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(54) Title: A COMPOSITION, PROCESS AND A KIT FOR SUGAR PROCESSING

(57) Abstract: The present disclosure relates to an enzyme based sulphur replacement product, an enzyme based sulphur replacement process and a kit applicable in sugar processing. The present disclosure also relates to a composition comprising an enzyme blend employed in the process for obtaining a sulphur replacement product; and a final product obtained after the sugar processing.

"A COMPOSITION, PROCESS AND A KIT FOR SUGAR PROCESSING"

TECHNICAL FIELD

The present disclosure relates to the field of industrial biotechnology. Particularly, the present disclosure relates to an enzyme based sulphur replacement product, an enzyme based sulphur replacement process and a kit applicable in sugar processing. The present disclosure also relates to a composition comprising an enzyme blend employed in the process for obtaining a sulphur replacement product; and a final product obtained after the sugar processing.

BACKGROUND OF THE DISCLOSURE

Colour of sugar is an important commercial attribute and colour removal is an important step in sugar manufacturing, considering the increase in demand for high quality white sugar.

A number of both traditional and new processes using chemicals are available to achieve good colour removal. Chief among them are carbonation, sulphitation, phosphatation, adsorption by activated charcoal and newer techniques like use of polymers for flocculation and ion exchange resins. Sulphitation is the most common method used for sugarcane juice clarification presently.

Sugarcane contains about 70% water, in which sucrose and other substances are held in solution, forming about 88% by weight of juice in the stem. The remaining 12% represents the insoluble cane fiber component. Sugarcane juice consists of a sucrose solution containing soluble and insoluble impurities (i.e. non sucrose species). The cane juice has an acidic pH ranging between 4.9 to 5.5.

Compounds imparting colour in sugarcane juice can be divided into 6 categories: plant pigments, colour precursors, melanoidins, caramels, alkaline degradation products of fructose (ADF) and polysaccharide colourant complex. The last four are factory produced colour pigments.

Turbidity in cane juice is due to presence of polysaccharides and hemicellulosic fiber material. In sugarcane, the common polysaccharides are indigenous sugarcane polysaccharide (ISP) and starch. Polysaccharides decrease filterability, increase boiling time and decrease recovery and can disrupt the sucrose crystal structure, resulting in elongated or other misshaped crystals, leading to problems in centrifugation, and end user products. Starch is found mostly in the leaves and growing points of the sugarcane plant, and is transferred to the raw juice when the sugarcane is crushed in the mill.

Sulphitation is the practice of adding sulphur dioxide (SO_2) or derivatives to process streams in a sugar factory. This is an important step in white sugar manufacturing. It is mainly carried out for three reasons:

a. Colour blocking
b. pH control
c. Biocide

The most commonly used method of sulphitation in tropical countries is the hot sulphitation method. In this process, the juice is first heated to 75°C then sulphited and limed, boiled, and settled. Harloff's process is a hot treatment procedure in which the juice is heated to 75°C and the lime and SO_2 are added simultaneously in such a way as to maintain the reaction acid to phenolphthalein and alkaline to litmus (pH about 7.4-7.8), except towards the end, when a quantity of lime is added to attain a strongly alkaline reaction (pH 10+), after which the sulphitation is completed to neutrality to litmus (pH about 7.2). As in all other similar processes, the juice is finally brought to boiling temperatures in juice heaters and settled. The juice is centrifuged to remove sludge and clear supernatant is obtained for further processing.

However, the limitations of the processes described above are as follows:

i. Sugar produced by double sulphitation contains > 20 ppm of sulphur which is beyond acceptable limits, hence quality is affected.

ii. Sugar produced by sulphitation does not form clear solution when dissolved due to presence of calcium sulphate and polysaccharides.

iii. SO_2 (sulphur di-oxide) does not dissolve completely during sulphitation and causes environmental hazards.

iv. The process gives rise to scaling problem in evaporators due to deposition of calcium sulphate.

v. In the product obtained by said process, there is secondary colour formation (colour return) upon storage of the product.

Crystallization by itself is helpful in partitioning colourants from sugar, but is not 100% efficient and is therefore supplemented with other physical and chemical methods of separating/removing colourant. Each process has its own advantages as well as limitations in terms of performance (phosphatation), capital investment (ion exchange and use of polymers) and effect on environment and consumer health (sulphitation and use of lime).

Though sulphitation is the most commonly used process involving the use of SO_2 gas to achieve removal of colourants in sugarcane juice clarification, periodic spikes in sulphur prices and sugar quality issues due to excess sulphur (> 10-20 ppm) precipitated in the sugar crystals have stimulated efforts to reduce or even eliminate its use and produce 'sulphur free' sugar. Development of a cost effective, compatible and environment friendly alternative to sulphitation is therefore necessary.

The present disclosure employs enzymes to replace/supplement current practices of colour removal during sugar manufacturing and therefore is an attractive alternative to the conventional physico-chemical methods.

The present disclosure also provides an enzyme based sulphur replacement product, a composition and a process for sugar processing which addresses the concerns associated with the existing sugar processing methods.

STATEMENT OF THE DISCLOSURE

Accordingly, the present disclosure relates to a composition comprising an enzyme blend, optionally along with excipient; a process for clarifying sugar juice, said process comprising acts of a) mixing the juice with enzyme blend to obtain a mixture, b) optionally holding-up followed by cooling the mixture, and c) neutralizing the mixture of step (a) or step (b) by adding a pH regulator, followed by re-holding-up and optional re-cooling, followed by optional adding of excipient or filtering or both,

to obtain the clarified sugar juice; clarified sugar juice obtained by the process as described above; a process for obtaining a composition comprising an enzyme blend optionally along with excipient, said process comprising act of combining plurality of enzymes selected from a group comprising amylase, xylanase, cellulase, hemicellulase, glucanase, galactosidase, glucose oxidase and non- enzyme component- ascorbic acid or any combination thereof optionally along with the excipient to obtain the enzyme blend; and a kit for clarifying sugar juice or for obtaining clarified sugar, said kit comprising enzymes selected from group comprising amylase, xylanase, cellulase, hemicellulase, glucanase, galactosidase, glucose oxidase and non- enzyme component- ascorbic acid or any combination thereof, pH regulator selected from a group comprising calcium oxide (lime), calcium hydroxide (milk of lime), orthophosphoric acid and inorganic calcium compounds or any combination thereof, and excipient selected from a group comprising anticaking agent, stabiliser and flocculent or any combination thereof along with an instruction manual.

BRIEF DESCRIPTION OF THE ACCOMPANYING FIGURES

In order that the disclosure may be readily understood and put into practical effect, reference will now be made to exemplary embodiments as illustrated with reference to the accompanying figures. The figures together with a detailed description below, are incorporated in and form part of the specification, and serve to further illustrate the embodiments and explain various principles and advantages, in accordance with the present disclosure wherein:

Figure 1 shows a process flow diagram of sugarcane manufacturing and points of enzyme application for replacing sulphur.

Figure 2 shows a bar graph depicting ICUMSA units for assessing reduction in colour in sugarcane juice without treatment, with sulphitation and in the presence of enzyme blend 'G' in sample 1.

Figure 3 shows a bar graph depicting the comparison of colour in sugarcane juice without treatment, sulphited clear juice and clear juice treated with enzyme blend 'G' in sample 2.

Figure 4 shows a bar graph depicting increase in purity of clear juice treated with enzyme blend 'G' as compared to conventional sulphitation process.

Figure 5 shows a bar graph depicting the colour of final sugar obtained by sulphitation process (87 ICUMSA Units) and enzyme blend 'G' application (91 ICUMSA Units).

Figure 6 shows a bar graph depicting the percentage recovery of final sugar obtained by sulphitation process (10.04%) and enzyme blend 'G' application (10.38%).

Figure 7 shows the process flow for manufacturing sugar from sugar beet.

Figure 8 shows the process flow for manufacturing sugar from raw sugar.

DETAILED DESCRIPTION OF THE DISCLOSURE

The present disclosure relates to a composition comprising an enzyme blend, optionally along with excipient.

In an embodiment of the present disclosure, the composition is a synergistic composition and wherein the enzyme blend comprises plurality of enzymes selected from a group comprising amylase, xylanase, cellulase, hemicellulase, glucanase, galactosidase, glucose oxidase and non-enzyme component- ascorbic acid or any combination thereof.

In another embodiment of the present disclosure, the amylase is selected from a group comprising α-amylase, β-amylase and γ-amylase or any combination thereof at a concentration ranging from about 0.1% to about 99% w/w, preferably from about 10% to about 20% w/w; the xylanase is selected from a group comprising exo-xylanase and endo-xylanase or a combination thereof at a concentration ranging from about 0.1% to about 99% w/w, preferably from about 20% to about 40% w/w; the cellulase is selected from a group comprising exo-cellulase, endo-cellulase and cellobiase or any combination thereof at a concentration ranging from about 0.1% to about 99% w/w, preferably from about 20% to about 40% w/w; the hemicellulase is at a concentration ranging from about 0.1% to about 99% w/w, preferably from about 15% to about 30% w/w; the glucanase is selected from a group comprising α-glucanase, β-glucanase, xyloglucan-specific endo-beta-1,4-glucanase, xyloglucan-specific exo-beta-1,4-glucanase and pullulanase or any combination thereof at a concentration ranging from about 0.1% to about 99% w/w, preferably from about 5% to about 30% w/w; the galactosidase is selected from a group comprising α-galactosidase and β-galactosidase or a combination thereof at a concentration ranging

from about 0.1% to about 99% w/w, preferably from about 5% to 10% w/w; the glucose oxidase is at a concentration ranging from about 0.1% to about 99% w/w, preferably from about 5% to 10% w/w; and the non-enzyme component- ascorbic acid is at a concentration ranging from about about 0.1% to about 99% w/w, preferably from about 0.015 to about 10 % w/w.

In yet another embodiment of the present disclosure, the composition is employed for clarifying sugar juice or obtaining chemical free sugar, and wherein the sugar juice is obtained from a source selected from a group comprising sugarcane, sugarbeet, raw sugar, and other substrate used for sugar processing or any combination thereof.

In still another embodiment of the present disclosure, the excipient is selected from a group comprising anticaking agent, stabiliser and flocculent or any combination thereof.

In still another embodiment of the present disclosure, the anticaking agent is selected from a group comprising fumed silica, bentonite and talc or any combination thereof; wherein the stabilizer is selected from a group comprising sodium chloride, fumed silica, sucrose, maltodextrin, trehalose, lactose, arabinose and cellulose or any combination thereof; and wherein the flocculent is an anionic flocculent.

In still another embodiment of the present disclosure, the excipient is in concentration ranging from about 0.01% to about 20%, preferably about 1% to about 10%.

The present disclosure also relates to a process for clarifying sugar juice, said process comprising acts of:

a. mixing the juice with enzyme blend to obtain a mixture;
b. optionally holding-up followed by cooling the mixture; and
c. neutralizing the mixture of step (a) or step (b) by adding a pH regulator, followed by re-holding-up and optional re-cooling, followed by optional adding of excipient or filtering or both, to obtain the clarified sugar juice.

In an embodiment of the present disclosure, the sugar juice is obtained from a source selected from a group comprising sugarcane, sugarbeet, raw sugar, and other substrate used for sugar processing or any combination thereof.

In another embodiment of the present disclosure, the enzyme blend comprises plurality of enzymes selected from a group comprising amylase, xylanase, cellulase, hemicellulase, glucanase, galactosidase, glucose oxidase and ascorbic acid or any combination thereof; and wherein the amylase is selected from a group comprising α-amylase, β-amylase and γ-amylase or any combination thereof at a concentration ranging from about 0.1% to about 99% w/w, preferably from about 10% to about 20% w/w; the xylanase is selected from a group comprising exo-xylanase and endo-xylanase or a combination thereof at a concentration ranging from about 0.1% to about 99% w/w, preferably from about 20% to about 40% w/w; the cellulase is selected from a group comprising exo-cellulase, endo-cellulase and cellobiase or any combination thereof at a concentration ranging from about 0.1% to about 99% w/w, preferably from about 20% to about 40% w/w; the hemicellulase is at a concentration ranging from about 0.1% to about 99% w/w, preferably from about 15% to about 30% w/w; the glucanase is selected from a group comprising α-glucanase, β-glucanase, xyloglucan-specific endo-beta-1,4-glucanase, xyloglucan-specific exo-beta-1,4-glucanase and pullulanase or any combination thereof at a concentration ranging from about 0.1% to about 99% w/w, preferably from about 5% to about 30% w/w; the galactosidase is selected from a group comprising α-galactosidase and β-galactosidase or a combination thereof at a concentration ranging from about 0.1% to about 99% w/w, preferably from about 5% to 10% w/w; the glucose oxidase is at a concentration ranging from about 0.1% to about 99% w/w, preferably from about 5% to 10% w/w; and the non-enzyme component- ascorbic acid is at a concentration ranging from about about 0.1% to about 99% w/w, preferably from about 0.015 to about 10 % w/w.

In yet another embodiment of the present disclosure, the mixing is carried out by adding the enzyme blend to the sugar juice having a temperature of about 35˚C to about 45˚C or wherein the mixing is carried out by adding the enzyme blend to the sugar juice to obtain a mixture followed by boiling the mixture to a temperature of about 65˚C to about 75˚C.

In still another embodiment of the present disclosure, the holding-up of step (b) is at a temperature ranging from about 50°C to about 80°C for a time period ranging from about 5 minutes to about 15 minutes.

In still another embodiment of the present disclosure, the cooling is at a temperature ranging from about 25°C to about 30°C.

In still another embodiment of the present disclosure, the neutralizing is carried out by addition of pH regulator selected from a group comprising calcium oxide (lime), calcium hydroxide (milk of lime), orthophosphoric acid and inorganic calcium compounds or any combination thereof.

In still another embodiment of the present disclosure, the re-holding-up of step (c) is for a time period ranging from about 20minutes to about 90 minutes, at a temperature ranging from about 90°C to about 110°C or is for a time period ranging from about 10minutes to about 20minutes, at a temperature ranging from about 90°C to about 110°C.

In still another embodiment of the present disclosure, the filtering is carried out by method selected from a group comprising membrane filtration, filter press and settling or any combination thereof.

In still another embodiment of the present disclosure, the clarified sugar juice can be further processed to obtain clarified sugar.

In still another embodiment of the present disclosure, the process comprises acts of:

a. adding the enzyme blend to the sugar juice having a temperature of about 30°C to about 45°C to obtain a mixture;
b. optionally holding-up at a temperature ranging from about 50°C to about 80°C for a time period ranging from about 5 minutes to about 15 minutes followed by cooling the mixture at a temperature ranging from about 25°C to about 30°C; and
c. neutralizing the mixture of step (a) or step (b) by adding a pH regulator, followed by re-holding-up for a time period ranging from about 20minutes

to about 90minutes, at a temperature ranging from about 90°C to about 110°C and optional re-cooling, followed by optional adding of excipient or filtering or both, to obtain the clarified sugar juice.

In still another embodiment of the present disclosure the process comprises acts of:

a. adding the enzyme blend to the sugar juice to obtain a mixture followed by boiling the mixture to a temperature of about 65°C to about 75°C;
b. optionally holding-up at a temperature ranging from about 50°C to about 80°C for a time period ranging from about 5 minutes to about 15 minutes followed by cooling the mixture at a temperature ranging from about 25°C to about 30°C; and
c. neutralizing the mixture of step (a) or step (b) by adding a pH regulator, followed by re-holding-up for a time period ranging from about 10minutes to about 20minutes, at a temperature ranging from about 90°C to about 110°C and optional re-cooling, followed by optional adding of excipient or filtering or both, to obtain the clarified sugar juice.

The present disclosure also relates to clarified sugar juice obtained by the process as mentioned above.

In an embodiment of the present disclosure, the juice can be further processed to obtain clarified sugar.

The present disclosure also relates to a process for obtaining a composition comprising an enzyme blend optionally along with excipient, said process comprising act of combining plurality of enzymes selected from a group comprising amylase, xylanase, cellulase, hemicellulase, glucanase, galactosidase, glucose oxidase and non-enzyme component- ascorbic acid or any combination thereof optionally along with the excipient to obtain the enzyme blend.

The present disclosure also relates to a kit for clarifying sugar juice or for obtaining clarified sugar, said kit comprising enzymes selected from group comprising amylase, xylanase, cellulase, hemicellulase, glucanase, galactosidase, glucose oxidase and non-enzyme component- ascorbic acid or any combination thereof; pH regulator selected

from a group comprising calcium oxide (lime), calcium hydroxide (milk of lime), orthophosphoric acid and inorganic calcium compounds or any combination thereof; and excipient selected from a group comprising anticaking agent, stabiliser and flocculent or any combination thereof along with an instruction manual.

Due to the disadvantages of the prior known and existing processes in the art pertaining to sugar processing and considering the current requirement of a 'greener process' of manufacturing sugar, an enzymatic method is designed in the present disclosure, to replace chemicals such as sulphur in sugar processing.

The use of enzymes over other known techniques for sugar processing provides benefits such as non-requirement of capital investment (as compared to employing column ion exchange resins), generation of low amount of sludge (as compared to employing activated charcoal and carbonisation) and relatively more cost effective (as compared to employing expensive techniques by using resins and polymers).

The enzymes work on formed colourants in substrate, preferably sugarcane juice; hydrolyses hemicellulosic matter or fibers or polysaccharides in the substrate and prevents oxidation of phenolic precursors. Thus, the present disclosure finds application in production of sulphur free sugar and clarification of sugar cane juice, sugarbeet juice or raw-sugar in the sugar industry.

Accordingly, the present disclosure relates to an enzyme based sulphur replacement product, a composition comprising components to obtain enzyme based sulphur replacement product, a process of clarifying sugar juice and a process for preparing sulphur free sugar- both for obtaining an enzyme based sulphur replacement product.

In an embodiment of the present disclosure, the substrate used in sugar processing is selected from a group comprising sugarcane juice (from sugarcane) and other substrates used for sugar processing including, but not limiting to sugarbeet juice (from sugar beet), raw sugar etc, and combination thereof.

In another embodiment of the present disclosure, the enzyme is selected from a group comprising xylanase (exo-xylanase, endo-xylanase), cellulase (exo-cellulase, endo-

cellulase, cellobiase), hemicellulase, glucanase (α-glucanase, β-glucanase, xyloglucan-specific endo-beta-1,4-glucanase, xyloglucan-specific exo-beta-1,4-glucanase, pullulanase), galactosidase (α-galactosidase, β-galactosidase), glucose oxidase, amylase (α-amylase β-amylase, γ-amylase) and ascorbic acid or any plurality of combination thereof.

In yet another embodiment of the present disclosure, each of the enzymes are at concentration ranging from about 0.1% to about 99%; preferably ranging from about 5% to about 99%, from about 10% to about 99%, from about 5% to about 80%, from about 10% to about 80%, from about 5% to about 60%, from about 10% to about 60%, from about 5% to about 50%, from about 10% to about 50%, from about 5% to about 40%, from about 10% to about 40%, from about 5% to about 20%, or from about 10% to about 20%, more preferably at about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 99%.

In still another embodiment of the present disclosure, the amylase is selected from a group comprising α-amylase, β-amylase and γ-amylase or any combination thereof at a concentration ranging from about 0.1% to about 99% w/w, preferably from about 5% to about 50% w/w and most preferably from about 10% to about 20% w/w; the xylanase is selected from a group comprising exo-xylanase and endo-xylanase or a combination thereof at a concentration ranging from about 0.1% to about 99% w/w , preferably from about 10% to about 50% w/w and most preferably from about 20% to about 40% w/w; the cellulase is selected from a group comprising exo-cellulase, endo-cellulase and cellobiase or any combination thereof at a concentration ranging from about 0.1% to about 99% w/w, preferably from about 10% to about 50% w/w and most preferably from about 20% to about 40% w/w; the hemicellulase is at a concentration ranging from about 0.1% to about 99% w/w, preferably from about 5% to about 50% w/w and most preferably from about 15% to about 30% w/w; the glucanase is selected from a group comprising α-glucanase, β-glucanase, xyloglucan-specific endo-beta-1,4-glucanase, xyloglucan-specific exo-beta-1,4-glucanase and pullulanase or any combination thereof at a concentration ranging from about 0.1% to about 99% w/w , preferably from about 2% to about 50% w/w and most preferably

from about 5% to about 30% w/w; the galactosidase is selected from a group comprising α-galactosidase and β-galactosidase or a combination thereof at a concentration ranging from about 0.1% to about 99% w/w , preferably from about 2% to about 50% w/w and most preferably from about 5% to 10% w/w; the glucose oxidase is at a concentration ranging from about 0.1% to about 99% w/w, preferably from about 2% to about 50% w/w and most preferably from about 5% to 10% w/w; and the ascorbic acid is at a concentration ranging from about about 0.1% to about 99% w/w, preferably from about 2% to about 50% w/w and most preferably from about 0.015 to about 10 % w/w.

In still another embodiment of the present disclosure, the enzymes are combined to obtain an enzyme blend/enzyme system, which is employed in the sugar processing procedure (sulphur-free process/enzyme-based sulphur replacement process) of the instant disclosure.

In still another embodiment of the present disclosure, the enzyme blend comprises combination of enzymes selected from a group comprising xylanase and cellulase; xylanase, cellulase and hemicellulase; xylanase, cellulase, hemicellulase and glucanase; xylanase, cellulase, hemicellulase, amylase and glucanase; xylanase, cellulase, hemicellulase, amylase, glucanase, galactosidase and glucose oxidase; and xylanase, cellulase, hemicellulase, amylase, glucanase, galactosidase, glucose oxidase and ascorbic acid, optionally along with excipients.

In still another embodiment of the present disclosure, the dosage of enzyme blend employed is in the range of about 1ppm to about 50ppm, preferably about 2ppm to about 30ppm, depending upon the nature of colourants and matter contributing to turbidity in the juice.

In still another embodiment of the present disclosure, the process of obtaining sulphur free sugar or clarifying sugar juice is carried out by addition of enzyme in juice collection tank or juice heater or primary sulphitation tank or third evaporator during sugarcane processing as shown in Figure 1. The enzyme blend of the present disclosure can also be added during the crushing of sugarcane or sugar beet.

In still another embodiment of the present disclosure, the process of obtaining sulphur free sugar or clarifying sugar juice is carried out by addition of enzymes and optionally excipient(s), including but not limiting to anticaking agents and stabilisers. Since the formulation is a powder and composed of proteins (i.e the enzyme blend), anticaking agents such as fume silica, bentonite or talc are added to the product to prevent formation of lumps and keep the product free flowing making it easy to pack, transport and apply. Stabilizers such as sodium chloride are added to help enzymes maintain activity in solution.

In still another embodiment of the present disclosure, the excipient is employed in concentration ranging from about 0.01% to about 20%, preferably about 1% to about 10%, with optimum being around 5%.

In still another embodiment of the present disclosure, the stabilizers are selected from a group comprising sodium chloride, fumed silica, sucrose, maltodextrin, trehalose, lactose, arabinose and cellulose and or any combination thereof.

In still another embodiment of the present disclosure, proper mixing of components during sugar processing is ensured by means of agitators, aerators etc, in the tank.

The present disclosure, employing enzymes work on the following principles:

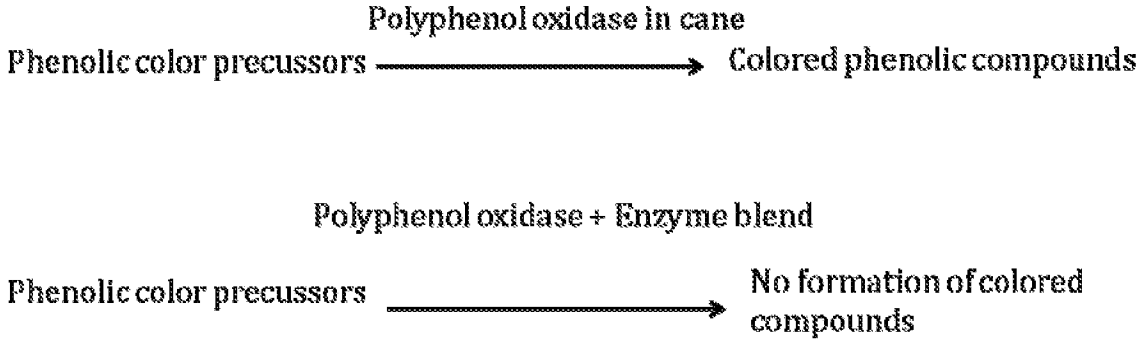
i. Enzymes targeted against formed colourants in sugarcane juice/sugar juice: Enzymes that work towards hydrolyzing, oxidizing/reducing, destabilizing bonds in the colourant molecule (pigments, phenolics, caramel, melanoidins etc.) are effective in reducing juice colour.

ii. Enzymes that hydrolyse hemicellulosic matter or fibers or polysaccharides in sugarcane juice/sugar juice: Fibers and hemicellulosic plant matter impart turbidity to the juice. Enzymes catalyze the conversion of hemicellulosic polymers to monosaccharides thereby removing turbidity and enhancing juice clarity. This also prevents the formation of colourant-polysaccharide complex which will reduce colour.

Most of the turbidity and colour in sugarcane juice is attributed to polysaccharides which combine with phenolic compounds. Enzymes break the bond between phenolic compounds and polysaccharides and further reduce the polysaccharides to oligo and mono saccharides which prevent formation of colourant polysaccharide complex.

iii. Enzymes which prevent oxidation of phenolic precursors: Enzymes that degrade phenolic precursors prevent colour formation in juice during processing. Also, crushing of the substrate releases an enzyme called polyphenol oxidase (PPO). This enzyme catalyzes the oxidation of o-diphenols to produce o-quinones, the polymerization of which produces the typical black, brown or red pigments. Inhibition of PPO by inorganic or biological inhibitors reduces formation of colour in juice.

Enzymes such as polyphenol oxidase in juice convert colourless precursors to coloured phenolics. Inhibitors of polyphenol oxidase (enzyme blend of the instant disclosure) prevent this reaction by blocking active site on polyphenol oxidase and hence reduce colour.



Advantages of the instant disclosure in sugar processing:

Sulphitation in cane juice is done in two steps: Sulphitation at juice stage and sulphitation at syrup stage. 70% of total sulphur consumed is used for juice sulphitation and rest 30% is consumed for syrup sulphitation. If juice sulphitation is replaced by the enzyme based sulphur replacement process of the present disclosure, sulphur accumulation in final sugar is eliminated. Although the process is not sulphur free, the end product would be sulphur free sugar. This is because, the sulphur used in

syrup sulphitation is much lower than what is used at juice stage, which gets eliminated during the enzyme-based sulphur replacement process and does not end up in the final sugar. Hence the end product is sulphur-free.

The enzyme blend of the present disclosure can also be added during the crushing of sugarcane or sugar beet.

Recent trials with this disclosure have shown that sulphitation in juice is completely replaced with enzymes. The role of sulphur in syrup sulphitation is as a bleaching agent. A combination of enzymes and alternate aids to remove colour in syrup such as chemicals, peroxide etc. will make the process completely sulphur free.

By employing the procedure of sugar processing detailed in the present disclosure, upto 70% of sulphur consumption has been reduced with the production of sulphur free sugar.

The benefits of replacing sulphur with enzymes of the instant disclosure are as follows:

i. Preparation of 'sulphur-free' sugar which is high in quality and selling price.
ii. Reduction in sulphur levels in final sugar to acceptable limits.
iii. Decrease in scaling of evaporators and pipelines and reduction in equipment corrosion.
iv. Reduction in sulphur levels in molasses which improves quality.
v. Reduction in sulphur levels in effluent thereby improving bio-methanation rates.
vi. The process of the present disclosure does not require addition of a unit operation or additional heating/retention time/change in pH than what is normally used in sugarcane processing.
vii. pH control: In conventional sugar manufacturing process, lime is added in large quantities in order to facilitate precipitation of impurities during clarification step. This raises the pH of juice to 9.0-10.0. Sulphur dioxide gas is then pumped into the juice in order to bring down the pH to 7.0 and also facilitate precipitation of impurities

by reacting with lime to form calcium sulphate. When enzymes are used, lime is added such that the pH of juice is maintained at 7.0. Hence the need for sulphur to maintain pH does not arise in the process of the present disclosure.

viii. Sugar is obtained after crystallization and is composed of pure sucrose. Enzymes compared to chemicals, cannot co-crystallize with sucrose. Moreover, enzymes get denatured during the processing of sugar which involves boiling and precipitation and therefore are not retained in the final product (sugar). Therefore, the application of enzymes for sugar processing/clarification does not cause any adverse health effects in the consumer.

A more complete understanding can be obtained by reference to the following specific examples, which are provided for purposes of illustration only and are not intended to limit the scope of the disclosure.

EXAMPLES

Example 1:

Extraction of sugar juice:

Sugarcane juice/sugar juice extraction is a continuous process usually involving three mills/crushers. The mills can be of roller type or mallets. During crushing, the nodes of the cane are broken down and stem is flattened to extract the juice. Juice is collected from each mill at first crushing and is called as primary juice. Poor juices collected in subsequent milling are reprocessed and hot water is usually applied at the last mill to maximise extraction. Mixed juice is the combination of juices collected along with primary juice in subsequent crushing.

Cane juice prepared by any other method of extraction is suitable for and can be employed in this disclosure.

Preparation of enzyme blend:

The process of preparing the enzyme blend, broadly include the following steps of:

1. Analysis of individual enzyme samples to confirm activity
2. Weighing of enzyme samples as per composition

3. Blending of enzymes along with excipient and additives to obtain the enzyme blend.

Alternately, commercially available multi-enzyme preparation composed of individual enzymes can be used directly. Such a product is usually obtained after co-culture of few selected microbial strains, or a single recombinant strain with plasmid encoding for multiple enzymes, or a single strain grown on multiple substrates producing the enzyme consortium.

In the present disclosure, the individual enzymes described above are analysed for enzyme activity. 1g of each enzyme is weighed out, to prepare a mixture of various combination of enzymes. Each combination of enzymes is homogenized well to give the enzyme blend. The enzyme blend comprises plurality of enzymes selected from a group comprising xylanase (exo-xylanase, endo xylanase or both), cellulase (exo cellulase, endo cellulase, cellobiase or a combination thereof), hemicellulase, β-glucanase (xyloglucan-specific endo-beta-1,4-glucanase, xyloglucan-specific exo-beta-1,4-glucanase or both) optionally along with glucose oxidase, amylase (α-amylase, β-amylase, γ-amylase), galactosidase (α- galactosidase, β-galactosidase or both) and α-glucanase or its isomer pullulanase and ascorbic acid or any combination thereof.

The purpose of employing the enzyme blend, is to break down polysaccharide-colourant complex, into forms that are readily dispersed into a colourless and soluble component and to prevent oxidation of phenols to coloured counterparts.

The enzymes may be obtained commercially in a solid or liquid form. However, the powder form is preferred in the instant disclosure for purpose of stability and to inhibit protein-protein interaction in solution.

Preferred enzymes are obtained from microbial strains and have sufficient activity per gram of enzyme protein to economically solubilize and remove colourants and matter contributing to turbidity within the available retention time.

Table 1: Microbial sources of enzymes employed in the enzyme blend.

Component	Source
Cellulase, xylanase, betaglucanase	Trichoderma strains specifically T. reesei or T. longbrachiatum. Other strains include Chrysosporium species and Humicola.
α-amylase	*Bacillus licheniformis, Bacillus amyloliquefaciens, Bacillus subtilis, Bacillus stearothermophilus* and other Bacillus species. Fungal sources include Aspergillus strains. The strains can be commercially procured from culture banks like National Collection of Industrial Microorganism.
α-glucanase	Trichoderma sp.
Galactosidase	Aspergillus, Bacillus, Penicillum, Trichoderma, Saccharomyces, E. Coli, Debaryomyces, Humicola, Lactobacillus
Glucose oxidase	*Penicillium chrysogenum, Penicillium italicum Penicillium variabile Aspergillus niger Penicillium amagasakiense Talaromyces flavus, Aspergillus niger Aspergillus niger*

The ascorbic acid which is a non-enzyme component employed in the enzyme blend of the instant disclosure can be produced by microbial fermentation or synthetically manufactured.

The commercial sources of enzymes are from enzyme manufactures like Novozyme, Advanced enzymes, Genencore, Dyadic, DSM, Meiji Seiko.

Table 2: The constituents of the enzyme-blend compositions A to G

	% (w/w) Individual Enzymes in the blend						
Enzymes	**A**	**B**	**C**	**D**	**E**	**F**	**G**
Xylanase (exo	50	40	40	30	30	25	25

xylanase or endo xylanase)							
Cellulase (exocellulase or cellobiohydrolase)	50	40	40	30	30	25	20
Hemicellulase		20	10	20	15	15	15
Alpha amylase				10	20	10	10
β glucanase			10	10	5	15	15
Galactosidase (alpha galactosidase)						5	5
Glucose oxidase						5	5
Ascorbic Acid							0.1
Excipients (maltodextrin or fume silica)							4.9

Example 2:

Procedure to obtain sulphur-free sugarcane juice/sugar juice in lab-scale:

a) Using enzyme-blend composition 'A':

100ml of sugarcane juice/sugar juice is aliquoted in two conical flasks. Initial juice of pH is recorded. About 30ppm of the enzyme blend 'A' which comprises 50% w/w of xylanase (exo-xylanase or endo xylanase) and 50% w/w of cellulase (exocellulase or cellobiohydrolase), optionally along with excipients, is added to one of the flasks. The other flask is taken as control. Both the flasks are placed in a water bath maintained at 70°C till temperature of juice reaches about 70°C. The flasks are held at about 70°C for about 10 minutes. After about 10 minutes, the flasks are cooled to about 28°C (ambient temperature) and thereafter the test and the control flask is neutralised (at pH7) using calcium oxide (lime). The flasks are thereafter plugged and allowed to settle at 100°C for 45-50 min. The flasks are thereafter cooled and the contents therein filtered and checked for ICUMSA (International Commission for Uniform Methods of Sugar Analysis).

b) Using enzyme-blend composition 'B':

100ml of sugarcane juice/sugar juice is aliquoted in two conical flasks. Initial juice of pH is recorded. About 30ppm of the enzyme blend 'B' which comprises 40% w/w of xylanase (exo xylanase or endo xylanase), 40% w/w of cellulase (exocellulase or cellobiohydrolase), 20% w/w of hemicellulase, optionally along with excipients, is

added to one of the flasks. The other flask is taken as control. Both the flasks are placed in a water bath maintained at 75°C till temperature of juice reaches about 75°C. The flasks are held at about 75 °C for about 15 minutes. After about 15 minutes, the flasks are cooled to about 30°C (ambient temperature) and thereafter the test and the control flask is neutralised (at pH7) using calcium oxide (lime). The flasks are thereafter plugged and allowed to settle at 100°C for 45-60 min. The flasks are thereafter cooled and the contents therein filtered and checked for ICUMSA (International Commission for Uniform Methods of Sugar Analysis).

c) Using enzyme-blend composition 'C':

100ml of sugarcane juice/sugar juice is aliquoted in two conical flasks. Initial juice of pH is recorded. About 30ppm of the enzyme blend 'C' which comprises 40% w/w of xylanase (exo xylanase or endo xylanase), 40% w/w of cellulase (exocellulase or cellobiohydrolase), 10% w/w of hemicellulase and 10% w/w of β- glucanase, optionally along with excipients, is added to one of the flasks. The other flask is taken as control. Both the flasks are placed in a water bath maintained at 65°C till temperature of juice reaches about 65°C. The flasks are held at about 65°C for about 10 minutes. After about 10 minutes, the flasks are cooled to about 25°C (ambient temperature) and thereafter the test and the control flask is neutralised (at pH7) using calcium oxide (lime). The flasks are thereafter plugged and allowed to settle at 100°C for 45-60 min. The flasks are thereafter cooled and the contents therein filtered and checked for ICUMSA (International Commission for Uniform Methods of Sugar Analysis).

d) Using enzyme-blend composition 'D':

100ml of sugarcane juice/sugar juice is aliquoted in two conical flasks. Initial juice of pH is recorded. About 30ppm of the enzyme blend 'D' which comprises 30% w/w of xylanase (exo xylanase or endo xylanase), 30% w/w of cellulase (exocellulase or cellobiohydrolase), 20% w/w of hemicellulase, 10% w/w of alpha amylase and 10% w/w of β- glucanase, optionally along with excipients, is added to one of the flasks. The other flask is taken as control. Both the flasks are placed in a water bath maintained at 70°C till temperature of juice reaches about 70°C. The flasks are held at about 70°C for about 10 minutes. After about 10 minutes, the flasks are cooled to about 28°C (ambient temperature) and thereafter the test and the control flask is

neutralised (at pH7) using calcium oxide (lime). The flasks are thereafter plugged and allowed to settle at 100°C for 45-50 min. The flasks are thereafter cooled and the contents therein filtered and checked for ICUMSA (International Commission for Uniform Methods of Sugar Analysis).

e) Using enzyme-blend composition 'E':

100ml of sugarcane juice/sugar juice is aliquoted in two conical flasks. Initial juice of pH is recorded. About 30ppm of the enzyme blend 'E' which comprises 30% w/w of xylanase (exo xylanase or endo xylanase), 30% w/w of cellulase (exocellulase or cellobiohydrolase), 15% w/w of hemicellulase, 20% w/w of alpha amylase and 5% w/w of β- glucanase, optionally along with excipients, is added to one of the flasks. The other flask is taken as control. Both the flasks are placed in a water bath maintained at 70°C till temperature of juice reaches about 70°C. The flasks are held at about 70 °C for about 10 minutes. After about 10 minutes, the flasks are cooled to about 28°C (ambient temperature) and thereafter the test and the control flask is neutralised (at pH7) using calcium oxide (lime). The flasks are thereafter plugged and allowed to settle at 100°C for 45-50 min. The flasks are thereafter cooled and the contents therein filtered and checked for ICUMSA (International Commission for Uniform Methods of Sugar Analysis).

f) Using enzyme-blend composition 'F':

100ml of sugarcane juice/sugar juice is aliquoted in two conical flasks. Initial juice of pH is recorded. About 30ppm of the enzyme blend 'F' which comprises 25% w/w of xylanase (exo xylanase or endo xylanase), 25% w/w of cellulase (exo cellulase or cellobiohydrolase), 15% w/w of hemicellulase, 10% w/w of alpha amylase, 15% w/w of β- glucanase, 5% w/w of alpha galactosidase and 5% w/w of glucose oxidase, optionally along with excipients, is added to one of the flasks. The other flask is taken as control. Both the flasks are placed in a water bath maintained at 70°C till temperature of juice reaches about 70°C. The flasks are held at about 70 °C for about 10 minutes. After about 10 minutes, the flasks are cooled to about 28°C (ambient temperature) and thereafter the test and the control flask is neutralised (at pH7) using calcium oxide (lime). The flasks are thereafter plugged and allowed to settle at 100°C for 45-50 min. The flasks are thereafter cooled and the contents therein filtered and

checked for ICUMSA (International Commission for Uniform Methods of Sugar Analysis).

g) Using enzyme-blend composition 'G':

100ml of sugarcane juice/sugar juice is aliquoted in two conical flasks. Initial juice of pH is recorded. About 30ppm of the enzyme blend 'G' which comprises 25% w/w of xylanase (exo xylanase), 20% w/w of cellulase (cellobiohydrolase), 15% w/w of hemicellulase, 10% w/w of alpha amylase, 15% w/w of β- glucanase, 5% w/w of alpha galactosidase and 5% w/w of glucose oxidase, 0.1 w/w ascorbic acid and 4.9% w/w of excipients (maltodextrin, fume silica), is added to one of the flasks. The other flask is taken as control. Both the flasks are placed in a water bath maintained at 70°C till temperature of juice reaches about 70°C. The flasks are held at about 70 °C for about 10 minutes. After about 10 minutes, the flasks are cooled to about 28°C (ambient temperature) and thereafter the test and the control flask is neutralised (at pH7) using calcium oxide (lime). The flasks are thereafter plugged and allowed to settle at 100°C for 45-50 min. The flasks are thereafter cooled and the contents therein filtered and checked for ICUMSA (International Commission for Uniform Methods of Sugar Analysis).

Example 3:

Procedure for Estimating ICUMSA:

For analysis of colour, ICUMSA Method GS 1/3-7 is employed. Sugarcane juice/sugar juice samples are diluted to attain Brix 5.0. pH is adjusted to 7.0 with dilute HCl/NaOH. The samples are filtered through 0.45 µm membranes and absorbance is measured at 420 nm against distilled water as blank.

Colour is then estimated as per the formula:

ICUMSA colour = {Absorbance/ (path length of cuvette x concentration of sugar cane juice/sugar juice)} x 1000.

Sugar colour is an indication of impurities present in sugar. Higher the ICUMSA higher is the impurities in sugar and hence lower is the quality. The limits of ICUMSA for plantation or milled white sugar is < 150 as per Codex Standard for Sugars 212-1999.

ICUMSA Results (of clarified juice treated with enzyme blend 'G' in lab-scale experiments as described above):

Preliminary results of this disclosure show that the colour in untreated sugarcane juice/sugar juice is 28758 ICUMSA units and this is reduced to 14466 ICUMSA units after sulphitation. The reduction in colour is 49.7%. With the addition of enzyme blend 'G', the colour in sugarcane juice/sugar juice is reduced to 9561 ICUMSA units which represents 66.7% reduction in colour when compared with untreated juice (Figure 2). The additional reduction in colour with enzymes (as compared to sulphitation) is 18%. This indicates that the instant disclosure can be used to replace sulphur in the process of manufacturing sugar and also facilitate better colour removal.

Table 3: Comparison of sugarcane juice parameters treated with sulphur and with enzyme blend 'G'.

Parameters	**Mixed juice treated with sulphur**	**Enzyme Treated Juice**
Brix	0.2	0.6
Absorbance @ 720 nm (Turbidity)	0.492	0.287
Initial ICUMSA (colour)	28758	28758
Final ICUMSA (colour)	14466	9561
% Reduction in colour	49.7 %	66.7 %
Purity (%)	77.27	78.32

Example 4:

a) Validation of the enzyme-based sulphur replacement process at plant-scale using enzyme blend composition 'A':

Large scale trial tests are carried out at three enzyme blend dosage levels: 15, 30, and 45 ppm (w/v). The enzyme- blend comprising xylanase (exo xylanase or endo xylanase) and cellulase (exo cellulase or cellobiohydrolase) at a concentration of 50% (w/w) each, i.e. enzyme blend composition A (Table 2) is added to the mixed juice

following milling to maximize the reaction time at dosages ranging from 1 ppm to 70 ppm with optimum dosage between 15 ppm to 45 ppm. The juice is thereafter processed as per typical clarification procedures.

Juice extraction is conducted in a five-mill tandem with a standard imbibition system. Juice from the crusher and the first mill (undiluted cane juice) is combined to form the primary juice. The enzyme blend is added to the mixed juice in the juice collection tank when the temperature is around 40 ^{o}C. The juice is then heated and it takes around 2-3 minutes to reach sulphitation tank where the temperature is about 60^{o}C to 70^{o}C. At prevailing operating conditions, the mixed juice requires between 2 and 2.5 minutes to enter the sulphitation tank. Residence time in the sulphitation tank is about 7-10 minutes and not more than 15 minutes and temperature ranges between 60^{o}C to 70^{o}C and not more than 80^{o}C or not less than 50^{o}C.The juice is then cooled to about 28˚C (ambient temperature). Milk of lime (calcium hydroxide) is added to raise the juice pH from approximately about 5.0- 5.5 to about 7.1 to 7.2. Thus the enzyme-blend has about 12- 15 minutes reaction time at an activity level of essentially 100%. No sulphur is added. The limed juice is then pumped to holding tanks and then to the juice heaters, where the temperature is increased to about 100^{o}C -105^{o}C thereby inactivating the enzyme-blend. Available reaction time at pH of about 7.1 to 7.2 and not more than 7.5 is about 14 to 16 minutes. Commercially available anionic flocculent (BASF) is added to speed the settling of mud and other particulate and colloidal matter in the clarifier. Clarifier residence time is about 80 to 90 minutes. This step is followed by an optional step of filtering by membrane filtration, using filter press or centrifugation or any combination thereof. The clear supernatant (clarified juice) is then pumped to the evaporator station to be concentrated before sugar boiling begins.

Samples of mixed juice, clear juice and final sugars are collected and analysed for various parameters. These parameters are compared with samples collected during sulphitation. Significant improvements in yields are obtained along with reduction in press mud without compromising the colour of final sugar. Sulphur is completely eliminated in the juice clarification step, resulting in additional saving as low energy is employed and lesser amounts of lime are used to achieve outcomes similar to sulphitation.

b) Validation of the enzyme-based sulphur replacement process at plant-scale using enzymne blend composition 'B':

The enzyme- blend comprising xylanase (40% w/w of exo xylanase or endo xylanase) , cellulase (40% w/w or exocellulase or cellobiohydrolase) and hemicellulase (20% w/w), i.e. enzyme blend composition B (Table 2) is added to the mixed juice following milling to maximize the reaction time at dosage of 15ppm, 30 ppm and 45ppm. The juice is thereafter processed as per typical clarification procedures.

Juice extraction is conducted in a five-mill tandem with a standard imbibition system. Juice from the crusher and the first mill (undiluted cane juice) is combined to form the primary juice. The enzyme blend is added to the mixed juice in the juice collection tank when the temperature is around 40 ^{o}C. The juice is then heated and it takes around 2-3 minutes to reach sulphitation tank where the temperature is about 60. At prevailing operating conditions, the mixed juice requires between 2 and 2.5 minutes to enter the sulphitation tank. Residence time in the sulphitation tank is about 7minutes and temperature is about 60^{o}C.The juice is then cooled to about 25°C (ambient temperature). Milk of lime (calcium hydroxide) is added to raise the juice pH from approximately about 5.0- 5.5 to about 7.1 to 7.2. Thus the enzyme- blend has about 12minutes reaction time at an activity level of essentially 100%. No sulphur is added. The limed juice is then pumped to holding tanks and then to the juice heaters, where the temperature is increased to about 100^{o}C thereby inactivating the enzyme-blend. Available reaction time at pH of about 7.1 to 7.2 and not more than 7.5 is about 14 minutes. Commercially available anionic flocculent (BASF) is added to speed the settling of mud and other particulate and colloidal matter in the clarifier. Clarifier residence time is about 80 minutes. This step is followed by a step of filtering by membrane filtration, using filter press or centrifugation or any combination thereof. The clear supernatant (clarified juice) is then pumped to the evaporator station to be concentrated before sugar boiling begins.

Samples of mixed juice, clear juice and final sugars are collected and analysed for various parameters. These parameters are compared with samples collected during sulphitation. Significant improvements in yields are obtained along with reduction in press mud without compromising the colour of final sugar. Sulphur is completely eliminated in the juice clarification step, resulting in additional saving as low energy

is employed and lesser amounts of lime are used to achieve outcomes similar to sulphitation.

c) Validation of the enzyme-based sulphur replacement process at plant-scale using enzymne blend composition 'C':

The enzyme- blend comprising xylanase (40% w/w of exo xylanase or endo xylanase), cellulase (40% w/w of exo cellulase or cellobiohydrolase), hemicellulase (10% w/w) and β glucanase (10% w/w) i.e. enzyme blend composition C (Table 2) is added to the mixed juice following milling to maximize the reaction time at dosage of about 15ppm, 30 ppm and 45ppm. The juice is thereafter processed as per typical clarification procedures.

Juice extraction is conducted in a five-mill tandem with a standard imbibition system. Juice from the crusher and the first mill (undiluted cane juice) is combined to form the primary juice. The enzyme blend is added to the mixed juice in the juice collection tank when the temperature is around 40 ^{o}C. The juice is then heated and it takes around 2-3 minutes to reach sulphitation tank where the temperature is about 70^{o}C. At prevailing operating conditions, the mixed juice requires between 2 and 2.5 minutes to enter the sulphitation tank. Residence time in the sulphitation tank is about 10 minutes and temperature is about 70^{o}C.The juice is then cooled to about 30˚C (ambient temperature). Milk of lime (calcium hydroxide) is added to raise the juice pH from approximately about 5.0- 5.5 to about 7.1 to 7.2. Thus the enzyme- blend has about 15 minutes reaction time at an activity level of essentially 100%. No sulphur is added. The limed juice is then pumped to holding tanks and then to the juice heaters, where the temperature is increased to about 105^{o}C thereby inactivating the enzyme-blend. Available reaction time at pH of about 7.1 to 7.2 and not more than 7.5 is about 16 minutes. Commercially available anionic flocculent (BASF) is added to speed the settling of mud and other particulate and colloidal matter in the clarifier. Clarifier residence time is about 90 minutes. This step is followed by an optional step of filtering by membrane filtration, using filter press or centrifugation or any combination thereof. The clear supernatant (clarified juice) is then pumped to the evaporator station to be concentrated before sugar boiling begins.

Samples of mixed juice, clear juice and final sugars are collected and analysed for various parameters. These parameters are compared with samples collected during

sulphitation. Significant improvements in yields are obtained along with reduction in press mud without compromising the colour of final sugar. Sulphur is completely eliminated in the juice clarification step, resulting in additional saving as low energy is employed and lesser amounts of lime are used to achieve outcomes similar to sulphitation.

d) Validation of the enzyme-based sulphur replacement process at plant-scale using enzymne blend composition 'D':

The enzyme- blend comprising xylanase (30% w/w of exo xylanase or endo xylanase), cellulase (30% w/w of exo cellulase or cellobiohydrolase), hemicellulase (20% w/w), alpha amylase (10% w/w) and βglucanase (10% w/w) i.e. enzyme blend composition D (Table 2) is added to the mixed juice following milling to maximize the reaction time at dosage of about 15ppm, 30 ppm and 45ppm. The juice is thereafter processed as per typical clarification procedures.

Juice extraction is conducted in a five-mill tandem with a standard imbibition system. Juice from the crusher and the first mill (undiluted cane juice) is combined to form the primary juice. The enzyme blend is added to the mixed juice in the juice collection tank when the temperature is around 40 ^{o}C. The juice is then heated and it takes around 2-3 minutes to reach sulphitation tank where the temperature is about 60^{o}C to 70^{o}C. At prevailing operating conditions, the mixed juice requires between 2 and 2.5 minutes to enter the sulphitation tank. Residence time in the sulphitation tank is about 15 minutes and temperature is about 80^{o}C.The juice is then cooled to about 28˚C (ambient temperature). Milk of lime (calcium hydroxide) is added to raise the juice pH from approximately about 5.0- 5.5 to about 7.1 to 7.2. Thus the enzyme- blend has about 12- 15 minutes reaction time at an activity level of essentially 100%. No sulphur is added. The limed juice is then pumped to holding tanks and then to the juice heaters, where the temperature is increased to about 100^{o}C -105^{o}C thereby inactivating the enzyme-blend. Available reaction time at pH of about 7.1 to 7.2 and not more than 7.5 is about 14 to 16 minutes. Commercially available anionic flocculent (BASF) is added to speed the settling of mud and other particulate and colloidal matter in the clarifier. Clarifier residence time is about 80 to 90 minutes. . This step is followed by an optional step of filtering by membrane filtration, using filter press or centrifugation or any combination thereof. The clear supernatant

(clarified juice) is then pumped to the evaporator station to be concentrated before sugar boiling begins.

Samples of mixed juice, clear juice and final sugars are collected and analysed for various parameters. These parameters are compared with samples collected during sulphitation. Significant improvements in yields are obtained along with reduction in press mud without compromising the colour of final sugar. Sulphur is completely eliminated in the juice clarification step, resulting in additional saving as low energy is employed and lesser amounts of lime are used to achieve outcomes similar to sulphitation.

e) Validation of the enzyme-based sulphur replacement process at plant-scale using enzymne blend composition 'E':

The enzyme- blend comprising xylanase (30% w/w of exo xylanase or endo xylanase), cellulase (30% w/w of exo cellulase or cellobiohydrolase), hemicellulase (15% w/w), alpha amylase (20% w/w) and β glucanase (5% w/w) i.e. enzyme blend composition E (Table 2) is added to the mixed juice following milling to maximize the reaction time at dosage of about 15ppm, 30 ppm and 45ppm. The juice is thereafter processed as per typical clarification procedures.

Juice extraction is conducted in a five-mill tandem with a standard imbibition system. Juice from the crusher and the first mill (undiluted cane juice) is combined to form the primary juice. The enzyme blend is added to the mixed juice in the juice collection tank when the temperature is around 40 ^{o}C. The juice is then heated and it takes around 2-3 minutes to reach sulphitation tank where the temperature is about 60^{o}C to 70^{o}C. At prevailing operating conditions, the mixed juice requires between 2 and 2.5 minutes to enter the sulphitation tank. Residence time in the sulphitation tank is about 7-10 minutes and not more than 15 minutes and temperature is about 50^{o}C.The juice is then cooled to about 28˚C (ambient temperature). Milk of lime (calcium hydroxide) is added to raise the juice pH from approximately about 5.0- 5.5 to about 7.1 to 7.2. Thus the enzyme- blend has about 12- 15 minutes reaction time at an activity level of essentially 100%. No sulphur is added. The limed juice is then pumped to holding tanks and then to the juice heaters, where the temperature is increased to about 100^{o}C -105^{o}C thereby inactivating the enzyme-blend. Available reaction time at pH of about 7.1 to 7.2 and not more than 7.5 is about 14 to 16 minutes. Commercially available

anionic flocculent (BASF) is added to speed the settling of mud and other particulate and colloidal matter in the clarifier. Clarifier residence time is about 80 to 90 minutes. . This step is followed by an optional step of filtering by membrane filtration, using filter press or centrifugation or any combination thereof. The clear supernatant (clarified juice) is then pumped to the evaporator station to be concentrated before sugar boiling begins.

Samples of mixed juice, clear juice and final sugars are collected and analysed for various parameters. These parameters are compared with samples collected during sulphitation. Significant improvements in yields are obtained along with reduction in press mud without compromising the colour of final sugar. Sulphur is completely eliminated in the juice clarification step, resulting in additional saving as low energy is employed and lesser amounts of lime are used to achieve outcomes similar to sulphitation.

f) Validation of the enzyme-based sulphur replacement process at plant-scale using enzymne blend composition 'F':

The enzyme- blend comprising xylanase (25% w/w of exo xylanase or endo xylanase), cellulase (25% w/w of exo cellulase or cellobiohydrolase), hemicellulase (15% w/w), alpha amylase (10% w/w) and βglucanase (15% w/w), alpha galactosidase (5% w/w) and glucose oxidase (5% w/w) i.e. enzyme blend composition F (Table 2) is added to the mixed juice following milling to maximize the reaction time at dosage of about 15ppm, 30 ppm and 45ppm. The juice is thereafter processed as per typical clarification procedures.

Juice extraction is conducted in a five-mill tandem with a standard imbibition system. Juice from the crusher and the first mill (undiluted cane juice) is combined to form the primary juice. The enzyme blend is added to the mixed juice in the juice collection tank when the temperature is around 40 ^{o}C. The juice is then heated and it takes around 2-3 minutes to reach sulphitation tank where the temperature is about 60^{o}C to 70^{o}C. At prevailing operating conditions, the mixed juice requires between 2 and 2.5 minutes to enter the sulphitation tank. Residence time in the sulphitation tank is about 7-10 minutes and not more than 15 minutes and temperature ranges between 60^{o}C to 70^{o}C and not more than 80^{o}C or not less than 50^{o}C.The juice is then cooled to about 28˚C (ambient temperature). Milk of lime (calcium hydroxide) is added to raise the

juice pH from approximately about 5.0- 5.5 to about 7.1 to 7.2. Thus the enzyme-blend has about 12- 15 minutes reaction time at an activity level of essentially 100%. No sulphur is added. The limed juice is then pumped to holding tanks and then to the juice heaters, where the temperature is increased to about 100°C -105°C thereby inactivating the enzyme-blend. Available reaction time at pH of about 7.1 to 7.2 and not more than 7.5 is about 14 to 16 minutes. Commercially available anionic flocculent (BASF) is added to speed the settling of mud and other particulate and colloidal matter in the clarifier. Clarifier residence time is about 80 to 90 minutes. This step is followed by an optional step of filtering by membrane filtration, using filter press or centrifugation or any combination thereof. The clear supernatant (clarified juice) is then pumped to the evaporator station to be concentrated before sugar boiling begins.

Samples of mixed juice, clear juice and final sugars are collected and analysed for various parameters. These parameters are compared with samples collected during sulphitation. Significant improvements in yields are obtained along with reduction in press mud without compromising the colour of final sugar. Sulphur is completely eliminated in the juice clarification step, resulting in additional saving as low energy is employed and lesser amounts of lime are used to achieve outcomes similar to sulphitation.

g) Validation of the enzyme-based sulphur replacement process at plant-scale using enzymne blend composition 'G':

The enzyme- blend comprising xylanase (25% w/w of exo xylanase), cellulase (20% w/w of cellobiohydrolase), hemicellulase (15% w/w), alpha amylase (10% w/w) and β glucanase (15% w/w), alpha galactosidase (5% w/w) a glucose oxidase (5% w/w), ascorbic acid (0.1% w/w) and excipient- maltodextrin, fume silica (4.9% w/w) i.e. enzyme blend composition G (Table 2) is added to the mixed juice following milling to maximize the reaction time at dosage of about 15ppm, 30 ppm and 45ppm. The juice is thereafter processed as per typical clarification procedures.

Extraction is conducted in a five-mill tandem with a standard imbibition system. Juice from the crusher and the first mill (undiluted cane juice) is combined to form the primary juice. The enzyme blend is added to the mixed juice in the juice collection tank when the temperature is around 40 °C. The juice is then heated and it takes

around 2-3 minutes to reach sulphitation tank where the temperature is about 60°C to 70°C. At prevailing operating conditions, the mixed juice requires between 2 and 2.5 minutes to enter the sulphitation tank. Residence time in the sulphitation tank is about 7-10 minutes and not more than 15 minutes and temperature ranges between 60°C to 70°C and not more than 80°C or not less than 50°C.The juice is then cooled to about 28°C (ambient temperature). Milk of lime (calcium hydroxide) is added to raise the juice pH from approximately about 5.0- 5.5 to about 7.1 to 7.2. Thus the enzyme-blend has about 12- 15 minutes reaction time at an activity level of essentially 100%. No sulphur is added. The limed juice is then pumped to holding tanks and then to the juice heaters, where the temperature is increased to about 100°C -105°C thereby inactivating the enzyme-blend. Available reaction time at pH of about 7.1 to 7.2 and not more than 7.5 is about 14 to 16 minutes. Commercially available anionic flocculent (BASF) is added to speed the settling of mud and other particulate and colloidal matter in the clarifier. Clarifier residence time is about 80 to 90 minutes. This step is followed by an optional step of filtering by membrane filtration, using filter press or centrifugation or any combination thereof. The clear supernatant (clarified juice) is then pumped to the evaporator station to be concentrated before sugar boiling begins.

Samples of mixed juice, clear juice and final sugars are collected and analysed for various parameters. These parameters are compared with samples collected during sulphitation. Significant improvements in yields are obtained along with reduction in press mud without compromising the colour of final sugar. Sulphur is completely eliminated in the juice clarification step, resulting in additional saving as low energy is employed and lesser amounts of lime are used to achieve outcomes similar to sulphitation.

Figures 3 and 4 compare the colour and purity of clear juice treated with enzyme blend 'G' against clear juice subjected to sulphitation process. Figures 5 and 6 compare the colour and percentage of recovery of final sugar obtained by sulphitation process and that obtained by enzyme blend 'G' application.

Example 5:

Comparison of the enzyme-based sulphur replacement process with sulphitation process for clarifying sugar juice (at plant-scale).

The enzyme based sulphur replacement process of clarifying sugar juice is advantageous over sulphitation, in the aspects of decreasing the scaling of evaporators and pipelines, reduction in equipment corrosion, improving biomethanation rates and quality of sugar.

Table 4: Comparison of the enzyme process with sulphitation process

Parameter	**Sulphitation**	**Enzymatic method employing enzyme blend 'G'**
Clear juice purity (%)	82.78	85.39
Calcium oxide in clear juice (ppm)	1180	1050
Rise in calcium oxide from mixed juice to clear juice (ppm)	370	227.5
Quantity of filter cake (in tonne)	277.43	96.4
Lime consumption (in tonne)	0.16	0.1
Recovery (%)	10.04	10.58

The above table shows that the amount of lime consumed and amount of lime present in clear juice has been significantly reduced in the process of the instant disclosure employing enzymes. Consequently, there will be reduction of scaling on pipelines and equipments in the process of the instant disclosure, caused due to deposition of salts of sulphur, potassium/calcium salts and reaction of sulphur with potassium and other ions.

Example 6:

Effect of dosage of enzyme-blend 'G' on reduction in juice colour: The effect of dosage of enzyme blend is studied on reduction of colour and enhancement of purity in juice. Enzyme blend is dosed at 15 ppm, 30 ppm and 45 ppm on mixed juice at

plant scale. Clear juice obtained after filteration is analysed for parameters. Data is represented in the table below:

Table 5: Analysis of mixed sugar juice and clear sugar juice

S.No	Particulars	No enzymes	With Enzyme blend 'G'		
			15 ppm (w/v)	30 ppm (w/v)	45 ppm (w/v)
1	Mixed Juice Analysis				
	Brix	14.29	14.42	15.02	14.48
	Pol	11.77	11.9	12.49	12.3
	Purity	82.38	82.52	83.15	83.78
2	Clear Juice Analysis				
	Brix	14.46	15.74	14.7	14.72
	Pol	11.97	13.79	12.52	12.44
	Purity	82.78	84.48	85.17	87.61
3	Calcium Oxide in clear juice in ppm		1180	1090	1010
4	Sugar colour (ICUMSA)	132	136	132	107
5	Clear juice transparency	17.3	20.3	20.6	24.2
6	Clear juice turbidity	20.1	17.5	11.4	11.4
7	Clear juice ICUMSA	12810	12750	9649	8594
8	Purity rise from mixed juice to clear juice	0.4	1.96	2.02	3.83

From the above table, it is evident that with an increase in the dosage of the enzyme-blend, colour and turbidity of the clarified sugar juice reduces and the purity increases.

Example 7:

Effect of individual enzymes on reduction in colour of sugar juice in comparison with synergistic effect of enzyme blend on juice colour:

In order to study the effect of individual enzymes on juice colour, in the test samples, enzymes are added to fresh sugarcane juice at about 30 ppm dosage, incubated for about 15 minutes at a temperature of about 50°C. pH of the juice is adjusted to about 5.0 before enzyme addition. Following incubation, the juice is cooled and lime is added to neutralize the pH and the juice is heated to about 100°C for about 50

minutes. The solution is then filtered and colour is estimated by the standard ICUMSA method.

Table 6: Estimation of colour of sugar juice treated with individual enzymes

	Test	Vol. of juice(ml)	Enzyme (ppm) %(w/w)	ICUMSA	% Reduction
1	Control (No enzyme)	25	0	29000	-
2	Cellulase	25	30	28800	0.6
3	Amylase	25	30	28278	2.4
4	Hemicellulase	25	30	27579	4.9
5	Xylanase	25	30	27953	3.6
6	Beta Glucanase	25	30	29415	-0.5
7	Alpha Galactosidase	25	30	28188	2.8
8	Glucose Oxidase	25	30	27753	4.3

The above results show that addition of individual enzymes has no significant effect on reduction in colour of juice. This can be attributed to each enzyme acting on a specific component, reduction of which has no significant effect on the colour of juice.

Synergistic effect of enzymes is studied by adding enzymes in various plurality of combinations to the juice. Enzyme combinations/enzyme blends are added to 25 ml of fresh sugarcane juice at about 30 ppm dosage, incubated for about 15 minutes at about 50 ^{o}C. pH of the juice is adjusted to about 5.0 before enzyme addition. Following incubation, the juice is cooled, lime is added to neutralize the pH and the juice is heated to about 100 ^{o}C for about 50 minutes. The solution is then filtered and colour is estimated by the standard ICUMSA method.

Table 7: Analysis of colour and turbidity in sugar juice treated with the enzyme blend compositions A to G

Sl. No.	Enzyme blend Name	Dose (ppm)	Turbidity	ICUMSA	% Reduction
1	Control (no enzymes)	0	0.0333	29000	0

2	A	30	0.0307	25520	12
3	B	30	0.027	24650	15
4	C	30	0.0286	26680	8
5	D	30	0.0246	24070	17
6	E	30	0.028	24070	17
7	F	30	0.0232	22620	22
8	G	30	0.022	22040	24

From the above results, it is evident that the percentage of reduction in ICUMSA values of the clarified juice is much higher in case of the combination of enzymes-A,B,C,D,E,F,G when compared to the ICUMSA values of the sugar juice that is clarified using individual enzymes. This shows that the said combinations of enzymes are synergistic compositions and not mere additive compositions.

Example 8:

Effect of ascorbic acid in combination with carbohydrate depolymerising enzymes on colour removal in sugarcane juice/sugar juice

In the test sample, enzymes from blend F mentioned above is added to fresh sugarcane juice at about 30 ppm dosage, incubated for about 15 minutes at about 50 ^{o}C along with ascorbic acid at various concentrations. pH of the juice is adjusted to about 5.0 before enzyme addition. After about 15 minutes, lime is added to neutralize the pH and the juice is heated to about 100 ^{o}C for about 50 minutes. The solution is then filtered and colour is estimated by the standard ICUMSA method.

Control for this experiment consisted of juice that is treated with lime but no enzymes.

Table 8: Analysis of colour of sugar juice treated with enzyme blend F and various concentrations of ascorbic acid

Test	ICUMSA	% Reduction
Control- No enzymes	30112	0.0
Control- enzyme blend F	23908	20.6

Enzymes +0.015% w/v Ascorbic acid	22794	24.3
Enzymes + 0.03% w/v Ascorbic acid	23005	23.6
Enzymes +0.05% w/v Ascorbic acid	22855	24.1
Enzymes +0.07% w/v Ascorbic acid	23186	23.0
Enzymes +0.1% w/v Ascorbic acid	22794.78	24.3
0.1% Ascorbic acid	29690	1.4

Ascorbic acid at 0.015% w/v to 0.1% along with enzymes has been found to be most effective in reducing colour in sugarcane juice.

Example 9:

Process for manufacture of sugar from sugarbeet involving the employment of enzyme-blend (Figure 7)

Sugar beets are washed, sliced, grated, the juice is extracted using hot water at a temperature range of about 50 °C to about 70 °C, wherein the pH of the juice is about 7 and the retention time is about 2 to about 5 minutes. The juice is then filtered. Instead of following the step of subjecting the sugar juice to the conventional process of sulphitation with sulphur dioxide gas, any of the enzyme-blend composition of the instant disclosure is added to the sugar juice to remove colour, turbidity and other impurities, wherein the temperature at which this step is followed, is at about 80 °C for a duration of about 10 minutes and wherein the sugar juice is at a pH of 7. The resulting clear/clarified juice is subjected to multiple stage evaporation. The multiple stage evaporation process is carried out to concentrate sucrose in juice by removing water. This is typically carried out in a series of 5 evaporators. The first evaporator is heated with steam from boilers and steam from water evaporated in first evaporator is used to heat second evaporator and so on. Since there is consequent decrease in heat due to heat loss from

from evaporator to evaporator there is decrease in pressure. This allows juice to boil at lower temperatures as it passes on.

After evaporation, thick juice is obtained which has sucrose concentrations of around 55%-65%.Thereafter, the resulting syrup is optionally treated with any of the enzyme-blend compositions 'A' to 'G', and further crystallized to obtain white sugar.

Process for manufacture of sugar from raw sugar involving the employment of enzyme- blend (Figure 8)

Raw sugar is firstly subjected to affination or washing at a temperature of about 85°C for about 2 to about 3 minutes, wherein the pH of the raw sugar is about 7.2 to about 8. The washed raw sugar is dissolved in water to obtain sugar juice which is then purified with milk of lime at about 100°C for about 2 to about 3 minutes, wherein the pH of the juice is about 8. The purified juice is then filtered. The filtered juice is clarified by treatment with any of the the enzyme-blend compositions 'A' to 'G', at a temperature of about 70 °C for a duration of about 10 minutes, wherein the pH of the juice is about 7, instead of being subjected to conventional processes like sulphitation and usage of ion- exchange resins. The resulting clarified juice is boiled to obtain the syrup which is again optionally treated with any of the enzyme-blend compositions of the instant disclosure. Thereafter, the clarified syrup is crystallized and centrifuged to obtain white sugar.

The examples provided herein should not be construed to be limited to the enzyme-blend combinations *per se* as disclosed in these sections. These examples are provided for purposes of illustration only and should be read in conjunction with the detailed description of the instant disclosure. Hence, a person skilled in the art will be able to extend the workability of similar enzyme blend or other varied combination of enzyme blends in addition to the enzyme blend combinations as provided in the examples herein.

We claim:

1. A composition comprising an enzyme blend, optionally along with excipient.
2. The composition as claimed in claim 1, wherein the composition is a synergistic composition and wherein the enzyme blend comprises plurality of enzymes selected from a group comprising amylase, xylanase, cellulase, hemicellulase, glucanase, galactosidase, glucose oxidase and non-enzyme component- ascorbic acid or any combination thereof.
3. The composition as claimed in claim 2, wherein the amylase is selected from a group comprising α-amylase, β-amylase and γ-amylase or any combination thereof at a concentration ranging from about 0.1% to about 99% w/w, preferably from about 10% to about 20% w/w; the xylanase is selected from a group comprising exo-xylanase and endo-xylanase or a combination thereof at a concentration ranging from about 0.1% to about 99% w/w, preferably from about 20% to about 40% w/w; the cellulase is selected from a group comprising exo-cellulase, endo-cellulase and cellobiase or any combination thereof at a concentration ranging from about 0.1% to about 99% w/w, preferably from about 20% to about 40% w/w; the hemicellulase is at a concentration ranging from about 0.1% to about 99% w/w, preferably from about 15% to about 30% w/w; the glucanase is selected from a group comprising α-glucanase, β-glucanase, xyloglucan-specific endo-beta-1,4-glucanase, xyloglucan-specific exo-beta-1,4-glucanase and pullulanase or any combination thereof at a concentration ranging from about 0.1% to about 99% w/w, preferably from about 5% to about 30% w/w; the galactosidase is selected from a group comprising α-galactosidase and β-galactosidase or a combination thereof at a concentration ranging from about 0.1% to about 99% w/w, preferably from about 5% to 10% w/w; the glucose oxidase is at a concentration ranging from about 0.1% to about 99% w/w, preferably from about 5% to 10% w/w; and the non-enzyme component- ascorbic acid is at a concentration ranging from about about 0.1% to about 99% w/w, preferably from about 0.015 to about 10 % w/w..
4. The composition as claimed in claim 1, wherein the composition is employed for clarifying sugar juice or obtaining chemical free sugar, and wherein the sugar juice is obtained from a source selected from a group comprising

sugarcane, sugarbeet, raw sugar, and other substrate used for sugar processing or any combination thereof.

5. The composition as claimed in claim 1, wherein the excipient is selected from a group comprising anticaking agent, stabiliser and flocculent or any combination thereof.

6. The composition as claimed in claim 5, wherein the anticaking agent is selected from a group comprising fumed silica, bentonite and talc or any combination thereof; wherein the stabilizer is selected from a group comprising sodium chloride, fumed silica, sucrose, maltodextrin, trehalose, lactose, arabinose and cellulose or any combination thereof; and wherein the flocculent is an anionic flocculent.

7. The composition as claimed in claim 5, wherein the excipient is in concentration ranging from about 0.01% to about 20%, preferably about 1% to about 10%.

8. A process for clarifying sugar juice, said process comprising acts of:
   a. mixing the juice with enzyme blend to obtain a mixture;
   b. optionally holding-up followed by cooling the mixture; and
   c. neutralizing the mixture of step (a) or step (b) by adding a pH regulator, followed by re-holding-up and optional re-cooling, followed by optional adding of excipient or filtering or both, to obtain the clarified sugar juice.

9. The process as claimed in claim 8, wherein the sugar juice is obtained from a source selected from a group comprising sugarcane, sugarbeet, raw sugar, and other substrate used for sugar processing or any combination thereof.

10. The process as claimed in claim 8, wherein the enzyme blend comprises plurality of enzymes selected from a group comprising amylase, xylanase, cellulase, hemicellulase, glucanase, galactosidase, glucose oxidase and ascorbic acid or any combination thereof; and wherein the amylase is selected from a group comprising α-amylase, β-amylase and γ-amylase or any combination thereof at a concentration ranging from about 0.1% to about 99% w/w, preferably from about 10% to about 20% w/w; the xylanase is selected from a group comprising exo-xylanase and endo-xylanase or a combination thereof at a concentration ranging from about 0.1% to about 99% w/w, preferably from about 20% to about 40% w/w; the

cellulase is selected from a group comprising exo-cellulase, endo-cellulase and cellobiase or any combination thereof at a concentration ranging from about 0.1% to about 99% w/w, preferably from about 20% to about 40% w/w; the hemicellulase is at a concentration ranging from about 0.1% to about 99% w/w, preferably from about 15% to about 30% w/w; the glucanase is selected from a group comprising α-glucanase, β-glucanase, xyloglucan-specific endo-beta-1,4-glucanase, xyloglucan-specific exo-beta-1,4-glucanase and pullulanase or any combination thereof at a concentration ranging from about 0.1% to about 99% w/w, preferably from about 5% to about 30% w/w; the galactosidase is selected from a group comprising α-galactosidase and β-galactosidase or a combination thereof at a concentration ranging from about 0.1% to about 99% w/w, preferably from about 5% to 10% w/w; the glucose oxidase is at a concentration ranging from about 0.1% to about 99% w/w, preferably from about 5% to 10% w/w; and the non-enzyme component- ascorbic acid is at a concentration ranging from about about 0.1% to about 99% w/w, preferably from about 0.015 to about 10 % w/w.

11. The process as claimed in claim 8, wherein the mixing is carried out by adding the enzyme blend to the sugar juice having a temperature of about 35°C to about 45°C or wherein the mixing is carried out by adding the enzyme blend to the sugar juice to obtain a mixture followed by boiling the mixture to a temperature of about 65°C to about 75°C.

12. The process as claimed in claim 8, wherein the holding-up of step (b) is at a temperature ranging from about 50°C to about 80°C for a time period ranging from about 5 minutes to about 15 minutes.

13. The process as claimed in claim 8, wherein the cooling is at a temperature ranging from about 25°C to about 30°C.

14. The process as claimed in claim 8, wherein the neutralizing is carried out by addition of pH regulator selected from a group comprising calcium oxide (lime), calcium hydroxide (milk of lime), orthophosphoric acid and inorganic calcium compounds or any combination thereof.

15. The process as claimed in claim 8, wherein the re-holding-up of step (c) is for a time period ranging from about 20minutes to about 90minutes, at a temperature ranging from about 90°C to about 110°C or is for a time period

ranging from about 10minutes to about 20minutes, at a temperature ranging from about 90°C to about 110°C.

16. The process as claimed in claim 8, wherein the filtering is carried out by method selected from a group comprising membrane filteration, filter press and settling or any combination thereof.

17. The process as claimed in claim 8, wherein the clarified sugar juice can be further processed to obtain clarified sugar.

18. The process as claimed in claim 8, wherein the process comprises acts of:

a. adding the enzyme blend to the sugar juice having a temperature of about 30°C to about 45°C to obtain a mixture;

b. optionally holding-up at a temperature ranging from about 50°C to about 80°C for a time period ranging from about 5 minutes to about 15 minutes followed by cooling the mixture at a temperature ranging from about 25°C to about 30°C; and

c. neutralizing the mixture of step (a) or step (b) by adding a pH regulator, followed by re-holding-up for a time period ranging from about 20minutes to about 90minutes, at a temperature ranging from about 90°C to about 110°C and optional re-cooling, followed by optional adding of excipient or filtering or both, to obtain the clarified sugar juice.

19. The process as claimed in claim 8, wherein the process comprises acts of:

a. adding the enzyme blend to the sugar juice to obtain a mixture followed by boiling the mixture to a temperature of about 65°C to about 75°C;

b. optionally holding-up at a temperature ranging from about 50°C to about 80°C for a time period ranging from about 5 minutes to about 15 minutes followed by cooling the mixture at a temperature ranging from about 25°C to about 30°C; and

c. neutralizing the mixture of step (a) or step (b) by adding a pH regulator, followed by re-holding-up for a time period ranging from about 10minutes to about 20minutes, at a temperature ranging from about 90°C to about 110°C and optional re-cooling, followed by optional adding of excipient or filtering or both, to obtain the clarified sugar juice.

20. Clarified sugar juice obtained by the process as claimed in claim 8.

21. The clarified sugar juice as claimed in claim 20, wherein the juice can be further processed to obtain clarified sugar.

22. A process for obtaining a composition comprising an enzyme blend optionally along with excipient, said process comprising act of combining plurality of enzymes selected from a group comprising amylase, xylanase, cellulase, hemicellulase, glucanase, galactosidase, glucose oxidase and non-enzyme component- ascorbic acid or any combination thereof optionally along with the excipient to obtain the enzyme blend.

23. A kit for clarifying sugar juice or for obtaining clarified sugar, said kit comprising enzymes selected from group comprising amylase, xylanase, cellulase, hemicellulase, glucanase, galactosidase, glucose oxidase and non-enzyme component- ascorbic acid or any combination thereof; pH regulator selected from a group comprising calcium oxide (lime), calcium hydroxide (milk of lime), orthophosphoric acid and inorganic calcium compounds or any combination thereof; and excipient selected from a group comprising anticaking agent, stabiliser and flocculent or any combination thereof along with an instruction manual.

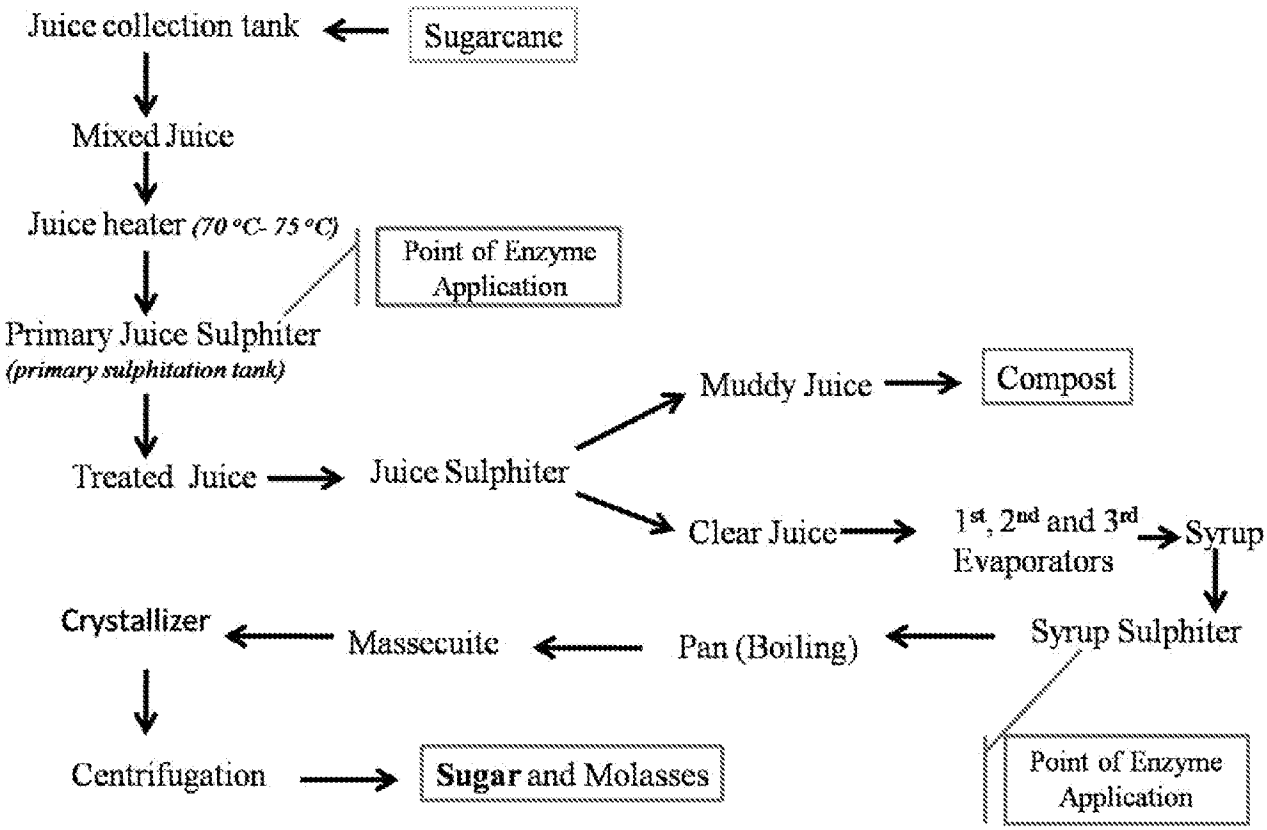
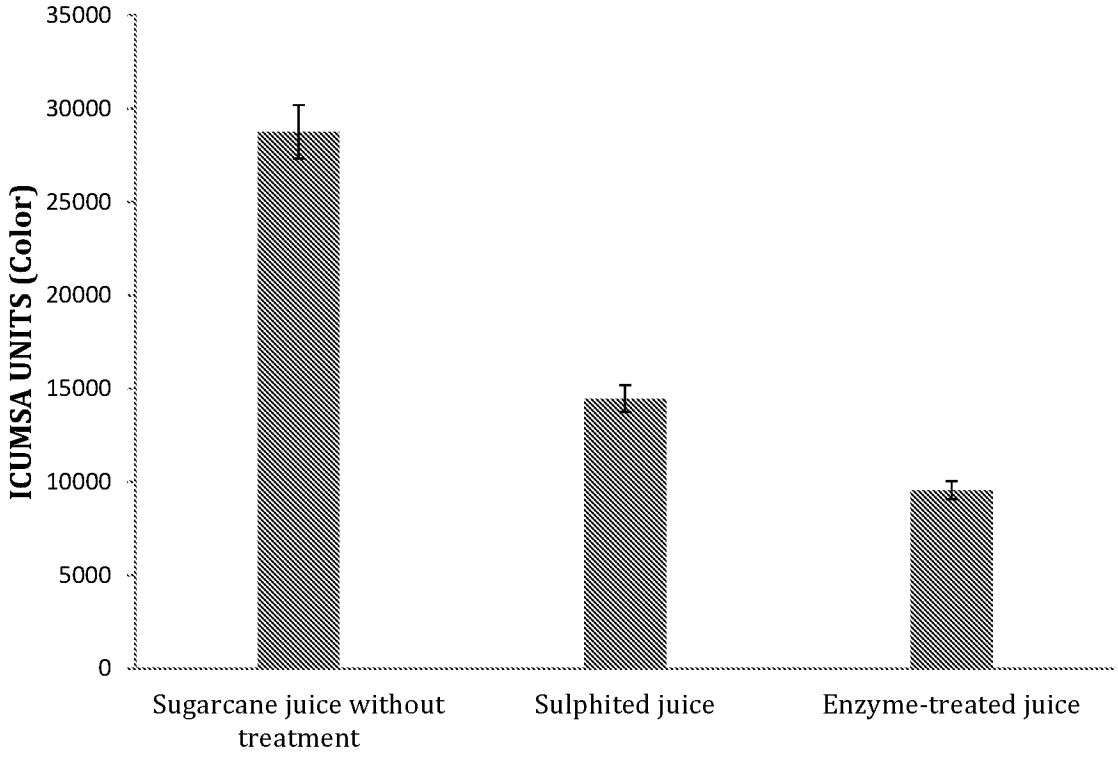

Juice collection tank
Sugarcane
Mixed Juice
Juice heater (70 °C- 75 °C)
Point of Enzyme Application
Primary Juice Sulphiter
(primary sulphitation tank)
Muddy Juice
Compost
Treated Juice
Juice Sulphiter
Clear Juice
1st, 2nd and 3rd Evaporators
Syrup
Crystallizer
Massecuite
Pan (Boiling)
Syrup Sulphiter
Centrifugation
Sugar and Molasses
Point of Enzyme Application

FIGURE 1


35000
30000
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15000
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ICUMSA UNITS (Color)
Sugarcane juice without treatment
Sulphited juice
Enzyme-treated juice

FIGURE 2

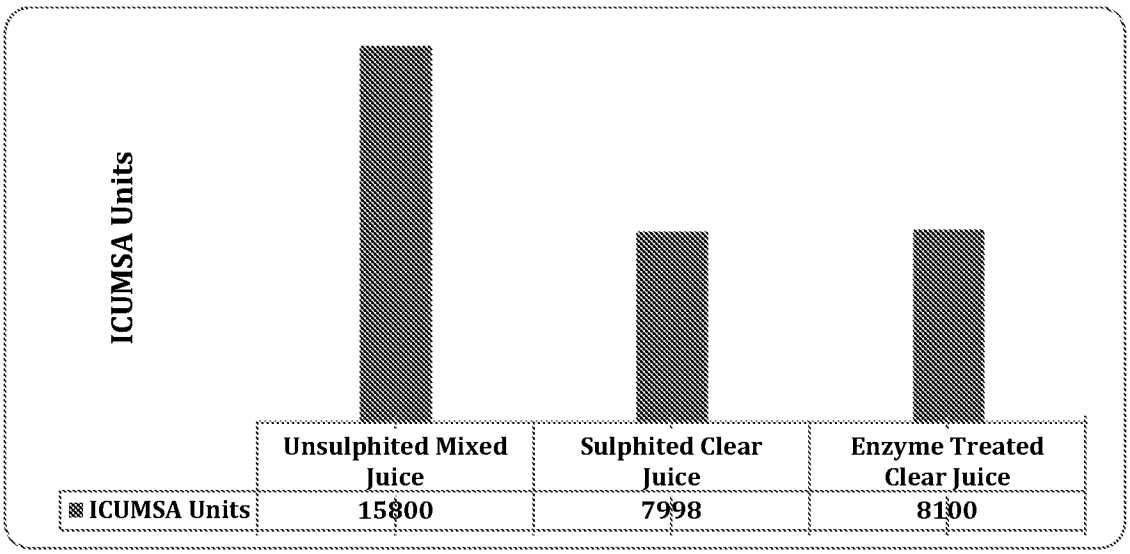

FIGURE 3

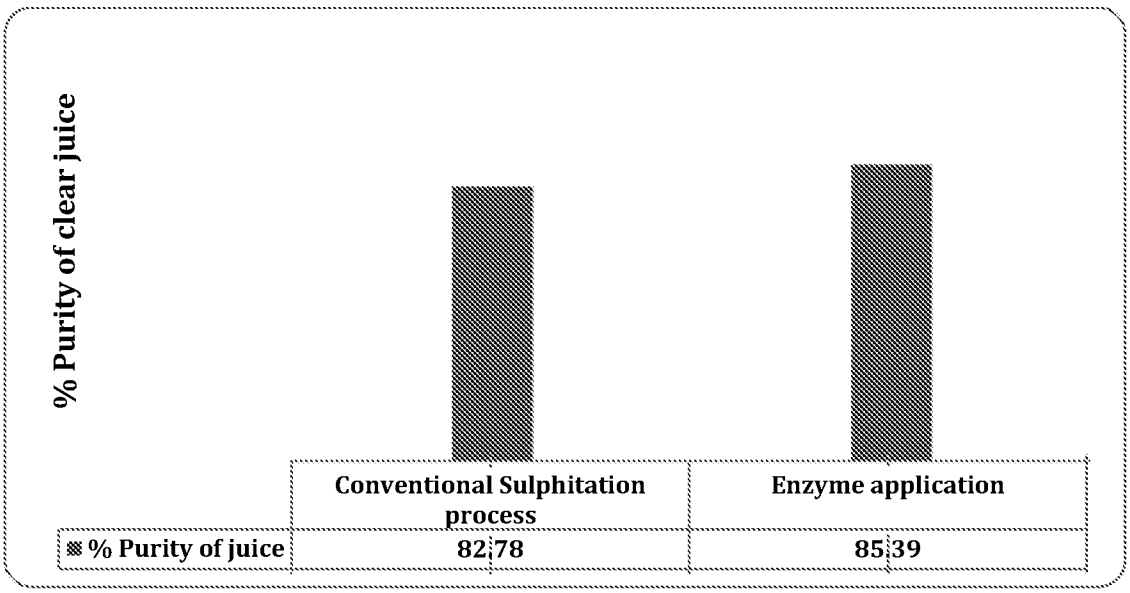

FIGURE 4

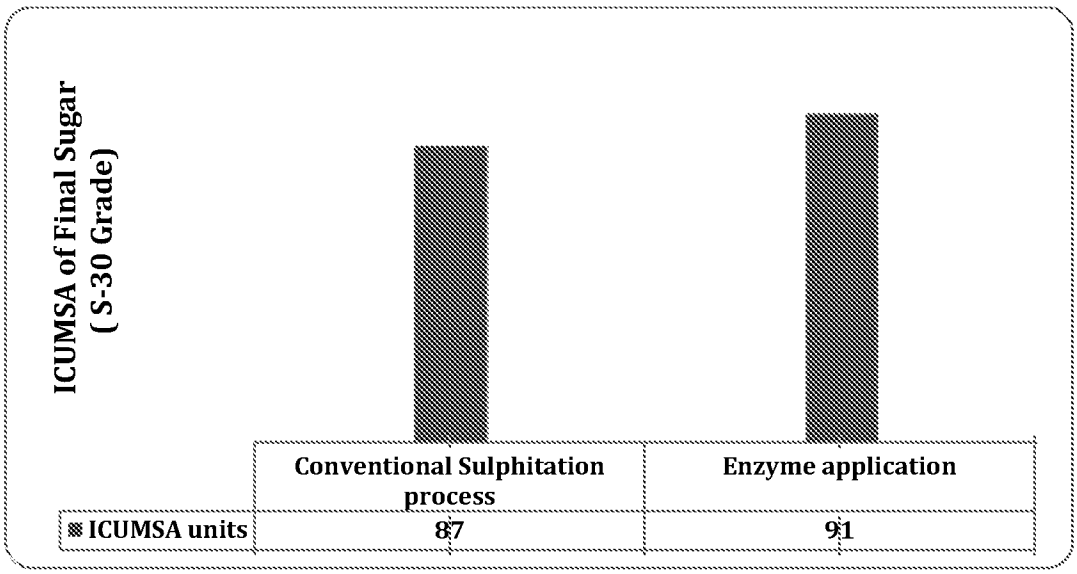

FIGURE 5

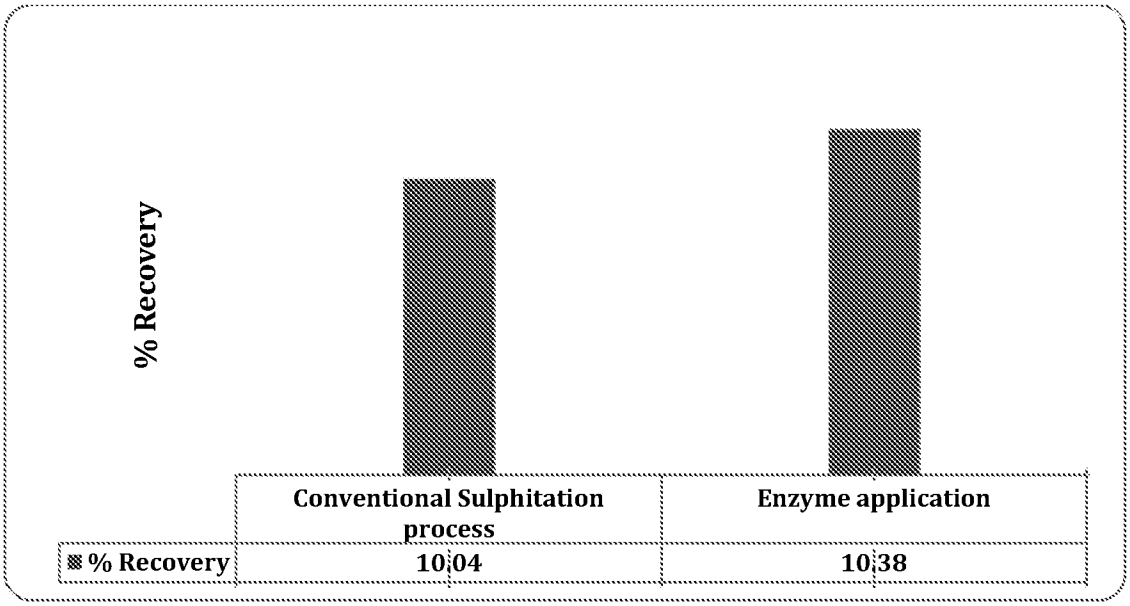

FIGURE 6

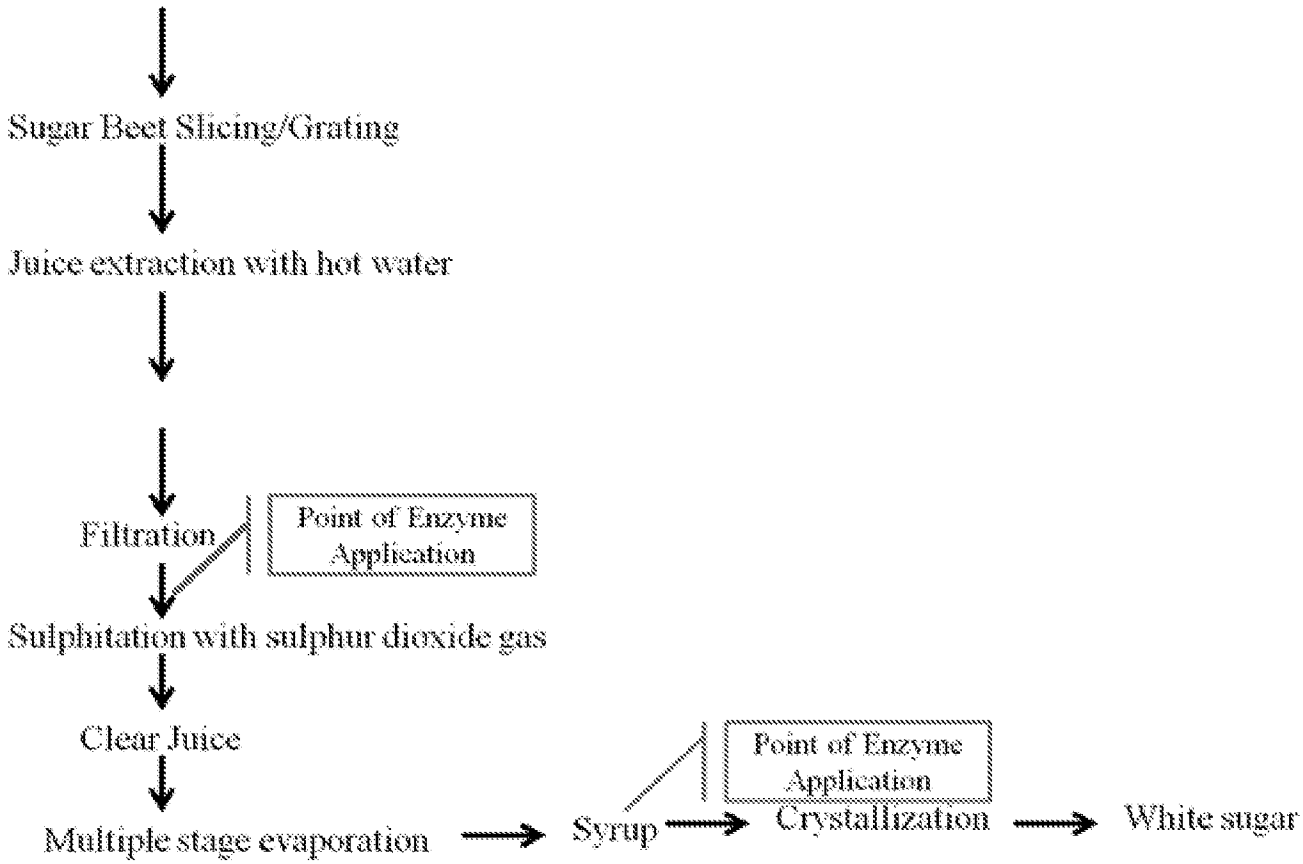

FIGURE 7

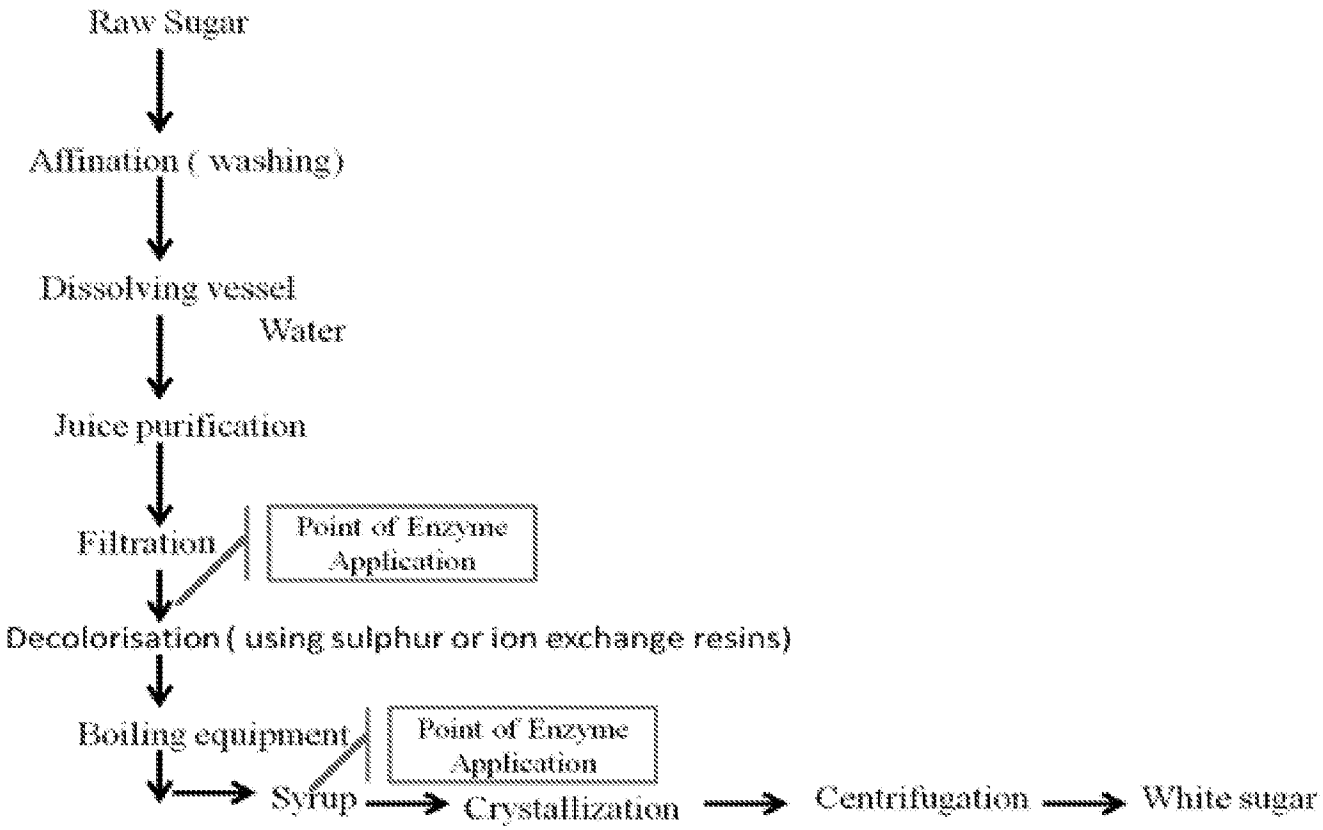

FIGURE 8

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2014/061982

A. CLASSIFICATION OF SUBJECT MATTER
INV. C13B20/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C13B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, COMPENDEX, EMBASE, FSTA, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Marianne Mckee ET AL: "Effect of Commercial Enzymes on Color and Total Polysaccharide Content in Sugarcane and Sugarbeet Juice" In: "Industrial Application of Enzymes on Carbohydrate-Based Material", 30 August 2007 (2007-08-30), American Chemical Society, Washington, DC, XP055138617, ISBN: 978-0-84-122096-6 vol. 972, pages 88-100, DOI: 10.1021/bk-2007-0972.ch007, page 97 - page 99; tables	1-23
X	CN 1 936 024 A (TANG MING [CN] MING TANG [CN]) 28 March 2007 (2007-03-28) the whole document	1-23

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 September 2014	19/09/2014

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Smeets, Dieter

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2014/061982

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6 156 563 A (KAMPEN WILLEM H [US]) 5 December 2000 (2000-12-05) column 3, line 29 - column 5, line 39 -----	1-23
X	WO 2006/063467 A1 (LOGEN ENERGY CORP [CA]; FOODY BRIAN [CA]; RAHME ZIYAD [CA]; TOLAN JEFF) 22 June 2006 (2006-06-22) paragraph [[0061]] - paragraph [[0067]]; claims; example 1 -----	1-23

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2014/061982

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