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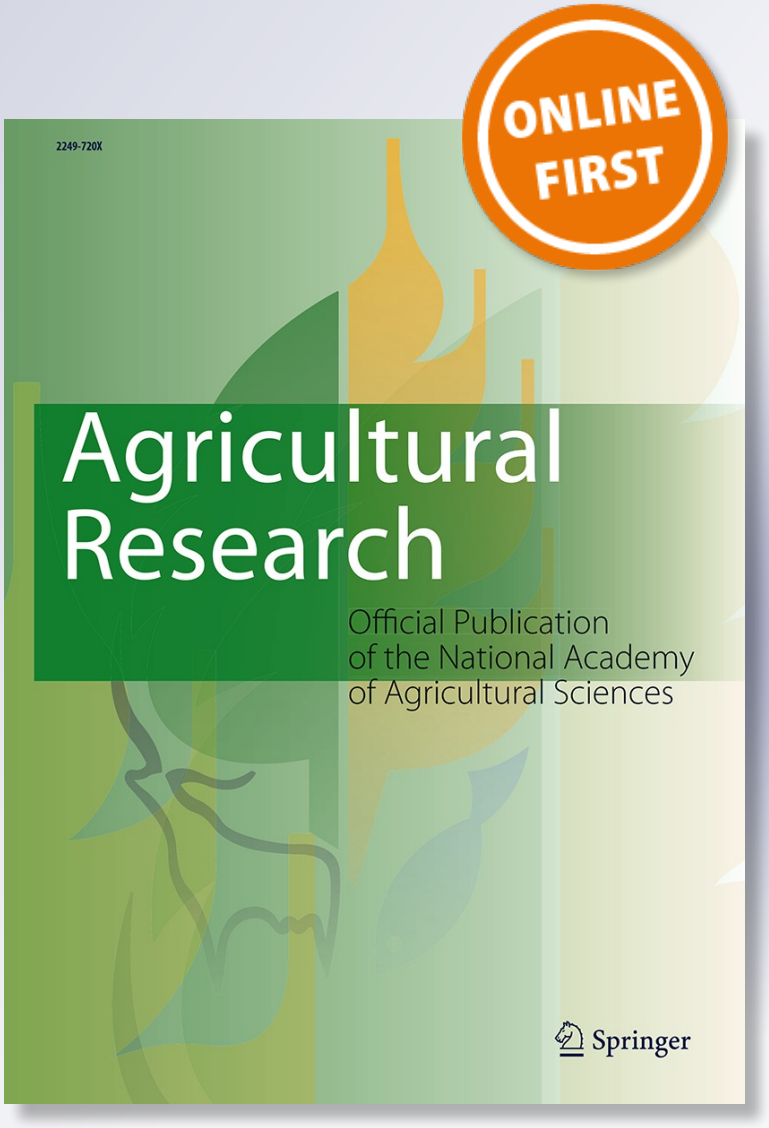

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FULL-LENGTH RESEARCH ARTICLE

Enzyme-Assisted Extraction of Carotenoid-Rich Extract from Red Capsicum (*Capsicum annuum*)

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Abstract Development of aqueous carotenoid-rich extract (ACE) is a major challenge for the food industry looking for natural colourants. Red capsicum an excellent source of carotenoids has been explored as a novel source for development of ACE through enzymatic liquefaction (EL). Three carbohydrases enzymes viz. viscozyme L, pectinase and cellulase were tested for their liquefaction effects and ability to recover higher carotenoids in aqueous extract. EL significantly ($p < 0.05$) improved the extract yield and total soluble solids by 2.5-fold to threefold in comparison with unliquefied extract. Incremental increase in dosage of enzymes significantly ($p < 0.05$) improved the extract yield, total carotenoids, phenolics, ascorbic acid content and antioxidant activity. Viscozyme and pectinase caused significantly higher recovery of carotenoids and other bioactives than cellulase. Viscozyme at dosage of 0.3 % at 60 °C gave the best results. Processing residue or pomace, a spin-off from the EL, was dried to capsicum pomace powder (CPP) and developed as a functional ingredient. The ACE and CPP had higher carotenoid content ranging from 41.72 to 279.83 mg/100 g, respectively. Valorization of capsicum through EL is a promising approach to recover concentrates as valuable food ingredient with reduced processing waste and thus providing sustainability to environment through green processing.

Keywords Red capsicum · Aqueous carotenoid-rich extract · Viscozyme · Valorization · Functional ingredient

Introduction

Consumption of carotenoid-rich fruits and vegetables has been strongly advocated for reducing the risk of cancers or cardiovascular disorders. Overwhelming epidemiological evidence suggests the positive role of dietary carotenoids as antioxidants, anti-carcinogenic, anti-inflammatory and anti-allergic agents [24]. Red capsicum, among commonly consumed vegetables, has rich bioactive composition, being a rich source of carotenoids, ascorbic acid, phenolics, flavonoids and capsaicin. It is a unique source of oxygenated carotenoids, capsanthin and capsorubin, which are exclusive to this genus, and acts as precursors of β-carotene [10].

Among vegetables, red capsicum has the highest content of total carotenoids (30.37 mg/100 g fw), which far exceeds that of any other preferred sources of vegetable for carotenoids such as carrots (8.0–10.0 mg/100 g) and tomato (4–8 mg/100 g) [33]. Considering its dense carotenoid composition, it is a suitable candidate vegetable for extraction of natural pigments and other bioactives.

Vegetable sources are being seen as promising candidates for extraction of natural colourants, considering increasing consumer concern over synthetic colourants. Commercial interest in methods for production of natural carotenoid pigments is increasing. Restrictions on several certified food colours have stimulated further interest in this subject. The main advantage of using natural food colourants is that they need not be certified and thus can be listed as ingredients simply as "colourants".

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There are numerous reports on extraction of carotenoids from vegetables sources; however, the preferred sources for extraction have been mainly tomato peels [25] and carrots [45]. The extraction procedures are usually solvent assisted and thus unsafe for human consumption posing safety hazards, toxicological concerns, environment requiring risk high-energy input while producing low product quality. Growing demand for green and healthy processes has given rise to alternate processes which are mild, eco-friendly and efficient, reduce by-products and avoid several operational conditions [31]. Green technology is the need of the hour keeping in view the increasing environmental issues such as pollution and global warming [37]. EL ensures faster extraction, higher recovery, reduced solvent usage and lower energy consumption when compared to non-enzymatic methods (solvent extraction or supercritical fluid extraction). EL helps in improving the extraction yields of bioactive compounds by disrupting the cell walls of the plant tissues and helps in releasing bound pigments, phenolics and flavonoids that otherwise would be unavailable and lost in press residues [2, 41].

In the light of the above considerations, EL of red capsicum was explored for developing an ACE. Three carbohydrases enzymes viz. viscozyme L, pectinase and cellulase were tested for their liquefaction ability in terms of recovery of total carotenoids, total phenolics, total flavonoids and ascorbic acid in the extract. The intent was valorization of red capsicum for developing extracts to be used as natural colourant and as functional ingredient in foods.

Materials and Methods

Raw Materials

Mature fruits of *Capsicum annuum* L. commonly known as sweet pepper cultivar "Bomby" (soluble solids 8–9 %; moisture content 92–93 % (wb)) at the red ripe stage grown under the green house of the Indo-Israel project at the Indian Agricultural Research Institute (IARI), New Delhi, were selected.

Upstream and Downstream Process for Enzymatic Liquefaction

Sweet pepper fruits of uniform colour and size were used for enzymatic liquefaction. Each replicate comprised of randomly selected 10 healthy fruits free from visible blemishes or disease. Fruits were thoroughly washed under running water, cut into pieces (3×2.5 cm^2) and immediately blanched at 90 °C for 2 min in a thermostatically controlled water bath for softening and inactivating endogenous enzymes to prevent autoxidation of phenolic compounds. This step also hastens the softening of the subsequent macerate for quick release of bioactives from the fibrous matrix. Blanched softened fruits were macerated followed by heating at 90 °C for 1 min to inactivate the endogenous polyphenol oxidase. The enzymatic liquefaction was performed as described by Khandare et al. [16] with slight modifications. Briefly, the crushed macerate was poured to amber coloured bottles and mixed with relevant enzyme preparations, namely pectinase (from *Aspergillus niger, P 2611,* Sigma-Aldrich, >3800 U/ml), viscozyme L (from *Aspergillus aculeatus,* V 2010, Sigma-Aldrich, FBU/g ≥100) and cellulase (from *Trichoderma reesei* ATCC 26921, C 2730, Sigma-Aldrich, >700 U/g). These enzyme preparations were added at the three dose levels (enzyme/mash ratio: 0.10, 0.20 and 0.30 %), mixed thoroughly and placed in a thermostatically controlled incubator with shaker (Innova 42, New Brunswick Scientific) for 1 h at 60 °C. Viscozyme incubation time and temp have been optimized through response surface methodology and those are reported in separate paper. Liquefaction was carried out at the intrinsic pH of red capsicum (4.5), which falls within optimum activity of enzymes (3.3–5.5) [3]. Control sample was incubated without enzymes in water bath at 60 °C. At the end of the incubation period, the liquid and solid (non-degraded solid) phases were separated by pressing in a stainless-steel hydraulic press (Johnston Automation, India) using nylon filter bags under 2600 lb/m^2 pressure for 60 s. The extract was heated at 90 °C for 1 min and packed in clean sterilized glass bottles. Unliquefied extract without enzymes served as control. The extract yield was subsequently used for analysis of bioactives and functional properties.

Development of Capsicum Pomace Powder

The processing residue generated during EL (moisture content, 70.39 % wb) was comminuted in a domestic blender (Inalsa, India), loaded in aluminium trays with a tray load of 0.30 g/cm^2 and air-dried in a convective drier (MAC®, Macro scientific works®, Delhi, India) operating at 60 °C. Residue was physically encapsulated with 3 % maltodextrin (based on the preliminary trials), uniformly spread with the help of a steel roller and dried to moisture content of 8.0 ± 0.5 % wb. The dried residue or capsicum pomace powder (CPP) was crushed in a domestic blender (Inalsa, India), vacuum packed (Indvac, Ahmedabad, India) and stored at ambient temperature (25–30 °C) till analysis.

Proximate Analysis

Chemical attributes of capsicum pomace powder (8.0 ± 0.5 %, wb) such as crude fibre, moisture content,

crude protein, lipids and water activity were evaluated using Association of Official Analytical Chemists [4] standard protocols. Water activity (a_w) was measured at 25 °C using Rotronic Hygrolab C1, USA. Total soluble solid (°Brix) was measured with a hand-held refractometer (Atago, Tokyo, Japan) and was calibrated with distilled water. Ascorbic acid was measured as described by Klopotek et al. [17].

Extraction of Phenolics and Antioxidants

Capsicum was cut into small pieces and sequentially homogenized in a domestic blender (Inalsa, India) for 2 min. For extraction of total phenolics, 5 g of homogenized samples was extracted twice with 30 ml of ethanol (80 %), by stirring and sonicating for 30 min in the dark. This was followed by centrifugation for 15 min at 10,000×*g* at 4 °C. The supernatant was then vacuum concentrated at 40 °C in a rota-evaporator and stored at − 20 °C. The concentrated sample was used as sample extract for estimation of total phenolics and hydrophilic antioxidant activity.

Total Phenolic and Flavonoid Content

Total phenolics were estimated spectrophotometrically using Folin–Ciocalteu reagent [39]. Results were expressed as gallic acid equivalent (mg GAE/100 g). Flavonoid content was measured using aluminium chloride method [46]. The absorbance of the solution was measured using UV–Vis spectrophotometer (Varian Cary 50). The results were expressed as quercetin equivalent (mg QE/100 ml).

Extraction and Analysis of Total Carotenoids

Total carotenoid content was determined by extracting the samples twice with acetone until the extract was colourless and filtered through a Whatman filter paper No. 1. The filtrates were combined and transferred to separating funnel containing 50 ml of 4 % aqueous NaCl and 100 ml of petroleum ether (BP 40–60 °C). Absorption of the petroleum ether layer was measured at 470 nm in dim light using UV–Vis spectrophotometer (Varian Cary 50).

Estimation of β-Carotene Content

Extracts obtained by solvent extraction were analysed using HPLC fitted with 600 quaternary pump, with auto-injector (20 µL loop), and a 2998 photodiode array detector (Waters Corp., Milford, MA, USA) and a 250 × 4.6 mm diameter and 5-µm C30 YMC column (YMC Co. Ltd., Ireland). The mobile phase comprised of isocratic mixture of MTBE: methanol (80:20, v/v), and it was run at a flow rate of 1 ml min^{-1}. Carotenoids were monitored at a wavelength of 450 nm.

Antioxidant Activity

FRAP assay was performed according to the procedure described by Benzie and Strain [7]. The cupric ion reducing antioxidant capacity of berries was determined according to the method of Apak et al. [5]. Standard curve was prepared using different concentrations of Trolox (100–1000 µM). The results for CUPRAC were expressed as µmol TE/100 ml, using molar absorptivity of Trolox as 1.67×10^4 mol^{-1} cm^{-1}. Antioxidant activity in terms of TEAC was measured using ABTS decolouration method using radical ABTS (2,2-azino-bis-(3-ethylbenzthiazoline-sulphonic acid) [34]. DPPH (2,2-diphenyl picrylhydrazyl) assay was carried out on the basis of scavenging ability of antioxidants towards the stable radical DPPH [8].

Functional Properties

Water retention capacity (WRC) and swelling water capacity (SWC) were measured according to the procedure of Robertson et al. [35]. Oil-holding capacity (OHC) was measured according to the procedure of Sosulsky and Cadden [40].

Statistical Analysis

The results of experiments were subjected to one-way ANOVA followed by post hoc analysis using Tukey's method. All measurements were taken in triplicate.

Results and Discussion

Extract Yield and Total Soluble Solids

Higher extract yield and total soluble solids are always desirable for food processing and pharma industry. In the present study, effect of three cell wall degrading enzymes on extract yield and total soluble solids was investigated (Figs. 1, 2). Viscozyme L and pectinase caused significantly ($p < 0.05$) higher liquefaction as evident by the increased extract yield (80–87 %). However, no apparent liquefaction was observed with cellulase. Significant improvements in percentage yield of aqueous extract and total soluble solids (TSS) were observed with increasing dosage of all enzymes ($p < 0.05$). Viscozyme L at 0.3 % resulted in highest yield of 87.5 % vis-à-vis 32.5 % in ULE depicting a 2.5-fold increase. Concomitant increase in TSS (°brix) was also observed along with increased extract yield. ULE had TSS of 3.23° in comparison with 9.11°brix

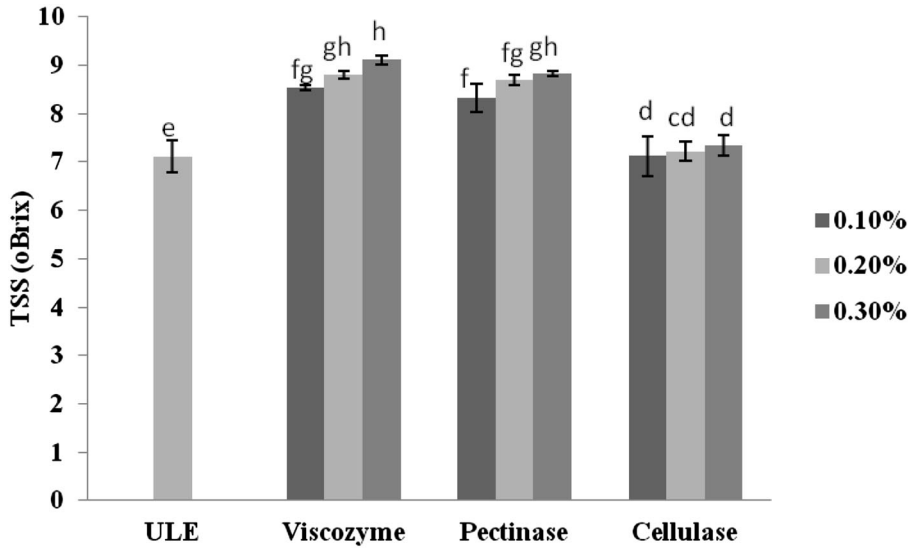

Fig. 1 Effect of different carbohydrases enzymes on total soluble solids (TSSs) of capsicum extract

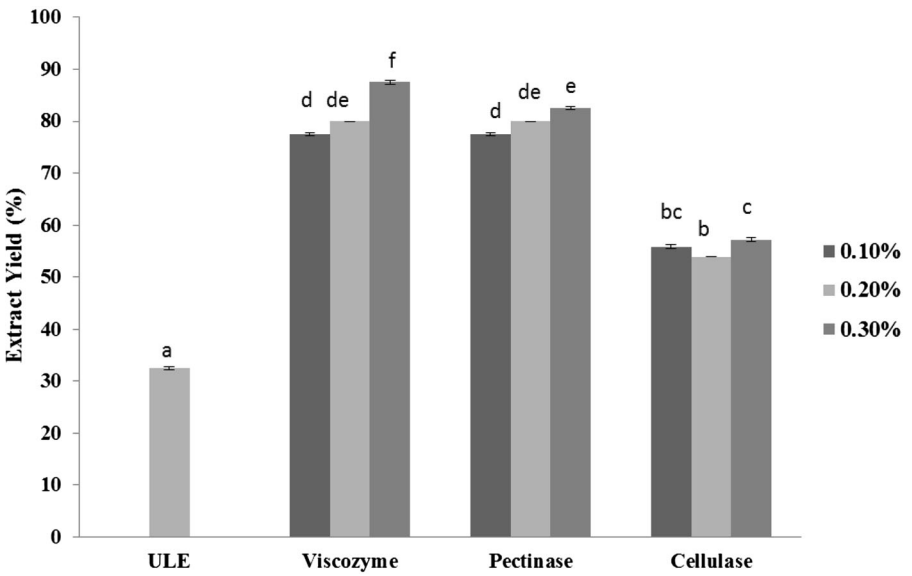

Fig. 2 Effect of different carbohydrases enzymes on extract yield of capsicum extract

in liquefied extract. These results are in agreement with the reports of previous researchers where pectolytic activity has been shown to increase extract/juice yield and TSS in carrot, Zizyphus and dates [1, 16, 18]. Extraction efficiency has been shown to improve through enzyme-assisted extraction. In a study by Kulshreshtha et al. [19], commercial proteases and carbohydrases enzymes significantly improved ($p \leq 0.001$) the biomass yield (40–70 % dry matter) as compared to aqueous extraction (20–25 % dry matter). Enzymatic liquefaction promotes in catalysing degradation of pectin and middle lamella in the plant cell wall, which acts as putty and binds to water. Significant pectin-degrading activity due to the presence of pectin esterase, pectin lyase and polygalacturonase catalyses the degradation of the smooth regions of the pectic substance and release of bound water and results in free flowing juice.

Total Carotenoid and Ascorbic Acid

Carotenoids are the major lipophilic antioxidants present in capsicum and perform many health-promoting functions in human body system. EL significantly ($p < 0.05$) improved the recovery of total carotenoids in the aqueous extract (Fig. 3). The content in ULE was 19.04 mg/100 g, which increased to 41.37 mg/100 g in extract liquefied with viscozyme L. (@ 0.3 %), depicting an overall increase by twofold. Among the three enzymes evaluated, viscozyme L and pectinase gave the highest recovery of total carotenoids

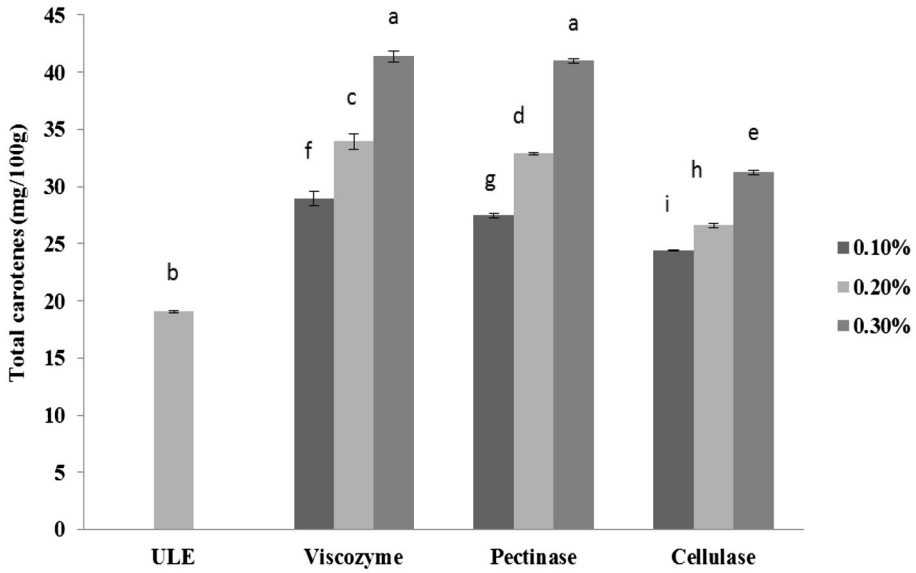

Fig. 3 Effect of different carbohydrases enzymes on total carotenes of capsicum extract

followed by cellulase. Increasing dosage levels of viscozyme L from 0.1 to 0.3 % registered significant increase by 33 % (Fig. 3). The liquefaction capacity in decreasing order was viscozyme L > pectinase > cellulase. Improved recovery of total carotenoids through EL in ACE may be due to coupled effect of enzymatic hydrolysis, homogenization, grinding and thermal processing, which helps in softening the plant tissues and breaking cell wall and releases the carotenoid pigment from protein–carotene complex, thus increasing its extractability [11]. Our results are in agreement with the study conducted by Cinar [9] on effects of enzyme concentrations and extraction time on the colour yield of carotenoid pigments from orange peel, sweet potato and carrot using cellulase and pectinase combinations. Similarly, Santamaria [38] reported that two-stage enzymatic process in chili guajillo puya resulted in extraction of 83 % carotenoids. Increased extractability also improves bio-accessibility and bio-availability [43]. A report by Strati et al. [42] showed maximum content of total carotenoids (127 mg/kg d.w.) and lycopene (89.4 mg/kg d.w.) in samples treated with enzymes in combination with ethyl lactate (solvent:solid = 10:1 ml:g) in comparison with control sample. The present results demonstrate the enhanced ability of cell wall degrading enzymes on extraction of bioactive carotenoids from cell matrix. All carotenoids have colour enhancing and functional properties. As colour is crucial marker for capsicum-based product, enzyme-tailored processing could be suitable eco-friendly alternative to present method of product development hitch to not only enhance colour, but also improved bioactivity too.

A marginal increase in ascorbic acid content was also observed in the extract liquefied with enzyme (Fig. 4). The ULE had ascorbic acid content of 138.38 mg/100 g, which increased to 160.96 mg/100 g on liquefaction with 0.3 % viscozyme. Occurrence of high ascorbic acid content in conjunction with high carotenoids in ACE is beneficial, as the presence of natural ascorbic acid acts as an antioxidant and helps circumvent the problems associated with oxidation of carotenoids, thus improving their stability. Numerous reports on positive effects of added ascorbic acid on stability of carotenoids support our results [27]. Carotenoid extract obtained by different ASE conditions was estimated by HPLC and characterized by LC–ESI–MS. Lutein and β-carotene were resolved at 29.9 min (Fig. 5).

Total Phenolics Content and Total Flavonoid Content

Phenolic compounds are important contributors to functional quality and have most important role in counteracting ROS, thus minimizing molecular damage. EL significantly ($p < 0.05$) improved the recovery of total phenolic content in the ACE, and liquefied extract had twofold higher content than the ULE (Fig. 6). Increasing dosage level of viscozyme L, pectinase and cellulase caused sharp increase in the total phenolic content; however, the order in decreasing manner was viscozyme L > pectinase > cellulase. Highest recovery of total phenolics (61.75 mg GAE/100 g) was observed in extract liquefied with 0.3 % viscozyme, vis-a-vis a content of 33.91 mg GAE/100 g in ULE. Unlike phenolics, the total flavonoid content showed a progressive moderate increase with EL. Viscozyme L enhanced the content to 57.81 mg/100 g from initial value of 51.23 mg/100 g in ULE (Fig. 7).

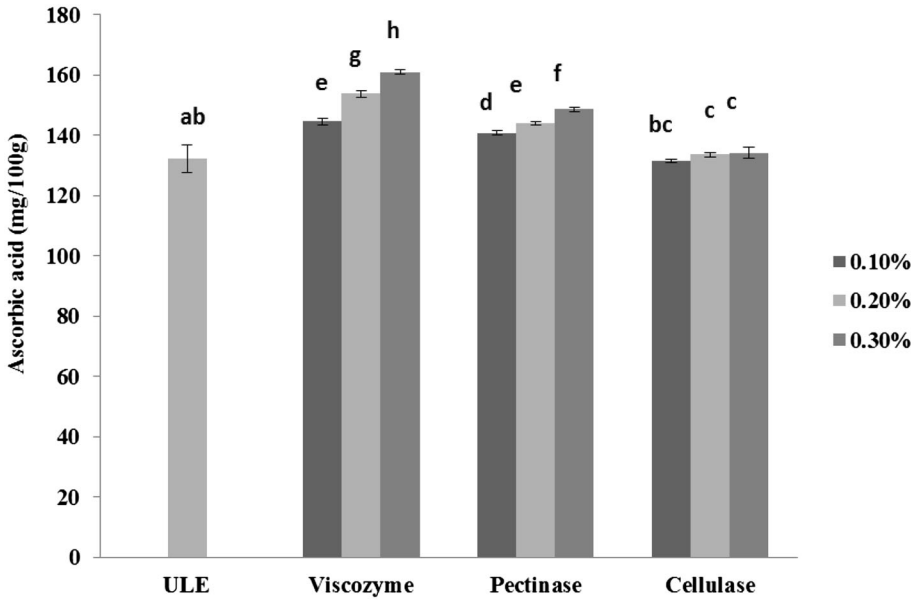

Fig. 4 Effect of different carbohydrases enzymes on ascorbic acid content of capsicum extract

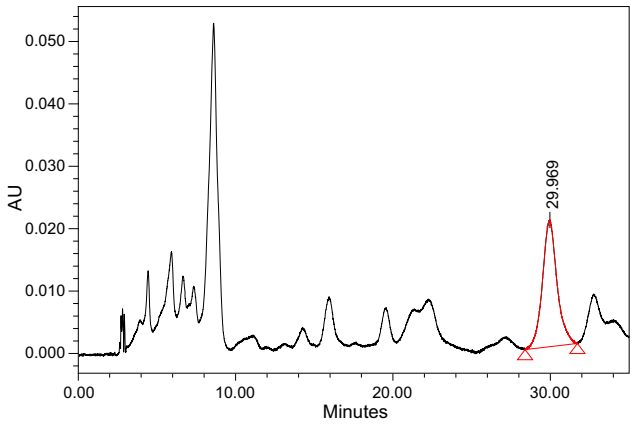

Fig. 5 HPLC chromatogram of red capsicum extract

These results demonstrated that EL releases bound phenolics from fibrous plant matrix, accounting for their increased content in the extract. Phenolic compounds complex with cellulose, hemi-cellulose and pectin through hydrophobic interaction and hydrogen bonding, which are subsequently released due to the hydrolytic activity of enzymes. In this context, different pectinolytic enzyme preparations have been used in processing industry and in general found that they are the most efficient at degrading polysaccharides and release of these bound phenols [28]. In the present study, three enzyme preparations performed well with different degrees of efficiency. The difference in the efficiency of enzyme in degrading the cell wall may be due to their inherent enzymatic activity. Cellulase enzyme acts on cellulose matrix of cell wall, whereas other two enzymes have multi-enzyme activities. Pectinase mainly has pectin trans-eliminase, polygalacturonase, pectinesterase activities with small amount of cellulase and hemi-cellulase activities. Viscozyme is a multi-enzyme preparation, consisting of arabanase, cellulase, Q-glucanase, hemi-cellulase and xylanase activities. As plant cell wall consisted of mainly pectin, cellulose and hemi-celluloses, the enzyme preparation having cellulases, hemi-cellulases and pectinases activity can efficiently hydrolyse the bonds in plant cell polysaccharides and released bound bioactive compounds. Similar reports on grapes and apple pomace confirm our findings [23]. Results on viscozyme and pectinase corroborate with findings of Koley et al. [18] and Oszmianski et al. [26] in Zizyphus juice and apple pomace.

Overall, viscozyme L gave the best results and caused twofold increase in total carotenoid and total phenolic content in ACE. Evans et al. [12] also reported fourfold high pectinase activity in viscozyme when compared to other enzymes. Synergistic actions of these together probably account for a greater degree of tissue breakdown, releasing more soluble components, higher extract yield, TSS and ascorbic acid in ACE. EL disrupts numerous hydrophilic and hydrophobic bonds of cellular matrix exposing cell wall sites for easy recovery of bioactives such as polyphenolics and carotenoids in the extract [28].

Antioxidant Activity

The evaluation of antioxidant activity in food sample is becoming increasingly important in the field of nutraceutical research as it provides useful information with regard to health-promoting functional quality of food material without the analysis of each antioxidant compound [18]. In the present study, four in vitro assays, namely FRAP, CUPRAC, TEAC and DPPH, have been used. Antioxidant activity of juice also

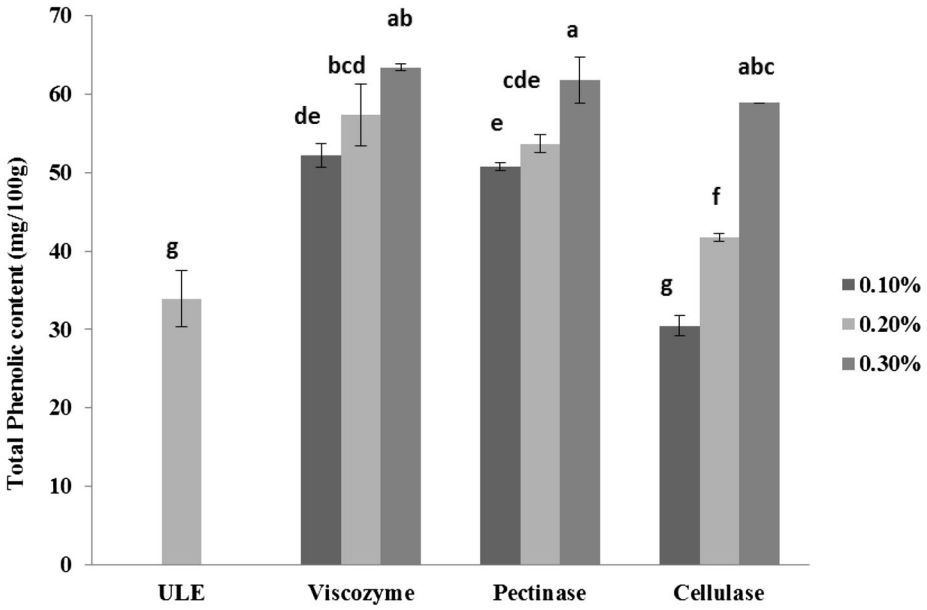

Fig. 6 Effect of different carbohydrases enzymes on total phenolic content of capsicum extract

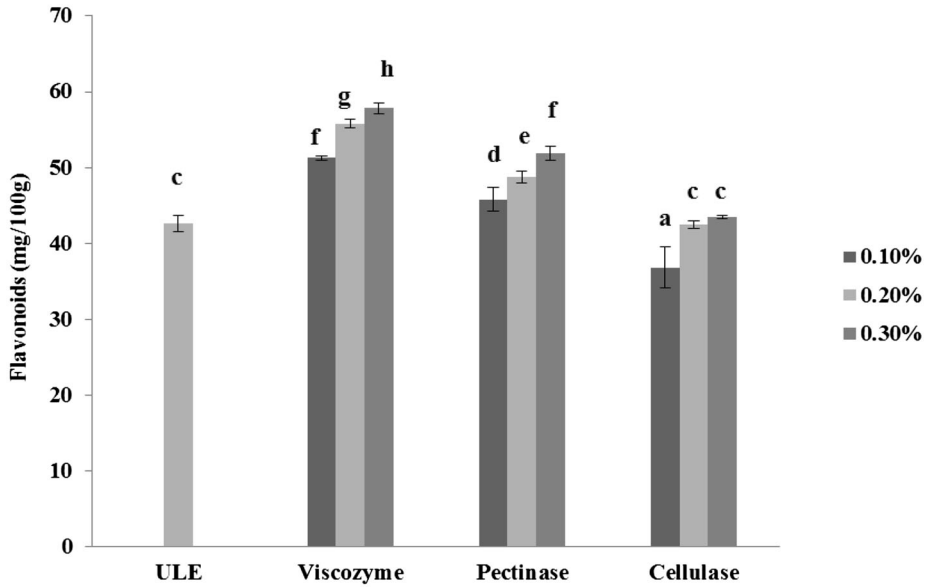

Fig. 7 Effect of different carbohydrases enzymes on flavonoid content of capsicum extract

dramatically improved after enzyme-assisted processing. The values ranged from 49.1 to 182.8 µmol/ml, respectively, in ULE and juice treated with viscozyme at 0.3 % depicted 3.7-fold increase over control (Table 1). Almost similar trend was observed for other enzymes. The efficiency of enzyme in increasing antioxidant potentiality of juice is in the order of viscozyme > pectinase > cellulase. High AOX in enzyme-assisted extract can be attributed to high recovery of hydrophilic antioxidants as reported in many other crops viz. ber (*Ziziphus mauritiana*) [18] and black carrot (*Daucus carota* subsp. *sativus*) [16].

It is interesting to compare the values of the AOX activities of the different liquefied extracts obtained by different assays. The values in the decreasing order were TEAC > CUPRAC > FRAP > DPPH. The difference in the results may be due to their mechanism of reaction. Although the above four methods were based on electron transfer reaction-based methods, FRAP and CUPRAC assays reflect the metal reducing power and DPPH and TEAC assays reflect organic radical scavenging power of dietary antioxidants [14]. Therefore, it is wise to compare the results of FRAP *vs* CUPRAC and TEAC *vs* DPPH.

Overall values of antioxidants in the CUPRAC assay were significantly higher than FRAP values. The similar difference in the value also has been reported recently for fruits [18] and vegetables [15]. High antioxidant activity in

Table 1 Effect of different carbohydrases enzymes on antioxidant capacity of ACE

	FRAP			CUPRAC		
Enzyme concentration (%)	0.1	0.2	0.3	0.1	0.2	0.3
Enzymes						
Viscozyme	165.8 ± 0.026^{g}	174.9 ± 0.008^{h}	182.8 ± 0.006^{i}	247.9 ± 0.064^{j}	257.7 ± 0.037^{k}	265.7 ± 0.032
Pectinase	147.1 ± 0.016^{f}	165.1 ± 0.029^{g}	175.3 ± 0.032^{h}	205.8 ± 0.004^{g}	217.1 ± 0.012^{h}	229.5 ± 0.018^{i}
Cellulase	117.8 ± 0.013^{c}	126.5 ± 0.002^{d}	135.8 ± 0.019^{e}	149.1 ± 0.021^{d}	156.8 ± 0.021^{e}	196.4 ± 0.008^{f}
Amylase	65.4 ± 0.002^{b}	67.4 ± 0.003^{b}	64.7 ± 0.004^{b}	101.76 ± 0.002^{b}	112.4 ± 0.003^{c}	114.7 ± 0.004^{c}
Control (ULE)	49.1 ± 0.051^{a}			94.8 ± 0.013^{a}		
	TEAC			DPPH		
Enzyme concentration (%)	0.1	0.2	0.3	0.1	0.2	0.3
Enzymes						
Viscozyme	292.8 ± 0.012^{i}	311.3 ± 0.025^{j}	341.6 ± 0.121^{k}	69.6 ± 0.031^{e}	76.4 ± 0.002^{f}	83.4 ± 0.004^{g}
Pectinase	252.9 ± 0.055^{g}	276.4 ± 0.038^{h}	294.5 ± 0.100^{i}	68.4 ± 0.029^{e}	70.4 ± 0.009^{e}	79.1 ± 0.029^{f}
Cellulase	168.5 ± 0.025^{d}	199.9 ± 0.071^{e}	224.9 ± 0.056^{f}	51.7 ± 0.039^{c}	58.7 ± 0.011^{d}	67.1 ± 0.038^{e}
Amylase	149.7 ± 0.003^{bc}	159.3 ± 0.002^{cd}	145.7 ± 0.004^{b}	26.5 ± 0.004^{ab}	27.5 ± 0.003^{b}	25.8 ± 0.001^{ab}
Control (ULE)	121.2 ± 0.123^{a}			23.1 ± 0.009^{a}		

Mean ± SD (μmol TE/100 ml)

Values are mean of three replicates ± standard deviation. Means with the same superscript do not differ significantly from one another ($p > 0.05$)

FRAP ferric reducing antioxidant power, *CUPRAC* cupric ion reducing antioxidant capacity, *TEAC* Trolox equivalent antioxidant capacity, *DPPH* 2,2-diphenyl-1-picrylhydrazyl, *ULE* control, unliquefied extract

CUPRAC may be attributed due to the presence of flavonoids such as quercetin and kaempferol in capsicum extract. The antioxidant potency of flavonoids is roughly proportional to the total number of –OH groups and is positively affected by the presence of an o-dihydroxy moiety in the B-ring [5]. Moreover, FRAP is electron transfer (ET)-based method. In ET methods, the reactivity is based on the deprotonation and ionization potential of the reactive functional group. In general, ionization potential values decrease with increase in pH, reflecting electron-donating capacity with deprotonation [29]. FRAP assay works on a low pH range. Therefore, in acidic conditions, the reducing capacity may be restrained due to protonation of antioxidant compounds [13]. Thus, low antioxidant activity is obvious when it measured through FRAP when compared with CUPRAC. Beside this, in FRAP assay, fixed end-point determination always results in underestimation of antioxidant potential of many compounds. Although FRAP assay relies on the hypothesis that redox reaction proceeds so rapidly that all reactions are complete within 4 min, for many phenolic compounds like caffeic acid, ferulic acid and quercetin, the absorption does not stop at 4 min; instead, it continued to increase even after several hours of reaction time [30]. However, CUPRAC method provides sufficient time to phenolics or other complex molecule for completion of their reaction.

Similar to metal reducing power, the overall values in TEAC assay were higher than those in DPPH assay. This may be accounted to matrix interference from coloured compounds (carotenoids) in extract. According to Arnao [6], higher the sample colour, the smaller the absorbance decrease and lesser is the measured AOX, even when working with minimal sample volumes (5–10 μl). Since the capsicum carotenoids absorbed maximally 427–478 nm, colour interferences with DPPH chromogen, which has an absorption maxima at 515 nm, likely resulted in the relatively low measured activity. Beside this, Huang et al. [13] reported that many antioxidants that react quickly with peroxyl radicals may react slowly or may even be inert to DPPH. Thus, there are chances of presence of antioxidants, which react with ABTS but remain inert to DPPH. Moreover, many phenolics may react slowly to DPPH compare to ABTS. Thus, fixed end-point determination may lead to underestimation of antioxidant potentiality measured through DPPH assay. In addition to above fact, solubility difference among the chromogen, i.e. DPPH and ABTS, resulted in different observations. ABTS ions dissolve in both aqueous and organic solvents. However, DPPH is soluble only in organic media (especially in alcoholic media) but not in aqueous media. As a result of presence of certain amount of water content in the solvent, the antioxidant capacity is decreased consequently part of

DPPH get coagulated thereby becoming inaccessible for reaction with antioxidants [21]. Additionally, DPPH chromogen is accessible to only those antioxidants compounds, which dissolve in alcoholic media, whereas ABTS chromogen is accessible to all antioxidants dissolved in alcohol and aqueous media.

In the present study, it is very difficult to judge regarding the suitability of specific assay to evaluate the antioxidant potentiality of enzymatically liquefied product. In fact, any above four methods neither accurately reflect the mechanism of action of all radical nor accessible to all antioxidants in a complex food system. But they give a clear understanding that, on liquefaction with increased enzyme concentration, antioxidant potentiality of extract has increased. The difference in AOX with respect to enzyme applied can be due to their inherent ability to degrade pectinaceous food matrix, thus releasing antioxidants with different proportion.

Composition of Capsicum Pomace Powder

Processing residue or discard from red capsicum generated as a spin-off during EL process was found to be a rich source of carotenoids as evident from its high colour values (L*: 38.78, a*: 42.78 and b*: 40.59; Table 2). The residue was thus dried to yield powder and thereof referred to as capsicum pomace powder (CPP).

Proximate Composition

Interestingly, CPP could be a useful functional ingredient, on similar lines with fibre ingredient developed from tomato and carrot peels [25]. Table 3 summarizes the proximate composition and functional quality of the CPP. It was found that CPP had lower protein and lipid content and higher fibre content as compared to tomato peels [25]. High proportion of dietary fibre in CPP can be used as an indigestible insoluble ingredient in food industry.

Bioactive Compounds

The total carotenoid content of CPP was 279.83 mg/100 g, depicting nearly 90 times higher content than those reported in other fibres viz. tomato and carrot peels (3–4 mg/100 g). The high carotenoid content of CPP comprising of β-carotene and capsanthin makes it an attractive source of colouring pigment in terms of nutraceutical supplement and natural colourant for increasing food value. The ingredient has already been tested in our laboratory for functionalizing and developing carotenoid-rich bread, chapatti (flat bread), papad, muffins, yoghurt, tomato soup and sauce (unpublished data). In all cases, enrichment resulted in high colour values and increased carotenoid content. Considering the fact that there is widespread prevalent malnutrition and VAD (vitamin A deficiency) in India, the ingredient seems to have enormous commercial value for various food applications.

The ascorbic acid content in CPP was 346.07 mg/100 g, which is almost in range (318.4–400 mg/100 g) of the content reported in dried aonla powder, considered to be the richest source of ascorbic acid [32]. The presence of high ascorbic acid in CPP is an added advantage as its antioxidant action imparts higher oxidative stability to colouring pigments carotenoids.

Phenolic content in CPP was 384.60 mg GAE/100 g dwb, which is nearly twofold the content reported in tomato peel fibre (158.1 mg GAE/100 g) [25] and other sources, for example orange (*C. aurantium* cv. Canoneta) (0.51 mg GAE/100 g), orange (*C. sinensis* cv. Valencia) (0.16 mg GAE/100 g), and lime (*C. urantifolia* cv. Persa) (0.35 mg GA/100 g) [20].

Table 2 Colour attributes of fresh capsicum, enzyme recovered juice and CPC

Samples	*L**	*a**	*b**
Raw capsicum (RC)	30.87	29.82	10.22
Capsicum juice (CJ)	45.20	39.58	64.19
Capsicum powder concentrate (CPC)	38.78	42.78	40.59

Table 3 Chemical, bioactive, antioxidant activity and functional properties of capsicum pomace powder

Chemical composition	
Moisture (%)	8.0 ± 0.35
Water activity (aw)	0.45 ± 0.003
Lipids (%)	2.93 ± 0.07
Protein (%)	6.63 ± 0.72
Crude fibre (%)	31.90 ± 0.13
Bioactive compounds	
	0 Months
Total carotenoids (mg/100 g)	279.83 ± 8.38
Total phenolic content (mg/100 g)	384.60 ± 5.19
Ascorbic acid (mg/100 g)	346.07 ± 4.65
Antioxidant activity (μmol TE/g)	
DPPH	14.33 ± 0.356
FRAP	9.42 ± 0.090
CUPRAC	11.64 ± 0.475
ABTS	31.39 ± 0.567
Functional properties	
Oil-holding capacity (g oil/g)	1.62 ± 0.025
Swelling capacity (ml water/g)	1.40 ± 0.055
Water retention capacity (g water/g)	0.468 ± 0.003

Data are mean values of triplicate determination ± standard deviation

Antioxidant Activity

The AOX activity of CPP ranged from 9.42 (FRAP) to 31.39 (TEAC) μmol TE/g. (Table 3). As expected, the values were higher than those reported in fibres from tomato, carrot, passion fruit, pineapple and guava (2.5–6.0 μmol TE/g) [22, 25]. High AOX is a consequence of higher phenolic compounds, ascorbic acid and carotenoid content, which are major contributors to AOX.

Functional Properties

Functional quality evaluated in terms of WRC, OHC and SWC relates to the physical and chemical structures of polysaccharide and dietary fibre components such as cellulose and hemi-celluloses in the materials (Table 3). WRC of CPP, an indicator of insoluble dietary fibre, was 46.8 g/g, which is in close agreement with the values reported elsewhere [44]. The OHC value of CPP was again in close agreement 1.62 (g oil/g) with that reported for banana fibre-rich powder (2.2 g oil/g fibre) [36] and tomato peel fibre (1.46 g oil/g fibre) [25]. SWC of CPP, an indicator of cellulose, was 1.40 ml water/g sample, on par with guava fibre (4.60 water/g sample) but lower than mango (1.4 ml water/g sample) [22].

Based upon the rich functional quality, it seems that CPP can be used as a promising functional ingredient for fortification of foods such as bakery and extruded snacks. Incorporation of this ingredient in different foods will not only enrich the food in terms of its provitamin-A carotenes but also impart rich vibrant colour with high sensory scores and hence higher consumer acceptability.

Optimized Process for Enzyme-Assisted Liquefaction of Red Capsicum

It is evident from above results that maximum liquefaction was observed with viscozyme L. This enzyme alone resulted in highest recovery of all bioactive compounds including total carotenoid content, total phenolic content, total flavonoids and total antioxidant activity with high TSS and extract yield. Viscozyme L at 0.3 % resulted in more than 87 % juice yield as compared to ULE (32.5 %), showing 2.5-fold increase over control. Simultaneous increase in TSS (°brix) was also observed along with increased juice yields. ULE had TSS of 3.23° in comparison with 9.11°brix in liquefied extract. Total carotenoids in ULE were 19.04 mg/100 g, which increased to 41.37 mg/100 g in extract liquefied with viscozyme L at 0.3 %, thereby showing an overall increase by twofold. Ascorbic acid content in ULE was 138.38 mg/100 g, which increased to 160.96 mg/100 g on liquefaction with 0.3 % viscozyme L. Extract liquefied at 0.3 % viscozyme L gave highest recovery of total phenolics 61.75 mg GAE/100 g *vis-a-vis* a content of 33.91 mg GAE/100 g in ULE. Antioxidant activity also revealed same results after enzyme-assisted processing. The values ranged from 49.1 to 182.8 μmol/ml, respectively, in ULE and juice treated

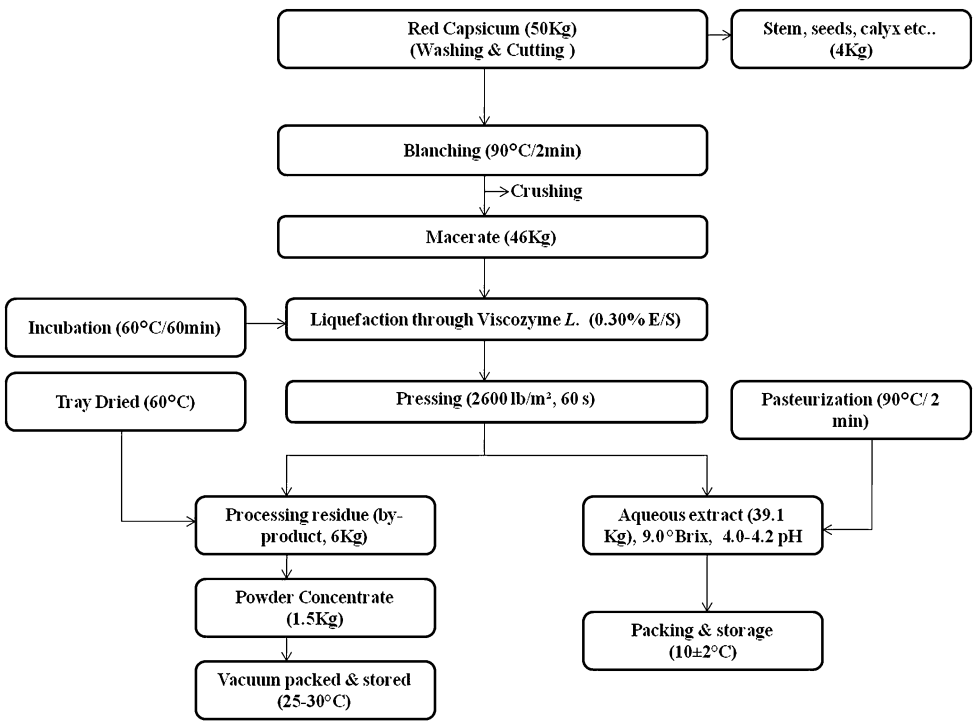

Fig. 8 Optimized scheme for total valorization of red capsicum for development of functional ingredients

with viscozyme at 0.3 % depicted 3.7-fold increase over control. Also, the CPP developed as a part of the process can serve as a promising food ingredient in bakery products, extruded snacks, traditional foods such as murukku and sev as evident from above results.

Based upon these observations, a pilot-scale process was optimized for 50 kg red capsicum (*var.* Bomby) as a raw material. The process flow chart has been illustrated in Fig. 8. The process gave around 39.1 kg of ACE and 1.5 kg of capsicum pomace powder.

Conclusions

The results of this study demonstrate that viscozyme L with multi-enzyme preparations (pectinolytic, cellulolytic and hemi-cellulolytic activities) is a promising enzyme, which can effectively facilitate liquefaction of red capsicum, and allows the recovery of carotenoids and other bioactive components from the vegetable under mild process conditions for enhanced yields and thus recovery of a ACE with high antioxidant activity. Keeping in view the current problem of huge waste disposal arising from food processing industry, this study also demonstrates that the discard generated during EL can be converted into a functional ingredient to be used as a source of natural colourant for development of nutraceutical food products with high fibre content. Thus, the study underscored that transformation of red capsicum into value-added products could not only be an economic opportunity for the agri-food sector, but also contribute to environmental protection.

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