Preliminary extraction of catechin in cashew testa

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Abstract. The water extraction procedure (WE) was critically selected to extract (+)-catechin and (-)-epicatechin in cashew testa. The effects of different extracting factors, including multiple extraction, extracting temperature, extracting duration, and solvent–material ratio, on catechin amounts were evaluated. Under optimal conditions, a gram of water extract (WE1) contained 151.2±4.7 mg of (+)-catechin and 85.2±2.3 mg of (-)-epicatechin. These values were nearly three-folded compared to raw material, 55.0±1.4 and 31.0±0.6 mg, respectively. By using fractional extraction with ethyl acetate, the amounts of (+)-catechin and (-)-epicatechin per gram of ethyl acetate extract (WE2) in the present extracts respectively increased to 219.4±16.5 and 123.6±8.9 mg.

1. Introduction
The desire for having better health and living a healthy lifestyle in modern life is increasing day by day. Therefore, people care about antioxidants, especially natural products which can use as nutraceuticals. They contain natural antioxidants found in plant-based foods, which have positive effects on health and immune systems. The biological activities of Anacardium occidentale L (commonly known as cashew), such as antidiabetic, anti-bacterial, anti-fungal, anti-oxidant and anti-inflammatory activities [1-4], have been widely reported. Cashew testa is the externally thin and reddish-brown-colored skin, which accounts for about 1 – 3% of the total weight of cashew nuts [5]. In the food industry, especially confectionery and bakery plants [6], cashew testa is usually removed at barely high temperatures (up to 200 °C) after shelling to avoid its astringent or bitter taste [5]. Currently, this testa is used as fuels like cashew nutshell [7].

Several academic studies showed that cashew testa is a rich source of tannins [5] with (+)-catechin and (-)-epicatechin as primary polyphenols [8]. The maximum amount of the catechin found in aqueous extract of cashew testa was up to 13.65% by using Soxhlet extractor [9]. The remarkable amounts of these catechins in cashew testa were higher than green tea [10] and dark chocolate [11].

Although several studies widely reported the pharmacological activities and antioxidant properties of cashew testa, testa's nutrition and health benefits have been undervalued [12]. Currently, there is no information on the catechin composition of cashew testa removed from the food industry or evaluating the effects of extracting conditions on catechin amounts in testa extract. Hence, the present study was conducted to characterize a suitable procedure to extract catechins and evaluate the effects of different extracting conditions on catechin amounts in cashew extracts. Furthermore, the correspondence between total flavonoid contents (TFC) and the amount of catechins was evaluated.

2. Materials and methods
2.1. Sample collection and preparation

The raw material for extraction was toasting cashew testa (figure 1) collected from cashew nut factories in Binh Phuoc, Vietnam. To avoid the development of molds and microorganisms, the moisture of toasted testa was reduced to a level below 10% [13]. It was then put in zip bags in the presence of desiccant bags for better storage conditions. Before conducting experiments, this sample would be ground to increase its contact area, which leads to a more effective extraction.

![Figure 1. The dried cashew testa.](image)

2.2. Chemicals

Absolute ethanol and ethyl acetate were supplied by Chemsol (Vietnam). Formic acid, n-hexane, sodium nitrite, aluminum chloride, and sodium hydroxide were supplied by Xilong (China). Standards of quercetin, (+)-catechin, (-)-epicatechin, HPLC grade methanol, acetonitrile and formic acid were supplied by Merck (Germany).

2.3. Analysis of catechin constituents of cashew testa extracts

Absolute ethanol and ethyl acetate were supplied by Chemsol (Vietnam). Formic acid, n-hexane, sodium nitrite, aluminum chloride, and sodium hydroxide were supplied by Xilong (China). Standards of quercetin, (+)-catechin, (-)-epicatechin, HPLC grade methanol, acetonitrile and formic acid were supplied by Merck (Germany).

2.3.1. TFC analysis

The aluminum chloride colorimetric method was modified from the procedure reported by Chang and Yang [15]. First, a preliminary 50±10 mg of the dried extract (DE) was dissolved in 10 mL of MeOH. Next, 0.05 mL of this solution would be added into 2 mL of distilled water, then 0.15 mL of NaNO2 5% was added. After 5 minutes, 0.15 mL of AlCl3 10% was added to the following mixture. Then 1 mL of NaOH 1M and 1.2 mL of distilled water were added to the mixture after 1 minute. Finally, the samples were measure at \( \lambda = 425 \) nm by spectrophotometer (GENESYS™ 30 Visible, Thermo Fisher Scientific, America) with the calibration curve of quercetin (TFC = 0.0011 × OD + 0.0469, R² = 0.9971).

2.3.2. HPLC analysis

Due to the method introduced by Trox and coworkers [5], 100±20 mg of ground testa extract were mixed into 10 mL of methanol – water mixture (40:60, v/v) with 1% v/v formic acid, then being centrifuged for 10 minutes at 13000 rpm to collect the supernatant. This supernatant was diluted in the initial solvent mixture (3:7, v/v) for measurements by HPLC-UV. For analytical HPLC, 10 µL of sample solution was injected into a HPLC (Agilent 1100) under chromatographic conditions, including C18 AQ column (5 µm, 150×4.6 mm, Phenomenex, America) at 40 °C, solvent A (formic acid : distilled water, 5:95, v/v), solvent B (formic acid : distilled water : acetonitrile, 5:10:85, v/v/v).
The catechin amounts were calculated through calibration curves of (+)-catechin ($C = 2.6556 \times A - 1.8213, R^2 = 0.9998$) and (-)-epicatechin ($C = 2.6520 \times A + 2.8134, R^2 = 0.9983$) at the wavelength of 278 nm.

The gradient program was as follows 5 to 26% B (12.38 min), 26 to 100% B (17.22 min), 100% B isocratic (10.00 min), and 5% B isocratic (10.40 min) with a flow-rate of 0.8 mL/min.

2.4. Analysis of catechin constituents of cashew testa extracts

The cashew testa was separately processed with two different procedures, including Water Extraction (WE) and Ethanol Extraction (EE).

For WE procedure, each batch containing 20.00±0.10 g of dried and ground cashew testa would be extracted with 200 mL of aqueous formic acid (1% v/v) [5]. The mixture was stirred at 700 rpm with a magnetic hot plate stirrer at 70 °C for 30 minutes. This process was repeated five times before eliminating the solvent under vacuum pressure to collect water extract (WE1). Following then, WE1 was converted into ethyl acetate extract (WE2) by using five-time ethyl acetate (EA) extraction (2:1 solvent–material ratio, 30 extracting minutes each time). The organic layer was collected and removed solvent under vacuum to obtain WE2, stored at -20 °C for the following analytical experiments.

For EE procedure, ethanol crude extract (EE1) would be collected through the similar experiments described in the WE procedure. However, the solvent for preliminary extraction was a mixture of 200 mL of aqueous ethanol (70% v/v) and 2 mL of formic acid [5]. Next, EE1 was also converted into ethyl acetate extract (EE2) after five-time hexane extraction (2:1 solvent–material ratio, 30 minutes for each time, collecting top layer) and five-time EA extraction (2:1 solvent–material ratio, 30 minutes each time, collecting top layer). After solvent removal, EE2 was obtained and stored at the same conditions of WE2.

These experimental procedures were graphically summarized through block diagrams in figure 2.
Figure 2. Procedures of WE (left) and EE (right).

2.5. Screening the effects of extracting conditions on catechin amounts in water extracts

Four specific factors would be considered and evaluated through the One-factor-at-a-time (OFAT) method, a traditional approach of designing experiments to evaluate the impact of an individual factor on a specific objective function [15]. These were multiple extraction, extracting temperature, extracting duration, and solvent–material ratio (table 1 for more details).

| Factors                        | Lower limit | Upper limit | Step |
|--------------------------------|-------------|-------------|------|
| Multiple extraction (times)    | 1           | 5           | 1    |
| Extracting temperature (°C)    | 40          | 80          | 10   |
| Extracting duration (min)      | 30          | 75          | 15   |
| Solvent–material ratio (mL/g)  | 8/1         | 16/1        | 2/1  |

The procedure in this part was pretty similar to that of WE. About 20.00±0.10 g of dried and ground cashew testa would be extracted in aqueous formic acid (1% v/v). For the first factor (multiple extraction), the initial point was conducted at determined extracting conditions: 70 °C, 30 minutes per time, 10:1 (mL/g) solvent–material ratio.
After removing the solvent, water extracts (WE1) would be collected and converted into EA extracts (WE2) by three-time EA extraction (2:1 solvent–material ratio, 30 extracting minutes for each time) at ambient temperature. These extracts were stored in a freezer for analysis.

3. Results and discussion

3.1. Procedure selection

In this study, WE and EE procedures were essentially evaluated through (+)-catechin and (-)-epicatechin contents. Water was used as the initial extracting solvent in WE procedure because it is considered the most inexpensive solvent in the chemical industry [16], non-flammable, non-toxic, readily available, and heavily used in the food industry [17]. Furthermore, water is the most environmentally benign solvent in extraction and chemical reactions [18]. Besides, it also has low oxygen solubility [19], suitable for extracting air-sensitive compounds, including all types of catechin.

On the other side, ethanol is a relatively safe solvent for humans [20] and is widely used in bioactive compound extraction industries. In EE procedure, hexane was exclusively used to remove fatty compounds such as stearic acid, oleic acid, and linoleic acid [5] because the solubilities of these above fatty acids were infinitive in hexane [21]. Both mentioned procedures used EA, a polar aprotic solvent [22], which has low toxicity to human health and the environment. Moreover, EA has been practically used to purify the catechin compounds in Korean tea extracts [22].

There are three main aspects needed to discuss in the present section, including extraction yield (Equation 1), TFC, and catechin amounts.

![Equation 1](image)

3.1.1. Extraction yield and TFC analysis

Based on Table 2, the difference in extraction yields between the two given procedures was considerable, around 41% for WE1 and 59% for EE1. These results confirmed that the extraction using water alone yielded low efficiency compared to polar organic compounds [23]. The amount of water in the mixture, which accounts for 30% by volume, is enough to expand the carbohydrate matrix [5] in dried material. In the presence of ethanol – a good solvent for extraction, the surface tension of solvent declines [24], strengthening the extraction effectiveness, which means the penetration of ethanol and water molecules to plant cells is effortless. Furthermore, ethanol is the best solvent for phenolic extraction [25] because of its inherent properties, allowing this solvent to dissolve both polar and non-polar substances [26]. In ethanol extracts, however, there were both polar impurities (polar phenolic compounds and carbohydrates, for example) and non-polar impurities (fatty acids and carotenoids, for example) [5] that made the rise in extract weight became meaningless. For EA extracts (WE2 and EE2), the differences between their extraction yield were trivial, about 32 and 31%, respectively. Because EA was used to effectively enrich catechins in crude extracts, these results showed the pre-eminence of WE procedure compared to the other one. However, TFC and HPLC analysis should be taken into account to choose the most suitable procedure.

**Table 2. TFC comparison in extracts collected from two different procedures.**

| n | EY | TFC<sub>c</sub> | TFC<sub>d</sub> |
|---|---|---|---|
| WE1 | 40.98±0.03 | 401.46±27.21 | 163.36±9.03 |
| WE2 | 32.27±0.02 | 446.30±46.50 | 185.31±11.52 |
| EE1 | 59.25±0.13 | 432.67±41.78 | 252.27±10.10 |
| EE2 | 30.74±3.44 | 615.670±3.21 | 216.19±0.21 |

*a: Procedure name; b: Extraction yield (%); c: TFC in dried extract (mg QE/ g DE); d: TFC in raw material (mg QE/ g RM)*
However, total flavonoid contents held different values in practical application that led to the need for TFC analysis. According to total flavonoid content in dried extract (TFC1) and total flavonoid content in raw material (TFC2) results in table 2, the EA extraction step led to an increase of TFC in WE2 and EE2, which confirmed the ability of EA to purify flavonoid compounds in the preliminary extracts. For details, TFC signals in DE increased between 1.7 to 2.8 times compared to raw material. After preliminary extraction steps, TFC2 in EE1 was considerably higher than WE1, slightly above 1.5 times. However, through fractional extraction with EA, TFC2 in EE2 was negligibly higher than WE2, about 1.16 times. With this result, the usage of extraordinary solvents, including ethanol and hexane, could lead to a trivial difference between WE2 and EE2. Besides, EE also consumed much capital cost (equipment and related instruments) and operating cost (primarily for mixing process and removing solvent).

3.1.2. HPLC analysis

According to results in table 3, the amounts of catechins obtained from two given procedures were similar, approximately 54 mg (+)-catechin/ g RM and 31 mg (-)-epicatechin/ g RM. It again confirmed the equal effectiveness between WE and EE procedures. The amounts of (+)-catechin and (-)-epicatechin in the present study were pretty in accordance with that of Trox et al. research, which was respectively about 57.0 and 44.6 mg per gram of raw material [5]. The differences between these values were mainly caused by material, sample collection and preparation. The cashew tests in the present work was toasting cashew tests, which was collected from an industrial cashew nut process in Vietnam (roasting temperature was up to 200 °C in a cashew roasting machine). In comparison, the sample in the Trox report was manually removed from the kernels (drying 3 hours at 45 °C in a heating block) [5]. As a result, the exposure time to high temperature on an industrial scale led to decomposition and other reactions such as polymerization and epimerization [24,25], which caused the considerable decline in catechin and epicatechin contents.

Table 3. Comparison of (+)-catechin and (-)-epicatechin amounts.

| Procedure | (+)-catechin | (-)-epicatechin |
|-----------|--------------|----------------|
| WE        | 167.6±0.6    | 54.1±0.2       |
|           | 93.3±3.9     | 30.1±1.3       |
| EE        | 172.4±2.7    | 53.0±5.0       |
|           | 99.8±0.8     | 30.7±3.1       |

a: Procedure name; b: Concentration (mg catechin/ g DE); c: Concentration (mg catechin/ g RM)

Besides, HPLC results were disparate from that of TFC. For details, the amounts of catechin obtained by HPLC analysis showed the trivial difference between WE2 and EE2. However, TFC analysis gave a much higher TFC signal in EE2 than WE2. It also proved the poor correspondence between TFC signals and catechin contents given by HPLC analysis.

In conclusion, the dominance of the WE procedure was certainly proved through inherent advantages of water, avoidance of using extraordinary organic solvents such as ethanol and hexane, and time-saving process. Their amounts of (+)-catechin and (-)-epicatechin per gram of RM were around 54 and 31 mg, respectively. Furthermore, TFC and HPLC analysis gave different results because of their different problem-solving approaches.

3.2. Screening the impacts of extracting conditions on water extraction through OFAT method

In this study, WE and EE procedures were essentially evaluated through (+)-catechin and (-)-epicatechin.

3.2.1. Multiple extraction

Logical trends for both (+)-catechin and (-)-epicatechin contents in figure 3 showed that multiple extraction was more efficient than single extraction. The increase in extraction times led to the increasing amounts of solvent. Thus, extraction yield for catechins would increase [23].
Figure 3. Impacts of multiple extractions on the amounts of catechins.

Compared with single extraction, two-time extraction also gave a clear difference with 20.0% of (+)-catechin and 20.6% of (-)-epicatechin. Furthermore, two-time extraction was slightly smaller than three-time extraction, around 11% in (+)-catechin and (-)-epicatechin amounts. As a result, other multiple extractions above two-time extraction appeared to be not economically reasonable because they required more solvent volume for extraction, energy, and other costs for solvent removal.

In figure 4, the low accuracy and repeatability of TFC analysis compared to HPLC were clarified. This phenomenon strongly depended on how flavonoid compounds reacted with other substances in Aluminum Chloride Colorimetric Method. Furthermore, TFC signals were not only for catechins but also combined with other flavonoid compounds, which gave a higher value of quercetin equivalent per gram of RM. In other words, there was no selectivity for (+)-catechin and (-)-epicatechin through TFC analysis.

Figure 4. The impacts of multiple extraction on TFC in RM.

A specific trend showed that the increase in extraction times led to the rise in TFC in extracts, reaching a plateau when extraction times were further increased to four. This trend was similar to HPLC analysis in the above part but at different rates.

In conclusion, two-time extraction should be chosen due to the results of HPLC and TFC analysis.

3.2.2. Extracting temperature

Overall, two-time extraction at 60 °C gave the highest amounts of both (+)-catechin and (-)-epicatechin through figure 5.
Figure 5. Impacts of extracting temperature on the amounts of catechins.

The extracting temperature rise from 40 to 60 °C caused the decrease of water viscosity [27] and the increase of flavonoid solubility in the hydrophilic solvent [28]. As a result, the temperature rise led to the rise in catechin contents, which averagely peaked at 60 °C with 52.8 mg (+)-catechin and 29.6 mg (-)-epicatechin per gram of RM. On the contrary, the temperature rise between 60 and 80 °C showed a gradual decrease of both catechins contents. It could be reasonably explained through the dominant occurrence of complex reactions such as oxidation, polymerization, and epimerization [24,25]. The reaction rates, especially oxidation and polymerization, would gradually rise when the extracting temperature increased. As a result, the content of the catechin monomers would be decreased [29]. For epimerization reaction, especially if the temperature was higher than 80 °C, the more non-epistructured catechins would be formed from the originally epistructured ones [24,25]. Thus, despite the increase of flavonoid solubility when the extracting temperature reached above 60 °C, these reactions still caused the decline of thermal-sensitive flavonoid contents, especially (+)-catechin and (-)-epicatechin.

In contrast to HPLC analysis, TFC results in figure 6 peaked at 50 °C. Furthermore, the general trend obtained from TFC analysis was utterly different from that of HPLC. It once more confirmed the poor correspondence between TFC and HPLC results.

Figure 6. The impacts of extracting temperature on TFC in RM.

Based on HPLC results, 60 °C was the best temperature to extract the highest amounts of (+)-catechin and (-)-epicatechin in cashew testa.
3.2.3. Extracting duration
From 30 to 75 minutes in figure 7, the increase of extracting duration caused the rise in both (+)-catechin and (-)-epicatechin contents. This trend followed Vuong’s review, which stated that the total extraction yield of catechins rose when the extraction time increased and reached a plateau [30]. In the present study, the average amounts of catechins per gram of RM continuously increased, from 50.3 to 55.9 mg for (+)-catechin and from 27.7 to 31.1 mg for (-)-epicatechin.

![Figure 7. Impacts of extracting duration on the amounts of catechins.](image)

Through, 45-minute extraction was higher than 30-minute extraction, around 5.8% for (+)-catechin and 7.2% for (-)-epicatechin. These differences were more considerable than others, 60-minute extraction versus 45-minute extraction and 75-minute extraction versus 60-minute extraction in particular. In other words, the increase in extracting duration after 45-minute extraction could be time-consuming and energy-consuming but could not achieve significant differences in catechin levels. Hence, 45-minute extraction would be the most suitable extracting duration.

3.2.4. Solvent – material ratio
The figure 8 generally showed a similar trend for these two catechin amounts with the increase in the solvent–material ratio. For the amounts of (+)-catechin and (-)-epicatechin per gram of RM, they started at around 50.8 and 28.9 mg and ended at 54.7 and 31.2 mg. These figures showed that the rise in the amount of solvent caused the increase in catechin extraction yield [23].

![Figure 8. Impacts of solvent–material ratio on the amounts of catechins.](image)
In the presence of a large solvent amount, the extraction efficiency for catechins would increase [23]. The amounts of catechins per gram of RM obtained from 14:1 ratio, about 54.4 mg (+)-catechin and 30.8 mg (-)-epicatechin, were in second place and considered to have reached the plateaux. It implied that the increase in solvent–material ratio after 14:1 ratio could not bring any significant differences in catechin amounts. As a result, 14:1 ratio would be chosen. However, the primary solvent in this extraction step was water. The solvent removal for water was complicated, energy-consuming, and time-wasting. Hence, the amount of water used for extraction should be considerably calculated with more objective functions such as capital and operating costs.

3.2.5. Summarization of results
The four-factor investigation was temporarily ended with these most appropriate points for water extraction, including two times, 60 °C, 45 min/time, and 14:1 mL/g. Under these determined conditions, table 4 showed that the purity of (+)-catechin and (-)-epicatechin in water extracts were respectively about 15.1 and 8.5%. These percentages were nearly three-folded compared to raw material, at around 5.5% and 3.1% for (+)-catechin and (-)-epicatechin.

Table 4. TFC comparison in extracts collected from two different procedures.

|       | (+)-catechin | (-)-epicatechin |
|-------|--------------|----------------|
| EY    | C1^a         | C2^b           | C1^c         | C2^d           |
|       | 36.35±0.40   | 151.2±4.7      | 55.0±1.4     | 85.2±2.3       | 31.0±0.6       |

a: Extraction yield (%); b: Concentration (mg catechin/g DE); c: Concentration (mg catechin/g RM)

3.3. Fractional extraction with ethyl acetate
After the water extraction step, the purity of (+)-catechin and (-)-epicatechin was about 15.1 and 8.5%, respectively, which were relatively low for practical application. Hence, other purification methods should be evaluated to increase the amounts of these two catechins. Following research about the recovery of catechin compounds from Korean tea [22], ethyl acetate (EA) was used in this study to purify catechins in water extract (WE1). WE1 obtained from water extraction steps were converted into EA extract (WE2) by three-time EA extraction (part 2.5 for detailed procedure). The extraction yield, catechin amounts, and TFC in these two extracts were illustrated in table 5.

Table 5. TFC comparison in extracts collected from two different procedures.

| n^a  | EY^b  | C1^c  | C2^d  | TFC^e  |
|------|-------|-------|-------|--------|
| WE1  | 36.35±0.40 | 151.2±4.7 | 85.2±2.3 | 1022.86±15.77 |
| WE2  | 25.15±2.06 | 219.4±16.5 | 123.6±8.9 | 1456.02±151.80 |

a: Sample name; b: Extraction yield (%); c: (+)-catechin concentration (mg catechin/g DE); d: (-)-epicatechin concentration (mg catechin/g DE); e: TFC in DE (mg QE/g DE)

Overall, the amounts of catechins and TFC in WE2 were higher than WE1, which confirmed the ability of EA in flavonoid purification. For details, both catechin amounts and TFC in WE2 rose nearly 1.5 times compared to WE1. Besides, the extraction yield of WE2 was lower than that of WE1, which observed that fractional extraction will decrease extraction yield. Most compounds with suitable polarity compared to EA, especially (+)-catechin and (-)-epicatechin, would selectively separate from the water phase and transfer to the EA phase. Other impurities, including polyphenols, carbohydrates, carotenoids, tocopherols, or fatty acids [5], would partially dissolve in the EA phase. Thus, in WE2, the amounts of desired compounds would increase while impurities content would decrease. As a result, the purity of (+)-catechin and (-)-epicatechin in the present extracts was respectively about 21.9 and 12.4% on average. These values could not compare to other studies because the worldwide references for the present research were still limited.

4. Conclusion
This study indicated the dominance of WE procedure because of the inherent advantages of water and avoiding the use of extraordinary organic solvents such as ethanol and hexane. This procedure was good for the environment, humankind, and sustainable development. The effects of extracting...
conditions on catechin amounts were evaluated to determine the most suitable ones, including two-time extraction, extraction at 60 °C, 60-minute extraction, and 10:1 mL/ g for solvent–material ratio. Under these conditions, WE1 was obtained and converted into WE2 by using fractional extraction with EA. In comparison with the original extract, catechin amounts in WE2 increased 1.5 times, which implied the effectiveness of this method in purifying flavonoid compounds.

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References

[1] Mota M, Thomas G and Filho J 1985 J. Ethnopharmacol. 13 289–300
[2] Gracilene S, Ricardo M, Celuta A and Costa S 2005 J. Ethnopharmacol. 96 563–8
[3] Pierre K, Selestian S, Paul M, Pierre W, Hermine J and David L 1998 J. Ethnopharmacol. 62 95–9
[4] Nizer M, Irene R, Paul R, Suhaila M and Mahinda A 2002 J. Agric. Food Chem. 50 3693–97
[5] Jennifer T, Vellingiri V, Walter V, Wolfgang S, Dietmar K, Reinhold C, Veronika S, Ute G, Donatus N and Hans B 2011 Food Chem. 128 1094–99
[6] Neel C and Feredoon S 2011 J. Agric. Food Chem. 59 5006–14.
[7] Singh N, Jena U, Patel J and Sharma A Renew. Energy 2006 31 481–7
[8] Jaiswal Y, Tatke P, Ashok V and Gabhe S 2010 Indian J. Nat. Prod. 26 17–22
[9] Jaiswal Y, Tatke P, Gabhe S and Vaidya A 2010 Res. J. Pharmacogn. Phytochem. 2 372–6
[10] Khokhar S and Magnusdottir S J. Agric. Food Chem. 2002 50 565–70.
[11] Natsume M, Osakabe N, Yamagishi M, Takizawa T, Nakamura T, Miyatake H, Hatano T and Yoshida T Biosci. Biotechnol. Biochem. 2000 64 2581–87.
[12] Oliveira N, Leal R and Dantas T 2015 Am. Int. J. Contemp. Res. 2 09–41
[13] Amadi J and Adebola 2008 Afr. J. Biotechnol. 7 4591–94
[14] Chang C, Yang M, Wen H and Chern J J. Food. Drug. Anal. 2002 10 178–82
[15] Khadiga A and Galal G 2018 Ann. Agric. Sci. 63 173–80.
[16] L. Luis B and Carmen N Angew. Chem. 2002 41 179–81.
[17] Liu Y, Shi J and Langrish T Chem. Eng. Sci. 2006 120 203–09
[18] Manish S, Luke A and Gennadi O Comput. Chem. Eng. 1999 23 1381–94
[19] Truesdale G, Downing A and Lowden G J. Appl. Chem. 1955 5 53–62
[20] Shi J, Nawaz H, Pohorly J, Mittal G, Kakuda Y and Jiang Y Food Rev. Int. 2005 21 139–66
[21] Hoerr C and Harwo H J. Phys. Chem. 1952 56 1068–73.
[22] Row K and Jin Y Bioresour. Technol. 2006 97 790–93
[23] Amra P, Mojca S, Zeljko K, Bernd W, Frank O and Sabine G Food Chem. 2006 96 597–605
[24] Biscay F, Ghoufi A and Malfreyt P J. Chem. Phys. 2011 134
[25] Li B, Smith B and Hossain M Sep. Purif. Technol. 2006 48 182–88
[26] Danlami J, Arsad A and Zaini M J. Taivan Inst. Chem. Eng. 2015 47 99–104
[27] Kestin J, Sokolov M and Wakeham W J. Phys. Chem. Ref. Data 1978 7 941–48.
[28] Chebil L, Humeau C, Anthoni J, Dehez F, Engasser J and Ghoul M J. Chem. Eng. Data 2007 52 1552–56
[29] Ünflü A, Prasad B, Anavekar K, Bubenheim P and Liese A Prep. Biochem. Biotechnol. 2017 47 918–24.
[30] Vuong Q, Golding J, Statopoulos C, Nguyen M and Roach P J. Sep. Sci. 2011 34 3099–106