



**EXPLORING BIOACTIVE COMPOUNDS, NATURAL ANTIOXIDANTS, AND EXTRACTION TECHNIQUES FROM WATERMELON (*CITRULLUS LANATUS*) FOR HEALTH AND FOOD APPLICATIONS**

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**ABSTRACT**

Watermelon (*Citrullus lanatus*) is a globally cherished fruit celebrated for its succulent sweetness. This article delves into the bioactive potential of watermelon, spotlighting its antioxidant-rich seeds, rind, and skin byproducts. Researchers are increasingly exploring the extraction of natural antioxidants from these byproducts due to their therapeutic potential. Watermelon's vibrant color and robust nutritional profile are attributed to compounds such as lycopene, carotenoids, phenolic compounds, and flavonoids, which act as crucial defenders against oxidative stress and its implications in various diseases. Various extraction methods are discussed, with ultrasound-assisted extraction (UAE) standing out for its efficient cavitation-driven mechanism. The pivotal role of phenolic compounds, particularly flavonoids, in plant antioxidant defense systems is underscored, exploring the distinct contributions of flavonoid and non-flavonoid phenolic compounds to plant health and coloration. Carotenoids like  $\beta$ -carotene and lycopene not only lend watermelon its vivid hues but also offer considerable health benefits. Techniques for evaluating antioxidant capacity, such as the DPPH assay, are explored, along with the application of bioactive natural compounds to enhance the stability of plant-based oils, addressing oxidation-related quality issues. The article also illuminates the potential anti-inflammatory and anti-diabetic properties of cucurbitacins, oxygenated steroidal triterpenes found in watermelon. Various extraction techniques, including maceration, infusion, percolation, and decoction, are briefly explored. In essence, this study highlights the significance of bioactive compounds in promoting human health and improving food quality, contributing to the harnessing of natural compounds from watermelon for health and food applications.

**1. Introduction**

Watermelon, scientifically known as *Citrullus lanatus*, belongs to the Cucurbitaceae family. Its name is derived from the Greek word "citrus," referring to the fruit, and the Latin term "lanatus," owing to the tiny hairs on its stems and leaves, giving it a woolly appearance (Mathew, *et al.*, 2014). Where about 117 million tonnes of watermelon were produced last year (FAOSTAT, 2016). Due to its sweet and refreshingly juicy flavor, it is consumed all over the world including in Asian countries (Mushtag, *et al.*, 2015).

Watermelon contains approximately 92% water and a significant number of byproducts, including seed, skin, and rind; these products may cause environmental hazards if not handled properly (Saeid, *et al.*, 2016). These byproducts are rich in bioactive compounds. Watermelon seeds have powerful antioxidant and radical-scavenging properties and phenolic compounds which showed health benefits against various diseases including cancer, inflammation, viral infections, and skin flakes (Manivannan, *et al.*, 2020; Yi, *et al.*, 2013).

Other beneficial properties such as rancidity inhibition and lipid oxidation are also associated with these byproducts (Prochazkova, *et al.*, 2011). Due to the potential for therapeutic and other beneficent implementation of such natural antioxidants and bioactive compounds, such as the development of functional foods, the extraction, and use of these health-promoting compounds from watermelon byproducts are an important area of research. (Chua, *et al.*, 2019). The watermelon fruit is a source of multiple minerals, vitamins, and proteins that are present in the skin, pulp and seeds (Zayed, *et al.*, 2021). Watermelon, with cucumbers, squashes, luffas, and melons, is a vital vegetable

crop in the cucurbit family (Zhang, *et al.*, 2020) *Citrullus lanatus* rind is an abundant source of natural antioxidants, lycopene, citrulline, and numerous polyphenols. Watermelon skin has a peppery and astringent flavor and calming impact. Watermelon skins along with seeds and rinds are also used as livestock feed (Dranca, *et al.*, 2016).

Watermelon fruit is divided into three major components including flesh, seed, and rind (Fig. 3). Flesh, also called pulp, rind, and seeds account for 68%, 30%, and 2% of the total biomass, respectively (Dietrich, *et al.*, 2016; Jawad, *et al.*, 2018).



Figure 1. Watermelon

WATERMELON <i>Calories</i>	
<b>Nutrition Facts</b>	
<i>Watermelon (1 cup - 154g)</i>	
Total calories 46	Fat 2g
Monounsaturated Fat 0.1g	Polyunsaturated Fat 0.1g
Carbohydrates 116g	Sugar 9.5g
Dietary Fiber 0.6g	
Vitamin A 18%	Vitamin C 21%
Lycopene 8%	

Figure 2. Total Calories in Watermelon

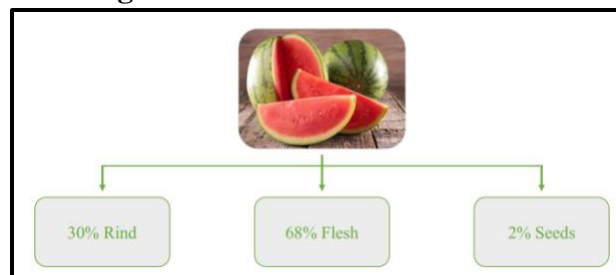
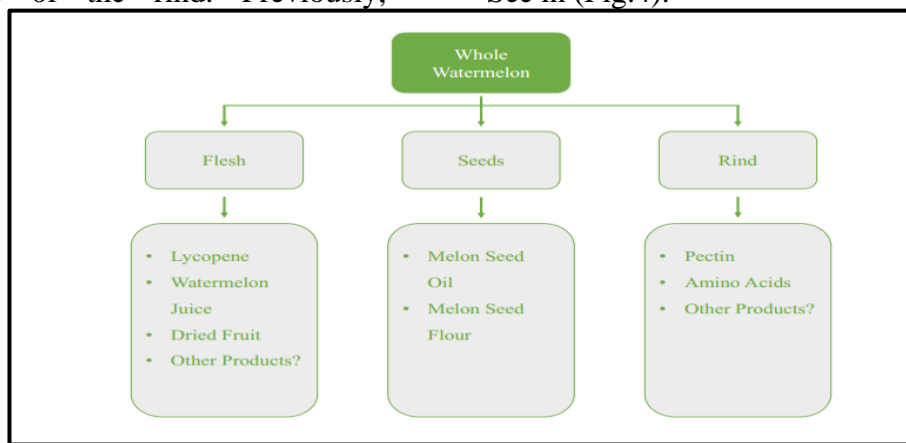


Figure 3. Breakdown of watermelon biomass

## 1.2. Bio refinery for watermelon

Almost all of the components of the watermelon can be used to make different valuable products, thus bio refinery of watermelon would be feasible. Previous authors have documented various benefits associated with the techniques for watermelon biorefinery (Perkins-Veazie, *et al.*, 2002). It is a fact that 1/3<sup>rd</sup> biomass of watermelon weight belongs to its rind, therefore, it can be as used for biomass refinery. This is the reason why rind gets the main focus. The foremost function of the bio refinery is the extraction of pectin which is a major constitute of the rind. Previously,

(Montesano, *et al.*, 2018), reported that this fruit is very useful for the extraction of pectin. However, the extraction of pectin from watermelon rind also gets much attention (Petkowicz, *et al.*, 2017; Montesano, *et al.*, 2018). Moreover, after the extraction of juice, the waste is also used for that purpose. To make the bio refinery concept more valuable in watermelon crops, considering value-added products for each of its components is essential. Numerous value-added products that can be derived from the harvest of watermelons are discussed previously (Montesano, *et al.*, 2018). See in (Fig.4).



**Figure 4.** Overview of watermelon bio refinery concept

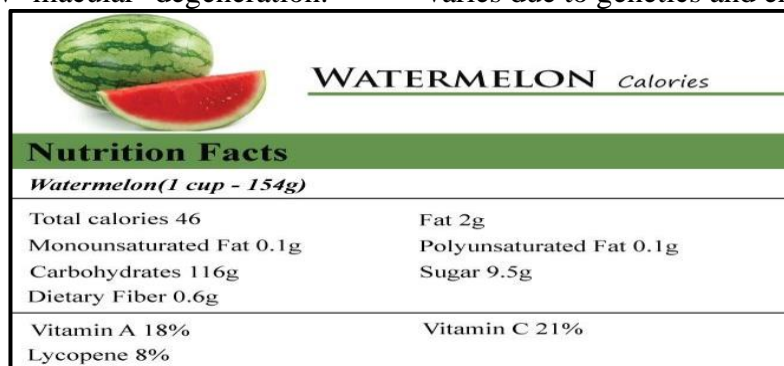
The flesh of watermelons can be produced to array of products with added value. Up to 60% of lycopene, also known as carotenoid with strong antioxidants, is present in watermelon flesh followed by typical tomato flesh (39%). Lycopene has numerous health benefits. It is commonly used as a nutraceuticals. (Montesano, *et al.*, 2018). As mentioned above, the market for watermelon flesh is expanding; flesh juice would be a new addition in this respect. Numerous studies have evaluated the viability of pasteurizing and commercializing watermelon juice, without extracting seeds (Montesano, *et al.*, 2018).

Even though watermelons with seeds continue to dominate the market. But, still, it is important to look into the watermelon's seed content. A number of studies on the viability of seed reported the manufacturing of flour for protein powders and oil production and stated that the biorefinery concept can be used to utilize the seed component for high return.

Proteins from watermelon seeds would be a beneficial addition to the majority of protein sources other than cereals (Mahindrakar, *et al.*, 2020; Montesano, *et al.*, 2018). Watermelon seeds are frequently used on a modest scale in many countries as a source of oil and protein. The flour of watermelon seeds contains an excellent amount of soluble protein it can be used in food products that are protein ideal. Moreover, it can also be used for the stabilization of the colloidal food system. Studies also stated that the extracted protein from watermelon can be used for cowpeas as a protein supplement as well. The protein assimilation of a blend of cowpea and watermelon proteins is about 80%, somehow lower than the lysine accessibility of watermelon flour which is 93%. Lycopene, a potent antioxidant found in tomatoes watermelon and other sources, has demonstrated efficacy against diseases like -

efficacy against diseases like cancer and cardiovascular issues. It's used as a food additive, supplement, and natural food coloring. However, it degrades due to factors like temperature and light during processing and storage. Carotene, an important antioxidant and precursor to vitamin A, also has benefits for skin health and may slow macular degeneration.

Challenges with its use in food include instability and poor solubility. Nanoencapsulation improves solubility. Xanthophylls like zeaxanthin and lutein aid ocular health. Red-fleshed watermelons are rich in lycopene, especially cis-isomers which are easily absorbed. Watermelon's lycopene content varies due to genetics and environment.



**WATERMELON** *Calories*

**Nutrition Facts**

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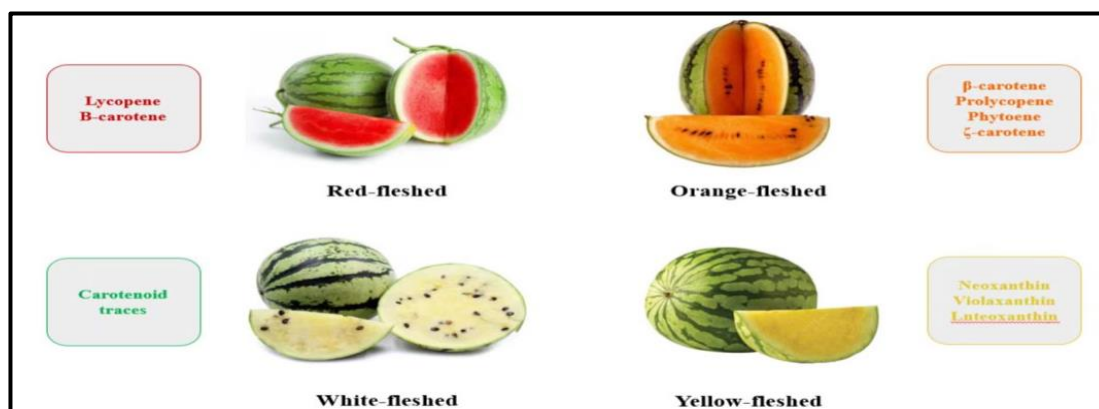


Figure 6. Main carotenoids in different color watermelons

Table 1. Studied carotenoid distribution in various watermelon cultivars, focusing on lycopene isomers and β-carotene

	Variety cultivar		Carotenoids	Amount mg/kg Fresh	References
	Xite (Hazera 6007)	Fruit pulp	Lycopene (90% all <i>trans</i> - and 10% <i>cis</i> isomers)	98.84	Kim (2014)
	Hazera 5109	Fruit pulp	Cumulative lycopene	96.01	Naz (2013)
	Extazy (HZ6008)	Fruit pulp	Cumulative lycopene	91.92	Naz (2006)
	Crimson sweet	Pulp	Cumulative lycopene	45.50	Fiedor (2014)

	Variety cultivar		Carotenoids	Amount mg/kg Fresh	References
	Giza	Pulp	Cumulative lycopene	61.98	Fiedor (2014)
	Dumara	Pulp	Cumulative lycopene	45.90	Naz (2013)
	P403	Pulp	Cumulative lycopene	45.04	Fiedor (2014)
	P503	Pulp	Cumulative lycopene	63.95	Fiedor (2014)
	PWM25-4	Matured fruits	Cumulative lycopene	72.90	Naz (2013)
	Kiran	Matured fruits	Cumulative lycopene	75.95	Naz (2013)
	Kareena	Matured fruits	Cumulative lycopene	79.43	Nazet (2013)
	Zaohua (red-ripe)	Pulp	Cumulative lycopene	36.90	Fiedor (2014)
	var. Ole ´	Pulp	Provitamin A	9.40	Fiedor (2014)
	Minipool	Flesh	Provitamin A	8.90	Naz (2006)
	Mielhart (Hazera 5133)	Flesh	Provitamin A	9.98	Fiedor (2014)
	–	Juice	Provitamin A	0.25	Naz (2013)
	–	Rind	Provitamin A	0.65	Naz (2013)
<i>Solanum lycopersicum</i>		Consumable portion	Cumulative lycopene	103.97	Suwanaruang (2016)
		Raw fruit	Provitamin A	4.3	Kim (2014)
Papaya		Consumable portion	Total lycopene	46.02	Naz (2013)
Psidium guajava, Horana Red variety		Fruit	all <i>trans</i> -lycopene	42.99	Kim (2014)
Carrot	var. Florida F1	Consumable portion	Provitamin A	264.08	Kim (2014)
<i>Carrot</i>	var. Nevis F1	Consumable portion	Provitamin A	244.98	Kim (2014) ´

**Table 2.** Research on carotenoids from various *Citrullus lanatus* sources highlights their diverse health benefits, including antioxidant, antiproliferative, cardio-protective, and potential antihyperglycemic properties.

Source	Pigment	Role	Research Findings	Reference
Watermelon fruit	Carotenoid compound	Antioxidant, antiproliferative, cardio-protective, antihyperglycemic effects.	Watermelon: Abundant source of cis-isomeric lycopene	Naz (2013)
Watermelon lycopene extract	Carotenoid compound	Antioxidative and anti-inflammatory	Watermelon lycopene extracts counter free radicals, inhibiting iNOS and COX-2 mRNA expressions and proteins in a dose-dependent manner.	Kim(2014)
Watermelon juice and lycopene extract	Carotenoid compound	Antioxidant	Watermelon juice and lycopene extract exhibited antioxidant activity against free radicals.	Naz (2014)
Watermelon fruit	Lycopene, carotenes, and xanthophylls	Antiproliferative	Lycopene and other carotenoid-rich fruits and vegetables form the basis of a healthy diet. watermelon, resulted in protection against prostate cancer	Naz(2014)
Watermelon fruit	Lycopene	Antioxidant	Watermelon is rich in lycopene, exhibiting potent antioxidant properties in vitro. However, a three-week supplementation study on middle-aged adults found no impact on antioxidant or cholesterol levels.	Pinto(2011)
Watermelon juice	Lycopene	ND		Collins(2004)
Watermelon juice	Lycopene	Antioxidant, antidiabetic	Watermelon's lycopene-rich content showed strong antioxidant and antidiabetic properties through $\beta$ -carotene bleaching and inhibition of $\alpha$ -amylase and lipase.	Naz (2014)

Numerous techniques are being used for watermelon rind to create products with added value. Although the rind alone can be used to extract juice, no known commercial uses have been found (Müller, *et al.*, 2014; Medeiros, *et al.*, 2019). Recently, USDA ARS started to create a patent that is used to obtain the rind

citrulline, an amino acid that helps remove nitrogen from the blood and convert it into urine (Montesano *et al.*, 2018). Other studies have also stated the use of rind as a component in cheese and pickles (Chen *et al.*, 2015; Montesano *et al.*, 2018).

**Table 3.** Mean values of Seeded, Seedless, Personal, Excursion, Fascination, Exclamation, and Captivation Varieties

Sample	Excursion	Fascination	Exclamation	Captivation	Seedless	Seeded	Personal
Melon	4.78a	4.00 <sup>a</sup>	4.24 <sup>a</sup>	4.85a	4.30 <sup>a</sup>	4.16 <sup>a</sup>	4.62 <sup>a</sup>
Fresh	4.85a	4.35 <sup>a</sup>	4.67 <sup>a</sup>	5.00 <sup>a</sup>	4.55 <sup>a</sup>	4.43 <sup>a</sup>	4.60 <sup>a</sup>
Green*	3.52 <sup>b</sup>	3.3 <sup>a,b</sup>	3.3a, <sup>b</sup>	3.41a, <sup>b</sup>	2.82a, <sup>b</sup>	2.9 <sup>a,b</sup>	2.37 <sup>a</sup>
Ripe	3.90 <sup>a</sup>	4.26 <sup>a</sup>	4.21 <sup>a</sup>	3.95 <sup>a</sup>	3.87 <sup>a</sup>	3.76 <sup>a</sup>	5.00 <sup>a</sup>
Seedy	3.17a, <sup>b</sup>	3.67 <sup>b</sup>	3.3a, <sup>b</sup>	2.9 <sup>a,b</sup>	2.94 <sup>a,b</sup>	3.2 <sup>a,b</sup>	2.55 <sup>a</sup>
Sweet***	4.76 <sup>b,c</sup>	3.76 <sup>a</sup>	4.26 <sup>a,b</sup>	5.0 <sup>b,c</sup>	4.4a, <sup>b,c</sup>	4. <sup>a,b,c</sup>	5.28 <sup>c</sup>
Sour***	2.72 <sup>c</sup>	2.2 <sup>b,c</sup>	2.14 <sup>b,c</sup>	2.6 <sup>b,c</sup>	2.37 <sup>b,c</sup>	2.0 <sup>a,b</sup>	1.43 <sup>a</sup>
Astringent	2.09 <sup>a</sup>	2.10 <sup>a</sup>	2.22 <sup>a</sup>	2.1 <sup>a</sup>	2.03 <sup>a</sup>	2.05 <sup>a</sup>	2.01 <sup>a</sup>
Refreshing***	6.82 <sup>b,c</sup>	5.44 <sup>a</sup>	6.03 <sup>a,b</sup>	7.21 <sup>c</sup>	6.66 <sup>b,c</sup>	6.0 <sup>a,b</sup>	6.2 <sup>a,b,c</sup>
Wateriness	7.41 <sup>a</sup>	6.75 <sup>a</sup>	6.97 <sup>a</sup>	7.5 <sup>a</sup>	7.06 <sup>a</sup>	7.02 <sup>a</sup>	6.96 <sup>a</sup>
Crispness***	5.82 <sup>c</sup>	4.5a, <sup>b</sup>	5.06a, <sup>b,c</sup>	5.6 <sup>b,c</sup>	5.4 <sup>a,b,c</sup>	4.47 <sup>a</sup>	5.05 <sup>ab,c</sup>
Mealiness*	3.75 <sup>a,b</sup>	5.08 <sup>b</sup>	4.41 <sup>a,b</sup>	3.53 <sup>a</sup>	3.62 <sup>a,b</sup>	4.6 <sup>a,b</sup>	4.36 <sup>a,b</sup>

Distinct letters in the same row indicate significant differences (\*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001).

**1.3. The source & effects of free radicals, as well as benefits of antioxidants to human health**

Free radicals, containing unpaired electrons, can harm cells. Reactive oxygen and nitrogen species (ROS and RNS) also contribute. Overproduction of radicals is linked to tissue damage, affecting lipids, DNA, and proteins. Environmental factors like toxins, radiation, and pollution generate free radicals, causing complications. ROS like oxygen radicals cause cell structure damage, nucleic acid alterations, and lipid peroxidation. Antioxidants counter oxidative damage by neutralizing radicals. Cells use endogenous and exogenous antioxidants for protection. Enzymatic (SOD, catalase) and non-enzymatic (vitamins) defenses exist. Free radical impact on health is illustrated in (Fig7). Watermelon, rich in vitamins and phytochemicals, particularly lycopene, acts as

potent antioxidant that defends against oxidative stress.

Lycopene, the red carotenoid pigment, supports normal metabolism, guarding against cancer and degenerative diseases. With its versatility, watermelon can be enjoyed in various ways – from breakfast to snacks – while offering substantial vitamin C and β-carotene content that enhances its potential to combat cancer through antioxidant prowess (Caillet, *et al.*, 2012).

**1.4. Oxidation of Plant-Based Oils**

Volatile oils (VOs) and fats are essential nutrients for humans, derived from plants or animals. Vegetable oil production is substantial at 207 million metric tonnes globally (2021/22). Volatile oils are obtained from oleaginous seeds and fruits through solvent extraction or mechanical expulsion. Quality is

determined by both sensory and compositional factors, influencing consumer preferences (Fadda, *et al.*, 2007). Off-flavors and odors in

oils arise from triglyceride oxidation or hydrolysis, as depicted below.

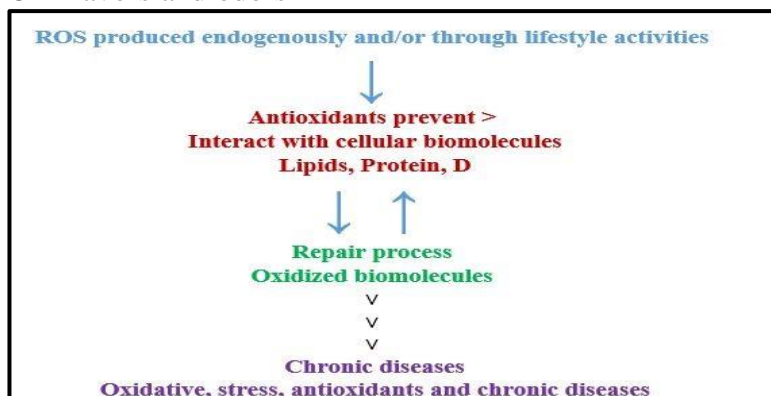


Figure 7. Oxidative Imbalance and its Link to Chronic Conditions

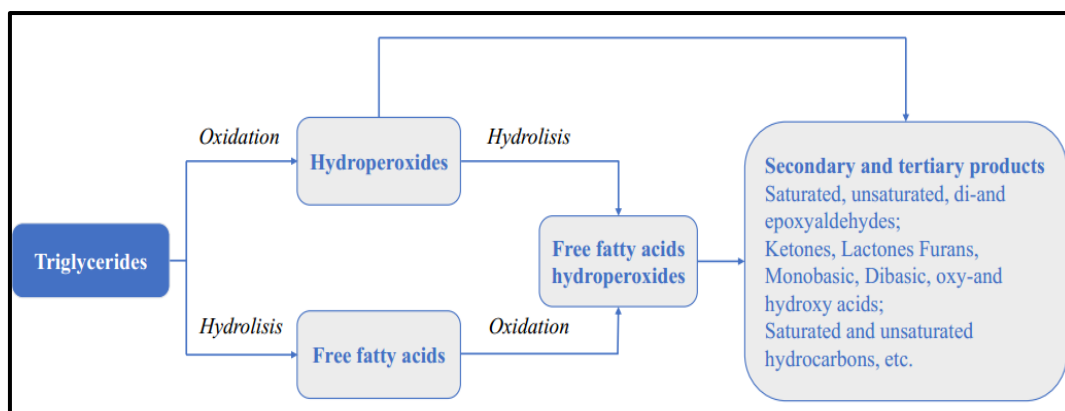


Figure 8. Oxidative and hydrolytic degradation reaction

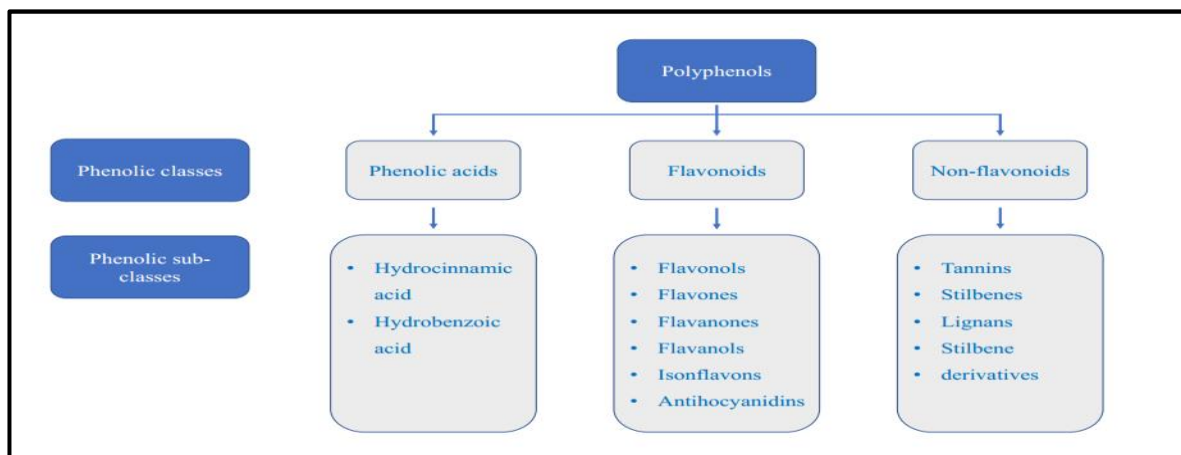


Figure 9. Phenolic classes and sub-classes

### 1.5 Phenolic Substances

Phenolic compounds are prominent in plants, found in various parts like roots, leaves, and fruits. Plants produce these secondary metabolites for protection and growth. Phenolic

compounds are classified based on their subunits, leading to phenolic acids, flavonoids, and non-flavonoids. They serve as antioxidants, scavenging free- radicals and inhibiting their production. Phenolic acids, like hydroxybenzoic



and hydroxycinnamic acids, are vital subcategories. These compounds have bioactive potential and contribute significantly to plant health (Ghasemzadeh, *et al.*, 2011; Azwanida, *et al.*, 2015).

### 1.6 Flavonoid Substances

Flavonoids (pronounced "flavus" in Latin; it signifies "yellow") is derived from the Latin word "flavus," which means "yellow". It imparts color to the flower's shoots, petals, or fruits. They give red or blue color (Sandhar, *et al.*, 2011; Vuolo, *et al.*, 2019). They are physiologically active plant compounds with

potential health benefits (Greenwell, *et al.*, 2015). The antioxidant processes of flavonoids have been investigated through chelation abilities, straight forward neutralization of reactive Oxygen species and inhibition of oxygen species and inhibition of oxidative enzymes. (Karak, *et al.*, 2019). Flavonoids are classified as flavonols, flavones, flavanones, Isoflavones, and anthocyanins based on species, environmental factors, plant tissues, growth circumstances, and level of maturity (Wang, *et al.*, 2018)

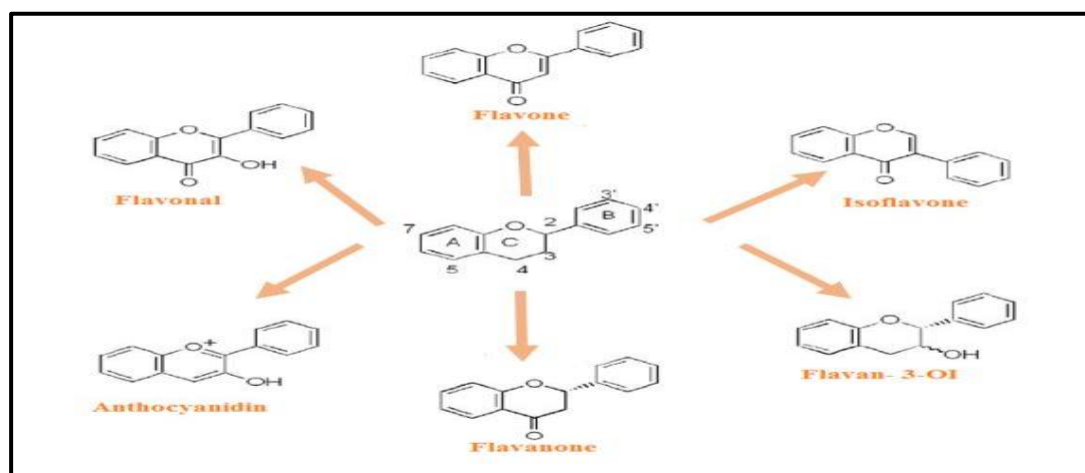


Figure 10. Flavonoid Substances

Table 4. Concentrations of phenolic compounds and flavonoids found in Modified Atmospheric Packaging

Vegetation	Substances	Levels	Reference
Licorice	Phenolics & terpenoids	4.94±0.42 g/100 g	Karak (2019)
Indian Snakeroot	Alkaloids	2.06 ± 0.13 g/100 g	Karak (2019)
Bloody Cranesbill	Catechins and proanthocyanidines	2.02 mg/kg	Naz (2014)
Moldavian Balm	Rosmarinic acid	245.92 ± 24.67 mg/g	
Moldavian Balm	Chlorogenic acid	1.39 ± 2.68 mg/g	Naz (2014)
Moldavian Balm	Pigenin-7-O-glucoside	6.72 ± 2.30 mg/g	
<i>Ficus microcarpa</i> L. fil	Protocatechuic acid	6.80 ± 0.10 mg/g dry extract	

-	Catechol P-vinylguaiacol	10.91 ± 0.01mg/g dry extract 3.90 ± 0.06 mg/g dry extract	Karak (2019)
-	Vanillin	4.27 ± 0.02 mg/g extract	
-	Syringaldehyde	8.96 ± 0.29 mg/g extract	
<i>Hibiscus cannabinus</i> L.	Flavonoid content	82.11 mg/g extract	Patel (2010)
<i>Trigonella arabica</i> Delile.	Tannin content	2 ± 0.47 mg TA/g	Jaradat(2010)
<i>Trigonella berythea</i> Boiss. & Blanche		9 ± 0.47 mg TA/g	Jaradat (2016)
<i>Origanum vulgare</i> L.ssp. hirtum (Link)	Rosmarinic acid Carvacrol	116.7 grams per kilogram of dried extract 94.6 ± 21.16 grams per kilogram of dried extract	Karak (2019)
<i>Origanum vulgare</i> L.	Rosmarinic acid	12.88 mg/g plant	
	Chlorogenic acid Hyperoside	2.10 milligrams per gram of plant 1.05 milligrams per gram of dried extract	Karak (2019)
	Isoquercitrin	0.69 mg/g dry extract	
<i>Satureja thymbra</i> L.	Salvianolic acid A Cafeic acid	66.4 grams per kilogram of dried extract (± 1.7 g/kg) 2.69 grams per kilogram of dried extract (± 0.1 g/kg)	Karak (2019)
<i>Thymus capitatus</i> (L.) Hoffm.	Taxifolin Eriodictyo	4.28 grams per kilogram of dried extract (± 0.03 g/kg) 2.36 grams per kilogram of dried extract (± 0.12 g/kg).	Oreopoulou (2019)

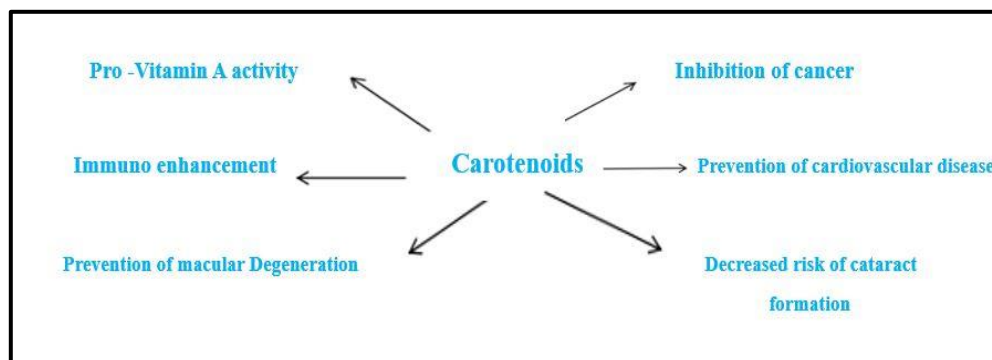


Figure 11. Non-Flavonoid Phenolic Substances

### 1.7 Non-Flavonoid Phenolic Substances

Tannins, also known as tannic acid found in numerous plants. They are non-phenolics. (Vuolo, *et al.*, 2019). Tannins are frequently divided into two chemical categories: hydrolyzable tannins and condensed tannins (Vuolo, *et al.*, 2019). Tannins have a more complex and homogenous structure than hydrolyzable tannins. Another type of phenolic chemical found in plants is stilbenes, lignans, and stilbene derivatives. All of these diverse compounds exhibit exceptional properties in plants like antioxidants or free radical scavenging (Oreopoulou, *et al.*, 2019; Tabaraki, *et al.* 2011; Balboa, *et al.*, 2014). Existing naturally in plants are numerous bioactive phenolic compounds with antioxidant properties

they include phenolic substances, carotenoids, coenzyme Q, lycopene, and vitamins (A, C, and E) (Küçük, *et al.*, 2017).

### 1.8 Carotenoids and their health benefit

Carotenes, present in both plants and animals, span yellow to red shades. These pigments, carotenoids, comprise lengthy aliphatic polyene chains with eight isoprene units. Found naturally in red, yellow, and orange hues, carotenoids abound in leafy greens and yellow-orange fruits. Notably concentrated in vibrant foods like plants, bird feathers, crustaceans, and marigold petals, their significant applications are shown in (Fig.12) (Eldahshan, *et al.*, 2013; Taeymans *et al.*, 2014).

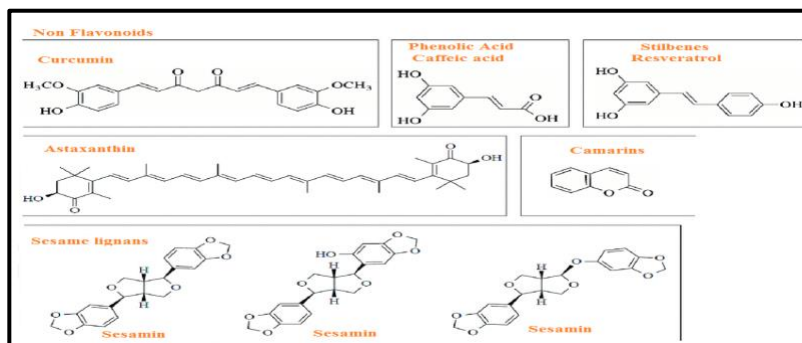


Figure 12. The Beneficial Roles of Carotenoids in Promoting Health

Fruits and Vegetables: Rich Sources of Vibrantly Colored Carotenoids in Human Diets Carotenoids are linked to health benefits like preventing cardiovascular diseases and cancer, and they provide essential vitamin A. Nature has over 600 carotenoids, but around 40 are typical in diets, with about 20 found in human tissues. Common carotenoids include  $\beta$ -carotene,  $\alpha$ -

carotene, lycopene, lutein, and cryptoxanthin. (Sethi, *et al.*, 2020). These molecules share structural features with double bonds and symmetry. Isomerization to cis-trans isomers is possible due to double bonds, with Tran's isomers being more prevalent and stable. The complete implications of carotenoid

isomerization on human health remain uncertain. (Eldahshan, *et al.*, 2013).

### 1.9 Evaluation of radical scavenging capacity (DPPH)

Antioxidant activity is assessed by measuring the ability to scavenge free radicals, turning them yellow. The extraction process is influenced by factors like temperature, solvent concentration, pH, and time. Extraction solvents depend on the compound's polarity; water for water-soluble antioxidants and organic solvents for fat-soluble ones. New methods combine ethyl acetate, hexane, and acetone, alongside traditional approaches like Soxhlet extraction and modern techniques such as ultrasound and microwaves. These newer methods require substantial energy input to enhance efficiency (Li, *et al.*, 2018; Zhao, *et al.*, 2013).

### 1.10 Usage of natural antioxidants to enhance oil property

Oil and fats are generally considered unstable because of oxidation but they do have many antioxidants such as tocopherols, carotenoids, tocotrienols, and sterols. When the oil is not oxidative stable it starts giving off many health problems that are serious. The quality of this type of oil becomes low and they do causes then cancers, mutagenicity, colds, CVD, and others (Farooq, *et al.*, 2021). Many techniques have been introduced that

Are been used to stable the quality if oil and increase oxidative stability. The one mostly used technique is to use synthetic antioxidants like TBHQ, TBHA, PG, and BHT (Zamuz, *et al.*, 2021; Samaram, *et al.*, 2015). Synthetic compounds are causing health hazards. However, natural compounds are effective (Castelo-Branco, *et al.*, 2016). Plants' volatile oil is found to be useful to enhance the quality of oil as well as flavor for oily products (Zheng, *et al.*, 2019). The oils that have been rich in volatile oils and some other kinds of products are also been tried to get used as functional foods. As the fortified food product is enhanced with nutrients, the flavor can also be enhanced by the addition of aromatic compounds (Naknaen, *et al.*, 2016). This strategy is rapidly spreading across the globe (Zheng, *et al.*, 2019). A variety of flavored

oils containing various ingredients have been marketed (Van Belzen, *et al.*, 2017; Oliveira, *et al.*, 2018). Leaf, roots, kernels, or flowers can be considered as by-products for the plant which contain high concentrations of bioactive compounds. These bioactive compounds are used as natural antioxidants to stabilize the oil against oxidation. These are phenolic acids, flavonols, and anthocyanidins (Galano, *et al.*, 2015). Several studies on the ability of plant extracts to inhibit oxidative degradation have been conducted previously. Olive leaves (Zahran, *et al.*, 2020), aromatic plants (Saoudi, *et al.*, 2016), rosemary, Rambutan, and fruit skin (Phuong, *et al.*, 2020), grape seed (Freitas, *et al.*, 2017), cocoa bean shell (Patricia, *et al.*, 2017), coffee husk (Ribeiro, *et al.*, 2017), peanut skin (Franco, *et al.*, 2018), and *Cressa cretica* (L.) leaves (Afshari, *et al.*, 2018), extracts commonly used. Extract-enhanced soybean oil had a lower peroxide value as well they do contain a smaller amount of secondary product (Zahran, *et al.*, 2020). The results come with fortified oil that contains a low amount of TBA. *Thymus vulgaris* extracts are normally used to delay the degradation process as reported previously (Kozowska, *et al.*, 2018; Phuong, *et al.*, 2020; Yang, *et al.*, 2016). *Rosmarinus officinalis* (L.) and coffee husk also do works as antioxidants. In addition, they reduce free fatty acid production and peroxide value while boosting antioxidant activity (Kozowska and Isabel, *et al.*, 2017). These studies indicated that these plant extracts could be used as a substitute for synthetic antioxidants.

### 1.11 Additional bioactive substances in watermelon.

There is a class of oxygenated steroidal triterpenes. This class is known as "cucurbitacins" as they do have a curcubitane skeleton. They possess antitumor, anti-inflammatory, and anti-diabetic properties (Kaushik, *et al.*, 2018). Both the watermelon flesh and leaves are utilized for extracting Curcubitacin B, C, D, E, I, and curcubitacin L 2-O-b-glucoside. (Hassan, *et al.*, 2011). It has been demonstrated that numerous anticancer activities inhibit cell proliferation, cell cycle arrest, and apoptosis (Chen, *et al.*, 2011). Despite having

the same molecular structure, the cucurbitacins listed above can induce distinct cell cycle arrest stages. Cell cycle arrest induced by cucurbitacin was frequently associated with apoptosis (Chen, *et al.*, 2011). The above-mentioned cucurbitacins inhibited cyclooxygenase (COX) 2 enzymes (Jayaprakasam, *et al.*, 2003a). Inhibiting TNF- $\alpha$  along with some other inhibitors like nitric oxide synthase-2, cyclooxygenase-2, and dihydrocucurbitacin B also showed their activity against inflammation (Escandell, *et al.*, 2007; Jayaprakasha, *et al.*, 2003). Cucurbitacins unquestionably contain the ability to initiate agonistic action. Cucurbitacin B exhibited hypoglycemic effects in differentiated enter endocrine L cells by initiating the AMPK and increasing GLP-1. This molecule improved hyperglycemia in diabetic mice with the help of activating intestinal AMPK and causing plasma GLP-1 (Li, *et al.*, 2018). The translocation is considered an important step to make glucose entry into the body cells by acting at the AMPK pathway level. AMPK activation is crucial to numerous metabolic processes because it increases fatty acid oxidation, inhibits lipid synthesis, and enhances the action of insulin (Tan, *et al.*, 2008; Ruderman, *et al.*, 2005). One of the potential antioxidants to neutralize the free radicals is vitamin C which has the capability to convert an iron state that is high in oxidation to  $Fe^{+2}$ . Lanatus is considered to be a good source of vitamin C. Its flesh provides more than rind and seeds (Rahman, *et al.*, 2013). The weight can be varied depends on the environmental conditions, genotype as well as pre-harvest and post-harvest conditions (Alka, *et al.*, 2018; Ilahy, *et al.*, 2019). Watermelon studies reported that the bioactives isolated from *C. lanatus* are an active source for the health of humans (Deshmukh, *et al.*, 2015; Ekene, *et al.*, 2014; Messaoudi, *et al.*, 2014).

### 1.12 Maceration, infusion, percolation, and decoction

Plants either coarse or powdered are soaked in a container that has been solvent-sealed also called a menstruum. For at least three days at room temperature with continuous agitation until the soluble materials have dissolved. The

mixture is then filtered, and the majority of the occluded solutions are extracted from the solid residue. The combined filtered and pressed liquid is then filtered to remove contaminants (Jovanovic, *et al.*, 2017). The liquid that has been filtered is evaporated and concentrated. Similar to maceration, infusion, and decoction are used to dip in hot water or cold water. Infusion, however, has a shorter maceration period. In general, maceration and infusion contain more fat-soluble compounds than decoction. The percolator is a one-of-a-kind piece of equipment used in the percolation extraction technique (Ribeiro, *et al.*, 2017).

### 1.13 Ultrasound-assisted extraction (UAE)

UAE technique is being used widely in the field of pharmaceutical and food industries during the last thirty years. They are considered an important part to increase the efficiency of extraction (Esclapez, *et al.*, 2011). The mechanism is based on the cavitation phenomena. Ultrasound propagates, by a successive approach in the waves of compressional and rarefaction present in a system of liquid (Chemat, *et al.*, 2012; Soria, *et al.*, 2010). When a few cycles pass the diameters of those bubbles increase. It starts to expand until it does not reach the critical threshold. This point is the one at which the bubbles mix with each other and releases energy in a high amount. When the energy releases the temperature rises up to 5000k from the room temperature and pressure up to 1000 atm. During this technique plant cell walls can be damaged because of the presence of high temperature and high pressure. Due to this, from the plant cell wall bioactive compounds start coming out. (Rodsamran, *et al.*, 2019; Muniz-Márquez, *et al.*, 2013). This is how the mass transfer rate improves. Temperature, pressure, and frequency are the factors that affect the frequency and yielding amount of extraction. For a thriving extraction, the type of solvent, solvent's volume, property of a sample like particle size, and their moisture content level (Talmaciu, *et al.*, 2015). Ultrasonic extraction has a variety of benefits over conventional techniques in contexts of extraction efficiency and time. Methods of

operation concerning extraction yields and extraction durations (Virost, *et al.*, 2010; Zhang, *et al.*, 2020).

**Table 5.** Examples of extraction methods of natural antioxidants

Extraction Method	Plant	Main Compounds	Main Results (Extract)	Reference
Soxhlet extraction	Spearmint ( <i>Mentha spicata</i> L.)	Flavonoids	Catechins = 0.144 mg/g	Bimakr (2011)
Maceration	Summer savory ( <i>Satureja hortensis</i> L.)	Phenols Flavonoids Anthocyanins	TPC = 125.34 ± 0.13 mg GAE/g TFC = 16.27 ± 0.34 mg RU/g TAC = 115.21 ± 0.95 mg C3G/g	Maškovi (2017)
Micro-waves assisted extraction	<i>Pistacia</i> leaves ( <i>Pistacia lentiscus</i> L.)	Polyphenols	TPC = 148.79 ± 8.22 mg GAE/g	Dahmoune (2014)
(UAE)	Rosemary leaves ( <i>Rosmarinus officinalis</i> L.)	Polyphenols	TPC = 2030 ± 38 ppm GAE TPC = 35.0 mg GAE/g	Bellumori (2016)
Supercritical Fluid extraction	Rosemary ( <i>Rosmarinus officinalis</i> L.)	Antioxidant compounds	EC <sub>50</sub> (DPPH) = 0.22 mg/mL	Babovic (2010)
Pressurized liquid extraction	Spinach ( <i>Spinacia oleracea</i> L.)	Vitamin E forms	α-T = 282 ± 12 μg/kg β-T = 8 ± 0.1 μg/kg γ-T = 82.9 ± 2 μg/kg	Viñas (2014)
High hydrostatic pressure extraction	Green tea ( <i>Camellia sinensis</i> L.) leaves	Polyphenols	Yield of polyphenols at 4 min = 29.96 ± 0.6%	Shen (2009)
Pulsed electric field	Norway spruce ( <i>Picea abies</i> L.)	Polyphenols	TPC = 9.20 g GAE/100 g	Bouras (2016)
Enzyme-assisted extraction	Stevia ( <i>Stevia rebaudiana</i> (Bert.))	Bioflavonoids	Catechins = 86–103 g/100 g	Puri (2012)

GAE stands for gallic acid equivalent, EC denotes effective concentration, TPC represents total phenolic content, TFC includes total flavonoid content, TAC measures total antioxidant capacity, and DPPH is 2,2-diphenylpicrylhydrazyl. Additionally, α-T, β-T, and γ-T stand for α-, β-, and γ-tocopherols.

Response surface methodology (RSM) is one of the most powerful and useful statistical procedures for optimizing multifaceted

operations and evaluating the impact of process variables and their interactivity (Fiedor, *et al.*, 2014).The past few years, this technique has

been extensively utilized to enhance the extraction of biologically active compounds from a variety of plant materials (Ghafoor, *et al.*, 2009; Zhang, *et al.*, 2016). However, only a few numbers of studies involving the optimization of UAE for the determination of total phenolic content (TPC) and antioxidant activity of watermelon skin (WMP) and seed (WMS) (Dranca, *et al.*, 2016). Therefore, the present study was conducted to determine the influence of sonication temperature, sonication time, and ethanol concentration on the TPC and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity of WMP and WMS extracts using RSM based on the Box-Behnken design (BBD). In addition, phenolic acids in WMP and WMS extracts were quantified using gas chromatography-mass spectrometry under optimal conditions (GC-MS). In this work, the following hypothesis was tested: a designed experiment based on variable combinations of UAE process conditions may aid in establishing optimal conditions for maximum recovery of phenolic bioactive from watermelon byproducts. In this work, response surface methodology (RSM) was also used to simulate the impact of distinct variables (specifications) on phenolic compound extraction from different plants, as described previously (Mas'ud, *et al.*, 2017; Rebollo-Hernanz, *et al.*, 2021; Wang, *et al.*, 2013). The results obtained were compared to percolation as a traditional extraction technique (Rebollo-Hernanz, *et al.*, 2021)

#### 1.14 Classification & preparation of hydrogels

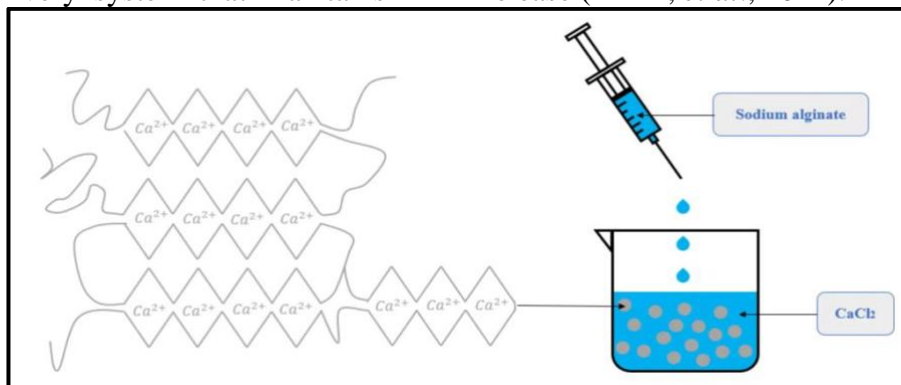
In the literature, numerous hydrogel classifications and perspectives are presented. According to the ionic species on the bound units, hydrogels are classified as cationic, anionic, or neutral. According to the sources, there are two major types of hydrogels: those made of natural polymers and those made up of synthetic polymers. However, some authors have also used other terms such as physical, chemical, and biological hydrogels (Silva, *et al.*, 2009). Changes in variables such as temperature, ionic concentration, and pH can cause physical hydrogels to transform from

liquid to gel (Ahmad, *et al.*, 2019). Chemical hydrogels, unlike other low-strength materials, rely on covalent bond formation to provide biomechanical stability and resistance to degradation. In biochemical hydrogels, biological agents, enzymes, and amino acids assist the gelation process. There are numerous other types of hydrogels, including crystalline, semi-crystalline, amorphous, and hydrocolloid clusters (Silva, *et al.*, 2009; Ullah, *et al.*, 2015).

Hydrogels are a three-dimensional polymer network that expands when exposed to water while retaining their mechanical integrity. Similar to extracellular matrices, hydrogels can hold vast quantities of water. The hydrophilic functional groups attached to the polymer membrane provide hydrogels with their water-retention capacity, while the cross-links between network chains give them the stability to resist degradation (Ullah, *et al.*, 2015). Hydrogels are gaining popularity due to their ease of production, vast range of applications, and biocompatibility. Hydrogels, both natural and synthetic, are cross-linked hydrophilic polymeric materials in nature (Li, *et al.*, 2006). Due to their high compatibility with human tissues, these polymers could be utilized in biomedical applications. In drug delivery, hydrogels offer numerous advantages including durability and sensitivity without side effects. Due to their well-established biocompatibility, hydrogels are frequently employed as drug-delivery hosts (Ullah, *et al.*, 2015). Hydrogels are also used for spatial and temporal delivery of various medicinal substances, macromolecular pharmaceuticals, tiny drug molecules, and cells. Due to their controllable physical characteristics, variable degradation rate, and ability to preserve unstable compounds from deterioration, hydrogels serve as a foundation for a variety of physical and chemical interactions to regulate the release of the enclosed drugs (Wang, *et al.*, 2019). Hydrogel beads are one of the hydrogel system's expansions. Beads are spherical objects that serve as a solid base for coating or encapsulating medicine within their core, in this way providing a controlled release. Moreover, beads are also known for their high bioavailability in

formulated medications. Gastro-retentive beads solve the problem of developing a gastro-retentive drug delivery system that maintains

drug release and prolongs the dosage form's stomach residence until the desired time of drug release (Amiri, *et al.*, 2021).



**Figure 13.** Preparation and cross-linking of hydrogel beads

### 1.15 Beads of oil

Oil beads contain liquid oil as their core material, created through dispersing vegetable oil into a wall material, resulting in a solution with consistent consistency. Ratios of oil-to-wall weight can reach up to 50% or more. Various proteins, carbohydrates, and gums are used as wall components, with  $\alpha$ -Cyclodextrin being utilized due to its amphiphilic nature. Alginate and pectin are common choices for gel formation. Encapsulation efficiency varies with oil content and wall materials. Combining emulsifiers with ionic gelation can enhance oil loads and encapsulation efficiency. Formulation adjustments allow control over bead size, oil content, and mechanical characteristics (Lin, *et al.*, 2020; Silva, *et al.*, 2019).

### 1.16 Botanical Description of Watermelon (*Citrullus lanatus*)

Watermelons belong to the Cucurbita citrullus species, sharing a genus with pumpkin, squash, and bottle gourd. Its scientific name originates from Greek and Latin roots, "Citrullus" referencing the fruit and "lanatus" denoting "woolly." It's a summer crop with sprawling stems, pinnately lobed leaves, and shallow roots. The round to cylindrical fruits have edible endocarp and weigh between 8 to 35 lbs, with smaller Asian varieties weighing 2 to 8 lbs. Male and female flowers exist on the same plant. Watermelon seeds are obovate to elliptical, yellow to black, maturing with the

fruit's ripening. The seeds lack dormancy, germinating in 2 days to 2 weeks at temperatures above 60 °F.

### 1.17 Application of natural antioxidants

Natural antioxidants have been applied for years to preserve food, counteracting oxidation-related flavor, color, and texture changes in fat and oil-containing products. Oxidation generates free radicals during the process, leading to unwanted alterations in the product's quality and nutritional value. Unsaturated fatty acids are particularly vulnerable to oxidation, with double bonds accelerating the process. Efforts to enhance product stability have involved antioxidants, bioactive treatments, and controlled extraction methods. Synthetic antioxidants like BHT, BHA, TBHQ, and PG are used but raise health concerns. (Müller, *et al.*, 2010). Oxidation can be categorized as physico-chemical autoxidation or enzymatically catalyzed lipoxidation. Synthetic antioxidants can harm health, while natural antioxidants from plants offer a sustainable solution to food preservation. Modern extraction methods like accelerated solvent extraction, microwave-assisted extraction, and ultrasound-assisted extraction are efficient for obtaining active components from plant organs. Ultrasound-assisted extraction (UAE) is particularly noteworthy due to its effectiveness and shorter time requirements, driven by acoustic wave-induced cavitation. UAE's benefits include



enhanced mass transfer and high extraction efficiency, although challenges like sample contamination and foam generation exist (Yao, *et al.*, 2020; Wang, *et al.*, 2013).

## 2. Conclusions

Watermelon, scientifically known as *Citrullus lanatus*, belongs to the Cucurbitaceae family and has a Greek-Latin name derived from "citrus" and "lanatus," meaning wooly. It is consumed worldwide for its sweet, juicy flavor. The fruit consists of flesh, seeds, and rind, with byproducts rich in bioactive compounds. Watermelon seeds have antioxidant properties and phenolic compounds, showing health benefits against various diseases. The potential therapeutic uses of these natural antioxidants have led to research on their extraction from watermelon byproducts. Watermelon's rind, seeds, and skin can be utilized in biorefinery processes. Lycopene, an antioxidant, is abundant in watermelon and is beneficial for various health aspects. Carotenoids like  $\beta$ -carotene and xanthophylls contribute to watermelon's nutritional value. Different varieties exhibit varying carotenoid profiles. Free radicals, formed during metabolic processes and due to environmental factors, can cause oxidative damage to cells and tissues. Antioxidants, found in watermelon and other sources, neutralize free radicals and protect against diseases. Watermelon's rich vitamin and antioxidant content, including lycopene and carotenoids, offer potential health benefits. Plant-based oils are essential nutrients obtained from seeds and fruits, with quality influenced by sensory and compositional factors. Oxidation and hydrolysis processes can cause off-flavors and odors in oils. Various aspects of phenolic compounds, flavonoids, non-flavonoid phenolic substances, carotenoids, antioxidants, and the usage of natural antioxidants in enhancing oil properties. It also highlights the presence of bioactive substances in watermelon and different extraction methods.

The importance of phenolic compounds in plants, including their classification into phenolic acids, flavonoids, and non-flavonoids, and their role as antioxidants. It explains the

significance of flavonoids in plant pigmentation and physiological activity. The concentration of phenolic compounds and flavonoids in different plants, particularly in modified atmospheric packaging, is provided. The distinction between flavonoid and non-flavonoid phenolic compounds is outlined, mentioning tannins, stilbenes, and other antioxidants found in plants. Carotenoids, such as  $\beta$ -carotene and lycopene, are discussed for their role in providing color to fruits and vegetables, as well as their health benefits. The text also covers the evaluation of radical scavenging capacity using DPPH, the use of natural antioxidants to enhance oil stability, and the potential of plant extracts to substitute synthetic antioxidants. Oxygenated steroidal triterpenes, called cucurbitacins, found in watermelon, are explored for their anti-inflammatory and anti-diabetic properties. Maceration, infusion, percolation, and decoction extraction methods are explained briefly. Overall, the text emphasizes the bioactive compounds present in plants and their potential health benefits, as well as techniques for utilizing these compounds to enhance food quality and stability. Various techniques and methods used in the fields of extraction, encapsulation, and natural antioxidants. Ultrasound-assisted extraction (UAE) is highlighted as an efficient method for extracting bioactive compounds from plant materials using cavitation-induced energy release. The benefits of UAE include improved mass transfer and high extraction efficiency. Response Surface Methodology (RSM) is mentioned as a statistical approach for optimizing extraction processes. The passage also introduces the concept of hydrogels, three-dimensional polymer networks that can hold water while maintaining their structure. Different methods of oil encapsulation, including beads and capsules, are explained, with a focus on emulsion extrusion. The nutritional value and potential uses of watermelon seeds are discussed, highlighting their high protein content and essential amino acids. The passage concludes by providing botanical descriptions of watermelon and its growth characteristics. Overall, the passage covers a range of

techniques and applications related to natural compounds, extraction, encapsulation, and their potential benefits.

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