



A Review on Extraction and Chromatographic Techniques for Organophosphate Pesticidal Residue Analysis in Paddy and Rice Bran

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Authors' contributions

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

Article Information

DOI: 10.9734/JABB/2024/v27i5845

Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://www.sdiarticle5.com/review-history/116414>

Review Article

Received: 22/02/2024
Accepted: 26/04/2024
Published: 26/04/2024

ABSTRACT

Organophosphorus pesticides (OPPS) are extensively employed in agricultural techniques and may expose people to them via food intake. We offer an analytical approach for detecting OPPS in paddy, rice grains and or rice bran that combines solid-phase extraction with a high-performance liquid chromatography detector. Acetonitrile extraction was used before SPE–HPLC–MSMS to isolate specific residues, which helped decrease matrix disruptions and identify a broad array of numerous residues in rice grains. Accordingly, ranges of quantitative determination ranged from 0.01mg/kg to more than 1.0 mg/kg, with coefficients extending from 0.995 to more than 0.999. At three stimulating intensities, high recuperation was reported, suggesting excellent precision. The relative SD of all residues confirmed the technique's accuracy. Using this, various OPPS were quantified, ranging from just below to maximum permissible limits to exceeding the limit. According to a sample study of different rice grain varieties accessible in the international market. This study

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focuses on the techniques of organophosphate pesticide residue extraction from paddy and rice bran. The study further discusses chromatographic techniques for the same.

Keywords: *Organophosphorus pesticides (OPPs); mass spectrometry; rice bran; chromatographic techniques; extraction techniques.*

1. INTRODUCTION

Ecological contamination is among the globe's most critical problems. It has become a worldwide concern in the past years due to a considerable rise in ecological contaminants and a shortage of preventative procedures or adherence to environmental regulations. The increasing population increase and the rising rate of food intake have compelled food companies to step up their attempts to expand food production [1]. Producers, among the most important parts of this system, are likewise employing increasingly large quantities of pesticides to safeguard their agriculture. Pesticide residues in a foodstuff have been a source of worry for consumers and environmental organizations due to pesticides' extensive use [2-6]. Pesticides are commonly employed to eliminate insects that are farming pests or pose a hazard to human health by transmitting harmful illnesses. Whereas such chemicals make anthropogenic agricultural use easier, they often endanger biotic organisms and degrade the living human habitat [7-10].

Organochlorine pesticides are a key type of insecticide utilized by local farmers in poor nations for decades due to low costs and insufficient financial facilities [11-14]. Since the early 1950s, dichlorodiphenyltrichloroethane has been widely used in agricultural and malaria repellents in Thailand, but it was phased out in 1994. In Thailand, the use of hexachlorocyclohexanes, drins, and chlordane were prohibited in 2000. Before Thailand, China had explicitly forbidden the use of DDTs as well as HCHs in 1983. The greater pesticide residue levels found in rice and soil samples from India contradicted the nation's pesticide management policies [15]. The long-term effects of unlawful Organochlorine pesticides are still affecting their remains in the ecosystem, posing a concern to human and ecological safety [16,17].

Because of their wide range of action, lesser persistence, and lower toxicity, numerous types of organophosphorus insecticides have been replaced for OCPs in recent years. On the other hand, these insecticides are long-lasting, hydrophobic, lipophilic, and bioaccumulative. Soil

is a primary receiver of pesticides that, through disintegration, desorption, diffusion, transpiration, desorption, and aggregation, mistakenly penetrate different trophic level species [18-20]. Certain herbicides may affect the acetylcholinesterase enzyme, linked to nerve damage and reproductive function disturbance [21,22,23]. Furthermore, its teratogenicity, mutagenicity, and carcinogenicity are harmful to people. Kids in rural India are at risk because of the widespread use of OPPS. In emerging economies, the status of the ecosystem and the behavior of pesticides in rice and soil have become complex challenges.

2. EXTRACTION OF PESTICIDAL RESIDUE IN PADDY AND RICE BRAN

2.1 Green Extraction Techniques

To optimize the quantity and purity of something like the extract, prevent operational degradation, effectively achieve a food-grade result, the extraction approach for high added-value chemicals from agricultural residues must be carefully designed [24-27]. The so-called "5-Stages Universal Recovery Process," which consists of macroscopic and microscopic level techniques, can be identified to achieve these goals: "i) macroscopic pre-treatment, ii) separation of macromolecules and micro-molecules, iii) extraction, iv) isolation and purification, and v) product yield". The most significant and vital of the five phases is extraction, which is a transportation systems phenomenon including the movement of a solute from the substrate to the solvent.

Hydraulic compression or solvent extraction are the most common methods for extracting RBO (rice bran oil). Notwithstanding its human and ecological hazards, hexane is the most often used solvent due to its outstanding dissolving capability, low normal boiling, as well as inexpensive expense.

International awareness of human and environmental security issues has risen in recent years. The science world has responded to this problem by developing new options for industrial operations in the food, cosmetics, and

pharmaceutical industries. The publishing of the 12 concepts of green chemistry as well as the 12 ideas of green design is a first step toward addressing these issues explicitly [28]. These twenty-four principles outline the most effective “green” approaches for improving chemical and industrial equipment and achieving more sustainable operations.

In the subject of green extraction, six additional concepts have indeed been established, which advocate enhancing current methods, innovations, switching to novel non-conventional procedures and using substitute solvents.

Numerous trials have already been conducted to extract the oil from this rice by-product to improve current techniques or invent new technologies while adhering to the 6 green removal criteria. Rice Bran Oil (RBO) has indeed been removed effectively utilizing non-

conventional solvents or technological advances, including “ultrasounds, microwaves, sub-critical water, supercritical fluids, or enzymatic extraction”. All of the processes examined in this research have significant benefits, particularly in terms of the environment [29]. Indeed, the scientists completed the extraction using bio-solvents or water instead of standard solvents, which are volatile, combustible, and often emitted into the environment, leading to air quality and greenhouse gases. Furthermore, many of the strategies discussed in this study allow for lower energy usage by improving or aiding current operations with intensifying methods or developing novel processes targeted at reducing the number of extraction stages. As a result, response time. According to Chemat and Galanakis, certain green extraction procedures may save up to 80% of the procedure heat power while lowering extraction yield by 90% without affecting the extract characteristics.

Table 1. γ -orizanol and RBO Extraction techniques

Extraction technique	Solvent	γ -Orizanol yield	Parameters	Oil yield	References
Solvent extraction	Isopropanol	3005 mg/kg (dry rice bran)	• 61 °C • 2:1 w/w • .1 hr	202.2 g/kg (dry rice bran) 24% (dry rice bran)	Mishra, et.al. (2012)
	Acetone	n.r.	• 31 °C • 4:1 w/w • 1.2 hr	21.05 g/100 g (fresh rice bran)	Snyder, F. et.al. (2017)
		4154 mg/kg (fresh rice bran)	• 83.4 °C • 4:1 w/w • 2.9 hr	22,58% (dry rice bran)	Guo, W. (2014)
	Ethanol with 6% of water	n.r.	• 175 °C • 4:1 w/w • 1.3 hr		Liu, W.-X. et.al. (2012)
Supercritical fluid extraction	CO ₂	n.r.	• 27 MPa • 71 °C • 2.1 h	72.1 mg/g (dry rice bran) 21.4%	Zhou, Q. et.al. (2013)
	CO ₂	n.r.	• 1000 Psi • 101 °C • 1.39 hr	5.49 mg/g (fresh rice bran)	Maryam, A. et.al. (2018)
	CO ₂	675.6 mg/g (RBO)	• 681atm • 49 °C • 0.3 hr or 0.16–0.19 hr • 241 ml/min • 340 bar		Akoto, O. et.al. (2015)
	CO ₂	n.r.	• 61 °C • 1.6 L/min • 2.9 hr	16% (dry rice bran)	Pan, H.W.; et.al. (2019)
Microwave-assisted extraction	Isopropanol	n.r.	• 119 °C • 2:1 w/w • 0.16 hr	0.25 g/100g (fresh rice bran)	Manirakiza, P. et.al. (2003)

Extraction technique	Solvent	γ -Orizanol yield	Parameters	Oil yield	References
	Isopropanol	2.246 ppm	<ul style="list-style-type: none"> • 81 °C • 2.2 bar • 96 W • 2:1 w/w 	24.6 g/ 100 g (dry rice bran)	Stadlinger, N. et.al. (2016)
	Methanol	84 ppm	<ul style="list-style-type: none"> • 0.29 hr • 37 °C • 810 W • 2:1 w/w • 1.1 hr 	95.03% (recoverable RBO)	Babina, K.; et.al. (2012)
	Ethanol	n.r.	<ul style="list-style-type: none"> • 129 °C • 4:1 w/w • 0.19 hr 	16.2% (dry rice bran)	
Ultrasound-assisted extraction	Water	n.r.	<ul style="list-style-type: none"> • 13 kHz • 299 W • 46 °C • 1:9 w/w • 0.29 hr 	20% (dry rice bran)	Tawatsin, A. (2015)
	Water	n.r.	<ul style="list-style-type: none"> • 59 kHz • 26 °C • 1:9 w/w • 1.1 hr • pH 	1230% (dry rice bran)	Han, Y. et.al. (2017)
	Ethanol 86%	n.r.	<ul style="list-style-type: none"> • 34 kHz • 130 W • 59 °C • 20:1 w/w • 0.27 hr 	19.73% (dry rice bran)	Ghanbari, F.; et.al. (2017)
Subcritical water extraction	Water	n.r.	<ul style="list-style-type: none"> • 239 °C • 0.11 hr 	250 mg/g (dry rice bran)	Fang, Y.; et.al. (2018)
Enzyme-assisted aqueous extraction	Water	n.r.	<ul style="list-style-type: none"> • 61 °C • pH 4.4 • 4:1 w/w 	21.6% (dry rice bran)	Zhang, Q. et.al. (2017)
	Water	1.66% (RBO)	<ul style="list-style-type: none"> • 49 °C • pH 8 • Alcalase • 1.9 h 	77% (recoverable RBO)	Tri, J. et.al. (2017)

2.2 Non-conventional Solvent Extraction

Organic chemicals originating from non-renewable resources are often used as solvents in extraction operations. The most frequent solvent used throughout the removal of RBO is hexane. Although such solvent is inexpensive and effective. It is a petroleum-related solvent that is igneous, instable, as well as hazardous to both persons and the ecosystem. Isopropanol, ethanol, ethyl acetate, and D-limonene are the most widely used non-solvents in RBO (rice bran oil) extraction. Such chemicals are all bio-based solvents that can be made from farming residue.

Numerous recommendations, including those from "GlaxoSmithKline (GSK), Pfizer, Sanofi, AstraZeneca, and the Green Chemistry Institute-Pharmaceutical Roundtable", classify certain solvents as suggested or favored. Such handbooks cover solvents from the standpoints of security, health in the workplace, ecology, technological constraints, and price.

In summary, the experiments mentioned above show that green solvents, rather than hexane or even other harmful solvents, may be used to extract the rice bran oil (RBO). Regarding RBO (rice bran oil) yield and quantity of γ -oryzanol removed, isopropanol and

ethanol produced the greatest results. Nevertheless, additional research is needed before green solvents may be utilized to extract RBO.

2.3 MAE (Microwave-assisted Extraction)

Microwave-assisted extraction appears to be among the most enticing rapidly evolving green innovations for extracting various substances in various matrices due to its potential to attain extreme temps in mild reaction conditions while using fewer solvents and power, resulting in high returns sanctity in oil and highly bioavailable substances.

Microwaves are electromagnetically comprised of two perpendicular oscillating forces, namely magnetic and electrical. In the environment, molecular dipoles are in a chaotic condition; whenever a continual electrostatic attraction is provided, a dipole alignment develops [30]; if a strong radiation magnetic field is present, such dipoles begin to spin constantly, as seen in Fig. below. Ionic conductivity combined dipole revolution both in the solvent and the matrix creates heat from the magnetic field.

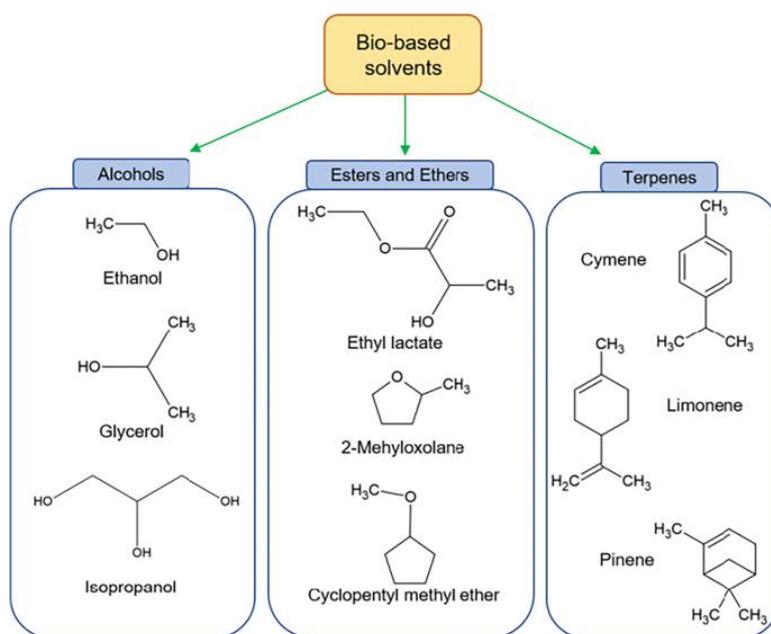


Fig. 1. Outline of the core bio-based solvents

Source: <https://link.springer.com/article/10.1007/s13399-020-00846-3>

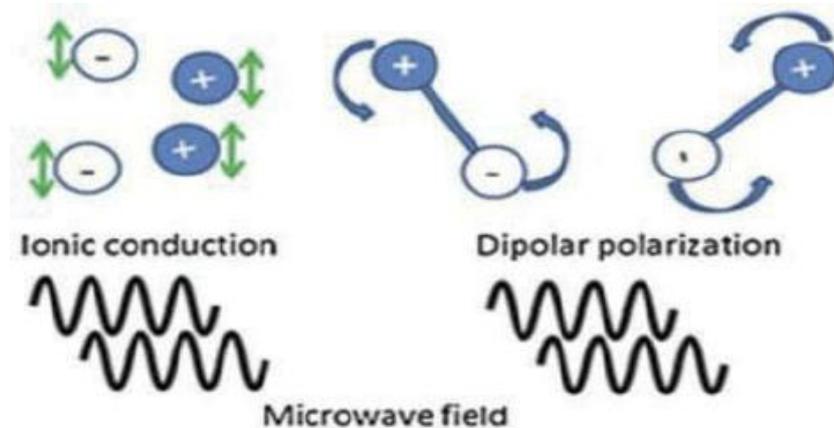


Fig. 2. The movements of molecular dipoles a microwave

Source: https://www.researchgate.net/figure/Ionic-conduction-and-depolarization-under-microwave-condition-86-Reproduced-with_fig4_280875804

Fast heating creates a rise in tension within the plant cells, which might also contribute to cell wall rupture, speeding mass transport between both the solvent and bioactive chemicals in detecting plant matrix extraction.

2.4 UAE (Ultrasound-Assisted Extraction)

The use of ultrasound in extraction procedures has grown significantly in recent years, notably in the agricultural and natural substance industries, for both traditional and environmentally novel extraction approaches. Ultrasound-assisted extraction is a low-cost, environmentally friendly method that adheres to all green chemistry standards. Compared to traditional extraction processes, UAE provides for a decrease in extraction duration and energy and solvent usage while maintaining the high quality of the result.

Ultrasounds (US) have frequencies ranging from “20 kHz to 10 MHz”, and thus are divided into three categories: intermediate-power or medium frequency, high-power or low frequency, and low-power or high frequency [31]. The ultrasonic pool and the probe-type ultrasonic apparatus, both used in research and industrial applications in the Ultrasounds, create such frequencies. The efficient extraction technology is a high-power ultrasonic probe, and its commercial and

industrial capabilities have developed over the previous years.

As Chemat points out, the UAE (Ultrasound-assisted extraction) is the total amount of numerous physical and chemical properties that happen both in the solvent and in the composite, including pressure changes, shear forces, cavitation, agitation, radical formation, microjets, erosion, fragmentation, capillarity, and sonoporation. The primary cause of the sonication effect appears to be acoustic cavitation [32]. This occurrence happens when ultrasonic waves cause a series of rarefaction and compress cycles in a material, such as a solvent, resulting in microbubbles. Because of the rapid and continual pressure fluctuations, these bubbles eventually burst. Critical aspects develop on the cell membrane whenever the cell membrane implosively collapses. The widespread transmission of atoms between both the solvent rises and the matrix while their diffusion increases. These characteristics make this process excellent for extracting various bioactive chemicals from various matrices using various solvents, including polyphenols, antioxidants, anthocyanins, or even other organic molecules. However, with all of these benefits, only a few research on the UAE of RBO and -oryzanol have been undertaken.

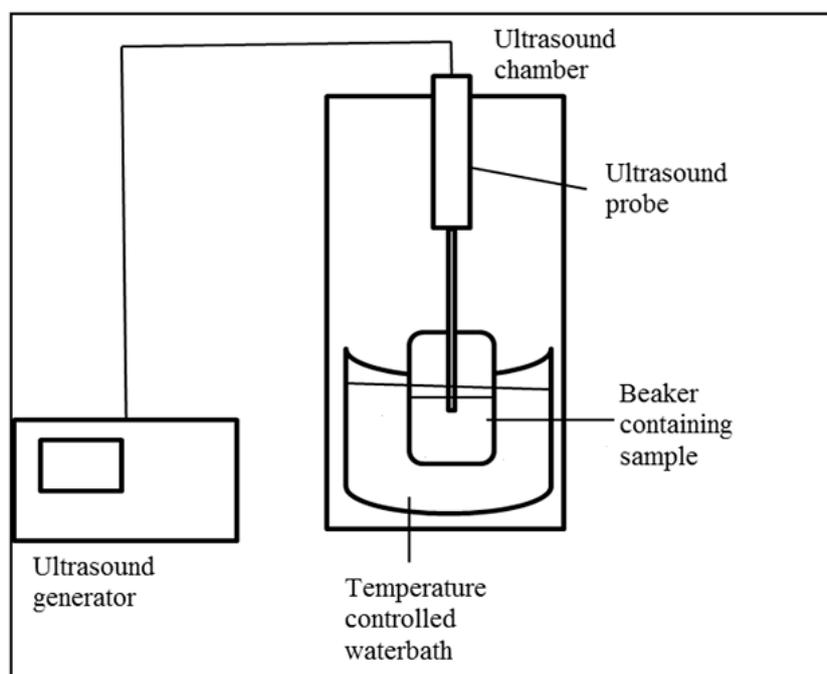


Fig. 3. The main Ultrasound-assisted extraction structures.

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2.5 SFE (Supercritical Fluid Extraction)

Over the past four decades, awareness of supercritical fluid extraction (SFE), particularly supercritical carbon dioxide extraction, has risen. It has grown into a green, environmentally favorable technique, notably for food, after its first industrial use in decaffeination and beer manufacturing.

Fluids having low viscosities, high population densities, and diffusivities that lie between liquids and gases are used in SFE at pressures and temperatures over their crucial junctures. In comparison to organic solvents, supercritical fluids have better transport capabilities and a greater matrix penetration power, allowing for a quicker and more accurate extraction.

It is feasible to control the dissolving power and selection of supercritical fluids by adjusting the pressure and temperature [33]. By simply lowering the density of the fluid and lowering the temperature and pressure, it is feasible to improve the liquified chemicals and create an unpolluted and solvent-free extract.

The majority of SFE systems extract different chemicals with supercritical carbon dioxide rather

than an organic solvent with a low critical temperature and pressure and are often utilized in the food processing industry to extract lipids from various matrices.

This gas is non-toxic, non-flammable, inexpensive, non-corrosive, and accessible in high purity grades; it can also be collected and reused [34]. It's a non-polar solvent; however, applying highly polar co-solvents, including such ethanol and water, during the extraction method might change its polarity and preference for certain compounds.

Investigators have been operating on RBO extraction for about four decades. Still, it is only lately that they have begun to concentrate on the antioxidants in the extracts, including such - oryzanol.

The majority of the studies looked at pressure, temperature, Carbon dioxide flow, extraction duration, and raw - materials size as the main factors in optimizing the sc CO₂ of RBO. Such operating circumstances were examined using several apparatus setups to determine the greatest balance of extraction productivity, quality of products, and procedure operating costs.

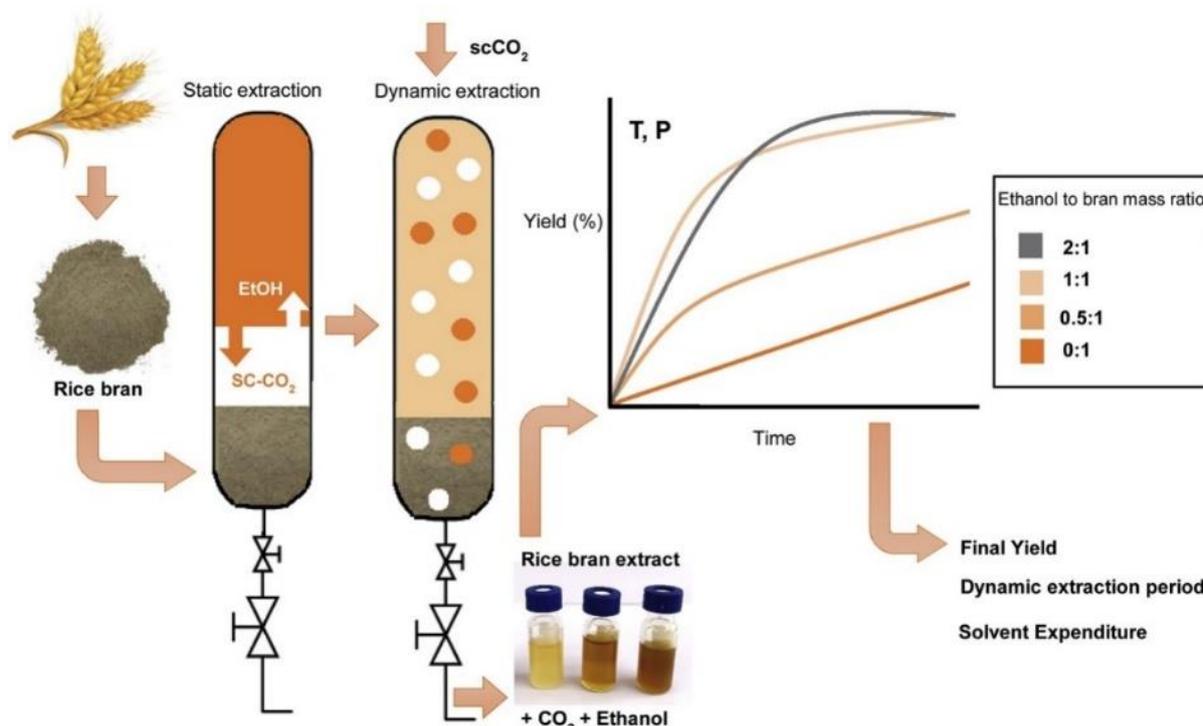


Fig. 4. The Carbon dioxide phase

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2.6 EAAE (Enzyme-assisted Aqueous Extraction)

The extraction of “aqueous enzymatic oil” is a new green technique. This method allows oil to be released in water extraction operations without the utilization of organic solvents. It’s worthwhile mentioning that hemicellulose, cellulose, lignin, pectin, and protein are the primary elements of plant cell membranes. The RBO accumulates in organelles known as “lipid bodies.” A phospholipid monolayer surrounds the organelles, including a family of structural proteins known as “oleosins.” Enzymes may aid in the disruption of this membrane by enabling oil to escape the cell. “Enzyme-assisted aqueous extraction (EAAE)” uses less energy as well as a solvent than traditional solvent removal and produces a high-quality product that doesn’t need any additional refinement. Pectinase, Cellulase, and protease are the key enzyme types used in Rice Bran Oil extraction.

Bhattacharyya and Sengupta were the very first to use Enzyme-assisted aqueous extraction to study the yield and quality of Rice Bran Oil made using “pectinase and cellulase” like a product of

temperature, response time, enzyme levels, and rice bran levels. Several researchers demonstrated that superior RBO may be created with properties comparable to those of traditional solvent removed oil with this process [35]. They pointed out that Enzyme-assisted aqueous extraction enables various portions of bran to be fractionalized, such as protein and fat, preventing the creation of by-products all through the extraction method since such elements can be readily parted and utilized for different reasons. Nonetheless, Enzyme-assisted aqueous extraction alone did not give a large harvest of Rice Bran Oil, necessitating a second hexane removal stage. Hernandez validated such findings, claiming that using amylase accompanied by hexane extraction resulted in a 5 % improvement above standard solvent extraction.

2.7 SWE (Subcritical Water Extraction)

SWE is a novel green removal technology, particularly for those that use food and vegetation as a beginning matrix. Subcritical water extraction uses water, the most environmentally friendly solvent-accessible since

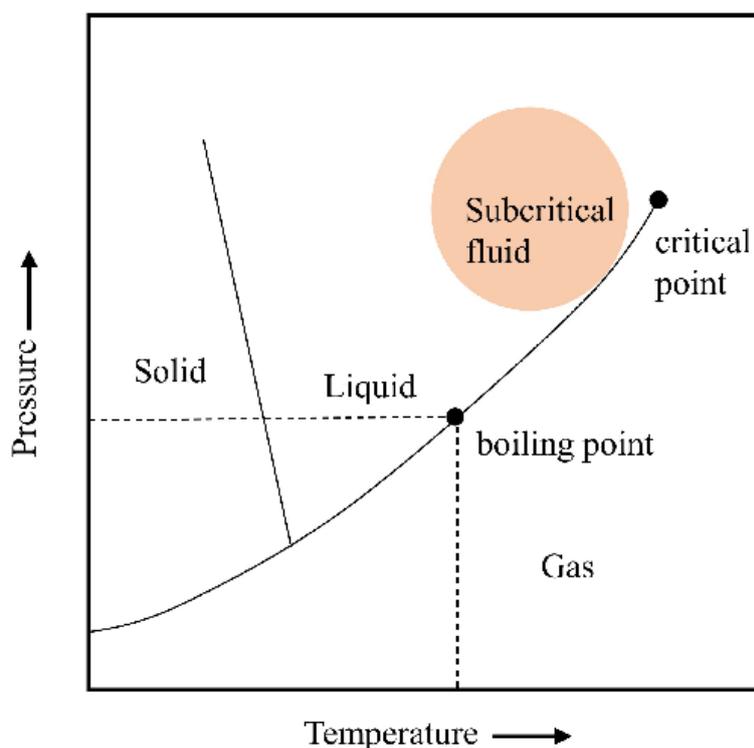


Fig. 5. Water phase diagram

Source: https://www.researchgate.net/figure/Schematic-phase-diagram-of-substance-showing-subcritical-fluid-area_fig2_329519018

it is non-toxic, non-flammable, readily available, and inexpensive. Water is a strongly polar molecule that cannot absorb most organic molecules under specific ecological circumstances [36]. Water is heated at temperatures over its boiling point, often between 101 to 375 °C, under elevated heat in SWE to retain its liquid condition. Under subcritical circumstances, water's dielectric properties and polarity change, allowing it to function somewhat like a non-polar chemical solvent. The dielectric constant of water is comparable to organic solvents like ethanol or methanol at temperatures over 200 °C. One of the most important parameters improving the effectiveness and accuracy of an extraction method is temperature. Water's permittivity, viscosity, and surface tension drop as the temperature rises, but diffusivity rises. As a consequence of these variables, it has enhanced matrix penetration ability and extraction effectiveness, and velocity [37]. The maximum effective extraction temperature must be specially picked based on the substances used in the procedure to prevent the destruction of the compounds of interest. PH, pressure, drift degree, removal duration, and the analytes' chemical composition are other factors to consider.

Regrettably, even though this technique has produced outstanding consequences for the removal of extra oils, including soybean, cottonseed, jojoba oil, or sunflower, and certain functional substances, including such essential oils, polyphenols, flavonoids, carotenoids, flavor, and fragrance compounds, it is not widely used for RBO extraction. Moreover, as opposed to traditional procedures, SWE offers numerous notable advantages, including using water rather than organic solvents, quicker extraction times, cheaper processing costs, and higher quality extracts.

3. CHROMATOGRAPHIC METHODS

Chromatography is a method for extracting the molecules, or solutes, of a mixture, based on the proportional quantities of each solute dispersed between a flowing fluid flow, known as the mobile stage, as well as a solid phase that is contiguous. The portable stage may be a liquid or a gas, whereas the immovable stage might be a solid or a liquid. Solute particles are continually exchanged between the two stages due to kinetic molecular movement. Whenever the dispersion prefers the flowing fluid for a given solute, the particles will spend most of their time moving

with the flow [38]. They will be carried away from those other species whose particles are maintained by the immobile stage for a more extended period. The division coefficient is a measure of the periods spent in the mobile and immobile zones divided by the levels in such areas for a particular species. In a limited space or restricted zone, a mixture of solute molecules is added to the network. The various species are carried at varying paces in the directions of flowing fluid. The flowing fluid is the main driver for solute movement. The solute attraction for the static stage is the resistance factor; the analyzer manipulates these forces to generate the segregation. Differential mobility from a limited starting region is characterized as chromatography, one of the various extraction procedures. Another part of this category is electrophoresis. The key driver in this example is an electromotive force, which imposes varied pressures on solutes with varying ionic charges. The viscosity of the non-flowing liquid is the resistive strain. Such forces combine to produce good ion mobility that is unique to each solute.

For the detection of volatile chemicals, gas chromatographic (GC) techniques are extensively utilized, either through headspace examination or by direct injection. Gas chromatographic is used to quantify lipid oxidation by-products, including such 2-decanal, 2-heptanal, 2,4-decadienal, hexanal, 2,4-heptagonal, and pentane [39]. The oxidation of lipids is exacerbated in sample processing and chromatography at high temperatures, which is a drawback of GC. This has an impact on the findings' consistency. Another chromatographic approach for measuring hydroxides, hydroperoxides, and secondary oxidation products is phase high-performance liquid chromatography. The HPLC approach has the benefit of reducing the breakdown of somewhat labile hydroperoxides and other oxidative products. Due to low concentrations of hydroperoxides and other compounds, HPLC techniques have limited sensitivity in identification. These techniques typically use UV and post-column response detection devices [40]. The wavelength of the UV detector has been tuned at 234 nm, which is the maximum sensitivity for polyunsaturated fatty acid hydroperoxides. DeMeyer used a reversed-phase column to quantify 13-hydroxyoctadecadienoic, two hydroperoxides, and 15-hydroxyeicosatrienoic acid. Nevertheless, no HPLC measurement of four linoleic acid hydroperoxides has been published [41]. Some

chemicals are employed in post-column response detection methods to interact with the oxidation products, which dissolve from the HPLC column to form high absorbent compounds. Straight UV monitoring has a slower response than these approaches.

4. CONCLUSION

In summary, our research demonstrates that pesticide residue levels typically decrease throughout rice production and exposure to such residues is linked to various health risks, including cancer and hormonal disruptions. We recommend future research focus on raising awareness of pesticide impacts and developing residue-minimizing, nutrient-preserving processing methods. Our study developed an effective method using an enhanced QuEChERS procedure and UPLC for detecting organophosphate pesticide residues, achieving excellent selectivity, repeatability, and precision, while also reducing time, labor, and solvent use. We observed that the pesticide broflanilide dissipates fastest in water and its residues were largely undetectable by harvest, suggesting its safe use at approved levels. This technique can be used for routine monitoring in rice and potentially other media, offering insights into the safe application of this herbicide in agriculture and the ecosystem.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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