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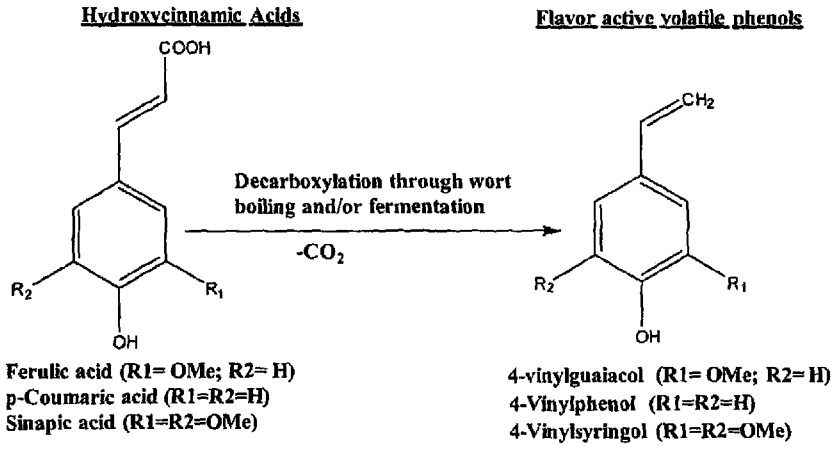


Figure 1

(57) **Abstract:** Press cake material is extracted with 80-100C water for up to 5 hours. The liquid is separated from the solid material, and the liquid is fermented/decarboxylated with suitable micro-organisms and/or suitable enzymes. The treated liquid is then separated from microorganisms and/or enzymes. The treated liquid can be processed further to recover the desired compound.

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METHOD FOR PRODUCTION OF CANOLOL

PRIOR APPLICATION INFORMATION

The instant application claims the benefit of US Provisional Patent Application 62/780,407, filed December 17, 2018 and entitled "Method for Production of Canolol", the entire contents of which are incorporated herein by reference for all purposes.

BACKGROUND OF THE INVENTION

Numerous investigations have been carried out on large scale synthesis of vinylphenols (example canolol) through microbial or chemical decarboxylation of cinnamic acids such as p-coumaric acid, ferulic acid, sinapic acid and caffeic acid from plant sources. However, efficient extraction of these compounds from natural plant sources are rare and not very successful at meeting the increasing demand due to 1) the limited availability of these compounds; 2) low yield; 3) high costs of extraction or purification; and 4) environmental concerns.

This patent application is a continuation of our previous work (Khattab et al. 2014) and a presentation entitled "Production of canolol from Canola meal, presented by U. Thiyam/ 2010 Canola Workshop, RCFFN; the first work dealing with the production of canolol from canola seed matrix (not oil). When our research work on canolol started in 2007, there was no existing knowledge of canolol occurring in toasted canola or rapeseed meal or press cake; hence it was critical to emphasize the importance of hydrolysis and heating, for example, by toasting or microwaving. Since 2010 several articles have proved that canolol, obtained through hydrolysis, is an anti-inflammatory and anticancer agent.

Press cake is particularly rich in phenolic compounds of biological activity. Earlier work (Khattab, R. Y., Eskin, M. N. A., & Thiyam-Holländer, U. (2014) Production of canolol from canola meal phenolics via hydrolysis and microwave-induced decarboxylation. *JAOCS, Journal of the American Oil Chemists' Society*, *91*(1), 89–97; Thiyam-Holländer, U., Aladedunye, F., Logan, A., Yang, H., & Diehl, B. W. K. (2014), Identification and quantification of canolol and related sinapate precursors in Indian mustard oils and Canadian mustard products. *European Journal*

of Lipid Science and Technology, *116*(12), 1664–1674) described the treatment of the press cake (Cruciferae) and particularly de-oiled by-product seeds of the species Brassica such as press cake, which includes: (1) separation of the phenolic molecules from the de-oiled seed by methods which do not destroy the naturally occurring phenolics in the press cake; (2) hydrolysis of the esters, glycosides, glucosides and bound complexes to release the cell-wall bound phenolics present in the seed by solvent, alkali treatment and/or acid treatment; (3) heat and microwave treatment of the seed prior to solvent extraction of phenolics; and (4) separation of the liquids from the solid press cake to produce a phenolic extract. While the process described in the aforesaid publications produces a phenolic extract which is rich in canolol, the procedure outlined suffers from certain operational disadvantages. For example, a) it requires a substantial amount of alkaline and acid hydrolysis treatment and b) is not sustainable because of the extensive use of solvents. The use of excessive amounts of solvents for the treatment or extraction of press cake is unacceptable by today's green and environmentally friendly standards. Furthermore, microwave and oven heat treatment are followed by alkali and acid treatments and an extraction procedure, meaning that multiple, complicated steps are required. Moreover, extraction yields and extraction time are not acceptable. Finally, any trace of solvent such as for example methanol in the end product renders the antioxidant product toxic.

Odinot et al (2017, Microorganisms 5, 67) describe a process for the enzyme feruloyl esterase type-A is incubated with raw meal at 55C and sinapic acid recovered by resin purification.

SUMMARY OF THE INVENTION

According to an aspect of the invention, a method is provided for producing a vinyl phenol compound or ethyl phenol compound comprising:

extracting a quantity of a press cake material with water at a temperature of 80-100C, thereby producing an aqueous extract and a sediment;

separating the aqueous extract from the sediment;

subjecting the aqueous extract to suitable microorganisms and/or enzymes to

ferment and decarboxylate the aqueous extract, thereby producing an aqueous extract comprising a vinyl phenol compound or an ethyl phenol compound.

The invention described is in reference to the treatment of press cake as a preferred example. It should be understood that this invention is applicable to other seeds of the genus Cruciferae. However, it is particularly applicable to the seeds of for example canola, Oriental or Yellow mustard press cakes and Camelina as such seeds are particularly rich in hydroxycinnamic acids.

In some embodiments, this process can include wort or brewing sugar or brewing enhancers and heated oilseed substrates or hydrolyzed oilseed extracts.

In some embodiments, this process can also be used to produce sinapic or other hydroxycinnamic acids from bound phenolics prior to the transformation to produce phenols for example volatile phenols.

As discussed herein, starting material is extracted with 80-100C water for up to 5 hours. The liquid is separated from the solid material, and the liquid is fermented/decarboxylated, for example, with suitable micro-organisms and/or suitable enzymes. The treated liquid is then separated from microorganisms and/or enzymes. In some embodiments, the treated liquid is processed further to recover the desired compound.

The starting material may be press cake or defatted press cake which has been crushed. As will be appreciated by one of skill in the art, as used herein, "press cake" and/or "defatted press cake" may include a heat-treated, a pressure-treated, an enzyme-treated, an alkali-treated or an acid-treated press cake or an extract derived from a press cake.

In some embodiments, the starting material is extracted in water at 80-100C for at least1-30 min (not necessarily up to 30 mins, but up to 2 days).

In some embodiments, the liquid comprising the microorganisms and/or enzymes is incubated at room temperature, plus or minus 10 degrees Celsius, that is, between 12C-32C.

The enzymes may comprise at least one of xylanase, a cell-wall degrading enzyme, a cinnamoyl esterase, a feruloyl esterase, and a ferulase decarboxylase.

The suitable micro-organisms may comprise at least one of xylanase, a cell-wall degrading enzyme, a cinnamoyl esterase, a feruloyl esterase, and a ferulase decarboxylase. That is, the suitable microorganisms may be microorganisms that express at least one of these enzymes.

The suitable microorganisms may be selected from the broad group consisting of yeast, for example, commercial yeast, active yeast, beer yeast, wine yeast, Brettanomyces, , Saccharomyces and combinations thereof, and lactic acid bacteria.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic diagram showing conversion of hydroxycinnamic acids to flavor active phenols.

Figure 2 shows a series of representative chromatograms indicating position of compounds of interest. HPLC-DAD chromatograms depicting the position of Hydroxycinnamic acids (Fig 2A, 320 nm) and Canolol (Fig 2B, 270 nm).

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned hereunder are incorporated herein by reference.

As used herein, "purified", in all of its grammatical forms, does not necessarily mean "absolute purity", but refers to an increase in concentration of the compound by for example, 10 fold. In some embodiments, a purified compound may comprise 80% or more of a given solution. That is, a purified composition may comprise 80% or more of the compound of interest.

Described herein is a green sustainable two-step treatment of a starting material such as for example but by no means limited to a press cake material, for example but by no means limited to, a press cake or defatted oilseed. The source of

the press cake or defatted oilseed may be for example but by no means limited to seeds of the genus Cruciferae. Suitable examples of the genus Cruciferae include but are by no means limited to the group consisting of Camelina sativa, Chinese mustard (Brassica juncea), black mustard (B. nigra), white mustard (Sinapis alba), horseradish (Armoracia rusticana), radish (Raphanus sativus) and B. oleracea (vegetables broccoli, Brussels sprouts, cabbage, cauliflower, kale, and kohlrabi). The processing of all these species (primarily oilseeds) to prepare a press cake is very similar. They are first de-oiled, extracted and pressed (crushed) in order to obtain the oil and a left-over by-product (the press cake). Press cakes made from these different species have different protein, amino acid, fat and phenolic compound profiles. However, in general they tend to have an oil content ranging from 8-20%.

If, for instance, oil content is over 40%, then it would still be valid for phenolic extraction, but oil particles may interfere with an aqueous extraction and effective phenolic extraction may be compromised.

In some embodiments, the method of the invention is particularly applicable to the seeds of canola, Oriental mustard press cakes and Camelina as such seeds are particularly rich in hydroxycinnamic acids. As discussed herein, a press cake is preferable since the phenolics are maximized by weight and other unwanted fractions could be reused. Oilseeds are generally pressed at 45-95°C, but this range is impacted by the type of instrumentation technique used to press and extract the oil. For example, cold-pressing at approximately or about ~50°C while expeller pressing above 55°C.

As discussed herein, esterified or cell wall bound hydroxycinnamic acids endogenous to oilseeds and plants are released from an insoluble and less active form to a free, active, flavour-active, decarboxylated form and are part of a super antioxidant product as a result of an aqueous extraction with water at 80-100C. In some embodiments, this is preceded by a dry or wet roasting process (80-200C/1-45 min). The compound formed may be either canolol, or other flavor active vinyl phenols and ethyl phenols depending on the procedure followed or the source of the press cake material.

As will be appreciated by one of skill in the art, another advantage of the press cake is that it comes with minimal oil content which could interfere with the aqueous extraction. As discussed herein, the press cake has been crushed or ground, thereby exposing the seeds to facilitate phenolic extraction in a high temperature and aqueous system, as discussed herein.

Canolol (4-vinylsyringol, VS), a strong antioxidant and alkylperoxyl radical scavenger originally discovered in crude canola oil (rapeseed), is produced by decarboxylation of sinapic acid (SA), a hydroxycinnamic acid, during canola seed roasting. Hydroxycinnamic acids, usually cell-wall bound in oilseeds or as tartaric acid esters in fruits, are released by hydrolysis. The conversion of hydroxycinnamic acids such as, for example, but by no means limited to, sinapic acid, p-coumaric acid, ferulic acid and caffeic acid, into volatile phenols involves decarboxylation.

It was previously believed that these compounds would be damaged when exposed to heat or boiling which is necessary in order to liberate the compounds.

Disadvantages inherent in the prior art are avoided by the: (1) direct extraction of the press cake, either defatted or containing up to 20% oil residue, by aqueous (water) microbial, enzymatic and heat treatment with the eventual separation of the volatile phenols from the extract by a suitable method; (2) water extraction of the as is press cake at or near the boiling point for releasing precursor hydroxycinnamic acids; (3) separating the liquid from solids after water extraction (recovered solids are potential animal feed products or soil fertilizers, which would not be possible if non-aqueous solvents were used); (4) treatment of the liquid to recover either canolol, or other flavor active vinyl/ethyl phenols, depending on the procedure selected, as discussed below.

Also described is process for separately generating and recovering canolol, or other flavor active vinyl or ethyl phenols, and phenolic antioxidants from press cake which comprises: crushing the press cake; extracting the resulting solid with water at a temperature between 80°C and 100°C for at least about one minute to a half hour (STEP1) (this is extendable to up to 2 days); separating the liquid from the solids

present after said extraction; incubating the separated liquid with suitable microorganisms and/or enzymes (STEP2); and isolating the compounds of interest.

As discussed herein, the process for hydrolyzing the complex hydroxycinnamates is carried out by the thermal treatment during the aqueous extraction and by enzymes such as xylanase, cell-wall degrading enzymes and cinnamoyl or feruloyl esterases.

The suitable microorganisms are preferably microorganisms that comprise enzymes capable of decarboxylation of hydroxycinnamic acid (for production of the compounds of interest) and cell wall degradation (for release of the hydroxycinnamates). Suitable microorganisms are well known in the art and include but are by no means limited to microorganisms used in the preparation of wine and/or beer. Specifically, beer yeast, wine yeast or related Brettanomyces yeasts are part of the process of beer making and can drastically influence the aroma and character of these products through the production of volatile phenols. A high amount of volatile phenols is mostly regarded as off-flavour in beers and wine and can mask the natural fruity flavours of a wine. Yeast strains are known to influence the type and formation of the vinyl phenols (4VG, 4VS/canolol) and can also differ in their ability to produce 4-vinylphenol and 4-vinylguaiacol. The fermentation aspect is critical, as microorganisms (yeast) and enzymes such as ferulase decarboxylase and esterases will decarboxylate to yield the vinyl phenols in a different manner.

As will be appreciated by one of skill in the art, the separated liquid may be considered to be fermented and decarboxylated by the suitable microorganisms and/or enzymes. Accordingly, there is provided a process for separately recovering decarboxylated products of the complex precursor hydoxycinnamic acid namely canolol, or other flavor active vinyl phenols and related antioxidants and the product is referred as brewed canolol & related phenols. In this manner, decarboxylated products of hydoxycinnamic acid such as for example canolol, or other flavour-active vinyl phenols and related antioxidants can be produced. This product can then be used as an end-product and sold as a vinyl phenol containing product in a liquid, spray, or freeze-dried (powder) form.

Similarly, in this manner, phenolic products of hydroxycinnamic acid such as sinapic acid or sinapine and related antioxidants can be enriched or degraded or produced. This product can then be used as an end-product and sold as a crude extract product in a liquid, spray, or freeze-dried (powder) form.

The liquid and solid phase can be separated by any suitable means known in the art, for example: sedimentation; or filtration using for example a standard cheese cloth or filter paper (such as for example but by no means limited to a coffee filter, or Whatman paper). The separation of the solid and liquid is required in order to access the active hydroxycinnamic acid ingredients in the liquid, as discussed herein.

In a preferred embodiment, the starting material undergoes an aqueous extraction at a temperature between 80 to 85°C for about one minute to one half hour. The liquid and solid phases present after the extraction are separated and the separated liquid is subjected to fermentation and decarboxylation, as discussed above.

The decarboxylated and fermented liquid comprises vinyl and ethyl phenols which can be further isolated using any of a variety of means known in the art. For example, the compounds of interest may be isolated by: column chromatography or preparative chromatography based on the retention time of the compound; and/or steam distillation or molecular distillation based on the volatility, molecular structure or molecular weight of the compound of interest.

It is noted that techniques for quantification and isolation of hydroxycinnamic acids and vinyl phenols have been extensively published and are well known in the art.

As will be appreciated by one of skill in the art, commercial scale-up through simplified bioreactors or fermenters can be adapted for the invention and exploited in several technologies and synthetic applications for health, food and cosmetics. Accordingly, the invention provides a novel, inexpensive, cost-effective and commercially adaptable process for producing brewed canolol, or other flavour-active vinyl phenols and related antioxidants.

In some embodiments, ratio of starting material (solid) to water (liquid) range may be from 1 to 5 to 1 to 100 (weight/volume).

As discussed herein, this process can include wort or brewing sugar and brewing enhancers, as discussed in the examples below.

In some embodiments, suitable wood chips, for example oak chips, is an additive (additional additive) precursor containing phenols. This is a precursor and contributes to the enhancement of vinyl phenols. Either roasted or non-roasted oak chips can be applied as enhancers for brewed canolol & related phenols for few minutes to hours during the STEP 1 and 2.

In some embodiments, lactic acid bacteria can be applied as enhancers for brewed canolol & related phenols (STEP 2). The lactic acid bacteria are associated with some antimicrobial, yield and beer quality. Enhancement is expected in the quality of the end product. The expected end result is an increase in the yield of canolol or vinyl phenols.

Other wood species (for example chip mixes made from pine (Pinus sylvestris) and birch wood (Betula verrucosa/B. pubescens) - might generate lignin in the course of the alkaline cooking process (50-170C/100min) (REF Alen et al. US patent 5032223, 1991). Vanillic acid is a lignin monomer and decomposition products from the cooking could be phenolic monomers consisting of guaiacol, vanillin, syringic acid, syringol, and trans-sinapyl alcohol. It is of note that a wide range of volatile compounds is transferred to beer (Fermentation 2018, 4, 20) and other beverages aged in barrels or on wood chips due to wood seasoning, ageing or toasting.

These and other objectives will become apparent from the following the description which highlights several methods for achieving the stated objects:

The invention will now be further elucidated and explained by way of examples. However, the invention is not necessarily limited to the examples.

Source of cake: Vittera canola press cake, Oriental and yellow mustard cake from GS Dunn and Sakai Spice.

The crushed cake is extracted in water at 80-100°C for 1-30 minutes. The press cake is extracted in water at 80-100°C for 1-30 minutes (ideally); this time can be extended up to 5 hours.

The solid to liquid range is 1 to 5 to 1 to 100 (weight/volume); the separation of the solid and liquid is required in order to access the active hydroxycinnamic acid ingredients in the liquid.

The above stated procedures achieved with a starting material having a moisture content of 2.5-12 percent.

Discussed below, Example 1 is critical to a continuous commercial process, because at a lower level there is incomplete enzymatic hydrolysis of the hydroxycinnamic acids. Furthermore, there is also the risk of bacterial contamination if the moisture is too high.

The above stated procedures were achieved with a hydroxycinnamic acid content and related derivative as low as 3 mg to about 25 mg per gram weight of solid matter (cake).

Example 1

Wort was prepared using 26 g steeping roasted cracked malt and 52 g of Pilsner DME in 500 ml water. Ground defatted press cake (ground using a coffee grinder or manually with a mortar and pestle) was prepared and 15 gram of defatted ground cake was slowly added with agitation to 500 ml (by weight) of boiling wort for two minutes and immediately brought to cool down and then filtered. Filtration eliminates nearly all the solid coarse impurities. The extract contains hydrolyzed and liberated hydroxycinnamic acids that are readily available for further treatment.

Specifically, the filtrate solution contains the class of hydroxycinnamic which can be converted to canolol, or other flavour active vinyl phenols. The filtrate was separated into 50 ml to 100 ml batches for monitoring the formation of the target phenols.

Potentially this process could produce up to 1000 micrograms or milligrams or grams of canolol, or other flavor active vinyl /ethyl phenols depending on the initial weight of the cake or water used in the process.

Example 2

Wort was prepared using 26 g steeping roasted cracked malt and 52 g of Pilsner DME in 500 ml water. 50 ml to 100 ml batches were prepared for monitoring the formation of the target phenols. To 50 ml of hot wort were added: 1) 2.7 mg of free Sinapic acid and 2) 2.4 mg of trans-Ferulic acid, 1.6 mg p-Coumaric acid and 3.6 mg of syringeldehyde and immediately brought to cool down. Yeast was added in a ratio to reflect 11.3 g per 20 liter.

This results in a few-fold to thousand-fold increase in canolol concentration by decarboxylation through enzymes or wine or beer yeast when incubated at least overnight, or up to seven days, depending on the temperature.

Proceeding in this fashion about 1 to 1000 mg of canolol, or other flavor active vinyl phenols, and related phenolics can be produced.

This experiment validated that pure standard compounds (SA) will liberate canolol or the respective vinyl phenol as proof of concept.

Example 3

The press cake (in kg) is added slowly to hot, near boiling water brought to a boil as before, with mild agitation using manual stirring. The two minute exposure time was immediate brought to a boil and then cooled.

Proceeding in this fashion about 1 to 1000 mg of canolol, or other flavor active vinyl phenols, and related phenolics can be produced.

Extraction time of the cake may be completed in about five minutes or slightly longer, using hot water as the extractant.

Example 4

The cake (in kg) is added heated water as described previously. After extracting the hydroxycinnamic acids, the solids and liquids were separated by either centrifugation or filtration. To the separated liquid there was added 0.9 to 1.1 mg (per 50 ml wort) Saccharomyces or Brettanomyces or Saccharomyces species, based on the weight of the solution.

Specifically, the liquid was held at boiling temperature for one minute, immediately cooled and transferred to sterilized environment and yeast mixtures were added to ferment and decarboxylate hydroxycinnamic acids to form canolol, or other flavor active vinyl phenols.

EXAMPLE 5

Wort was prepared using 100 g smoked cracked malt and 50 g of Pilsner DME in 500 ml water. 50 gram of heated defatted ground mustard flour was slowly added with agitation to 500 ml (by weight) of boiling wort for ten minutes and immediately brought to cool down and then filtered. The extract contains a complex mixture of hydroxycinnamic acids that are readily available for further hydrolysis, decarboxylation or other structural transformation from the original structure.

The filtrate was separated into 20 ml to 40 ml batches for monitoring the changes in the structure of the original hydroxycinnamic acids.

Potentially this process could produce up to 1000 micrograms or milligrams or grams of canolol, or other flavor active vinyl /ethyl phenols depending on the initial weight of the cake or water used in the process and other phenolic cinnamic acid or benzoic acid or related phenols as by-products.

While the preferred embodiments of the invention have been described above, it will be recognized and understood that various modifications may be made therein, and the appended claims are intended to cover all such modifications which may fall within the spirit and scope of the invention.

CLAIMS

1. A method of producing a vinyl phenol compound or ethyl phenol compound comprising:

extracting a quantity of a press cake material with water at a temperature of 80-100°C, thereby producing an aqueous extract and a sediment;

separating the aqueous extract from the sediment;

subjecting the aqueous extract to suitable microorganisms and/or enzymes to ferment and decarboxylate the aqueous extract, thereby producing an aqueous extract comprising a vinyl phenol compound or an ethyl phenol compound.

2. The method according to claim 1 wherein the vinyl phenol compound or the ethyl phenol compound is purified and/or isolated.

3. The method according to claim 1 wherein the vinyl phenol compound is canolol.

4. The method according to claim 1 wherein the aqueous extract comprises hydroxycinnamic acid.

5. The method according to claim 1 wherein the press cake material and the water are mixed at a ratio of 1:5 to 1:100 (weight/volume).

6. The method according to claim 1 wherein the press cake material is extracted for less than 5 hours.

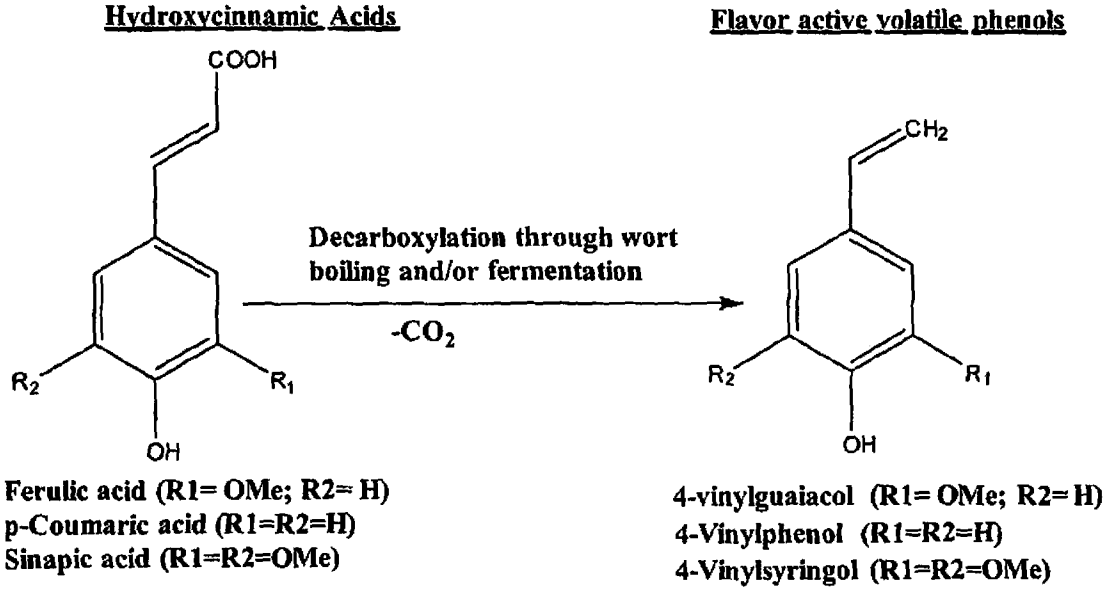
7. The method according to claim 1 wherein the press cake material is extracted for 1 minute to 30 minutes.

8. The method according to claim 1 wherein the press cake material is from the genus Cruciferae.

9. The method according to claim 1 wherein the enzymes comprise at least one of xylanase, a cell-wall degrading enzyme, a cinnamoyl esterase, a feruloyl esterase, and a ferulase decarboxylase.

10. The method according to claim 1 wherein the suitable micoorganisms comprise at least one of xylanase, a cell-wall degrading enzyme, a cinnamoyl esterase, a feruloyl esterase, and a ferulase decarboxylase.

11. The method according to claim 1 wherein the suitable microorganisms are selected from the group consisting of beer yeast, commercial yeast, wine yeast, Brettanomyces, lactic acid bacteria, Saccharomyces and combinations thereof.



Ferulic acid (R1= OMe; R2= H)
p-Coumaric acid (R1=R2=H)
Sinapic acid (R1=R2=OMe)

4-vinylguaiacol (R1= OMe; R2= H)
4-Vinylphenol (R1=R2=H)
4-Vinylsyringol (R1=R2=OMe)

Figure 1

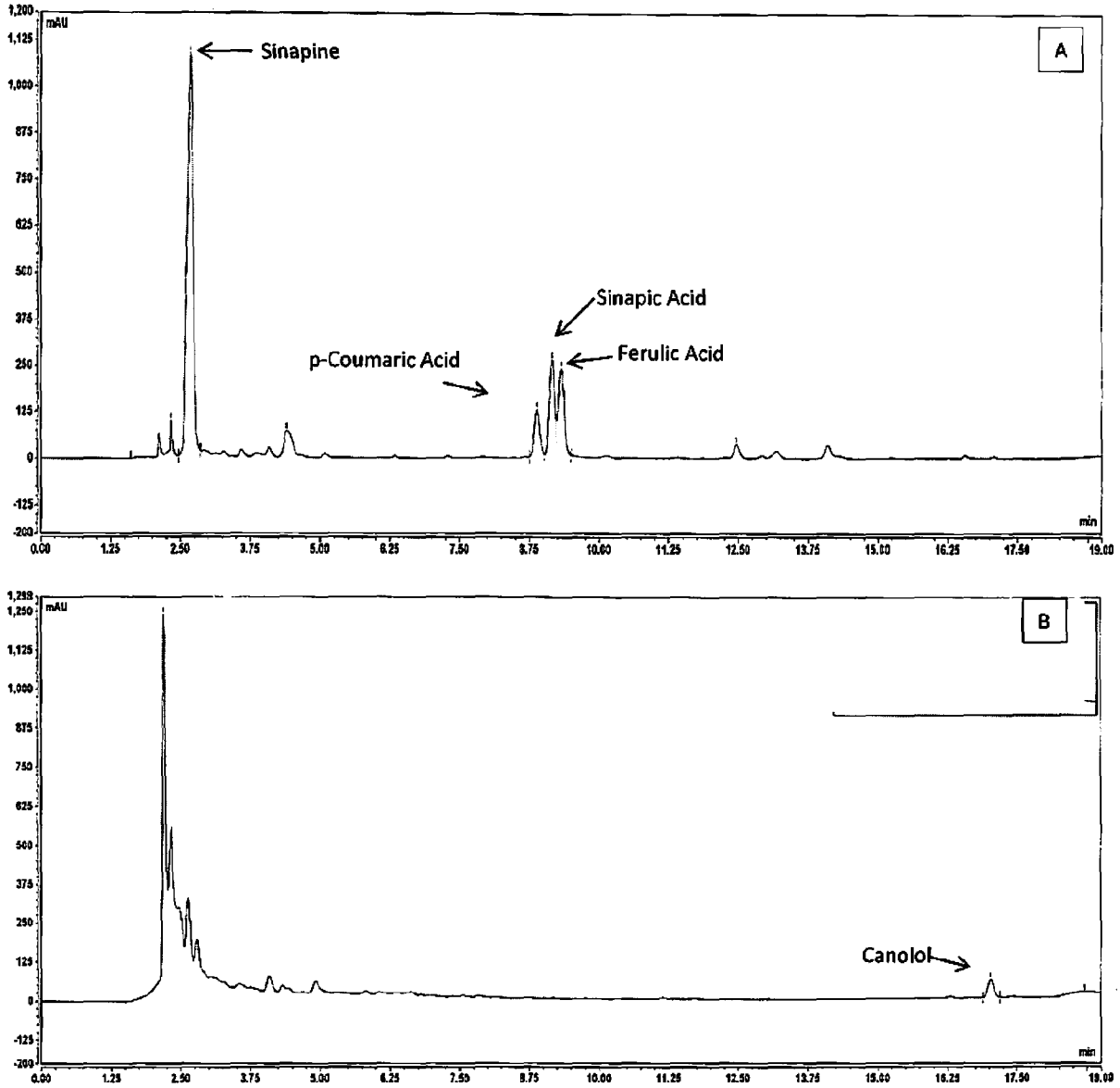

mAU
Sinapine
A
Sinapic Acid
p-Coumaric Acid
Ferulic Acid
min
mAU
B
Canolol
min

Figure 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2019/051800

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***C12P 7/22*** (2006.01) , ***A23L 27/24*** (2016.01) , ***C07C 39/20*** (2006.01) , ***C07C 43/295*** (2006.01) , ***C12P 1/00*** (2006.01) , ***C12P 7/02*** (2006.01)

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)
Not searched by classification

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
None

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Scopus, Canadian patent database, Google Patent, Google Scholar
Keywords: phenol, canola, rapeseed, antioxidant, extraction, yeast, canolol, heat, microwave, authors names, cruciferae, seed, polyphenol, press cake, enzyme, vinyl phenol, ethyl phenol, fungus, oil,

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ZAGO E. et AL, "*Influence of rapeseed meal treatments on its total phenolic content and composition in sinapine, sinapic acid and canolol*". Industrial Crops and products, 15 December 2015 (15-12-2015), Vol. 67, pp. 1061-70, ISSN 0926-6690 (abstract) [online] [retrieved on 31 January 2020 (31-01-2020)].	1 – 11
A	WO2017072450A1 (LOMASCOLO A. et AL) 04 May 2017 (04-05-2017)	1 - 11

☐ Further documents are listed in the continuation of Box C. ☑ See patent family annex.

*	Special categories of cited documents:	"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
"A"	document defining the general state of the art which is not considered to be of particular relevance	"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
"D"	document cited by the applicant in the international application	"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
"E"	earlier application or patent but published on or after the international filing date	"&"	document member of the same patent family
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Information on patent family members

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