

Review Article

Extraction and Encapsulation Methods for Pomegranate Seed (P. granatum) Oils, Review

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Abstract

The interest in the functional use of bioactive components of fruit products in a variety of fields is constantly on the increase. Lately, research is ongoing to develop new technologies such as simple mixing, pressureassisted extraction, enzymatic extraction, ultrasound-assisted extraction, pulsed electric fields, high hydrostatic pressure, ohmic heating, and microwave-assisted extraction. This research is aimed at designing equipment, improving the efficiency of extracting bioactive compounds with organic solvents, and developing extraction methods. Research has shown that pomegranate fruits, arils, and seeds contain a variety of bioactive constituents, such as ellagitannins, hydroxycinnamic acids, hydroxybenzoic acids, flavones, flavonol-3-ols, anthocyanidins, anthocyanins, and conjugates. Consequently, encapsulation techniques preserve the quality of the oil and other components of pomegranate seeds. The technique of encapsulation enables the safe delivery, preservation, and regulation of the targeted release of bioactive constituents in food systems. Natural botanical conservation, which not only extends the shelf life of products but also has unique biochemical qualities, is increasingly being used in many sectors, including food, cosmetics, and pharmaceuticals. This study provides information on the encapsulation techniques used to protect sensitive compounds and their benefits. Currently, there are increasing problems related to food quality, safety, and reliability that cause undesirable effects on product quality. These techniques are expected to be effective in achieving food safety and reliability goals in the future.

Keywords: Punica granatum, Pomegranate seed oils, Bioactive components, Encapsulation, Extraction methods

Nar Çekirdeği (P. granatum) Yağlarının Ekstraksiyonu ve Enkapsülasyon Yöntemleri, Derleme

Özet

Bitkisel ürünlerin biyoaktif bileşenlerinin çeşitli alanlarda fonksiyonel kullanımına olan ilgi sürekli olarak artmaktadır. Son zamanlarda, Basit Karıştırma, Basınç Uygulamalı Ekstraksiyon, Enzimatik Ekstraksiyon, Ultrases Destekli Ekstraksiyon, Darbeli Elektrik Alanlar, Yüksek Hidrostatik Basınç, Ohmik Isıtma, Mikrodalga Yardımcı Ekstraksiyon gibi yeni teknolojilerin geliştirilmesine yönelik araştırmalar devam etmektedir. Bu araştırmalar sayesinde, ekipman tasarımı, biyoaktif bileşiklerin organik çözücüler ile ekstraksiyon etkinliği ve ekstraksiyon yönteminin geliştirilmesi amaçlanmaktadır. Nar meyvesinin, kabuğunun ve çekirdeğinin, ellagitanninler, hidroksisinamik asitler, hidroksibenzoik asitler, flavonlar, flavonol-3-oller, antosiyanidinler, antosiyaninler ve konjugeler gibi çeşitli biyoaktif bileşiklere sahip olduğu araştırma literatürlerinde belirtilmiştir. Bu derleme nar çekirdeğinin yağ komposizyonları ve bileşenlerinin kalitesini koruyan enkapsülasyon tekniklerine yönelik yapılan çalışmaları özetlemektedir. Kapsülleme, gıda sistemlerinde biyoaktif bileşenlerin güvenli bir şekilde verilmesini, korunmasını ve hedeflenen salım düzenlenmesini sağlayan bir tekniktir. Bu çalışma, hassas bileşikleri ve faydalarını koruyan enkapsülasyon teknikleri hakkında bilgi vermektedir. Günümüzde, gıda kalitesi, güvenliği ve güvenirliğiyle ilgili sorunların artması ürün kalitesinde istenmeyen etkilere neden olmaktadır. Bu tekniklerin ileride gıda güvenliği ve güvenilirlik konusunda etkili olması beklenmektedir.

Anahtar Kelimeler: Punica granatum, Nar çekirdek yağı, Biyoaktif bileşenler, Enkapsülasyon, Ekstraksiyon yöntemleri

1.INTRODUCTION

Approximately 500 species of *Punica granatum* of various sizes, shapes, flavors, and colors are cultivated worldwide. The pomegranate is a spherical fruit with an 8–12 cm diameter. The fruit's rinds can be thick or thin, varying in color from pale yellow to crimson. The fruits of the superior varieties have strong planting and fleshy textures, juicy but thinner than those of the lesser varieties [1].

Pomegranate (*P. granatum L.*) is grown in tropical and subtropical regions [2]. The Silk Road introduced the pomegranate, widely cultivated in Central Asia, to China. The spread of medical knowledge throughout China is key to enriching conventional medical theory [3]. Ancient Mediterranean cultures have played a significant role in pomegranate fruit cultivation, which has occurred for over 4,000 years due to its medicinal and nutritional qualities. Pomegranate, which has an annual global production of 2.5-3 million tons, is used in various culinary items such as juice, concentrate, jam, sugar, sauce, preserves, and fresh food. The food and pharmaceutical industries have developed food additives, medicines, supplements, and extracts from pomegranate processing waste. Previous research reports that pomegranate's chemical constituents are mainly tannins, alkaloids, organic acids, and flavonoids [3]. The bioactive components of pomegranates include anthocyanin, ellagic acid, ellagitannin, punicic acid, flavanol, flavan-3-ol, and flavone [4].

Flavonoid compounds such as flavonols, anthocyanins, and flavan-3-ol are mainly present in pomegranate seeds [2]. Juice and seeds are the main components of pomegranate waste, yielding up to 20% oil [5]. Owing to its inhibitory effect on lipid peroxidation, punicic acid, the main component of pomegranate seed oil, may inhibit cancer cell growth [3]. An alternative oil is pomegranate seed oil, which contains nutraceutical functional components such as sterols and punicic acid [2]. Accordingly, these oils have been evaluated for their high content of tocopherols, phytosterols, and phenolic constituents, especially for pharmaceutical purposes [5]. Furthermore, pomegranate seed oil has a high concentration of bioactive conjugated alphalinolenic acid isomers, accounting for approximately 85% of the oil's fatty acids [6]. Moroccan pomegranate seed oil is prone to oxidation due to its high conjugated α -linolenic acid concentration [7]. As storage time increases, the oxidative stability of pomegranate seed oil also decreases [2]. It is a valuable oil with characteristics and an abundance of bioactive molecules [8], such as terpenoids, polyphenols, alkaloids, and other nitrogen-containing substances [9]. It is also used for its biocompatibility [10], neuroprotective effects [11], and as a byproduct in animal feed [12, 13]. Polyphenols are an important class of phytochemicals used to treat diabetes [14, 15, 16]. Integrating essential oils into natural polymer packaging is considered an innovative method for food preservation [17]. Many studies have shown that dried pomegranate seeds and oil have therapeutic effects [18].

Research on the function of fatty acids, which have a wide variety of pharmacological effects, particularly anti-bacterial and anti-viral effects, glucose and lipid metabolism regulation, anti-tumor effects, cardiovascular improvement, and immunomodulation, has provided a basis for further research on pomegranate fruit [3]. Pomegranate seed oil's punicic acid concentration has been associated with biological benefits such as anti-diabetes, anti-obesity, anti-carcinogenic, and anti-inflammatory properties [6].

2.EXTRACTION OF POMEGRANATE SEED (Punica granatum) OIL

The pomegranate fruit's seeds yield pomegranate seed oil, a valuable natural resource. The importance of this oil is based on its unique composition and function of fatty acids, antioxidants, vitamins, and bioactive compounds [19]. The extraction method and solvent significantly affect the oxidation parameters in the extraction of pomegranate seed oil [20], and sustainable extraction methods are preferred [21].

The quality and yield of pomegranate seed oil varies depending on the cultivar, extraction methods, etc. Boiling pomegranate seeds before extraction, which also increases oil yield, is thought to reduce the overall oxidation of fatty acids [22]. The pomegranate seed oil has a high punicic acid concentration and a low omega-6/omega-3 ratio [23]. Microwave pretreatment of seeds before cold pressing significantly increases palmitic, oleic, and linoleic acids while decreasing the level of punicic acid [24]. In addition, cooking and digestive processes affect the compounds in pomegranate oil [25].

Conjugated fatty acids are present at different concentrations in numerous vegetable oils, which comprise 12-20% of the pomegranate seeds. Furthermore, polyunsaturated fatty acids with alternate double bonds are geometric isomers. This fatty acid is particularly interesting because of its positive physiological effects in various diseases [1]. These oils are carboxylic acids with long, saturated, or unsaturated aliphatic hydrocarbon chains. In addition, they are derived from triglycerides, or phospholipids, which contain aliphatic hydrocarbon chains. Based on the number of carbon atoms in the chain, there are three types of hydrocarbon chains: small, medium, and long. Ultimately, the small chains include 4-6 carbon atoms, the medium chains include 8–18 carbon atoms, and the long chains include more than 18 carbon atoms. The oil content in seeds varies in both quantity and quality, depending on the ripeness and geographical location of the pomegranate fruit grown [1].

Seeds possess significant quantities of insoluble bound phenolics, which play a role in stiffening the thick walls of sclerotia cells. In situ synthesis from linoleic acid, present at lower levels in seed oil, yielded punicic acid and, to a lesser extent, oleic, palmitic, and stearic acids in the study. Thus, it synergistically contributes to the antioxidant activity of pomegranate fruit. Boron, iron, and copper are the major trace elements found in juice, seeds, and peels, respectively, whereas silver and molybdenum are present in the seeds of all varieties. This demonstrates that the insoluble phenolics were mostly connected with structural polysaccharides that contribute to the cell wall membrane and protoplasts that accumulate/extract potentially hazardous metal ions [26].

The lipid content of the Zivzic cultivar was investigated, and five fatty acids were identified: stearic, palmitic, linoleic, oleic, and punicic acids. However, the proportion of polyunsaturated fatty acids in pomegranate seed oil has numerous health benefits [27]. Given its remarkable ability to combat inflammation and provide antioxidant benefits, it has preventive effects against estrogen-sensitive breast cancer. Furthermore, it is effective against pancreatic, prostate, and colon cancer. Researchers have also discovered that they can withstand high levels of flavonoids, potentially beneficial for individuals suffering from rheumatoid arthritis. Among the omega-5 fatty acids found in pomegranate seeds, conjugated linolenic acid is the most abundant [28].

The total fatty acids content of pomegranate seed, 22.63 g/100 g dry weight was cultivated in Morocco. The most basic fatty acid consisted of punicic acid (75.1 g/100 g), followed by catalpic acid, and linoleic acid. Beta-cytosterol was the sterol marker, while palmitic acid and stearic acid were saturated fatty acids. In addition, the total tocopherol content was 332.44 mg/100 g, with β -tocopherol being the main component, followed by alpha-tocopherol and gamma-tocopherol [7].

The fruit seeds and seed oils of 10 pomegranate cultivars growing in central Morocco were analyzed, finding oil outputs ranging from 17.59% to 24.69%. These studies identified high levels of polyunsaturated fatty acids. The presence of chlorophyll and pheophytin in oils, and the amount of antioxidants is also high [29]. Costa et al. (2021) determined the chemical composition, bioactivity, and quality of three marketed pomegranate seed oils from Turkey and Israel. The amount of punicic acid in all the oils was the same, but the amount of total conjugated linolenic acid was higher in Israeli species based on quality indicators and lipid types. Bioactive compounds included total phenolics, tocopherols, β -carotene, and conjugated linolenic acid, tocopherols (α , γ , ε), β -carotene, and total conjugated linolenic acid. This oil exhibits a unique phenolic profile, comprising three primary classes: phenolic acids, flavonols, and flavanones, with vanillic acid being the sole component present in all lipids. Comparisons with gas chromatography analysis using both flame ionization detectors and mass spectrometry detectors revealed eight different conjugated linolenic acid isomers in the retention times of the standards and mass spectrometric fragmentation profiles. Principal component analysis of pomegranate seed oil's phenolic content identified γ -tocopherol as the primary component, particularly in Turkish pomegranates [6].

The spectrophotometric method was employed to measure the overall phenolic, flavonoid, and antioxidant activity of the pomegranate seed oil extracts. Titration was employed to analyze tannins, while pH differences were utilized to determine anthocyanins. The conventional extraction approach produced the most total phenolics, expressed in gallic acid equivalents, whereas the sonication system produced the most total flavonoids, represented in catechin equivalents. Total phenolics were extracted more effectively using conventional methods (figure 1), and the novel large whole cultivar had the greatest bioactivity of the cultivars tested [30]. Table 1 shows the methods used to obtain pomegranate seed oil in the food sector and its uses [31].

[].	Table 1. Pomegranate seed oil				
	Extraction Methods	<i>P. granatum</i> Oil Components	Used in the Food Industry		
	Soxhlet	Stearic acid	Packaging		
	Hot and cold pressing	Oleic acid	Animal feed		
	Supercritical fluid	Phytosterol	Functional component		
	Aqueous extraction	Arachidonic acid	Antimicrobial agent		
	Enzymatic extraction	Punicic acid	Drug applications		
	Microwave-assisted	Aliphatic alcohols	Fat substitutes		
	Ultrasound-assisted	Linolenic acid			

Tocopherol Palmitic acid

Scientific research has indicated that pomegranate oil contains conjugated linolenic acids and punicic acid, a key fatty acid known for its notable biological activity [32]. The major sterol base was β -cytosterol, followed by campesterol, and delta-5 avenasterol. They are abundant in polyunsaturated fatty acids, including primary linoleic acid (omega-6), linolenic acid (omega-3), bioactive conjugated linolenic acid (omega-5), and eicosapentaenoic acid. Punicic acid constitutes 81.29% of the total fatty acids, while oleoresins, phenolic constituents, and sterols are other bioactive molecules that exhibit antioxidant activity. In addition, the oil contained high concentrations of rutin, quercetin, and caffeic acid. Oils with strong phenolic components have higher levels of gallic acid, catechin, chlorogenic acid, and caffeic acid than other vegetable oils [33]. New methods are being developed to replace conventional extraction methods so that they can be safely applied to foods in the future (Figure 1). Synergies between bioactive components combined with encapsulation techniques offer a bright future for safe and high-quality food [34]. Some of the extraction methods are explained in the next sections.

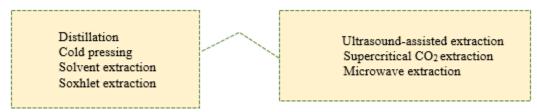


Figure 1. Conventional methods and new methods

2.1 Soxhlet Extraction

Although both Soxhlet and subcritical extraction methods provided high yields, the ultrasonic extraction and Soxhlet (shaking extraction) methods extracted greater amounts of squalene compounds. Squalene is a beneficial compound with numerous health benefits. The squalene content of pomegranate seed oil varies between 1.20 and 1.72 mg/g, depending on the oil extraction method. Pomegranate seed oil contained three tocopherol species (α , y, and δ), ranging from 348.2 to 359.8 mg/100g. This study also determined ten different fatty acids in varying amounts. Punicic acid was found in high quantities among the fatty acids

measured using these techniques, ensuring the desired quality [30].

The fatty acid content of oils extracted by ultrasonic, soxhlet, and Bligh and Dyer methods was converted to fatty acid methyl esters. The results indicated that the timed extraction approach was quick and efficient. When compared to two conventional procedures for extracting pomegranate seed oil, soxhlet extraction, and the Bligh and Dyer method, the approach proved effective [28]. Solvent extraction is an environmentally harmful method [35].

2.2 Ultrasound-assisted Extraction

Ultrasound is a hybrid technology owing to its high throughput and low cost. The least expensive configuration for bioactive extraction and enzyme applications is ultrasonic baths [36]. In addition, ultrasound pretreatment increased the extraction of ellagic, chlorogenic, and gallic acids from both seeds and peels compared with the untreated sample [37]. Ultrasonication is a system used in various studies aimed at maximizing the cavitation activity in a sample matrix with different experimental parameters, such as duration, temperature, frequency, and power [38].

Seed oils of three pomegranate species (Jebali, Testouri, and Gabsi) cultivated in Tunisia were extracted via ultrasound to determine their physicochemical characteristics, fatty acid content, and sterol composition. It identified 81.53% punicic acid from Testouri and 86.41% from Gabsi as the major fatty acid component of pomegranate seed oil, while β -cytosterol emerged as the dominant phytosterol. Although the antioxidant activity of seed oils of two pomegranate species (Jebali, Testouri) decreased, the proanthocyanidin content increased. The extract of Gabsi pomegranate seeds has the greatest concentration of polyphenols and flavonoids. The extracts from Testouri and Gabsi species contained total polyphenols, 318.04±1.75mg and 82.19±1.77mg of catechin equivalents, respectively. Jebali species yielded the maximum oil, followed by Testouri and Gabsi. Consequently, oil from Tunisian varieties, especially Jebali, has become more important for human consumption in terms of fatty acids than other varieties [39].

Pomegranate seed oil research has enhanced the use of ultrasound to evaluate the concentration of carotenoids, tocopherols, sterols, phenols, and flavonoids. Furthermore, the extracts' antioxidant and antibacterial activities were assessed. Pomegranate seed fatty acid composition is also known for its potent bioactivity. The ultrasonic approach also characterized the carotenoids, tocopherols, sterols, phenols, and flavonoids found in pomegranate seed oil. Therefore, the antioxidant and antimicrobial activities of these compounds were determined. In addition, a high concentration of oleoresin in the oil containing bioactive molecules were also detected. Phenols and antioxidants were more abundant in these oils than in the standard vegetable oils [33].

Pomegranate seeds and arils that were extracted with ultrasound had better bioactivity, antimicrobial activity, and fewer phytotoxic compounds than seeds and arils that were extracted with ethanol solvents alone. The standardized extracts were rich in phenolics, ranging from (0.16-0.73 mg GAE/mg extract) gallic acid equivalent [40]. The ultrasound-assisted extraction method produced a high content of antioxidants, flavonoids, and anthocyanins [41]. On a dry basis, cyanidin-3-glucoside concentrations ranged from 0.06-0.60 μ g/mg, whereas catechin concentrations ranged from 0.019-0.068 mg CATE/ mg extract, equivalents. Furthermore, the antioxidant activity was measured at 0.01-0.20 mg/ml. The process is successful against gram-positive bacteria and yeast but less against gram-negative bacteria [40].

This assessed the bioactivity, anti-bacterial activity, and phytotoxicity of peel and seed extracts from the Acco, Big Full, and Superb pomegranate cultivars using standard pure ethanol solvent-assisted and ultrasound-assisted extraction procedures. In the cold-pressing investigation, the temperature of the extracted oil was less than 40°C, and the yield was 4.29%. Therefore, the industry proposes the co-extraction of oil from a mixture of plant materials and seed oils as a reliable, cost-effective, and time-efficient method. Pomegranate seed oils are considered to be of good quality and have high oxidative stability [42].

The hydrophilic components from pomegranate seed oil were extracted using pulsed ultrasonic extraction with water as the solvent. The resulting extracts contained (750mg/100g and 600mg/100g, respectively)

hydrolyzable tannins and vitamin C compounds [43].

2.3 Supercritical CO₂ Extraction

Pomegranate seed oil was extracted utilizing a more sustainable, ecologically green technology known as supercritical fluid extraction rather than conventional solvent extraction with n-hexane and carbon dioxide. Supercritical carbon dioxide extraction showed the highest lipid peroxidation inhibition capability (16.43%) when compared to n-hexane solvent extraction [44].

Byproduct seed pastes from *Vitis vinifera*, *Foeniculum vulgare*, *Cannabis sativa*, and *Punica granatum* were extracted using two different techniques: supercritical CO_2 and n-hexane. The remaining samples were extracted using 10% or 20% ethanol and water. Matrix identification was accomplished using high-performance liquid chromatography, a diode array detector, liquid chromatography, and high-resolution mass spectrometry. As a result of the study, the organic acids and the kaempferol flavonol from the pomegranate seed paste were determined and antioxidant activity was correlated with extract solubility. [45].

Supercritical CO₂ extraction [46] and modified freeze crystallization techniques based on different melting points of oils were used [47]. Furthermore, the ultrasound-assisted method with enzymatic pretreatment yielded a higher oil yield than other techniques [48]. Studies have shown that pomegranate seed oils treated at high temperatures exhibit strong oxidative stability. Modifying the tocopherol compounds in pomegranate oil is effective for this purpose [49]. There are many bioactive compounds in pomegranate fruits, arils, and seeds. These include flavones, flavonol-3-ols, ellagitannins, anthocyanidins, anthocyanins, hydroxycinnamic acids, hydroxybenzoic acids, and conjugates (Table 2).

Extraction Methods	Analysis Equipment	Identified Components	Solvents	References
Cold solvent extraction	Oxidation tests, FTIR ^e ,Schaal oven operation, spectrometer, GC ^e	Linoleic acid,oleic acid, palmitic acid, stearic acid, tetracosanoic acid, eicoseanoic acid, arachidic acid and dihimo-gamma- linolenic acid	<i>n</i> -Hexane, <i>n</i> -Heptane (after cold methylation with 2 N KOH in methanol)	[2]
Conventional and ultrasound extraction,	UV/Vis ^d , pH differential	Total flavonoids expressed as catechin equivalents; anthocyanins expressed as cyanidin- 3- glucosides and antioxidant activity	Ethanol-water, Tewen80, methanol	[40]
Cold pressing	UV/Vis ^d	Pheophytin, punicic acid, phenolic acids, quercetin, and naringenin	Ethanol, gallic acid, acetone, ethanol	[42]

Table 2. Some of the extraction analysis methods to identify to pomegranate seed of component

Table 2. Continue on					
Cold pressing	GC-FID ^a , HPLC ^b	Palmitic acid, stearic acid, arachidic acid, <i>n</i> -Hexane, <i>n</i> -Heptane, catalpic acid, linoleic _{ethanolic} potassium acid, β -sitosterol, δ -hydroxide (2 N) tocopherol			
Soxhlet extraction, Cold pressing	GC°	Palmitic acid, stear acid, oleic aci linoleic acid, punic acid	d,KOH with methanol	[1]	
Ultrasound extraction	HPLC, UV–Vis ^d , fluorescence spectrophotometry	Ellagic, chlorog nic, and gallic acids	e Methanol, formic acid, water	[37]	
Hot water extraction	ⁿ HPLC ^b , GC ^c	Eicosapentaenoic acia Docosahexzaenoic acid, Linoleic acid	d, Tween80, 2,2- Diphenyl 1-1- picryl hydroxyl and Trolox,	[50]	
Ultrasound- assisted extraction	HPLC ^b	Pectin, phenolic compounds Carotenoids	Ethanol, gallic acid	[51]	

^a Gas chromatography-flame ionization detector (GC-FID). ^b High-performance liquid chromatography (HPLC). ^c Gas chromatography (GC). ^d Ultraviolet-Visible Spectroscopy (UV/Vis). ^eFourier transform infrared spectroscopy (FTIR).

3.ENCAPSULATION

Encapsulation (Figure 2), which provides a protective peel barrier to functional ingredients with numerous advantages, is an effective preservation method. Encapsulation of functional ingredients can be achieved using various techniques. This new technology ensures the resilience of these functional constituents and enables their incorporation into diverse food matrices. [52, 53, 18].

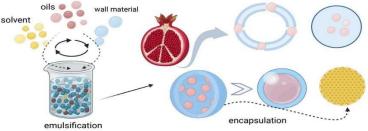


Figure 2. Emulsion preparation and encapsulation technology

Micro- and nanoemulsions are the most scientifically investigated encapsulation technologies due to their simplicity of usage and cheap production costs. They are characterized as a nano-sized dispersion of a liquid phase in another undissolved phase, with 10⁻⁶ micrometers and 10⁻⁹ nanometers. Despite the differences in terminology, both micro- and nanoemulsion oil droplets are nanometric in size. This is because microemulsions are thermodynamically stable, whereas nanoemulsions are kinetically stable. Another reason is the low surface tension during microencapsulation. Nanoencapsulation has a lower free energy; however, micro- and nanoemulsions do not differ qualitatively in composition from the aqueous phase. Encapsulation studies consist of an oily phase that includes an active oil component, a surfactant, and

sometimes a cosurfactant, alone or in combination with other oils. The most widely used surfactants nowadays are sucrose esters, sorbitan fatty acid esters, glycerol fatty acid esters, polyoxyethylene sorbitan fatty acid esters, and polyoxyethylene ether surfactants. Surfactants like Tween 80 and Tween 20 are widely employed in developing oil-based colloidal systems. Natural proteins and polysaccharides are preferred in this regard. Saponins and rhamnolipids are natural surfactants that are used in research. Cosurfactants are commonly used in microemulsion formulations, with short- to medium-chain alcohols being the most widely used. Other short- or medium-chain alcohols utilized in comparable studies include glycerol, propylene glycol, polyethylene glycol derivatives, and sorbitol [54].

Research on pomegranate seed oil found 16 fatty acids, and after 30 days, whey protein isolate produced the best linoleic, oleic, punicic, α -eleostearic, catalpic, and β -eleostearic acid formulations. However, the encapsulation process did not affect the oil composition. The oil encapsulated with modified starch affected the formation of more volatile constituents than other oils [55]. Alginate concentration significantly affected the microspheres' oil loading, size, shape, and strength. The optimal process parameters for microencapsulating pomegranate seed oil were 10% oil loading, 350 μ nozzle diameter, and 3.23% alginate concentration. Encapsulation of pomegranate seed oil in calcium alginate may have advantageous effects on cosmetic, medicinal, and culinary applications. Furthermore, alginate content, oil loading, and feed size all substantially affected the microspheres' size, shape, and strength [56]. In research on pomegranate seed oil with a smaller emulsion droplet size, there was a greater degree of encapsulation, and different wall materials and their compositions affected particle size. In addition, the density of particles and the level of porosity have a direct effect on the stability of the encapsulation against oxidation. The relationship between the volume of interstitial air and liquid content and particle density is directly proportional [57].

A functional microcapsule containing ω -5 fatty acids and necessary amino acids was created by covering it with pomegranate seed protein isolate. The microcapsules were formed by loading different coating materials, such as pomegranate seed oil [58], gum arabic, gelatin, and pomegranate seed protein isolate, followed by spray drying. In addition, the microencapsulation efficiency was determined to be low, while the pomegranate seed protein isolate exhibited the highest hydrophilicity. The combination of gum arabic and pomegranate seed protein isolate led to the formation of microcapsules with highly desirable morphologies. All microcapsules exhibited heterogeneous particle size distributions. All microcapsules were analyzed using x-ray diffraction, Fourier transform infrared spectroscopy, and nuclear magnetic resonance. The thermal investigation found a deformation temperature of greater than 250 °C. Gum arabic and pomegranate seed protein isolates outperformed gelatin in reducing the negative effects of temperature on oxidation [59].

The pomegranate seed oil contains conjugated linolenic acids and can be oxidatively damaged due to its unsaturated fatty acids. In addition, these oils have nutritional value and numerous functional properties. In this investigation, microencapsulation effectiveness varied from 93.3 to 96.6%, making it appropriate for spray drying and suggesting that most oils were adequately encapsulated. Particle density and porosity are essential physical parameters influencing food powder stability during storage. Furthermore, the lack of fractures in the microcapsules is important since it minimizes permeability and protects the core components. Spray drying had no detrimental impact on peroxide concentrations. During storage at 25° C, all samples showed an increase in peroxide content, and the trend continued at 60°C. However, oxidation intensity was higher than in treatments maintained at $25 \,^{\circ}$ C [60].

Nanocapsules, nanoemulsions, micelles, and nanogels allow insecticides to outperform traditional pesticides while causing minimum harm to the environment. The active ingredients of the nanocapsules were protected. Stimulus-responsive nanocapsules control pesticide release by responding to pH, temperature, light, enzymes, or oxidative reactions [61]. Moreover, many oils have been incorporated into nanoemulsions, increasing the utility of natural substances [62]. Pullulan-nano-based pomegranate seed oil was also developed [63]. In research using pomegranate seed oil, loaded nanomaterials with an average diameter of 327 nm and 97.6% encapsulation effectiveness were created. The fish fillets and cheese samples covered with nanomats had reduced lipid oxidation than the control [64]. Nanotechnology is one of the most

promising methods for evaluating the bioactive components of byproducts [65].

Microwave extraction yielded an extract that contained O-H bonds, the most abundant phenolic compounds. The type and amount of extractive compounds in the extract also had a big effect on how well the nanocapsules worked and how the particles' sizes were spread out on average. Furthermore, essential oilloaded nanoparticles exhibit decreased volatility, chemical inertness, sustained release, and storage stability [66].

Microwave pretreatment increased the water absorption capacity, texture, and rheological characteristics of ovalbumin-inulin-carrageenan-pomegranate seed oil emulsion gel. Pomegranate oil's oxidation was shown to be inhibited [67]. Gellan gum [68], propolis [69], geleol [70], chitosan-capric acid [71], maltodextrin, gum arabic [72], whey protein isolate [73], and alpha-tocopherol [74] were used in this study. Whey protein isolates microgels, and whey protein isolates in their original form, gum arabic, maltodextrin, modified starch, and whey protein isolate mixtures were all employed as encapsulation barriers [57].

The wall material and temperature strongly influence the encapsulation effect. Thus, air, temperature, and light all contribute to oil oxidation, which reduces its consistency and nutritional value. However, as the density of many small particles increases, round or irregular particles become less dense than flat particles. The peroxide concentration of bulk and encapsulated oils increased with increasing storage time. Thus, its value rises at high temperatures or over time. Understanding the impact of hygroscopicity on encapsulated powders and food shelf life is crucial. Water intake promotes the oxidation of fats and lipids, increasing the fluidity of the powder, which is damaging to food. During drying, higher temperatures result in a higher moisture content because the powder particles remain inside for a shorter time. The particle size was determined by the drying method, wall material, and core/wall material ratio [56].

It has been reported that the encapsulation process reduces the formation of trienes and dienes and protects oils from oxidation [50]. Organic solvents should be extracted using microwave-assisted and ultrasonic-assisted extraction methods. Additionally, the equipment used for this extraction poses challenges for large-scale industrial production applications. Subcritical extraction has greater application potential in oil extraction than other methods [30]. Complexation or encapsulation of pomegranate extracts with metal nanoparticles or biopolymers has led to the development of food supplements. In the studies conducted thus far, hydrodynamic cavitation has not yet been used for pomegranate fruit. However, these methods are important because of their relatively high extraction rates of bioactive compounds [75]. Plant and fruit extracts are also used in the cosmetic industry via electrohydrodynamic processes [76]. Integrating essential oils into natural polymer packaging is considered an innovative method for food preservation [17]. Some studies on this topic are presented in Table 3.

Tuble 5: Extraction studies summarised than encupsulation methods					
Extraction Methods	Analysis Equipment	Identified Components	Solvents	Methods and Wall Materials	References
Ultrasound extraction with ethanol	GC ^c , SEM ^f , Attenuated total reflectance-FTIR ^e , DCS ^g , UV-Vis ^d	Punicic acid, oleic acid, linoleic acid, palmitic acid, stearic acid	Hexane, ethyl acetate, ethanol, isopropanol	Microencapsulation; Spray drying, succinylated taro starch	[77]
Soxhlet extraction		Oils		Microencapsulation; freeze drying, spray drying, maltodextrin, whey protein	[78]

Table 3. Extraction studies summarised than encapsulation methods

Table 3. Continue on					
Ultrasound extraction	UV/Vis ^d , Aluminum chloride colorimetric method, HPLC ^b , FID ^h , electron microscopy	tocochromanol, sterols, phenolics, flavonoids, β - sitosterol, campesterol, lutein, gallic acid, polyunsaturated fatty acids	Petroleum ether, <i>n</i> -Hexane, methanol, acetonitrile, tributyl-methyl ether, toluene	Encapsulation Maltodextrin, gum arabic	[33]
Solvent extraction	UV–Vis ^d	Total phenolic compounds $349,52 \pm 0,01 \text{ mg GAE/g}$	Ethanol, deionized water	Nanocapsulation; gelation technique, calcium chloride, sodium alginate	[79]
Solid-liquid extraction	FID ^h , HPLC ^b , SEM ^f	Fatty acids composition, phenolic compounds catechin, protocatechuic, rutin, <i>p</i> -coumaric	Tween80, acetonitrile, methanol chloroform, Ethanol	Encapsulation in double emulsions (direct, membrane)	[80]
Pulsed ultrasound- assisted extraction, aqueous extract analysis	UV–Vis ^d , HPLC ^b	Malic acid, cinnamic acids, cinnamic acids (<i>γ</i> -terpinene), succinic acid, vitamin C	Water	Microencapsulation; spray-dried, pectin	[43]
Soxhlet Extraction	SEM ^f , FT-IR ^e , DSC ^g , FID ^h	polyphenols	Deep eutectic solvent	Microencapsulation; complex coacervation, chitosan and zein	[81]

^fScanning electron microscopy (SEM), ^gDifferential scanning calorimetry (DSC), ^hFlame ionization detector (FID

4. ENCAPSULATION METHODS

4.1 Coacervation

Coacervation microparticles were made from highly soluble wall materials, but the coacervation procedure and a hydrophobic core lowered their solubility. The high temperature most likely caused dehydration of the microparticles, which raised stress on their structure and hastened fracture and pore development. This is visible in the increased concentration of punicic acid, α - and β -eleostearic and catalpic acids, which are also isomerized. The chemical classes include esters, aldehydes, alcohols, ketones, carboxylic acids, ethers, and hydrocarbons. As shown by the buildup of aldehydes, lipid oxidation has increased. These structural changes, which included a loss of gamma-tocopherol and total conjugated linolenic acid, in addition to increased hygroscopicity of wall components, didn't enhance lipid oxidation [6,82].

Pomegranate rind extract and pomegranate seed oil microencapsulation research have focused on preserving and delivering active components. Pomegranate arils and seeds have been shown in studies to have technologically favorable benefits on several dishes, including meat, baked items, and dairy products [5]. An ionic gelation technique was developed in this study. This results in adsorption-generated pectin-starch

beads with higher microstructural stability and slower release rates [83]. Natural botanical conservation, which extends the shelf life of products and has unique biochemical qualities, is increasingly used in many sectors, including food, cosmetics, and pharmaceuticals.

4.2 Spray-Drying

Microencapsulating pomegranate seed oil from Mexican crops involved spray drying succinylated taro starch and β -cyclodextrin. The technique with the highest encapsulation efficiency for pomegranate seed oil was favored using a central composite design. These oil-bearing microparticles, prepared with 15% feed solids at an inlet air temperature of 190°C, exhibited little water solubility, water activity, or hygroscopicity, enabling their processing, storage, and incorporation into essentially oil-phase food matrices. Nevertheless, the experiment yielded poor encapsulation and a high concentration of surface oil. Furthermore, the materials can be spray-dried for a reduced duration without compromising the quality of the oil, resulting in yields ranging from 23.1% to 48.5%. The effect of the β -cyclodextrin-taro starch microparticles loaded with pomegranate seed oil ranged from 22.81% to 61.09%. Microparticle solubility regulates the type of food matrix into which they can be put, whereas hydrophobicity affects storage conditions by influencing lipid oxidation. The hydrophobicity of taro starch stops it from becoming more soluble and water-attracting, which is good for using and storing microparticles. Furthermore, pomegranate seed oil-loaded microbeads are better suited to low-water or high-oil food matrixes, and pomegranate seed oil supplementation enhances microbead structural disorder [77].

Pomegranate seed oil is encapsulated using the most common spray-drying technology to retain its bioactive constituents and lengthen its shelf life, as it contains conjugated fatty acids prone to degradation. This study used whey protein only at a 1:4 weight ratio with maltodextrin. Input emulsion amount, drop size, encapsulation efficiency, moisture content, bulk density, particle morphologies, size, hygroscopicity, and solubilizing power were all measured. The spray drying procedures were as follows: intake temperature 125-150 °C, output temperature 60-67 °C, airflow 40-42 m³/min, feed rate 5.2g/m, and pump speed of 40%. As a result, the particles were spherical, with notches on their surfaces. Following 15 days of oxidation stability at 60 °C (8 h/day), 35% of the total solids showed a decreased size of droplets. Using only whey protein resulted in better encapsulation and oxidative stability than using a mixture of whey protein and maltodextrin as a wall material [78].

Spray drying was used to microencapsulate natural and enhanced extracts of pomegranate (*P. Granatum*) in the epidermis, which are rich in polyphenols such as punicalagin and ellagic acid. The microparticles made with biopolymer-based gum arabic, pectin, and modified chitosan bore were usually between 2.55 and 6.86 μ m in size. The gum arabic-based carriers had the quickest release. The Korsmeyer-Peppas technique was used for the experimental release profiles, and pectin-based microparticles demonstrated the slowest release profile. Cancer cells' vitality was somewhat reduced when coated [84].

Albumin had the least bioactivity retention among the wall materials used in the encapsulation process, while the combination of maltodextrin and pure gum arabic provided better bioactivity retention. However, the freeze-drying technique produces capsules with higher hygroscopicity than the spray-drying technique [85]. The encapsulation technique, used as a micro- and nanostructure for pomegranate seed oil, conserved the bioactive constituent oleoresin and reduced mycotoxins. Accordingly, applying this oil to fungal media reduced aflatoxin and zearalenone levels by up to 63% and 78.5%, respectively. The method described in this study enabled the incorporation of oleoresin into this oil, which protected against aflatoxin-induced hepatotoxicity and had no cytotoxic effect on an isolated murine hepatocyte cell line [33].

An instant caffè latte drink containing pomegranate seed oil microparticles (30%) was formulated, the microparticles were applied, and their physical properties were evaluated. Depletion of γ -tocopherol led to a reduction in conjugated linolenic acid concentration compared to complicated coacervation spray drying. Direct spray drying of pomegranate seed oil emulsions was a more efficient, dynamic, and cost-effective encapsulating approach for producing more oxidation-resistant particles. Coacervation somewhat raised the moisture content. However, homogenization is critical in this procedure [6,82].

A spray-drying microdispersion approach with pectins as a polymeric matrix was utilized to stabilize the overall polyphenolic content to create water-soluble bioactive molecules. Consequently, powders were encapsulated (approximately 50%) while retaining their functional qualities. In addition, the extraction of water-soluble bioactive compounds from these byproducts was studied utilizing pulsed ultrasound-assisted extraction with just water and spray-drying microdispersion with low-methoxyl pectin [43].

4.3 Emulsification

A high-energy emulsification approach was used to create an oil/water emulsion and a combination of ultrasonication and homogenization processes. This study combined chitosan, sodium alginate, whey protein concentrate, and maltodextrin wall components with seed oil and distilled water. The mixture was ultrasonically processed for 3 minutes before the pH of the emulsion was corrected using sodium hydroxide and hydrochloric acid. The emulsion was placed in an ultrasonic bath for six minutes to obtain a homogeneous mixture. All microencapsulated emulsions were lyophilized and kept at -18 °C. Equation 1 was used for calculating microencapsulation efficiency:

Encapsulation efficiency (%) =
$$\frac{Total \ oil - Surface \ oil}{Total \ oil} x \ 100$$
 (1)

The microencapsulation procedure produced excellent yield, low release, good moisture, and stability of balanced (*Lallemantia royleana*) seed oil extracted by ultrasonic incubation in polysaccharide and protein matrices. Emulsions were found to have good stability and purity. Under harsh conditions, this technique effectively preserves the produced oil. They can also be utilized extensively in water-based food items. The structure of the optimal sample indicates that the emulsification and microencapsulation operations were successful [86].

In a study where pomegranate seed oil was encapsulated using conventional and Pickering emulsion methods, low-viscosity emulsions were obtained, and capsules with a droplet size of $1.39-2.55 \mu m$ were formed by spray drying. The emulsions obtained in this study formed particles with encapsulation efficiencies between 56.28 and 73.83% and between 28.07 and 93.99%. The encapsulated oil provided better oxidative protection than the non-encapsulated oil due to its smaller particle sizes (ranging from 9.86 to 22.60 μm). However, experts believe that a combination of whey protein isolate, modified starch, and the non-degradability of pomegranate seed oil would make the ideal wall materials for future food industry applications. They can be spheroidal or oblong, depending on the heating conditions and pH [57].

Several researchers have successfully encapsulated and stabilized Pickering and conventional emulsions via emulsification with pomegranate seed oil. Furthermore, recent research has revealed that Pickering emulsions are more stable than traditional emulsions and that both emulsions have appropriate qualities for stabilizing oil-water systems. Their high stabilities facilitate the ability of emulsions to dry and be used in food products. Spray-dried emulsions generated powders with useable and storable physicochemical features, such as excellent encapsulation efficiency, yield, and resistance to oil oxidation [55].

4.4 Extrusion

Extrusion techniques include hot melt, melt injection, co-extrusion, and electrostatic extrusion. Encapsulating more bioactive compounds aids in reducing particle size. Thus, reducing the particle size of the extrudate promotes an increase in the shelf life of the encapsulated bioactive substance, as well as a controlled release at the desired spot in the body. Furthermore, this technique preferentially uses wall materials rather than spray-drying [87]. Conventional extraction techniques are often unfavorable owing to their energy-intensive and time-consuming nature. They use organic solvents that harm the environment and human health. In contrast, new technologies have been identified as more advantageous than traditional methods. The majority of conjugated linoleic acid is obtained from pomegranate seeds [88]. It is utilized as a functional component in foods and drinks, a fat replacement, and a preservative in food packaging [31]. Phenolic constituents play an insignificant role in sustaining oil quality. Additionally, the kind of antioxidant utilized had no significant effect on the quantity of punicic acid, the oil's primary fatty acid, or the radical

scavenging capacity of pomegranate seed oil. According to research, pomegranate rind extract can help stabilize pomegranate seed oil [4]. Cold-pressed pomegranate seed oil extract contains bioactive compounds that can be antioxidants [89]. Encapsulated oil liposomes preserve the oil's *ant*ioxidant and *anti*-inflammatory effects. Punicic acid also offers a regulated release mechanism [44]

Microencapsulated bioactive pomegranate extracts appear to be useful in enhancing plant biological activity. Encapsulation is an effective approach for improving the biological activities of bioactive substances, enhancing their bioavailability and stability, and overcoming application limitations [60].

5. CONCLUSIONS

Pomegranate seed oil is an oil with a wealth of bioactive compounds that have nutritive, antioxidant, and anti-mycotoxigenic activities. It also has many health benefits due to the presence of bioactive compounds. However, due to the high concentration of unsaturated fatty acids, it is subjected to very rapid oxidative degradation. Microencapsulation can be an excellent alternative technique to modify the reactivity, stability, sensitivity, and photosensitivity of these natural compounds. The benefits of pomegranate oil, a non-traditional type of oil, have been confirmed by scientific studies to date. This research aims to summarize recent research, analytical and extraction techniques used to recover bioactive constituents, especially punicic acid, from pomegranate seed oil, the waste product of pomegranate fruit, as well as a literature review related to the analytical and extraction techniques used.

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