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(54) Title: PROCESS FOR OBTAINING CAFFEYOYLQUINIC ACIDS-RICH EXTRACTS FROM HELIANTHUS ANNUUS

(57) Abstract: The present invention relates to extracts of deoiled *Helianthus annuus* seeds which are useful for the prevention and treatment of dyslipidaemia, hyperglycaemia and hypertension, metabolic syndrome and type 2 diabetes. The present invention also relates to the process for preparation of said extracts and compositions containing them. The extracts according to the invention, when added to carbohydrate-based foods, reduce the glycaemic index and postprandial absorption of glucose, and induce a modification of the lipid profile.



PROCESS FOR OBTAINING CAFFEOYLQUINIC ACIDS-RICH EXTRACTS FROM
HELIANTHUS ANNUUS**Field of invention**

The present invention relates to extracts of deoiled *Helianthus annuus* seeds which are useful for the prevention and treatment of dyslipidaemia, hyperglycaemia and hypertension, metabolic syndrome and type 2 diabetes.

5 The present invention also relates to the process for preparation of said extracts and compositions containing them. The extracts according to the invention significantly reduce the postprandial and baseline blood glucose levels, and the blood triglyceride levels in overweight or obese patients. When the extracts according to the invention, complexed with macromolecules, are
10 added to foods rich in starchy carbohydrates, their glycaemic index is reduced.

Prior art

Helianthus annuus extracts have been little used in traditional and allopathic medicine; however, *Helianthus annuus* seeds are widely used for the industrial production of oil, and the exhausted residue of the biomass is
15 mainly used as forage in animal feed or biogas production.

Helianthus annuus oil is an excellent seed oil characterised by an appreciable content of glycerides, which modulate the intestinal absorption of fats. When the seeds are intact, or deprived of their outer shell, they contain variable amounts of caffeoylquinic acids in the form of mono- and diesters of
20 quinic acid, of which chlorogenic acids form the preponderant part.

Description of the invention

It has now surprisingly been found that, thanks to the extraction process described below, it is possible to obtain extracts characterised by a high

content of caffeoylquinic acids, which possess potent hypoglycaemic activity on the postprandial and baseline blood glucose levels.

The present invention therefore relates to *Helianthus annuus* extracts, the process for their preparation, and compositions containing them.

5 The process according to the invention comprises:

a) extraction of industrial residues of *Helianthus annuus* with aqueous mixtures of aliphatic alcohols;

b) concentration under vacuum of the water-alcohol solution from step a) until complete elimination of the alcohol solvent, and filtration of any
10 insoluble matter and residual fatty phases;

c) adjustment of the pH of the aqueous solution from step b) to values around 4.5 ± 1 ;

d) ultrafiltration of the aqueous solution from step c) through a 400 Da organic membrane;

15 e) chromatography or nanofiltration of the solution from step d);

f) concentration of the retentate from step e) under vacuum or by atomisation.

In step a), "industrial residues of *Helianthus annuus*" means extracts of *Helianthus annuus* seeds obtained by hot extraction with hexane followed by
20 elimination of the solvent ("desolvation") at temperatures exceeding 100°C.

According to a preferred aspect of the invention, the extraction of step a) is performed with aqueous mixtures of ethanol/water, preferably 80% v/v, in the presence of organic or inorganic acids able to maintain a pH of less than 2, preferably dilute sulphuric acid, until the mono- and dicaffeoylquinic acids
25 are exhausted.

According to a preferred aspect of the invention, in step c), the pH of the aqueous solution is adjusted to values around 4.5 ± 1 with calcium carbonate.

The aqueous solution originating from step c) undergoes absorption resin chromatography using a polystyrene resin and/or an ion exchange and absorption resin or nanofiltration on ceramic membranes with a 400 to 600 Da cut-off, to remove salts and undesirable low-molecular-weight products. The retentate retains caffeoylquinic acids, while salts and sugars remain in the permeate.

The process of the invention is of particular industrial interest, as the availability of biomasses is substantially unlimited and available at negligible cost, with evident benefits to the economy of process and the final cost of the extract obtained.

The extracts obtained by the process of the invention are characterised by a high caffeoylquinic acid content, and exert a potent hypoglycaemic activity on the postprandial and baseline blood glucose levels. Said effect is also maintained if the product is added in suitable amounts to foods rich in carbohydrates, which is the major application of this novel extract in the dietary field.

Heat treatment used in desolvation together with acid treatment at the extraction step induces structural modifications that lead to improved biological activity of the extract in terms of its antioxidant and metabolic effect. The treatment cleaves bonds with protein structures, wherein caffeoylquinic acids, changing to the quinone form, bind to the SH groups of proteins with the Michael reaction or reactions with amino groups which often accompany the fate of polyphenols in plants.

The *Helianthus annuus* extract obtained by the process according to the invention preferably has a caffeoylquinic acid content ranging from 40 to 80%, preferably from 50 to 60%.

The extract of the invention can be advantageously formulated for human treatment as oils enriched with diglycerides, in the presence or absence

of phospholipids as surfactant carrier, or incorporated in foods such as bread, all types of biscuits, and foods in general which do not undergo aqueous washing at high temperature, because the active ingredients are freely water-soluble. In view of the latter aspect, the caffeoylquinic acids could be made
5 insoluble in water by forming complexes with vegetable or animal proteins which, when denatured by heat, incorporate them in a stable manner. The active products are released in the intestine by enzymatic hydrolysis of the protein, where they can interact with other substrates and modify the absorption of glucose, inhibiting the enzyme 6-phosphate synthetase.

10 It has been observed that the addition of the extract to a food rich in starchy carbohydrates significantly reduces the postprandial blood glucose level.

According to the present invention, the amount of extract to be administered as such in nutraceutical formulations generally ranges between
15 50 and 500 mg, preferably 250 mg, at each meal at which starchy carbohydrates are eaten.

The results of the clinical trial are set out below.

Postprandial blood glucose level

The subjects were given, under controlled clinical trial conditions, a
20 mixed Mediterranean meal containing 60% carbohydrates, 25% lipids and 15% proteins, together with 250 mg of the extract according to the invention. An 18% reduction in the postprandial blood glucose level was observed ($p \leq 0.05$) (12 volunteers vs. placebo).

Baseline blood glucose level

25 The trial subjects, who were healthy volunteers, were treated for one month with three capsules containing 250 mg of extract (at breakfast, lunch and dinner), which they took with a standard Mediterranean diet (see above), which was equal for the different subjects in the placebo-controlled crossover

study. At the end of the month's treatment, a 15% reduction in the baseline blood glucose level was observed (subjects with a borderline baseline blood glucose level of 110 ± 5).

Enhancement of postprandial and fasting hypoglycaemic activity makes
5 these extracts a useful modulator of the body weight and metabolic syndrome in all cases wherein an incorrect diet or dysmetabolism associated with age has created health problems.

A reduction in the blood triglyceride level was also observed In the treated patients. In separate clinical tests on subjects suffering from liver
10 disease with elevated transaminase values, the treatment reduced said parameters to normal, with an evident reduction in liver steatosis.

As already mentioned, under suitable conditions the extracts according to the invention can react rapidly with macromolecules, especially glycoproteins, which involves two advantages. Firstly, the extracts complexed
15 with macromolecules are protected against bacterial attack and oxidation and are released, after their enzymatic or bacterial demolition, in sites where they can perform their hypoglycaemic and antioxidant activity. Secondly, the extracts complexed with macromolecules can also be used in aqueous environments. In this way, they can be added to foods like pasta (which must
20 be cooked in water) without any appreciable loss of active ingredients.

The extracts of the invention can also be added to bread, pizza, rusks, biscuits, drinks and foods in general, including those based on proteins.

According to another preferred aspect, the extracts of the invention are formulated as conventional or gastroprotected capsules or tablets so as to
25 promote topical local activity, leaving the digestive function unchanged at stomach level. According to a preferred aspect, the formulations containing the extracts according to the invention will be supplemented with oils rich in diglycerides.

According to a further aspect, the compositions according to the invention can also contain other substances with a useful or complementary activity.

The compositions according to the invention are formulated by conventional methods, such as those described in “Remington’s Pharmaceutical Handbook”, Mack Publishing Co., N.Y., USA. In particular, the compositions according to the invention are formulated by conventional formulation techniques used for vegetable ingredients, which require particular care to be taken to avoid interactions with the excipients and the capsule matrices. Examples of oral formulations are tablets, dragées, soft and hard gelatin capsules, and cellulose capsules.

The examples set out below further illustrate the invention.

Example 1 - Preparation of *Helianthus annuus* extract by nanofiltration

10 Kg of deoiled *Helianthus annuus* seeds is pelleted and extracted with an 85% v/v mixture of ethanol/water containing a amount of H₂SO₄ sufficient to maintain the pH at 2.5, until the caffeoylquinic acid content is exhausted. Extraction is performed at a temperature of 40°C. The water-alcohol solution is concentrated to 10 L “until complete elimination of ethanol”, and products insoluble in water are then filtered. The aqueous solution is alkalinised to pH 5 and then subjected to ultrafiltration using a 10 KDa flat organic membrane. The perfectly clear solution containing all the caffeoylquinic acids, flavonoids and other polyphenols in small amounts then undergoes nanofiltration through a ceramic membrane with a 400 Da cut-off. The caffeoylquinic acids are concentrated in the retentate, while the permeate, which contains salts, sugars and undesirable low-molecular-weight products, is discarded. The retentate is concentrated to a dry residue of 10% and atomised. 1.2 kg of a pale beige extract is obtained, which has a

caffeoylquinic acid content of 56%, measured by HPLC, and a chlorogenic acid content of 32%. This extract is used to prepare capsules or tablets, or can be added to various foods in suitable doses.

Example 2 - Preparation of *Helianthus annuus* extract by chromatography

50 Kg of deoiled *Helianthus annuus* seeds is pelleted and extracted with an 85% v/v mixture of ethanol/water containing a amount of H₂SO₄ sufficient to maintain the pH at 2.5, until the caffeoylquinic acid content is exhausted. Extraction is performed at a temperature of 40°C. The residual biomass is discarded, and the water-alcohol solution is concentrated until the ethanol is eliminated. The aqueous solution is concentrated to 10 L, and the water-insoluble products are filtered. The aqueous solution is alkalinised to pH 5 and subjected to ultrafiltration through an organic membrane with a 10 KDa cut-off. The clear aqueous concentrate is absorbed on 50 L of a polystyrene absorbing resin from which the active extract is subsequently recovered by elution of the resin with 90% ethanol/water.

After concentration until dry, about 4 kg of extract containing 56% caffeoylquinic acids, expressed as chlorogenic acids, is obtained.

Example 3 - Cellulose capsules

Type 0 cellulose capsules are filled with the following ingredients:

Unit composition:

<i>Helianthus annuus</i> extract	250 mg
Soya lecithin	10 mg
Sunflower oil	q.s. for 700 mg

Example 4 - Tablets

Unit composition:

<i>Helianthus annuus</i> extract	200 mg
Microcrystalline cellulose	300 mg
Lactose	190 mg
Silicon dioxide	5 mg
Magnesium stearate	5 mg

Example 5 - Food preparation (pizza)

- 5 About 200 g of flour is mixed with 10 g of brewer's yeast, salt, oil and 50 ml of water. The ingredients are kneaded, 500 mg of *Helianthus annuus* extract is added, and the dough is left to stand for 2 h. The dough is then rolled out, cheese and other desired ingredients added, and the pizza is cooked in a hot oven at 200°C until ready. The glycaemic index of this pizza was
- 10 compared with that of a pizza prepared with the same ingredients but without the addition of *Helianthus annuus* extract, and the glycaemic index was 15% lower.

CLAIMS

1. A process for the preparation of extracts of *Helianthus annuus*, which comprises:

- 5 a) extracting with aqueous mixtures of aliphatic alcohols the *Helianthus annuus* seeds obtained by extraction with hexane followed by elimination of the solvent at temperatures above 100°C;
- b) concentrating the water-alcohol solution from step a) under vacuum to complete elimination of the alcohol solvent, and filtering any
- 10 residual insolubles and fatty phases;
- c) adjusting the pH of the aqueous solution from step b) to pH 5;
- d) subjecting the aqueous solution from step c) to ultrafiltration on 10 kDa organic membranes;
- e) subjecting the solution from step d) to chromatography or
- 15 nanofiltration;
- f) concentrating the retentate from step e) under vacuum or by atomisation.

2. The process of claim 1, wherein in step a) the extraction is carried out with ethanol/water mixtures, in the presence of organic or inorganic acids

20 capable of maintaining a pH below 2.

3. The process of claim 2, wherein in step a) the extraction is carried out with 80% v/v ethanol/water mixtures in the presence of dilute sulphuric acid.

4. The process of claim 1, wherein in step c) the pH of the aqueous solution is adjusted to values around 5 using calcium carbonate.

25 5. The process of claim 1, wherein in step e) the solution is subjected to chromatography on absorption resin using a polystyrene resin and/or ion exchange and absorption resin.

6. The process of claim 1, wherein in step e) the solution is subjected to

nanofiltration using a ceramic membrane with cut-off from 400 to 600 Da.

7. Extracts of *Helianthus annuus* obtained with the process of claims 1-6.

8. The extracts of *Helianthus annuus* of claim 7, having a caffeoylquinic acid content ranging from 40 to 80%, preferably from 50 to 60%.

5 9. The extracts of *Helianthus annuus* of claims 7 or 8, complexed with vegetable or animal proteins.

10. Formulations comprising the extracts of *Helianthus annuus* of claims 7-9.

11. The formulations of claim 10 containing 50 to 500 mg of extracts of
10 *Helianthus annuus*.

12. The formulations of claims 9-11, also containing oils enriched with diglycerides and optionally surfactants.

13. The formulations of claims 10-12 in the form of conventional or gastro-protected capsules or tablets.

15 14. Foods based on carbohydrates containing the extracts of claims 7-9.

INTERNATIONAL SEARCH REPORT

International application No

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A. CLASSIFICATION OF SUBJECT MATTER

INV. A23L1/30 A61K36/28 A61P3/10
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A23L A61K

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	----- -/--	1-6



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

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INTERNATIONAL SEARCH REPORT

International application No

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
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INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2013/070928

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

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权利要求书1页 说明书4页

(54) 发明名称

由向日葵获得富含咖啡酰奎宁酸的提取物的方法

(57) 摘要

本发明涉及用于预防和治疗血糖异常、高血糖和高血压、代谢综合征和2型糖尿病的去油向日葵 (*helianthus annuus*) 种子提取物。本发明还涉及制备所述提取物的方法及含有该提取物的组合物。当将本发明的提取物加至以碳水化合物为基本成分的食物中时,该提取物可以降低血糖指数和餐后葡萄糖的吸收,并引起脂质特征发生改变。

1. 制备向日葵提取物的方法, 该方法包括:
 - a) 用脂肪醇水混合物提取通过己烷提取得到的向日葵种子, 然后在高于 100℃ 的温度下除去溶剂;
 - b) 真空浓缩得自步骤 a) 的水-醇溶液直至完全除去醇溶剂, 并且滤除残留的不溶物和脂相;
 - c) 将得自步骤 b) 的水溶液的 pH 调节至 5;
 - d) 用 10kDa 的有机膜对得自步骤 c) 的水溶液进行超滤;
 - e) 对得自步骤 d) 的溶液进行色谱或纳滤;
 - f) 真空或通过雾化浓缩得自步骤 e) 的截留物。
2. 权利要求 1 的方法, 其中在步骤 a) 中, 提取是在能够将 pH 保持在 2 以下的有机酸或无机酸存在下采用乙醇 / 水混合物进行的。
3. 权利要求 2 的方法, 其中在步骤 a) 中, 提取是在稀硫酸存在下采用 80% v/v 乙醇 / 水混合物进行的。
4. 权利要求 1 的方法, 其中在步骤 c) 中, 使用碳酸钙调节水溶液的 pH 至大约 5。
5. 权利要求 1 的方法, 其中在步骤 e) 中, 使用聚苯乙烯树脂和 / 或离子交换和吸附树脂在吸附树脂上对溶液进行色谱。
6. 权利要求 1 的方法, 其中在步骤 e) 中, 使用截留值为 400 — 600Da 的陶瓷膜对溶液进行纳滤。
7. 采用权利要求 1 — 6 的方法得到的向日葵提取物。
8. 权利要求 7 的向日葵提取物, 所述提取物含有 40% — 80%、优选 50 — 60% 的咖啡酰奎宁酸。
9. 权利要求 7 或 8 的向日葵提取物, 该提取物与植物和动物蛋白复合。
10. 制剂, 该制剂含有权利要求 7 — 9 的向日葵提取物。
11. 权利要求 10 制剂, 所述制剂含有 50 — 500mg 向日葵提取物。
12. 权利要求 9 — 11 的制剂, 所述制剂还包含富含甘油二酯的油和任选的表面活性剂。
13. 权利要求 10 — 12 的制剂, 所述制剂为常规形式的或肠溶胶囊或片剂。
14. 基本成分为碳水化合物的食物, 该食物含有权利要求 7 — 9 的提取物。

由向日葵获得富含咖啡酰奎宁酸的提取物的方法

技术领域

[0001] 本发明涉及用于预防和治疗血脂异常、高血糖和高血压、代谢综合征和 2 型糖尿病的去油向日葵 (*helianthus annuus*) 的种子提取物。本发明还涉及制备所述提取物的方法及含有所述提取物的组合物。本发明的提取物可以显著降低超重或肥胖患者的餐后和基线血糖水平以及血液甘油三酯水平。当本发明的提取物与大分子复合,并加至富含淀粉性碳水化合物的食物中后,可以降低其血糖指数。

背景技术

[0002] 向日葵提取物极少用于中医和对抗疗法中;但是,向日葵种子却被广泛用于油的工业化生产中,生物质耗尽的残渣主要被用作动物饲料或用于生产沼气。

[0003] 向日葵油是一种极佳的种子油,特征在于甘油酯含量非常可观,它可以调节脂肪的肠吸收。当种子为完整的或剥离了其外壳后,它们包含了变量的以奎宁酸的单和二酯形式存在的咖啡酰奎宁酸,其中绿原酸占优势。

发明内容

[0004] 现已惊奇地发现,通过以下所述的提取方法,可以得到咖啡酰奎宁酸含量较高的提取物,该提取物具有有效降低餐后血糖和基线血糖水平的活性。

[0005] 因此,本发明涉及向日葵提取物、其制备方法及含有该提取物的组合物。

[0006] 根据本发明的方法包括:

[0007] a) 用脂肪醇水混合物对向日葵的工业残渣进行提取;

[0008] b) 真空浓缩得自步骤 a) 的水醇溶液至乙醇溶剂完全除去,然后滤除不溶物和残余的脂相;

[0009] c) 将得自步骤 b) 的水溶液的 pH 值调节至约 4.5 ± 1 ;

[0010] d) 通过 400Da 的有机膜对得自步骤 c) 的水溶液进行超滤;

[0011] e) 对得自步骤 d) 的溶液进行色谱或纳滤 (nanofiltration);

[0012] f) 真空或通过雾化浓缩得自步骤 e) 的截留物。

[0013] 在步骤 a) 中,“向日葵的工业残渣”指用己烷进行热提取、随后在 100℃ 以上的温度下去除溶剂 (“去溶剂化”) 后得到的向日葵种子提取物。

[0014] 根据本发明的一个优选方面,步骤 a) 的提取过程在能够将 pH 值保持在 2 以下的有机酸或无机酸 (优选稀硫酸) 存在下用乙醇 / 水混合物进行至单 - 和二 - 咖啡酰奎宁酸耗尽,优选 80% v/v 的乙醇 / 水混合物。

[0015] 根据本发明的一个优选方面,在步骤 c) 中,水溶液的 pH 值采用碳酸钙调节至约 4.5 ± 1 。

[0016] 得自步骤 c) 的水溶液,在使用聚苯乙烯树脂和 / 或离子交换和吸附树脂上进行吸附树脂色谱,或者采用截留值为 400 ~ 600Da 的陶瓷膜进行纳滤,以除去盐和不需要的低分子量产物。截留物中保留咖啡酰奎宁酸,而盐和糖则保留在渗透物中。

[0017] 本发明的制备方法具有特别的工业意义,因为生物质实质上是可以无限获得的而且其成本可以忽略不计,所以从方法的经济学而言具有显著的意义并可显著降低获得提取物的最终成本。

[0018] 通过本发明方法得到的提取物的特征在于该提取物具有高含量的咖啡酰奎宁酸,对餐后和基线血糖水平显示有效的降糖活性。如果将适量的本产品加至富含碳水化合物的食物中,所述作用仍可被保持,此为这种新提取物在饮食领域中的主要应用。

[0019] 去溶剂化中使用的热处理方法和提取步骤中的酸处理可以使结构得以修饰,由此使提取物的生物学活性在抗氧化和促新陈代谢效应方面得到提升。处理方法裂解了蛋白结构之间的化学键,转变为醌形式的咖啡酰奎宁酸在 Michael 反应中与蛋白质的 SH 基团结合或与常常存在于植物多酚中的氨基反应。

[0020] 优选按照本发明的方法获得的向日葵提取物中的咖啡酰奎宁酸含量范围为 40%—80%,优选 50%—60%。

[0021] 本发明的提取物可被方便地配制为富含甘油二酯的油用于人类疾病的治疗,制剂可以含有或不含有磷脂作为表面活性剂载体,或者将该提取物加至各种食物中,例如面包、各种类型的饼干和一般不能耐受高温水洗的食物,这是由于活性成分可自由地溶于水。有鉴于此,咖啡酰奎宁酸可以通过与植物或动物蛋白质形成复合物变得不溶于水,这些蛋白通过热变性后可以稳定形式掺入。蛋白质被酶水解后在肠中释放出活性产物,该活性产物可以在肠内与其他底物相互作用并改善糖的吸收,抑制 6 磷酸合成酶。

[0022] 已经观察到,当将提取物添加至富含淀粉性碳水化合物的食物中时,该提取物可以显著降低餐后血糖水平。

[0023] 根据本发明,提取物以营养制剂的形式与含有淀粉性碳水化合物一起每餐服用时的剂量范围通常为 50—500mg,优选 250mg。

[0024] 临床试验结果如下。

[0025] 餐后血糖水平

[0026] 在有对照的临床试验条件下,给予实验对象混合的地中海食物 (Mediterranean meal),该食物含有 60%碳水化合物、25%脂质和 15%蛋白质以及 250mg 本发明的提取物。可以观察到餐后血糖降低了 18% ($p \leq 0.05$) (12 名志愿者,安慰剂)。

[0027] 基线血糖

[0028] 实验对象为健康志愿者,于早、中、晚餐时服用三颗含有 250mg 提取物的胶囊一个月,同时服用标准地中海食物 (见上文),这和安慰剂对照交叉实验组的不同实验对象的饮食是一样的。在一个月治疗结束后,可以观察到实验对象的基线血糖水平降低了 15% (实验对象的基线血糖边界线为 110 ± 5)。

[0029] 可以增强餐后和空腹降糖活性使这些提取物成为体重和代谢综合征的有效调节剂,在这些情况中,由年龄引起的饮食不当或代谢失调已经产生了健康问题。

[0030] 还可以观察到接受治疗的患者血中甘油三酯水平的降低。在另一组由于转氨酶值升高而遭受肝病折磨的患者的临床实验中,治疗后所述参数恢复到正常,肝脂肪变性有明显降低。

[0031] 如前文所述,在适当的条件下,本发明的提取物可以与大分子、特别是糖蛋白快速发生反应,这具有两方面的优势。首先,与大分子复合的提取物可以免受细菌攻击并且不被

氧化,在被酶或细菌解离后,可以在原位释放以发挥它们的降血糖和抗氧化活性。其次,与大分子复合的提取物也可在含水环境中使用。这样,可以将它们添加至例如意大利面(必须在水中烹饪)的食物中而活性成分不会显著丢失。

[0032] 还可以将本发明的提取物添加至面包、比萨、甜面包干、饼干、常规饮料和食物中,包括基本成分为蛋白质的那些。

[0033] 根据另一个优选方面,本发明的提取物可配制为常规的或肠溶(gastroprotected)胶囊或片剂以提高其局部活性,同时不改变胃的消化功能。根据另一优选的方面,可以向含有本发明的提取物的制剂中加入富含甘油二酯的油。

[0034] 根据再一方面,本发明的组合物也可以包含其它具有有用或互补活性的物质。

[0035] 本发明的组合物可以通过常规方法配制,如“Remington's Pharmaceutical Handbook(雷明顿药物手册)”,Mack Publishing Co., N.Y., USA 所述。具体而言,本发明的组合物通过用于植物活性成分的常规制剂技术配制,需要特别注意的是,避免与赋形剂和胶囊基质之间发生反应。口服制剂的实例为片剂、糖衣药丸、软和硬明胶胶囊以及纤维素胶囊。

[0036] 以下实施例进一步说明本发明。

[0037] 实施例 1:通过纳滤制备向日葵种子提取物

[0038] 将 10Kg 去油的向日葵种子制成小粒(pelleted),并用 85% v/v 乙醇/水混合物萃取,直至咖啡酰奎宁酸耗尽,所述混合物含有足量的硫酸以将 pH 保持为 2.5。提取在 40℃ 温度下进行。浓缩所述水-乙醇溶液至 10 升“直到完全除去乙醇”,接着滤除不溶于水的产物。碱化水溶液至 pH 为 5,接着使用 10KDa 的平有机膜对其进行超滤。含有咖啡酰奎宁酸、黄酮类和少量多酚的完全透明的溶液通过截留值为 400Da 的陶瓷膜进行纳滤。浓缩截留物中的咖啡酰奎宁酸,同时丢弃含有盐、糖和不需要的低分子产物的渗透物。浓缩截留物至 10% 的干残渣并雾化。获得 1.2kg 淡米色提取物,其中包含使用 HPLC 测得的含量为 56% 的咖啡酰奎宁酸和 32% 的绿原酸。将提取物用于制备胶囊或片剂,或者适量加至各种食物中。

[0039] 实施例 2:通过色谱法制备向日葵提取物

[0040] 将 50Kg 去油的向日葵种子制成小粒,并用 85% v/v 乙醇/水混合物提取,直至咖啡酰奎宁酸被耗尽,所述混合物含有足量的硫酸以将 pH 保持为 2.5。萃取在 40℃ 温度下进行。丢弃残余的生物物质,浓缩水-乙醇溶液直至除去乙醇。浓缩水溶液至 10L,滤去不溶于水的产物。碱化水溶液至 pH 为 5,之后通过截断值为 10KDa 的有机膜对其进行超滤。将清澈的水浓缩液吸附于 50L 的聚苯乙烯吸附树脂中,随后通过用 90% 乙醇/水洗脱树脂回收活性提取物。

[0041] 浓缩至干燥后,可获得大约 4kg 包含 56% 以绿原酸形式表示的咖啡酰奎宁酸的提取物。

[0042] 实施例 3:纤维素胶囊

[0043] 将下列成分填充至 0 型纤维素胶囊中:

[0044] 单位组成:

[0045]

向日葵提取物	250mg
大豆卵磷脂	10mg
葵花油	适量至 700mg

[0046] 实施例 4 :片剂

[0047] 单位组成 :

[0048]

向日葵提取物	200mg
微晶纤维素	300mg
乳糖	190mg
二氧化硅	5mg
硬脂酸镁	5mg

[0049] 实施例 5 :食物制备 (比萨)

[0050] 将大约 200g 面粉与 10g 啤酒酵母、盐、油和 50ml 水混合。将各成分捏合,加入 500mg 向日葵提取物,之后将面团放置 2 小时。然后铺开面团,加入起司和其它所需成分,在 200℃ 热烤箱中烘烤比萨直至妥当。将加入了向日葵提取物的比萨的血糖指数与含有相同成分但是不含向日葵提取物的比萨的血糖指数进行比较,发现血糖指数降低 15%。

Abstract

The present invention relates to extracts of deoiled *Helianthus annuus* seeds which are useful for the prevention and treatment of dyslipidaemia, hyperglycaemia and hypertension, metabolic syndrome and type 2 diabetes. The present invention also relates to the process for preparation of said extracts and compositions containing them. The extracts according to the invention, when added to carbohydrate-based foods, reduce the glycaemic index and postprandial absorption of glucose, and induce a modification of the lipid profile.