Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 12 [6] May 2023 : 246-261

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

REVIEW ARTICLE **OPEN ACCESS**

Importance of pectinase in preparation of enzymatic cocktails for valorization of horticultural wastes into natural carotenoids: an updated overview

Ghadge Amit Babasaheb
Department of Science
Smt. Ratnaprabhadevi Mohite Patil College of Home Science for Women, Akluj, Tal-Malshiras, Dist-Solapur
Email: amitghadge9921@gmail.com

ABSTRACT

One industry that generates massive amounts of solid waste is agriculture. These wastes can either be used as raw materials for the bio-economy or permitted to accumulate indiscriminately and endanger global health and food security. Agro-wastes are produced by farming activities like horticulture, seed production, dairy farming, animal breeding, grazing, nursery plots, market gardens, and forestry or woodland output. An effective management technique is to recycle agricultural solid wastes to produce useful items. The growing demand for foods with health advantages encourages the production of bioactive chemicals from a variety of byproducts, supporting the sustainable exploitation of natural resources. The scientific community is actively researching various extraction techniques for the extraction of various useful substances from these agro-wastes and limiting their detrimental effects on the environment. Carotenoids can be extracted using a practical alternative method that involves pretreating the substrate with enzymes. Enzymolysis has been investigated as a potential improvement over conventional extraction methods or cutting-edge methods like supercritical fluid extraction, ultrasound-assisted extraction, microwave-assisted extraction, or surfactant-assisted extraction. The synthesis of carotenoids by microorganisms is a rapidly growing sector because of the allure of large-scale production employing various types of cultivation systems and consumers' preference for natural products over synthetic ones because of increased health consciousness.

Keywords: *Pectinases, carotenoids, extraction, fermentation, agro-wastes*

Received 02 .04.2023 Revised 05.05.2023 Accepted 15.06.2023

INTRODUCTION

Due to the adverse effects on the environment, economy and society, agricultural and food wastes are a major problem on a global scale. The management of such waste is the concern of several scientific investigations. The rise in agricultural land since the 1960s, which was triggered by technological advancements brought about by the green revolution, yields have been enhanced to satisfy the demands of a fast-expanding global population. Global agriculture currently generates an average of 23.7 million tonnes of food every day [1].Global agricultural yield has put a lot of pressure on the environment, harming soil, air, and water resources and endangering both human health and ecological sustainability. Agricultural waste production is thought to be around 998 million tonnes each year[2].

Agricultural solid wastes are produced in significant quantities every year, with an average annual growth rate of 7.5%[3].A sustainable course for agriculture has yet to be discovered, with the expected increase in global population to almost 10 billion by 2050. Due to natural resource limitations, this must be accomplished through utilizing fewer fossil fuels, reducing emissions, and reducing solid waste. Agricultural wastes (agro-wastes) are the leftovers from cultivating crops and/or from the first processing of raw agricultural products, such as vegetables, fruits, dairy products, meat, poultry, and other items.Farming operations such as horticulture, seed production, dairy farming, animal breeding, grazing, nursery plots, market gardens, and forestry or woodland production all result in the creation of agro-wastes[4].Agro-wastes make up close to 30% of all agricultural products generated worldwide. Raw agricultural goods may contain animal and plant remnants as residues (e.g., manure, different crop residues, wastes from activities like pruning, harvesting, growing, and fertilization).Using sustainable management technologies, several studies have quantified the generation of agro-wastes and the environmental effects of these wastes, such as global nutritional and environmental losses due to agro-wastes, with a focus on reducing wastes as a more environmentally and economically sound alternative

than treatment[5, 6, 7]. The Ministry of New and Renewable Energy (MNRE) in India estimates that the country produces 500 million tonnes of agro-wastes annually. According to the same research, the bulk of this crop residue is actually used as fodder and fuel for home or industries. Still, there is a surplus of 140 million tonnes, of which 92 million tonnes is burned annually. The volume of agro-waste burnt in India annually is larger than the total production of agro-waste in some of the other countries [8].
Agriculture is one such sector which produces solid wastes in enormous quantities, which can either be exploited as raw materials for the bio-economy or left to pile indiscriminately and pose a threat to world health and food security [9, 10]. Recycling agricultural solid wastes has numerous advantages, including lowering greenhouse gas emissions and reducing the need for fossil fuels. It also has a positive impact on the growth of new green markets, the creation of jobs, the production of bioenergy, and the bioconversion of agricultural solid wastes into a variety of high value products. Efforts are needed to combat the threat posed by improper management of agricultural solid wastes because of the considerable impact that agricultural solid wastes have on human health, animal health, and the environment.In many areas of developing nations, agricultural solid wastes are carelessly disposed of or burned in public spaces, causing air pollution, soil contamination, the release of hazardous gases, smoke, and dust, and the possibility that the residue will end up in a water source, polluting the water and aquatic environment. In order to prevent global warming that would cause temperatures to rise by at least 35.6°F on average, greenhouse gas emissions must be severely curtailed [3]. Uncontrolled burning of agricultural solid wastes releases emissions that are relevant to the climate. The improper management of agricultural solid wastes has an impact on climate change, which in turn hinders the production of food.

How to deal with these wastes?

There are different options for handling agricultural solid wastes. Some of these agricultural solid wastes could be added to cement mixtures, the creation of water glass, paper, ethanol, animal feed, the production of electricity and biogas, the removal of heavy metals, mulching, organic fertilizers, and compost. Recycling agricultural solid wastes to create valuable goods is an efficient management strategy. Avoiding the production of garbage is the greatest strategy for waste management. Reusing and recycling agricultural waste to create compost, organic fertilizer, and other value-added products is the second-best strategy.Instead of employing conventional methods, such as thermal management, landfilling, and decomposition, the sustainable biorefinery can manage agro-waste in a sustainable manner by collecting wastes and valorizing or recycling them into useful products[11, 12]. A circular economy model makes it possible to recycle or reuse goods and reintroduce them into the supply chain, allowing for both economic growth and limiting adverse environmental effects. This is done by valorization of fruit and vegetable waste [13] (Hussain et al., 2020).Numerous researches have reported on the management of agro-industrial wastes utilizing solid state fermentation to create bioactive chemicals[14, 15].By-products of the agricultural industry are regarded as a sustainable source for producing a variety of bioactive chemicals [16].

Different Carotenoids that can be extracted from such wastes

The increasing demand for foods with health benefits drives the use of various byproducts to obtain bioactive substances, which supports the sustainable use of natural resources. Agro-industrial residues contain high concentrations of substances including fibres, lipids, carbohydrates, peptides, carotenoids, phenolic compounds, and other substances that have a variety of bioactivities and functions and can be used as ingredients in other products [17, 18]. The extraction of high added-value molecules from fruits and vegetables can be done using a variety of biotechnological techniques and green processing technologies, such as microwave, ultrasound, supercritical fluid, pulsed electric field, and enzyme-assisted extraction, which will meet consumer expectations and leave a little adverse environmental impact [19, 20]. These bioactive components can be used as antimicrobials, antioxidants, natural colors, fortifying ingredients, texture enhancers and for other purposes. Vegetable and fruit pigments, such as carotenoids, betalains, anthocyanins, and chlorophyll, are thought to be healthy and safe due to their capacity for colouring as well as their antioxidant, anti-inflammatory, and anti-mutagenic properties [21]. Carotenoids have a range of colors from yellow to red and also have antioxidant properties and are typically used as natural food colorants. By utilizing their inherent qualities, such as immune system modulation, the reduction of the risk of cancer and cardiovascular disease, as well as vitamin A precursors, they can also be used as food supplements [22]. The need for natural carotenoids that are pure, stable, well-characterized, and affordable keeps rising, in part because of the potential benefits to human health. According to estimates, their market will increase from $1.5 billion in 2019 to $2.0 billion in 2026 [23].Large quantities of tomato peels and seeds that are currently used as animal feed, fertilizer, or disposed away in landfills It is nonetheless abundant in vitamins, phenolic compounds, carotenoids, and

other highly advantageous phytochemicals. Lycopene, which makes up 80–90% of the tomato's total pigments, is the most significant antioxidant present.

Agricultural wastes used for enzymatic extraction of carotenoids

Large quantities of agro-industrial raw materials are produced worldwide and are primarily used for human and animal use as well as the generation of energy [24, 25]. But, according to estimates, losses of up to 50% of the agricultural raw materials occur most frequently during harvest, post-harvest, slaughter, transport, processing, storage and consumption [26]. These raw material losses can amount to 1.3 billion tonnes of material a year that is no longer used or processed through the proper processes [27]. These agro-wastes can prove thebest resources of treasured substances. Due to their composition, residues sometimes exhibit slow degradability, which can lead to accumulation and have a harmful influence on the environment [25]. Therefore, different extraction methods are widely investigated in the scientific community for extraction of various beneficial compounds from these agro-wastes and reducing their harmful effects on the environment. Significant concentrations of substances such fibres, lipids, carbohydrates, peptides, carotenoids, phenolic compounds, and other substances are present in agro-industrial wastes. These substances have a variety of biological activities and functionalities and can be used as ingredients in other products [17, 18]. New approaches have to be designed to convert such agro-wastes into value-added marketable products like carotenoids. In order to maximize the value of important substances found in agricultural food wastes, information on the extraction procedures and their optimization can be a great way to design and improve novel and useful products like carotenoids at the industrial level. Despite substantial recent attempts to increase the recovery yield of carotenoids from agro-wastes, research is now being done to produce green and sustainable techniques.

Enzyme-assisted extraction (EAE) uses hydrolytic enzymes to break down the cell wall or other parts of the cell. This facilitates the solvent's penetration into the plant material, facilitating the elution of the metabolites. This technology is gaining popularity as it is thought to be a more cost- and environmentally-friendly alternative to traditional or contemporary extraction techniques. Many studies have reported on its promising use in enhancing the recovery of various classes of bioactive metabolites (such as polyphenols, carotenoids, polysaccharides, proteins, components of essential oils, and terpenes). Many researchers discuss the extraction of carotenoids from vegetable sources, with tomato peels and carrots being the most favored sources. But studies are also being done to search for other plant sources.

High volumes of byproducts from tomato processing are both a source of bioactive substances and a nuisance to farmers in terms of the environment [28]. In a study to investigate the enzyme assisted extraction of lycopene-containing chromoplasts, unsold tomatoes were used and the yield was reported to be 5.43 ± 0.04 (mg_{Lyc}/Kg_{tomato})/U [29]. The tomato waste obtained from a local market in Mexico was used for extraction of lycopene assisted by the use of cellulose and pectinase [30]. Catalkaya and Kahveci [31] successfully used industrial waste of tomatoes for extraction of lycopene. Tomato paste production waste was used and 11.5 mg lycopene/g yield was observed. The highest total carotenoid extraction yields (55.15 mg/100 g d.w.), β-carotene extraction yields (35.85 mg/100 g d.w.), and lycopene extraction yields (15.44 mg/100 g d.w.) were obtained in the peels of the Bulgarian tomato cultivar 'Stela'[32]. Response surface methodology (RSM) was used to optimize the extraction of lycopene from tomato processing waste. In this case, ultrasound and enzyme assisted extraction of lycopene was studied [33].Maximum lycopene extracted from the tomato skin, waste, and paste was 80 ± 2.4 mg/kg, 42 ± 1.3 mg/kg and 60 ± 1.2 mg/kg, respectively, using the immobilized cellulase and pectinase [34]. Due to the high insoluble fibre content, tomato peels have a limited bioavailability of lycopene despite the high content. The extraction of lycopene and soluble dietary fibre (SDF) from tomato peels could be greatly improved by enzymatic treatment to the tomato peels [35]. Optimization of the extraction of lycopene from waste tomato peels using pectinase and tri-solvents was performed by Munde et al [36]. A commercial tomato waste was used for lycopene extraction by Phinney et al. [37].In an experiment by Ladole et al. [38]tomato peels were pre-treated with a mixture of ultrasonic and enzyme-immobilized amino-functionalized magnetic nanoparticles (AMNPs) for the effective release of lycopene. Tomato pomace is the major byproduct of production of tomato paste and is a rich source of lycopene. It is solid waste stream from food processing units. Allison and Simmons [39] used this tomato pomace for lycopene extraction.

Solid-state fermentation was used as a bioprocess for carotenoid extraction and recovery in tomato waste using *Aspergillus nigerGH1*[14]. Using enzymatic pretreatment and supercritical CO_2, lycopene and beta-carotene were extracted as oleoresin from a Tunisian industrial tomato peels by-product [40]. Tomato (*Solanum lycopersicum* L.) fruit paste and waste were tested for the extraction of carotenoids with pretreatment of digestive enzymes [14, 41].

Carrot pomace powder was used in pectinase-assisted extraction of carotenoids and the extraction efficiency was found to be 90% [42]. Carotenoids were also extracted from the enzyme treated carrot

pomace in flaxseed oil [43]. Enzyme-assisted extraction of carotenoids was also studied using sunflower wastes (petals and florets) and it was observed that 335 ppm of carotenoids could be extracted [44].Red capsicum extract was used in the Viscozyme®, pectinase and cellulase assisted extraction of total carotenoids [45]. When buckwheat husk was used for extraction of lycopene with commercial preparations of xylanase, it was observed that the yield was improved 4 to 5 times [46]. Palm kernel expeller was also used for the extraction of carotenoids [47].

Bio-pigment from pumpkin (*Cucurbita maxima* Duch) is a natural way to increase the nutritional content and aesthetic appeal of food products. The enzymatic pretreatment with cellulase and pectinase was done to the pumpkin for extraction of carotenoids and it was observed that yield of β-carotene was 61.75% under the optimal conditions [48]. Pericarp of ripe bitter melon was pretreated with enzyme preparations and then supercritical fluid extraction was done to obtain β-carotene yield up to 90.12% [49]. By combining ultrasonic and enzymatic methods and extraction with ethanol solvent, the extraction of β-carotene from orange processing waste was studied [50]. Another agro-industrial waste, longan (*Dimocarpus longan*) peel, was pretreated with cellulase, α-amylase, protease or β-glucosidase for extraction of carotenoids [51].There is potential for commercial production of β-carotene after it was recovered from orange processing waste using ultrasonic and enzymatic methods with pectinase and ethanol solvent [50]. Tuntiteeraboon et al. [52] investigated the pectinase-assisted extraction of carotenoids from the date palm and effects of different processing conditions on the juice quality. Macadagdag et al. [53] studied the extraction of carotenoid extraction from the peels of lemon through xylanase and pectinase treatment.

Although ripe pumpkin's (*Cucurbita maxima*) fluffy portion and fibrous strands contain a considerable amount of β-carotene, they are regarded as waste. It was observed that when this waste portion of ripened pumpkin was pretreated with enzyme, the yield of β-carotene was improved [54].Carrot residues were used for extraction of α- and β-carotenes and lutein by high power ultrasound enzyme assisted extraction [55]. Pomegranate (*Punica granatum* L.) peel was tested for enzymatic extraction of β-carotene [56]. The by-product of juice production from persimmon was investigated for the pectinase assisted extraction of bioactive carotenoids [57].The sweet corn cob is always regarded as agricultural waste. It was reported that sweet corn cob also contains carotenoids such as β-carotene, zeaxanthin and lutein [58].Papaya (*Carica papaya* L.) seeds and peels, which are 20% of the total fruit weight, are considered as wastes. These peels have been used for the extraction of carotenoids [59]. A study was conducted on the enzymatic pretreatment of different cultivars of pomegranate seeds. Ultrasound-assisted and ethanol extracted pomegranate seed oil was found to contain the carotenoids [60]. Enzymatic hydrolysis of watermelon fruits yielded improved amount of lycopene [61]. Pontillo et al. [62]reported the enzyme assisted extraction of carotenoids from the leaves of rosemary. Carotenoid content was found to be improved in the pectinase assisted extraction of oil from rice bran [63].In another study by Rosero Henao et al. [64], guava pulp was used for pectinase assisted extraction of lycopene.

Use of microbial enzymes for valorization of agricultural wastes into carotenoid extraction

The plant material is pretreated with enzymes like protease, pectinase, pectinesterase, cellulase, hemicellulase, cellobiase, α-amylase, and fructosyltransferase in order to hydrolyze the cell walls and release the phytochemicals that are bound to lipid and carbohydrate chains within the cell in EAE. For the purpose of extracting volatile compounds, hydrophilic and hydrophobic pigments, phenolic compounds, and other bioactive compounds from plant samples, this pretreatment is followed by solvent (water) extraction or pressurized hot water extraction [65].

The following elements are necessary for the EAE: the measurement, sort, and needed phase of the enzymes; a coordination of reaction's temperature and time; chemical constitution, size of particle, plant material's water content; solvent to solid ratio. There are various advantages of Enzyme-assisted extraction, like efficiency of scale up processes, increased extraction yield, a value-added quality of extract and green extraction (asnatural origins like enzymes and water utilized), and a reduced need for additional extract filtration and purification [66].

Enzymes have been used particularly for the treatment of plant material prior to conventional methods for extraction. Enzymes such as cellulases, pectinases and hemicellulase are often required to disrupt the structural integrity of the plant cell wall, thereby enhancing the extraction of bioactives from plants. These enzymes hydrolyze cell wall components thereby increasing cell wall permeability, which results in higher extraction yields of bioactives. Enzymes can be derived from bacteria, fungi, animal organs or vegetable/fruit extracts.

To use enzymes most effectively for extraction applications, it is important to understand their catalytic property and mode of action, optimal operational conditions and which enzyme or enzyme combination is appropriate for the plant material selected. In order to recover food and non-food byproduct

biomolecules from agriculture, both traditional and innovative extraction methods are used. Some of the conventional methods used for this purpose are maceration with organic solvents, solid-state fermentation [25], Soxhlet extraction, and hydro-distillation. Lycopene and other carotenoids can be extracted from their natural sources using the laborious extraction method known as maceration. This method is typically employed for samples for which carotenoid makeup is unknown. Maceration is a cheap and easy process. Unfortunately, this method is arduous, time-consuming, and heavily dependent on organic solvents. Therefore, maceration is not favored method to be used on a large scale commercially. As a result, novel extraction techniques are being researched to replace traditional ones. These novel techniques have the benefits of quicker extraction times, more bioactives recovery, and potential for commercial use [38].

Enzymatic pretreatment of the substrate is a feasible alternative method for extraction of carotenoids [36]. Enzymolysis has been researched as a potential upgrade to traditional (such as maceration and distillation) or contemporary extraction techniques such as supercritical fluid extraction, ultrasound-assisted, microwave-assisted, or surfactant-assisted extraction [67, 68, 69, 70]. The accessible polysaccharides are joined to the cellulose, hemicellulose, pectin, protein, and phenolic substances that make up the cell wall through hydrogen and hydrophobic bonds. To break down the cell wall structure, a variety of enzymes can be used, including cellulases, pectinases, and hemicellulases [71]. Pectinase treatment of tomato peels was found to improve the recovery of lycopene under gentle extraction conditions by breaking down the pectin network of the cell walls.

To extract lycopene from tomato peels, ultrasound assisted extraction (UAE) has been utilized in conjunction with enzymes (pectinase and cellulase) co-immobilized with magnetic nanoparticles. With this method, the lycopene yield could be increased 1.3 times more than with a regular extraction method [38].

In a study wherein tomato waste was used as substrate for extraction of lycopene and assisted by pectinase and cellulase, it was reported that 0.93 mg/g of fresh tissue was the quantity of lycopene recovered from tomato waste when cellulase was used and for pectinase, lycopene recovery was 0.48 mg/g of fresh tissue at a pH = 5, a temperature of 50°C, and a time reaction of 80 min [30].

Another enzymatic mixture was shown to be effective in improving the extraction of lycopene from tomato industrial waste. It was found that higher lycopene yields were obtained when enzymolysis was done with Celluclast: Pectinex in the ratio 1:1, enzyme to substrate ratio 0.2 mL/g, temperature 40^0C, pH 4.5 for 5-hour treatment and extraction solvent ethyl acetate was used [31].

Optimization of the enzyme-assisted extraction of carotenoids from carrot by response surface methodology was studied and Box-Behnken design was used for the optimization of extraction conditions from fresh carrot. The response (yield) was quantified using a spectrophotometric method. The highest yield from the experimental result was 393.4 Âµg/mL followed by 335.4 Âµg/mL whereas 198.0 Âµg/mL represents the least. [72].

In an experiment of enzymolysis of beetroot (*Beta vulgaris*), a mixture of cellulase, xylanase and pectinase (37%, 35% and 28%respectively) was used to extract betacyanin, and betaxanthin. It was found that yield of betaxanthin was 11.37 ± 0.45 mg/mL U and that of betacyanin was 14.67 ± 0.67 mg/mL U [73].

In a study by Prokopov et al. [32], comparison between the enzyme-assisted extraction of carotenoids and non-enzymatic extraction process was done using canning industry waste tomato peels of the Bulgarian cultivar “Stela”. It was observed that when the tomato peels were pretreated with enzymes like pectinase, cellulase and endo-xylanase, the extraction yields of total carotenoid, lycopene, and β-carotene were more than that of non-enzyme pretreated tomato peels. Type and concentration of enzyme used for extraction of carotenoids affected the extraction yields in this study. When the tomato peels were pretreated with mixture of cellulase (100 U g^{-1}) and endo-xylanase (400 U g^{-1}), maximum yields of total carotenoids (55.15 mg/100 g), beta-carotene (35.85 mg/100 g) and lycopene (15.44 mg/100 g) were obtained.

Lombardelli et al. [29]reported the application of tailored enzyme mix of polygalacturonase, pectin lyase, cellulase and xylanase to recover the carotenoid-containing chromoplasts from the tomato waste. RSM analysis was done for the optimization of physicochemical parameters. It was observed that maximum yield of carotenoid-containing chromoplasts was about 4.30 ± 0.08 (mg_{Lyc}/Kg_{tomato})/U and that of total carotenoid was 5.43 ± 0.04 (mg_{Lyc}/Kg_{tomato})/U.

Munde et al. [36] carried out the optimization of tri-solvent mediated and pectinase-assisted extraction of lycopene from the waste tomato peels in which the pectinase pretreatment was done with 2% enzyme concentration at pH 5.5, temperature of 45^0C and 4-hour incubation at 150 rpm. In comparison to single solvent, enzyme-treated, and tri-solvent extraction methods, the improved method increased lycopene extraction and recovery by 7.38, 4.65, and 1.59 times, respectively.

In another investigation [42]carrot pomace powder was pretreated with commercial pectinase (61 U/mL, 60 min) and it was observed that β-carotene extraction efficiency was improved up to 90%.Enhanced extraction of carotenoids from radish (***Raphanus sativus*** L.) was attempted with the application of enzyme-pretreatment to breakdown the starch and complex polysaccharides like cellulose, pectin and hemicellulose present in the plant cell wall. This was achieved by using enzyme-cocktail of cellulase, amylase, pectinase, glucanases and hemicellulases. Maximum extraction yields were reported at 3.53% (w/w) of enzyme concentration at pH 6.1, 46.08^{0}C temperature and incubation time of 66.08 min[74]. In order to extract the polyphenolic antioxidants from dried saffron tapes, a binary mixture of the enzymes cellulase and heicellulase (1:1) was utilized. It was reported that the yields were 45% and 38% greater than those of the untreated control sample [75].

Shahram and Dinani [50] combined the use of ultrasonic and enzyme-assisted processes with ethanol as solvent for the extraction of β-carotene from orange waste. Response Surface Methodology (RSM) was applied for the optimization of extraction parameters. Highest β-carotene extraction was achieved at pectinase concentration (0.40% w/w), ultrasound exposure for 115.55 min and pH 5.11. Scanning electron microscopy revealed that the combination of ultrasound and pectinase pretreatment and ethanol extraction considerably ruptured the substrate tissue. FTIR analysis also indicated that no functional groups of the extracts were degraded due to such pretreatment.

Tomato waste was used for the extraction of lycopene in a study [30] in which cellulase and pectinase enzymes were used at five different levels of temperature, pH and for time period of 120 min. Complete randomized design model was applied with three replications per treatment and analysis of data was done with one-way ANNOVA. A mixture of hexane and acetone was used for after enzymatic action. The amount of lycopene extracted was measured spectrophotometric at 520 nm. For cellulase pretreated tomato waste the amount of lycopene recovered was 0.93 mg/g of fresh tissue at pH 5, temperature 60°C and reaction time of 60 min. The extraction and recovery of Lycopene from pretreated tomato waste by using pectinase enzyme was observed 0.48 mg/g of fresh tissue at 50°Ctemperature, 5 pH and RT (reaction time) of 80 min.

Cellulase and pectinase enzymes were used for the extraction of carotenoids from pumpkin [48]. In this investigation, the use of Surface graph and Derringer's desired function methodology was done for finding the optimal parameters for extraction of carotenoids. It was found that maximum carotenoids extraction was achieved when cellulase/pectinase ratio was 0.97w/w, 42.54 °C incubation temperature, pH 4.8 and incubation time of 91.58 min. Under the optimal conditions, extraction yield of β-carotene was found to be 61.75%.

Enzyme-assisted aqueous extraction (EAAE) method was used to measure total carotenoids content in the mesocarp of virgin palm. It was observed that when mesocarp was treated with pectinase from ***Aspergillus niger,*** the total carotenoid obtained was 35 and 40 mg/100 g of the sample [76].

Microbial cellulase and pectinase were applied to extract the carotenoids from peels of lemon (Citrus limo). It was reported that when lemon peels were treated with cellulase (20U/mL) and pectinase (60 U/mL) at pH 5.0 for 120 minutes, the maximum yield of carotenoids was 86.95 ug/g [77].

Nath et al. [45]investigated the extraction of carotenoids from sweet peppers with the use of enzymatic treatment of viscozyme L, pectinase, and cellulase. It was reported that the extraction yields of carotenoids were more with viscozyme L and pectinase than cellulase. The recovery of carotenoids from sweet peppers was found to be 2.3 times more with enzyme treatment than with the solvent extraction using n-hexane.

Enzyme-assisted extraction was combined with ultrasound extraction of α- and β-carotenes from discarded carrots (*Daucus carota L.*) in a study by Encalada et al. [55].It was reported that yield of these carotenes was highest when enzyme hemicellulase was used for extraction.

Due to their lower cost and quicker carotenoid extraction processing time as compared to commercial enzymes, the use of raw enzymes has various advantages [78].

Significant improvement in the total carotenoids from lemon peels by dual-enzyme-assisted extraction of total carotenoids in comparison with solvent-only extraction was reported by Macadagdag et al. [53]. Highest yield (36.16 ug phytofluence equivalent/g lemon peel) of total carotenoids was achieved from 50:50 xylanase: pectinase treatment for 90 min incubation time.

Gómez-Linton et al. [79] evaluated the use of efficiency of hydrolyzing enzymes, sonication and green solvents to extract total carotenoids from tomato. Quantification of total carotenoids was done with spectrophotometric method and HPLC. It was reported that the use of hydrolyzing enzymes and sonication increased the carotenoid content in extraction process, when these were applied individually. It was also reported that when these extraction methods were used together and isopropyl acetate was used for solvent extraction, the highest carotenoid extraction was achieved.

Comparison of the simple solvent extraction method and enzyme-assisted extraction of lycopene from tomatoes, tomato paste and pulp, higher lycopene extraction from tomato pulp was observed through enzyme-assisted extraction technique [41].
Reshmitha et al. [80] reported that the amount of lycopene extracted from tomato or tomato peel increased from 3.82% to 4.9% when cellulase and pectinase were used in combination.
The peels of Kinnow mandarin were used for extraction of carotenoids by applying purified cellulolytic enzymes. It was observed that maximum carotenoid yield (8.60 ± 0.44 mg/100 g peel) was obtained when 250 IU both of carboxymethyl cellulase and pectinase per 100 g of kinnow peels were used [81].
Lycopene yield was observed to rise by 38.65% (40.08 ug/g of fresh pulp) in an experiment to measure the lycopene from guava pulp using a pectinase-assisted method, when pectinase concentration of 0.02% v/w of guava pulp was employed, incubation period of 45 minutes, and temperature 28^0C and pH 4.5[64].
Adadi and Barakova [82] applied the RSM to optimize the enzyme-assisted extraction of carotenoids from fresh carrots. Maximum extraction yield obtained experimentally was 393.4 μg/mL.
In another study of extraction of lycopene from watermelon fruits, cellulase enzyme was used at the concentration of 2.4% (v/v) for 60 min and highest yield of lycopene obtained were 14,125±0.271 μg/ml [61].

Use of microbial fermentation for obtaining pectinase

Solid- state fermentation:

Production of pectinase and pectin lyase enzymes by *Bacillus subtilis SAV-21* in a solid-state fermentation using combination of orange peel and coconut fiber (4:1) as substrate with moisture content of 60%, pH 4.0, temperature 35^0C and incubation period of 4 days and 8 days respectively was studied. It was observed that pectinase yield could be increased by supplementation with galactose and yeast extract to the basal medium [83].
In a study to compare the pectinase production by *Bacillus licheniformis KIBGE-IB4* using algal biomass of *Ulva lactuca* as substrate through Solid State Fermentation (SSF) and Submerged Fermentation (SmF), it was reported that higher pectinase production (2457 ± 3.31 U mg^{-1}) was achieved by SmF. *B. licheniformisKIBGE-IB4* required 10.0 g L^{-1}*U. lactuca* as a biomass in the medium with a pH 7.0 when incubated at 37°C for 24 hours, according to parametric optimization of pectinase synthesis [84].
Solid state fermentation method was studied using *Bacillus* sp. *TMF-1* strain and screening of agricultural by-products such as soybean meal, sunflower meal, maize bran, maize pericarp, olive oil cake and wheat bran for the production of proteases, α-amylases, cellulases and pectinases. It was reported that pectinase yield was highest (64.90 IU g^{-1}) on untreated soybean meal [85].
Optimization of medium composition for solid state fermentation for pectinase production with the use of *Aspergillus* ***niger*** *ATCC 1640* was performed by Ahmed et al. [86]. Plackett-Burman design (PBD) studies revealed that the Satkara peel, urea, and $(NH_4)_2SO_4$ were the factors affecting the pectinase production. The statistical optimization of these parameters by RSM showed that predicted response for production of pectinase was in good agreement with the experimental results. The highest pectinase yield of 0.6178 μmol/mL was reported.
A solid-state fermentation method was used for the production of pectinolytic enzyme by fungi *Aspergillus niger* and *Aspergillus flavus* usinggrapefruit (*Citrus parasidis*) peels as substrate. Highest pectinolytic activity of 13.32 μmol/mg/min for *Aspergillus niger* and 11.32 μmol/mg/min for *Aspergillus flavus* was noted at the day third of the incubation [87].
A solid-state fermentation was used for pectinase production by a fungal strain ***Aspergillus niger*** *IBT-7*[88]. Optimization of pectinase production through different physical and nutritional parameters showed maximum pectinase production in a medium containing rice bran which was moistened with Czapek's nutrient medium. It was also reported that the maximum pectinase yield (39.1 U/ml/min) was obtained at moisture content 30 ml, pH 5.0, 72 hours incubation at 30^0C and xylose (1.5%) and yeast extract (1%) as supplemented carbon and nitrogen sources.
The ability of the new fungal strain *Aspergillus cervinusARS2*to give higher yield of pectinase using agricultural waste was studied [89]. According to the OFAT observations, peptone (a nitrogen source) had the highest pectinase activity of 45.05 IU/mL, followed by orange peel (a carbon source) at 44.51 IU/mL, and NaH_2PO_4 (a mineral salt) at 43.21 IU/mL. The optimization results by Response Surface Methodology (RSM) and Central Composite Design (CCD) showed that 10.63 g of orange peel, 3.96 g/L of peptone, 2.07 g/L of KH_2PO_4, and 2.10 g/L of NaH_2PO_4 had the highest pectinase activity of 105.65 0.31 IU/mL.
Using a sequential design approach, a *Saccharomyces cerevisiae* strain from a Brazilian sugarcane liquor vat was used to optimize the secretion of pectinases on passion fruit residual flour (PFRF) using solid-state fermentation [90]. In this study, a factorial design and two rotational central composite designs were used to identify the influencing factors. The validated experimental result was of 7.1 U mL-1 at pH 5,

30 °C for 24 hours, static SSF, and 50% PFRF (w/w). There were 3.5, 0.08, 3.1, and 0.8 U mL^{-1} of polygalacturonase, pectin methyl esterase, pectin-lyase, and pectate-lyase activities, respectively.

In solid state fermentation, *Aspergillus oryzae* produced polygalacturonase (PG) and pectin lyase (PL) on a variety of agricultural and industrial bioproducts [91]. In solid-state fermentation, the wheat bran, Orange Pulp and peels and lemon peelshave been utilized as substrate for the maximum enzyme production. 67.2% enzyme purification was made possible with the use of ammonium sulphate precipitation, dialysis and ion exchange chromatography.

The solid-state fermentation of orange pomace by *A. niger* was investigated for its potential to produce polygalacturonases. The tray bioreactor produced exo- and endo-pectinases at 45% and 37% higher yields than the rotating-drum bioreactor, respectively. Comparing the water sorption isotherms for bagasse and orange pomace revealed that adding bagasse to orange pomace for water preservation in the fermentation was very beneficial. It was determined through experimentation in a tray bioreactor that bagasse increased the production of pectinases from orange pomace. The results showed that adding bagasse (40 g bagasseg[1]dry solid) to orange pomace increased the exo- and endo-pectinase activity by 17 and 23%, respectively [92].

A solid-state fermentation method for the production of pectinases from a wild strain of *Aspergillus sp*. was studied in tray-type bioreactors in which dehydrated coffee waste like pulp and husk were used as substrates. After applying the response surface methodology, in a 3-level factorial design for optimization of the physicochemical parameters, it was observed that maximum Enzymatic Activity (EA) of 29.9 IU/g was achieved at 79% relative humidity and 35°C incubation temperature [93].

Coffee pulp was also utilized in a solid-state fermentation for the production of pectinase by *Aspergillus* sp. *VTM5*. Enzyme assay was performed by Somogyi-Nelson Method and it was reported that the optimum temperature and incubation period for pectinase production were 30°C and of 72 hours, respectively [94].

Optimization of the fermentation conditions for maximum production of pectinase using guava waste powder in solid state fermentation, *Aspergillus niger NCIM 616* and pectinases-producing filamentous fungi (designated as P1) was carried out and it was reported that maximum pectinase production was achieved at fermentation time of (144 and 120 hours), temperature of 30^0C, pH (8 and 6), moisture content (160% and 100%), inoculum volume of (3 ml) and age of inoculum of (120 and 144 hours) for *Aspergillus nigerNCIM 616* and pectinases-producing filamentous fungi, respectively [95].

Oumer and Abate [96] studied the production of pectinase by *Bacillus subtilis* strain *Btk 27* using wheat bran as substrate. It was reported that the yield of pectinase increased from 800.0 ± 16.2 U/g to 1272.4 ± 25.5 U/g in solid state fermentation with initial medium pH of 6.5, incubation temperature of 37^0C and supplementation of the medium with 3 mM $MgSO_4.7H_2O$.

Labrath and Gaikar [97] used *Aspergillus species NCIM -1432* in solid state fermentation of fresh orange peels to obtain the pectinase enzyme. Enzyme activity 155 U/ g was reported in this study. When solid state fermentation method was used to study the production of pectinase by *Penicillium sp*, *Pichia kudriavzevii F2-T429-5* and *Aspergillus niger* using the substrates like banana peels, wheat bran, corn cobs, orange peels, fruit peels and leaves of sweet prayer plant, it was reported that higher pectinase production was obtained by *Aspergillus niger* with the use of orange peels as substrate [98].

Pathania et al. [99] studied the production of extracellular pectinase by brown rot fungus *Rhizopus delemar* F_2 in a solid state fermentation of apple pomace pretreated with microwave at 450 W for 1 min. Enhanced pectinase production of 61.50 U g^{-1} was reported at 30 °C for 6 days incubation with moisture content of 1:4.5.*Aspergillus giganteusNRRL10* strain was used in a solid-state fermentation method in which a mixture of lemon peels, orange peels and wheat bran was used as substrate. Statistical analyses revealed that pH, temperature, and $CaCl_2$ concentrations were the major factors affecting the pectinase yield. Different aeration flow rates were also studied in this experiment and it was reported that the maximum enzyme activity was achieved at 20 L min−1 per kilogram of dry substrate (kgds).

Ketipally et al. [100] studied the polygalacturonase production in solid state fermentation from *Aspergillus nomius MR103*. It was reported that maximum pectinase production of 4.83 IU/mg could be achieved with the use of orange peels as substrate at pH 7.0, 37^0C for 7 days. It was also observed that supplementation of the substrate with sucrose as carbon and peptone as nitrogen source improved the pectinase yields. Maximum polygalacturonase production of 1440.57 U/g was achieved when apple pomace was used as substrate in a solid-state fermentation using *Penicillium expansum* 3.5425. In this study, rotational orthogonal design (ROD) experiment was used for the optimization fermentation conditions and it resulted in 2.72-fold increase in the enzyme production. The purified polygalacturonase had specific activity of 19269 U/mg and molecular weight of 30 kDa [101].

Submerged fermentation

The production of pectinase by *Bacillus subtilis BKDS1* using pineapple stem extract (PSE) medium by submerged fermentation (Smf) showed that 12.5% of the extract in distilled water was the optimal concentration for producing pectinase enzyme. Plackett-Burman design (PBD) and response surface methodology (RSM) were used to try and improve pectinase production in terms of media optimization. Only four of the nine factors evaluated by PBD—yeast extract, $CaCl_2$, $CaCO_3$, and inoculum were found to significantly influence pectinase production. The data showed that, compared to unoptimized medium, optimized medium yields a larger yield of enzyme. Additionally, it was reported that fermenters produce enzymes at a quicker pace than shaker flasks [102].

Pectinase production by SmF using three fungi strains were tested. The outcomes showed that every strain is able to produce pectinase. The most pectinase activity was found in *Penicillium chrysogenumMF318506* at 0.214 U/ml on the suggested medium for 6 days at 30^0C and 150 rpm. As a carbon source, various agricultural byproducts (orange peel, banana peel, potato peel, pomegranate peel, wheat bran, and rice bran) were screened. With 3% orange peel, 2.0 g/l $(NH_4)_2SO_4$, 6.0 g/l peptone, 6.0 g/l KH_2PO_4, 6.0 g/l K_2HPO_4, 0.1 g/l $MgSO_4.7H_2O$, 0.5 g/l KCl, and 0.1 g/l $FeSO_4$, the maximum pectinase activity of 1.057 U/ml was produced. Using the PBD, the Box-Behken design (BBD), which employs the response surface methodology (RSM), was used to further optimize the chosen parameters. At 2.5% orange peel and 4.5 g/l peptone, 1.292U/ml of pectinase activity was the highest ever recorded. Statistical optimization increased pectinase production from orange peel waste by 6.04 times [86].

Optimization of different cultural conditions (one factor at a time) like pH, temperature, inoculum size and substrate concentration for pectinase production in a SmF by two bacterial strains *Bacillus subtilis TYg4-3* and *Bacillus amyloliquefaciens SW106* revealed that the highest levels of pectinase activity was found at pH 6.0, which gave *Bacillus subtilisTYg4-3* and *Bacillus amyloliquefaciensSW106*, respectively, 1.4550.095 U/mL and 1.6950.01 U/mL. With 0.955 0.006 U/mL for *Bacillus subtilisTYg4-3* and 1.098 0.012 U/mL for *Bacillus amyloliquefaciensSW106*, the optimal temperature for a higher yield was attained at 37^0C. While maltose produced 1.3430.062 U/mL and KNO_3 1.0750.077 U/mL activity for *Bacillus amyloliquefaciensSW106*, lactose produced 1.6550.046 U/mL and NH_4Cl 1.6030.005 U/mL activity for *Bacillus subtilisTYg4-3*. *Bacillus subtilisTYg4-3* produced the most activity at 72 hours, giving 1.275 0.006 U/mL, and *Bacillus amyloliquefaciensSW106* produced the most activity at 36 hours, giving 1.345 0.033 U/mL. At 1.5% and 2.0% of inoculum size and substrate concentration, respectively, *Bacillus subtilis* TYg4-3 produced 2.0830.008 U/mL and 2.0150.036 U/mL. The best enzyme activity was produced by *Bacillus amyloliquefaciensSW106* at 2.0% of inoculum and substrate concentration, yielding enzyme activities of 2.193 0.175 U/mL and 2.256 0.067 U/mL, respectively [103].

Using pectin agar plates to screen seven isolates from damaged fruits and vegetables for pectinase synthesis, the most productive bacterial strain, MPTD1, was discovered to be *Bacillus sonorensis.* Plackett-Burman and Box-Behnken designs were used to optimize various process parameters, and it was discovered that factors like yeast extract, K_2HPO_4, incubation period, $NaNO_3$, and KCl have a negative effect on pectinase production. The synthesis of pectinase is positively influenced by variables including pH, $MgSO_4$, and pectin mass fractions. The highest enzyme activity measured was 2.43 (M/mL)/min. This is the first account of *Bacillus sonorensis* pectinase production by SmF. According to optimization studies, pectinase production was significantly influenced by pH, $MgSO_4$, and pectin mass fractions. The Pareto chart shows that potassium chloride has a negative effect on pectinase synthesis by *Bacillus sonorensis,* but yeast extract, dipotassium hydrogen phosphate, incubation period, and sodium nitrate have a lower impact. With 2% K_2HPO_4, 0.75% $MgSO_4$, 2% yeast extract, 0.5% $NaNO_3$, and 15% pectin, at pH-8.0 and a 24-hour incubation period, the highest pectinase production rate of 2.434 (M/mL)/min was noted. Maximum pectinase production was observed under the following conditions: pH-7.0, 25% pectin, and 0.88% MgSO4 mass fractions, according to optimization utilizing the Box-Behnken design [104]. By using a cup-plate assay, pectinase production by a fungus *Piriformospora indica* was studied. Six days of culture resulted in the highest dry cell weight (10.21 g/L), growth yield (0.65 g/g), specific growth rate (0.56 day1), and pectinase activity (10.47 U/mL) on pectin-containing media (P+). All the parameters were lower in the pectin-free medium (P-) than in the P+ media. *P. indica* produced 2.7 times more pectinase on P+ than it did on P-. For maximum polygalacturonase activity, a pH of 5 and a temperature of 50°C were optimal [105].

Optimization of different physic-chemical parameters for production of pectinase by the two active pectinolytic fungi (P_1 and P_2) and extracted mango peels pectin as a growth substrate under submerged fermentation was carried out and it was reported that the optimal pH, incubation period, temperature and substrate concentration for the highest yield of pectinase were found to be 6, 4 days, 35^oC and 1.5% respectively [106].

Mucor circinelloides and *M. hiemalis* strains were used in a submerged fermentation of tangerine peel to produce pectinase. Temperature, pH, incubation time, inoculum size, and substrate concentration were the five variables that were the subject of experiments that were designed using a Box-Behnken design, response surface methodology (RSM), and analysis of variance. Both *M. circinelloides* and *M. hiemalis* provided high pectinase at their optimal values (38.02 U/mL and 39.76 U/mL, respectively). Pectinase from *M. circinelloides* and *M. hiemalis* was partially purified and the results were 1.74- and 1.99-fold purification with 31.26 and 31.51% recovery, respectively. The maximum activity was seen for pectinase from *M. circinelloides* and *M. hiemalis* at neutral pH and 50 and 60°C, respectively [107].

Two agro-industrial wastes, apricot and peach pomace, used along with orange peels for the production of polygalacturonase from a koji mold *Aspergillus sojae* in a SmF. These three agroindustrial wastes were individually subjected to a Doehlert response surface approach design in shake flasks, which produced 60–80 U mL^{-1} polygalacturonase activity. In bioreactor investigations using a mixture of apricot pomace and orange peel, oxygen limitation issues were resolved and polygalacturonase activity levels of 380 U mL^{-1} were attained by fixing the stirrer speed to 600 rpm and cascading airflow to the dissolved oxygen tension up to 1.7 vvm [108].

It was reported that *Aspergillus sp*. *Gm* isolated from soil sample produced pectinase enzyme in submerged state fermentation using citrus pectin as substrate at a 48-h incubation period, 1% substrate concentration, and 30 °C temperature. The maximum enzyme activity was observed at 30 °C (75.4 U/mL), 5.8 (72.3 U/mL), and 0.5% (112.0 U/mL), respectively, for temperature, pH, and substrate concentration [109].

In a study, pectinase production from *Bacillus subtilisPSE-8* was optimized utilizing response surface methods with cassava peels as the substrate for submerged fermentation. Four independent variables such as temperature, incubation time, and cassava peel concentration pH, were examined at each of 5 levels. The maximum pectinase synthesis (117.5 U/mL), according to data from RSM, occurred at pH 9, 45°C, 3% w/v of cassava peel concentration, and a 3-day incubation period [110].

The production of pectinase was maximized in an investigation by co-culturing *B. pumilus* and*B. Subtilis* utilized apple pomace in submerged fermentation as source of carbon in an optimized solid load and pH for. According to the Box-Behnken response surface methodology, the ideal pectinase activity was obtained with 15% apple pomace (solid load), pH 9.0, and 1/4 of the culture ratio (*B. subtilis/B. pumilus*) at 30°C after 24 hours of fermentation. It was shown that co-culturing *Bacillus* spp. led to a two-fold increase in pectinase synthesis when compared to earlier monoculture *Bacillus* study [111].

SmF method was used for pectinse production by *Bacillus subtilis MF447840* and optimum process parameters were studied. It was reported that maximum pectinase yield of 345 ± 12.35 U was achieved at 5% inoculums, 25% volume ratio, 37°C, pH (7.4) and agitation rate of 120 rpm and 4 days incubation period [112].

In an investigation of production of pectinases from soil actinomycetes, it was observed that the isolate E5 produced maximum yield of pectinases (159.65U/ml) at an initial medium pH of 6, at 35^0C for 6 days and with 5ml of the 5-day old culture. It was reported that incorporation of ammonium sulfate as nitrogen source enhanced the pectinase activity to 187.79U/ml. Optimization by RSM revealed maximum yield of pectinase of 202.7955 U/ml [113].

Production of Polygalacturonase from AR2 pectinolytic bacteria isolated from orange peel and vegetable waste by a SmF using *Raja nangka* banana peel as source of pectin was studied and it was reported that polygalacturonase was produced by AR2 pectinolytic bacteria with the addition of various carbon sources (1% glucose, 1% galactose, 1% lactose) and variation of pectin concentrations (5%; 7.5%; 10%). The highest polygalacturonase activity was obtained by production of polygalacturonase on 1% glucose and 10% pectin medium with a cultivation temperature of 55°C and an agitation speed of 144 rpm for a 48-h incubation time [114].

Present-day market potentials and commercial applications of microbial carotenoids

The COVID-19 crisis is likely to cause the global market for carotenoids to rise at a compound annual growth rate (CAGR) of 2.6%, from its projected value of US$1.7 billion in 2020 to US$2 billion by 2027. The market for astaxanthin, the carotenoid with the highest value due to its potent antioxidant activity, was valued at US$192.5 million globally in 2020 and is anticipated to grow to US$228.4 million by 2027. Due to the low cost of chemical synthesis, carotenoids acquired through the synthetic approach make up the majority of the market, primarily for feed and cosmetics. This is in addition to the use of nutraceutical foods (made of natural carotenoids) as a method of disease prevention and treatment. Natural carotenoids are preferred for human consumption because synthetic ones may have negative health effects [115]. Numerous carotenoids, including carotenes and xanthophylls, are used extensively in the food, feed, nutraceutical, and cosmetics sectors [116].

They have advantageous qualities. They are vitamin precursors, which are necessary nutrients for the maintenance and operation of biological activities including as immunity, reproduction, and eyesight [117]. The use of microbial carotenoids as food colouring, additives, flavours, and functional nutrients in commercial food products is growing, especially in Asia. These applications range from the traditional fermentation of rice by *Monascus* sp. to produce a traditional reddish colour [118].The use of carotenoids as colorants for wine, cheese, meat, and fish. *Brevibacterium linens* produced carotenoids have been employed to enhance the sensory quality of French cheese [119]. In addition to using carotenoids in food, administering bioactive carotenoids in their natural state is another way to promote health. This method reduces the number of free radicals that are produced as a result of reactive oxygen species (ROS) produced during cellular metabolism as a defence against infectious and chemical agents that cause chronic diseases like Alzheimer's, leukaemia, cardiovascular, and eye diseases [120]. The number of businesses producing microbial carotenoids through biotechnology has grown over the past few decades, yet they are still losing money on the market to makers of synthetic carotenoids (Novoveska et al. 2019). This situation arises from the fact that very little natural carotenoid is produced by microorganisms and subsequently extracted from them [121], whereas carotenoid can be produced quickly by synthetic route using cheap chemicals, low-cost process excluding downstream units (extraction and purification) responsible for most of the energy consumption on the process [122]. Natural carotenoids are primarily in trans form, while synthetic carotenoids can also arise in cis and are typically generally racemic (although isomerization can also be done). Due to the attractive potential for large-scale production using various types of cultivation systems (raw materials), and consumers' preference for natural products over synthetic ones due to rising health consciousness, the production of carotenoids by microorganisms is a rapidly expanding industry [123]. However, due to the high cost and low production of natural carotenoids, synthetic counterparts must be produced in order to meet consumer demand. In addition to increasing release yield, using crude enzymes reduces the need for large amounts of organic solvents and ensures that hydrolyzed extracts have a higher antioxidant potential [125]. The release of bioactive compounds like carotenoids from plant tissues by enzyme-assisted hydrolysis is a good biotechnological option because the cost of producing crude enzymes is inexpensive.

Conflict of interest: Author is having no conflict of interest

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CITATION OF THIS ARTICLE

Ghadge Amit Babasaheb. Importance of pectinase in preparation of enzymatic cocktails for valorization of horticultural wastes into natural carotenoids: an updated overview. Bull. Env.Pharmacol. Life Sci., Vol 12[6] May 2023: 246-261