

Biological Functions of Volatile Compounds Extracted from the Rice Bran- A Review

Tejaswi Jasti¹, Rama Krishna Chintala² and Viswanatha Chaitanya Kolluru^{1*}

¹Department of Biotechnology, GITAM Institute of Technology, GITAM University, Visakhapatnam-530045, India.

²Department of Environmental Studies, GITAM Institute of Science, GITAM University, Visakhapatnam-530045, India.

*Corresponding Author E-mail : viswanatha.chaitanya@gmail.com

<http://dx.doi.org/10.13005/bpj/1879>

(Received: 11 December 2019; accepted: 25 March 2020)

Rice is the staple crop that serves as the main source of food for the three fourth of the world's population. Rice grain is surrounded by a brown layer known as bran, which serves the purpose of nourishing the seed during its initial stages of development. Bran is rich in nutrient compounds such as oryzanols, phytosterols, tocopherols, tocotrienols, coumaric acid, and oil. It is also a rich source of volatile compounds (VOCs). A number of VOCs with nutraceutical properties are extracted from the rice bran, which is believed to play a pivotal role in increasing the nourishment as well as enhancing the human health benefits. The present review provides the physical, chemical, biological properties and various extraction methods of VOCs isolated from the rice bran. Biological functions of the VOCs such as antioxidant and anti-cancerous properties has been provided along with its critical impact on the endocrine and central nervous system.

Keywords: Aroma; Antioxidants; Biological Functions; Rice Bran; Volatile Compounds.

Rice is a cereal crop consumed as the staple food by most of the world's population, mainly by the people of Asia, America, and Africa Continents. Almost 18,000 varieties of rice are available in the global market and more than 100 countries are involved in the production of rice. Among them, Asia is the leading producer of rice, which alone produces 90% of the total rice output worldwide. The average size of a rice grain is 5.51 to 6.60mm in length. Mainly, it is made up of Theendosperm, husk, Bran and Germ. A rice grain consists of 70% endosperm and its by-products include 20% husk, 8% bran and 2% germ.¹ Rice grain is surrounded by a brown layer constituting the bran. Rice bran

is composed of an aleurone layer and a pericarp. It consists of 50% carbohydrate, 15% protein, 20% oils, oryzanols, phytosterols, tocopherols, tocotrienols, pectin, and other dietary fibers.² They are well known for their anti-cancerous, antioxidant, anti-diuretic, anti-allergic and many other nutraceutical properties. For example, 2-acetyl 1-pyrroline is the major component of rice that significantly contributes to the aroma, known as popcorn aroma.³ The phytochemicals such as phenolic compounds, β -cryptoxanthin, and zeaxanthin displays antioxidant properties, which helps in reducing the free radicals.⁴ Linoleic acid, a polyunsaturated oil isolated from the rice bran

has the ability to lower plasma LDL cholesterol, whereas folic acid lowers plasma homocysteine.⁵ Coumaric acid is a volatile compounds (VOC) hat has anti-tumorous activity against human malignant tumors⁶. Flavonoids are considered to have anti-allergic, anti-inflammatory and gastroprotective effects.⁷ Lignins help in inducing the cell cycle arrest, in-vitro apoptosis and also plays an active role in the inhibition of colon cancer.⁸

Volatile Organic Compounds (VOCs)

Chemical compounds with a low boiling point and high vapor pressure (> 0.1 millimeter mercury) at room temperature are known as volatile organic compounds (VOCs). The low boiling point possessed by these compounds permits them to evaporate easily as they are held by weaker intermolecular forces. VOCs are basically emitted from two major sources into the atmosphere.⁹ They are (1) Anthropogenic sources such as incinerators, piping, flares, wastewater systems, heaters, catalytic cracking units, etc. and (2) Biogenic sources that are the natural sources that emit VOCs through microbes present in the soil, vegetation and also through abiotic processes such as lightning and burning of biomass. These biogenic sources are main sources for the emission of non-methylated volatile organic compounds (NMVOCs) and nitric oxide.¹⁰ VOCs are large in number and ubiquitous in nature. Alkanes, alkenes, alkynes, alcohols, aldehydes, ketones, aromatic hydrocarbons are the various types of volatile organic compounds that are found to be present in the rice bran^{11,9}. Nowadays, a large number of these compounds are isolated from foods and beverages also. For example, many volatile organic compounds are present in wheat bran, rice bran, oat bran, etc. As these compounds are reported to play an important role in fighting against many diseases, the consumption of cereal foods is increasing day by day.

Physical and Chemical Properties of VOCs

Physical and chemical properties of volatile organic compounds include intermolecular forces, boiling point, and bond dissociation energy, solubility in water, melting point, vapor pressure, the dissociation constant in water, critical temperature, molecular mass, and molar volume, Henry's law constant, fugacity, and octanol-water partition coefficients.¹¹ As volatility means having the ability to escape into the atmosphere, these

volatile organic compounds must be formed by the molecules bound by weak intermolecular forces so that the bonds present between them can be broken easily.¹² The temperature at which the vapor pressure of a liquid equals the pressure of the surrounding and liquid gets converted into vapor is known as the boiling point. Based on the boiling point volatile organic compounds are classified into 3 categories:

1. Very volatile organic compounds (VVOCs) -boiling point between 50° and 100° C
2. Volatile organic compounds (VOCs) -begin to boil from 50° to 100° C and end boiling at 240° and 260° C
3. Semi volatile organic compounds (SVOCs) – begin boiling at 240° and 260° C and end boiling at 380° to 400° C.

The pressure exerted by a liquid at equilibrium on its vapor phase is known as the vapor pressure of the liquid. The VOCs are compounds having a high vapor pressure. According to recent research, organic compounds with low vapor pressure are also found to be volatile.¹³ As the melting point of a substance depends on the intermolecular forces, it follows the same trend as the boiling point. The solubility of a given compound depends on the degree of branching, hence it is not the same for all VOCs. Henry's law constant explains about the partitioning of a chemical between the two phases in a binary system at equilibrium. More of Henry's law constant, more is the volatility.¹⁴ These are some of the physical and chemical properties of volatile organic compounds.

Biological Properties of VOCs

The biological property of a compound is defined as a natural extension to the physical and chemical properties that provide energy for various reactions in an organism. These organic chemicals have made our lives much easier by taking part in our day to day activities. Biological properties of VOCs include anti-infective, anti-biotic, anti-tumor, anti-allergic, anti-ulcer, anti-inflammatory, anti-arthritic and nutraceutical properties. They are also well known for their anti-oxidative, cardiovascular activities and can also treat many diseases, which are degenerative.¹⁵ Apart from this VOCs also help in the prevention of oxidative damage to nuclear DNA and other vital tissues in the human body. Compounds such as citral,

Z-ligustilide, and eugenol are known for their anti-infective nature against malaria and many other diseases.¹⁶ Antioxidation is the deactivation of free radicals, which are natural by-products of oxidative metabolisms. g-oryzanol is found to be one of the best anti-oxidant compounds present in the rice bran.¹⁷ VOCs are also believed to be cholesterol-lowering agents for many years as they maintain low-density lipoprotein (LDL) cholesterol levels in the body¹⁷. Encompassing cereal-based foods to the diet helps in lowering blood pressure and improves insulin sensitivity resulting in reducing the risk of coronary heart disease (CHD)⁵. Based on the experiments conducted, it was found that cyanidin-3-glycoside is metabolized into cyanidin and protocatechuic acid. These metabolites help in the inhibition of lipopolysaccharideLPS-induced inflammation.¹⁸ Being highly volatile, they can be easily absorbed through the skin. For example, tocotrienols stabilize the free radicals produced in the skin, when it is exposed to harmful oxidative rays and repairs the skin by protecting it from the UV rays. Hence, they are referred to as anti-aging agents. Reduced production of insulin by pancreas results in impaired entry of glucose into the cells (hyperglycemia). This problem can be solved by g-oryzanol, which stimulates the production of insulin.¹⁹ As ECG has both ups and downs, volatile organic compounds also have their own beneficiary and detrimental issues. These chemicals affect various organs and systems of the human body when comes in contact with them through ingestion, inhalation, and dermal contacts. An experiment was conducted in which a group of people was exposed to volatile organic compounds for a specific period of time. As a result, they developed symptoms like irritation in throat, nose, eye, headache, mental illness, and respiratory problems.²⁰ In recent studies, it was proved that overexposure to volatile organic compounds leads to neurobehavioral disorders in humans, as some of them bind to hemoglobin and hampers its capacity of transferring oxygen to various body parts, leading to heart and brain disorders.²¹ It was also stated that VOCs take part in an immunosuppressive activity, resulting in auto-immune diseases. VOCs such as propane, butane, etc. pose a variety of human health hazards, as they are believed to act as endocrine disruptors, reproductive and liver toxins.²²

Biological Functions of Volatile Organic Compounds Present in Rice Bran Antioxidants

As a result of various biochemical reactions that occur in the human body, many reactive oxygen species (ROS) and reactive nitrogen species (RNS) are formed in the form of free radicals and released into the body system. The majority of these compounds are oxygen-derived, often referred to as pro-oxidants. These free radicals produced are toxic in nature and are said to attack the macromolecules like lipids, proteins, and DNA thereby resulting in the damage of cells and tissues in the body. They also lead to the cause of many diseases like neurodegenerative, cardiovascular diseases (CVD), aging, diabetes, liver malfunction, kidney problems, and many other chronic disorders.²³ It is imperative to suppress the generation of these free radicals and to properly dispose them out of human body, which is done by special compounds or scavengers known as antioxidants. These are produced within the body or taken from the exogenous sources.²³ Generally, the ratio of pro-oxidants and antioxidants in the body is proportionate to each other. Under some circumstances, antioxidants get diminished leading to a surge of pro-oxidants, ultimately causing oxidative stress. Type 2 diabetes is mainly due to the oxidative stress that is developed within the body. It is evident that the antioxidants are produced endogenously by various mechanisms that involve metal-binding proteins and various enzymes. a-tocopherol is one of the most important antioxidants that is produced by the human body. Unfortunately, the amount of the endogenously produced a-tocopherol is not sufficient to deactivate all the pro-oxidants that are released to prevent the damage. Consider a case where the DNA gets damaged by the continuous action of pro-oxidants, amount of antioxidants that are produced is unable to compensate for the incurring damage, leading to the formation of several kinds of cancers. In order to avoid such complications, antioxidants are to be supplemented through regular accomplished with food supplements, fruits, vegetables, and many grains enriched with antioxidants in the diet for maintaining good health and well-being. Out of these rice bran is considered to be the richest source of these compounds with antioxidant properties (Table 1). Vitamin E is one of the major

antioxidants that are present in the rice bran. The amount of vitamin E present in the rice bran is 300mg per kg. It is composed of α -tocopherol, α -tocotrienol, γ -tocopherol, and γ -tocotrienol. Tocotrienols consist of more antioxidant activity than tocopherols. The contents of tocopherols, tocotrienols, and oryzanol in rice bran are ranging from 378 -503 ppm, 343 – 478 ppm and 511- 802 ppm respectively.²⁴

Another major compound in rice bran with antioxidant properties is γ -oryzanol. It is a mixture of 10 ferulate esters of triterpene alcohol and is considered to have the highest antioxidant activity when compared to all other VOCs present in the bran. Antioxidant properties of γ -oryzanol are due to the presence of ferulic acid, a strong antioxidant, in its basic structure. 3000mg per kg of γ -oryzanol is present in the rice bran, which is much greater than α -tocopherol.²⁵ Phytosterols and its derivatives also contribute to their antioxidant properties.²⁶ Phenolic compounds are also efficient antioxidants. High concentrations of low molecular weight phenolic compounds such as ferulic acid and coumaric acids are found in the rice brans, whose pericarp is light in color. Rice brans with red pericarp are rich in high molecular weight phenolic compounds including cyanidin-3-O-b-D-glucoside, peonidin-3-O-b-D-glucoside, malvidin, ferulic, protocatechuic acids, etc.²⁷

Antioxidants play a key role in improving human health. Therefore, it is very important to consume foods that are rich in antioxidant reserves. Apart from this, antioxidants also involved in improving the shelf life of various foods and their by-products. Shelf life is the time period up to which food products can be consumed. The bio products present in the food gets degraded after a certain period of time, which results in their spoilage. In addition to this, the effect of various factors like oxidation, rancidity, temperature, crystallization of the molecules, loss of volatile organic compounds from the food may also result in reducing the shelf life of a particular food product.²⁸ It will be of no use in consuming foods after the completion of their shelf life period as the nutrients, vitamins, and proteins will no longer be in their active state. Change in color, smell, taste, and texture are the major indications of a food product crossing its shelf life period. In order to regulate the effect of these reactions and also to

increase their shelf life, antioxidants are to be added to the foods at the time of their processing. Coumarins, flavonoids, phenolic compounds, tocopherols, and tocotrienols were the various antioxidants present in the rice bran. As already discussed in the previous section, tocopherols and tocotrienols are mainly involved in the prevention of lipid oxidation, which in turn reduces rancidity. While transporting fragile vegetables such as tomatoes, there is a high chance of depletion due to many factors such as temperature and pressure acting on them. If they are transported to long distances, then factors such as rancidity and oxidation will also come into play. It is the responsibility of a food processor to incorporate the required antioxidants to the foods, while they are getting packaged. Antioxidants present in the rice bran helps in enhancing the shelf life of foods thereby maintaining their nutritional quality.

Anti-Cancerous Properties

The incidence of cancer is rising day by day. It is estimated that 55% of the deaths worldwide are due to different types of cancers, developed due to the uncontrolled growth of cells inside the body.²⁹ These abnormal cells are known as cancerous cells or tumor cells. Many types of cancers were identified, but the major ones include colon, breast, prostate and lung cancers, which occurs mainly due to the mutations in the genes, DNA lesions and, cell division, which is the critical factor. Endogenous damage involves the damage of DNA by the oxidation products of various reactions. In the absence of antioxidants, the damage is much more amplified, leading to the formation of cancer. Poor diet, pollution, prolonged exposure to the sun, occupation, hormones, medical interventions are some of the other factors that cause cancer. The major symptoms of cancer are fatigue, dry mouth, weight loss, early satiety, pain and weakness, anorexia, constipation, etc. When it comes to young people, depression, anxiety, sleeplessness, bloating, vomiting, etc. are also included.²⁹ Cancer can be controlled by taking an appropriate diet which includes fruits, vegetables and more importantly bran based foods rich in bioactive phytochemicals. These phytochemicals are known to be cancer-fighting and also take part in enhancing the immune system.³⁰ One of the main reasons for the formation of a tumor is due to the damage that is done to the DNA by the

oxygen species. This problem can be resolved by including the rice bran products in the diet as it is rich in carotenoids and phenolic compounds which are effective antioxidants. Components of rice bran including α -tocopherol, γ -tocopherol, phytosterols like stigmasterol, lycopene and β -sitosterol, γ -oryzanol and other micronutrients such as calcium, magnesium, vitamins and amino acids which are all effective chemo preventive agents (Cho *et al.* 2009). Earlier studies demonstrate that the fermentation of rice bran with *Saccharomyces baoulardii* has produced more amounts of ferulic acid, which helps in lowering the lymphoma cell division when compared to the normal rice bran. A reduction in the formation of tumors was observed

in the mice that were fed with 30% rice bran along with the regular diet. Tricin, a flavone isolated from rice bran was proved to be as effective as the non-steroidal drugs used against cancer. It is a chemopreventive and chemotherapeutic agent, involved in the protein regulation of the cell cycle pathway and arresting the growth of human breast cancer cells.³¹ MGN-3 or bio bran (modified arabinoxylan) is a fermented product of rice bran that helps in regulating the growth of T-regulatory cells, which are involved in immunosuppression, thereby maintaining the immune responses within the body of cancer patients.³² γ -tocotrienol and cisplatin (a chemopreventive agent) have shown a great effect on the viability of H28 mesothelioma

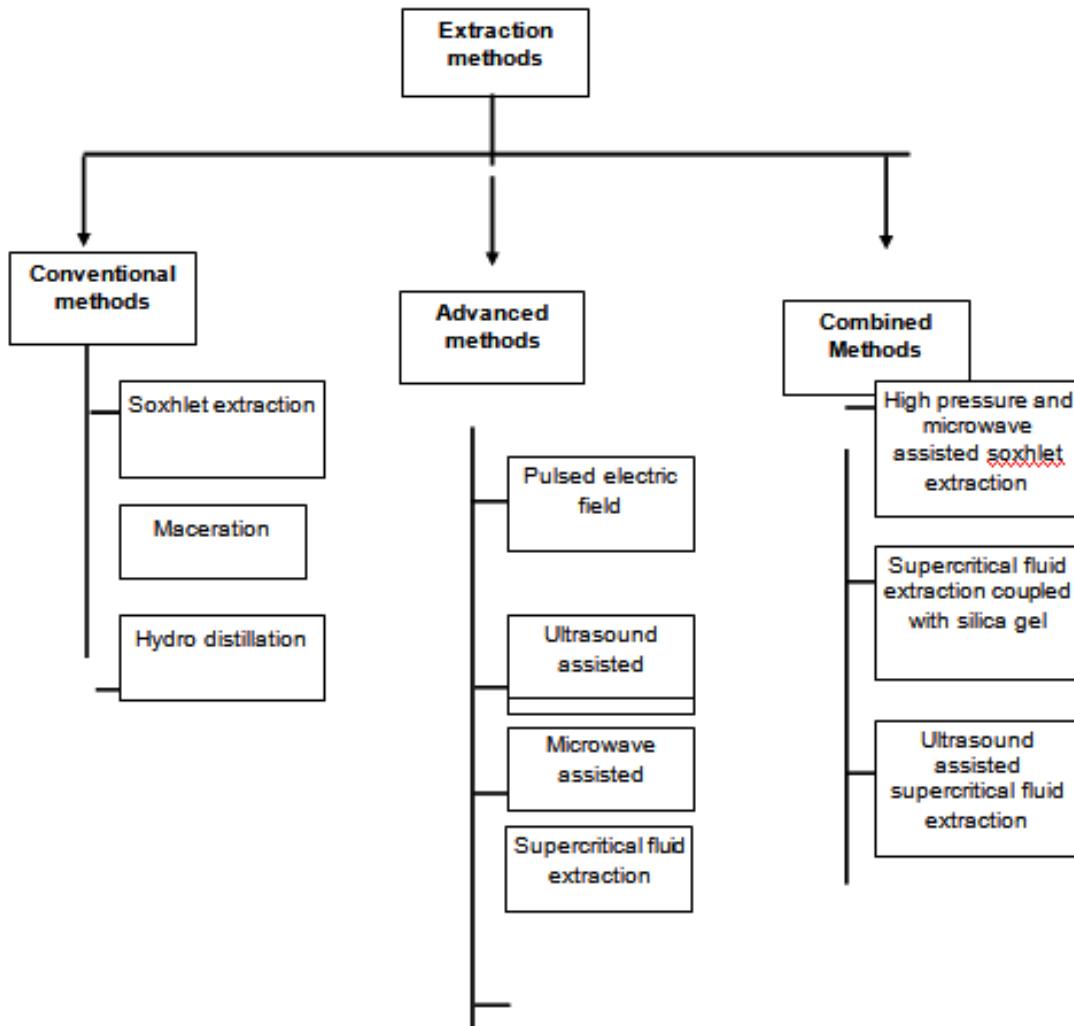


Fig. 1. Techniques for the extraction of VOCs from rice bran

cancer cells.³³ Bioactive compounds present in rice bran have exhibited chemo preventive properties in enhancing or inhibiting the immune responses. DNA mutations get accumulated inside the body due to overexpression of COX-2, responsible for the development of tumors. The coumaric acid

and other components can regulate such immune responses. Phytic acid, a chelator of many metals like zinc, magnesium, etc. present in rice bran has been reported for its anti-cancerous property. It is also involved in the suppression of colon carcinogenesis induced by azoxymethane.³⁴

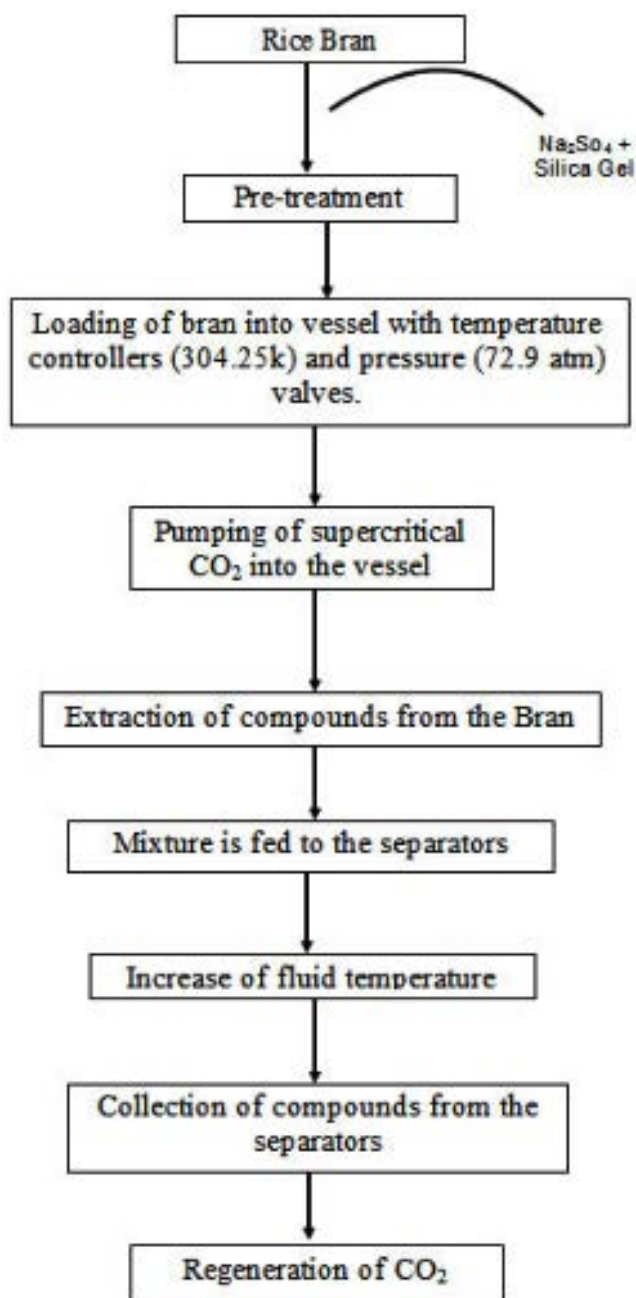
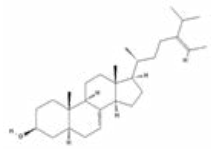
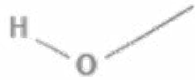
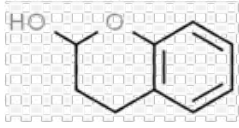
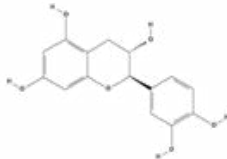

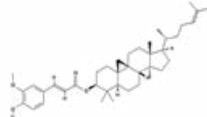
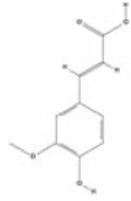
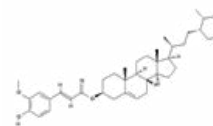


Fig. 2. Supercritical CO₂ extraction of VOCs from rice bran

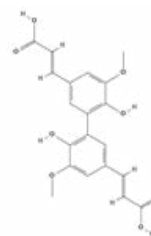
Table 1. List of volatile compounds isolated from rice bran with antioxidant properties

Compound Name	Antioxidant Properties	Structure
Avenasterol	Isomerization of allylic free radical to stable radicals	
Methanolic compounds	Scavenges DPPH and ABTS free radicals	
Chromanol	Prevention of lipid oxidation	
Catechins and rutin	Scavenges the peroxy free radicals	
Phytic acid	Inhibits superoxide radical dependent DNA damage	
cycloartenylferulate	Reduction of oxidative stress	
ferulic acid	Protection of skin from UV radiation	

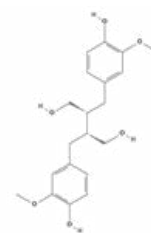
24-methylenecycloartanyl Prevents lipid peroxidation and reduces the free radical ferulate and α -sitosterol production ferulate



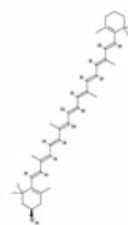
Diferulic acid lipo protein Prevents the oxidation of low density



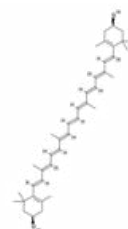
Secoisolariciresnol High antioxidant property



b-cryptoxanthin Inhibits the formation of radicals



Zeaxanthin Inhibits reactive oxygen Species (ROS) formation

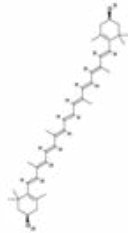
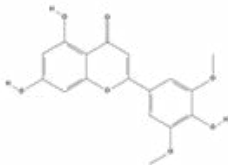
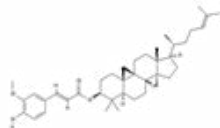
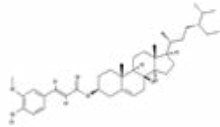
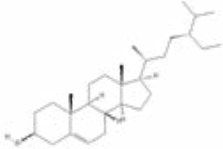
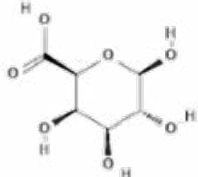


Anthocyanins like cyanidin-3-glucoside and peonidin-3-glucoside are involved in the inhibition of growth and apoptosis in breast cancer cells.³⁵ From all these shreds of evidence, it is posited that consumption of rice bran or its products will help in the prevention of chronic diseases such as cancer (Table 2).

Aromatic Properties

Every human being is sensitive to smell. It is a well-known fact that rice produces a characteristic aroma when getting cooked. Flavour is very important and primary to the consumer. Even a small change to this sensory property will make a particular variety of rice unacceptable

Table 2. Anti cancerous properties of volatile compounds isolated from rice bran

Compound Name	Anti-cancerous properties	Structure
Ferulic acid	reducing the lymphoma cell growth	
Tricin	reducing intestinal adenoma number	
Cycloartenylferulate(CF)	prevents skin cancer by activating caspase 8	
b-sitosterol	Prevents colorectal, stomach and breast cancers	
Phytosterol	Prevention of colon cancer	
Pectin	Suppression of colonic tumorigenesis	

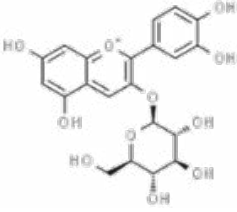
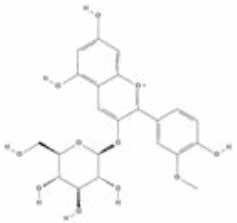
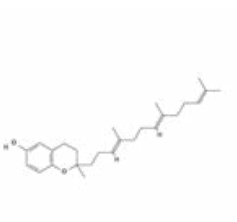
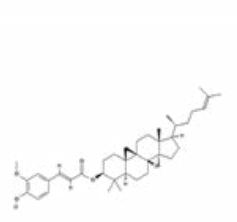
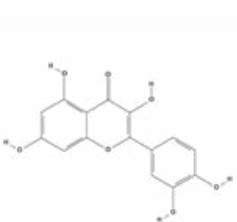
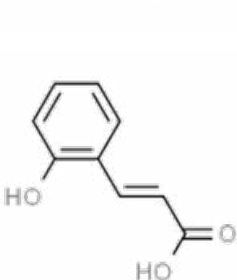
Cyaniding-3-glucoside	Inhibits the growth of breast cancer cells	
Peonidin-3-glucoside	Prevents the growth of breast cancer cells	
Tocotrienols carcinogens	Involved in the protection of mammary	
Cycloartenylferulate	Prevents skin cancer	
Dition and quercetin	Regulator of cell cycle	
Coumaric acid	Acts against malignant tumors	

Table 3. Volatile compounds of rice bran with characteristic aroma

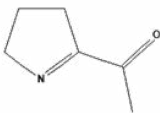

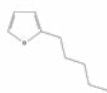


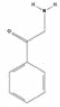
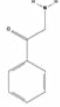


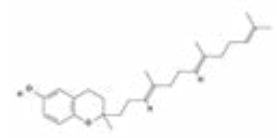
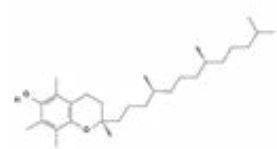
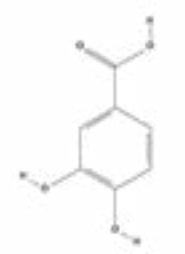
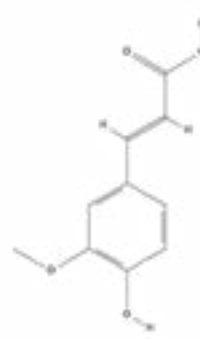
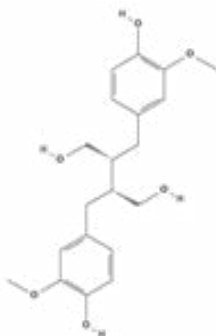
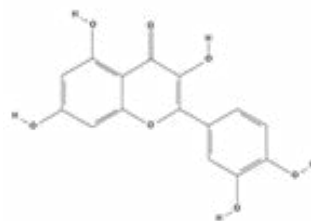
Compound	Aroma Properties	Structure
2-acetyl-1-pyrroline	Popcorn aroma	
Hexanal	Grassy flavor	
2-pentylfuran	Beany flavor	
2-Nonenal	Fancid flavor	
Octanal	Fatty flavor	
2-amino acetophenone	medicinal phenolic	
pentadecan-2-one, hexanol, and 2-pentylfuran	Responsible for aroma in basmati rice	
Guaiacol	Responsible for smoky aroma in black rice	
Isovalericacid	Major odor active compound present in both red and black rice bran	

Table 4. Volatile compounds of rice bran with other health benefits

Compound	Biological Properties	Structure
g- tocotrienol	Peroxisome proliferator activated receptor	
á- tocopherol	Prevents Alzheimer's disease and allergies	
Protocatechuic acid	potent inhibitor of inflammatory mediators	
Ferulic acid	Reduces gastric inflammation and inhibits Melanin production	
Secoisolariciresnol	Oestrogenic activity	

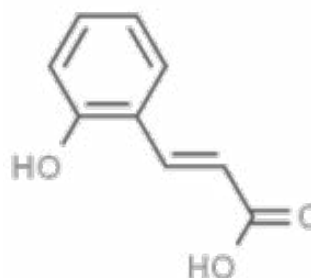
Quercetin

Influences the cell cycle pathway



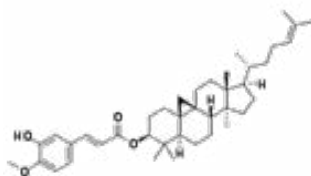
Coumaric acid

Controls pro-inflammatory immune signaling



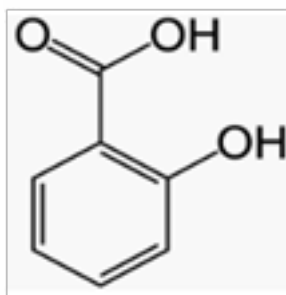
g-oryzanol

Hypolipidemic effect, promotes growth, stimulates hypothalamus, inhibits tumor growth, anti-blood cholesterol agent



Phenolic acid

Lowers blood glucose level and enhances plasma insulin level



by the consumer. This odor is mainly due to the bran, comprising a number of volatile organic compounds (Table 3). The bran is generally removed depending on the extent to which the rice grains are milled in the industry. After milling process, the entire composition of the rice grain will be changed, having an impact on the odor of cooked rice. It was demonstrated that the rice grains subjected to greater extent of milling produced less aroma when compared to the ones

that are milled to a lesser extent. The major volatile organic compound responsible for the aroma in rice bran is 2-acetyl 1-pyrroline (commonly known as the popcorn aroma).

There are many approaches to find out the volatile organic compounds contributing to the aroma of rice. The first approach is by calculating the aroma value (odor threshold). The more the aroma value of a volatile organic compound, the more is its contribution to the flavor³⁶. Compounds

having a lower odor threshold value are aldehydes such as 2-decenal, nonanal, decanal, octanal, etc. likely contribute to the rice aroma. Aldehyde hexanal is found to have a higher threshold value when compared with other compounds. The second approach is by finding their dilution value (DV) which is related to the corresponding aroma value. Based on this dilution value, 2-amino acetophenone, a medicinal phenolic is considered to be an important odorant³⁷. Basmati rice popular for its characteristic and strong fragrance. The compounds responsible for fragrance in basmati rice are hexanol, 2-pentyl furan, and pentadecan-2-one. In addition to these, other volatile organic compounds such as benzaldehyde, pentanol, 2-acetyl pyrroline, octanal, hexadecanol, 6,10,14 trimethyl pentadecan-2-one, (E)- hept-2-enal also help in distinguishing between the fragrant and non-fragrant rice varieties³. Red and black rice are the two varieties of rice gaining importance especially due to their color and flavor. The presence of guaiacol in black rice is the major difference between the two varieties. The black smoky aroma in the black rice is due to the presence of this volatile compound. Some of the aroma contributors in red rice are myristic acid, nonanal, 6, 10, 14trimethyl pentadecan-2-one³.

Health Benefits of VOCs

Besides controlling diabetes and reducing the risk of getting chronic diseases, rice bran also has many other health benefits. The extracts also possess antibiotic properties as they have the ability to inhibit the growth of many bacteria like *E. coli*, *Vibrio cholerae*, and *Salmonella* species, etc. They were found to be effective against *V.cholerae* strain O1³⁹, thus involved in the treatment of diarrhea. Modified arabinoxylan rice bran was found to enhance the phagocytosis of *E.coli* bacteria³⁸. Sulfated glucans extracted from the rice bran are known to protect against viral infections as they stop the entry of megaloviruses into primary human fibroblasts.³⁹ By-products of rice bran are also well known for their cholesterol-lowering properties. They include goryzanol, b-sitosterol, tocopherols, and some other unsaturated fatty acids. Amongst these, g-oryzanol was present in higher content and was also found to be very effective in reducing animal serum cholesterol levels. When exposed to some allergens like peanut proteins, pollen spores, etc., the human body reacts to them by releasing

histamine from the basophilic mast cells. This results in inflammation or swelling. Arabinoxylan rice bran has the anti-allergic property as it activates human monocyte dendritic cells in vitro. Tricin rich rice hull, an allelochemical has the ability to protect plants against phytopathogens. It reduces the risk of rice seedling rot disease caused due to many soil-borne pathogenic fungi. Other components extracted from rice hulls are effective in preventing the growth of duckweed.⁴⁰ Cereals rich in dietary fiber are very efficient in reducing the risk of coronary heart disease. The phytosterols present in them aid in increasing lecithin cholesterol acyltransferase levels in the blood which thereby improves sequestration of cholesterol in the hydrophobic core of HDL cholesterol. Rice bran also possesses anti-aging properties. theoryzanol component act against UV light-induced lipid peroxidation and is referred to as a potential sunscreen agent. Hair growth can be stimulated by ferulic acid and its esters present in g-oryzanol. The elevated serum levels in hypothyroid patients could be reduced by g-oryzanol in rice bran. It has a significant impact on menopause by maintaining the menopause symptoms like hot flashes etc. Bone loss can be reduced in women who are suffering from post-menopausal osteoporosis by consuming oryzanol containing rice bran foods.⁴¹

Effect of VOCs on the Central Nervous System

Volatile organic compounds produced by the rice brans show a great impact on the functioning of the central nervous system. When exposed to high concentrations of VOCs they enter the body through the respiratory tract and in the long run, they may pose problems like respiratory failure initially and then leads to depression of central nervous system. there was a considerable increase in the number of transcripts with the increased level of exposure to toluene,⁴² displaying a shred of genomic evidence on the involvement of neuronal pathways in synaptic transmission and plasticity, which were greatly affected by volatile organic compounds. Apart from these side effects, some VOCs are also useful for the functioning of CNS. For example, vitamin E or tocopherol present in rice bran is regarded as the chain-breaking antioxidant of the body. When it is orally supplemented through diet, it has the ability to reach the cerebrospinal fluid and brain, in turn, demonstrating its central role in

maintaining neurological structure and function.⁴³ Cycloartenolferulic acid ester, a component of gamma oryzanol present in bran serves as a new plant-derived cerebral activator which possesses a wide range of pharmacological actions.

Effect of VOCs on the Endocrine System

It is believed by athletes that consumption of volatile organic compounds through diet would elicit anabolic effects such as production and release of testosterone, which stimulates the release of human growth hormone in the body. However, for a matter of fact, VOCs like oryzanol and phytosterols are poorly absorbed from the intestinal tract. They are released as feces and they have not been shown to elicit anabolic activities. When g-oryzanol is injected intravenously or subcutaneously, there was a reduction in the release of growth hormone and enhanced production of catecholamines. Ferulic acid shows an anti-androgenic effect on the prostate gland. Some *in vitro* studies have also suggested that phytosterols may suppress immune system responses. Ethyl acetate fraction and ferulic acid help in regulating blood glucose levels by increasing glucokinase activity and production of glycogen in the liver, thereby controlling type-2-diabetes.⁴⁴ Linoleic acid helps in modulating the immune system by enhancing b-lymphocyte proliferation, which may have some anti-allergenic properties.⁴⁵ The health benefits of VOCs extracted from rice bran have been displayed in Table 4.

Methods Employed for the Extraction of VOCs

Though chemistry helped us in preparing a variety of compounds that are useful to us, we still cannot make them as efficiently as nature does. For example, plants synthesize a large number of natural compounds having unique functions when used in their pure form. These compounds are known as secondary metabolites. To obtain them commercially from plant sources, a large number of extraction techniques have been developed. These extraction techniques vary from traditional methods such as solvent extraction, condensation, and soxhlet extraction (Fig. 1) to advanced methods that make use of supercritical carbon dioxide, subcritical water and much more for the extraction (Fig. 2). Thus they are considered as eco-friendly techniques. Organic solvent extraction is a method that is used to separate compounds based on their solubilities in water and an organic solvent. In

this technique, the plant material has to be pre-treated by using methods like cryogenic milling, which helps in increasing the surface area, thereby reducing the loss of volatile organic compounds. The limitations of this method include the usage of low temperatures, which is not suitable for the extraction of certain VOCs of interest that require relatively high temperatures during the evaporation process. This also led to the emission of highly toxic gases into the environment. The final product was found to contain some impurities due to higher boiling. After the extraction process, the recovery of the product is also very important and this can be done by using the latest technique known as flash desolventizing. It removes the solvent residues with high-velocity superheated solvent at low temperatures. In addition to the liquid-liquid extraction methods, there are vapor phase extraction techniques that are more efficient as the vapor diffuses easily into the plant material when compared to the liquid solvents. Steam distillation is one of the widely used vapor phase extraction procedures in which steam is continuously passed through the material absorbing the VOCs due to low partial pressure. To overcome the narrow range of temperatures and the low contact of material with the solvent, another method known as soxhlet extraction is used. In this method, a thimble holder containing the sample is filled with the solvent and when the liquid reaches the overflow level, a siphon removes the liquid into distillation flask for extraction. Sample throughput can be increased as it brings the liquid continuously in contact with the solvent and is relatively inexpensive. The drawback is, it is an old-fashioned and manual technique apart from being laborious and time-consuming. Among the latest extraction methods, microwave-assisted extraction has been commonly used for the past 10 years. This method uses microwaves, which disrupts the membranes and penetrates inside the cell helping in the extraction of the compound. This is used for the extraction of essential oils, terpenes like linalool, nerol, carotenoids and volatile oils from the brans. This is considered to be an optimized method and is effective than the soxhlet extraction.⁴⁶ Pressurized solvent extraction is an alternative to soxhlet extraction and other traditional methods. This method utilizes a solvent at elevated temperatures and pressures. An increase in temperature enhances the extraction

kinetics and an increase in the pressure maintains the solvent in a liquid state, ensuring a safe and rapid extraction process.⁴⁷ As both vapors and gases have low solubilizing ability, extractions are performed at very high temperatures and pressures. Hence this technique is known as supercritical extraction. This procedure helped in improving the solubilizing properties, which are comparable to that of liquids keeping their diffusion coefficient high. If the pressure is decreased compounds can be separated and gas can be re-pressurized for further use. Carbon dioxide is chosen as the medium due to its inert and nontoxic nature. It has low polarity and allows fractionated separation. It is known as supercritical carbon dioxide extraction. Supercritical water extraction is a powerful technique that maintains the temperature around 300 degrees centigrade and high pressure so that the solvent is in a liquid state. Water has unique properties like high polarity, high dielectric constant and high boiling point. This is the fastest and cheapest method than conventional methods.⁴⁸ Ultrasound-assisted extraction involves the production of acoustic cavitations by passing ultrasonic waves. This is advantageous as it exerts mechanical effect and allows easy penetration of the solvent into the sample material, it also increases the contact between the solid and liquid phases. As chemicals are not used, the degradation of the compounds of interest is greatly reduced.⁴⁹ The parameters used for the extraction of phenolic compounds from the wheat bran using this method include 25 min of extraction time and 60 degrees centigrade of extraction temperature.⁵⁰ Enzyme assisted extraction is recently used for separating bioactive compounds from the plants. This uses enzymes that can degrade or disrupt the cell membrane and enables better release and extraction of compound.⁵¹ The advantages of this method include less extraction time and reduced use of solvents which improves the quality of the product. Major limitations of this method are (a) The available enzymes cannot hydrolyze cell walls completely, and (b) this method is not preferred for industrial scale as the conditions for enzymes have to be maintained, which is an herculean task.

Identification and Quantification of VOCs Isolated from the Rice Bran

Rice bran is a rich source of a large variety of aromatic volatile organic compounds.

They are given a lot of importance today due to antioxidant properties and health benefits. After the extraction process, the product obtained had a large number of these volatile compounds. The identification of the extracted compounds is very important. Initially, gas-liquid chromatography was used along with a mass selective detector but the sample should be derivatized before quantification. HPLC coupled with UV spectrophotometry can be used for analyzing the sample without the derivatization. This technique can be applied for the simultaneous separation and quantification of the compounds. Using a UV multiwavelength detector, identification of each and every compound is possible because different compounds get absorbed at different positions in the ultraviolet region. However, the UV-visible spectrum will not provide sufficient power that is required for the identification.⁵² To overcome this problem gas chromatography-mass spectrometry (GC-MS) was used as it provides the power that is required for identification and could give accurate results for the analysis of plant extracts. The identification was done based on the name, structure, and weight of the molecular components. Then the peak area of the component will be compared to the total peak area thus obtaining the relative percentage of each component. The final step in the identification process is to compare the spectrum of the unknown component with the spectrum of the known component present in the NIST library.⁵³ This is a fast and direct method for the identification of terpenoids and steroids. Liquid chromatography-mass spectrometry (LC-MS) is also an extensively used technique due to the separation capabilities of liquid chromatography and the high power of mass spectrometry. Conventionally flame ionization detector was also used but due to the lack of information about the spectrum, they were replaced by the mass spectrometric detectors. Single quadrupole and tandem mass spectrometry are the two types of MS of which the tandem mass spectrometers are advantageous because of their high scan rate. The main disadvantage of GC-MS was the low time resolution and artifacts in the chromatogram.⁵⁴ So proton transfer mass spectrometry was introduced, which involves the transfer of protons to the VOCs and sending these protonated molecules to the analyzer followed by detecting a signal.⁵⁵ It provides high sensitivity

with real-time monitoring. If the spectrum of an unknown compound does not coincide with any spectrum present in the databases, then the primary thing that is to be done is structure elucidation. The determination of the structure of unknown molecules plays a key role in organic chemistry. Nuclear magnetic resonance (NMR) spectroscopy helps in doing this.⁵⁶ The first step is to interpret 1D and 2D NMR spectra and find out the correlations between all pairs of atoms inside the molecule. And then all the structures with the coupled pair of atoms are found out. Finally correct and best structure can be determined by a structure verification step which makes use of NMR chemical shift prediction.⁵⁷

ACKNOWLEDGMENTS

The research lab of K.V. Chaitanya is funded by grants from the Department of Science and Technology and University grants commission (UGC), Govt. of India.

REFERENCES

- Nagendra Prasad MN, Sanjay KR, Shrivya Khatokar M, Vismaya MN, Nanjunda Swamy S. Health benefits of rice bran—a review. *J Nutr Food Sci.*; **1**(3):1-7 (2011).
- Hernandez, N., *et al.* “Enzymatic treatment of rice bran to improve processing.” *Journal of the American Oil Chemists’ Society*, **2**: 177-180 (2000).
- Sukhonthara, Sukhontha, Chockchai Theerakulkait, and Mitsuo Miyazawa. “Characterization of volatile aroma compounds from red and black rice bran.” *Journal of oleo science*, **3**: 155-161 (2009).
- Okarter, Neal, and Rui Hai Liu. “Health benefits of whole grain phytochemicals.” *Critical reviews in food science and nutrition*, **3**: 193-208 (2010).
- Truswell, A. S. “Cereal grains and coronary heart disease.” *European journal of clinical nutrition* **1**: 1 (2002).
- Garrat, Ghislain, *et al.* “Gastrointestinal absorption and urinary excretion of trans-cinnamic and p-coumaric acids in rats.” *Journal of agricultural and food chemistry* **8**: 2944-2950 (2006).
- Harborne, Jeffrey B., and Christine A. Williams. “Advances in flavonoid research since 1992.” *Phytochemistry* **6**: 481-504 (2000).
- Qu, Hongyan, *et al.* “Lignans are involved in the antitumor activity of wheat bran in colon cancer SW480 cells.” *The Journal of nutrition*, **3**: 598-602 (2005).
- Reimann, Stefan, and Alastair C. Lewis. “Anthropogenic VOCs.” *Volatile organic compounds in the atmosphere*: 33-70 (2007).
- Guenther, Alex, *et al.* “Natural emissions of non-methane volatile organic compounds, carbon monoxide, and oxides of nitrogen from North America.” *Atmospheric Environment* **12-14**: 2205-2230 (2000).
- A. Barberes, R.P. Reis, A.L.D. Spigolon, P.E. Fonseca, C.B. Mello, M.T. Barata, *Geosciences* **8**:1-18 (2018).
- Tonne, Cathryn, and Paul Wilkinson. “Long-term exposure to air pollution is associated with survival following acute coronary syndrome.” *European heart journal* **17**: 1306-1311 (2013).
- Shin, Hyeong-Moo, Thomas E. McKone, and Deborah H. Bennett. “Contribution of low vapor pressure-volatile organic compounds (LVP-VOCs) from consumer products to ozone formation in urban atmospheres.” *Atmospheric Environment* **108**: 98-106 (2015).
- Hodzic, Alma, *et al.* “Volatility dependence of Henry’s law constants of condensable organics: Application to estimate depositional loss of secondary organic aerosols.” *Geophysical Research Letters* **13**: 4795-4804 (2014).
- Devi, R. Renuka, and C. Arumughan. “Phytochemical characterization of defatted rice bran and optimization of a process for their extraction and enrichment.” *Bioresource technology* **16**: 3037-3043 (2007).
- Stien, D., *et al.* “Experimental design optimization of antimicrobial mixtures of active VOCs.” *Planta Medica* **16**: P1M3 (2014).
- Patel, M., and S. N. Naik. “Gamma-oryzanol from rice bran oil—A review.” (2004).
- Hassimoto, Nezu Mariko Aymoto, Maria Inés Genovese, and Franco Maria Lajolo. “Absorption and metabolism of cyanidin-3-glucoside and cyanidin-3-rutinoside extracted from wild mulberry (*Morus nigra* L.) in rats.” *Nutrition research* **3**: 198-207 (2008).
- Minatel, Igor Otavio, *et al.* “Antioxidant activity of ³-oryzanol: A complex network of interactions.” *International journal of molecular sciences*, **8**: 1107 (2016).
- Koren, Hillel S., Delores E. Graham, and Robert B. Devlin. “Exposure of humans to a volatile organic mixture. III. Inflammatory response.” *Archives of Environmental Health: An International Journal* **1**: 39-44 (1992).
- Otto, David A., *et al.* “Exposure of humans to a volatile organic mixture. I. Behavioral

- assessment." *Archives of Environmental Health: An International Journal* **1**: 23-30 (1992).
22. Rudel, Ruthann A., and Laura J. Perovich. "Endocrine disrupting chemicals in indoor and outdoor air." *Atmospheric Environment* **1**: 170-181 (2009).
 23. Irshad, M., and P. S. Chaudhuri. "Oxidant-antioxidant system: role and significance in human body." (2002).
 24. Iqbal, Shahid, M. I. Bhangar, and Farooq Anwar. "Antioxidant properties and components of some commercially available varieties of rice bran in Pakistan." *Food chemistry* **2**: 265-272 (2005).
 25. Xu, Zhimin, Na Hua, and J. Samuel Godber. "Antioxidant activity of tocopherols, tocotrienols, and ³-Oryzanol components from rice bran against cholesterol oxidation accelerated by 2, 2'-Azobis (2-methylpropionamide) dihydrochloride." *Journal of Agricultural and Food Chemistry* **4**: 2077-2081 (2001).
 26. Wang, Tong, Kevin B. Hicks, and Robert Moreau. "Antioxidant activity of phytosterols, oryzanol, and other phytosterol conjugates." *Journal of the American Oil Chemists' Society* **12**: 1201-1206 (2002).
 27. Kaur, Kiranpreet, and Ashwani Kumar. *Development of Milk Substitute from Peas, Sesame Seeds and Flaxseeds*. Diss. Lovely Professional University, (2018).
 28. Jung EH, Ha TY, Hwang IK. Anti-hyperglycemic and antioxidative activities of phenolic acid concentrates of rice bran and hydroxycinnamic acids in cell assays. *The Korean Journal of Food And Nutrition* **23**:2: 233-9 (2010).
 29. Walsh, Declan, Sinead Donnelly, and Lisa Rybicki. "The symptoms of advanced cancer: relationship to age, gender, and performance status in 1,000 patients." *Supportive Care in Cancer* **3**: 175-179 (2000).
 30. Verschoyle, R. D., *et al.* "Evaluation of the cancer chemopreventive efficacy of rice bran in genetic mouse models of breast, prostate and intestinal carcinogenesis." *British journal of cancer* **2**: 248 (2007).
 31. Cai, Hong, *et al.* "The rice bran constituent triclinin potently inhibits cyclooxygenase enzymes and interferes with intestinal carcinogenesis in ApcMin mice." *Molecular cancer therapeutics* **9**: 1287-1292 (2005).
 32. Lissoni, Paolo, *et al.* "Modulation of the anticancer immunity by natural agents: inhibition of T regulatory lymphocyte generation by arabinoside in patients with locally limited or metastatic solid tumors." *Cancer Ther* **6**: 1011-1016 (2008).
 33. Nakashima, Keisuke, *et al.* "The tocotrienol rich fraction from rice bran enhances cisplatin induced cytotoxicity in human mesothelioma H28 cells." *Phytotherapy research* **9**: 1317-1321 (2010).
 34. Tan, Bee Ling, and Mohd Esa Norhaizan. "Scientific evidence of rice by-products for cancer prevention: chemopreventive properties of waste products from rice milling on carcinogenesis in vitro and in vivo." *BioMed research international* (2017).
 35. Chen, Hao, Yuegang Zuo, and Yiwei Deng. "Separation and determination of flavonoids and other phenolic compounds in cranberry juice by high-performance liquid chromatography." *Journal of Chromatography A1-2* : 387-395 (2001).
 36. Lam, H. S., and A. Proctor. "Milled rice oxidation volatiles and odor development." *Journal of Food Science* **9**: 2676-2681 (2003).
 37. Jezussek, Magnus, Bienvenido O. Juliano, and Peter Schieberle. "Comparison of key aroma compounds in cooked brown rice varieties based on aroma extract dilution analyses." *Journal of Agricultural and Food Chemistry* **5**: 1101-1105 (2002).
 38. Ghoneum M, Agrawal S. Activation of human monocyte-derived dendritic cells in vitro by the biological response modifier arabinoside rice bran (MGN-3/Biobran). *International journal of immunopathology and pharmacology* **24**:4 :941-8 (2011).
 39. Ghosh, Tuhin, *et al.* "Anti-cytomegalovirus activity of sulfated glucans generated from a commercial preparation of rice bran." *Antiviral Chemistry and Chemotherapy*, **2**: 85-95 (2010).
 40. Kong, Chui Hua, *et al.* "Allelochemical triclinin in rice hull and its aurone isomer against rice seedling rot disease." *Pest management science* **9**: 1018-1024 (2010).
 41. Wilson, Thomas A., *et al.* "Rice bran oil and oryzanol reduce plasma lipid and lipoprotein cholesterol concentrations and aortic cholesterol ester accumulation to a greater extent than ferulic acid in hypercholesterolemic hamsters." *The Journal of nutritional biochemistry* **2**: 105-112 (2007).
 42. Hester, Susan D., *et al.* "Acute toluene exposure alters expression of genes in the central nervous system associated with synaptic structure and function." *Neurotoxicology and teratology* **5**: 521-529 (2011).
 43. Sen, Chandan K., Savita Khanna, and Sashwati Roy. "Tocotrienol: the natural vitamin E to defend the nervous system?." *Annals of the New York Academy of Sciences* **1**: 127-142 (2004).
 44. Jung, Eun Hee, *et al.* "Hypoglycemic effects of

- a phenolic acid fraction of rice bran and ferulic acid in C57BL/KsJ-db/db mice." *Journal of Agricultural and Food Chemistry* **24**: 9800-9804 (2007).
45. Sierra, Saleta, *et al.* "Increased immune response in mice consuming rice bran oil." *European journal of nutrition* **8**: 509-516 (2005).
46. Kaufmann, Béatrice, and Philippe Christen. "Recent extraction techniques for natural products: microwave assisted extraction and pressurised solvent extraction." *Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques* **2**: 105-113 (2002).
47. Dunford, Nurhan Turgut, and Minquan Zhang. "Pressurized solvent extraction of wheat germ oil." *Food Research International* **9-10**: 905-909 (2003).
48. Miller, David J., and Steven B. Hawthorne. "Solubility of liquid organics of environmental interest in subcritical (hot/liquid) water from 298 K to 473 K." *Journal of Chemical & Engineering Data* **1**: 78-81 (2000).
49. Rostagno, Mauricio A., Miguel Palma, and Carmelo G. Barroso. "Ultrasound-assisted extraction of soy isoflavones." *Journal of Chromatography A*, **2** : 119-128 (2003).
50. Wang, Jing, *et al.* "Optimisation of ultrasound-assisted extraction of phenolic compounds from wheat bran." *Food Chemistry* **2**: 804-810 (2008).
51. Pinelo, Manuel, Anis Arnous, and Anne S. Meyer. "Upgrading of grape skins: Significance of plant cell-wall structural components and extraction techniques for phenol release." *Trends in Food Science & Technology* **11**: 579-590 (2006).
52. Chen, Pei-Ni, *et al.* "Cyanidin 3-glucoside and peonidin 3-glucoside inhibit tumor cell growth and induce apoptosis in vitro and suppress tumor growth in vivo." *Nutrition and cancer* **2**: 232-243 (2005).
53. Sermakkani, M., and V. Thangapandian. "GC-MS analysis of Cassia italica leaf methanol extract." *Asian J Pharm Clin Res* **2**: 90-94 (2012).
54. Cappellin, Luca, *et al.* "PTR-MS in Italy: a multipurpose sensor with applications in environmental, agri-food and health science." *Sensors* **9**: 11923-11955 (2013).
55. Lubes, Giuseppe, and Mohammad Goodarzi. "Analysis of volatile compounds by advanced analytical techniques and multivariate chemometrics." *Chemical reviews* **9**: 6399-6422 (2017).
56. Elyashberg, Mikhail. "Identification and structure elucidation by NMR spectroscopy." *TrAC Trends in Analytical Chemistry* **69**: 88-97 (2015).
57. Elyashberg, M. E., A. J. Williams, and G. E. Martin. "Computer-assisted structure verification and elucidation tools in NMR-based structure elucidation." *Progress in Nuclear Magnetic Resonance Spectroscopy* **1-2**: 1-104 (2008).