



ANALYSIS OF METABOLITES PRESENT IN ULTRASONICALLY TREATED KARUPU KAVUNI BY USING GC-MS/FID

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The Investigation of metabolites present in the ultrasonically treated kavuni rice are determined by the GC-MS/FID. In this study the karupu kavuni rice is ultrasonically treated at the temperature of 30^o C & 45^o C and examined for the metabolites by using the GC-MS/FID more than twenty active compounds are identified and found at the different peak heights. At ultrasound treatment 30^o C and 45^o C, the highest peak was found at the 18.1min and metabolite found as cis-13-Octadecenoic acid. In untreated kavuni rice similar types of the compounds was identified. Hence this study proves that the ultrasound treatment on the rice, retains the metabolites, present in the kavuni rice

Keywords: Karupu kavuni; ultrasound treatment; GC-MS/FID.

1. INTRODUCTION

The black rice which is called as “Karuppu Kavuni” in tamil is a type of rice variety which is naturally fortified with immense proportion of nutrients and antioxidants. In ancient days, it was believed that the regular consumption of black rice will prevent ageing and increase the longevity of the royal society. Due to this belief, the consumption of black rice was

restricted only to the royals of ancient kingdoms and was forbidden to common civilians and thus got several names like Forbidden Rice, Imperial Rice, King Rice and Prized Rice. Moreover, due to its rich anthocyanin content on its outer layer of the kernel, it is also called as Black Rice or Purple Rice [1]. Several researches in the recent decade have found that the black rice have excellent antioxidants that effectively quenches the reactive oxygen species

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(ROS) in the body and thus, by acting as functional food, it reduces the incidence of diabetes, obesity, cardiac attack etc., and also by acting as a potential chemo preventive agent, it hinders the cancer growth [2]. Many studies have linked some of these beneficial health effects with the anthocyanin content of the black rice. Among several anthocyanin pigments, the Cyanidin 3-glucoside (which constitutes 93% of its anthocyanin content) and Peonidin 3-glucoside are found to be predominant pigments identified in black rice [3]. It is also observed that one tablespoon of black rice equals or exceeds the amount of anthocyanin present in blueberries. Some established health effects of black rice (Karuppu Kavuni) are as follows: Rich in antioxidants particularly anthocyanin, Iron and Protein. Natural gluten free grain enriched with beneficial phytochemicals comparatively more than blueberries. Apart from its health benefits, the black rice is adored by the common people for its stunning black coloured outer bran and, the high anthocyanin and fiber content along with a pack of aromatic natural phytochemicals present in the black rice gives as an excellent and appealing visual and textural feel and a specific appetizing aroma after cooking. Though the rice is the predominant staple food in several countries, its potential contribution in the prevention or management of several chronic diseases is not so widely recognized. In contrary, several researches prove that the consumption of fermented traditional pigmented rice varieties helps in bringing immense health benefits and makes the consumers to lead a nutritious life. Fortunately, the research on the effects of black rice consumption has gained popularity in recent decade or so and thus, enabling us with good research data to know its effects on different organs of the body. A huge number of scientific studies have shown that, the black rice powder is one of the nature's most well balanced super food and its abilities are truly remarkable. In some studies, the quality of the black rice can be improved by the appropriate technologies and treatments [4]. when compared to other methods such as pre-gelatinization, enzymatic treatment, germination treatment, and soaking treatment, ultrasound treatment on rice will reduce the whole process time, enhance cooking and eating properties of white and brown rice varieties [5]. The main advantage of ultrasound is, it requires only a minimal processing time, inflated productivity with lower energy utilization. So it is highly recommended to several processing methods such as extraction process, emulsification methods, homogenization, crystallization, filtration, separation, viscosity alteration, defoaming, and extrusion. For the effective solubilization, modification, and purification of starch, the ultrasound treatment is highly suitable. In this study the phytochemical screening of the

ultrasonically treated kavuni rice was analyzed and compared with untreated rice samples.

2. MATERIALS AND METHODS

2.1 Sample Collection

The freshly milled Karupu kavuni (*Oryza sativa L. indica*) rice is procured from the farmer in Thiruvavur district, Tamilnadu, India. After the procurement, the grains are stored at room temperature sealed in polyethylene bags for future analysis.

2.2 Ultrasonic Treatment

Karupu kavuni rice of 250g is soaked in 300 ml of water. Then the soaked grains are sealed in the zip lock polyethylene pouches. After packing, the PE pouches are placed inside the ultrasonic equipment (SONICA 45L EP S3 *) treated at the temperature of 30° C and 45° C for 30 mins after the treatment, rice samples are kept at room temperature.

2.3 GC-MS Analysis

2.3.1 Sample extraction

The ultrasonically treated samples are grinded and extracted with methanol, and analyzed through Gas Chromatography – Mass Spectrometry flame ionization detector for identification of different metabolites.

2.3.2 GC methods

The 8890GC/5977B GC/MSD equipped with Column Rtx-5MS (5% Diphenyl / 95% Dimethyl polysiloxane), 30m x 0.25mm ID x 0.25µm. The carrier gas was flowed at the rate of 1ml per min, with the Split ratio of 10:1. The Single Quadrupole Mass Spectrometer was used as the detector with the sample injection volume of 2µl, the Oven temperature Programmer was set at 110° C hold for 3.50 min Up to 200° C at the rate of 10 ° C/min, No hold Up to 280 ° C at the rate of 5° C / min followed by 12 min hold. For the data analysis OpenLab CDS Software was used. The temperature of the injector is 280° C.

3.3.3 Methods for detection

NIST Version-2020 was used as the library. For the detection, the Inlet line temperature is 290° C with source temperature of 250 ° C, Electron energy at 70 eV, the ionization was happens at 50-550 amu Mass scan (m/z) . 0 - 3.5 min is needed for the solvent

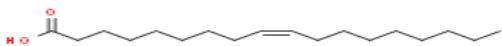
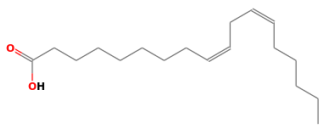
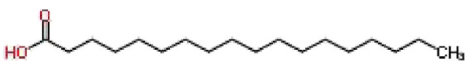
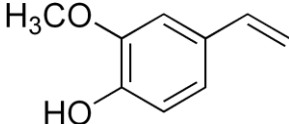
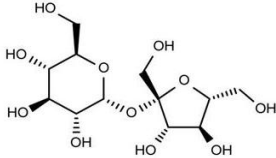
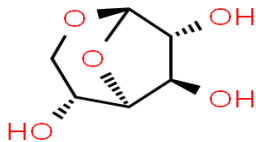
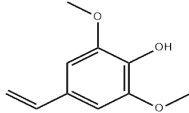
delay, the Total GC- MS running time is about 40.50 min.

4. RESULTS AND DISCUSSION

The signal provided by a compound that elutes from the GC column into the detector is represented by each peak in the chromatogram. The peak size is related to the amount of comparable compounds in the

examined samples. The results are tabulated in the below tables, Table 1 represent the compounds present in the untreated sample. Table 2 and Table 3 represents the compounds present in the ultrasound treated kavuni rice at the temperature of 30⁰ C and 45⁰ C respectively. For the untreated and treated kavuni rice, the highest peak was at the retention time of 18.2 min, 18.1min and 18.1min the identified compound as cis-13-Octadecenoic acid.

Table 1. Structure of metabolites present in the untreated kavuni rice (*Oryza sativa L. indica*)

S.no	Name of the compound	structure
1	Oleic Acid	
2	9,12-Octadecadienoic acid (Z,Z)-	
3	n-Hexadecanoic acid	
4	2-Methoxy-4-vinylphenol	
5	Sucrose	
6	β -D-Glucopyranose, anhydro	1,6- 
7	Phenol, dimethoxy-	4-ethenyl-2,6- 

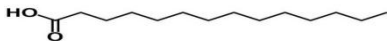
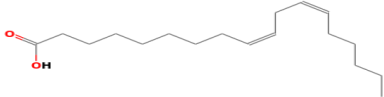
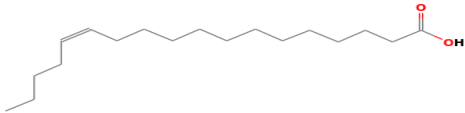
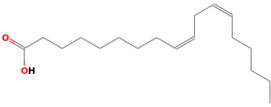
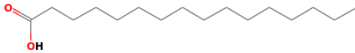
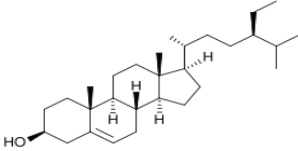
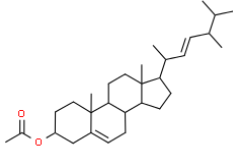
S.no	Name of the compound	structure
8	Tetradecanoic acid	
9	Pentadecanoic acid, methyl-, methyl ester	
10	Hexadecanoic acid, methyl ester	
11	9,12-Octadecadienoic acid (Z,Z)-	

Table 2. Structure of metabolites present in the ultrasonically treated at 30 ° C kavuni rice (*Oryza sativa L. indica*) at different retention time

S.no	Name of the compound	Retention time	structure
1	cis-13-Octadecenoic acid	18.18	
2	9,12-Octadecadienoic acid (Z,Z)-	18.11	
3	n-Hexadecanoic acid	15.69	
4	β-Sitosterol	37.49	
5	Ergosta-5,22-dien-3-ol, acetate, (3β,22E)-	35.93	

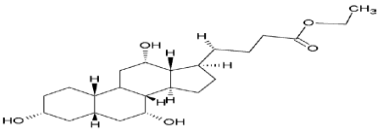
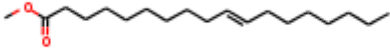
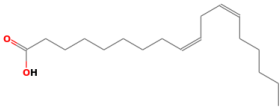
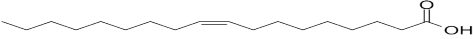
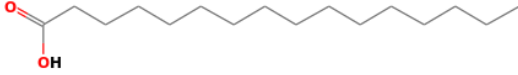
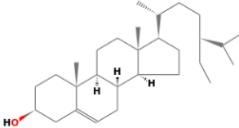
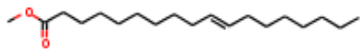
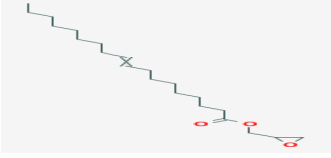
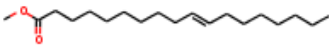
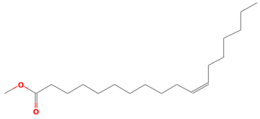
6	Ethyl iso-allocholate	35.10	
7	10-Octadecenoic methyl ester	acid, 17.6	
8	Oleic Acid	18.4	
9	8,11-Octadecadienoic acid, methyl ester	17.5	

Table 3. Structure of metabolites present in the ultrasonically treated at 45⁰ C kavuni rice (*Oryza sativa L. indica*) at different retention time

S.no	Name of the compound	Retention time	structure
1	9,12-Octadecadienoic acid (Z,Z)-	18.17	
2	Oleic Acid	18.24	
3	n-Hexadecanoic acid	19.68	
4	γ-Sitosterol	37.50	

S.no	Name of the compound	Retention time	structure
5	10-Octadecenoic acid, methyl ester	17.61	
6	9-Octadecenoic acid (Z)-, oxiranylmethyl ester	23.04	
7	10-Octadecenoic acid, methyl ester	17.6	
8	8,11-Octadecadienoic acid, methyl ester	17.5	
9	Oleic Acid	16.2	

The majority of the compounds are similar to the treated and the untreated sample. For both treated and untreated kavuni rice there are totally 20 active metabolites compounds are found. The molecular formula for the oleic acid is $C_{18}H_{34}O_2$ and it is used for the replace of saturated fats in the diet and also it involves in lowering cholesterol and inflammation. 9, 12-Octadecadienoic acid (Z,Z) which helps in the production of the mammalian nutrition [6] n-Hexadecanoic acid is called as palmitic acid, which

act as the food additive and surfactant [7]. Tetradecanoic acid act as the antibacterial and it has the role as human metabolite. Methyl esters are act as the excellent solvent. Ethyl iso-allocholate is act as the potent inhibitor for Dihydropteroate synthase. Sterols are used in the pharmaceutical industry used in the formation of the progesterone and corticoids. The Fig. 1. represents the obtained chromatogram of GC-MS analysis for control sample.

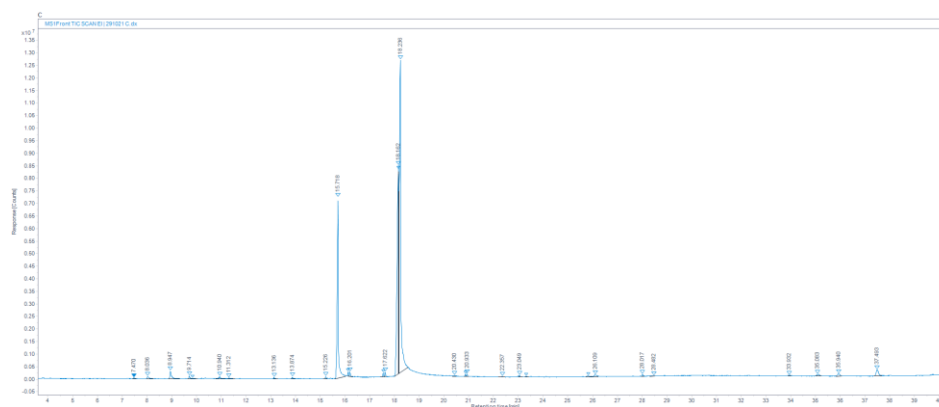


Fig. 1. Phytochemical screening of untreated sample

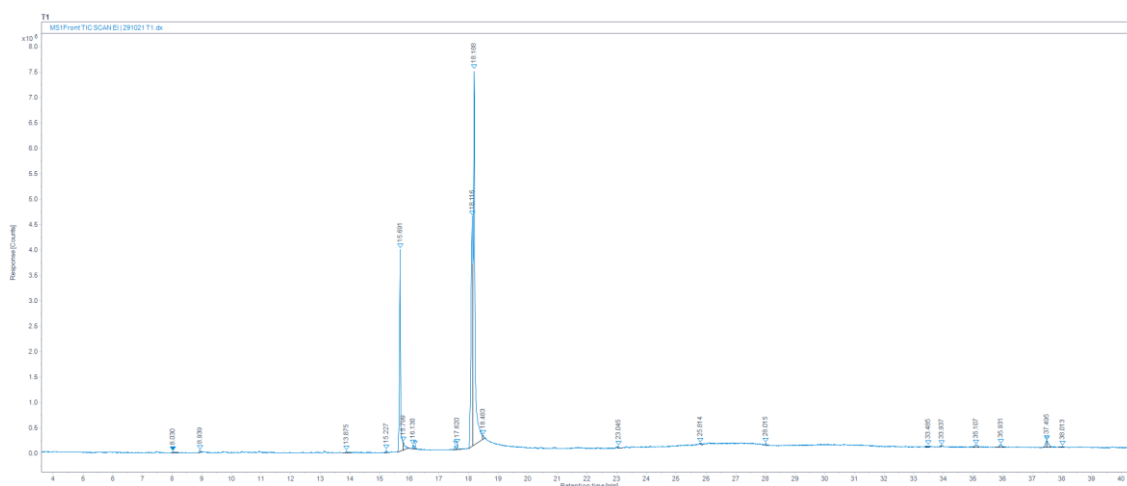


Fig. 2. Phytochemical screening of ultrasonically (30^o c) treated

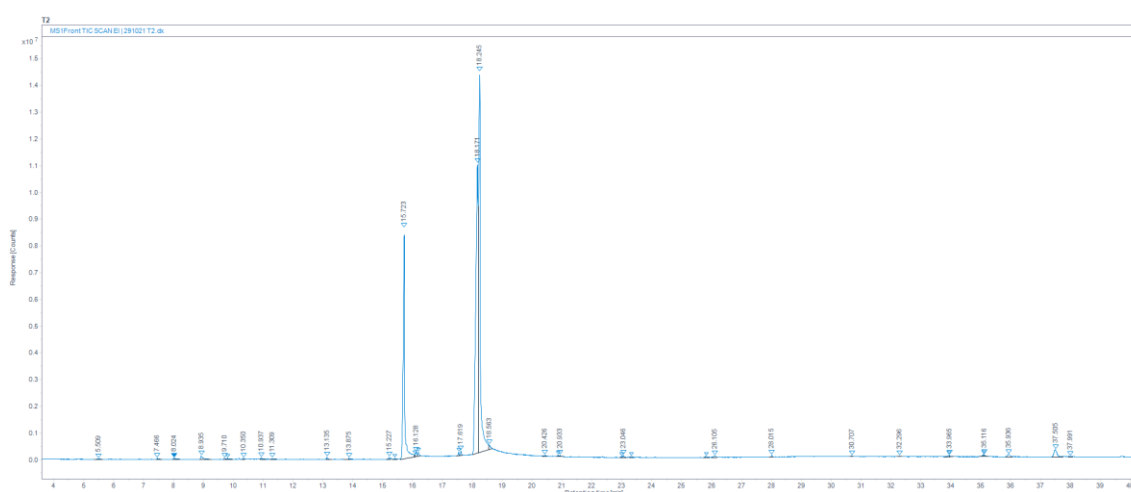


Fig. 3. Phytochemical screening of ultrasonically (45^o c) treated sample

For both ultrasonically treated and untreated samples the metabolites present in the kavuni rice are almost similar, the increase of temperature in the ultrasound sound treatment does not affect the major metabolites present in the kavuni rice. Hence the ultrasound treatment is recommended to processing and development of food products.

5. CONCLUSION

The black rice is immensely enriched with the lot of metabolites, which act as the sanative food for various types of illness. The ultrasound treatment is the non-thermal green technology which effectively retains the various metabolites. The GC-MS analysis of karupu kavuni rice of treated and untreated kavuni rice samples, have found similar metabolites are identified. Hence this method of green technology is highly recommended for the processing of rice

varieties and development of value added products from the kavuni rice.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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