The International Journal of Materials and Engineering Technology (TIJMET) (2024) 7(1)
http://dergipark.gov.tr/tijmet
ISSN: 2667-4033

RESEARCH ARTICLE

Ultrasound-assisted ethanolic extraction of oil from Pistacia vera l. using response surface methodology

Ezgi Kalkan[*1], Medeni Maskan[1]

[1]*Food Engineering Department, Engineering Faculty, Gaziantep University, Şehitkamil, Gaziantep, Türkiye*

Article Info
Article history:
Received 05.12.2023
Revised: 13.02.2024
Accepted: 11.03.2024
Published Online: 18.04.2024
Keywords:
Pistachio Oil
Extraction
Ultrasound
Optimization

Abstract
In this study, an ultrasound-assisted ethanolic extraction technique was used for the first time with success to extract oil from *Pistacia vera L*. The influence of extraction temperature (25, 37.5, 50°C), extraction time (10, 20, 30 min), and solvent-to-solid ratio (2:1, 4:1, 6:1) on oil efficiency was studied. Ultrasound-assisted extraction is a sustainable extraction technology that uses non-toxic organic solvents like ethanol instead of traditional solvents. According to numerical optimization, the ideal extraction parameters were 32.74°C extraction temperature, 29.47 min extraction time, and 5.94:1 extraction solvent to solid ratio. At these ideal conditions, oil yield is predicted to be 23.54% and experimentally obtained to be 22.04%. The study discovered that linear and quadratic levels of extraction time, as well as interaction levels of extraction time and solvent-to-solid ratio, had a significant effect on oil yield. It can be stated that the ultrasound-assisted ethanolic method is effective in providing high oil yields compared to traditional methods, reducing extraction time and temperature, and allowing the use of alternative green solvents.

1. Introduction

The pistachio nut *(Pistacia vera L.)* is one of the world's favorite nuts [1]. Pistacia genus are members of the cashew family (Anacardiaceae) and include at least eleven species. It most likely originated as the term "pistak" in Avestan, an ancient Persian language [2]. Pistachios are a native of western Asia, and traders brought them to the Middle East, Mediterranean nations, and Europe. The Neanderthals consumed pistachios 300,000 years ago, according to evidence. Charred pistachio residues have been discovered in the Mousterian layers of Israel's Kebara Cave [3]. The United States of America, Iran, Türkiye, and China are the main pistachio producers. Türkiye's annual production is the most third with 119.335 tonnes, according to the most recent data [4].

Pistachios have characteristic organoleptic properties and high nutritional quality. They contain a significant amount of fat (between 50-70%) and are abundant in monounsaturated fatty acids They are also a good supplier of protein, minerals, and bioactive constituents [5]. Nutritionally, seeds are an excellent source of essential unsaturated fatty acids, particularly omega-3 (linolenic acid) and omega-6 (linoleic acid), as well as valuable bioactive compounds such as vitamin E, phenolic acids, flavonoids, phytosterols, tocopherols, and carotenoid pigments.These phytonutrients are used in the treatment of many diseases. Immunity support, lipid metabolism, and protection against nephrotoxicity, including in chronic diseases such as diabetes and cardiovascular disease, have been associated with some important seed oils. [6,7].

In recent times, oil extraction from oilseeds has been performed by conventional methods such as solvent extraction, steam distillation, or mechanical pressing. The main disadvantages are the high consumption of non-environmental and poisonous organic solvents, the complexity and long hours of the process, and the need for extra purification steps. The drawbacks of existing classical separation methods have prompted the industry to develop green extraction techniques, switching from organic solvents to alternative green solvents, while simultaneously boosting the yield and quality of the oil [8].

No studies have been done on the procedure and optimization of oil extraction from pistachios using a novel approach, such as the ultrasound technique. For the production of improved quality and greater yield products for use in food systems, optimization is required. The extraction process conditions must be developed to the industrial production of high-quality oil. In this research, ultrasound-assisted ethanolic obtaining of oil via extraction methodology from *Pistacia vera L.* are produced using Response Surface Methodology (RSM). The goal of this study was to ascertain the ideal extraction conditions using RSM and to assess the impact of extraction temperature from 25 to 50°C, extraction time as from 10 to 30 min, and solvent-to-solid ratio (2–6) on the oil yield.

2. Materials and Methods

2.1. Material and Reagents

Fresh pistachio nuts harvested in 2023 were supplied from the local market in Gaziantep, Türkiye. The pure ethanol and hexane employed were of analytical grade. All chemicals were purchased from Sigma Aldrich Co (St. Louis, MO).

2.2. Sample Preparations

The pistachio nuts are manually dehulled and dried in an oven at 45 °C during the 24 h to remove the moisture content

Corresponding Author: Ezgi Kalkan
E-mail: ezgisoganci93@hotmail.com

How to cite this article:
Kalkan, E., Maskan, M., Ultrasound-assisted ethanolic extraction of oil from Pistacia vera l. using response surface methodology, The International Journal of Materials and Engineering Technology (TIJMET), 2024, 7(1): 7-11

from approximately 14 % to 2.6 %. Dried pistachio nuts were then ground in a blender and kept in sealed polyethylene bags at 4°C to further analysis.

2.3. Conventional Soxhlet Extraction

Soxhlet extractor was used for the extraction of eight gr of pistachio powder. This method was referred by Association of Official Analytical Chemists (AOAC). A measured quantity of powdered pistachio was added to a cellulose thimble, which was then put into a glass solvent flask. 150 mL of hexane was added to the flask. The extraction procedure ran at 180°C for 3 hours. When the extraction was finished, the thimble was withdrawn from the extraction system, and the oil was gently poured out of the bottom of the flask. The experiment was conducted according to the procedures in Food Quality Control Lab.

2.4. Ultrasound-Assisted Extraction

80 gr of dried pistachio powder was mixed with determined ratio of ethanol (2-6) in a 250 ml plastic beaker. The temperature of the ethanol was adjusted before starting the ultrasound treatment. An ultrasound probe (Soniprep 150, MSE (UK) Ltd, United Kingdom) was immersed into the beaker and sonicated for a determined duration (10-30 min) at the frequency of 15 kHz and power of 100 W by modifying the method from [9]. A magnetic stirrer was placed under the beaker to allow the ultrasound to penetrate and mix all the particles. Ultrasound was applied at 1.5 minute intervals, and adjusted to the desired temperature while controlling with the thermometer. The solution was subsequently centrifuged for 10 minutes at a constant speed of 10000 rpm. The residue was washed with 20 ml ethanol and centrifuged one further time. The supernatant was filtered with Whatman paper and evaporated by rotary evaporator (IKA RV 10 digital, IKA, Germany) at 50 rpm, 50°C. Obtained oil samples were kept in amber glass bottles in the refrigerator at 4 °C.

2.5. Moisture Content Determination

According to the method of [10], an adequate amount of pistachio powder was analyzed by a moisture analyzer (Mettler Toledo MJ33, Greifensee, Switzerland) after dehulling and before ultrasound treatment.

2.6. Extraction Yield (EY) Determination

The yield of pistachio oil was calculated according to Equation (1) on dry basis. *EY* was the weight ratio of extracted oil ($M_{initial}$) after evaporation and mass of sample weight (M_{final}) before ultrasound treatment [11].

$$EY(\%) = \frac{Minitial\,(g)}{Mfinal\,(g)} \text{ x } 100 \qquad (1)$$

2.7. Experimental Design, Modeling and Statistical Analysis

In this experiment, RSM was carried out to examine ultrasound-assisted ethanolic oil extraction from pistachio nuts. To achieve this objective, a three-factor and level design of Box-Behnken, designed using Design Expert version 13 (Minneapolis, USA), was implemented to develop the design matrix, analyze experimental data, and optimize the results. The experimental design consisted of 17 experiments, including 5 replications of the center point. Three distinct levels were used for every single independent variable: +1, 0, and 1. Yield was determined as the response in this study. The experimental results acquired for the dependent variable were converted as a function of into quadratic equations of the independent variables as given in Equation (2).

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{11} x_1{}^2 + \beta_{22} x_2{}^2 + \beta_{33} x_3{}^2 + \beta_{12} x_1 x_2 + + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 \qquad (2)$$

The dependent response variable, Y, is determined by the independent variables of extraction temperature (X_1), extraction time (X_2), and solvent-to-solid ratio (X_3); β_0 is a constant coefficient; β_1, β_2, and β_3 denote the linear regression coefficients; β_{11}, β_{22}, and β_{33} refer to the squared regression coefficients; and β_{12}, β_{13}, and β_{23} represent the interaction coefficients of independent variables. The significance of response model terms was carried out as an analysis of variance (ANOVA) with 95% confidence ($p \leq 0.05$). Additionally, Table 1's depiction of the independent variables and their levels describes the model's quality and sufficiency.

Table 1. Independent and dependent variables for the experiment

Independent variables	Level			
	Code	Low (- 1)	Central (0)	High (+1)
Extraction temperature, °C	X_1	25	37.5	50
Extraction time, min	X_2	10	20	30
Solvent to solid ratio	X_3	2	4	6

2.8. Process Optimization

Numerical and graphical (3D plots) optimization was performed using the superposition of RSM. The response is the extraction yield. By assigning desired values to all parameters, optimum numerical values for the response were obtained. Independent variables of extraction temperature, extraction time, and solvent-to-solid ratio were maintained within set range. The extraction yield as a parameter is targeted to the maximum. Table 2 exhibits the Box-Behnken response surface design of three independent variables (X_1, X_2, X_3) with their three levels and response values (Y_1).

Table 2. Independent variables of design of three-factor, three-level Box-Behnken and response value

Run	*X1*	*X2*	*X3*	*Y1,(%)*
1	25	20	6	22.41
2	25	30	4	14.13
3	25	10	4	11.7
4	25	20	2	11.82
5	37.5	10	2	14.37
6	37.5	20	4	15.03
7	37.5	20	4	15.2
8	37.5	20	4	14.98
9	37.5	30	6	23.51
10	37.5	20	4	14.96
11	37.5	30	2	11.82
12	37.5	10	6	18.64
13	37.5	20	4	15.4
14	50	10	4	13.28
15	50	20	6	17.18
16	50	20	2	12.43
17	50	30	4	16.69

3. Result and Discussion

3.1. Data Analyses and Model Fitting

The mathematical model reflecting the EY response of the extracted pistachio oil as a function of extraction temperature (X_1), extraction time (X_2) and solvent-to-solid ratio (X_3), which are independent variables of the ultrasound-assisted ethanolic extraction process, is shown by the following mathematical equation.

$$EY = 15.114 - 0.06 * X_1 + 1.02 * X_2 + 3.9125 * X_3 + 0.245 * X_1X_2 - 1.46 * X_1X_3 + 1.855 * X_2X_3 - 1.1445 * X_1^2 - 0.0195 * X_2^2 + 1.9905 * X_3^2 \quad (3)$$

The influence of these three independent factors was assessed using RSM. Table 2 contains the results of 17 different experimental runs with 5 center points according to the Box-Behnken Design. The P-value of ANOVA was significant, i.e. ≤ 0.05, proving that the Box-Behnken Design model was successfully applied to the data to optimize the extraction conditions. The model summary and the analysis of variance of the results are shown in Table 3. The more closely the empirical data match the real data, the greater the R^2 score. The purpose of the p-values is to evaluate each parameter's statistical significance. Model terms are considered relevant when P values are less than 0.05.

Table 3. Model summary and the variance analysis of the results for *EY*

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	174.59	9	19.40	11.99	0.0018
X_1-Temperature	0.0288	1	0.0288	0.0178	0.8976
X_2-Time	8.32	1	8.32	5.14	0.0576
X_3-Ratio	122.46	1	122.46	75.68	< 0.0001
X_1X_2	0.2401	1	0.2401	0.1484	0.7115
X_1X_3	8.53	1	8.53	5.27	0.0554
X_2X_3	13.76	1	13.76	8.51	0.0225
X_1^2	5.52	1	5.52	3.41	0.1074
X_2^2	0.0016	1	0.0016	0.0010	0.9758
X_3^2	16.68	1	16.68	10.31	0.0148
Residual	11.33	7	1.62		
Lack of Fit	11.19	3	3.73	108.17	0.0003
Pure Error	0.1379	4	0.0345		
Cor Total	185.92	16			

3.2. Effect of Process Variables on the Response EY

As it is demonstrated in Table 2, *EY* response is ranged between 11.7% and 23.51 %.In Table 3, according to the ANOVA result, the quadratic model was significant for *EY*. There are three significant terms which are the linear and quadratic level of solvent to solid ratio (X_3,X_3^2) and interaction term of extraction time and solvent to solid ratio (X_2X_3). However, there was no other significant terms. In addition, the terms of X_3, solvent to solid ratio was highly significant on response. R^2 and adjusted R^2 values are 0.9391 and 0.8607 respectively. It indicates that the combination of RSM and Box-Behnken Design is a suited model for ultrasound-assisted ethanolic extraction of oil from pistachio nuts.

Ultrasound-assisted extraction is a relatively new technique in food and bioprocess engineering. This applications have been successfully tested for the extraction of oils from a diverse range of plant sources[8]. Also, ultrasound-assisted extraction holds great potentiality for minimizing both the economic and environmental impact of oil production. The industry's goal is to limit the use of non-renewable resources, reduce process time and energy consumption, and in turn improve the quality of the final product - not necessarily to maximize yield [12].

In Figure 1, three-dimensional response surface plots visualize the correlation between the *EY* response and the independent variables.The impact of extraction time, temperature, and oil yield is shown in Figure 1a. The response extraction yield was greatly affected by extraction time, as demonstrated in Figure 1a. The extraction yield rises as the extraction duration is extended. Numerous studies have provided an explanation for how prolonged ultrasonic exposure might disrupt the cell membrane due to the enormous energy released by cavitated bubbles as they collapse. The oil transition from cell to solvent was accelerated as a result. This result was similar with Chen et al.[13] that samara oil yield was gradually increased with increasing time 20 min to 40 min. Additionally, Figure 1a illustrates how raising the temperature to 37.5°C increases the extraction yield. However, further raising has a negative impact on yield. This outcome was consistent with Senrayan and Venkatachalam's findings[14]. They stated that a higher oil solubility in the solvent was the cause of the rise in oil yield with temperature increase. Furthermore, they discovered that 30.73% was the maximum oil yield of *Coriandrum sativum L.* seed oil and that any additional growth would result in a decline in oil yield. This outcome matched the findings of the current research.

This relationship between extraction time and the solvent-to-solid ratio appears in Figure 1b. It has been shown that oil yield increased when the solvent-to-solid ratio increased. Moradi and Rahimi [15] showed that raising the solvent-to-seed ratio from 4:1 to 6:1 results in a higher production of sunflower oil. However, continued increasing leads to decline since the substrate concentration is dropping. They discovered that the 6:1 ratio generated the highest production, which is also relevant of the current study, which also finds the highest yield at this ratio. The relationships between the solvent-to-solid ratio and the extraction temperature are shown in Figure 1c. As can be observed, oil yield gradually increases as the solvent-to-solid ratio rises, peaking at a ratio of 6:1. However, as the temperature rises from 25 to 40 °C, there is a reasonable increase, but additional rises do not influence the oil output.

3.3. Overall Optimization and Model Validation

By choosing the independent variables in range and targeting for *EY* response maximum, the optimal extraction temperature (25-50°C), extraction time (10-30 min), and solvent-to-solid ratio (2:1-6:1) for pistachio oil using ultrasound-assisted ethanolic extraction were identified. Based on numerical optimization, the most suitable extraction parameters were an extraction temperature of 32.74°C, an extraction time of 29.47 min, and an extraction solvent to solid ratio of 5.94:1. It is expected that by implementing these conditions, 23.54% oil yield would be attained. In this investigation, the desirability value was determined to be 1, which signifies complete desirability. Experiments were conducted under the best possible conditions to verify the predicted values from the model. Oil yield for the experiment was 22.04% percent. As a result, as the predicted outcome (23.54%) did not significantly differ from the experimental value (22.04%), this supports the validity of the predicted model.

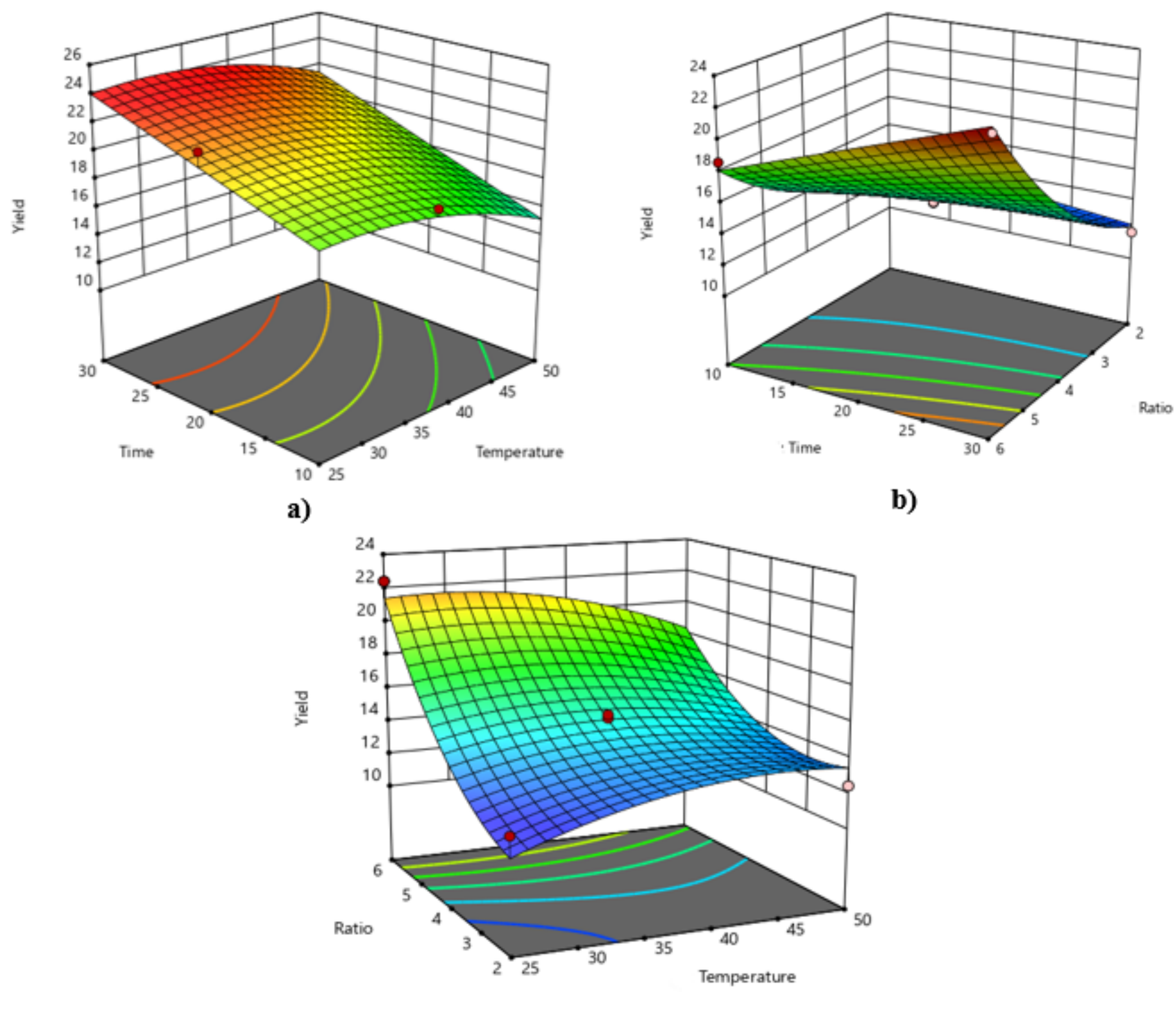

Figure 1. Three-dimensional plots showing the mutual effect of extraction temperature, extraction time, and solvent-to-solid ratio on the extraction yield (*EY*) of pistachio oil

3.4. Comparison of Oil Yield Obtained by Optimal Ultrasound-assisted Ethanolic extraction and Other Treatments

In order to assess the effectiveness of the ultrasound-assisted ethanolic extraction method, ethanolic extraction of oil without ultrasound, ultrasound-assisted hexane extraction, and hexane extraction without ultrasound were all performed under the optimal conditions (32.74°C extraction temperature, 29.47 min extraction time, 5.94:1 solvent to solid ratio). The influence of extraction methods carried out under these optimal circumstances on the yield of oil can be seen in Figure 2.

Experiments were conducted in the current study at the ideal conditions, which were determined by numerical optimization to be 22.04%, in order to validate the model. We can get an agreement that ultrasound treatment has a significant impact on oil yield by comparing the ethanol extraction with and without ultrasound. As it increased, the yield rose from 13.04% to 22.04%. Additionally, hexane experiments with and without ultrasound were conducted to examine the performance of different solvent types. It demonstrates that hexane exceeded ethanol in effectiveness. With ultrasound-assisted hexane extraction, the greatest oil efficiency of 50.05% was achieved. Additionally, hexane extraction without ultrasound treatment results in a 46.88% oil yield, which is lower than ultrasound-assisted hexane extraction but still more substantial than ethanolic extractions. Furthermore, using conventional soxhlet extraction, the pistachio oil yield is 55.8%.

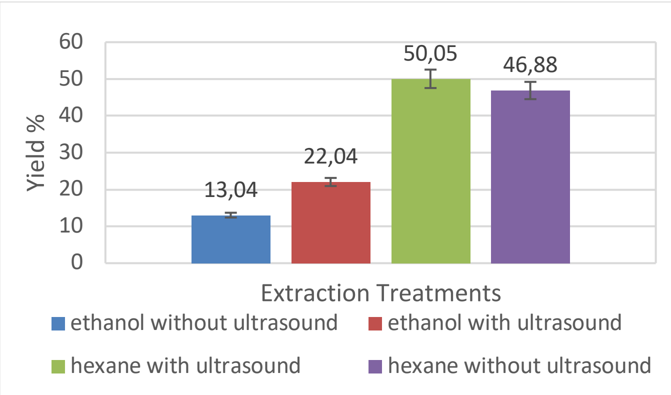

Figure 2. Effect of different extraction treatments at optimum conditions on oil yield

4. Conclusion

As a result, the optimal conditions for this investigation were determined and the yield of the pistachio oil was measured. According to numerical optimization, the ideal operating conditions were 32.74°C extraction temperature, 29.47 min extraction time, and 5.94:1 solvent to solid ratio. The greatest oil yield of 23.51% was found during ultrasound-assisted ethanolic extraction, notably at 37.5 °C, 30 min, and a solvent-to-solid ratio of 6:1. This study reveals that ultrasound has a substantial effect on oil yield and can be used as an alternative method for lowering the extraction duration and temperature of conventional soxhlet extraction. Aside from that, pistachio oil yield needs to be evaluated qualitatively in subsequent continuous investigations to highlight the advantages of ultrasound technology.

Acknowledgments

The research was made possible by the aid and technical support provided by Associate Prof. Dr. Derya Kapusuz Yavuz and Associate Prof. Dr. Abdulcabbar Yavuz, for which the authors are thankful.

Author contributions

Medeni Maskan: Conceptualization, methodology, data curation, writing—original draft preparation, Reediting editing, supervision, project administration, funding acquisition

Ezgi Kalkan: Conceptualization, methodology, data curation, software, validation, investigation, writing—original draft preparation.

References

1. Arena, E., Campisi, S., Fallico, B., Maccarone, E., Distribution of fatty acids and phytosterols as a criterion to discriminate geographic origin of pistachio seeds, Food Chem, **2007,** 104(1):403-408
2. Ferguson, L., Polito, V., Kallsen, C., The pistachio tree; botany and physiology and factors that affect yield. Pist Prod Manual, 4th Ed Davis, CA, USA, Univ Calif Fruit Nut Res Inf Cent, **2005**, 31(9)
3. Mandalari, G., Barreca, D., Gervasi, T., Roussell, MA., Klein, B., Feeney, M.J., et al. Pistachio nuts (Pistacia vera L.): Production, nutrients, bioactives and novel health effects. Plants, **2021**, 11(18)
4. Nations F-F and AO of the U. The production of fish meal and oil. FAO Fisheries Technical Paper 142, **1986**
5. Satil, F., Azcan, N., Baser, K.H.C., Fatty acid composition of pistachio nuts in Turkey. Chem Nat Compd, **2003**, 39:322–4
6. Hashemi, S.M.B., et al., Characteristics of wild pear (Pyrus glabra Boiss) seed oil and its oil-in-water emulsions: A novel source of edible oil. Eur J Lipid Sci Technol, **2018**, 120, 1700284
7. Sarwar, M.F., Sarwar, M.H., Sarwar, M., Qadri, N.A., Moghal, S., The role of oilseeds nutrition in human health: A critical. J Cereal, **2013**, 4, 97–100
8. Putnik, P., et al., Novel food processing and extraction technologies of high-added value compounds from plant materials. Foods, **2018**, 7, 106
9. Marwah, M.K., et al., Novel controlled-release polylactic-co-glycolic acid (PLGA) nanoparticles for sodium thiosulphate, a hydrogen sulphide donor, retains pro-angiogenic potential of hydrogen sulphide. J Exp Nanosci **2022,** 17, 197–213
10. Chen, F., et al., The Underlying Physicochemical Properties and Starch Structures of indica Rice Grains with Translucent Endosperms under Low-Moisture Conditions. Foods, **2022**, 11, 1378
11. Haji Heidari, S., Taghian Dinani, S., The study of ultrasound-assisted enzymatic extraction of oil from peanut seeds using response surface methodology. Eur J Lipid Sci Technol **2018**, 120,1700252
12. Pingret, D., Fabiano-Tixier, A-S., Chemat, F., Ultrasound-assisted extraction. Nat Prod Extr Princ Appl, **2013**, 21-89.
13. Chen, F., Zhang, Q., Fei, S., Gu, H., Yang, L., Optimization of ultrasonic circulating extraction of samara oil from Acer saccharum using combination of Plackett–Burman design and Box–Behnken design. Ultrason Sonochem **2017**, 35, 161–75
14. Senrayan, J., Venkatachalam, S., Optimization of ultrasound-assisted solvent extraction (UASE) based on oil yield, antioxidant activity and evaluation of fatty acid composition and thermal stability of Coriandrum sativum L. seed oil. Food Sci Biotechnol, **2019**, 28, 377–86
15. Moradi, N., Rahimi, M., Effect of ultrasound-and pulsed electric field-assisted enzymatic treatment on the recovery and quality of sunflower oil. Sep Sci Technol, **2019**, 54, 1043–54