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Research paper

Study Identification of Bioactive Compounds from Fractional Extracts of Cornlettes Using LC-TOF/MS

Nurraihana Hamzah[1], Saffanah Mohd Ab Azid[1], Wan Rosli Wan Ishak[*1]

Nutrition & Dietetics Department, School of Health Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia.
**Corresponding author E-mail: wrosli@usm.my*

Abstract

Cornlettes or baby corn is a type of vegetable. It can be easily found in Asian cuisines. Since previous reports indicated that the crude extracts of cornlettes showed some significant antioxidative scavenging properties, this vegetable may contain certain functional bioactive compounds. This study aimed to identify bioactive constituents in the fractional extract of local Malaysian cornlettes. The aqueous-alcoholic extract of cornlettes was fractioned with hexane and ethyl acetate, subsequently. The LC-MS system was used to elucidate different types of functional compounds from the ethyl acetate fraction. Both crude and the fractions were evaluated for total phenolic content and antioxidant activities. The LC-MS analysis of ethyl acetate fraction showed that it contains six phenolic compounds, four carboxylic acids and one for each of alkaloid, amino acid, ethyl ester and adenine. The ethyl acetate fraction exhibited the most significant DPPH radical-scavenging activity and the highest amount of total phenolic compounds**.** In summary, cornlettes may provide a rich source of phenolic compounds and functional nutrients including essential fatty acids.

Keywords: *Antioxidant activity; Cornlettes; Phenolic; Phytochemicals;* LC-MS

1. Introduction

Phytochemicals are the plant derived chemicals and naturally very diverse. They consist of a wide range of chemical compounds with different structures and functionalities that can be used as nutraceuticals, functional food and food additives.[1, 2] These bioactive compounds are beneficial to human health as they can prevent many chronic diseases such as cancer, diabetes, cardiovascular diseases, stroke and other illnesses.[3] Both fruits and vegetables are the most powerful natural sources of bioactive compounds. With this situation, escalation of attention has been focused on fruits and vegetables as they might play some potent significant roles in preventing a wide range of diseases such as cancer, diabetes, stroke, coronary heart disease, cataracts, arthritis, Alzheimer's disease and inflammatory bowel disease.[3-5]

The use and application of natural resources such as edible plants around the world in combatting various illnesses including non-communicable diseases are still occur. Until today, many population around the globe still rely on the prowess of fruits and vegetables as their routine staple therapeutic diet or food ingredient in rejuvenating and sustaining their health condition.
Cornlettes is a young cob corn (*Zea mays* L.), the newly developed corn which is a type of vegetable commonly found in most of the Asian cuisines and other parts of the world. This vegetable is commonly consumed by Asian populace in form of either salad or minimally cooked. This tender vegetable item is normally mixed with other types of vegetable in the preparation of soup. On the other practice, both sautéing and stir-frying techniques are commonly chosen by Asian populace including Malaysian. Alternatively, this young cob ear is sometimes being steamed or blanched before eaten with a certain type of condiments.

It appears yellow in colour with the size of our finger-length. It has a sight sweetness intensity and succulent taste. Cornlettes is a good source of nutrients such as protein, crude fibre, carbohydrate, dietary fibres and other essential nutrients. It also contains vitamin C, β-carotene and some essential minerals such as calcium, magnesium and phosphorus.[6] Zin, Robert [7] has shown that cornlettes powder incorporated in both baked-based foods namely cookies and muffins resulted in a lower value of the glycaemic index (GI) and reduces the postprandial blood glucose response. In addition, cornlettes extracts also showed high radical scavenging activity.[8]
There was a study which focused on the physicochemical properties and morphological characteristics of composite flour added with cornlettes (*Zea mays*) for functional food ingredient was done recently.[8] Composite flour of cornlettes has shown a uniformed wheat starch granules which clustered with the irregular shape of the cornlettes starch granule. Addition of cornlettes to partially replace wheat flour to form a blend or composite flour has resulted in improvement of swelling power and amylose composition while slightly altering the starches morphological structures. [8]

Very recently, our preliminary study revealed that cornlettes had demonstrated the promotion growth of certain probiotic bacteria. In addition, cornlettes was also found to show potential prebiotic properties (unpublished data). Many previous studies have also linked the health benefits of cornlettes with various therapeutic properties including nutritional and functional characteristics after

being incorporated in some food formulations. However, there is a scanty report on the chemical composition of cornlettes.

Thus, the aim of the present work is to investigate phytochemical profiles of cornlettes which are locally consumed in Malaysia and other Asian countries. Our analytical attention was focused on secondary metabolites particularly phenolic compounds which have never before been investigated and reported in the literature. Liquid chromatography coupled with time-of-light mass spectrometry (LC-TOF/MS) was chosen as the powerful method of chemical analysis to identify the metabolites in cornlettes extracts using liquid-liquid extraction (LLE).

2. Materials and Methods

2.1 Sample Preparation

Fresh young cornlettes was collected from Pasir Mas District, Kelantan, Malaysia. The cornlettes was detached from the fruit stalks and then oven dried at 50°C for two days. The dried cornlettes was ground into powdered form and then sieved with 150 µm size sieve shaker and stored in the airtight Duran bottle at 4°C before further analyses.

2.2 Fractional Extraction Preparation

Preparation of fractional extract was done according to our previous study.[9] Briefly, dried cornlettes was extracted with 60% methanol with ratio 1:10 (*w*:*v*) in a conical flask and was shaken at a constant rate using a water bath shaker for 30 min at 60°C. Then, the extract was filtered through a Whatman No. 1 filter paper. The filtrate was concentrated to dryness using a rotary evaporator and then lyophilized in freeze-dryer (Ilshin, Korea).

Five grams of the obtained crude extract was dissolved in 100 ml of distilled water and sequentially extracted thrice using 100 ml hexane and ethyl acetate. Then the solvent in each fraction was removed using a rotary evaporator. The cornlettes fractions then proceeded for total phenolic content, DPPH scavenging activities and phenolic identification using LC-TOF/MS systems.

2.3 Total Phenolic Content and DPPH Scavenging Activity Analysis

Both total phenolic content and DPPH (2, 2 -diphenyl-1-pycryl-hydrazyl) free radical scavenging of the crude and fractional extracts were determined according to our previous study.[9] There are 4 types of extracts/fractions of cornlettes which were used to determine the total phenolic content and DPPH scavenging activity, namely crude extract, hexane fraction, ethyl acetate fractions and water fraction.

2.4 Phenolic Compound Identification

Analysis of phenolic compounds in cornlettes extract using Liquid chromatography-time-of-light mass spectrometry (LC-TOF/MS) was done according to our previous study.[9] The extract was injected to an Agilent® 1290 Infinity LC system (Agilent Technologies, Inc., Santa Clara, CA, USA) coupled to an Agilent 6520 Accurate-Mass Q-TOF mass spectrometer with dual ESI source. The extract was separated using a C18 column (Agilent Zorbax Eclipse XDB-C18, 2.1 mm × 150 mm) using the following condition: a gradient consisting of a gradient mobile phase mixture system of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) as the mobile phase. The solvent gradient was as follows: 5% B held for 5 min, 5-100% B in 15 min, held for 5 min and 100-5% B in 5 min. The negative mode was used for MS. Data was analysed using the MassHunter Qualitative Analysis B.05.00 software. Molecular feature extraction (MFE) was performed in order to determine the molecular masses.

3. Results and Discussion

The results of total phenolic compounds and DPPH scavenging capacities of crude and fractions are given in Table 1.

Table 1: Total phenolic compounds (TPC) and DPPH scavenging activity of different extract/fractions obtained from cornlettes.

Sample	TPC, mg/mL	DPPH scavenging activity, %
Crude extract	18.86±1.14b	18.58±2.46b
Hexane fraction	ND*	6.45±3.87c
Ethyl acetate fraction	114.92±1.07a	84.18±1.61a
Water fraction	16.09±2.09c	16.59±4.26b

Results are means ± standard deviations for three replicates of samples. Values within columns with different letter are significant different at $p<0.05$. *ND = Not detected.

Generally, polar solvents are regularly used for the extraction of polyphenols from vegetables or fruits or other natural products. The common solvents used are aqueous mixtures containing acetone, ethyl acetate, ethanol and methanol. In the present study, ethyl acetate fraction of cornlettes showed the highest total phenolic content (114.9 mg/ml) while crude extract and water extract fraction recorded total phenolic content of 18.9 and 16.1 mg/ml, respectively. The ethyl acetate fraction of cornlettes also showed the highest DPPH radical-scavenging activity at 84.2% while crude extract, water extract fraction and hexane fraction recorded significantly lower percentage of DPPH radical-scavenging activity (18.6, 16.6 and 6.5%, respectively). This is due to the high amount of polyphenolic constituents present in the fraction.

Other previous studies had also shown that the anti-oxidative activities and free radical scavenging capacities have increased proportionally with the polyphenol content.[10-12] This is due to the fact that phenolic groups in phenolic compounds that are responsible for high antioxidant activity and their hydroxyl groups confer significant scavenging ability.[12]

The ethyl acetate fraction was further used for chemical compound analysis as it showed the highest DPPH radical scavenging activity and amount of total phenolic content. To know what is/are a responsible active compound in the fraction, the LC-TOF/MS was used. Using the molecular masses found and the suggested formula given by the MS software, tentative compound identification is listed in Table 2. The compounds assigned to the peaks in the chromatogram (Figure 1) were chosen based on molecular mass.

The high resolution and sensitivity of the TOF detector over the triple quadrupole assist the identification of the phenolic compounds in the cornlettes extract. With the advancement of technology, LC-TOF/MS systems is preferred due to the advantages in terms of its robustness, accuracy and sensitivity in identifying bioactive compounds specifically phenolic groups.

Fractional extract of cornlettes contained six phenolic compounds comprised of three phenolic acids, one each of flavonoid, lactone and hydroquinones. Phenolic acid found in this extract are D-α-Hydroxyglutaric acid (Peak 1), gallic acid (Peak 2) and 4- Hydroxyphenylpyruvic acid (Peak 3). Gallic acid is a compound that widely distributed in fruits and plants. It can be found in nutgalls, grape and various types of tea. It has anti-fungal and anti-bacterial properties.[13] It also significantly showed cytotoxicity to tumor cells.[14] Gallic acid extract was also reported to exhibit various pharmacological properties. This compound which prominently detected in ash gourd was linked with hypoglycaemic and cardioprotective properties.

This fractional extract also contained four carboxylic acids comprised of three fatty acids and one monocarboxylic acid. Fatty acids identified in this extract were methyl N-(a-methylbutyryl)glycine (Peak 9), 9S,10S,11R-trihydroxy-12Z-octadecenoic acid (Peak 11) and 9E,12Z,15Z-octadecatrienoic acid (Peak 15). The 9S,10S,11R-trihydroxy-12Z-octadecenoic acid was previously found in Tasmannia lanceolate.[15] While, 9E,12Z,15Z-octadecatrienoic acid was found in flaxseed oil, canola, soy, perilla, and walnut oils.[16]

Peak 14 was identified as Kukoamine B, an alkaloid found in Lycium chinense [17] and has been reported to have the potential to treat sepsis.[18] In addition, many compounds remained unidentified in all samples. The TOF instrument allows for the identification of unique masses even when the peaks in the chromatogram appear to be co-eluting as seen in figure 1. Indeed, the numerous unidentified compounds need additional comprehensive study.

Table 2. Compounds Identified in Fractional Extract Using LC-TOF/MS in the Negative Mode.

Peak No	Retention time (min)	Compound assigned	Unique mass	Class of compound
1	1.08	D-α-Hydroxyglutaric acid	148.037	Phenolic acid
2	1.60	Gallic acid	170.0217	Phenolic acid
3	7.71	4-Hydroxyphenylpyruvic acid	180.0426	Phenolic acid (carboxylic acid)
4	7.83	Methyldopa	211.0846	Amino acid
5	8.85	8-Hydroxyluteolin 7-glucoside	464.0943	Flavonoid
6	8.99	Monoethyl phthalate	194.0582	ethyl ester
7	9.44	5-Pyridoxolactone	165.0428	Lactone
8	9.59	6-Benzylaminopurine	225.101	Adenine
9	9.61	Methyl N-(a-methylbutyryl)glycine	188.1055	Carboxylic acid (fatty acid)
10	10.45	Sebacic acid	202.121	Carboxylic acid (dicarboxylic acid)
11	12.27	9S,10S,11R-trihydroxy-12Z-octadecenoic acid	330.2421	Fatty acid
12	14.41	Cytotrienin A	648.3403	Hydroquinones (phenol)
13	15.96	Kukoamine B	530.3126	Alkaloid
14	18.50	9E,12Z,15Z-octadecatrienoic acid	278.2254	Fatty acid

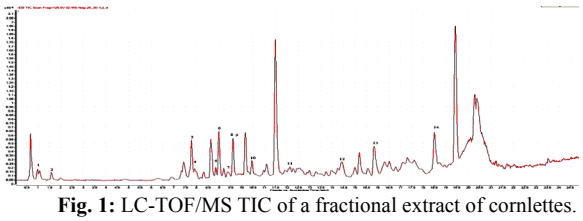

Fig. 1: LC-TOF/MS TIC of a fractional extract of cornlettes.

4. Conclusion

This study has indicated the presence of bioactive compounds and exhibition of antioxidant properties in the fraction of cornlettes. The compounds identified in the fractions are mainly phenolics. Therefore, this fraction could be exploited further for the possibility of its applicability in both nutritional and nutraceutical. Furthermore, studies in isolation and quantification of individual phenolic compounds are needed for further investigation. Mechanistic action of phenolic compounds in exhibiting significant protective effects on pre-clinical *in vivo* systems is suggested for future study.

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