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(71) Applicant and
(72) Inventor: **RAGHUNATH RAO, Jonnalagadda** [IN/IN]; 6-3-633/G, 1st Floor, Innovative House, Punjagutta, Hyderabad 500 082 (IN).

(74) Agent: **RAMESH KUMAR, Adsumilli**; 340, New Additional Law Cnamber, II Floor, High Court Buildings, Chennai 600 014 (IN).

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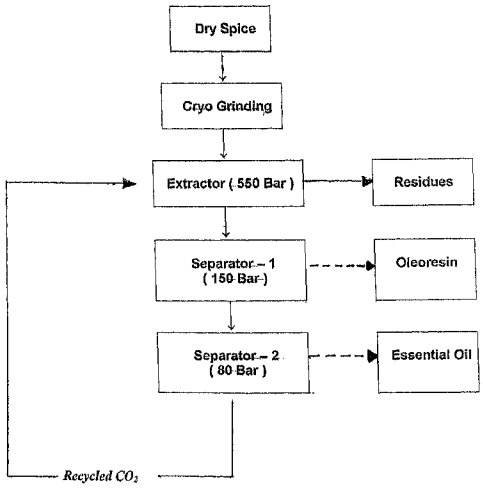
(54) **Title:** METHOD OF ISOLATING SOLANESOL EXTRACT FROM TOBACCO UTILIZING SUPER CRITICAL CO_2 FLUID EXTRACTION PROCESSING



(57) **Abstract:** The present invention relates to a naturally obtained solanesol of high molecular weight with primary monohydric aliphatic alcohols, which contains 45 carbon atoms. This invention also relates to the process for obtaining the solanesol with other lipids by extracting utilizing super critical fluid CO_2 from air dried Virginia tobacco leaves and concentrating utilizing organic solvents. The solanesol mixed with other lipids obtained has enhanced purity. The solanesol is useful in pharmaceutical compositions, and dietary supplement.

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Field of Invention

The present invention relates to a naturally obtained Solanesol of high molecular weight primary monohydric alipthatic alcohol $C_{45}H_{74}O$ from locally grown air dried Virginia tobacco leaves.

State of Art

Solanesol extracted from tobacco is the starting material for many high value bio-chemicals such as Vitamin-K analogues and **Co-enzyme Q10 (COQ10)**. Further, it is a potentiating agent in the medicines.

The research shows that after introducing solanesol radicals into the structure of some medicines, the effect are increased distinctly. With solanesol as its primary material Co-enzyme Q is useful in the treatment of heart diseases, cancers and ulcers.

Co-enzyme Q10 is extensively used as a supplement in Japan, USA and Europe. Co-enzyme Q10, also known as Ubiquinone, is a naturally occurring molecule, similar in structure to vitamin E. It is a co-factor in the electron transport chain, the biochemical pathway in cellular respiration from which ATP (adenosine tri-phosphate) and metabolic energy are derived. Since most cellular functions are dependent on availability of energy, Co Q10 is essential for the health of all human tissues and organs.

Defincies of this substance have been reported in a wide range of conditions including cardio-vascular disease, hypertension, and periodontal disease. Animal studies have shown that the decline in Co Q10 levels that occurs with age may be partly responsible for age related deterioration of the immune system.

In view of the increasing importance of Co Q10 for which solanesol is the starting material.

In view of the above mentioned health benefits, the current study was developed to carry out a process utilizing Super Critical Fluid Extraction (SCFE) to extract and separate natural solanesol along with other lipids from air dried Virginia Tobacco leaves

It is to be noted that the prior art description give in detail information of the technology, method, process and system known in the art. It explain the deficiency in the related art and the object of the invention being to overcome or surmount the problem associated with the prior art. This forms the essential feature and the object of the invention.

Summary of Invention

The present invention relates to a naturally obtained solanesol of high molecular weight with primary monohydric aliphatic alcohols, which contains 45 carbon atoms. This invention also relates to the process for obtaining the solanesol with other lipids by extracting utilizing super critical fluid CO_2 from air dried Virginia tobacco leaves and concentrating utilizing organic solvents. The

solanesol mixed with other lipids obtained has enchanced purity. The solanesol is useful in pharmaceutical compositions, and dietary supplement.

The inventors have made great effort to provide a process which meet the requirement of the industry and overcome the problem associated in the prior art.

To achieve the above object according to first aspect and feature of the present invention there is provided a process to improve, efficiency,

According to second aspect and feature of the invention in addition to the first feature the invention comprises a process, which is unique, rugged efficient, economical.

At the outset of the description which follows it is to be understood that ensuring description only illustrate a particular from of this invention. However, such particular form is only an exemplary embodiment without intending to imply any limitation on the scope of this invention. Accordingly, the description is to be understood as an exemplary embodiment and reading of the invention is not intended to be taken restrictively.

The above and other objects features and disadvantages will be clear from the following description of preferred embodiment taken in conjunction with accompanying drawings.

The foregoing description is outlined rather broadly preferred and alternative feature of the present invention so that those skilled in the art may better understand the detailed description of the invention that follows. Additional features of the invention will be described hereinafter that form the subject of claims of the invention. Those skilled in the art should appreciate that they can readily use the disclosed conception and specific embodiment as a basis for designing and modifying other structures for carrying out the same purposes of the present invention. Those skilled in the art should realize such equivalent conception do not depart from the spirit and scope of the invention in its broadest form.

Now the invention will be described in detail with reference to the following figures. The nature of the invention and the manner in which the invention is to be performed is clearly described in the following specification.

Fig.1 of the drawing shows the process of extracting the natural solanesol along with other lipids from air dried Virginia tobacco leaves.

Fig.2 of the drawing illustrate supercritical fluid extraction (SCFE)

Fig.3 of the drawing illustrate SCFE process block diagram.

The Process covers the following:

1. Drying of air dried Virginia tobacco leaves to bring down the moisture from 12% to 8 to 9%.

2. Extracting natural solanesol along with other lipids from air dried Virgnia tobacco leaves utilizing super critical fluid CO_2 processing at varied pressures and temperatures.

3. Dissolving of natural solanesol along with other lipids in SCF CO_2 under varying pressures from 150 to 425 bar at varying pressure intervals under varied temperature of 40 to 90°C at varied temperature intervals.

4. Separation of dissolved natural solanesol with 40 to 70% strength along with other lipids from the saturated SCFE stream in Separator – 1 from the saturated super critical CO_2 stream resulting in varied yields from 1.5 to 3.60% of the dried tobacco leaf.

5. Simultaneously separating in Separator-2, the lesser strength of 15 to 30% solanesol fraction with lipids resulting in varied yields from 1.0 to 1.8% of the dried tobacco leaf.

6. Optimizing extraction conditions around 175 bar pressure and at a temperature of 60°C resulting in two fractions one with solanesol of 40 to 43% strength and second fraction with solanesol of 15 to 30%.

7. Subjecting the second fraction with 15 to 30% solanesol strength to liquid – liquid fractionation to enhance the solanesol strength to 50% by separating the lipids.

8. The purity of the mixture of two fractions of solanesol was generally found to be 40 – 70% by HPLC analysis.

9. The natural solanesol of 40 – 70% strength refined by organic solvent process (unwanted lipids are removed) to have an enriched solanesol I (herein called refined solanesol) with purity up to 90 to 95% by HPLC method.

10. General solvents / solvent mixtures of varied proportions used in refining process of natural solanesol with lipids are isopropyl alcohol, methanol, hexane and acetone.
11. The yield of refined solanesol having constituent purity upto 90 to 95% by HPLC is varied from 1.2 to 2.4% of the tobacco leaves utilized.

The dried and milled tobacco is transferred into the basket which in turn is loaded into the extractor and subjected to SCFE processing. Refer flow diagram.

Initially the air in the extractor is vented off using low pressure CO_2 and then high pressure CO_2 is introduced for processing the biomass. The process of standardization o SCFE CO_2 extraction process, the pressure varies from 150 to 425 bar and at temperatures 40 to 90°C, and a mass flow of SCFE CO_2 at a range of upto 70 ks/kg of tobacco. Better solubility of solanesol along with the lipids is achieved at about pressures around 175 bar and at a temperatures of around 60°C, resulting in a yield of around 3 - 5% depending upon the quality of the tobacco leaves.

The high pressure SCFE CO_2 fluidizes the milled tobacco in the extractor.

The solanesol along with the lipids containined in the fluidized milled tobacco leaf are dissolved in SCFE CO_2 and reaches a saturation point by the time SCFE CO2 leaves the extractor and enters the Separator 1.

The Separator 1 is set up with different pressures and temperature parameters between 60 to 150 bars and temperatures at 40 to 60°C. Due to these variations and temperature in the Separator, the dissolved solanesol along with the other lipids from the supercritical CO_2 stream from the extractor, separates out from the CO_2 stream and collected in the Separator itself.

The supercritical CO_2 stream still containing lesser strength solanesol with lipids and moisture picked up from the tobacco leaves is vapourized to pressures of re-cycle stream system and enter Separator 2 at a pressure of 40 to 45 bar and temperatures at 25°C.

The moisture with some dissolved lipids separates out and collects in Separator 2 and the CO_2 gas is free of any dissolved material is recovered and re-cycled.

The extraction process is continued for a pre-determined period of time to ensure that the total quantity of CO_2 passed through the milled tobacco leaves to ensure that solanesol along with the lipids are dissolved and the tobacco is left with traces of solanesol.

At the end of the pre-determined time, the extractor is de-pressurized, initially recovering the high pressure CO_2 and subsequently venting the CO_2 which could not be re-cycled.

The basket containing the milled tobacco free from solanesol and other lipids is removed from the extractor and the tobacco leaves are evacuated using a vacuum system.

The process described above is carried utilizing a single extractor and two separators resulting a batch mode operation at the R & D center.

The process is also carried out utilizing two or more extractors in a continuous mode unlike with one extractor resulting in batch mode operation.

The process of extracting natural solanesol along with other lipids from tobacco leaves are carried out in two sets of processing units (i.e.) a Product Development Unit (PDU) and a Commercial Unit.

The lesser strength of solanesol along with the lipids is collected from Separator-2 is stored from batch to batch for subjecting liquid-liquid fraction. When adequate quantity of 600 to 1000 Ltrs. Is collected, the same is subjected to liquid-liquid fractionation process utilizing extractor 1 and extractor 2 one after the other, to enhance the solanesol strength to 40 – 70%.

The liquid-liquid extraction is optimized at super critical CO2 pressures at around 125 bar and at temperature around 50°C. In this process, lipids fractions are dissolved in fluid CO2 and carried out and separated in Separator-1. The concentrated solanesol with some % of lipids are retained in the extractor. Thus, the fraction with higher strength solanesol of about 40 to 70% is collected directly from the extractor for further processing.

The two fractions thus, obtained are mixed together and subjected to refining to dissolve the lipids and increase the purity of solanesol to 90 to 95%

The PDU has the following equipment and the processing parameters:

- An Extractor with 5 L capacity
- Two Separators
- High pressure carbon dioxide pump with 1000 bar pressure capacity and a carbon dioxide re-cycling system with heating and chilling units.
- The Commercial Plant has the following equipment:
- Two extractors with 800 L volume and basket of 600 L capacity.
- Two separators.
- High pressure carbon dioxide pump with 550 bar pressure and a carbon dioxide re-cycling system with heating and chilling units.

The natural solanesol along with other lipids contained in the tobacco leaves is subjected to refining to enrich solanesol percentage (herein called refined solanesol) with a purity upto 90% by HPLC method.

General solvents / solvent mixtures of varied proportions used in refining process of natural solanesol with lipids are isopropyl alcohol, methanol, hexane and acetone.

The yield of refined solanesol having constituent purity upto 90 to 95% by HPLC is varied from 1.2 to 2.4% of the tobacco leaves utilized.

Compressed and liquefied gases as solvents:

If the pressure is raised sufficiently, many substances which are gaseous at ambient pressure either liquefy or begin to behave like liquids in that they exert appreciable solvent power, even for solutes of low volatility. For example, at temperatures upto 31.0°C (the critical temperature) carbon dioxide can be liquefied by raising the pressure (Figure 2.1) and this liquid can be used to dissolve natural oils and quite a wide range of non-polar or slightly polar materials. Many of these are natural products. Based on solubility behavior, liquid CO_2 has been used commercially as a solvent for obtaining hop extracts since 1980.

Liquid propane has also been used for extracting natural products. Propane has the disadvantage of being a fire-hazard, but it is a more powerful solvent than carbon dioxide and the pressures required when using it as a solvent are usually lower.

The physical properties of the liquefied gaseous solvents in the applications deviate substantially from those of normal liquid solvents, due to the higher solvent reduced temperatures at which the extractions are carried out. These typically range from 1.0 own to 0.9 or slightly below, whereas the solvent reduced temperatures in normal liquid extraction operations do not normally exceed about 0.7. One consequence of this is that the isothermal compressibilities are higher. At a reduced temperature of 0.95 the compressibility is about 10 times as great as that for a liquid at the normal boiling point. At higher reduced temperatures the isothermal compressibility at the saturation point rises rapidly towards its theoretically infinite value at the critical point. This fact can cause complications when these liquids are pumped. Viscosities and diffusivities also differ from those in normal liquids and, as the reduced temperature rises towards unity, approach those in supercritical

fluids. The above effects start to become significant when the solvent reduced temperature rises above about 0.9 and liquids at reduced temperatures exceeding this value are described as 'near-critical' liquids.

Although, at temperatures above their critical temperatures, gases do not liquefy on raising the pressure, they can still exhibit liquid-like solvent properties if the pressure is sufficiently high for the density to approach a liquid-like value. The dissolving power of the solvent is then strongly density dependent and can be varied by changing the pressure.

This ability of the compressed gases to display solvent powers akin to liquids is termed the 'gas / fluid extraction' effect. The existence of this effect has been known for over 100 years. However, it was not until about 1978 that a commercial viable extraction process based entirely on the gas extraction effect came into operation, for the decaffeinating of coffee using compressed supercritical carbon dioxide.

Advantages of the use of near-critical solvents – Carbon dioxide:

1. Because of carbon dioxide good health and safety characteristics, it has been the solvent almost universally considered for applications in the food and related industries. There is a possibility of mixing this solvent with other components for substantial enhancements in solubility.
2. Control of pressure, a range of selectivities and dissolving powers can be obtained with carbon dioxide as a solvent at given temperature.
3. Because of diffusivity and viscosity behavior of supercritical carbon dioxide, it gives better penetration into pores and matrices and hence faster and more efficient extraction in extractant film controlled processes than do normal liquid solvents.
4. By careful design, the solvent recovery step can be made to require less energy than is the case with normal liquid extraction and the removal of the last traces of solvent from the product is not difficult.

   Where the product is to be used for human consumption, stringent food standards may well reduce the allowable amount of residual solvent to a level well below the required from an economic consideration of solvent losses. Under these conditions, supercritical CO_2 extraction with carbon dioxide as a solvent which is gaseous under normal condition has the advantage that nearly all the solvent (carbon dioxide) will automatically be expelled when the product comes to ambient pressure.

5. The comparatively low dissolving power of supercritical carbon dioxide tend to be associated with good selectivity, resulting in good quality pure products.

   Low extraction temperature associated with the use of liquid super critical CO_2 are also found to be helpful in minimizing thermal degradation of the product.

   Because of the above advantages the commercial application of super critical extraction techniques to natural products (particularly those of high market value) are in increase inspite of high investment cost.

SUPERCRITICAL FLUID EXTRACTION (SCFE) – Refer diagram – 2

PRINCIPLE and CO_2 as SUPER CRITICAL FLUID

- CO_2 in super critical state exists in liquid and gas form.
- Supercritical CO_2 is highly selective in dissolving and fractionation.
- Dissolving power of supercritical CO2 increases with increased pressure.
- Gentle extraction conditions –
- Extraction at high pressure (upto 550 bar) and Low temperature (45- 60c)
- Short extraction cycle (less than 2 hours)
- Single step process – simultaneous extraction and fractionation of OR & EO.
- Ensures no thermal and oxidation degradation – No artifacts.
- Pure and high quality full spectrum extracts.

ADVANTAGES OF SUPERCRITICAL CO_2 AS A SOLVENT

a. CO_2 is non-toxic, non-inflammable, inert and free of environmental hazards, extensively used in food processing.

b. Ensures no thermal and oxidation degradation, and no artifacts.

c. Due to low viscosity and high diffusivity, ensures concentrated extracts results in higher yields.

d. Residual CO_2 in the extracts vapourizes at room temperature, hence does not require de-solventing process.

e. Free of bacterial count

f. Free of heavy metals and pesticides residue

SCFE PROCESS BLOCK DIAGRAM – Refer diagram – 3

SCFE PROCESS ADVANTAGES:

- Extract of complete and true profile of the natural material.
- More top notes (i.e.) no loss of high volatile compounds.
- More heart (i.e.) high terpene ester contents
- More active ingredients (i.e.) no formation of artifacts.
- Extracts with high concentration (OR)
- Pure extracts free of polar pesticide residues and heavy metals
- Extracts of stable and long shelf life.
- Environmentally friendly process. No liquid and gaseous effluents
- Extracts classified as natural, categorized under GMP
- A state of the art process of current and futuristic relevance.
- Versatile and flexible process, wide application.

In general, carbon dioxide capabilities for extraction of natural products can be summarized:

- Lipophilic compounds such as hydrocarbons, ethers, esters, ketones and aldehydes are easily extractable.
- Polar substances such as sugars, polysaccharides, amino acids, proteins, phosphatides, glycosides and inorganic salts are not soluble.
- Fractionation is possible when the substances display differences in volatility, molecular weight or vapour pressure.

The solvation power of carbon dioxide may of course be altered by the presence of a co-solvent. This may be either a component, such as water, for example, which is present naturally in the substance as in the case of caffeine separation from coffee beans or teas.

The super critical CO_2 extracts can be generally classified into the following application areas:

- Extraction of edible oils, fats and waxes, with or without fractionation.
- Extraction of alkaloids from vegetable matter, particularly decaffeintion of coffee or tea.
- Extraction of flavors, spices and essential oils, particularly the extraction of hops.
- Purification of contaminated materials, such as production of pesticides free extracts or de-alcoholization of wines and beers.

SPECIFICATIONS

Raw Material : Tobacco

Physical Appearance	:	Air dried cured tobacco leaf - milled
Color	:	Dark Brown
Odour	:	Typical Virginia Tobacco
Moisture content	:	Max. 10%
Solanesol content	:	Min. 1.2 to Max. 2.8%
Nicotine	:	.....

Product specifications:

Name	:	Solanesol
Chemical Name	:	(2E,6E,10E,14E,18E,22E,26E,30E)-3,7,11,15,19,23,27,-31,35-Nonamethyl-2,6,10,14,18,22,26,30,34-Hexatriacontanonaen-1-ol
Molecular formula	:	C45H74O
Molecular weight	:	631.07
Appearance	:	light yellow or white solid matter
Purity by HPLC	:	Min. 90%
Solubility	:	In organic solvents and insoluble in water.
Storage	:	The product is stable stored at .....2-8□ and in a dampproof, airtight, lightresistant area for at least two years.
Stability	:	Stable, but may be sensitive – store cold. Combustible. Incompatible with strong oxidizing agents.

The invention has been explained in relation to specific embodiment. It is inferred that the foregoing description is only illustrative of the present invention and it is not intended that the invention be limited or restrictive thereto. Many other specific embodiments of the present invention will be apparent to one skilled in the art from the foregoing disclosure. All substitution, alterations and modification of the present invention which come within the scope of the following claims are to which the present invention is readily susceptible without departing from the spirit of the invention. The scope of the invention should therefore be determined not with reference to the above description but should be determined with reference to appended claims along with full scope of equivalents to which such claims are entitled.

CLAIM

1. A method for isolating solanesol along with the lipids from non-alcoholic and non-lipid compounds contained in the milled tobacco leaves wherein said method comprises of subjecting the milled tobacco leaves to super critical CO_2 fluid extraction process to obtain solanesol along with other lipids, and the mixture thus obtained are concentrated by using organic solvents.

2. A method for isolating solanesol along with the lipids from non-alcoholic and non-lipid compounds contained in the milled tobacco leaves as claimed in claim 1 wherein the process has the following steps:
   a. Drying of air dried Virginia tobacco leaves to bring down the moisture from 12% to 8 to 9%.

   b. Extracting natural solanesol along with other lipids from air dried Virgnia tobacco leaves utilizing super critical fluid CO_2 processing at varied pressures and temperatures.

   c. Dissolving of natural solanesol along with other lipids in SCF CO_2 under varying pressures from 150 to 425 bar at varying pressure intervals under varied temperature of 40 to 90°C at varied temperature intervals.

   d. Separation of dissolved natural solanesol with 40 to 70% strength along with other lipids from the saturated SCFE stream in Separator – 1 from the saturated super critical CO_2 stream resulting in varied yields from 1.5 to 3.60% of the dried tobacco leaf.

   e. Simultaneously separating in Separator-2, the lesser strength of 15 to 30% solanesol fraction with lipids resulting in varied yields from 1.0 to 1.8% of the dried tobacco leaf.

   f. Optimizing extraction conditions around 175 bar pressure and at a temperature of 60°C resulting in two fractions one with solanesol of 40 to 70% strength and second fraction with solanesol of 15 to 30%.

   g. Subjecting the second fraction with 15 to 30% solanesol strength to liquid – liquid fractionation to enhance the solanesol strength to 50% by separating the lipids.

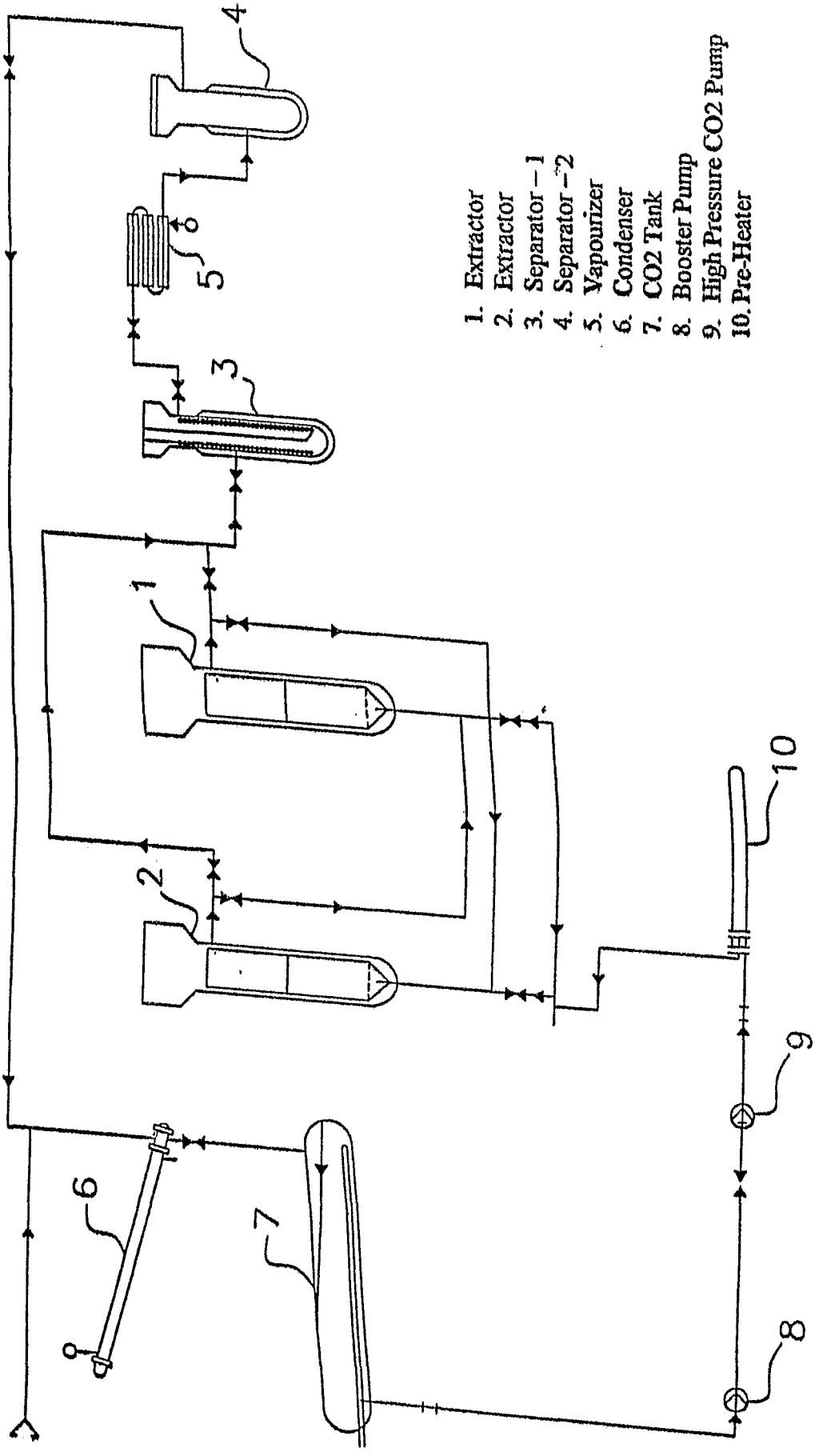
   h. The purity of the mixture of two fractions of solanesol was generally found to be 40 – 70% by HPLC analysis.

i. The natural solanesol of 40 – 70% strength refined by organic solvent process (unwanted lipids are removed) to have an enriched solanesol I (herein called refined solanesol) with purity up to 90 to 95% by HPLC method.

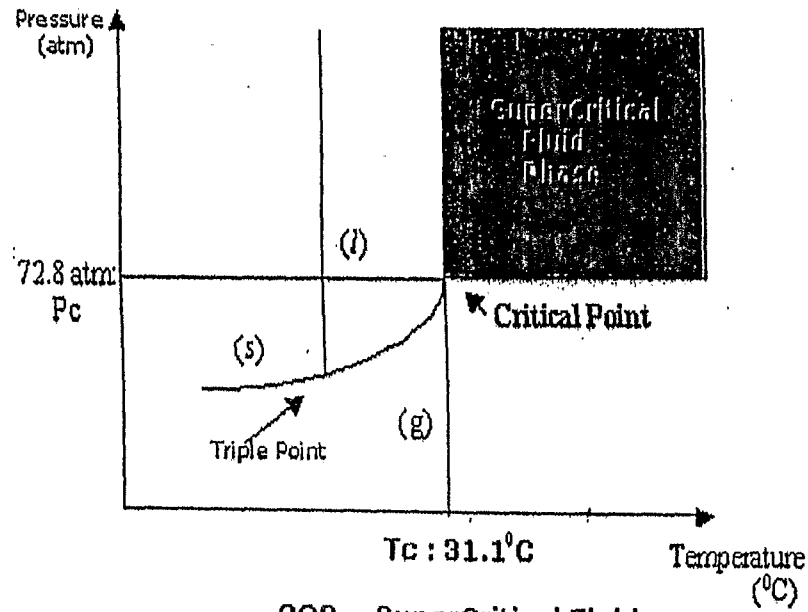
j. General solvents / solvent mixtures of varied proportions used in refining process of natural solanesol with lipids are isopropyl alcohol, methanol, hexane and acetone.

k. The yield of refined solanesol having constituent purity upto 90 to 95% by HPLC is varied from 1.2 to 2.4% of the tobacco leaves utilized.

FLOW DIAGRAM -



SUPER CRITICAL FLUID EXTRACTION - DIAGRAM - 2



CO2 - SuperCritical Fluid

SCFE PROCESS BLOCK DIAGRAM - 3