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(71) Applicants: **UNIWERSYTET PRZYRODNICZY W POZNANIU** [PL/PL]; ul. Wojska Polskiego 28, PL-60-637 Poznań (PL). **UNIWERSYTET MEDYCZNY W POZNANIU** [PL/PL]; ul.Fredry 10, PL-61-701 Poznań (PL).

(72) Inventors: **LEWANDOWICZ, Grażyna**; ul. Lecha 42, 117, PL-61-294 Poznań (PL). **KOWALCZEWSKI, Przemysław**; ul. Libelta 20c, 5, PL-62-100 Wągrowiec (PL). **OLEJNIK, Anna**; ul. Starowiejska 1g, 11, PL-61-644 Poznań (PL). **JODYNIS-LIEBERT, Jadwiga**; ul. Sosnowa 137, PL-62-002 Złotniki (PL). **KUJAWSKA, Małgorzata**; ul. Pobiedziska 14, 19, PL-61-049 Poznań (PL). **LESIECKI, Mariusz**; Góra 29, PL-98-290 Warta (PL).

(74) Agent: **RUMPEL, Alicja**; 29, lok. 11, al. Śmigłego-Rydza, PL-93-281 Łódź (PL).

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(54) Title: THE METHOD OF SUBSTANCE MANUFACTURING FROM POTATO'S JUICE AND ITS APPLICATION

Fig. 1

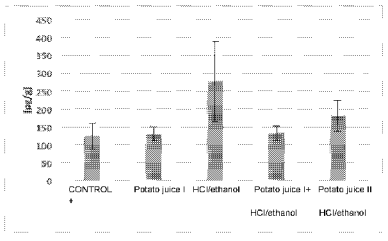
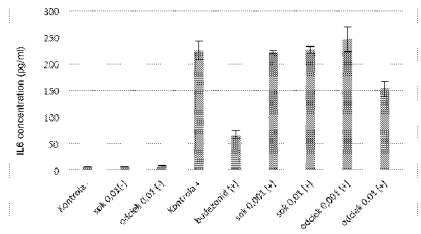


Fig. 2



EN	PL
sok	juice
odciek	eluate
budezonid	budesonide
Kontrola	Control

(57) Abstract: A method has been disclosed, regarding the production of an agent from potato juice, the agent intended for the therapy of prophylactics of inflammatory conditions, especially within the gastric tract.

WO 2015/112034 A1

The method of substance manufacturing from potato's juice and its application

The subject of the invention is a manufacturing method of a substance from potato's juice and the substance application for the manufacture of a medicinal agent to be used for treatment or prophylactics of inflammatory conditions, especially within the gastric tract.

Prior art

The potato industry is one of the major sewage producers in the food processing branch. This type of sewage is characterised by a high load of organic and biogenic substances, as well as by the campaign type of production, what creates huge problems in its management. The main source of potato starch industry sewage load is the juice water which, in the scale of entire Europe, is generated in the volume of 2 million tons [ZWIJNENBERG H.J., KEMPERMAN A.J.B., BOERRIGTER M.E., LOTZ M., DIJKSTERHUIS J.F., POULSEN P.E., KLOPS G.-H. 2002. *Native protein recovery from potato fruit juice by ultrafiltration.* Desalination 144, 331-334]. Water juice contains approximately 5% of dry substancje, including 4% of organic substance, consisting of raw protein (about 2%), carbohydrates (0.5-0.8%), fat (about 0.2%). The other organic substances include fibre suspension and fine starch granules – approximately 0.15% in total. Mineral components provide approximately 1% of juice water composition, where the majority belongs to potassium (about 0.6%) and phosphorus (about 0.3%) [NOWOTNY F. 1972. *Technologia przetwórstwa ziemniaczanego.* The Wydawnictwo Naukowo- Techniczne Publisher, Warsaw]. The chemical dem and of juice Walters for oxygen is, at least, 20,000 mg of O_2/dm^3 [ZWIJNBERG et al. 2002]. In the Polish potato industry, in order to reduce the sewage load with nitrogen compounds, a coagulation process of the potato protein is carried out, based on the technology, described in Polish patent No. 126 333. This method consists in acidification of juice water to obtain pH of, approximately, 5.0, to be then heated up to 105-120°C for the time period ≤ 10 minutes and then cooled down to 90-98°C with a maintained overpressure range between $1x10^5$ and $3x10^5$ Pa [MĘŻYŃSKI L., MĄCZYŃSKI M., URBANIAK G., KALISZAN Z., SZYMANKIEWICZ A., OTWOROWSKI M., KORONKIEWICZ J. 1985. *Sposób odzyskiwania białka z soku ziemniaczanego*. Patent PL No. 126 333]. Such a coagulate is then submitted to expansion, centrifuging and drying. This process is also used at potato processing plants in Holland and Germany, using various modifications, e.g., the use of membrane filtration before protein coagulation stage [OOSTEN B.J. 1976. *Ultra filtration of potato juice results in high yield of protein* Die Stärke 28, 135-137; MEUSER F., SMOLNIK H.D. 1976.*Möglichkeiten des Einsatzes der Ultrafiltration und der reversiblen Osmose zur Gewinnung und Aufarbeitung löslicher Inhaltsstoffe aus Prozeßwässern der Stärkeindustrie.* Die Stärke 28, 271-278]. In the potato protein coagulation processes, the reduction degree of nitrogen compounds does not exceed 50% and the generated, co-called- eluate has to be further neutralized in the processes of aerobic or anaerobic biodegradation [NOWAK J., LASIK M., MISKIEWICZ T., CZARNECKI Z. 2002. *Biodegradation of high temperature wastewater*

from potato starch industry. W: Almorza D., Brebbia C.A., Sales D., Popov V. (ed.): "Waste Management and the
Environment" WIT PRESS Southampton, Boston, ISBN 1-85312-919-4, 655-663; BARAMPOUTI E.M.P., MAI S.T., VLYSSIDES A.G. 2005. *Dynamic model of biogas production in an UASB reactor for potato processing wastewater treatment.* Chemical Engineering Journal 106, 53-58]. After potato protein coagulation, the eluate, from the physicochemical standpoint, is a specific solution with the dry mass content of 3.3-4.4%, mineral components, expressed as ash, (1.1-1.3%) and low-molecular proteins (1.3=1.6%) [Lubiewski Z., Śmigielska H., Lewandowicz G., Balcerek W. 2006. Charakterystyka odcieku po koagulacji białka pozyskiwanego w toku kampanii krochmalniczej. Zeszyty Problemowe Postępów Nauk Rolniczych, Issue No. 511, 617-626].

Freshly squeezed potato juice used to be applied in the traditional European folk medicine as a therapy for gastric ulcers. Maximilian Bircher-Benner, a Swiss physician, started using that substance already at the end of the 19th century, however, scientific research, concerning the therapeutic efficacy and safety of use of potato juice in medicine, has been initiated only in the 21st century. The possibility to use potato juice in medical treatment has been evaluated on the basis of a few clinical studies of short duration and with small patient numbers [Chrubasik S., Chrubasik S., Torda T., Madish A. 2006. Efficacy and tolerability of potato juice in dyspeptic patients: A pilot study. Phytomedicine 13, 11-15, Vlachojannis, J.E., Cameron M., Chrubasik S. 2010. Medicinal Use of Potato-derived Products. A Systematic Review. Phytotherapy Research 24, 159-162]. In US Patent No. 6723354, treatment or prophylactic methods are described, used against inflammations, caused by the irritating effects of faeces. The inventors claim that the therapeutic effect is provided by protease inhibitors, contained in potato juice. They are applied as ointments, creams, sprays, fluid or powders. [Ruseler-van Embden J. G. H., van Lieshout L. M. C., Laman J.D. 2004. Methods and
means for preventing or treating inflammation or pruritis. patent US 6,723,354]. The inhibitors of proteases are the main (50% of the whole composition) fraction of the proteins, contained in native potato juice. Beside protease inhibitors, the potato protein contains patatines (30-40%) and other proteins (10-20%) [Bartova V., Barta J. 2009. Chemical composition and Nutritional Value of Protein Concentrates Isolated form Potato (*Solanum tuberosum* L.) Fruit Juice by Precipitation with Ethanol or
Ferric Chloride. Journal of Agricultural and Food Chemistry 57, 9028-9034]. The medicinal application of potato juice was also postulated in Patent Application No. WO 2008053224 Bennet H., Roberts I.S. Treatment of gastrointestinal diseases]. The inventors assign the therapeutic efficacy of the juice to its antibacterial activity against bacteria of the *Helicobacter pylori* strain.

The essence of the invention

The methods of gastric inflammation treatment by means of agents, based on potato juice and known from the prior art description, consist either on the activity of protease inhibitors (what requires application of the proteins in their native, undenaturated form) or on the use of

specifically isolated fraction with elevated antagonistic activity against *Helicobacter pylori*. Since the biological activity of proteins is associated with their native structure, which may easily be damaged by detrimental effects of temperature, as well as by a number chemical factors to maintain the biological activity of protease inhibitors, contained in potato juice, it should be applied in a freshly isolated form. It imposes significant constraints on the use of the method in practice. However, it has unexpectedly turned out that potato juice, submitted to thermal or hydrothermal processing, demonstrates anti-inflammatory properties. Moreover, it has unexpectedly been found out that, protein fraction elimination, in result of acid-thermal coagulation (used, as standard, in potato starching plants), increases the anti-inflammatory activity of potato juice. In consequence, it is possible to produce thermally fixed agents with anti-inflammatory effects in the gastric system, obtained from the raw material, being either potato juice or an eluate after potato protein coagulation, both being the by-products of the starch production process.

The subject of the invention is a manufacturing method of a substance from potato juice, characteristic in that it includes the following stages:

a) juice is obtained from potatoes, especially from potato tubers, protein fracture, contained in the obtained potato juice, is preferably coagulated and an eluate is isolated, deprived, in principle, of protein fracture,

b) a solution, obtained in stage a), is fixed by dehydration or addition of osmoactive substances to obtain a ready-made preparation with dry mass content of, at least, 90% weight.

It is preferable that dehydration is carried out by, at least, one of the following methods: evaporation, lyophilisation, cryo-concentration, spray drying, microwave drying or lyophilisation.

It is preferable that saccharides, sugar alcohols and salts are used as osmoactive substances.

Another subject of the invention is the application of potato juice, whereby it is obtained by the method according to this invention to be used for the manufacturing of an agent for treatment or prophylactics of inflammations, especially within the gastric system.

The essential feature of the manufacturing method, conformable with this invention, consists in submitting potato juice, obtained either from starching process or as eluate after potato protein coagulation, to thermal processing, whereby, the finished product should be characterised by low water activity, protecting it against biochemical and microbiological changes. A product, obtained in such a way, contains all the valuable components of potato juice in the nature of peptides, carbohydrates, mineral salts or paravitamins, as well as, at least, some of the proteins. The product, obtained from the hydrothermal and/or thermal processing of potato juice, with the content of dry substance at the level, typical for potato juice or lower, and amounting to approximately 3.5-5%, is then submitted to the fixing process by means of subsequent processes, reducing water activity of the finished product, down to the level, not allowing for development of any microorganisms. The fixing process is carried out via dehydration and/or addition of osmoactive substances, such as saccharose or kitchen salt. The method acc. to this invention does not impose any limitations on the raw material dehydration.

Thus, dehydration is possible either by concentration or drying. Concentration is carried out with the use of evaporators and, preferably, of cryoconcentrators. The drying process involves the use of spray dryers, sublimation dryers or micro-vacuum types. The application of drying process allows to reduce the level of added osmoactive substances. Neither does the method, acc. to this invention, impose any constraints on the type of added osmoactive substances, unless they belong to the group of foodstuffs. As osmoactive substances, one may use various mono- and oligosaccharides, such as glucose, fructose, maltose, saccharose, maltodextrins, oligofructose, sugar alcohols, e.g., erythritol and mineral salts. The solid product, obtained by the method of this invention, is useful as a supplement of diet, supporting normal functions and preventing inflammatory conditions in the gastric system.

In order to better explain the essential feature of the method of this inventions, its description has been illustrated with enclosed figures.

Figure 1 presents the effects of potato juice on the level of TNF-α. The applied designations: potato juice I – potato juice in dose of 200 mg/kg m.c./day; potato juice II – potato juice in dose of 500 mg/kg m.c./day;*** the difference statistically significant for p<0.001; ▪▪ the difference statistically significant for p<0.01; ▪▪▪the difference statistically significant for p<0.01.

Figure 2 illustrates the effect of the products, obtained by the method of this invention, on Il6 expression. The applied designations: **statistically significant differences $^{a}p<0.05$; $^{b}p<0.01$; $^{c}p<0.001$ vs. the control (+).*

Figure 3 presents the effects of the products, obtained by the method of this invention, on TNF-α expression. The applied designations: **statistically significant differences $^{a}p<0.05$; $^{b}p<0.01$; $^{c}p<0.001$ vs. the control (+).*

The method, according to this invention, is illustrated by the following examples:

Example 1

Raw material concentration via cryoconcentration

The volume of 200 litres of fresh potato juice with a 6°Brix extract content, was placed in a Pilot Plant Cold Concentration Unit. After venting of the installation, the system was cooled down to 0°C. The process was carried out from the initial temperature of ice crystallisation in the product (-0.2°C) up to the target value of -1.1°C. The other parameters of the process: cycle duration: 40 s; compression duration: 10 s; temperature of ice deposit flushing water: 3.5°C; flushing pump operation duration: 15 s; the release valve opening and closure time periods in each cycle of the column operation: 9 s and 5 s, respectively. Concentrate volume of 40 litres was obtained, with 30.5°Brix extract concentration.

Example 2

Thermal coagulation of potato juice

The volume of 100 ml of potato juice, freshly isolated from potato tubers, is placed in a 250 ml conical bulb, closed with a cotton-wool cap, and loaded into an autoclave, where it is submitted to sterilisation process at the temperature of 121°C during 15 minutes. After cooling,

the obtained suspension is centrifuged at 10,000 RPM during 10 minutes. The obtained supernatant, also called effluent, is spray dried or lyophilised for fixing.

Example 3

Acidic-thermal coagulation of potato juice

Potato juice, obtained in starch production process, is heated in a plate heat exchanger to the temperature of 40°C, pumped over to a tank with a stirrer and acidified to pH=5.5 by means of concentrated hydrochloric acid. Then, the obtained solution is directed to a steam heater, supplied with water steam of 0.9 MPa pressure. The coagulation process is carried out at 0.15 overpressure during 7 minutes. The obtained suspension is cooled in the plate heat exchanger down to the temperature of 35° and delivered to centrifuges. The centrifuging process is carried out in such a way that the dry mass content in the deposit was ≥ 40%. The obtained supernatant, also called effluent, is then submitted to spray drying for fixing.

Example 4

Raw material concentration by evaporating

The volume of 50 dm^3 of the effluent after coagulation potato protein, obtained acc. to Examples 2 or 3, and with dry mass content of 3.47%, is submitted to concentration in a vacuum evaporator. The evaporating process is carried out at reduced pressure and within the temperature range of 60-80°C to achieve 8-fold concentration of the raw material.

Example 5

Raw material spray drying

The processing products, obtained according to Examples 1-4, or fresh potato juice, obtained in the starching process, were delivered in a continuous way to a Mobile Miner™ 2000 spray dryer (Niro A/S). The process is carried out at the temperature of 220°C at the cyclone inlet and of 80°C at its outlet. A product is obtained with the dry mass content of 90±0.5%.

Example 6

Raw material lyophilisation

The processing products, obtained acc. to Examples 1-4 or fresh potato juice, is directed to a lyophiliser. The dried material is placed on shelves in the lyophiliser chamber and then submitted to freezing in the temperature of -35° for 3 h. During the main drying cycle and an additional drying cycle the shelf temperature is 20°C. The main drying is carried out under pressure of 0.22 mbar for 17 h, while the additional drying lasts 4 h and is carried out under pressure of 0.005 mbar. A product is obtained with the dry mass content of 90±0.5%.

Example 7

Reduction of water activity by addition of osmoactive substances

The volume of 10 dm^3 of the eluate, obtained after potato protein coagulation, in placed in the tank with a stirring facility. At continuous stirring, 100 g of sodium chloride and 350 g of saccharose are added and brought to a solution. Such a product may also be used as a food product, e.g., a fluid spice for soups.

Example 8

Anti-inflammatory activity of spray dried potato juice

The potato juice, fixed by means of spray drying, is intragastrically given to rats in the form of water suspension, in two doses of 200 and 500 [mg/kg m.c./day] for 5 days. On the 5th day, two hours before autopsy, the rats received a mixture of 0.3 mM HCl / 60% ethanol to induce acute gastritis by direct irritating and mucosa damaging effects. Immediately after decapitation, the stomachs were dissected and washed in 50 mmol of phosphate buffer with pH of 7.4 [Caldas GF, do Amaral Costa IM, da Silva JB, da Nóbrega RF, Rodrigues FF, da Costa JG, Wanderley AG. Antiulcerogenic activity of the essential oil of Hyptismartiusii Benth.(Lamiaceae) J Ethnopharmacol. 2011;137:886-92]. The obtained lesions in the stomach were macroscopically classified (see Table 1). The level of proinflammatory cytokine – the tumour necrosis factor (TNF-α) was assayed by the immunoenzymatic method (R & D Systems) (see Fig. 1).

Table 1. Effects of dried potato juice on the number of ulcers

Group	Ulcer classification*
Control	0
Potato juice I	2
Ethanol/HCl	29
Potato juice I + HCl/ethanol	19
Potato juice II + HCl/ethanol	25

*Classification, accounting for the number and size of ulcers

Potato juice I – potato juice in dose of 200 mg/kg m.c./day

Potato juice II - potato juice in dose of 500 mg/kg m.c./day

Example 7

Anti-inflammatory activity of fresh potato juice and of potato protein coagulation effluent

Inflammatory condition in a RAW264.7 cell culture was induced with LPS (lyposaccharides from *E.coli* in dose of 5 ng/ml). The induction of inflammatory condition was carried out at presence of fresh potato juice and of the effluent after protein coagulation. Budesonide, an anti-inflammatory drug, was the substance of reference:

- fresh potato juice in dose of 0.001 and 0.01 mg s.m./ml
- effluent in dose of 0.001 and 0.01 ms s.m./ml
- budesonide – the anti-inflammatory drug

The samples, in which inflammatory condition was induced, were marked with (+).
The samples, in which inflammatory condition was not induced, were marked with (-).
In the studies, the effects of the analysed products were assayed on the levels of the following inflammatory condition markers: IL6 (interleukin 6), TNF-α (tumour necrosis factor alpha). The analysed products in the applied doses did not induce any changes in the life of RAW264.7 cells. The assays were performed by the immunoenzymatic method. The concentrations of the inflammation markers were provided per ml of the culture. In that volume, the number of live cells was 1.3×10^6 cells/ml.
See Fig. 2 doe the obtained results

A statistically significant decrease of IL6 level was observed in the cultures of LPS-stimulated macrophages, using budesonide 1 mM (↓70%) and the effluent in concentration of 0.01 mg/ml (↓32%). The statistical significance was analysed vs. the K+ control with LPS induction but without any anti-inflammatory agent. See Fig. 3 for obtained results.
The TNF-α level was reduced after budesonide (↓59%) and potato juice in concentration of 0.01 mg/ml (↓16%). The analysed derivatives do not affect the concentration of the factor in a culture of activated macrophages.

Patent claims

1. The method of manufacture of a substance from potato juice, characteristic in that it includes the following stages:
   c) juice is obtained from potatoes, especially from potato tubers, whereby protein fraction, contained in the obtained potato juice, is preferably coagulated and eluate is isolated which is, in principle, deprived of protein fraction,
   d) the solution, obtained in stage a), is fixed by dehydration or addition of osmoactive substances to obtained a ready-made agent with the dry mass content of, at least, 90% of weight.
2. The method according to Claim 1, characteristic in that the dehydration is carried out by, at least, one of the following methods: evaporising, lyophilisation, cryoconcentration, spray drying, microwave drying or lyophilisation.
   e) to obtained a ready-made agent with the dry mass content of, at least, 90% of weight.
3. The method according to Claim 1, characteristic in that saccharides, sugar alcohols and salts are used as osmoactive substances.
4. The application of the potato juice substance, obtained by the method, according to Claims 1-3, to produce an agent for the treatment or prevention of inflammatory conditions, especially these within the inflammatory tract.

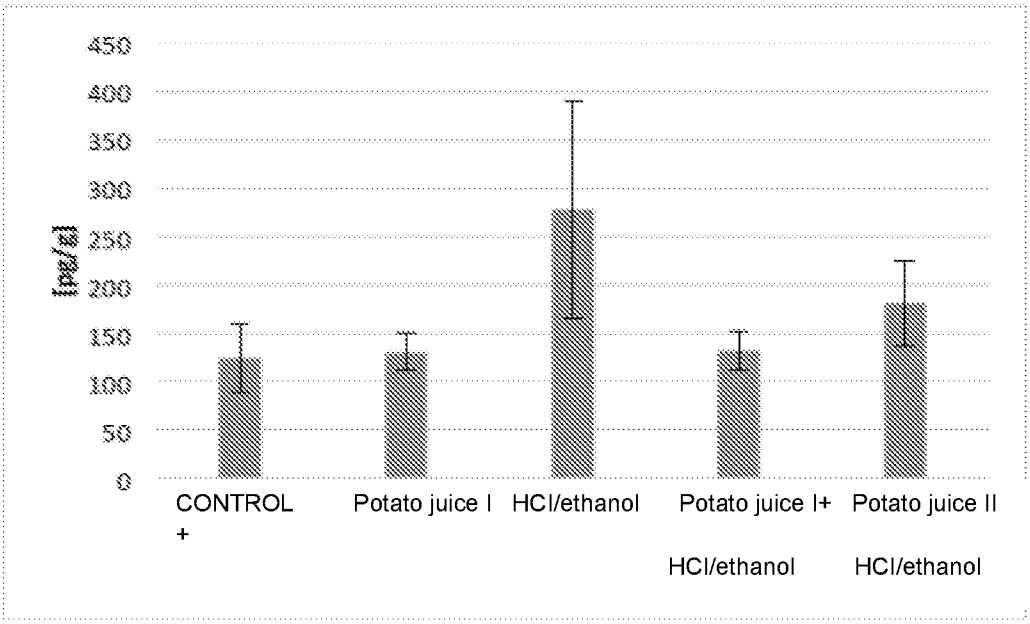
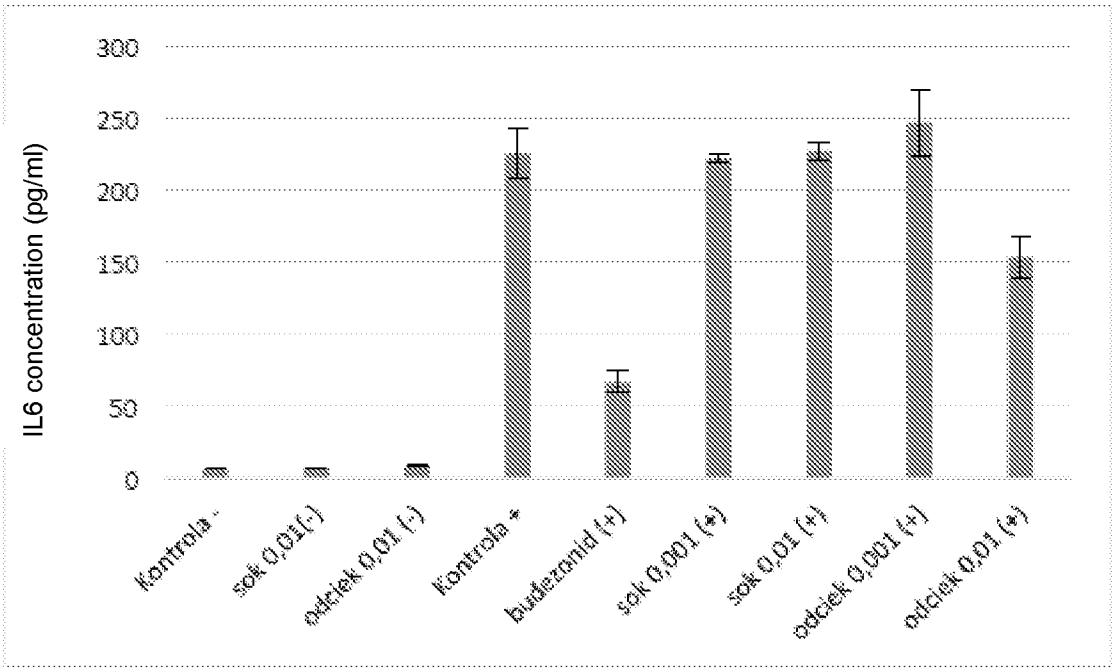


Fig. 1



EN	PL
sok	juice
odciek	eluate
budezonid	budesonide
Kontrola	Control

Fig. 2

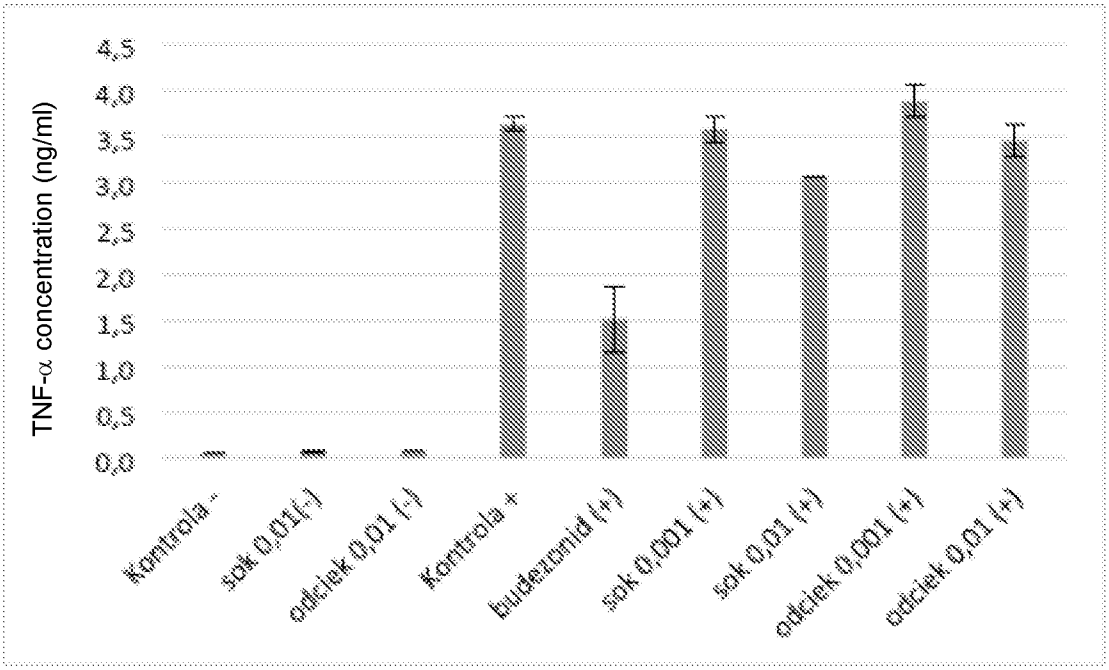

TNF-α concentration (ng/ml)
4,5
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
Kontrola -
sok 0,01(-)
odciek 0,01 (-)
Kontrola +
budezonid (+)
sok 0,001 (+)
sok 0,01 (+)
odciek 0,001 (+)
odciek 0,01 (+)

Fig. 3

INTERNATIONAL SEARCH REPORT

International application No
PCT/PL2015/050001

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K36/81 A61P29/00 A61P1/04
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)
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.
Y	WO 2009/061186 A1 (COOPERATIE AVEBE U A [NL]; GIUSEPPIN MARCO LUIGI FEDERICO [NL]; SPELBR) 14 May 2009 (2009-05-14) *cf. abstract, page 5, lines 29/30, page 6, lines 5-25, page 7, lines 8-27, page 9, lines 8-11, claims 1-5*	1-4
Y	WO 2010/062174 A1 (COOPERATIE AVEBE U A [NL]; KLIJNSTRA GERTJAN [NL]; GIUSEPPIN MARCO LUI) 3 June 2010 (2010-06-03) *cf. claims 6-9*	1-4
X	US 2004/166183 A1 (RUSELER-VAN EMBDEN JOHANNA G H [NL] ET AL) 26 August 2004 (2004-08-26) *cf.section [0002], sections [0022] to [0031] at page 3*	1-4

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 March 2015	07/04/2015

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) 340-3016	Stoltner, Anton

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/PL2015/050001

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2009061186 A1	14-05-2009	CA 2704845 A1	14-05-2009
		EP 2214498 A1	11-08-2010
		US 2010247710 A1	30-09-2010
		WO 2009061186 A1	14-05-2009
WO 2010062174 A1	03-06-2010	AT 523091 T	15-09-2011
		CA 2742165 A1	03-06-2010
		DK 2191731 T3	12-12-2011
		EP 2191731 A1	02-06-2010
		US 2011217436 A1	08-09-2011
		WO 2010062174 A1	03-06-2010
US 2004166183 A1	26-08-2004	NONE	