HIGH VALUE ADDED OF AMINO ACIDS, AROMATIC MATERIALS AND NUTRIENTS IN *Citrullus lanatus* RINDS – A WASTE TO WEALTH

Norma Hussin
Zaulia Othman
Samsiah Jusoh
Rosalizan Saleh
Muhammad Afif Dzulkarnain
Industrial Crop Research Centre,
Malaysian Agricultural Research and Development Institute (MARDI), 43400 Serdang, Selangor, Malaysia.
Email: normahus@mardi.gov.my

Mohamad Jani Saad
Teoh Chin Chuang
Masniza Saari
Amir redzuan Shamsulkamal
Engineering Research Center,
Malaysian Agricultural Research and Development Institute (MARDI), 43400 Serdang, Selangor, Malaysia

Hazniza Adnan
Food Science & Technology Research Centre,
Malaysian Agricultural Research and Development Institute (MARDI), 43400 Serdang, Selangor, Malaysia

Asraf Hadi Hadi Abu Samah
MARDI Kuala Linggi, Kuala Linggi, 78207 Kuala Sungai Baru, Melaka

ABSTRACT

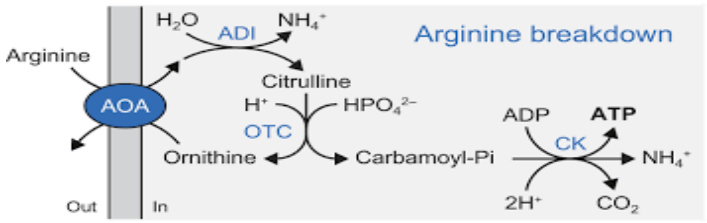
Watermelon (C. lanatus) is a tropical fruit with sweet taste and juicy fruits. The fruits can be taken as a nutritious juice which is very popular drinks in Malaysia. A consumption of watermelon is usually focused on their fruit pulp or flesh and the rest is discarded. The waste of fruit peels or rinds contain many of important nutrients such as amino acids, vitamins and other phytonutrients. The fruits consist of 60 % flesh and 40 % rinds, and the melon rinds contain approximately 92.50% moisture content. Other valuable content in this fruit is citrulline amino acid, which is cannot be produced by the body and it is very important as a substance for controlling blood pressure. In this research arginine was selected as a specialty amino acid, due to this amino acid is related to the citrulline amino acid in our body. The C. lanatus compounds can lower blood pressure by releasing two primary amino acids namely L-citrulline and arginine, and these amino acids can be converted into a nitric oxide in the body and stimulate the blood vessels, which is an essential process for regulating blood pressure. In this research, three samples of C. lanatus rinds were found at 17mg/100g and 46mg/100g (red types C. lanatus); and 23 mg/100g (yellow type C. lanatus) of arginine amino acid levels together with other essential amino acids. The main objectives of this research are to determine the nutritional properties, amino acid contents and aroma profiles in the C. lanatus rind extracts.

Keywords: Amino acid, aroma profiles, Citrullus lanatus, nutritional analysis and watermelon

INTRODUCTION

Watermelon (*Citrullus lanatus*) is a very famous short-term non-seasonal fruit in Malaysia. The fruit has been categorized under major fruits by the Ministry of Agriculture and Food Security, Malaysia. Watermelon can be taken as a nutritious food, juices and popular drinks in Malaysia. The consumption of watermelons is usually focused on their fruit pulp or flesh and the rest is discarded. The waste of rinds (exocarp) contains many important nutrients such as amino acids, vitamins and other phytonutrients. The fruits consist of 60 % flesh and 40 % rinds, and the melon rinds contain approximately 92.50 % of moisture content. Other valuable content in this fruit is citrulline amino acid, which cannot be produced by our body, however, it is very important as a substance for controlling blood pressure. In Figure 1, the existing of citrulline metabolisme is produced by an intake of arginine source via the arginine breakdown pathway. Even though there is an amount of citrulline reported in the watermelon, this study is more focused on the arginine content that is naturally derived from the waste of watermelon peels.

Figure 1: A schematic of the arginine breakdown pathway.



AOA, arginine/ornithine antiporter; ADI, arginine deiminase; OTC, ornithine transcarbamoylase; CK, carbamate kinase. Source: Tjeerd Pols et. al (2021)

The technologies for the determination of bioactives and producing extracts from watermelon rinds (by-products) were mentioned by Xiana et al. (2020). In our body, nitric oxide (NO) is involved in the hypertension and chronic kidney disease (CKD) and there is no synthase that can metabolize an L-arginine (ARG) to generate NO and L-citrulline (CIT), as mentioned by Ying-Jui Lin et al. (2013). In Figure 1, Tjeerd Pols et al. (2021) illustrated a detailed pathway of 'A Schematic of the Arginine Breakdown Pathway'. Other important byproducts such as ethanol can be produced by watermelon waste and rind parts. The valuable waste material of watermelon rinds can be utilized for production of amino acids, flavour and fragrance (F&F), phytochemical materials, and valuable fuels. Regarding Bichi et al. (2022), *C. lanatus* aqueous rind extract has bioactive constituents of pharmacological importance, and these compounds can be used in the pharmaceutical industry to design and develop novel lead drugs for treating diseases. Thus, these valuable watermelon rinds are very important to be utilized as a value-added supplement. The main objectives of this research are to determine the nutritional properties, amino acid contents and discover the aroma profiles in the *C. lanatus* rind extracts.

MATERIALS AND METHODS

Materials

Two types of red and yellow flesh of watermelons were purchased from a FAMA outlet at Putrajaya and a Mini Giant Store, Bangi Selangor, respectively. A hygienic collection of watermelon rind collections from the watermelon fruits was prepared from washing, cutting, separations of the pulp parts (edible), cutting the rinds, grinding and extracting the watermelon rind pulp. The extract samples were frozen until analysis. Nutritional contents: Determination of nutritional analyses (proximate) as referred to the Method of Analysis for Nutrition Labelling, 1993 & Pearson's, 1991. A total inverted sugar is with reference to total sugar (all monosaccharides and disaccharides) as described in the AOAC 923.09.

Amino acid analysis

The watermelon rind samples were processed at MARDI and the amino acid analysis was done at UNIPEQ, UKM Malaysia. Determination of the amino acids was conducted by using a HPLC-AccQ-Tag Waters Method as followed by UNIPEQ-UKM laboratory.

A semi-volatile aroma (ethanol extract)

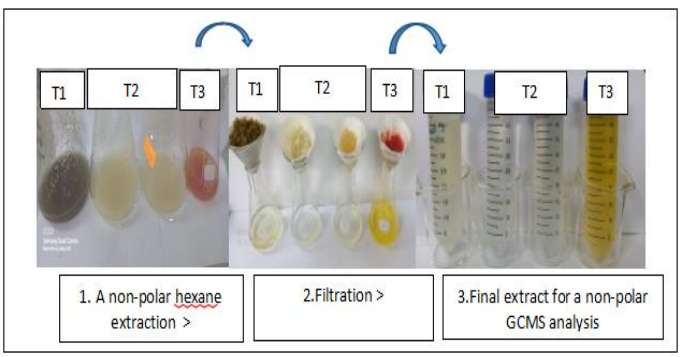
An analysis of watermelon rind parts was done in this research. The extracts were filtered using a Whatman no.1 and kept in a freezer until preparation for another GC-MS analysis for determination of semi-volatile compounds. The mass spectra results were analyzed and compared with a NIST 17 library database. The compounds with qualitative value ≥ 90 similarity were reported. The GC-MS running condition was set as followed:

GC Parameters	
Injection mode	Splitless
Pressure	31.383 psi
Oven	50 ºC (2min), 25 ºC/min to 325 ºC (3 min)
Column	Agilent DB-UI 8270D 20 m x 180 µm x 0.36 µm

A non-polar aroma constituents in watermelon parts

A three parts of watermelon aroma were extracted using the procedure in Figure 2. The composition of the volatile aroma fractions of watermelon part sample was investigated using a gas chromatography coupled to mass spectrometry analysis (GC-MS) using a non-polar column.

Figure 2: Watermelon part aroma extraction for GCMS analysis



RESULTS AND DISCUSSION

Nutritional analyses of watermelon rinds and pulp

In Table 1, four samples of watermelon rinds were evaluated for nutritional analyses. Carbohydrate, fibre, fat, ash, protein and moisture contents were ranged between 2.2-7.0 %, 2.6-2.8 %, 2.6-3.0 %, 0.3 -0.8 %, 0.1-0.3 %, 0.6-0.8 % and 92.0-93.1%, respectively. The sweetness of the rinds was less sweet, 1.0 - 3.8 %, compared to the watermelon pulp products (IT1). The energy levels based on the kcal for both watermelon rinds were between 5.5-7.0 kcal. As a reference, a red pulp of watermelon concentrated (IT1) produced by ER Centre MARDI was also determined by its nutritional values and the energy kcal/100g of the product sample. The pulp product (IT1) energy was higher than the rind parts due to its carbohydrate (inverted sugar) was higher at 8.8% and 9.4%, as compared to watermelon rinds (KT1MF, KT2C and KT3Y) that contain lower inverted sugar of 2.2%, 1.0% and 3.8% respectively. However, with a low amount of sugar level in the rind parts, these will benefit the watermelon waste processing industries especially for producing a stable dried powder of watermelon rinds for secondary product blending and processing.

Table 1: Nutritional analyses of watermelon rinds and concentrated watermelon pulp

Nutritional Contents	Methods	KT1MF	KT2C	KT3Y	IT1
Carbohydrates	OF/17-037	2.7	2.7	4.0	8.8
Carbohydrates	OF/17-035	5.5	5.8	7.0	9.4
Energy(kcal/100g)	OF/17-036	2.8	27	31	41.9
Energy(kJ/100g)	OF/17-036	116	113	130	176
Total Dietary Fibre	OF/17-014 (AOAC985.29)	2.8	3	2.6	0.7
Moisture Content	OF/17-038 (Moisture Analyzer)	93.1	92.7	92.0	89.3
Ash content	OF/17-002	0.3	0.8	0.3	0.2
Fat Content	OF/17-010	0.3	0.2	<0.1	<0.1
Total inverted sugar	OF/17-001 (AOAC923.09)	2.2	1.0	3.8	8.8
Protein Content	OF/17-006	0.8	0.6	0.7	1.0

Notes: KT1MF - Watermelon Rind (Red pulp fruit 1); KT2C - Watermelon Rind (Red pulp fruit 2); KT3Y - Watermelon Rind (Yellow pulp fruit) and IT1 - Watermelon pulp/juice concentrated

Semi aromatic profiles of watermelon rind extracts

Table 2 shows that 16 natural aroma chemicals derived from ethanol extract of the watermelon rinds were detected with a high probability of more than 90% mass library compounds, which comprised of 5-hydroxymethylfurfural and 2,3-dihydro-3,5-dihydroxy-6-metyl-4H-pyran-4-one, were at 33.45% and 17.61% respectively. Other important compounds also were obtained with a probability (Qual) of more than 91% with referred to the NIST Library. As reported by Karabonde et al. (2014), the GC-MS melon aroma analysis of n-hexane extract of *Citrullus vulgaris* flesh revealed 55 compounds, which include 5- hydroxymethyl furfural, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, and ethyl ester-hexadecanoic acid are reported as antioxidants. Other compound of (Z,Z)-3,6-nonadienal is the predominant watermelon odorant with several other aldehydes reported. One of these is (Z)-3-hexenal, the same compound largely responsible for the smell of fresh-cut grass. These two compounds are similar in their aroma.

Table 2: Semi-volatile compounds derived from the ethanol extract of watermelon rinds

Retention time (min)	Library Compound ID (NIST17)	Qual (Probability)	Retention time (min)
4.468	5-methyl-2-furancarboxaldehyde	94	0.28
4.671	2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	91	4.03
5.801	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	95	17.61
6.017	3,5-dihydroxy-2-methyl-4H-pyran-4-one	94	2.71
6.300	5-hydroxymethylfurfural	95	33.45
7.082	(*E*)-2-tetradecene	98	0.30
8.113	1-tridecene	90	0.24
9.761	*n*-hexadecanoic acid	99	4.83
9.874	Ethyl ester-hexadecanoic acid	99	1.30
10.349	Phytol	98	0.25
10.444	(*Z,Z*)-9,12-octadecadienoic acid	99	3.64
10.472	(*Z,Z,Z*)-9,12,15-octadecatrienoic acid	99	4.73
10.538	Ethyl ester linoleic acid	97	1.05
10.641	Ethyl ester octadecanoic acid	98	1.22
12.619	(*Z*)-13-docosenamide	95	0.32
14.936	(3β,5α,24*S*)-stigmast-7-en-3-ol	95	1.97

A non-polar aroma constituents in watermelon parts

The aroma compounds of the T1, T2 and T3 extracts obtained from n-hexane extracts were shown in Figure 3, 4 and 5, which contain aroma constituents with more than 0.5% constituents and 90% above of probability as referred to the mass library. The

constituents obtained were found more in alkane, fatty acids and other aromatic compounds, as seen in Figure 3, 4 and 5. The aroma of watermelon skin (T1 - dark green peel extract) has refreshing green aroma constituents, as shown in the GC-MS aroma profile (Figure 3). In a Figure 4 (T2 extract of light green rinds) contributed a fresh aroma of watermelon. The natural aroma of T3 extract of flesh pulp (Figure 5) presenting a sweet fruit aroma with a fresh melon-scented aroma characteristic and yellowish clear in colour. A finding by Bichi et al. (2022) mentioned that *C. lanatus* aqueous rind extract has thirty-one (31) bioactive constituents of pharmacological importance identified by GC-MS and FTIR analysis. These watermelon rind extracts are highly economic potential materials as value added aroma ingredients to be incorporated in fragrance and flavours (F&F materials), stimulants/enhancers in animal feeds, cosmetic-soap and aromatherapy ingredients and as fuel or gas substances.

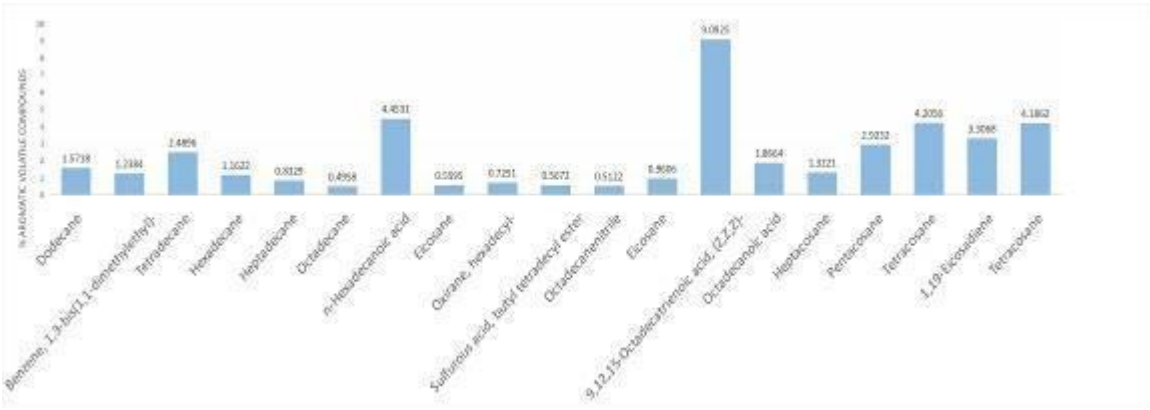


Figure 3: Tentative volatile aromatic compounds of watermelon peel skins (T1- green skin part) by GCMS analysis

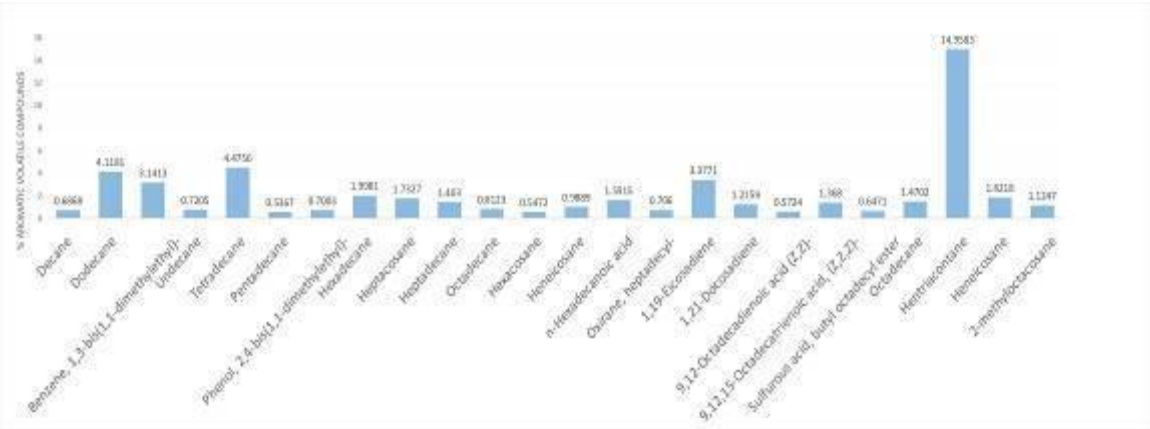


Figure 4: Tentative aromatic compounds of watermelon rinds (T2-green-whitish skin part) by GCMS analysis

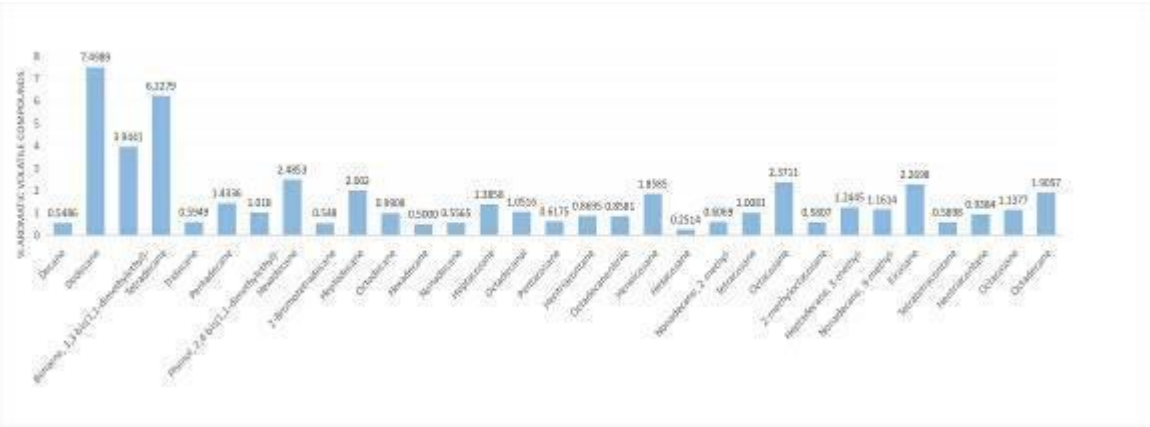


Figure 5: Tentative aroma compounds of watermelon flesh (T3-red) by GCMS analysis

Amino acid profiles

In this research, arginine amino acid was selected as a specialty amino acid, due to this amino acid is related to the conversion of citrulline in the body. The *C. lanatus* compounds can lower our blood pressure by releasing two primary amino acids namely L-citrulline and arginine, and these amino acids can be converted into nitric oxide in the body and stimulate the blood vessels, which is an essential process for regulating blood pressure. Watermelon rind (red pulp 1 and 2) contained up to 46 mg/g and 17 mg/g arginine amino acids, and watermelon rinds (with yellow pulp) contained 23 mg/g arginine amino acid. These amino acids were important as phytochemical in the human body and related to the conversion of arginine into citruline amino acid in the body. Thus, by consuming watermelon rinds will increase our arginine and citrulline contents, and its act to reduce our blood pressure as mentioned by many researchers.

CONCLUSION

Watermelon rinds are rich with nutritional value and amino acid nutrients, besides other industrial substances such as alkanes, acids, vitamins and other components. The aroma extract of watermelon rinds are valuable as industrial materials. The content of the amino acid of arginine can be referred to the important of citrulline, which is an important nutraceutical substance for controlling our blood pressure.

ACKNOWLEDGEMENT

This research was funded by the Malaysian Agricultural Research and Development Institute (MARDI) - PIC-521 (P21400010170521) fund under Engineering Centre, MARDI and also thanks to our Industrial Crop Research Centre R&D team, CRIMLab UKM and ALS Technichem (M) Sdn.Bhd. for their special services.

REFERENCES

Bichi S. A, Abdulmumin Y, Abdulmumin T.M, Muhammad I.A, Murtala M, Dalhatu M.M, Amina L. A, Sarki S.I , Danjaji H.I, Mustapha R.K. (2022). GC-MS and FTIR analysis of aqueous extract of Citrullus lanatus (Water Melon) rind. *Sch Int J Biochem*, 5(4): 57-66.

Karabonde A.U., Atiku M.K., Wudil A.M.and Buratai L.B. (2014). Antioxidant effect of Citrullus vulgaris (watermelon) extract against lipid oxidation in fish during ooking. *Journal of Pure and Applied Sciences*, 7(2): 105 – 110.

Method of analysis for nutrition labelling. (1993). AOAC International, Arlington, Va.

Pearson, M.E. (1991). Composition and Analysis of Foods. Ed. Ronald, S. Kirk and Ronald Sawyer, Ninth Edition. 640-642.

Tjeerd Pols,Shubham Singh,Cecile Deelman-Driessen,Bauke F. Gaastra,Bert Poolma 2021. Enzymology of the pathway for ATP production by arginine breakdown. *The FEBS Journal* 288 (2021) 293–309.

Xiana Rico Beatriz GullónJosé Luis Alonso Remedios Yáñez (2020). Review - Recovery of high value-added compounds from pineapple, melon, watermelon and pumpkin processing by-products: An overview. *Food Research International*. volume 132, June 2020, 109086.

Ying-Jui Lin 1, Chien-Ning Hsu, Mao-Hung Lo, Chien-Fu Huang, Shao-Ju Chien, You-Lin Tain. (2013). High citrulline-to-arginine ratio associated with blood pressure abnormalities in children with early chronic kidney disease. *Circ J*. .2013;77(1):181-7.