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(54) Title : METHOD FOR THE CONCENTRATION AND PURIFICATION OF THE EXTRACT OBTAINED FROM CASHEW
PSEUDOFRUIT WASTE AND PRODUCT WITH A HIGH CAROTENOID CONTENT

(54) Título : PROCESSO DE CONCENTRAÇÃO E PURIFICAÇÃO DE EXTRATO OBTIDO A PARTIR DE RESÍDUOS DE
PSEUDOFRUTO DE CAJU E PRODUTO DE ELEVADO TEOR DE CAROTENOIDES

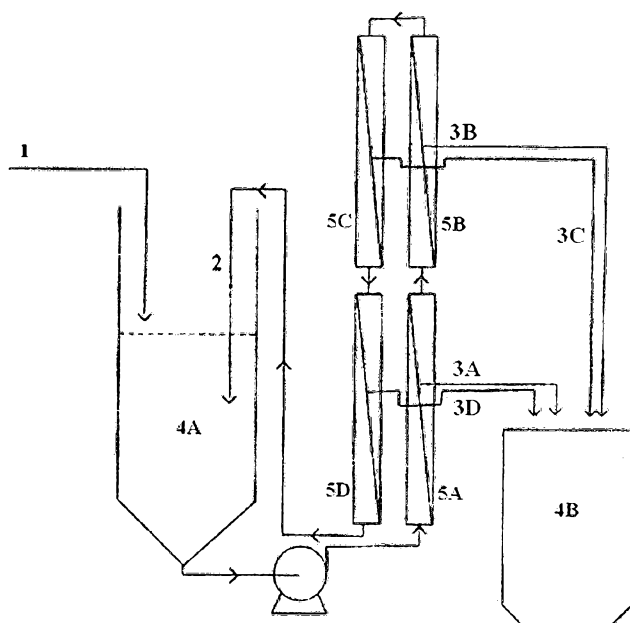


Fig. 4

(57) Abstract : The present invention discloses a method for the concentration and purification of carotenoids from waste fibres from true-cashew juice production, under controlled conditions and without the use of any organic solvent. The method involves pre-macerating the cashew fibres with the use of cell-structure-disaggregating enzymes that act on the fibrous tissue in combination with controlled pressing operations in successive aqueous-extraction cycles. The concentration of the unprocessed extract obtained by maceration/pressing takes place at ambient temperature with the use of tangential-flow microfiltration membranes. The concentrated product is then processed using diafiltration techniques in order to purify it, thereby removing the majority of the undesirable components and promoting the microbial and biochemical degradation thereof. The final concentrate has a potential use as a colouring in foods for human consumption and in animal feed, being applicable to the areas of ready-to-drink juices and beverages owing to its considerable solubility in water.

(57) Resumo :

(Continua na página seguinte)



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A presente invenção apresenta um processo para concentração e purificação de carotenoides a partir de fibras residuais da produção de suco de caju integral sob condições controladas sem uso de qualquer solvente orgânico. O processo envolve uma pré-maceração das fibras de caju com uso de enzimas desagregadoras de estruturas celulares, que atuam sobre os tecidos fibrosos em combinação com prensagens controladas em ciclos sucessivos de extração aquosa. A concentração do extrato bruto obtido por maceração/prensagem é realizada à temperatura ambiente com uso de membranas de microfiltração em fluxo tangencial. O produto concentrado é então tratado por técnicas de diafiltração para ser purificado, eliminando-se grande parte dos componentes indesejáveis e que favoreçam a sua deterioração microbiana e bioquímica. O concentrado final possui potencial de aplicação como corante para alimentos de consumo humano e ração animal, sendo aplicável nas áreas de sucos e bebidas prontas para o consumo devido à considerável solubilidade em água.

Specification of Patent of Invention: **"CONCENTRATION AND PURIFICATION OF EXTRACT OBTAINED FROM CASHEW PSEUDOFRUIT WASTES AND PRODUCTS WITH A HIGH CAROTENOID CONTENT".**

5 FIELD OF THE INVENTION

The present invention belongs to the field of industrial processes for the extraction and purification of molecules of commercial interest, describing the production of extracts containing carotenoids and other substances of interest from wastes of cashew processing by means of techniques that involve pressing, optionally enzymatic maceration and filtration (micro- or ultrafiltration), as well as diafiltration. The process proposed by the developed technology allows production of a yellow coloring extract, a product that can be obtained from industrial wastes of large-scale cashew juice production. In this way, such concentrated and purified extract of cashew carotenoids represents a value aggregation to a byproduct needed by the entire food sector to meet the world demands of natural colorings. Generally, yellow coloring used in Brazil have a synthetic origin and available natural yellows present an orange coloration, differently from what is obtained by means of the present technology, which is bright light yellow, owing to its composition containing xanthophylls, whose predominant color is light yellow.

20 BACKGROUND OF THE INVENTION

Industrial processes for the extraction and purification of molecules of commercial interest, as a coloring, an antioxidant or a molecule with specific properties for the use in human nutrition and diet have been the target of numerous scientific and academic works as well as of requests of applications for patents worldwide.

Patent document PI 0002359-0 discloses a process of high vacuum evaporation and distillation for extraction and concentration of liposoluble vitamins and provitamins, including carotenoids, starting from byproduct materials deriving from plant and animal product industrialization. This state of the art document describes a process to concentrate

non-saponifiable substances, comprising of liposoluble vitamins and provitamins, growing factors and plant and animal hormones, called "products of value", obtained from plant or animal products, or from wastes of the industrialization of such products, without the need for solvents. The process comprises extraction and concentration of said products by evaporation/distillation and the production of fatty acids and other high quality organic acids, by hydrolysis of the wastes obtained by distillation/evaporation.

Patent document PI 0507797-4 describes a process and equipment for diafiltration of thickened fruit juices. The product to be filtrated is submitted to a filtration process. A first flow of wash fluid (water) and a second flow of a product permeate itself (flow driven back through the filtering means used) are added, thus the product flow is diluted by the first and second flow before entering the membranes system.

Patent document WO 2004094350 also describes a method for carotenoids extraction from plant material by means of supercritical fluid extraction. In this document, supercritical fluid extraction is processed in two steps. The first extract includes β -carotene production; the second extract may have a controlled β -carotene concentration and furthermore it includes substantial quantities of lutein.

This process (WO 2004094350) exhibits significant differences from the process presented by the present invention, which does not include any heating or cryogenic step, and is a mass of industrial fibrous waste derived from cashew stalks pressing, however, it does not undergo trituration and/or enzymatic inactivation. The process presented describes a sequence of single operations and processes which, altogether, aims at concentrating and purifying a carotenoid-enriched extract under mild conditions which maximize the yield and purity of the final product. On the other hand, the application for patent under analysis refers to the production of a purified carotenoids extract from carrots and other triturated vegetables which are thermally treated for bleaching. In this case, a set of operations and processes is claimed, especially those for the production of a carotenoid-

enriched mass, mainly beta-carotene, by initially producing heating for enzymatic inactivation (bleaching), followed by successive triturations in different meshes to obtain a fine granulometric mass to be then treated with enzymatic liquefaction and by release of carotenoids in aqueous medium. In
5 the following phase, this mass is applied on membranes for pre-concentration and thus filtered by one or two volumes and water to eliminate hydrosoluble compounds. At the end of such diafiltration step, the resulting mass is frozen and immediately defrosted to attain the final carotenoids concentration and the final mass is treated with flash
10 pasteurization in order to acquire microbiological and biochemical stability.

Therefore, unlike the present invention, a trituration system of the vegetal material is described in the referred document, treating the material aggressively with respect to the content of interest and it should be noted that carotenoids are highly sensible to temperatures above 65 °C, even at low pH
15 as it is indicated in the description thereof. Furthermore, unlike the document itself, the present patent application does not include heating above this temperature and none of the steps of the process in general, as such procedure degrades carotenoids molecules significantly and promotes a loss of product quality. Moreover, in the process of the present invention there is
20 no need for cryoconcentration since, the process proposed by the present invention, which involves the filtration step on microporous membranes, promotes a volume reduction up to 30 times, and thus, it is more efficient.

Similarly, patent document PI 0605425-0 describes a process of extraction and purification in series of active substances with coloring
25 capacity from solid matrixes by using supercritical extraction with CO₂ for colorings production deriving from bixin from annatto. The referred process uses extraction and purification steps by means of adsorption and elution columns equipped with extractor and fractionator mounted in series that can be used in series or in batches. This process uses an aqueous solution as
30 solvent, which at the end of the process is evaporated at temperatures between 30 and 700°C in order not to degrade bixin.

The recovery of mixed carotenoids from microalgae (*Dunaliella*

salina) is described in patent document WO 98/28082. This document describes a process in which collected cells are broken and the algal suspension circulates in high pressure by vigorous pumping. Cells can be dehydrated by means of techniques of separation bubble adsorption, including a flotation circuit, having a thinning zone and a concentration zone. If a higher carotenoids concentration is required, the algae concentrate can be filtered in a tangential-flow microfiltration unit, free of flocculant agents without losing carotenoids in the permeate.

Patent document PI 0006126-3 presents an extraction process of carotenoids and other antioxidants from previously pressed and incubated plant tissues. The molecules of interest are recovered by precipitations and thermal treatments, mainly heating followed by quick cooling; in this way, an extract containing carotenoids is recovered as a precipitate.

Patent document PI 0416795-3 presents as subject matter an integrated process for the extraction and purification of tocopherols, carotenoids and sterols from plant oils by means of alcohol esterification processes. The specification of the referred patent reports as one of the advantage of the use of such a process the production of an enriched and purified product, free of factors of substantial decomposition of these components of interest, such as sugars that are eliminated by dialysis.

Besides the cited documents, patent document PI 0103885-0 presents as subject matter the production of a cashew bagasse extract rich in pigments, obtained by a process comprising humidification of cashew bagasse waste from juice extraction, pressing of the resulting mixture, separation of the extract from solids and concentration thereof by centrifugation and filtration operations.

The present application for protection represents a development of the technique presented in PI 0103885-0, having the same ownership and authorship of the present application, by pooling it with essential steps in order to obtain a product with higher quality and by employing technologies that allow a continuous optimization of its processing.

Therefore, unlike state of the art documents, the present

development intends to complement the production of a crude extract obtained from cashew wastes with new processing technologies that involve pooling the steps of pressing, enzymatic maceration and filtration techniques employing membranes in specific sequential steps, for the production of a
5 final concentrated and purified extract suitable for industrial use from which various substances of commercial interest are isolated, from colorings to dietary supplements. Furthermore, the state of the art does not introduce an equivalent solution for processing cashew wastes crude extracts and it fails to describe a synergic process, such as the one introduced by the present invention, in which
10 the fruit extract or waste undergoes integrated pre-treatments, such as pressing and enzymatic maceration aiming at optimizing the production of compounds of interest at the end of the process associated to final filtration and diafiltration processes, which generate a product of high purity.

SUMMARY OF THE INVENTION

15 The present invention describes a process for concentration and purification of carotenoids extracts from cashew stalk fibers for the use as natural yellow coloring with the use of microfiltration diafiltration membranes. The proposed process occurs under controlled conditions without using any organic solvent, involving, optionally, pre-maceration of the cashew fibers
20 with the use of cell-structure-disaggregating enzymes (pectinases, amylases, cellulases and hemicellulases), acting on the fibrous tissues in combination with controlled pressing operations in successive aqueous-extraction cycles. The concentration of the unprocessed extract obtained by maceration/pressing takes place at room temperature by using tangential-
25 flow micro and/or ultrafiltration ceramic membranes. The concentrated product is then treated with membrane diafiltration techniques, thereby removing the majority of the undesirable components and those favoring its deterioration. The final concentrate has a potential use as a coloring in foods for human consumption and in animal feed, being applicable in the areas of
30 ready-to-drink juices and beverages owing to its considerable solubility in water. Based on the properties of some carotenoids present in the final concentrated and purified extract, it is estimated that this material has a

potential applicability in the area of dietary supplements as provitamin A and antioxidants for food, pharmaceutical and cosmetic industries.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: it shows schematically the general steps of the process in a flowchart. The alphanumeric symbols displayed therein have the following correspondence:

E0: waste fibers from cashew stalk processing;

A: pressing;

M1: addition of enzymes for maceration of the extract to be pressed (optional);

E1: intermediate crude aqueous extract;

B: sieving;

E2: crude aqueous extract;

C: filtration on micro- and/or ultrafiltration and diafiltration porous membranes; and

E3: final concentrated and purified extract.

Figure 2: it shows schematically the general steps of the process in a flowchart with emphasis on the optimized step of pressing. Alphanumeric symbols displayed therein possess the following correspondence:

M0: cashew fiber mass for juice production;

A0: pressing for juice production;

S0: produced whole juice;

E0: waste fibers from cashew stalk processing;

M: Enzymatic maceration (optional);

M1: addition of enzymes for maceration of the extract to be pressed (optional);

A: pressing step of E0 extract;

A1; A2; A3; A4; A5 and A6: pressing cycles of E0 extract;

F1; F2; F3; F4; F5 and F6: waste fibers of each pressing cycle;

S1; S2; S3; S4; S5 and S6: aqueous extract of each pressing cycle;

E1: intermediate crude aqueous extract;

B: sieving;

E2: crude aqueous extract;

C: filtration on micro- and/or ultrafiltration and diafiltration porous membranes; and

5 E3: final concentrated and purified extract.

Figure 3: schematic representation of a screw press. Alphanumeric symbols displayed therein possess the following correspondence:

10 E0: waste fibers from cashew stalk processing;

R: waste fibers outlets of each pressing cycle;

E1: intermediate aqueous crude extract;

V: rotation speed control; and

F: applied force control.

15 Figure 4: it shows a scheme of the pilot unit of membrane diafiltration operation. The alphanumeric symbols displayed therein have the following correspondence:

1: water;

2: retained;

4A: feeding tank;

20 5A; 5B; 5C and 5D: membranes system unites;

3A; 3B; 3C and 3D: permeate outlets; and

4B: permeate recovery tank.

25 Figure 5: it shows a scheme of the pilot unit of membrane diafiltration operation detailing the equipment composing it. The alphanumeric symbols displayed therein have the following correspondence:

3A; 3B; 3C and 3D: permeate outlets;

4A: feeding tank;

5A; 5B; 5C and 5D: membranes system unites;

6A: fluid inlet of the heat exchanger;

30 6B: fluid outlet of the heat exchanger;

7A; 7B and 7C: manometer;

8: thermometer;

9A: purge valve;
9B: transmembrane pressure control valve;
10: heat exchanger; and
11: pump.

5 Figure 6: flowchart of the permeate ($\text{L.h}^{-1}.\text{m}^{-2}$) as a function of the volume reduction factor for different transmembrane pressures (T_{mp}).

Figure 7: flowchart of the permeate ($\text{L.h}^{-1}.\text{m}^{-2}$) as a function of the concentration factor for two different situations – with and without pectinase addition – during the whole concentration step (filtration micro- and/or
10 ultrafiltration porous membrane and dialysis).

Figure 8: it shows four charts, wherein (8-I) is the evolution of volume reduction factor (VRF), (8-II) or reduced diavolume (RD), (8-III) permeate flow (PF) density and (8-IV) the soluble solids content (SSC), respectively, as a function of time in the concentration process of E2 extract
15 for two different situations – with and without pectinase addition.

DETAILED DESCRIPTION OF THE INVENTION

The present patent application of invention differs from the state of the art in that it proposes a process combining concentration and purification by diafiltration with the usual techniques for extract purification
20 from cashew fiber crude extracts.

The present process allows the production on an industrial scale of a viscous and bright yellow liquid for different uses, such as natural coloring and antioxidant in food, cosmetic and pharmaceutical industry.

The subject matter of this patent application consists of a set of
25 operations that aims at obtaining a concentrate of carotenes and xanthophyll, with emphasis on auroxanthin in *cis* and *trans* form, β -cryptoxanthin, mutatoxanthin and zeaxanthin. Following the aforementioned, β -carotene and lutein come next in order of importance, as they have a composition enriched with several other carotenoids.

30 The proposed process comprises the following steps, corresponding to the information contained in Figure 1:

A) Obtaining a carotenoid crude aqueous extract, E1, from

waste fibers from cashew stalk processing, E0, by means of pressing. In this step, enzymatic treatment is optional and it can take place by addition of cell-structure-disaggregating enzymes (pectinases, amylases, cellulases and hemicellulases) (M1);

5 B) Sieving of the intermediate crude extract obtained (E1) aiming at an initial standardization to avoid issues concerning an excessive increase in viscosity in the final phases of membranes processing, thus E2 crude extract is obtained;

 C) Concentration of the obtained crude extract, E2, by means of
10 techniques of microfiltration on porous membranes, reaching values of concentration factor between 2 and 30 times; and subsequent elimination of the soluble dry extract (sugars, hydrosoluble minerals and vitamins) contained in the concentrated extract through a process of diafiltration on micro- or ultrafiltration porous membranes, thus increasing the purity of
15 present carotenoids and promoting the microbiological and biochemical stability. The resulting product of (C) is the final concentrated extract E3.

 Considering that the pH of the crude extract (E2) is already in a range between 3.5 and 5.0, there is no need for a correction of this value in order to attain a major stability of carotenoids present in the final
20 concentrated and purified extract.

 The first step of the process (A) for producing the crude extract is based on the optimization of the process proposed by Brazilian patent document PI 0103885-0. Therefore, the described technological development involves the use of such extract for the operation/process of concentration
25 and purification aiming to obtain a product with high carotenoid concentrations.

 Before the start of the process, cashews are harvested and taken to a reception area of the processing unit, where chestnuts are withdrawn immediately, and the stalks are separated to be selected and washed for
30 withdrawal from dirt and unwanted components for juice processing.

 Afterwards, the stalks are directly pressed in an expeller-type or screw press, typical of cashew juice and cajuína processing in processing

units in the Northeast of Brazil. In this step, about 80% of whole juice and 20% of waste fibers are produced, as described in PI 0103885-0. Thus, these fibers (E0) are humidified in the ratio from one to two parts of fibers for each part of water to be pressed.

5 After that, the process introduced by the present technology begins, with step (A) referring to a series of pressing on E0 in order to obtain the intermediate extract (E2). In this step, commercial enzymes with pectinolytic, amylolytic and cellulolytic activity may be added or not in order to increase the yield of pressing, if needed. The addition of enzymes may be
10 up to 0.5% of the mass of fibers, the used value usually being 0.2%.

 After crude extract E2 is obtained, it is taken to step (B), where it is treated in sieves with meshes between 0.1 and 1.0 mm, aiming at an initial standardization to avoid issues of excessive increase in viscosity in the final phases of membranes processing.

15 The processing of the crude extract by filtration on microporous membranes is then carried out in (C) aiming at initially reducing the water that was added during the enzyme-assisted extraction in the previous phase, if this has occurred. Afterwards, step (C) is processed into distinct phases: a first phase (C1) in which the volume of the feeding tank (1A) is kept constant
20 until the concentration of the crude extract reaches VRF (volume reduction factor) between 2 and 30 or until depletion of crude extract volume to be added, wherein in this moment it begins a phase (C2) of concentration with maximum reduction of the volume of concentrated extract within the recirculation system, herein called retained, until the lowest recirculation limit
25 in the set of membranes. In this situation, aeration of the retained should be avoided, and a volume that does not allow aeration by recirculation pump should be kept. Therefore, a cold pre-concentration is promoted, using the technology of filtration on aluminum oxide microporous membranes, or simply aluminum, wherein other materials may be possibly used. These
30 membranes have the capacity of totally retaining carotenoids and other hydrophobic molecules and, due to this characteristic, they are employed as a means of retention of the carotenoids contained in the extract.

Therefore, in this last step (C), following C1 and C2, in which an initial pre-concentration phase takes place and the concentration factor is increased until a recirculation within the system in the lowest limit of the feeding tank of the microfiltration unit, a phase C3 is processed, with the purification of the concentrated extract obtained after C1 and C2 by the execution of the process of diafiltration on microfiltration membranes.

Thus, what is retained at the end of (C2) undergoes diafiltration (C3) with volume of pure water (diavolume) added until the desired soluble solids content ($^{\circ}\text{Brix}$) is reached.

The entire operation is carried out under temperature conditions between 10 e 50°C aiming at maintaining the functional properties of carotenoids that will be obtained as a concentrated solution purified by diafiltration at the end of the process.

Due to the presence of low fermentable sugars concentration, the obtained product may be stored at refrigeration temperatures for later use.

The final product may also be thermally treated to provide a longer durability, although the use of heat is avoided along the entire process, as it deteriorates the carotenoid content, modifying its molecular profile and consequently its provitaminic and antioxidant activity. On the other hand, even if it is thermally treated, the final product has a high coloring power for use in the food and pharmaceutical field.

The carotenoid concentrate (E3) obtained has the characteristics of being an aqueous extract, in the form of a bright yellow emulsion with the capacity of coloring juices in a yellow and yellow-orange tonality for the use in food and beverages. In this way, the product may be used as a natural yellow food coloring; a natural antioxidant in the form of capsules (nutraceutics); provitamin A and fractioning to isolate carotenoid groups with specific functions is still possible.

With regard to step (C), it is important to characterize the pilot unit of tangential microfiltration, represented schematically in Figures 4 and 5, in which the used solvent is water.

The microfiltration unit has a set of four membranes, preferably made of aluminum oxide (Membralox[®]), where other types of membrane may be used optionally, in order not to limit the embodiment of the invention to this one. Its feeding tank (4A) has 3L to 10L capacity with four modules
5 arranged in series, each one containing a ceramic membrane with a filtration area from 0.0001 to 0.0900 m², in preferred embodiments at least 0.0055 m² and a porous diameter from 0.01 µm to 0.5 µm, ideally 0.2 µm. The pressures of the four microfiltration sections are: P1 = 2.75 bar; P2 = 2.25 bar; P3 = 1.75 bar and P4 = 1.25 bar, wherein all of them, as well as the
10 system pressure, can vary from 0.5 to 10.0 bar. Based on the presented data, the embodiments of the invention involve resizing on the basis of the same parameters, which use the base concept to treat the desired volumes of cashew stalk wastes.

A progressive cavity pump (Moineau) whose power attains at
15 least 1.0 CV, being higher or lower as a function of the amount of matter to be processed, is represented in item (11) of Figure 5, allows liquid pressurization and its tangential-flow in the system. Tangential speed is fixed at a value from 1 m.s⁻¹ to 10 m.s⁻¹ and what is retained is continuously circulated in the filtration system. The dead volume of the system is
20 calculated as 1.3 L for a feeding tank with a 3 L capacity, distributed in the construction elements (pumps, pipes, membranes and tray).

The microfiltration unit allows controlling a series of parameters, among which: transmembrane pressure, general temperature of the system, tangential speed and, as set out, the phase involving diafiltration itself follows
25 a model comprising three phases, equivalent to C1, C2 and C3 described before: (1) pre-concentration, at constant volume (C1); (2) concentration at variable volume (C2); and (3) diafiltration/purification at constant volume (C3).

The general processing conditions for carotenoids purification
30 from waste fibers from cashew stalk processing involve checks of different operational parameters. The temperature was checked by means of a heat exchanger (10), as indicated in Figure 5, installed in the recirculation circuit of

what is retained by power usage in a jacket external to stainless pipes with cold or hot water aiming at obtaining a temperature stabilization at the required range, which is from 38 to 42°C. When working with carotenoids, this temperature may be further broadened to a range varying from 10 to 85°C, depending on the purpose given to the final desired product. Based on metabolic pathways of carotenoid degradation, a further interest may exist in specific molecules that are degrading products possessing coloring activity that is more pronounced than in its natural state and, in other cases, the needs for maintaining the integrity of the molecule. Thus, according to the target product lower or higher temperatures are used. The tangential speed was fixed by means of rotation control of the engine of the used pump (11), also shown in Figure 5, wherein it can be controlled through the use of frequency inverters for increasing or decreasing this velocity as a function of the nature of the treated material (either more viscous or less viscous). And transmembrane pressure is controlled by means of a final control valve of inlet (9A) and outlet (9B) pressure of the pilot system, indicated in Figure 5. Depending on mechanical resistance of the used membrane, more or less flow speed of the retained may be used, thus increasing drag and, as a consequence, the self-cleaning process of the filtration surfaces, favoring an increase of average permeate flow. In this case, an average transmembrane pressure from 1 to 5 bar in the system, preferably 2.7 bar, is recommended, due to the characteristics of the system and of the pump driving the motive power; however, such pressure can be adjusted to other values according to the needs/capacities of the production system.

The option for diafiltration system at constant volume was established with a view to optimizing the use of water as a component of diafiltration (solvent). As this natural resource is scarce in those regions where cashew is grown and the presented system promotes a reduced use of water, there is a significant gain in the process. However, the use of the diafiltration system at a variable volume for extract and cashew fibers is not excluded.

Therefore, it is crucial to note that this technology is viable for a

great range of applications and equipment suitable for use, as well as operational parameters. Thus, they are not restricted to those presented in the examples and/or descriptions of the present invention.

EXAMPLES

5 The present invention is further explored by the following examples.

Example 1 – Production of purified carotenoid extract.

 A crude aqueous carotenoid extract was obtained, represented in Figure 1 as E1, from waste fibers from cashew stalks processing (E0) and by means of six consecutive pressing cycles, as indicated in Figures 1 and 2 by operation (A), which includes the addition of pectinases, as shown in Figure 2 by operation (M). Water was added to the mass of cashew fibers (M0) (1:1 mass/mass) and subjected to pressing in a INCOMAP-300 model press (300 kg.h⁻¹ nominal capacity) for juice production, which resulted in waste crude
10 extract (E0) and whole juice (S0). Afterwards, water and pectinases (500 mg.kg⁻¹ of pectinase Pectinex SP-L - Ultrazymes[®]) were added to waste fibers (E0) and the material was homogenized. The temperature was kept at values from 50 to 55°C and the same press was used (2500 N force) to submit the material to six consecutive pressing cycles, with the
15 reincorporation of the extracts (S1 to S5) and the mass of fibers (F1 to F6) obtained in each of the pressing (A) step (A1 to A6), as illustrated in Figure 2. Figure 3 shows a schematic representation of the press used.

 After pressing, an emulsion presenting a yellow coloration due to the presence of carotenoids was obtained. Such emulsion was filtrated on
25 stainless steel sieves with 0.30 mm mesh in order to remove suspended particles. The extract obtained after sieving, E2, is called crude aqueous extract, and was stored under refrigeration (-20°C) in packages of low density polyethylene for storage prior to concentration process (C).

 The subsequent concentration step (C) employs tangential
30 microfiltration techniques. A four-membrane system MEMBRALOX (PALL-EXEKIA), with 0.22 m² filtration area and 0.2 µm porous diameter, made of aluminum oxide of the monotubular type was used. The system pressure was

2.75 bar, the temperature being set at 40°C ($\pm 2^\circ\text{C}$). The process was carried out under a concentration factor equals to 13. The concentration by means of techniques of microfiltration on microporous membranes was conducted in three phases: a first phase in which the volume of the feeding tank is kept constant until the depletion of the volume of the crude extract, wherein in this moment a concentration phase begins with maximum reduction of the volume of the concentrated extract within the recirculation system, herein called “retained”, until the lowest recirculation limit in the set of membranes; and the third phase in which diafiltration on microfiltration microporous membranes takes place with the addition of pure water volume until a soluble solids content between 20 and 30 °Brix is reached.

The operation yield deriving from 1000 kg cashew stalks is about from 30 to 40 kg of concentrated diafiltrate (E3).

Total carotenoid contents of both the intermediate crude extract (E1) and the final concentrated extract (E3) were analyzed by liquid chromatography, as shown in Table 1.

Table 1 – Carotenoid content (mg.kg^{-1}) in the crude extract of cashew fibers (E1) obtained in six consecutive pressing cycles and in the final concentrated extract (E3).

Carotenoids	Crude Extract (E1)	Concentrated Extract (E3)
<i>Auroxanthin (cis and trans)</i>	3.08	16.10
<i>Mutatoxanthin trans</i>	0.62	2.50
<i>Lutein</i>	1.04	4.70
<i>Mutatoxanthin cis</i>	1.25	6.30
<i>Zeaxanthin</i>	1.10	5.60
<i>Cis antheraxanthin</i>	0.20	0.80
<i>β-Cryptoxanthin</i>	1.52	8.00
<i>13-cis-β-carotene</i>	0.13	0.70
<i>α-Carotene</i>	0.09	0.60
<i>β-carotene</i>	0.52	2.50

<i>Others</i>	0.85	6.40
<i>Total Carotenoids</i>	10.40	54.20

Example 2 – Comparison between extraction with and without the use of enzymes.

100 kg batches of crude fibers (E0) were used and pressed with an INCOMAP 300 press in six pressing cycles. The extracts were submitted to two processes: the first (I), with 500 mg.kg⁻¹ commercial pectinase (Pectinex SP-L - Ultrazymes[®]) and, the second (II), without addition of pectinase, the other operational conditions kept as the established optimized conditions provided by the process proposed by the present invention.

In both processes of this example (I and II) 20 L of crude extract resulting from the described pressing were used, which underwent three phases in (C): pre-concentration with a decreasing concentration of feeding tank volume and; a final diafiltration step. Diafiltration begins when the system attains a lower circulation volume of what is retained, and starting from this point, the circulating volume is kept constant until the end of the process. The system employed has a feeding tank of 3.0 L capacity.

Therefore, pre-concentration was initially carried at constant volume of feeding tank, wherein the concentration factor attained values of about 5 and after a second concentration phase with a decrease of tank volume to the least possible volume, when the concentration factor reaches its maximum value, but without damaging the carotenoid content. After reaching the maximum concentration factor in the microfiltration system, a final phase of purification by diafiltration of what is retained is conducted at the limit condition of circulating volume, which is about from 1.3 to 1.5 L in the microfiltration pilot unit.

In all the phases of the process, the adopted operational conditions were: feeding temperature of crude extract of 40°C (± 2), average transmembrane pressure of 2.75 bar (± 0,3) and speed of de 6.0 m.s⁻¹.

In this example, the parameter addressed in step (C) is explained. The used method consisted of an initial pre-concentration phase which increased the concentration factor to a system recirculation at the

lowest limit of the feeding tank of the microfiltration pilot unit, followed by the purification phase of the concentrated extract by diafiltration on microfiltration membranes, as presented above. Furthermore, samples for the analysis of the global process performance were collected every 10 minutes, wherein
5 aliquots of permeate were collected and the volumes in precision graduated test tubes submitted to physicochemical were measured. During the diafiltration phase, in each sample of permeate an equivalent volume of distilled water was replenished to keep total soluble solids content close to zero at the end of the process, so as to promote an increase in the purity of
10 carotenoids present in the final concentrate.

Both in (I) and (II), the global process for concentration and dialysis of the 20 liters used in each of the experiments took about 10 hours per essay. Such method led to the production of a concentrated extract that could reach concentration factors in the order of 20 to 30 times when
15 compared with the initial volume used in the concentration phase, with good performances of permeate flow kept even when high values of volume reduction factor (VRF) were reached.

Besides (I) and (II) essays, intermediate works were conducted in experimental phases which involved treatments with pectinolytic,
20 cellulolytic and amylolytic enzymes with the purpose of investigating the behavior of the process when the extract is submitted or not to a pre-treatment before carrying out the concentration operation. Permeate flows were high in all treatments; however, values were more significant in treatments with pectinase and amylase. Therefore, further works with
25 diafiltration step carried out with the use of enzymes are recommended. Permeate flows in this step still remained at the same highest levels, since diafiltration consists of adding a solvent (in this case, water was added, although other solvents may be used) to eliminate the hydrosoluble components that may produce subsequent problems, such as fermentations
30 and chemical and biochemical interactions with the components of interest. In this step, permeate flows remained in the range of $130 \text{ L.h}^{-1}.\text{m}^{-2}$ for treatments with enzyme and of $80 \text{ L.h}^{-1}.\text{m}^{-2}$ for treatments without enzymes.

Table 2 shows a global average of all the parameters observed in the process of concentration and purification by diafiltration of the aqueous extract rich in carotenoids obtained from waste fibers of cashew stalks of whole juice processing for (I) and (II).

- 5 Table 2 – Global average of all the observed parameters in the process of concentration and purification by diafiltration.

Parameters of the Process and Main Results	Containing 500 mg.kg⁻¹ of pectinase (I)	Without pectinase (II)
Feeding volume (mL)	20000.00	20000.00
Feeding tank volume (mL)	3000.00	3000.00
Volume in diafiltration (mL)	1320.00	1170.00
Volume of water already used in diafiltration (mL)	5889.20	5552.60
Average permeate flow (L.h ⁻¹ .m ⁻²)	130.80	80.40
Volume Reduction Factor – VRF	19.06	17.80
Initial Soluble Dry Extract (g.L ⁻¹)	52.00	52.00
Final Soluble dry Extract (g.100 mL ⁻¹)	2.00	5.00
Initial total dry matter (g.L ⁻¹)	64.80	64.70
Final total dry matter (g.L ⁻¹)	256.80	237.80
Initial insoluble dry matter (g.L ⁻¹)	12.80	12.50
Final insoluble dry matter (g.L ⁻¹)	244.00	223.00
Total carotenoids – initial (g.L ⁻¹) *	0.38 x10 ⁻²	0.33x10 ⁻²
Total carotenoids– final (g.L ⁻¹) *	0.73 x10 ⁻¹	0.53x10 ⁻¹

* Analyses in triplicate

- 10 Additionally, the variations of the physicochemical characteristics observed in the final product of diafiltration (final concentrated extract) by using (I) are shown in brief in Table 3. From the results, it was possible to reach in the final extract (E3) concentrations of its constituents up to 10 times higher than those contained in the crude extract (E1). Furthermore, it can be observed that the synergy between the optimized pressing processes in combination with the use of enzymes plus the use of microfiltration

techniques was positive, since it allowed an increment of up to 75% in total carotenoid concentration.

Table 3 – Physicochemical characteristics of the final extract obtained after diafiltration (E3).

Physicochemical parameters	Values
Total soluble solids (g.100 mL ⁻¹)	0.5 to 1.5
Total dry extract (g.L ⁻¹)	237.8 to 250.5
Insoluble dry extract (g.L ⁻¹)	223 a 260
Total carotenoids (mg.L ⁻¹)	0.053 to 0.53
pH	4.5 to 4.7
Ascorbic acid (g.100 mL ⁻¹)	Below detectable limit
Sugars total (g.100 mL ⁻¹)	0.2 to 1.3
Titrateable acidity in % of citric acid (g.100 mL ⁻¹)	1.26 to 1.78
Total polyphenols in tannic acid (mg.100 mL ⁻¹)	250 to 500

5 Example 3 – Application of purified extract as a coloring.

Concerning the application of the concentrated and purified extract obtained at the end of the process described in **Example 1**, it can be seen that an addition between 0.5 and 3.0% of concentrated extract to juices and beverages is enough to reach the typical yellow coloration.

10 Example 4 – Comparative studies on concentration step (C).

During the final step of the filtration process (C) using microporous membranes, permeate sampling was performed in the system without the substitution of the power source until the operational limit of the pilot equipment, with the least possible volume of about 1.5 liters. Starting
15 from this point, the diafiltration step began (C3), and thus volumes of water (diavolumes) were added, the soluble solids content (°Brix) being used as a control parameter every 10 minutes.

The conclusion of the process is indicated when the soluble solids control reaches a value ranging from 0 to 10.0 g.kg⁻¹, indicating that
20 water soluble materials (sugars, mineral salts and vitamins) were eliminated by the operation of diafiltration. The samples for the analysis were collected

in three steps: the feeding crude extract, the permeate and what was finally retained.

Figures 4 and 5 show the details of the operation of diafiltration – step (C), in which the solvent is water. Diafiltration, as shown in Figure 2, was conducted in three phases:

- First step: pre-concentration at a constant volume of the tank of 3.0 liters and crude extract feedback until depletion of total volume to be purified by diafiltration.

- Second step: the interruption of crude extract feedback occurs. The tank volume drops until the maximum concentration point is reached.

- Third step: this is the diafiltration phase itself, when the volume of each filtrate (permeate) is collected, and afterwards the tank is replenished with the same volume of demineralized water to reach the least soluble solids content (close to zero) in what is finally retained.

Permeate sampling was performed only by measuring permeate flow density. From the beginning of the diafiltration phase, an ATAGO digital refractometer was used to measure total soluble solids content ($^{\circ}\text{Brix}$), monitoring it to keep it at a value close to zero. All the soluble materials present in the permeate were eliminated and only the insoluble portion of the extract with water, enriched with carotenoids, was left inside the recirculation system.

The decision to perform diafiltration only in the final concentration phase was due to the fact that water consumption in these conditions may be important for the project of a factory at an industrial scale. It can be noted that, in conditions of least retention volume of the feeding tank, diafiltration is processed more rapidly and a lot of care should be taken for aeration of the manipulated product in these conditions.

During this phase, permeate extraction was compensated by the addition of distilled water to the feeding tank every 10 minutes. The solids content is the washing indicator which is measured in the permeate at each extraction. Diafiltration is interrupted when this parameter reaches values of 0 and 10 g.kg^{-1} in the permeate. During diafiltration, the retained volume

remains constant and the VRF does not undergo alterations.

The concentration step itself (prior diafiltration promotion) is divided into two phases: pre-concentration at constant volume of the tank, with feedback thereof with crude extract and a second step, with a continuous decrease of the extract volume to the lowest limit of the feeding tank of the pilot unit. The system normally operates diafiltration considering the intake of cashew fibers in the extract, obtaining a volume of about 1500 mL at the end of the concentration step. A volume slightly higher than the lowest limit of 1000 mL was used in the pilot unit in order to avoid unwanted contacts with air. For each one of the steps it is important to establish the volume reduction factor, as explained below.

Pre-concentration step: in order to calculate the corresponding volume reduction factor, the ratio between the volume of the extract supplied in the circuit and the retained volume was used. During this phase, the supplied volume with crude extract (V_A) equals the permeate volume (V_P) in all the process. The retained volume (V_R) corresponds to the tank volume (V_B). Therefore, in this phase the VRF is calculated as follows:

$$\text{VRF} = 1 + V_P / V_B, \text{ wherein}$$

V_P : permeate volume extracted at every sample collection (mL).

V_B : feeding tank volume (mL).

In this case, the tank volume is fixed at 3000 ml during all the pre-concentration phase.

Concentration step: during this phase, the tank volume varies and the permeate volume is extracted continuously every 10 minutes, although this extraction is not compensated by the addition of extract to the feeding tank. Thus, the volume of the circulating product decreases more rapidly and it concentrates in a non-linear fashion. At this point, the calculation of the changes in the VRF changes with respect to the pre-concentration step and it is calculated by taking into account that the circulating volume decreases of the same proportion collected in the permeate volume. This means that the circulating volume represents the initial volume minus the collected volume accumulated at each permeate

sampling during this phase. The calculation formula for each permeate sampling in this step is:

$$VRF = 1 + [V_{PC} / (V_B - V_P)] , \text{ wherein}$$

5 V_{PC} : accumulated volume of permeate during the whole process (mL).

V_B : feeding tank volume (mL).

V_P : accumulated volume of permeate during concentration phase (mL).

10 **Purification step (diafiltration):** during this phase there is no concentration and the volume of the tank remains constant until the end of diafiltration, with a soluble solids content from 0 to 10.0 g.kg⁻¹, measured by refractometer in each of the sample of diavolumes during diafiltration. At the end of this step, manipulation should be completed and the samples of the final retained collected for the control analysis and modeling.

15 As a result of the different analyses conducted during the essays, the evolution of the permeate flow as a function of the volume reduction factor during the step of micro- and diafiltration is presented in the chart of Figure 6, under different transmembrane pressures.

20 Additionally, as indicated in the prior example and presented in the chart of Figure 7, the sample submitted to enzyme pre-treatment had a 130 L.h⁻¹.m⁻² average stabilization and without pre-treatment it indicates a value lower than 80 L.h⁻¹.m⁻², thus suggesting a good industrial performance and applicability of the proposed process, even for pectinase-free samples.

25 Moreover, the analysis of the chart in Figure 7 indicates that pectinase treatment has a higher concentration factor, reaching values around 19, whereas the pectinase-free sample has a slightly lower value, with a concentration factor of around 17.8. Even if we take into account that the values of the concentration factor are very close, it is important to observe that the time needed for reaching such concentration level was very different for each case. The pectinase treatment showed a good performance of permeate flow that gets stabile in the pre-concentration phase (C1),
30 whereas the one without pectinase showed a stabilized flow only at the

beginning of the concentration phase (C2) with a reduction in circulating volume without feedback.

In order to better illustrate this fact, the charts of Figure 8 present the general behavior of extract concentration and diafiltration as a function of the duration of the process. In this way, Figure 8 shows four charts, wherein (8-I) is the evolution of the volume reduction factor, (8-II) is the reduced diavolume, (8-III) is the density of the permeate flow and (8-IV) is the soluble solids content, respectively, as a function of time, in the concentration process of extract E2An analysis of the behavior of the concentration factor as a function of time (Figure 8-I) shows that the curve referring to the enzyme pre-treatment process presents a growing concentration rate owing to the higher permeate flow during all the phases of the process, when compared with the essays without enzymatic pre-treatment. This fact can be attributed to pectinase action as a factor for reducing the viscosity and decreasing the average particle size in the product.

The behavior of total soluble solids content (Figura 8-IV) shows a small increment at the beginning of the process for both cases. It is observed that soluble solids content in both initial samples was approximately 5.2 °Brix. The reading of 5,1 a 5,2 °Brix in both cases may be attributed to the presence of water inside the microfiltration pilot unit, which is stabilized after the first crude extract feedback. The decrease in the total content of soluble solids content showed a typical behavior of diafiltration processes, wherein the final point of the adopted process corresponds to content values close to zero. In both cases, the end of the process took place when this parameter was enough low to avoid subsequent fermentations and not to cause any detriment to total carotenoid content.

The reduced diavolume (RD) is a value corresponding to a ratio between the added volume of water and the recirculation volume in the system. This value is normally used for system resizing, for scheduling of industrial units. Therefore, it shall be noted in Figure 8-II that this parameter grows linearly along time, indicating good diafiltration performance.

As the diafiltration phase was carried out after the maximum

concentration of the crude extract, it is observed that the volume of water needed for reducing soluble solids content is very low if compared with the initial volume of 20 liters of crude extract. Diafiltration in this example was conducted with a circulating volume of 1.45 L (calculated by the sum of
5 extracted permeates) and with the addition of 5.9 L of distilled water for pectinase treatment. The circulating volume calculated for diafiltration when there was no addition of pectinase in the previous pressing step was 1.2 L and 5.6 L of distilled water added to reach a soluble solids content close to
10 zer.

The obtained results prove that the proposed technology allows the production of volume concentration factors close to 20 and that the synergic use of pectinolytic enzymes as pre-treatment during pressing phase has a positive effect on the global performances of the process.

CLAIMS

1. Process for the concentration and purification of the extract obtained from cashew pseudofruit waste and product with a high carotenoid content comprising the steps of washing cashew processing waste; successive
5 pressing of the materials comprised of cashew waste fibers; and filtration of the total liquid phase; characterized in that it comprises the steps of:

A) optionally, enzymatic treatment of the crude aqueous extract during pressing (A);

B) sieving of the crude aqueous extract (B); and

10 C) concentration and purification of the aqueous extract on micro- and/or ultrafiltration microporous membranes (C).

2. Process according to claim 1 characterized in that the pressing step (A) takes place with 5 to 10 pressing cycles, preferably 6.

15 3. Process according to claim 1 characterized by the optional addition of commercial enzymes with pectinolytic, amylolytic, cellulolytic and hemicellulolytic activities in the pressing step (A).

4. Process according to claim 3 characterized in that the addition of enzymes comprises values between 0.01 and 0.5% out of the fiber mass (E0), preferably 0.2%.

20 5. Process according to claim 1 characterized in that sieving (B) takes place in sieves having mesh between 0.1 and 1.0 mm.

6. Process according to claim 1 characterized in that concentration (C) takes place by means of micro- and/or ultrafiltration microporous membranes, comprising steps of:

25 C1) addition of the extract (E2) to the feeding tank (4A) at constant volume conditions;

C2) concentration with maximum reduction of the volume of concentrated extract within the recirculation system until the recirculation limit of the set of membranes; and

30 C3) diafiltration on membranes with solvent addition until attaining soluble solids content from 0 to 10.0 g.kg⁻¹.

7. Process according to claim 6 characterized in that the feeding

tank is maintained at constant volume in (C1) until the crude extract concentration attains values of volume concentration factor between 2 and 30 and/or until depletion of the volume of the added crude extract.

5 8. Process according to claim 6 characterized in that the employed membranes have a porous diameter from 0.01 μm to 0,5 μm , preferably 0,2 μm .

9. Process according to claim 6 characterized in that the pressures of the microfiltration sections varies from 0.5 to 10.0 bar, preferably from 1.75 to 2.75 bar.

10 10. Process according to claim 6 characterized in that tangential speed is fixed in 1 to 10 m.s^{-1} , preferably 6.0 m.s^{-1} .

11. System for the concentration and purification of aqueous extracts characterized by comprising a set of microfiltration membranes, extract pumping equipment, heater exchanger(s), feeding and collection
15 tank(s), manometer (s), thermometer(s) and control valve(s).

12. Process according to claims 1 and 11 characterized in that the steps of concentration and purification of the extract take place at temperature between 10°C and 85°C, preferably 38°C to 42°C.

13. Product of high carotenoid content of cashew fibers
20 characterized in that it is obtained from the process of concentration and purification of cashew pseudofruit waste extract and in that it comprises the components auroxanthin; mutatoxanthin; lutein; zeaxanthin; antheraxanthin; β -Cryptoxanthin; 13-cis- β -carotene; α -Carotene and β -carotene and it may be optionally associated with other additives and/or carotenoids of interest.

25 14. Product according to claim 13 characterized for being used as a coloring and/or industrial additive.

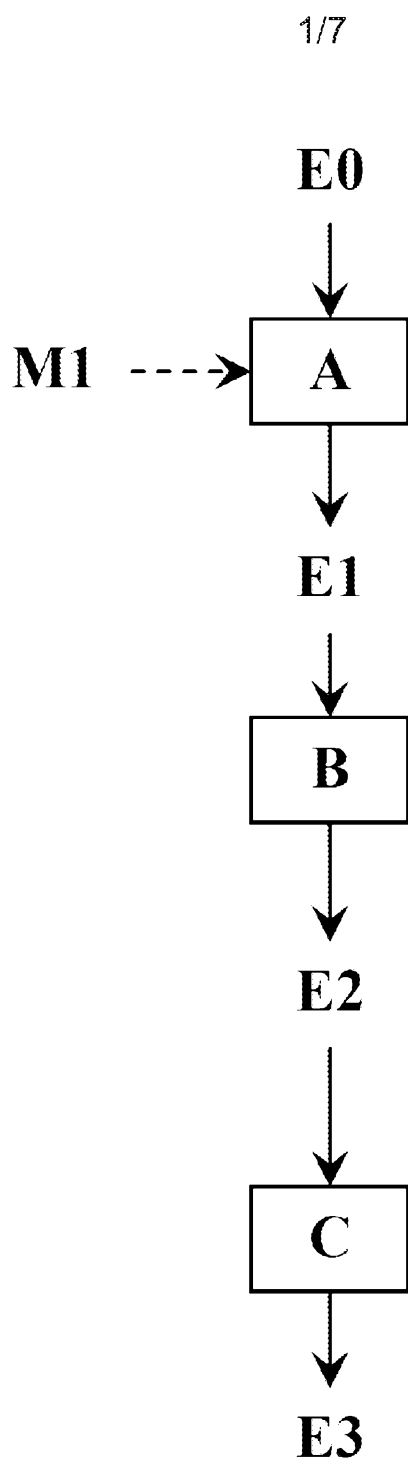


FIG. 1

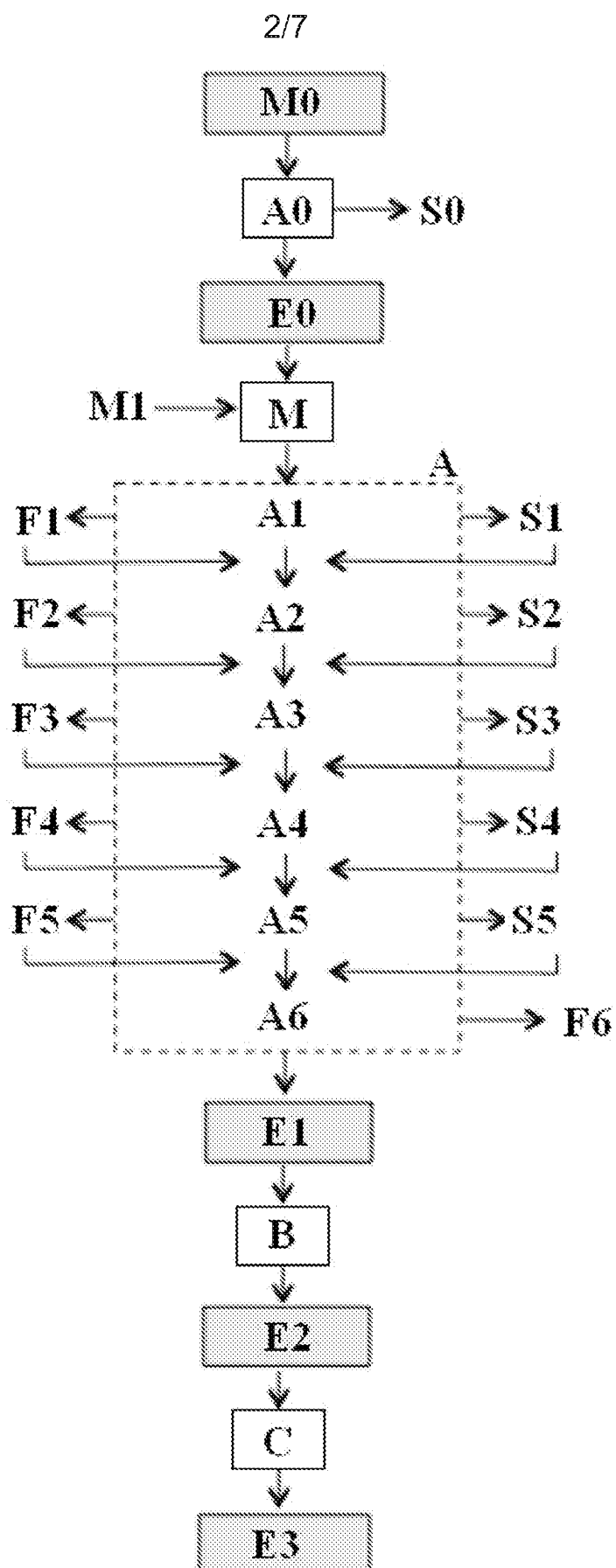


FIG. 2

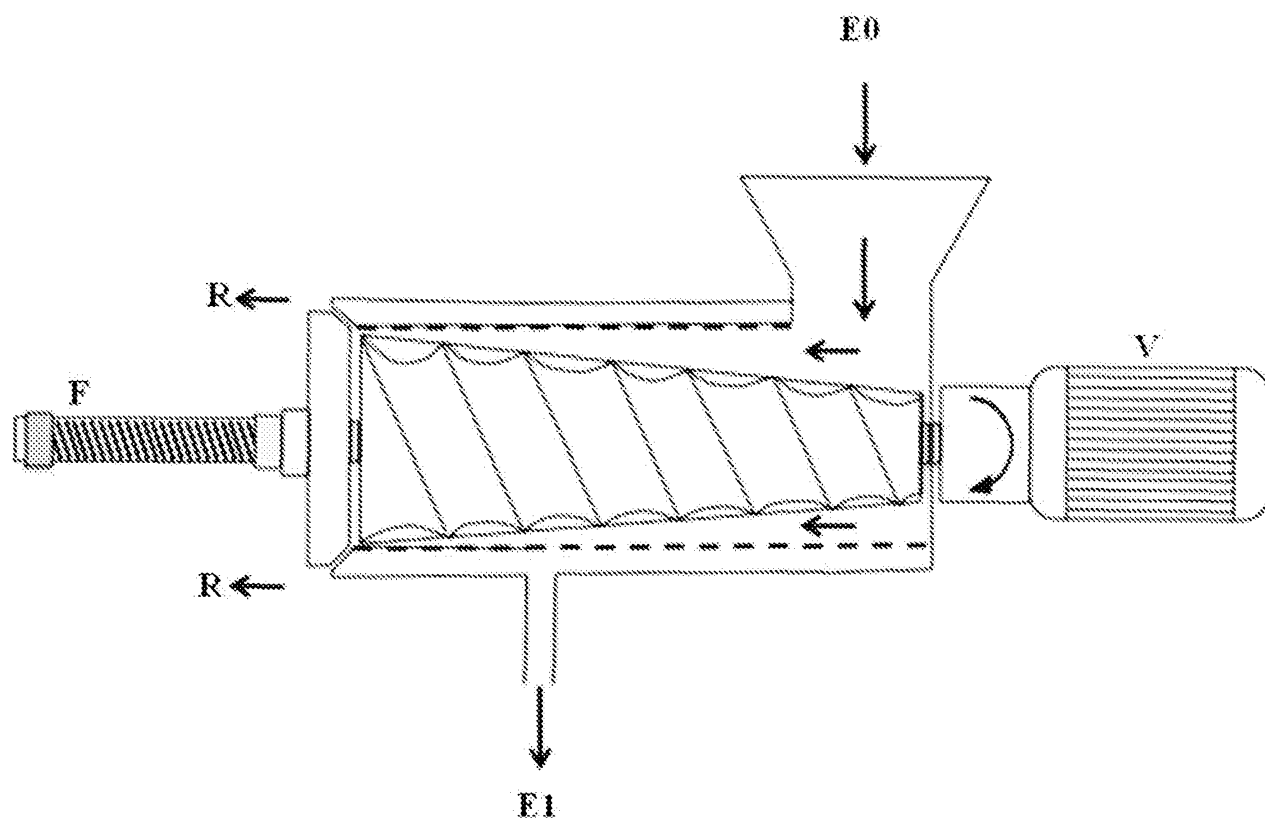


FIG. 3

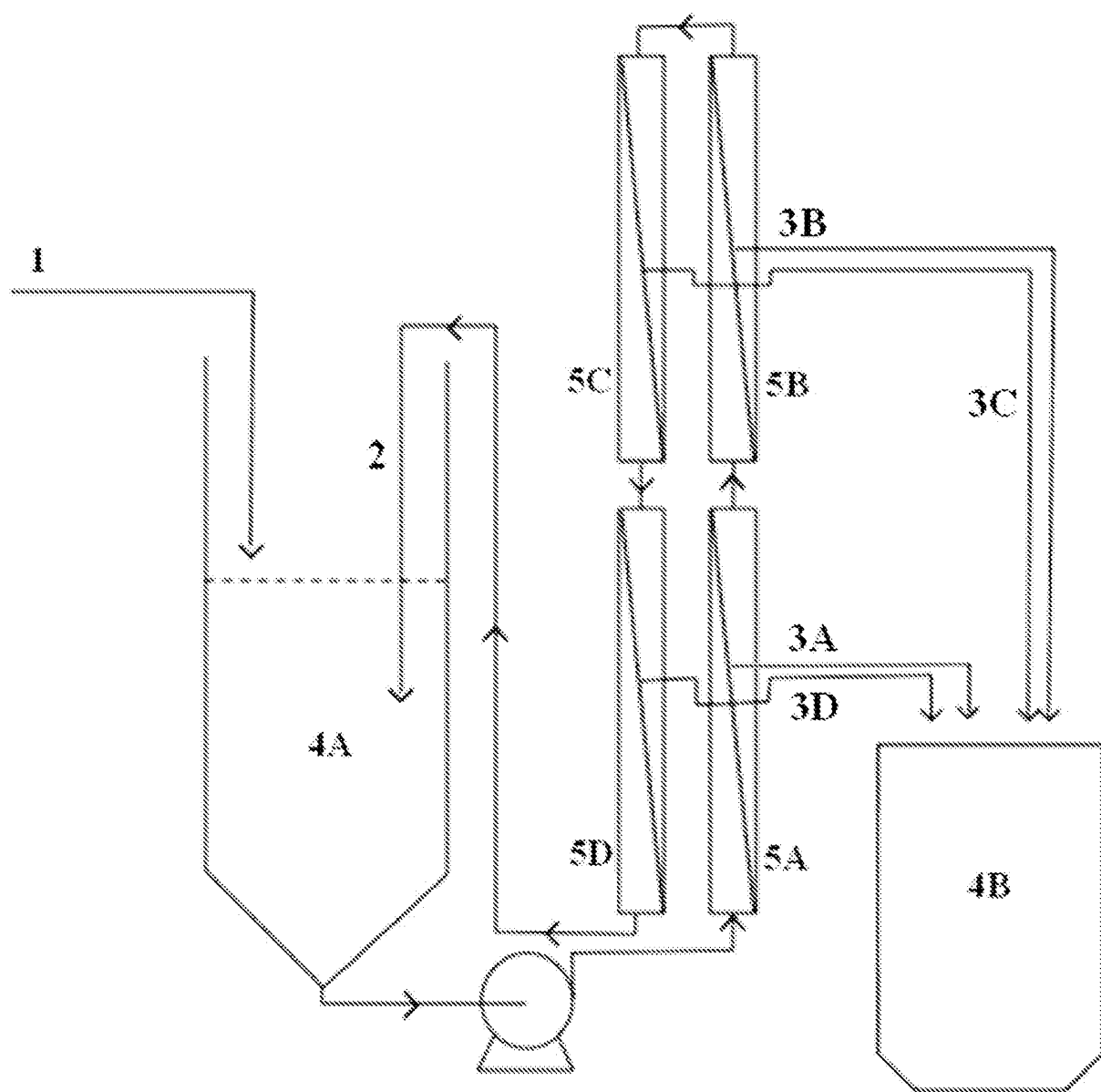


FIG. 4

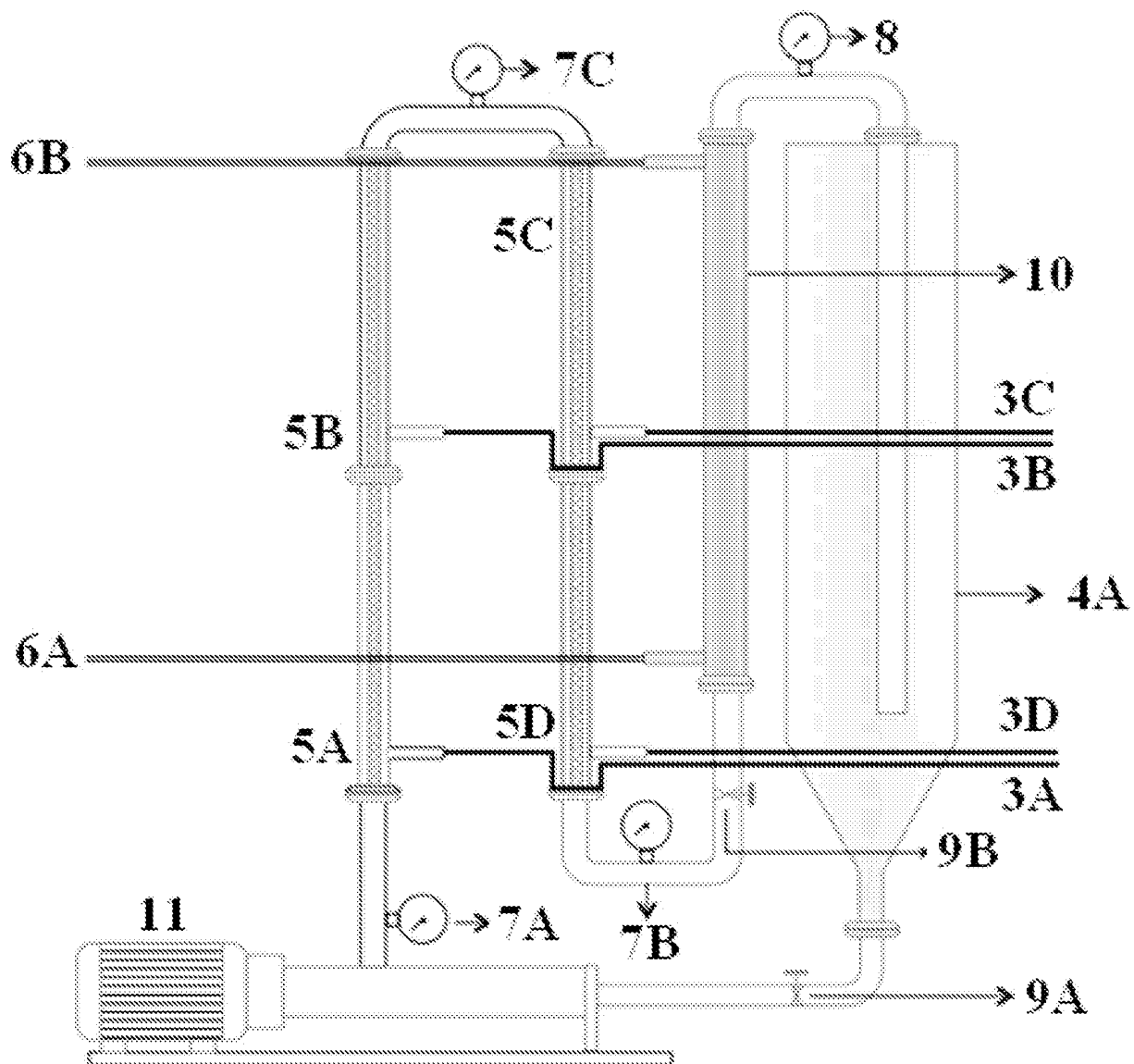


FIG. 5

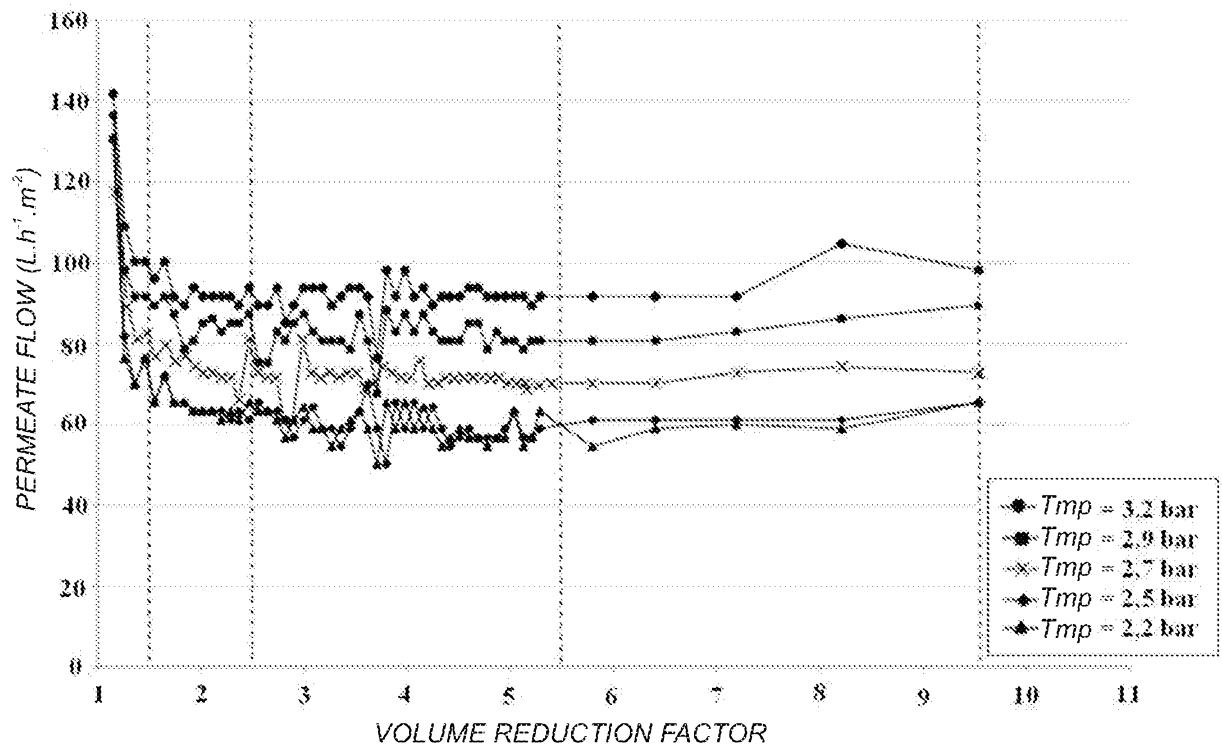


FIG. 6

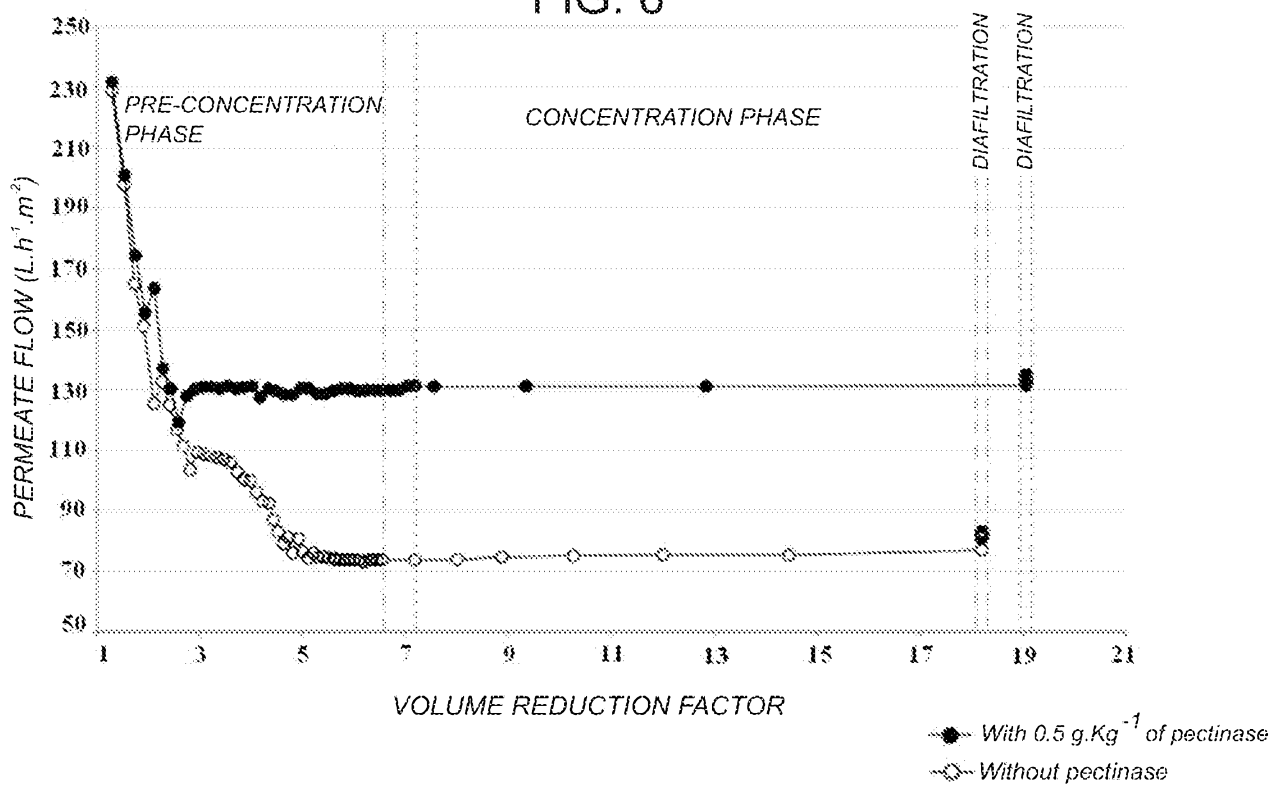


FIG. 7

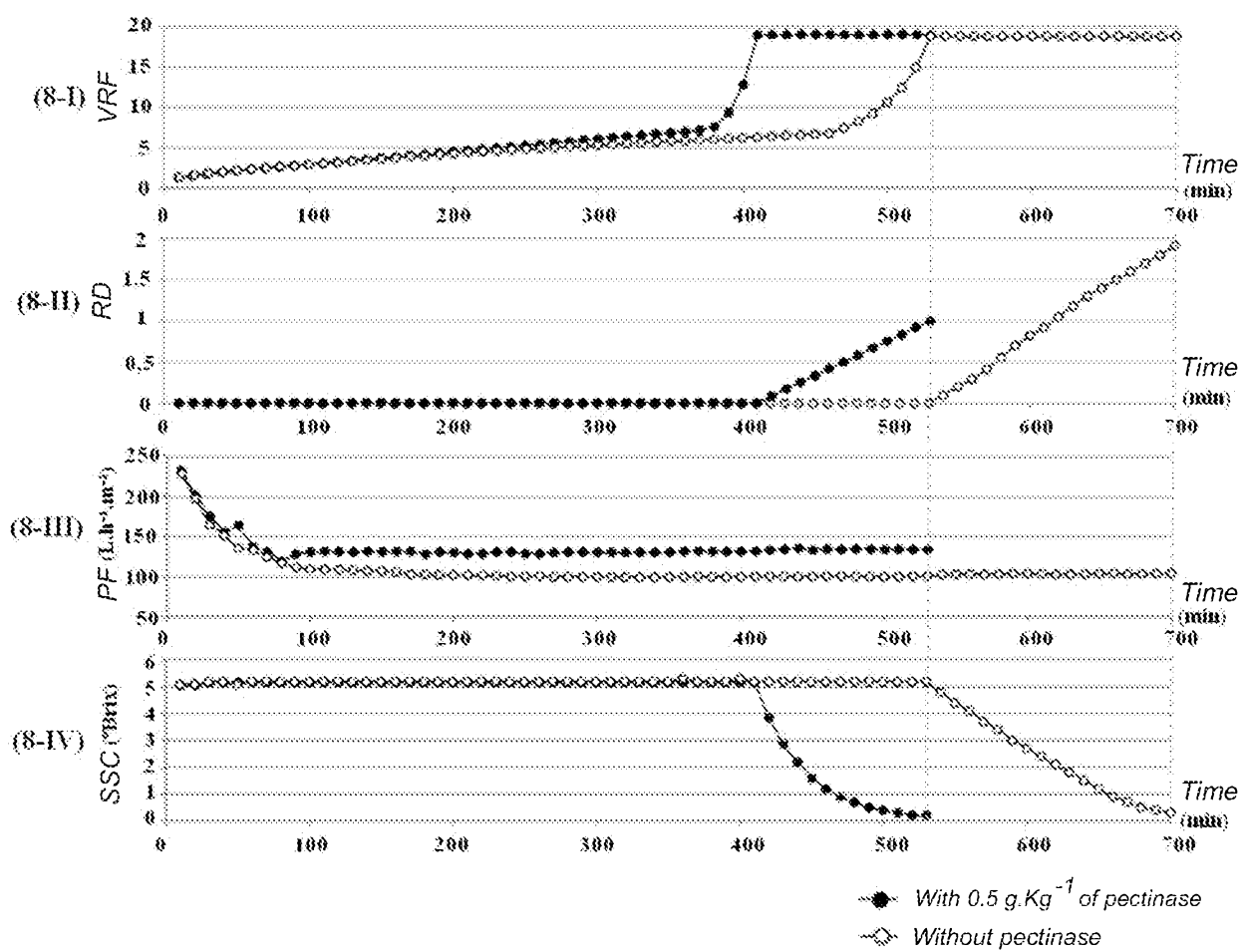


FIG. 8