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ABSTRACT

"CAROTENOID RICH COMPOSITION AND PROCESS OF ITS PREPARATION"

The present invention relates to heat stable carotene rich composition extracted through a green process. The process is solvent free and highly effective for extraction of the carotenoids and other bioactives from plant matrix. The process employs the use of chemical facilitators or cell wall degrading enzymes to increase extraction efficiency. More particularly, the invention relates to a process of preparation of carotene, phenolic and ascorbic acid enriched products from biological resources such as capsicum through a novel enzyme mediated process.

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To
The Controller of Patents
The Patent Office Branch, New Delhi

CLAIMS

We claim:

1. A process of preparation of stable carotenoid rich composition comprising the steps of:

   a. selecting, washing and cutting of biological source;

   b. blanching the said biological source at 90–95 °C for 1–3 minutes;

   c. maceration/communition of the blanched pieces obtained in step b ;

   d. treating the homogenate obtained in step c. with cell wall degrading enzymes;

   e. incubating the mixture of step d. at a temperature range of 55–65 °C for 60–90 minutes;

   f. pressing, clarifying the mixture of step e. to obtain juice;

   g. heat processing the juice of step f. at temperature range of 80–90 °C for 1 minute;

   h. concentrating the juice of step g. to 3–5 folds under vacuum at temperature range of 50–60 °C;

   wherein,

   the said enzymes used are selected from the group of but not limited to pectinases, viscozyme, amylases hemicellulase, cellulase, or combination thereof; and

   the said enzymes are used in the range of 0.2–0.8% to enhance juice yield and extractability of phenolic components from the fibrous cell wall matrix.

2. The process of preparation of stable carotenoid rich composition as claimed in claim 1, wherein the vegetables of the genus *Capsicum* comprises bell pepper (red).

3. The process of preparation of stable carotenoid rich composition as claimed in claim 1, wherein the said blanching is performed preferably at a temperature range of 90–95 °C preferably for duration of 1–3 minutes.

4. The process of preparation of stable carotenoid rich composition as claimed in claim 1, wherein the said incubation is performed preferably at a temperature range of 55–65 °C preferably for duration of 60–90 minutes.

5. The process of preparation of stable carotenoid rich composition as claimed in claim 1, wherein the said heat processing of the juice is performed preferably at a temperature range of 80–90 °C preferably for duration of 1 minute.

6. The process of preparation of stable carotenoid rich composition as claimed in claim 1, wherein the said concentration is achieved preferably at a temperature range of 50–60 °C.

7. A stable carotenoid rich composition having enhanced phenolic contents, ascorbic acid, such that the said composition is taste neutral, solvent free and has high nutraceutical value; characterized in that:

    a. the carotenoid content in the said composition ranges from 42.68–85.23 mg/100ml in single strength juice and concentrated composition respectively

    b. phenolic content in the said composition ranges from 101.90–165.67 mg GAE/100ml in single strength juice and concentrated composition respectively.

    c. the antioxidant activity of the said composition ranges from 1.67–4.52 µmol TE/ml (TEAC) single strength juice and concentrated composition respectively.

8. The stable carotenoid rich composition as claimed in claim 7, wherein the said composition is obtained from a vegetable of the genus *Capsicum* such as bell pepper (red).

9. The stable carotenoid rich composition as claimed in claim 7, wherein the said composition is used as functional and nutraceutical ingredient in bakery, beverages and confectionaries etc. with high degree of stability of carotenoids.

10. The stable carotenoid rich composition as claimed in claim 7, wherein the said composition is in the form of but not limited to juice, syrup, paste, powder, aqueous based concentrate, alcohol based concentrate or other similar formulations.

11. The stable carotenoid rich composition as claimed in claim 7, wherein the said composition is used as colorant ingredient in cosmetics.

12. A stable carotenoid rich composition and process of preparation of the same substantially as described herein with reference to the foregoing description, examples and drawings.

Dated this.....day of....., 2013

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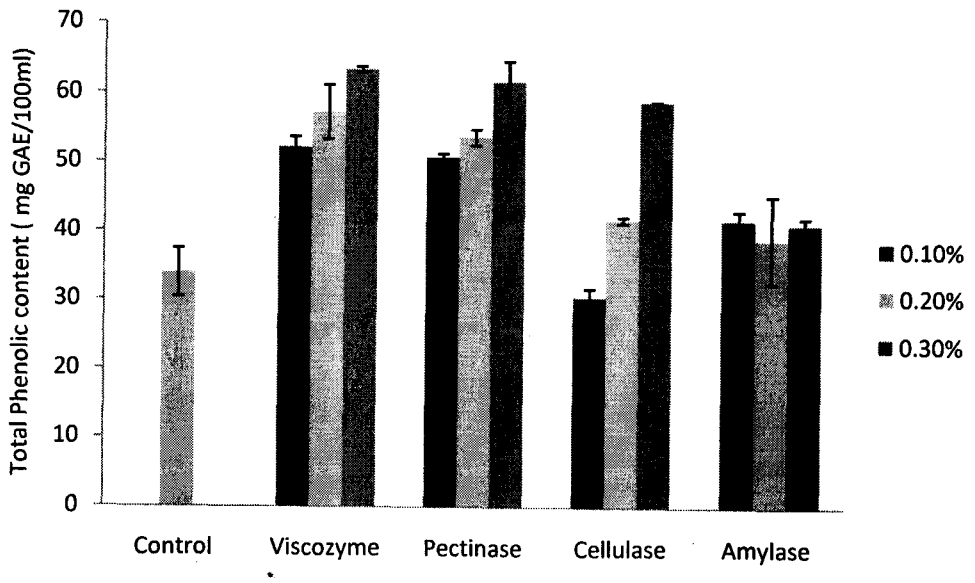


FIG. 1

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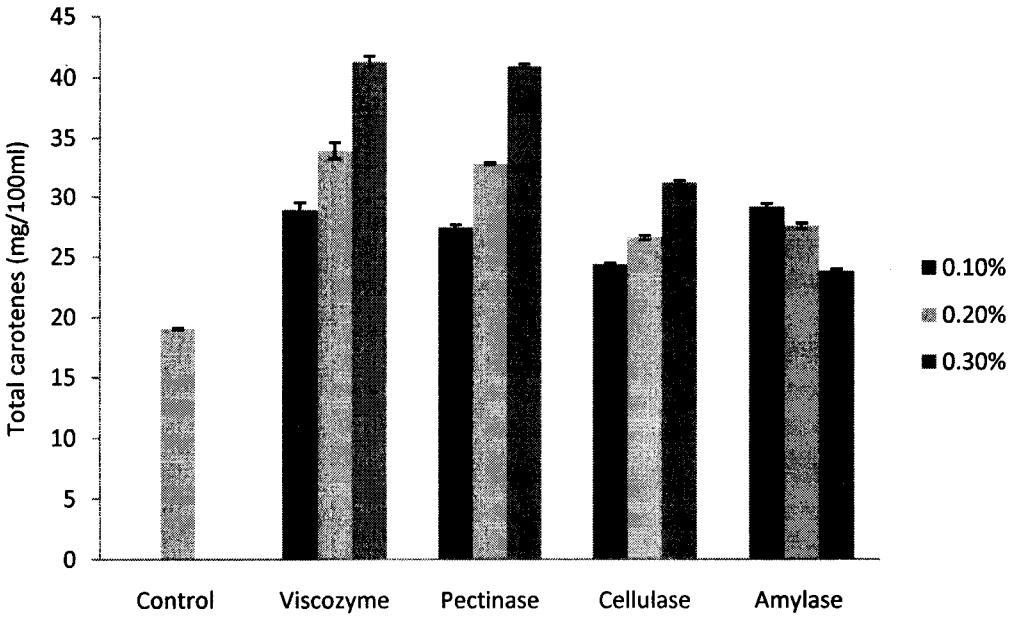


FIG. 2

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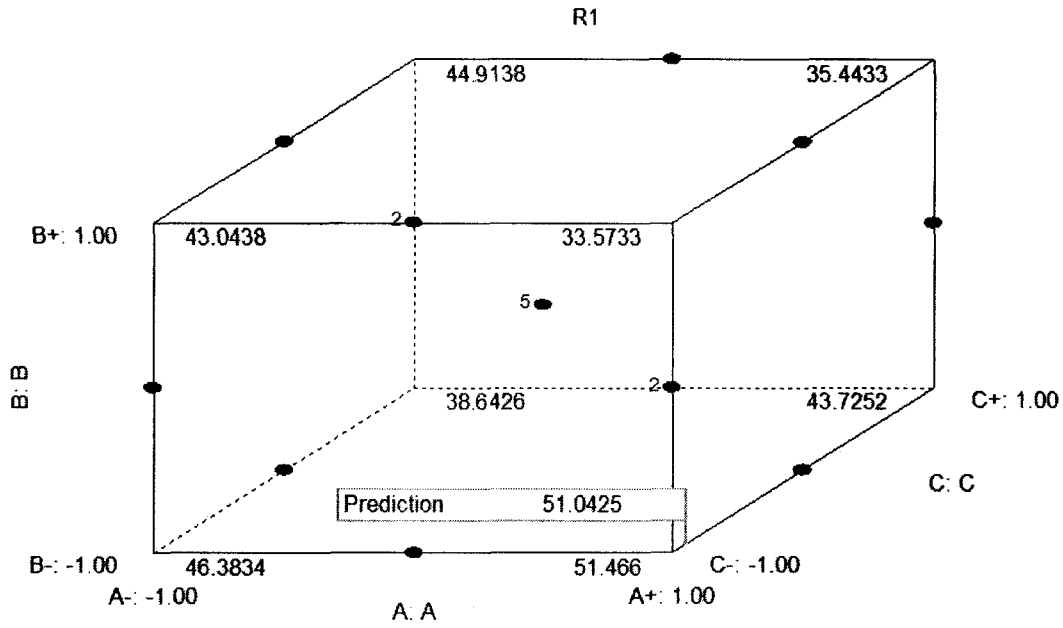


FIG. 3

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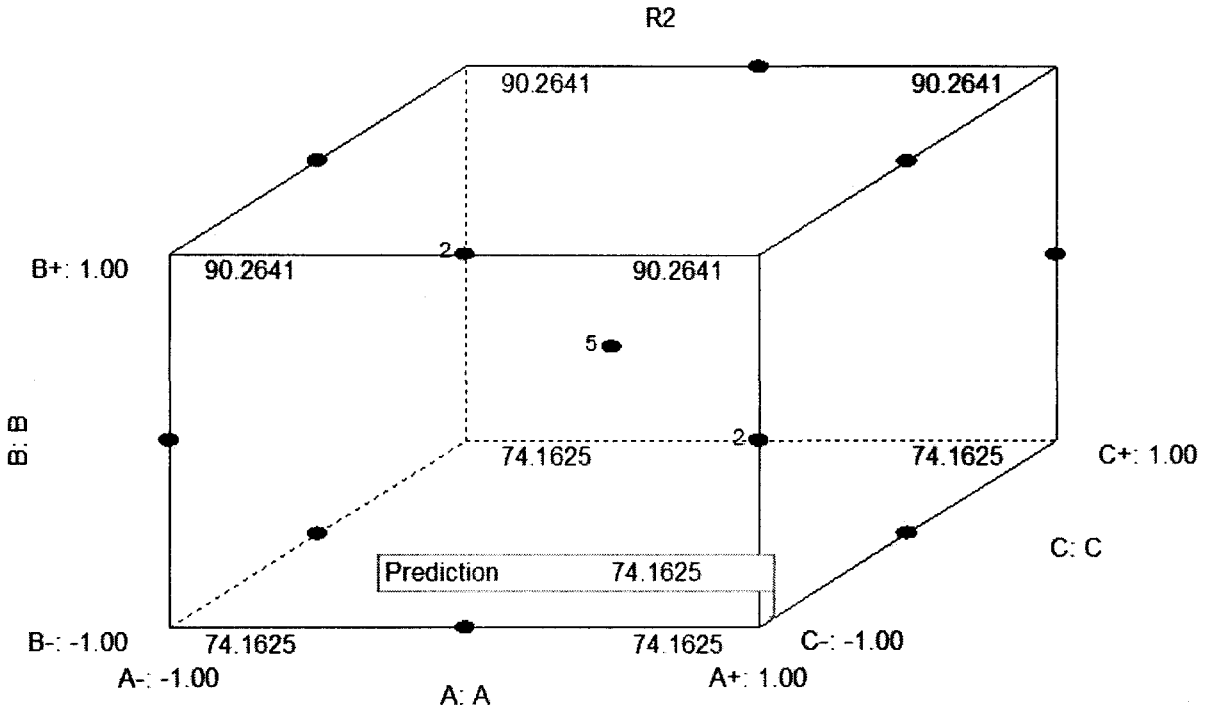


FIG. 4

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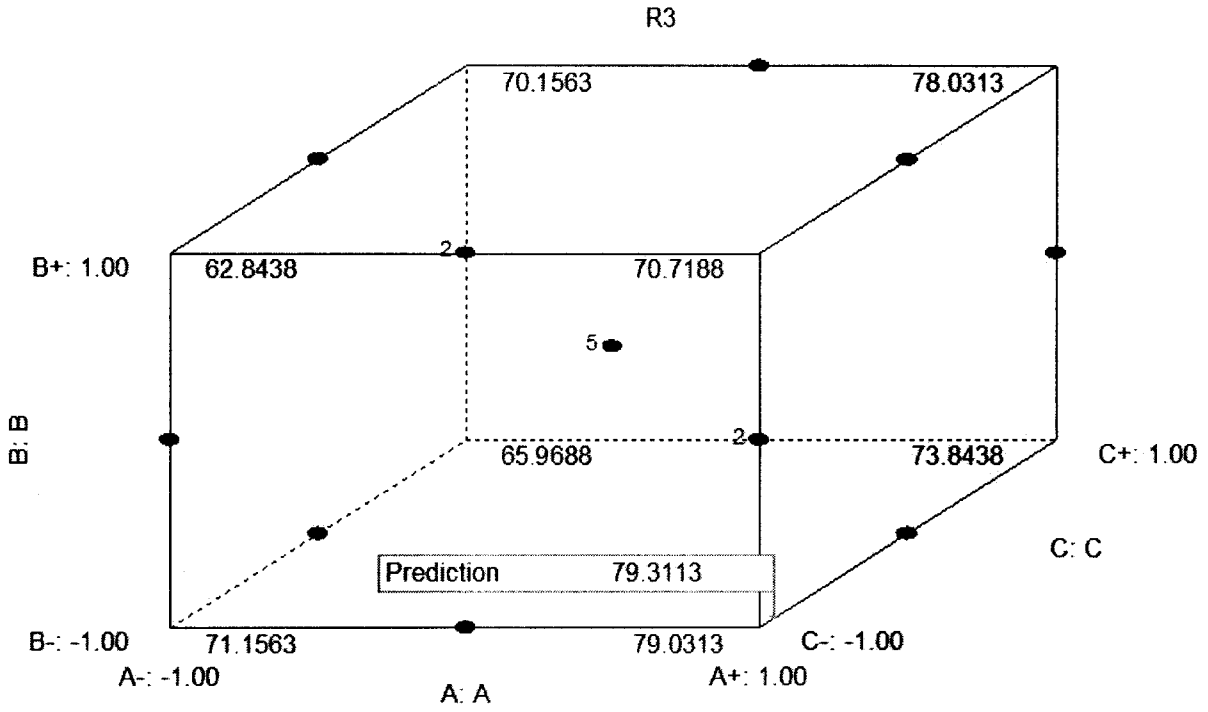


FIG. 5

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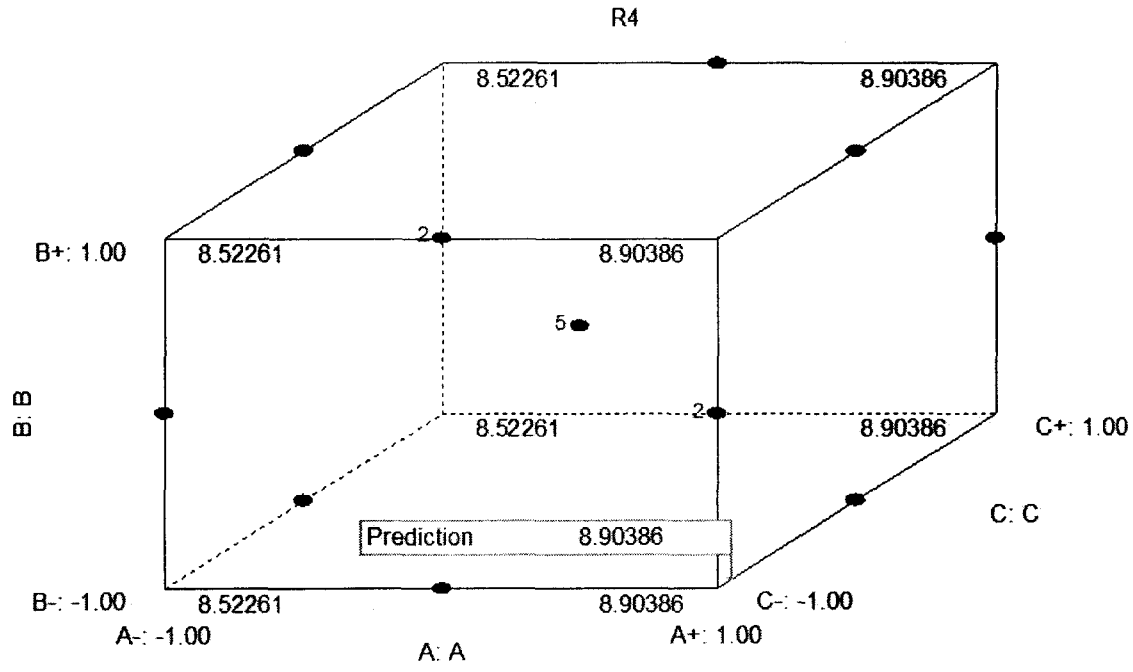


FIG. 6

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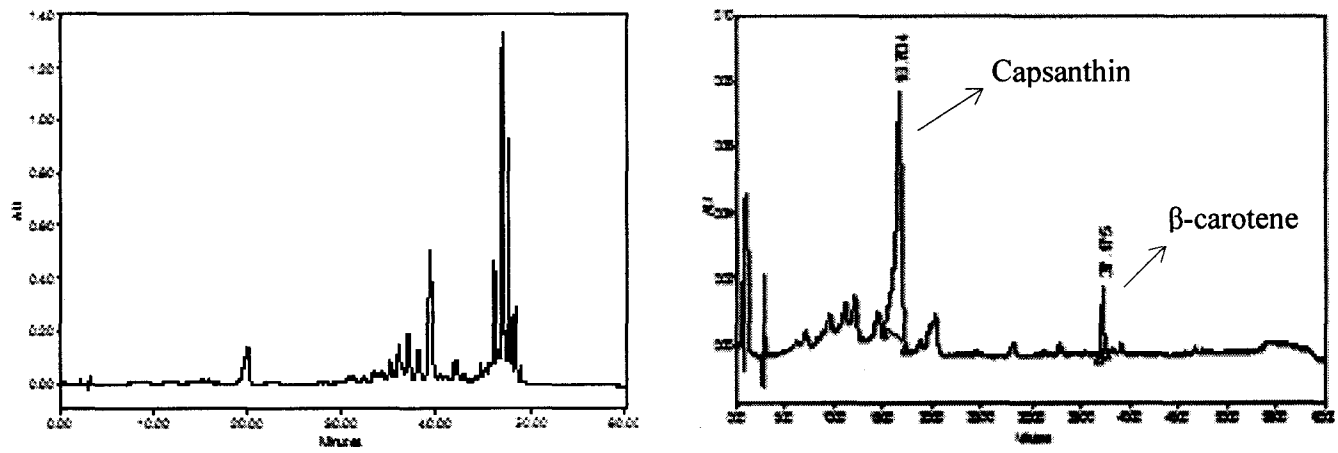


FIG. 7

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FIELD OF THE INVENTION

The present invention relates to carotenoid rich composition and its process of extraction from a vegetable source. More particularly, the invention relates to a solvent free extraction method for extraction of carotenoids and its product containing mixture of carotenoids such as β-carotene and capsanthin.

BACKGROUND OF THE INVENTION

The carotenoids form a group of pigments which have a yellow or red color and confer the characteristic colors on many food and cosmetic stuffs. Carotenoids are isoprenoid compounds, with an extensive conjugated double bond system. They are divided into two main classes; carotenes [acyclic (lycopene) and cyclic (beta-carotene)] and xanthophylls (e.g., capsanthin).

Overwhelming scientific evidence suggest that carotenoid-rich vegetables and fruits may provide a mechanism for preventing oxidative damage in vivo, and thus carcinogenesis. Epidemiological studies have moreover shown that frequent and regular consumption of carotenoids reduces the risk of chronic disorders, including cardiovascular disorders, and has a beneficial effect on cancer prevention. This protective function of the carotenoids is seen both in their action as antioxidants and, as in the case of beta-carotene, in their provitamin A activity [Journal of the American Dietetic Association, 97: 991-996 (1997)].

Among vegetables, rich in carotenoids, red capsicum has the highest content of total carotenoids (30.37 mg/100g FWB) which far exceeds that of any other vegetable; carrots (8-10 mg/100g), spinach (17.31 mg/100g), broccoli (1.56 mg/100g), cabbage (0.43 mg/100g), and asparagus (0.06 mg/100g) (Muller, 1997). Paprika is a unique vegetable

with a synergistic mix of carotenoids include β-carotene, zeaxanthin, lutein, capsanthin, capsorubin and cryptocapsin, which confer substantial nutritional and medical values.

Capsanthin occurs naturally together with some other xanthophylls (zeaxanthin, lutein and capsorubin) and β-carotene in Capsicum fruits. Capsanthin is hydrophobic and forms aggregates or adheres non-specifically to structural surfaces. A xanthophyll capsanthin, which is a typical and major carotenoid in red paprika (sweet pepper), inhibited more effectively the photooxidation of linoleic acid, polyunsaturated fatty acid, than β-carotene, lycopene, and lutein, and acts as an antioxidant against free radical attack and singlet oxygen exposure in plasma lipoprotein.

Capsanthin appears to rank highest among natural carotenoids in its capacity for quenching singlet oxygen and scavenging free radicals. Thus, it is supposed that capsanthin also has an anticarcinogenic potential as other antioxidants.

Epidemiological studies have shown that frequent and regular consumption of carotenoids, such as paprika carotenoids, reduces risks of chronic disorders, such as cardiovascular disorders (Kohlmeier L et al. 1995. Am. J.Clin. Nutr. 62: 137-146) or cancer (Murakoshi et al, 1992.Cancer Res. 52: 6583-6587; Levy et al.1995. Nutr. Cancer 24: 257-267; and Tanaka et al., 1994. Carcinogenesis 15: 15-19).

Paprika cultivars are used commercially to produce powders, oleoresins and concentrates as food colorants. These products are typically very rich in carotenoids. Paprika is not genotoxic and Grashorn et al. (2001) tested two preparations of paprika oleoresins and confirmed a linear increase in yolk color due to addition of paprika oleoresins.

The total carotenoids content in most known commercial paprika cultivars varies from 25 to 30 milligram per /100 gram fresh fruit weight (Crit. Rev. Food Sci. Nutr. 24:245-355; and Mingez-Mosquera et al. 1992. J. Agric. Food Chem. 40:2384-2388). In comparison, rich carotenoids tomato cultivars contain less than 0.15 milligram carotenoids per gram

commercial carrot cultivars do not contain more than 5-7 mg/100g fresh weight of total carotenoid content (Baranski et al, 2012).

KR 8905256 patent discloses a process for the preparation of a red pepper paste, where the process comprises: scalding raw red pepper in boiling water (85-100°C) for 4-8 minutes and cooling, cutting and mixing, adding cell-collapsing enzyme preparation composed of pectinase, cellulase and hemicellulase etc produced by *Aspergillus niger* and incubate, remove the skin of the above-treated red pepper by pulp finisher, heating to 90-100°C for 1-5 minutes, concentrate by ordinary method.

US 3615721 patent discloses treating vegetable and fruit material with enzymes having cellulase, hemi-cellulase and pectinase activity, mechanically treating the material to form a slurry or paste, followed by shaping and drying the material.

US 7572468 patent describe the methods for extraction of carotenoids from carotenoid-containing plant material using an extraction solvent comprising ethyl lactate. The method also includes the use of an ethyl lactate-ethanol blend as the extraction solvent. After extraction, the solvent containing the extracted carotenoids is separated from the extracted plant solids and treated to separate the dissolved carotenoids from the extraction solvent and obtain a carotenoid-containing concentrate.

EP 1423020 patent describes the extraction of carotenoids using solvent extraction from carotenoid-containing plant matter comprising of tomato paste, carrots, dried carrots, wolfberry fruit (*Cycium barbarum*), corn and *Dunaliala* biomass wherein the Brix in said plant matter is greater than 10 degrees brix, comprising of the following steps: (i) Mixing the plant matter with water to achieve brix not greater than 10 degrees. (ii) Crushing the mixture from stage (i) and separating the solids from the liquid to obtain two phases, pulp and serum. (iii) Extracting the pulp to obtain carotenoid-containing plant oleoresin.

EP 09498872 patent describes the extraction of carotenoids from paprika, red pepper, pungent chilli, or other spice plants, e.g., of the genus Capsicum containing carotenoid pigments, with an edible solvent in a series of mixing and high temperature and pressure mechanical pressing steps using edible solvent and a counter current extraction procedure.

Santamara et al., (2000) conducted solvent extraction of carotenoids from capsicum using ethanol as solvent. However, enzyme assisted extraction is still to be explored in this area.

Enzyme mediated extraction is the recent approach for extraction of important bioactives and health promoting compounds such as phytochemicals from plant matrix (Nattaporn et al., 2011) and this has proved to be a potential alternative to conventional solvent based extraction methods.

Use of solvents release only the most accessible and feebly attached phenols from the plant matrix whereas enzymes can serve as chemical agents applying conditions making phytochemicals more easily and efficiently extractable (Costaya et al., 2010).

The patent (Muller and Steinert, US2005/0129820A1), describes the composition from capsicum and process thereof. The invention describes a composition treated with a combination of enzymes for development of a composition puree/ paste with high content of vitamin C. The inventors describe the process for preparation of puree with high content of vitamin C, using enzymes assisted process. The main objective of enzyme assisted process was to facilitate maceration and softening of tissues, so as to develop a composition high in vitamin C. The invention makes no reference to the extraction of other bioactive compounds mainly carotenoids and phenolics which constitute a major phytochemicals of capsicum. The enzymatic efficiency was not achieved, as the level of enzyme used was lower than the optimum and was only to facilitate maceration and not liquefaction of the vegetable tissue.

On the basis of the above prior art, it is clear that there is an urgent need to develop a cost effective and solvent free method for processing of capsicum for extraction of not only carotenoids, but also other relevant phytochemicals such as capsaicin, ascorbic acid and phenolics.

The present invention describes the optimized upstream and downstream variables *viz.* enzymes used, enzymes concentration, incubation temperature and incubation time, for obtaining a composition/ aqueous extract rich in carotenoids, phenolics and other phytochemicals. The said composition is heat stable and shows high stability of bioactives when appropriately packed and stored. The said composition is different in terms of its original raw material product and the process of extraction. The inventive steps and enzyme composition involved are different in comparison to the mentioned prior art. Infact it is an aqueous extract free from organic solvents. The targeted vegetable is *Capsicum annuum* (red sweet pepper) which is distinctly different from pungent types. The developed composition also has carotenoid pigments in the natural state still bound to the proteins thereby imparting greater oxidative stability to the extract.

Furthermore the present invention also exemplifies the complete utilization of the pomace generated in the process by suitably converting the same into a valuable functional ingredient which further can be used as a natural food colorant and as a source of a dietary fibre concentrate, for enhancing carotenoid content and nutraceutical value of various foods such as bakery products, soups, sauces, yogurts and functional breads or muffins.

In addition, enzyme assisted processing exemplifies the following benefits accrued vis-à-vis pure extraction protocols:

1. **Single step process:** Aqueous extraction is a single step process followed by downstream operations to get a concentrated product.
2. **A food based approach:** An aqueous extract mimics a food, in the sense that it contains a synergistic blend of all phytochemicals as found in a food product which

2. **A food based approach:** An aqueous extract mimics a food, in the sense that it contains a synergistic blend of all phytochemicals as found in a food product which together results in higher antioxidant activity than a single pure compound or a supplement.
3. **Provides stable yellow/orange hue:** The food industry is looking for natural yellow/orange hues in foods such as bread, confectioneries, ice creams, and dairy food products. The industry is still utilizing synthetic colors and coal tar dyes for developing food products therefore there is an urgent need to develop colors from natural sources.

OBJECT OF THE INVENTION

The principal object of the present invention is to utilize a novel source of bioactive carotenoids, ascorbic acid, phenolics and capsaicin from genus *Capsicum* and to develop an enzyme mediated optimized process for a carotenoid rich concentrate. The developed concentrate can be used as a natural colorant, for phenolic and carotenoid enrichment. In addition the concentrate also improves the nutraceutical quality of the food products.

Yet another object of this invention is to utilize a unique and unconventional source of carotenoids other than tomatoes and carrots.

Yet another object of this invention is to obviate the disadvantages associated with the conventional, solvent mediated process of extraction of carotenoids from the natural sources and thereby developing a green process of extraction.

Yet another object of this invention is to provide an aqueous extraction method of carotenoids together with other phenolics and bioactives such as capsaicin and ascorbic acid.

Yet another object of this invention is to obviate the use of artificial or synthetic colors from food products.

Yet another object of this invention is to develop a functional carotenoid rich concentrate for improving the nutraceutical quality and consumer appeal of the food products.

Yet another object of this invention is to develop a fibre rich powder concentrate from the left out pomace by mixing a suitable carrier agent such as maltodextrin, cyclodextrin, arabic gum etc.

Still another object of this invention is to exemplify its use in bakery products and more specifically golden bread, muffins, yoghurts and ice-creams.

SUMMARY OF THE INVENTION

The present invention relates to heat stable carotene rich composition extracted through a green process. The process is green and solvent free for effective extraction of the juice rich in nutraceutical content. The process employs the use of chemical facilitators or cell wall degrading enzymes to increase extraction efficiency.

More particularly, the invention relates to a process of preparation of carotenes and phenolic enriched products from biological resources such as capsicum other than the most routine source (carrot & tomato) through a novel enzyme mediated process.

The process is governed by variables such as upstream and downstream process steps with optimized variables of pH, extraction time and temperature. The product obtained from this process has high nutraceutical content, high antioxidant activity, neutral in taste has characteristic sweet aroma of capsicum and enhanced stability.

The process utilizes a popular vegetable more specifically red capsicum with high carotene and nutraceutical properties. Since, the said pigment extracts have enhanced stability; it has huge potential for developing functionalized juices and bakery products. In preferred embodiments, the carotenoid rich extract of the novel extraction process has been used in the development of functional baked products such as breads, muffins, soups, pizza, sauces etc. Such products are pleasing, attractive and are endowed with significant higher antioxidant activity then there unfortified counter parts.

The present invention includes the method of preparing food product by applying an effective amount of a pigment, as described herein. The carotenoid rich composition can

be incorporated as a solid powder or concentrate at various stages during manufacture and/or processing of a food product.

The present invention includes process of preparation of food products having heat stable carotenoid rich composition with high nutraceutical value, as described herein. It is noteworthy that "food products" broadly include both solid foods, liquid beverages and other edible materials regardless of their specific form.

Indeed, the present invention is not limited to only foods and can include pigment systems for other products as well particularly those, such as cosmetics.

BRIEF DESCRIPTION OF DRAWINGS

The invention may be more completely understood in consideration of the following detailed description of various embodiments of the invention in connection with the accompanying drawings.

Fig.1 is a response of different carbohydrases enzymes on total phenolic content in capsicum juice

Fig.2 is a response of different carbohydrases enzymes on total carotenoid content in capsicum juice

Fig.3 is a predicted response obtained by Response Surface Methodology (RSM) of total carotenoid content (mg/100ml) from capsicum macerate extracted through enzyme assisted processing

Fig.4 is a predicted response obtained by Response Surface Methodology (RSM) of total phenolic content (mg GAE /100ml) from capsicum macerate extracted through enzyme assisted processing

Fig.5 is a predicted response obtained by Response Surface Methodology (RSM) of Juice Yield (%) from capsicum macerate extracted through enzyme assisted processing

Fig.6 is a predicted response obtained by Response Surface Methodology (RSM) of TSS (°brix) from capsicum macerate extracted through enzyme assisted processing

Fig.7 (A) Diethyl ether extract of *Capsium annuum* L. (B) Saponified extract of *Capsicum annuum* L.

DETAILED DESCRIPTION OF THE INVENTION

Reference will now be made in detail to exemplary embodiments of the present invention, examples of which are illustrated in the accompanying drawings Figs 1-7, Table 1&2. It is to be understood that other embodiments may be utilized without departing from the respective scope of the present invention.

The present invention involves enzyme mediated processing of capsicum employing chemical facilitators or cell wall degrading enzymes or carbohydrases to increase extraction efficiency. The process is green and solvent free for effective extraction of the juice rich in nutraceutical content. The process is governed by variables such as upstream and downstream process steps with optimized variables of pH, extraction time and temperature.

The mean carotenoid and phenolic content in straight pressed juice (without enzyme) was 19.04 mg/100ml and 33.91 mg GAE/100ml, which increased by two fold in juice extracted through identified enzyme mixtures. The response was variable when different carbohydrate enzymes and concentrations were used. However, viscozyme alone resulted in maximum recovery of total phenolics (TPH) and total carotenoids (TC) with its increasing concentration (0.1 to 0.3%). The TPH in capsicum juice treated with viscozyme showed significant increase ($p<0.05$). TPH content in capsicum juice increased from

showed significant increase ($p<0.05$). TPH content in capsicum juice increased from 52.14–63.37 mg GAE /100ml with increasing viscozyme L. dosage. Similar increase was observed with TC and increasing concentrations of viscozyme L. from 0.1–0.3% the TC increased from 28.92–41.37 mg/100ml (Fig 1&2).

The functional composition from capsicum possessed as high as of 101.92 mg GAE/100ml total phenolics in single strength juice which increased to 165.67 mg GAE/100ml in capsicum concentrate respectively depending upon the level of incorporations and assays used. Similar trend of 2 fold increase was found with total carotenoids which increased from 42.68 mg/100ml in single strength juice to 85.23 mg/100ml in the capsicum concentrate (18 °Brix) depending upon the initial raw material and final concentration achieved. The identified enzyme mixture also resulted in concomitant increase of total antioxidant activity ranging from 1.67 to 4.52 mmol/L in single strength juice and concentrate respectively (Table 1).

Muffins fortified with capsicum concentrate showed an appreciable increase in antioxidant activity (AOX), total phenolics and total carotenoids. The AOX ranged from 0.22–0.48 µmol Trolox/g fw in control muffins to 0.81–3.76 µmol Trolox/g fw in fortified muffins. Total carotenoid and total phenolic content was as high as 6.53 mg/100g and 66.52 mg GAE/100g respectively. Muffins thus serve as an excellent delivery mechanism for various bioactives (Table 2).

In order to verify the predictive capability of the model, optimum conditions were established by RSM and comparisons between predicted results and the practical values were done by experimental rechecking using those presumed optimal conditions. The response of juice yield and total carotenoid calculated at optimal extraction conditions, has been shown by cube plots in Figs. 3-6.

Carotenoids in *Capsicum* sp include mainly capsanthin, its esters and β-carotene. Other carotenoids viz. capsorubin, zeaxanthin, canthaxanthin were present in lesser concentration. Carotenoids were extracted by diethyl ether three times using ultrasonic assisted extraction technique. The capsicum extract showing the presence of capsanthin and β-carotene as can be seen in the HPLC chromatogram (Fig.7 a and b). The content of carotenoids was measured by comparing with the standard compounds.

The production of functional carotenoid rich composition from red capsicum via enzyme mediated process is summarized into following upstream and downstream processes

UP-STREAM PROCESS

The main upstream processes include:

Step 1 Selection of raw material: Selection of red capsicum variety with high total carotenoid content. Freshly harvested, medium sized with proper maturity with no green pigmentation (intermediate stage).

Step 2 Washing and cutting: Washing the whole fruits with water to wash off pesticides, soil and other foreign material. This should be performed 4–5 times repeatedly to ensure product free of dirt and dust. The fruits are then cut with a domestic cutter (prestige) to a size of 3.0 x 2.5cm^2

Step 3 Blanching: Subjecting the cut slices to a temperature of 90–95 ^{o}C for 1–3 min and inactivating endogenous PPO enzymes to prevent auto-oxidation of phenolic compounds. This step also hastens the softening for quick release of carotene pigment from the fibrous matrix. The temperature of the blanched pieces ranges from 55–65 ^{o}C

Step 4 Maceration/ Communition: The slices are then fine communited to a macerate in a waring blender to obtain a fine mass. The headspace in the blender or homogenizer should be minimized to prevent or minimize the aeration of the pulp during processing.

Step 5 Enzymatic liquefaction: The macerate obtained above is then subjected to enzymatic liquefaction with a commercially available cell wall degrading enzyme mixture such as Viscozyme L. (from *Aspergillus aculeatus),* 100 FBG/g activity (Novozymes) alone or in combination of one or more enzymes in the range of 0.2–0.6%.

Viscozyme L is a multienzyme complex containing a wide range of carbohydrases, including arabanase, cellulase, Q-glucanase, hemicellulase and xylanase. The enzyme also has activity against the branched pectin like substances found in soy bean cell walls. The enzyme preparation is produced from a selected strain of the *Aspergillus* group. The optimum conditions for the activities of this enzyme complex are pH 3.3–5.5 and a temperature of 40–50 °C.

Step 6 Incubation: The above macerate treated with enzyme is incubated at 55–65 °C for 60–90 min for liquefaction and maximum stability of carotenes.

DOWNSTREAM PROCESS

The following steps are involved in the downstream process:

1. **Pressing:** The macerate is then pressed in laboratory scale hydraulic press using nylon filter bags at a pressure of 2000 lb/m^2 to express the juice.
2. **Heat processing:** The juice thus obtained is heat processed at 80–90 °C for 1 min which ensures the inactivation of the enzyme which prevents further degradation of carotenoids, followed by acidification to maintain pH below 4.5 with 0.1–0.3% food grade acidulant selected from citric acid, ascorbic acid or lactic acid followed by hot filling or alternatively preserved by class-II preservatives (0.1–0.4% of potassium sorbate, potassium metabisulphite, or sodium benzoate).

3. **Concentration:** The juice is concentrated under vacuum (50–60 °C) to 3-5 folds.
4. **Storage:** Storage at 1–6 °C

Accordingly, the preferred embodiment of the present invention provides for a process of preparation of stable carotenoid rich composition comprising the steps of:

a. selecting, washing and cutting of biological source;
b. blanching the said biological source at 90–95 °C for 1-3 minutes;
c. maceration/communition of the blanched pieces obtained in step b ;
d. treating the homogenate obtained in step c. with cell wall degrading enzymes;
e. incubating the mixture of step d. at a temperature range of 55–65 °C for 60–90 minutes;
f. pressing, clarifying the mixture of step e. to obtain juice;
g. heat processing the juice of step f. at temperature range of 80–90 °C for 1 minute;
h. concentrating the juice of step g. to 3–5 folds under vacuum at temperature range of 50–60 °C;

wherein, the said enzymes used are selected from the group of but not limited to pectinases, viscozyme, amylases hemicellulase, cellulase, or combination thereof; and the said enzymes are used in the range of 0.2–0.6% to enhance juice yield and extractability of phenolic components from the fibrous cell wall matrix.

In another embodiment of the proposed invention, the vegetables of the genus *Capsicum* comprise bell pepper (red).

In yet another embodiment of the proposed invention, the said blanching is performed preferably at a temperature range of 90–95 °C preferably for duration of 1–3 minutes.

In yet another embodiment of the proposed invention, the said incubation is performed preferably at a temperature range of 55–65 °C preferably for duration of 65–90 minutes.

In yet another embodiment of the proposed invention, the said heat processing of the juice is performed preferably at a temperature range of 80–90 °C preferably for duration of 1 minute.

In still another embodiment of the proposed invention, the said concentration is achieved preferably under vacuum at a temperature range of 50–60 °C.

In yet another preferred embodiment is proposed a stable carotenoid rich composition having enhanced phenolic contents, ascorbic acid, such that the said composition is taste neutral, solvent free and has high nutraceutical value; characterized in that:

a. the carotenoid content in the said composition ranges from 46.28–85.23 mg/100ml in single strength juice and concentrated composition respectively.
b. phenolic content in the said composition ranges from 101.90–165.67 mg GAE /100ml in single strength juice and concentrated composition;
c. the antioxidant activity of the said composition ranges from 1.67– 4.52 µmol TE/ml (TEAC) respectively in single strength juice and concentrated composition respectively.

In still another embodiment of the proposed invention, the said composition is obtained from a vegetable of the genus *Capsicum* such as sweet bell pepper (red).

In yet another embodiment of the proposed invention, the said composition is used as functional and nutraceutical ingredient in bakery, beverages and confectionaries etc. with high degree of stability of carotenoids.

In yet another embodiment of the proposed invention, the said composition is in the form of but not limited to juice, syrup, paste, powder, aqueous based concentrate, or other similar formulations.

In still another embodiment of the proposed invention, the said composition is used as colorant ingredient in cosmetics.

Now, the present invention will be described in further detail with reference to Examples and various Tables. However, it should be understood that the present invention is by no means restricted by such specific Examples and Tables.

Example 1

Table: 1 Effect of Enzyme Assisted Processing on yield, total phenolics, and carotenoids in capsicum juice

Description	TSS (°Brix)	Juice Yield (%)	Total Phenols (mg GAE /100ml)	Total carotenoids (mg/100ml)	Antioxidant activity (TEAC) (mmol/L)
Straight Pressing (control)	7.05	28.75	54.34	23.00	0.31
Single strength Juice extracted with enzyme	8.85	79.78	101.92	42.68	1.67
Capsicum concentrate	16.78	NA	165.67	85.23	4.52

Enzyme assisted processing (EAP) efficiently improved the recovery of bioactives in comparison to straight pressed juice (without enzyme). The increase is 2 folds in case of single strength juice and around 3 folds in concentrate. The process thereof defines clear advantages of using EAP in comparison to conventional extraction procedures.

Example 2

Table: 2 Improvement in antioxidant activity in muffins fortified with capsicum concentrate

Description	Antioxidant activity (µmol Trolox/g fw)				Total Phenolics (mg GAE /100g)	Total Carotenoids (mg/100g)
	FRAP	DPPH	ABTS	CUPRAC		
Control (untreated without enzyme)	0.22	0.48	0.32	0.38	11.84	0.00
Fortified with capsicum concentrate	1.09	3.76	1.67	0.81	66.52	6.53

CUPRAC- cupric ion antioxidant reducing capacity, FRAP -Ferric reducing antioxidant power, DPPH - 2, 2-diphenyl-1-picrylhydrazyl, TEAC - Trolox equivalent antioxidant capacity, Total phenolics and total carotenoids in mg/100g

The present invention product may be applied into the bakery industry for developing carotenoid rich muffins; with enhanced phenolic and carotene content. In addition, the remarkable stability of the functional ingredient to withstand high baking temperature increases its economic feasibility.

As seen from the above cited example the fortified muffins show an high antioxidant activity and phenolic content. Such bakery products functionalized with carotenoids serve as a delivery mechanism for consumption of healthy pigments and other phenolics for health benefits

Example 3

The effect of enzyme assisted processing (EAP) with different carbohydrases enzymes on the recovery of total phenolics (TPH) and total carotenoid content (TC) of capsicum macerate (CM) is presented in Fig.1 & 2. There was a significant increase ($p<0.05$) in TPH in capsicum juice processed through EAP. The TPH in straight pressed juice was 33.91 mg GAE /100ml. Viscozyme L. was found to be more efficient in recovering the TPH than other carbohydrate enzymes employed in the present study. TPH content in capsicum juice increased from 52.14–63.37 mg GAE /100ml with increasing viscozyme L. dosage.

Pectinase performed equally good, yet the TPH was slightly lower (50.72–61.75 mg GAE /100ml) with increasing enzyme dosage. Overall EAP caused remarkable improvement in recovery of TPH, by nearly 2 fold over SP. In case of cellulase and amylase, only higher enzyme dosage catalyzed recovery (30.45–58.86 mg GAE /100ml), overall TPH remained lower than viscozyme and pectinase. The most dramatic effect of EAP was observed with respect to recovery of total carotenoids (TC) in capsicum juice. Carbohydrate enzymes remarkably improved the recovery of TC by 2.5 to 3.0 fold. Viscozyme L. and pectinases resulted in highest recovery of TC whereas; cellulase and amylase caused only marginal improvements. The TC in SP juice was 19.04 mg/100ml. With the increasing concentrations of viscozyme L. from 0.1–0.3% the TC increased from 28.92–41.37 mg/100ml. Pectinase showed equivalent good recovery ranging from 27.46–41.00 mg/100ml with increasing enzyme dosage (0.1–0.3%), however it was significantly lower than viscozyme L. Similar trend, yet marginal improvements were observed with cellulase and amylase (24.40–31.23 mg/100ml). Overall results indicated that viscozyme L. was more efficient than other carbohydrases employed in the study for causing liquefaction of CM.

Example 4

Table: 3 Nutraceutical quality of capsicum fiber concentrate from red capsicum compared with tomato fiber concentrate**

	Total carotenoids (mg/100g)	Total Phenolics (mg GAE /100g)	Ascorbic acid (mg/100g)	ABTS (µmol TEAC/g)
CFC	197.22-279.35	279.54-384.67	216.02-346.45	22.78-31.89
TFC*	3.25-4.00	29.22-108.56	NA	3.90

- CFC – Capsicum fiber concentrate
- TFC – Tomato fiber concentrate
- *(Gonzalez et al., 2011)

Capsicum fiber concentrate has been compared with tomato fiber concentrate. As clear from the data, CFC supports higher content of antioxidants (carotenoids, phenolic compounds and ascorbic acid). Due to health related properties of dietary fiber and associated bioactive compounds it could be used as a nutritional supplement.

Example 5

Table: 4 Functional quality of capsicum fiber concentrate from red capsicum compared with tomato fiber concentrate**

	Water absorption index (WAI) (g/g)	Water solubility index (WSI) (%)	Oil holding capacity (OHC) (g/g)	Crude fiber (%)	Glucose retardation index (GRI) (%)
CFC	5.4-6.0	1.6-2.0	1.35-2.0	85.56-87.56	56.02
TFC*	0.11	NA	1.46	86.15	39.22

(values represent mean of three replicates)

- CFC – Capsicum fiber concentrate
- TFC – Tomato fiber concentrate
- *(Gonzalez et al., 2011)

** the CFC is referred to the left over pomace after Enzyme Assisted Processing (liquefaction) of Capsicum

Water solubility index (WSI) and water absorption index (WAI) represents measure of starch degradation; it means higher values of WSI and WAI leads to higher degradation of starch and such condition leads to more numbers of soluble molecules. The constituents of fibre affect the gastrointestinal tract differently, ultimately affecting the nutrition of the animal. Crude fiber has a high water-holding capacity, which affects the speed at which the diet passes through the intestinal tract (transit time). Fibre constituents have extensive cation-exchange capacity, which can bind dietary minerals, thereby providing better absorbing ability. The glucose retardation index (GRI) of the glucose is directly related to the soluble fiber content and viscosity of the solution. Dietary fibers may delay the gastric emptying of ingested foods into the small intestine, resulting in a sensation of

fullness, which may contribute to weight control. Delayed gastric emptying may also reduce postprandial blood glucose concentrations and potentially have a beneficial effect on insulin sensitivity.

Example 6

Referring to Figs. 3-6, RSM was used to optimize and study the effect of independent variables such as enzyme concentration, incubation temperature and extraction time for increase in the recovery of total carotenoid content, total phenolics content, juice yield and TSS from capsicum. The experimental design and statistical analysis were performed using Stat-Ease software (Design-Expert 8.0.10 Trial, Delaware, USA Echip, 1993). A three-level three-factor Box–Behnken design was chosen to evaluate the combined effect of three independent variables enzyme concentration, temperature and time on the response. The variables and their levels were chosen, based on the results of preliminary experiments. The minimum and maximum values for enzyme concentration 0.2, 0.3 and 0.4 %, temperature were set at 50, 60 and 70 °C and extraction time was between 30, 60 and 90 min. The response values were total carotenoid content (mg/kg), total phenolics content (mg/kg), juice yield (%) and TSS (°Brix). Three experiments of each condition were carried out and the mean values were stated as observed responses. The cube diagrams in Figs 3-6 were used to flag the maximum response at optimum input points.

Example 7

Fig.7 represents the HPLC chromatogram for presence of capsanthin and β-carotene in capsicum extract. Carotenoids in *Capsicum* extract included mainly capsanthin, its esters and β-carotene. Other carotenoids viz. capsorubin, zeaxanthin, canthaxanthin were however, present in lesser concentration. Carotenoids were extracted by diethyl ether three times using ultrasonic assisted extraction technique. Extracted carotenoids in organic phase were partitioned between water and diethyl ether. Capsanthin was present in *Capsicum* sp in the form of mainly as esters of fatty acids. Saponification was done to get the total capsanthin. Organic phase was evaporated and dissolved in methanol (2ml) and methanolic NaOH (1N, 10ml) was added under N_2 atmosphere. The mixture was

sonicated at 50 °C for 45 minutes. Then the reaction mixture was partitioned between water and ether. Organic layer was separated and washed with water thrice to remove excess alkali present. Organic layer was evaporated and dissolved in tert-butyl methyl ether prior to LC analysis. HPLC was run using mobile phase comprising of mixtures of methanol, tert butyl methyl ether, and water at 81 : 15 : 4, v/v/v (A) and 6 : 90 : 4, v/v/v (B), starting with 10 min isocratic at 100% A, followed by a gradient to obtain 50% B at 40 min, 100% B at 50 min, 100% A at 55 min and isocratic 100% A from 55 min to 60 min at a flow rate of 1 ml/min. The injection volume was 20 ml. The content of carotenoids was measured by comparing with the standard compounds.

It will thus be seen that the objects set forth above, among those made apparent from the preceding description, are efficiently attained and, since certain changes may be made in carrying out the above process and steps set forth without departing from the spirit and scope of the invention is intended that all matter contained in the above description shall be interpreted as illustrated and not in a limiting sense.

It is also to be understood that the following claims are intended to cover all of the generic and specific features of the invention herein described and all statements of the scope of the invention in which, as a matter of language might be said to fall there between.