Novel Method of Essential oil Extraction from Spices

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Abstract:

Essential oils have a wide range of industrial and pharmaceutical applications hence different novel technologies are recently being investigated to study the extraction of essential oils, enzyme assisted extraction is one of the recent green technology which is being studied extensively to see its effect on the yield of various components like essential oils and bioactive compounds. This review article summarises the spices in which enzyme-assisted extraction has been employed and its effect on the yield of the essential oil as well as the effect on the quality parameters of the oil, various studies, however, confirm that enzyme-assisted extraction increased the yield of the essential oil, which becomes a potential energy-efficient and cost-effective process and can be used at the industrial scale for a wide range of food, pharmaceutical applications.

Key words: novel extraction technologies; eo extraction; spices; enzymes; enzymatic extraction; essential-oil extraction

Introduction

Spices are dried plant components used to enhance the flavor, color, aroma, and other culinary delights of food products and can be obtained from the seed, fruit, root, and bark, of the plants.(Singh & Yadav, 2022). Various spices and parts of the plant from which it is obtained is shown in Table.1. There are more than 100 different types of spices cultivated worldwide. Some of the major spices like cinnamon, clove, and ginger production is dominated by Asia, Western countries like Europe and America are also known for the production of some of the well-known spices like coriander, chives, thyme, and sesame seeds.(Gottardi et al., 2016) Essential oils also known as aromatic oils which give distinctive flavor and aroma to the spices are the constituents of the spice.(Dwivedy et al., 2017; Ribeiro-Santos et al., 2017) Spice essential oil (SEO) consists of various bioactive compounds and the yield of bioactive compounds extracted depends on the methods used for the extraction of the compounds, the technology used also affects the total yield of the essential oils(Fernandes et al., 2016; Tariq et al., 2019). The volatile oils, oleoresins, extracted from spices have a wide range of application in the food, pharma, and cosmetic industry due to the presence of beneficial bioactive compounds which consists of secondary metabolite including polyphenols vitamins, and minerals(Tariq et al., 2019). Hence various extraction methods are used for the extraction of these health-promoting compounds as well as to extract volatile oils and oleoresins from the spices. The extraction is the separation of dissolvable material from the insoluble residue, which might be solid or liquid, by utilizing different solvents(Hu et al., 2019; Pawlaczyk-Graja et al., 2019).

Figure. Fennel seeds

Different novel techniques are used for the extraction of volatile oils and bioactive compounds, as well as oleoresins ranging from Supercritical fluid extraction (SFE) technique to Enzyme assisted extraction (EAE), ultrasound-assisted extraction (UAE), and many more. When compared to other conventional methods like hydro distillation these novel techniques have shown increased efficiency in the extraction of the volatiles with the reduction in the time of the extraction as well as efficient use of energy leading to less energy consumption while at the same time complying with the Green Chemistry goals(Giacometti et al., 2018). This review paper discusses some of the novel technologies of EO extraction technologies in brief and detail information on the use of enzyme-assisted extraction technology in the extraction of spice EO in past few years and its prospect in the future has been discussed.

Figure. Fennel plant

Spices	Part of the plant from which it is derived	References
Turmeric	Rhizome	(Govindarajan & Stahl, 1980)
Rosemary	Leaves	(Nieto et al., 2018)
Red pepper	Fruit	(Yoshioka et al., 1999)
Fenugreek	Seeds	(Pandey et al., 2022)
Garlic	Bulb (underground stem)	(Ahmad Wani et al., 2022)
Onion	Bulb (underground stem)	(Golubkina et al., 2022)
Ginseng	Root	(Ptak et al., 2022)
Cardamom	Seeds	(Paul I.D., 2022)
Cumin	Seed	(Namjoo et al., 2022)
Pepper	Fruit	(Balakrishnan et al., 2022)
Fennel	Fruits	(N. et al., 2022)
Celery	Seeds	(Chauhan et al., 2021)
Cinnamon	Bark	(G. Das et al., 2022)
Ginger	Rhizome	(Laelago Ersedo et al., 2023)

Table 1: Different parts of the plant from which the respective spices are obtained

2. Some advanced emerging technologies

2.1 Cold atmospheric-pressure plasma (CAPP)

Atmospheric cold plasma is a nonthermal technology. The mechanism in atmospheric plasma involves the formation of a substantial, brief-lived, stable bubble during plasma bubbling leads to the distilled water disintegration, which leads to uniform distribution of the volatile compounds(Lokeswari et al., 2021). In an experiment Turmeric powder treated with cold atmospheric-pressure plasma (CAPP) showed both an increase and decrease in the profiling of the essential oil, the varying effect of cold plasma treatment was attributed to plasma-induced damage to the food tissues which released volatile oils easily but the presence of reactive species produced during the plasma treatment oxidized some of the compounds(Hemmati et al., 2021). Other studies conducted on Cuminum cyminum L. seeds of two different sizes showed an increase in the extraction of the essential oil by using the atmospheric plasma technique.

2.2 Ultrasound- assisted extraction (UAE)

UAE is a mechanical method based on changing the frequency and amplitude of sound waves. The shock waves produced during ultrasound treatment including the micro-jets, shear, and turbulence, destroy the cellular matrix, which causes an instant release of essential oil and its rapid release(Khadhraoui et al., 2021). One of the experiments used clove for an ultrasonic experiment where the essential oils obtained from the clove were found to be effective against gram+ve as well as gram -ve bacteria(Tekin et al., 2015).

2.3 Microwave-assisted extraction technology (MAE)

MAE works on rapid heating technology which causes vibrations of the compounds and molecules present in the spice which increases the temperature and pressure causing the rupture in the cell which leads to the release of essential oils from the damaged surface. Microwave-assisted extraction was performed in pepper using different factors for the essential oil extraction with an improved yield of the essential oil (Tran et al., 2020).

2.4 Supercritical fluid extraction (SFE)

SFE technique is used for the extraction of essential oils using solvents with pressure and also the temperature above the critical points. The use of supercritical fluids like co2 has been used as solvents for the extraction process and is a useful technique. (Widmann et al., 2022)were efficiently able to perform Supercritical Fluid Extraction with CO2 of Curcuma longa L. and revealed an optimum yield of all the constituents of the essential oil of turmeric.

Some of the other environmentally greener technologies being used are Ionic liquid technology where ionic liquids having melting points above 100 °C are used and extraction takes place with the interaction of anions and cations(Ramos et al., 2019). Another energy-efficient pulse electric field technology is being also used for the extraction by the generation of transient electrical pulses which results in the formation of pores in the wall of the cells hence improving the extraction process from the essential oils(Ranjha et al., 2021).

3. Enzyme Assisted technology and mechanism

3.1 Enzymes

Enzymes have been used traditionally for the different processing of the foods like making wine, ripening cheese, softening dough, enhancement of flavors in various foods, and baking processes (α-Amylase), in industries they have been serving different purposes like clarification of the juices by the use of enzyme pectinase(Micard et al., 1994; Saxena et al., 2001; Soffer & Mannheim, 1994). Enzymes are comprised of a globular protein component (biopolymer) and cofactors(Datta et al., 2013). They act as natural catalysts and increase the rate of biochemical reactions (Kurochkina, 2019). Enzymes can be intercellular or extracellular and have different protein structures ranging from secondary to quaternary. An enzyme consists of an active site where the substrate binds and a change in the shape of the enzyme takes place proceeding the reaction forward which results in the formation of the products without undergoing any change in the reaction itself (Mohamad et al., 2015). Enzyme activity is influenced by temperature, acidity, ionic strength, and the environment.

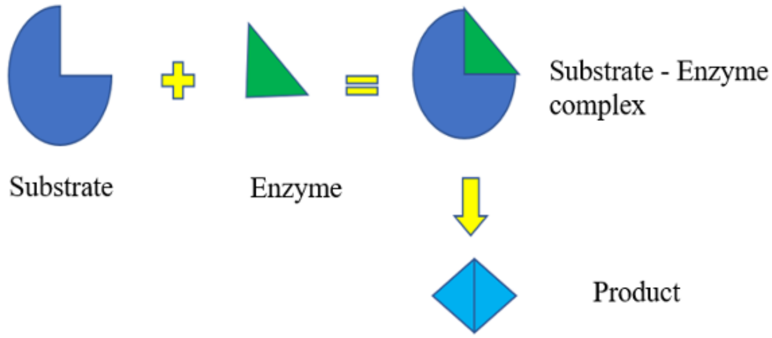

Figure 1: Mechanism of Enzyme Action

The activity of the enzyme is specific and depends on the structure and conformation of the active site(Bhuvarahamurthy et al., 1996). The Lock and Key model of enzyme activity describe that an enzyme is like a molecular lock that can be unlocked by a substrate of a specific molecular shape hence the activity of the enzyme has been described as specific. Low temperatures are preferred for enzymatic activity up to 45 degrees Celsius. Enzymatic productivity is a measurement of the rate at which a product forms or a substrate vanishes under specific reaction conditions, at a predetermined temperature(Dias Gomes & Woodley, 2019). It is the sole way to accurately assess the robustness and reaction yield (a measure of substrate conversion) of an enzymatic process Siddiqui et al., 2022). The process during the application of the enzyme has to be standardized to achieve maximum activity from the enzymatic process like the concentration of enzyme, incubation time, Ph, and temperature. Enzymes are generally used for the hydrolysis of the molecules like pectin starch, proteins, and cellulose(Leschine, 2005; Mansfield et al., 1999). They are also known as "green" biological catalysts. Enzymes are widely used in a variety of food processing industries, like dairy, vegetables, food, and oil processing(Robinson, 2015). It has been shown to enhance product consistency and quality, reduce reliance on raw materials for processing, substitute for chemical food additives, and prevent potentially dangerous food byproducts are just a few of the benefits(Schäfer, 2007).(Sharma et al., 2017)reviewed that utilization of the enzymes cellulases, pectinases, and amylases, as well as their combination, might result in higher production of fruit juice with greater quality. They are also being employed to improve the sensory and textural quality of the food.(Rastogi & Bhatia, 2019) Understanding the nature of the enzyme, its mechanism of action, and environmental conditions like acidity and the optimum temperature is crucial to use them in food applications

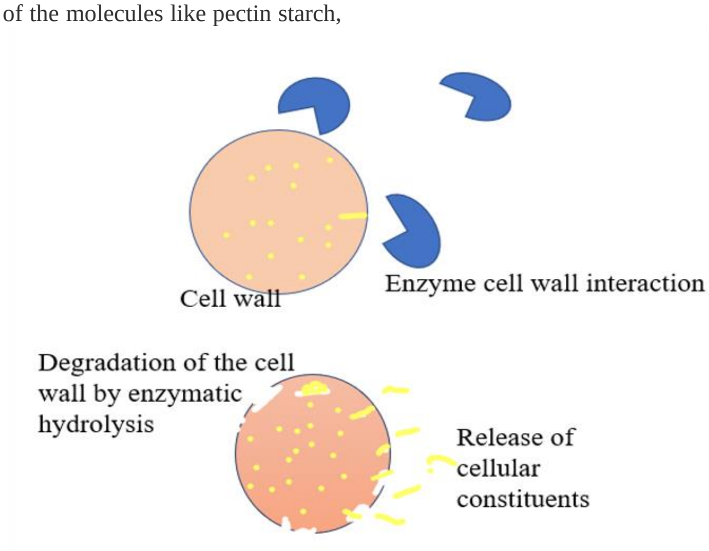

Figure 2: Mechanism of Enzyme-assisted extraction technology

3.2 Enzyme-assisted extraction of essential oil and mechanism

The intracellular components are released by utilizing the enzyme which hydrolyses the plant cell wall under ideal experimental conditions, this is the basic principle of the enzymatic extraction technique. The enzyme's active site is bound by the cell wall of the plant. As a result, the enzyme adapts its shape according to the substrate onto its active site, resulting in increased interaction, which causes the rupture of the cell wall causing the discharge of the components(Sheldon & van Pelt, 2013). Enzyme-assisted extraction processes use many types of carbohydrate hydrolyzing enzymes like cellulase, xylanase, and amylase, to assist in the processes of extraction and increase its yield(M'hiri et al., 2014; Sowbhagya & Chitra, 2010). Enzyme-assisted extraction methods have been shown to improve the yield of essential oils with less utilization and usage of energy, with an increased rate of extraction and minimal usage of the solvent(Adetunji et al., 2017; Puri et al., 2012a). The process of extraction depends on various factors like the concentration of the enzyme being utilized, the temperature, the total time of extraction, the size of the substrate, and finally the solvents being utilized for the process of extraction(Adetunji et al., 2017).

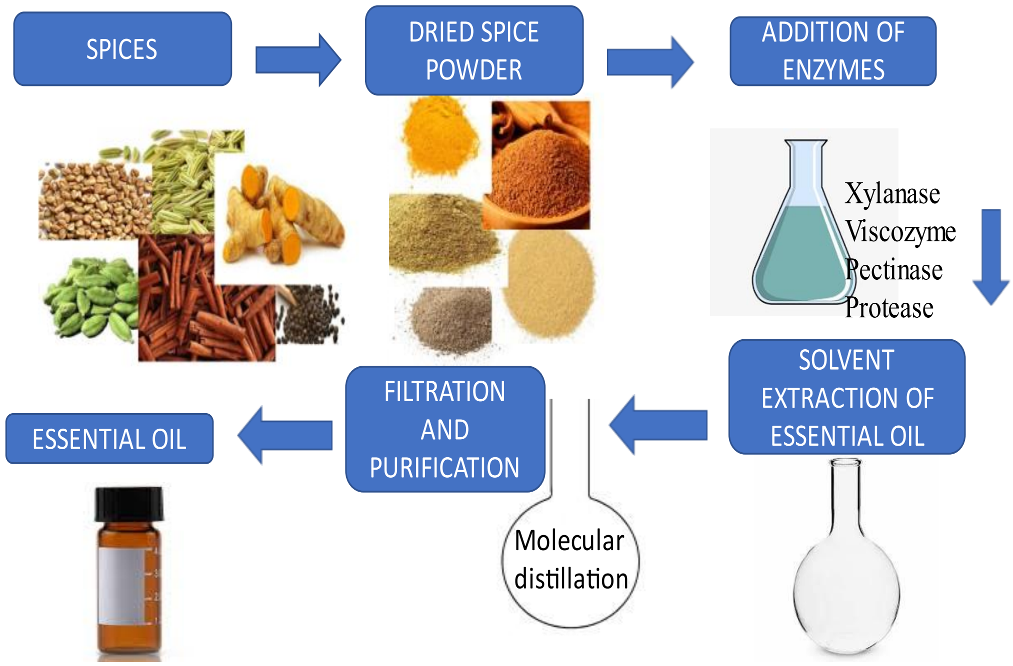

Figure 3: Flow chart of essential oil extraction using enzyme- assisted extraction technology

4.Enzyme assisted extraction technology in spices

Studies on enzymatic extraction of essential oils from various plant materials including spices and herbs have shown an increase in the yield as well as the retention of the flavor of the essential oils. Cellulases, hemicellulases, and pectinases are the major enzymes used for the prior treatment of cellulosic materials like plants and their parts(Costa et al., 2020; Mishra et al., 2005; Ranalli et al., 2004). Oil extraction methods utilize mainly the enzymes like pectinase, cellulase, and -amylase(Puri et al., 2012b).The following spices are being utilized for the extraction of the EO's with enzymes from the last 20 years and sufficiently has shown the increased yield and quality of the essential oils.

4.1 Turmeric

Turmeric is a rhizome herbaceous underground plant belonging to the family Zingiberaceae. Ancient Vedic scripture mentions it as "the herb of the sun". The volatile oil of turmeric is an orange-yellow, fluorescent liquid with a smell similar to tubers. The major flavoring compounds present in turmeric are turmerone and zingiberene used in the food industry. Turmeric has various traditional and medical uses with the treatment of various diseases like anorexia, cough, arthritis, and hepatic disorders, turmeric essential oil incurs many health benefits owing to the major compounds found in TO which include sesquiterpene, ketones, β-, and ar-turmerone(K. Das, 2016), many technologies Conventional and non -conventional are being used for the extraction of the EOs.Enzyme-assisted extraction is one such technology and its importance has been proved by (Kurmudle et al., 2013a) for enhanced yields of turmeric oleoresin and its constituents. They conducted an experiment where they extracted turmeric oleoresin using acetone as a solvent after exposing turmeric to various types of enzymatic pretreatment by optimizing various parameters such as concentration of the enzyme, pH, time of incubation, and time of extraction, treatment with α-amylase enzyme increased the yields of aromatic oil along with the curcumin and oleoresin, However, enzymes like Xylanase along with cellulase did not show any increase in the yield of the product because of the presence of starch as a major constituent in the turmeric and lower amount of cellulose and xylan α-amylase was found to be more effective.

4.2 Cardamom

Cardamom is of the *Zingiberaceae* family, and is popularly known as the 'queen of spices used as a flavoring agent in beverages like tea, is also used in the perfume industry because of its distinctive smell and in food to give aroma(Chempakam & Sindhu, 2008). Cardamom has been traditionally used for the prevention and treatment of disorders like asthma and digestive functions, cataracts, nausea, diarrhea, and cardiac disorders(G. Hamzaa & N. Osman, 2012; Gilani et al., 2008; Khan et al., 2011; Qiblawi et al., 2020). Cardamom capsules' distinctive aroma and usefulness as a functional food, medicinal, and nutraceutical are attributed to the essential oil and other bioactive metabolites present in them(Ashokkumar et al., 2020). Two major chemicals 1,8 cineole and α-terpinyl in cardamom oil promote flavor in the spice(Anwar et al., 2016).

(Baby & Ranganathan, 2016c) used enzymes for the treatment of the cardamom to study its effect on the yield of the essential oils and it was inferred that the yield of the essential oil increased as well as its flavor was preserved, the enzymatic pretreatment with ViscozymeL increased the yield of the EOs causing the lysis of the cell wall as was confirmed by the SEM analysis.

4.3 Cumin - The spice has a strong, sweet, and spicy aroma with a slight bitterness and pungency in the taste, especially used in the form of ground spice (Amin, 2012). The flavor component majorly present in the cumin is cumin aldehyde which gives it a warm, astringent, and spicy flavor. The seeds of this plant and the essential oil extracted from them are used for pharmaceutical processes, food processing industries, and beverage industries and its distinctive aroma makes it eligible to be used in perfumes(Sowbhagya, 2013). Cumin is commercially sold as both seeds and powder. Traditional uses of cumin include anti-inflammatory, diuretic, carminative, and antispasmodic. It has also been used to treat dyspepsia, jaundice, diarrhea, flatulence, and indigestion(Sharif et al., 2018).In an experiment oil yield from cumin, after pre-treatment with enzymes like cellulase, pectinase, protease as well as Viscozyme, increased the yield of oil and there was no effect on the sensory as well as the chemical quality of the oil(Sowbhagya et al., 2011a).

4.4 Pepper

The Sanskrit word pippali, meaning berry, is where the word "pepper" stems from. Pepper, also known as Piper nigrum L., is widely regarded as the "king of spices" due to its enormous trading share in the world market(Kumar et al., 2011; Srinivasan, 2007). Piperine, which adds pungency, and volatile oil, which is in charge of the scent and flavor, both contribute to the pepper quality. One of the most widely traded pepper products is oleoresin, it contains flavor (oil) and pungency (piperine) elements(Ravindran & Kallupurackal, 2012). Pepper oil is generally extracted through steam distillation of ground pepper and contains some compounds like limonene, myrcene, *cis*-ocimene, carvone, carventanacetone ,δ-cadinene,elemol, cubebol and csome of the other phenolic compounds like such as quercetin,isoquercetin, isorhamnetin 3-β-D-rutinoside(Pino et al., 1990). According to (Dutta & Bhattacharjee, 2015), in batch mode compared to continuous mode, alpha-amylase-assisted supercritical carbon dioxide extractions of black pepper dramatically enhanced the yields and phytochemical characteristics of extracts rich in piperine when compared to supercritical co2 extraction alone. The use of a mixture of enzymes before the extraction of essential oils from black pepper and cardamom improved the yield of the EO. The active chemicals present in the spices, such as -caryophyllene in black pepper and -terphenyl acetate in cardamom, significantly increase in the pretreated samples.

4.5 Fennel

The fruit of the fennel plant and its essential oil are used as spices to flavor nonvegetarian food like meat and fish and others like bread, biscuits, candies, and alcoholic beverages as well as in the production of fragrances, soaps, and they are also used in several therapies(Sayed Ahmad et al., 2018). Its volatile oil contains terpenic chemicals. The major compounds present in fennel oil include anethole, estragole, fenchone, d-limonene, and -pinene(Malhotra, 2012).In a study several enzymes, including Celluclast, Pectinex, Viscozyme, and Protease, increased the essential oil yield, also SEM analysis showed a significant difference in the structural cell wall of the enzyme-pretreated samples and there was no difference in the physio-chemical properties of the essential oil(Baby & Ranganathan, 2016d).

4.6 Green chillies

Chilies have a distinctive flavor, color, and pungency and is widely used for consumption all over the world(Chakrabarty et al., 2017). They provide essential antioxidants and vitamins A, C, and E and several other antioxidants to the body and consist of flavonoids and various other minerals like thiamine and niacin and also consist of various chemicals, two of them particularly include carotenoids and capsaicinoids. Chilli oleoresins and oils are also used for the preparation of snack foods, canned products, and flavoring of various food items in the food industry(Ahmed et al., 2002). A study conducted by (Baby & Ranganathan, 2016a) to study the yield and quality of oleoresin after the enzymatic treatment which suggested increased yield of the oleoresin after the treatment when compared to the control samples. Viscozyme-treated samples showed greater recovery of oleoresin when compared to the others. HPLC analysis was used to detect the quality of the capsaicinoids extracted and showed no alterations.

4.7 Celery seeds - Celery belongs to the family Umbelliferae and has many commercial applications. Celery seed powder can be mixed with salt to flavor delicacies like fish and eggs as well as juices and tomatoes, the essential oil produced is used by flavor and fragrance industries consisting of major compounds like limonene and β-selinene giving it a defining odor, the volatile oil also consists of coumarins, furanocoumarins (bergapten), and flavonoids(apiin)(Malhotra, 2006; Sowbhagya, 2014a). A study conducted on the enzymatic extraction of celery seeds showed that the compound limonene extraction increased as well as the yield of the essential oil increased with little change in the physical and chemical properties of the oil (Sowbhagya et al., 2010).

4.8 Cinnamon -Cinnamon belongs to the family of Lauraceae, it's used as a spice in food as it provides distinctive taste, aroma, and flavor to the food and is also used as a spice(Thomas & Kuruvilla, 2012). Cinnamon bark is powdered and used as spice commercially and gets its aroma and flavor from the essential oil present in the bark because of the presence of compounds like eugenol and cinnamaldehyde (80-90%)(Lee et al., 2013), which is dark yellow and is commercially used in the canned food and confectionery industries, it also has several applications in the fragrance industry(Cardoso-Ugarte et al., 2016). It is used as a carminative and used to treat gastrointestinal problems since ancient times and has several pharmacological benefits(Gruenwald et al., 2010). In one of the studies, the aqueous solution of alpha-amylase and viscozyme was used for the extraction of the cinnamon essential oil which showed increased yield in the content of the oil with viscozyme enzyme showing greater yield than the alpha-amylase treated samples(Sethunga et al., 2021). Hence again proving the fact of increased essential oil concentration when compared to the traditional methods.

4.9 Garlic- Garlic is used as a spice and has many health benefits, owing to compounds present in it like sulfides, saponins, and phenolic compounds. It has antioxidative and various health-benefiting properties as it consists of various compounds like sulfides, saponins and phenolic compounds, the presence of sulfides gives it a distinctive flavor(Diretto et al., 2017). It is used in powder form commercially, the oil from the garlic is extracted and has pharmaceutical benefits like curing diabetes and CVD(Bradley et al., 2016). A study conducted on the enzymatic treatment of garlic for extraction of essential oils showed an increase in the yield of essential oil, with very little change in the physical and chemical properties of the oil(Sowbhagya et al., 2009a).

4.10 Ginger -Ginger is the rhizome belonging to the Zingiberaceae family and has many fold applications as food, spice, and supplement(Ravindran et al., 2016). Ginger has been used mainly as a carminative agent and also as medicine for aiding digestion and alleviating colds(Baliga et al., 2011). The volatile oil of ginger consists of chemicals like sesquiterpenes, zingiberene, curcumin, and farnesene, and some monoterpenes, such as linalool(Kiyama, 2020). Ginger oil also has high therapeutic properties and anti-inflammatory, anti-analgesic, and anti-cancer properties(Rahmani et al., 2014). One of the studies assessed the application of crude multi-enzymatic extracts obtained from the fermentation of cocoa beans increased the yield of the ginger essential oils(dos Santos Reis et al., 2020).

SPICE	Component Extracted	Enzyme used	Most efficient enzyme	Control EO yield	EO Yield of enzyme treated sample	Percentage increase	Referances
Turmeric	Oleoresin	Xylanase, cellulase enzyme, glucoamylase α-amylase	α-amylase			70 %	(Kurmudle et al., 2013b)
Cardamom	Essential oil	Celluclast 1.5 L, Pectinex Ultra SP.L, ViscozymeL and Protease	ViscozymeL	6.73	7-16 %	-	(Baby & Ranganathan, 2016e)
Cumin	Essential oil	cellulase, pectinase, protease and Viscozyme	Cellulase	2.7%	3.2–3.3%	18–22%	(Sowbhagya et al., 2011b)
Fennel	Essential oil	Celluclast, Pectinex, Viscozyme, and Protease	Viscozyme	1.24%	1.38–1.5%	11.5% to 22.5%	(Baby & Ranganathan, 2016f)
Green chilli	Oleoresin	Celluclast 1.5 L, Pectinex Ultra SP. L, ViscozymeL, Protease and an equal combination of Celluclast 1.5 L, Pectinex Ultra SP. L and Viscozyme L	Viscozyme	0.65%	0.84% -1.05%	-	y & Ranganathan, 2016b)
Celery seeds	Essential oil	cellulase, pectinase, protease and viscozyme	cellulase	1.8%	2.2–2.3%	22–27%	(Sowbhagya, 2014b)
Garlic	Essential oil	cellulase, pectinase, protease and viscozyme		0.28%	0.39–0.51%	-	(Sowbhagya et al., 2009b)
Ginger	Essential oil	multi-enzymatic extracts			-	47.95 %	(dos Santos Reis et al., 2020b)

Table 2: Yield of essential oil after the enzymatic extraction of essential oil from different spices

5. Conclusion

There are various novel sustainable and green extraction technologies in demand for extraction of essential oil as a sustainable method. Enzymatic treatment is one such novel technology that is being used extensively for the extraction of various compounds from plants as well as animals like bioactive chemicals, colorants, pectins as well as essential oils from the spice. Its use has significantly increased in the food processing industry as it has proved to be an energy-efficient process and takes less time with improved yield when compared to the traditional processes of maceration, distillation, and decoction used for the extraction of essential oils. It is a greener alternative for the extraction process of essential oils and various studies have confirmed its potential use in industries for large-scale processing of food.

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