(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization

International Bureau





(10) International Publication Number WO 2016/207820 A1

(43) International Publication Date 29 December 2016 (29.12.2016)

(51) International Patent Classification: C12C 5/00 (2006.01) C12C 12/00 (2006.01) C12C 7/20 (2006.01)

(21) International Application Number:

PCT/IB2016/053730

(22) International Filing Date:

23 June 2016 (23.06.2016)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data: PV 2015-422

23 June 2015 (23.06.2015)

CZ

- (71) Applicants: MENDELOVA UNIVERZITA V BRNE, FAKULTA, ZAHRADNICKA **USTAV** LIZNOVE **TECHNOLOGIE** ZAHRADNICKYCH PRODUKTU [CZ/CZ]; Valticka 337, 69144 Lednice (CZ). VYZKUMNY USTAV POTRAVINARSKY PRAHA, V.V.I. [CZ/CZ]; Radiova 7, 10231 Praha 10 (CZ). CENTRUM VYZKUMU GLOBALNI ZMENY AV CR, V.V.I. [CZ/CZ]; Belidla 986/4a, 60300 Brno (CZ).
- (72) Inventors: HIC, Pavel; Devicany 217, Devicany (SK). BALIK, Josef; Sidliste osvobozeni 760, Lednice (CZ). KULICHOVA, Jana; M. Horakove 396, Trebic (CZ). SNURKOVIC, Petr; Smetanova 1183, Straznice (CZ). HOUSKA, Milan; Hradecka 7, Praha 3 (CZ). STRO-HALM, Jan; Slezska 87, Praha 3 (CZ). LANDFELD, Ales; Nesmen - Zasmuky 39, Nesmen - Zasmuky (CZ). TRISKA, Jan; Karla Stecha 14, Ceske Budejovice (CZ).

VRCHOTOVA, Nadezda; Netolicka 6, Ceske Budejovice (CZ).

- (74) Agent: NOVOTNY, Jaroslav; Rimska 45/2135, 12000 Praha 2 (CZ).
- (81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.
- Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))



Beer and beer-based beverage containing lignans, and method of their production

Technical field

The invention relates to the beer and beer-based beverage containing lignans, and the method of their production.

Beckground Arts

In economically highly developed countries, tumour and cardio-vascular diseases represent a significant cause of mortality, which stems from several factors. Primarily, these include a bad life style and unbalanced nutrition. Foodstuffs frequently contain only a small amount of health-beneficial substances, leading to failure to obtain the recommended daily amount of such matter from the accepted diet. Therefore, possibilities of increasing the content of heath-beneficial substances in foodstuffs have been sought.

Beer is one of the most consumed beverages in the Czech Republic. It is easy to digest and supports the appetite. Beer is produced by the fermentation of wort prepared from malt, water and hops. Beer-based beverages include beverages from unhopped wort using a technological process similar to the production of beer. These beverages are frequently mixed with another kind of soft beverage, fruit juice, herbal extract, etc. At present, there are attempts to add health-beneficial substances, and thus to increase their positive effects on the human organism. European patent No. 2369947 protects the addition of polyphenols and colloidal silicon to beer and beer-based beverages. Utility model No. 18928 protects the beer with an increased content of rutin.

Another group of health-beneficial substances involves lignans. As secondary metabolites of vascular plants they are distinguished by antioxidant, antitumour, antiviral, antibacterial, insecticide, fungicide, estrogenic, antiestrogenic effects and, last but not least, by effects protecting against cardiovascular diseases. Linseed is a significant source of lignans containing up to 3.7 g/kg of secoisolariciresinol diglucoside. However, it is consumed minimally in usual nutrition. Other sources of lignans are: soya, rice, cereals, nuts and fruits. But the content of lignans in the aforesaid foodstuffs is as much as 1,000 times lower than in linseed; therefore they represent only a minimum source of lignans. A high content of lignans has been found in knots of coniferous trees that contain 5 to 10 % of lignans by weight. Knots of the European spruce (*Picea abies*) contain 6 to 29 % of lignans by weight, mostly hydroxymatairesinol (HMR) and alpha-conidendrin (CONI). Invention disclosure PV 2014 -

870 describes a suitable procedure as to how to obtain lignan extract from this natural source. The extracts of lignans obtained in this way can be added to foodstuffs and beverages, resulting in their increased content of health-beneficial substances. However, these additions can cause both foodstuffs and beverages to have a slightly bitter taste and resin aroma. The issue of lignans is dealt with in the following documents: Slanina, J. Biologická a farmakologická aktivita lignanů. *Chemické Listy*, 94, 2000, 111-116. Harmatha, J. Strukturní bohatství a biologický význam lignanů a jim příbuzných rostlinných fenylpropanoidů. *Chemické listy*, 99, 2005, 622-632. Manach, C. et al. Polyphenols: Food sources and bioavailability. *The American Journal of Clinical Nutrition*, 79, 2004, 727-747. Holmbom B. et al. Knots in trees – A new rich source of lignans. *Phytochemistry Reviews*, 2, 2003, 331-340.

PCT/IB2016/053730

Disclosure of Invention

The aforesaid shortcomings are eliminated by beer and beer-based beverages containing lignans, and the method of their production according to this invention, the principle of which lies in the fact that beer and beer-based beverages contain 1 to 200 mg of lignans per litre of beverage and 0.01 to 1.0 g of granulated hops per litre of beverage. The method of production of beer and beer-based beverages consists in adding pre-treated alcohol extract from knots of the European spruce as a source of lignans to the basic ingredients of both beer and the beverage. The method of production of beer and beer-based beverages consists in adding pretreated aqueous extract from knots of the European spruce as a source of lignans to the basic ingredients of beer and the beverage. The method of production of beer and beer-based beverages consists in adding chips made from knots of the European spruce as a source of lignans to the basic ingredients of the beverage. The method of production of beer and beer-based beverages consists in adding either aqueous or alcohol extract from the European spruce at any production stage, but preferably at the initial stages of wort boiling, to ensure that the effect on the beer aroma and flavour will be minimal. The method of production of beer and beer-based beverages consists in adding chips made from knots of the European spruce at any production stage, but preferably at the final stages of wort boiling, to ensure that the effect on the beer aroma and flavour will be high.

For beer and beer-based beverages where an attempt is made to achieve minimal changes in product taste, it is appropriate to use aqueous or alcohol extract from chips of the European spruce as a source of lignans to be added at the initial stage of wort boiling. It is appropriate to add a reduced amount of hops at the end of wort boiling to develop the expected beer aroma.

PCT/IB2016/053730

For beer and beer-based beverages where an attempt is made to achieve the maximum changes in product taste and simultaneously a fine resin aroma, it is appropriate to use chips made from knots of the European spruce as a source of lignans to be added at the final stage of wort boiling.

An advantage of the technical solution to this invention is that the additions of aqueous or alcohol extracts of lignans, or of chips from the European spruce, as appropriate, can reduce their bitterish taste, or even to replace in full the amount of hops added in the course of wort boiling. It has been proved that lignans have many positive effects on human health, and the production of such beverages will result in increasing their content of these bioactive components significantly. This will also extend the range of beverages having health benefits. The bitterish taste and resign aroma of lignans eliminate the need of beer hopping. The beer and beer-based beverages feature a specific bitterish taste due to the production process and, therefore, are appropriate basic carriers of lignans extracted from knots of the European spruce.

Made for Carrying out the Invention

The listed examples only document, but do not limit the production potential in any manner whatsoever.

1. For brewing 20 litres of bottom fermented beer, 24 litres of unhopped wort containing 10 % extract by weight obtained by traditional mashing were used. During wort boiling, i.e. after 15 minutes of boiling, 10.6 ml of alcohol extract of lignans made from knots of the European spruce, with HMR and CONI concentrations of 172.78 mg/ml and 16.3 mg/m, respectively, are added. This corresponds to a total lignans concentration of 100 mg/l in the finished beverage. Then boiling takes place for 75 minutes. Subsequently, the hopped wort is cooled down and water is added to a value of 12 % extract. Then it is fermented using bottom fermentation brewery yeast. After five days of fermentation at a temperature of 13 °C, the beer is discharged to 20 l stainless steel barrels and allowed to mature at a temperature of 8 °C for 15 days. Subsequently analyses of the HMR and CONI content are made. The following values have been measured: HMR 73.1 mg and CONI 10.2 mg in one litre of beer.

- 2. For brewing 20 litres of bottom fermented beer, 24 litres of unhopped wort containing 10 % extract by weight obtained by traditional mashing were used. During wort boiling, i.e. after 15 minutes of boiling, 40 g of chips made from knots of the European spruce are added. This corresponds to a total lignans concentration of 100 mg/l in the finished beverage. Then boiling takes place for 75 minutes. Subsequently the hopped wort is cooled down and water is added to a value of 12 % extract. Then it is fermented using bottom fermentation brewery yeast. After five days of fermentation at a temperature of 13 °C, the beer is discharged to 20 l stainless steel barrels and allowed to mature at a temperature of 8 °C for 15 days. Subsequently, analyses of the HMR and CONI content are made. The following values have been measured: HMR 86.3 mg and CONI 9.74 mg in one litre of beer.
- 3. For brewing 20 litres of bottom fermented beer, 24 litres of unhopped wort containing 10 % extract by weight obtained by traditional mashing were used. During wort boiling, i.e. after 10 minutes, 5.3 ml of alcohol extract of lignans made from knots of the European spruce, with HMR and CONI concentrations of 172.78 mg/ml and 16.3 mg/m, respectively, are added. This corresponds to a total lignans concentration of 50 mg/l in the finished beverage. Then boiling takes place for 65 minutes. Subsequently 20 g of granulated hops with an alpha bitter acids concentration of 3.7 % are added to the hopped wort. The hopped wort is then boiled for another 15 minutes; thereafter, it is cooled down and water is added to a value of 12 % extract. Then bottom fermentation yeast is added. After five days of fermentation at a temperature of 13 °C, the beer is discharged to 20 l stainless steel barrels and allowed to mature at a temperature of 8 °C for 15 days. Subsequently, analyses of the HMR and CONI content are made. The following values have been measured: HMR 31.45 mg and CONI 5.06 mg in one litre of beer.
- 4. For brewing 20 litres of bottom fermented beer, 24 litres of unhopped wort containing 10 % extract by weight obtained by traditional mashing were used. During wort boiling, i.e. after 10 minutes of boiling, 20 g of chips made from knots of the European spruce are added. This corresponds to the total concentration of lignans in the finished beverage equalling 50 mg/l. Then, boiling follows for 65 minutes. Subsequently, 20 g granulated hops with a concentration of alpha bitter acids of 3.7 % are added to the hopped wort. The hopped wort is then boiled for another 15 minutes; thereafter, it is cooled down and water is added to a valued of 12 % extract. Then bottom fermentation yeast is added. After five days of fermentation at a temperature of 13 °C, the beer is discharged to 20 l stainless

WO 2016/207820 PCT/IB2016/053730 5

steel barrels and allowed to mature at a temperature of 8 °C for 15 days. Subsequently, analyses of the HMR and CONI content are made. The following values have been measured: HMR 31.45 mg and CONI 5.06 mg in one litre of beer.

5. For brewing 20 litres of bottom fermented beer, 24 litres of unhopped wort containing 10 % extract by weight obtained by traditional mashing were used. During wort boiling, i.e. after 10 minutes of boiling, 10 ml of aqueous extract from chips from the European spruce knots are added. This corresponds to a total lignans concentration of 50 mg/l in the finished beverage. Then boiling takes place for 65 minutes. Subsequently, 20 g of granulated hops with an alpha bitter acids concentration of 3.7 % is added to the hopped wort. The hopped wort is then boiled for another 15 minutes; thereafter, it is cooled down and water is added a value of 12 % extract. Then bottom fermentation yeast is added. After five days of fermentation at a temperature of 13 °C, the beer is discharged to 20 l stainless steel barrels and allowed to mature at a temperature of 8 °C for 15 days. Subsequently, analyses of the HMR and CONI content are made. The following values have been measured: HMR 33.21 mg and CONI 4.24 mg in one litre of beer.

Industrial Applicability

Beer and beer-based beverages with an increased content of natural lignans extend the present range of beverages. They also help increase the intake of health-beneficial substances, and thus positively influence the health condition of the population.

PATENT CLAIMS

6

- 1. Beer and beer-based beverages containing lignans, and the method of their production c h a r a c t e r i z e d by the fact that beer and beer-based beverages contain 1 to 200 mg of lignans per litre of beverage.
- 2. Beer and beer-based beverages according to Claim 1 c h a r a c t e r i z e d by containing also 0.01 to 1.0 g of granulated hops per litre of beverage.
- 3. The method of production of beer and beer-based beverages c h a r a c t e r i z e d by adding pre-treated alcohol extract from knots of the European spruce as a source of lignans to the basic ingredients of both the beer and beverage.
- 4. The method of production of beer and beer-based beverages c h a r a c t e r i z e d by adding pre-treated aqueous extract from knots of the European spruce as a source of lignans to the basic ingredients of both beer and beverage.
- 5. The method of production of beer and beer-based beverages c h a r a c t e r i z e d by adding chips from knots of the European spruce as a source of lignans to the basic ingredients of the beverage.
- 6. The method of production of beer and beer-based beverages according to Claims 2 and 3 c h a r a c t e r i z e d by adding either aqueous or alcohol extract made from the European spruce at any production stage, but preferably at the initial stages of wort boiling, to ensure that the effect on the beer aroma and flavour is minimal.
- 7. The method of production of beer and beer-based beverages according to Claim 5 c h a r a c t e r i z e d by adding chips made from knots of the European spruce at any production stage, but preferably at the final stages of wort boiling, to ensure that the effect on the beer aroma and flavour is high.

INTERNATIONAL SEARCH REPORT

International application No PCT/IB2016/053730

A. CLASSIFICATION OF SUBJECT MATTER INV. C12C5/00 C12C7/20 ADD.

C12C12/00

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) C12C

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

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GB 14515 A A.D. 1908 (KUMMLE ALBERT [DE]) 3 September 1908 (1908-09-03) the whole document	1-7
X	David Ackley: "Homebrew Spruce Beer Recipe", 11 October 2011 (2011-10-11), XP002761231, Retrieved from the Internet: URL:http://www.eckraus.com/blog/homebrew-spruce-beer-recipe [retrieved on 2016-08-30] the whole document	1-7

* 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		
"A" document defining the general state of the art which is not considered to be of particular relevance	the principle or theory underlying the invention		
"E" earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive		
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other	step when the document is taken alone		
special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is		
"O" document referring to an oral disclosure, use, exhibition or other means	combined with one or more other such documents, such combin being obvious to a person skilled in the art		
"P" document published prior to the international filing date but later than the priority date claimed	"&" document member of the same patent family		
Date of the actual completion of the international search	Date of mailing of the international search report		
7 October 2016	18/10/2016		
Name and mailing address of the ISA/	Authorized officer		
European Patent Office, P.B. 5818 Patentlaan 2			
NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040,			
Fax: (+31-70) 340-2040,	Diller, Reinhard		

INTERNATIONAL SEARCH REPORT

International application No PCT/IB2016/053730

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT						
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.				
X	Anonymous: "Spruce Beer, or, A Beer to Ward Off Scurvy", Small Beer Press	1-7				
	, 14 September 2009 (2009-09-14), XP002761232, Retrieved from the Internet: URL:http://smallbeerpress.com/not-a-journa 1/2009/09/14/spruce-beer/ [retrieved on 2016-08-30] the whole document					
Α	WO 2009/155887 A1 (AGRA GROUP AS [CZ]; STUCHLIK MILAN [CZ]; KOPENEC JIRI [CZ]) 30 December 2009 (2009-12-30) the whole document	1-7				
Α	IVON E J MILDER ET AL: "Lignan contents of Dutch plant foods: a database including lariciresinol, pinoresinol, secoisolariciresinol and matairesinol", BRITISH JOURNAL OF NUTRITION, vol. 93, no. 3, 1 March 2005 (2005-03-01), pages 393-402, XP002667326, CAMBRIDGE UNIV. PRESS, UK ISSN: 0007-1145, DOI: 10.1079/BJN20051371 [retrieved on 2007-03-08] the whole document	1-7				

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2016/053730

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB 190814515 A	03-09-1908	NONE	
WO 2009155887 A	30-12-2009	AU 2009264469 A1 BR PI0914180 A2 CA 2727197 A1 CN 102066364 A CZ 300846 B6 EA 201100091 A1 EP 2307405 A1 JP 2011525498 A UA 103490 C2 US 2011207805 A1 WO 2009155887 A1 ZA 201100265 B	30-12-2009 20-10-2015 30-12-2009 18-05-2011 26-08-2009 30-06-2011 13-04-2011 22-09-2011 25-10-2013 25-08-2011 30-12-2009 26-10-2011