CSCG contained TPC and antioxidant capacities; however, the content was varied according to the intensity of the brewing technique. The antioxidant crude extracts from CSCG, extracted by UAE at suitable conditions (50°C, 1:20 g/ml, and 40 min), showed a high correlation between TPC and antioxidant activity, while the correlation in antioxidant crude extracts from HSCG was moderated. This finding showed that antioxidant crude extracts from CSCG was mainly attributed to phenolic content. In addition, the TPC and antioxidant activities of antioxidant crude extracts from CSCG were higher than antioxidant crude extracts from HSCG. Hence, this sample was further used to investigate their application in low fat pork sausage. The addition of 1.00% w/w antioxidant crude extracts from CSCG in low fat pork sausage could reduce the TBARS during storage implied that the TPC in sample could reduce the lipid oxidation in meat product during storages which prolongs the shelf life and quality of the food product.

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Disclosure statement
No potential conflict of interest was reported by the author(s).

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