Appendix n° 2b

Joanna Harasym, PhD, MSc, Eng

SUMMARY OF PROFESSIONAL ACHIEVEMENTS

2016

1. Full name

Joanna Paulina Harasym

2. Diplomas, degrees - giving the name, place and year of their acquisition and the title of PhD dissertation.

1. MSc Eng, Master in biotechnology,
   Faculty of Fundamental Problems of Technology, Wrocław University of Technology, 1994, thesis entitled: *Microbial degradation of model petroleum waste water.*
2. Pedagogical Study Diploma,
   Wroclaw University of Economics, 1998.
3. PhD degree diploma of doctor of agricultural sciences in food technology and nutrition,
   Faculty of Food Science, University of Life and Enviromental Sciences in Wrocław, 2003, thesis entitled: *Studies on the extraction of citric acid and its salts from postfermentation broth*
4. Postgraduate diploma - Research and Development Project Manager,
   Higher School of Banking in Wrocław, 2013, thesis entitled: *Development of beer production process with strong antioxidant properties – a feasibility study*
5. Patent Attorney Application diploma,
   Polish Chamber of Patent Attorneys, Warsaw, 2015
6. Patent Attorney,
   Polish Chamber of Patent Attorneys, Warsaw, 2016

3. Information about the employment in the scientific research bodies

1994-1995	**Research assistant trainee** - Food Biotechnology Department, Faculty of Engineering and Economics of the Industry, Wroclaw Academy of Economics, Wrocław
1995-2003	**Research assistant** - Food Biotechnology Department, Faculty of Engineering and Economics of the Industry (from 2000 - Faculty of Engineering and Economics, Wroclaw Academy of Economics, Wrocław
2003-2004	**postdoctoral research assistant** - Food Biotechnology Department, Faculty of Engineering and Economics), Wroclaw Academy of Economics, Wrocław
2004-present	**assistant professor** - Department of Biotechnology and Food Analysis, (previously Food Biotechnology Department), Faculty of Engineering and Economics, Wrocław University of Economics
2011-2012	**Rector´s Delegate** of the commercialization of research results, Wrocław University of Economics
2016-2018	**senior investigador,** Faculdad de Ciencias Agrarias, Departamiento Ingeniería Agrícola y Forestal, University of Valladolid, Spain (under **Individual Fellowship Marie Sklodowska - Curie Actions, Horizon 2020**)

4. An achievement per article 16, paragraph 2, of the law of 14 March 2003 on scientific degrees and scientific title and degrees and title in the field of art (DZ.U. No. 65, item 595 as amended):

as the above achievement I indicate series of 5 publications - 2 national patents, 1 European patent and 2 original papers from JCR list journals under the common title:

The extraction process of oat β-glucan with varying molar mass and the evaluation of its impact on inflammation in the stomach

Publications included in the scientific achievements:

1. **Harasym J.,** Brach J.: *Sposób otrzymywania polisacharydu nieskrobiowego ze zbóż*, Patent RP – 217750 – 4 strony, Data zgłoszenia – 2008-03-03 nr 384594, Data wydania decyzji - 2014-01-22 - **25 pkt *.**
2. **Harasym J.,** Brach J., Czarnota J. L., Stechman M., Slabisz A., Kowalska A., Chorowski M., Winkowski M., Madera A.: *A method of production of beta-glucan, insoluble food fibre and oat protein preparation*, Patent europejski – EP 2515672 B1– 9 strony, Data zgłoszenia – 2010-12-22 nr PCT/PL2010/050063, 10809339.4, Data publikacji o przyznaniu patentu w Bulletin EPO – 2016-07-06 - **40 pkt **.**
3. **Harasym J.,** Tytuł: S*posób wyodrębniania beta-glukanu ze zbóż*, Patent RP –224430 – 4 strony, Data zgłoszenia – 2014-10-27 nr 409942, Data wydania decyzji - 2016-06-13 - **15 pkt**.**
4. **Harasym, J**., Suchecka, D., Gromadzka-Ostrowska, J. 2015. *Effect of raw material size reduction by freeze-milling on beta-glucan recovery process from oat bran*, Journal of Cereal Sciences, 61, str. 119-125. **35 pkt, IF (2015) = 2.402**
5. Suchecka, D., Błaszczyk, K., **Harasym, J.,** Gudej, S., Wilczak, J., Gromadzka-Ostrowska, J. 2017. *Impact of purified oat 1-3, 1-4-β-D-glucan of different molecular weight on alleviation of inflammation parameters during gastritis*, Journal of Functional Foods, 28, str. 11-18, **45 pkt, IF (2015) = 3.973**

** (per the regulation of the Minister of Science and Higher Education of July 13, 2012, on the criteria and procedures for granting category for scientific research bodies), ** (per the regulation of the Minister of science and higher education from 27 October 2015, on the criteria and procedures for granting category for scientific research bodies)*

Aggregated **IF** of the above presented achievement is **6.375** and total number of points per MNiSW list of journals and criteria for the evaluation of the scientific research bodies results in **160**. In all above patents and articles, except publication n°5, I was the originator of the hypotheses and research concepts and performer of the experimental part. All three patents have been implemented in business practice, and both articles presented as achievement resulting from the article. 16 paragraph. 2 of the Act of 14 March 2003 on scientific degrees and scientific title and degrees and title in the field of art (Dz. U. No. 65, item 595 with d.) are based on the results of the research carried out within the framework of a research project NN312427440 entitled *Effect of soluble fraction of oat beta-glucans on non-specific inflammation of the colon* funded by National Science Center, in which I was the main contractor (Task 1. Development of technologies of β-D-glucan preparations receiving) in collaboration with the entrepreneur owning the patented solutions. Additionally in all collective publications I was also responsible co-author for the correspondence tasks of publication (corresponding author).

An overview to the scientific objective of the work and the results achieved, together with a discussion of their possible use.

The aim of the research work presented as the achievement was to study the extraction process of highly purified 1-3, 1-4-β-D-glucan from oats with different molecular mass and to develop its manufacturing method for industrial use. In addition, as a result of literature study of the activity of 1-3, 1-4 -β - D-glucan and after analysis of the mechanism of its interaction with cellular receptors I have set a research hypothesis of the existence of activity of low molecular mass 1-3, 1-4-β-D-glucan from oats equally high as for high molecular mass one but characterized by a different specificity. The verification of this hypothesis based on the analysis of the preparation of various molar masses impact in inflammation of the stomach has become a natural complement to the range of research carried out in the framework of the work presented as an habilitation achievement. In cooperation with the concerned business operators have been developed the methods for obtaining highly purified 1-3, 1-4-β-D-glucan from oats with a high molecular weight, high purity and economically viable and environmentally friendly, while the research grant NN312427440 (attended with entity owning patents to receive highly purified 1-3, 1-4-β-D-glucan from oats with a high molecular weight) was developed a method of obtaining a polymer with a low molecular weight and was verified the hypothesis of a different activity of highly purified 1-3, 1-4 -β - D-glucan from oats, depending on the molar mass.

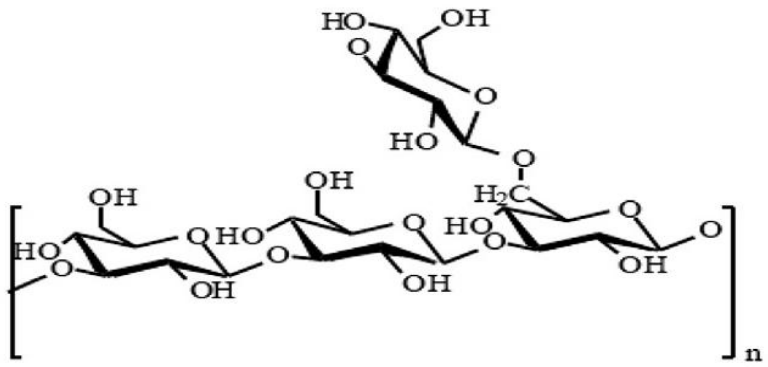
The achievement is a continuation of my research interest regarding the extraction of active substances and the extension of the scope of the research to the problems associated with the activity of a obtained substance *in vivo* in animal studies.

1. Introduction

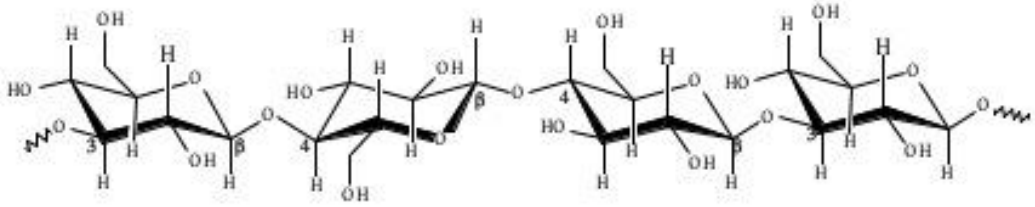
β-D-glucans are homopolymers built with D-glucose monomers linked with β-glycosidic bonding, which are characterized by the specific biological activity. Synthesized in many kinds of prokaryote and eukaryote, in which they are accumulated or secreted. For example are found in cell walls of fungi *Basidiomycota* and bakery yeast (*Saccharomyces cerevisiae*), algae, also occur in the form of bacterial exopolysaccharides and are accumulated in grains of certain cereals, mainly oats and barley [1, 2].

The arrangement of the structural polymer chain, the type of bond and the frequency of their occurrence is variable depending on the origin of the compound, which affects the scope of its biological properties. β-D-glucan from baker's yeast structurally is made up of linked with β-D-(1-3) and (1-6) linkages glucose monomers (Fig. 1a), cereal β-D-glucans are linked by (1-3) and (1-4) linkages (Figure 1b), and cellulose which also is β-D-glucan is connected only by (1-4) linkages (Fig. 1 c), bacterial β-D-glucan known as curdlan (secreted by bacteria *Agrobacterium sp*) are characterized by only (1-3) linkages (Fig. 1 d), β-D-glucan known as lentilan from mushroom (*Lentinula endodes* known as Shiitake Mushroom) is connected by β-D-(1-3) and (1-6) linkages, like other mushroom β-D-glucans as schizofylan (obtained from *Schizophyllum commune)* and grifolan (from *Grifola frondosa),* while β-D-glucan with lichens called lichenan is characterized by mixed (1-3) and (1-4) linkages (Fig. 1e) [3 , 4].

The way the glucopyranosyl units are connected expressed in the quantity and frequency of each type of linkages results in the formation of various macrostructures of the polymer such as helix, triple helix, a simple chain, random coil or cyclical (Fig. 1e) [4, 5, 6, 7, 8].



a)



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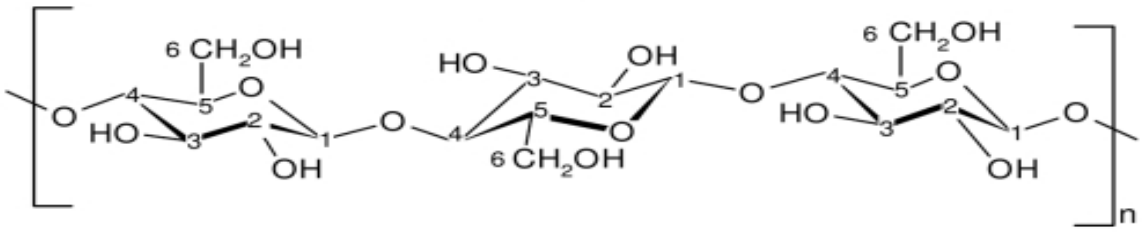

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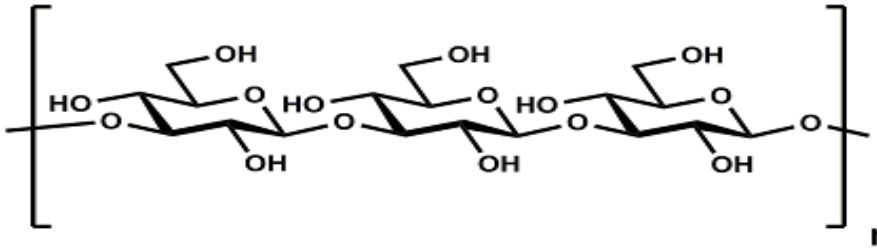
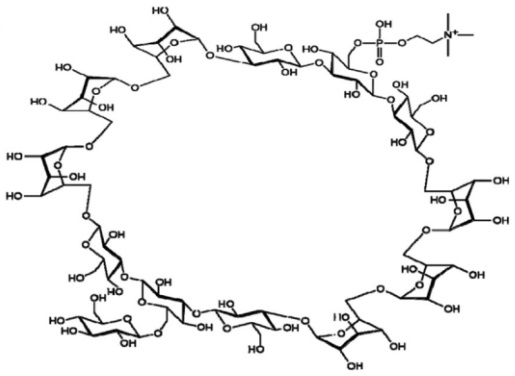


d)



e)

Fig. 1. Structure of β-D-glucan depending on the type of linkage between glucopyranosyl units, a) 1-3, 1-6 linkage; b) 1-3, 1-4 linkage; (c) 1-4 linkage; (d) 1-3 linkage; e) oxygen bridges forming cyclical β-D-glucan.

Notably intensive research on the possibilities of application of β-D-glucans, especially β-(l-3)-D-glucan are carried out from the beginning of the 21st century. This substance has biological activity aroused increasing interest in the pharmaceutical, cosmetics and dietary supplements industry. Due to the diversity of linkages, and thus the structure and physical-chemical properties, β-D-glucans are divided into several groups, and the factors varying between them are structural features, closely related to the biological material, from which they were extracted [9, 10]. The most investigated group are β-(1-3)-D-glucans and β-(l, 3) (l, 6)-D- glucans, which can have linear chains, branched-chain or cyclic ones. They are the components of the cell walls of fungi, or bacteria secreted exopolysaccharides. Such preparations can be obtained in an efficient way with a very high purity, making their industrial production viable.

While β- (l, 3) (l, 4)-D-glucans, found in considerable quantities in the cereals are now very intensively investigated, especially as highly purified fractions, with a view to their practical application in different branches of industry. The economic difficulties of extraction from plant raw materials were a major factor limiting their practical application in industrial production [10, 11].

β-D-glucans from yeast due to the structure of the polymer are insoluble in water, and β- D-glucans from fungi fruiting body consist from 53 to 83% of insoluble fraction. In turn, the cereal β-D-glucans are built from glucose molecules linked linearly with β (1-3) and β (1-4) linkages and this structural feature makes them soluble in water. Among cereals, highest content of β-glucan (in grams per 100 grams of dry mass) was noted for barley (2-20 g, of which 65% is the water-soluble fraction) and oats (2-11 g, of which 82% is the fraction soluble in water). Other cereals also contain β-D-glucans, but in much smaller quantities: sorghum 1.1-6.2 g, rye 1.3-2.7 g, corn 0.8-1.7 g, triticale 0.3-1.2 g, wheat 0.5-1.0 g, durum wheat 0.5-0.6 g, rice 0.13 g [12].

Cereal β-D-glucan is a linear homopolysaccharide of glucopyranosyl units connected with two linkage type - β-(1-4) and β-(1-3). The oligomeric units of cellulose linked with (1-4) are separated by single bonds (1-3). Although most of the cellulose segments is triple (containing three identical monomers) and quadruple (containing four identical monomers), the larger fragments of cellulodextrins are also present [13].

Cereal β-D-glucan shows considerable diversity in the structure. Despite the similarities between β-D-glucans that occur in different types of cereals, β-D-glucan from oats, barley and wheat is structurally diverse, which was showed by quantitative analysis by HPLC of the oligosaccharides made using lichenase [13]. The lichenase enzyme, which is (1-3)-(1-4)-β-D-4-glucanohydrolase, specifically cleaves the glycosidic linkage of (1-4) on the third position of the glucose unit in the β-D-glucan, resulting in three stages of oligomers polymerization (DP). Main products of hydrolysis of β-D-glucan from cereals is 3-0-β-celulobiozylo-D-glucose (DP3) and 3-0-β-celulotriozylo-D-glucose (DP4), but also occurs cellulodextrin-like units released at less (~ 5-10%), especially from the region of the polymer, containing more than three consecutively arranged 4-O-glucose units. Distribution of oligosaccharides inside these same types of cereals is very similar with the main difference occurring between the β-D-glucans of different botanical origin [13].

Literature data indicate emerging differences between relations of DP3:DP4 inside the β-D-glucans of the same cereal origin, which can be attributed to genotype and environmental parameters. β-D-glucan from varieties of waxy barley indicates a higher ratio of DP3:DP4 compared to the cultivation of non waxy barley. In addition, the ratio of tri-to tetrasaccharides in β-D-glucan from the aleuronic tissue of barley and oats is higher than the from starchy endosperm tissue [3].

β-D-glucan has all the functional properties of viscous and gel-forming food hydrocolloids, combined with all the physiological characteristics of fiber. For physical properties of β-D-glucan, such as solubility and rheological properties in the solution and gel state, the molecular characteristics is responsible, such as the presence of cellulose oligomers, a structural conformation, molecular weight, as well as temperature and concentration in the solution [14].

In the process of dissolution, the β-D-glucan absorbs a lot of water and creates a gel, which is not digested in the small intestine. For this reason, it is treated as a fiber. The ability of β-D-glucan to create viscous gels in the gastrointestinal tract is at the heart of the role that it plays in the prevention of coronary heart disease and type 2 diabetes. Effect of diet containing β-D-glucans from oats and barley on the regulation of blood glucose after a meal and blood cholesterol is very well known in the scientific literature and is the scientific basis for the justification of EU authorized health claims for products containing β-D-glucan, especially from oats, within a specified concentration of it [15].

It is necessary to indicate also that the β-D-glucans reveal different physicochemical properties, such as the molar mass, the degree of branching of polymer chain, and hence the water solubility or viscosity not only depending on the origin raw material, but also the extraction method used [13, 14].

The concentration of β-D-glucan in the whole grain of oats is typically in the range of 2 to 11% and from 6 to 16% in bran products [16, 17, 18]. There are also products offered on market that declare even higher concentrations of β-D-glucan – 20%, 22%, 30%, 34% [19, 20], which are usually correctly referred to as the bran concentrates or misspelled as "β-glucan from oats."

β-D-glucan in oats is located thorough the whole endosperm, but in the largest amounts is accumulated in the lining cells of aleurone and subaleurone bran layers, differing at the same time with ratio of tri -for tetrasaccharides. During the processing of the grain the bran layers can be separated from the endosperm by using conventional methods of grinding and sieving. However, the typical processes are usually not able to supply products containing β-glucan in high concentrations. In addition, the relatively low content of β-D-glucan in typical bran meal makes that they are used only in a limited number of functional food products.

β-D-glucan concentrates, containing higher concentrations of β-D-glucan than oat bran, allow to manufacture the products richer in β-D-glucan without impacting too much on texture and mouth feel [21, 22], whereas isolates of β-D-glucan produced by extraction are no longer restricted on the use and allow to create a wide range of products both food and pharmaceuticals.

Methods of extraction of β-glucan can be divided into the so-called wet and dry methods. Wet methods are methods that use the extraction process to extract the highly purified active substance - the β-D-glucan from raw material, while the dry methods are based on grinding, fractionation and rejecting the fraction poor in β-D-glucan, which results in its concentration in finally received concentrate.

Recovery of β-D- glucan from oats by wet extraction is usually based on the following operations:

(a) enzymatic hydrolysis of all components except β-D-glucan,

(b) precipitation with acid, alkaline or solvent,

(c) and wet sieving of water-insoluble solids.

Discussion of the results and their use

As a result of the cooperation established with the economic operator concerned with the development of technology for the extraction of β-D-glucan from oats and the start of production on the Polish market the technology has been developed of recovering β-D-glucan from oats of high molar mass which was presented in publication No 1.

1. Harasym J., Brach J., *Sposób otrzymywania polisacharydu nieskrobiowego ze zbóż*, Patent RP – 217750, Decision date of patent granting - 2014-01-22

It is known that the used extraction method affects the molar mass of received polysaccharide. That's if the health impact of the β-D-glucan from oats based on researches documenting its beneficial effect on the level of triglycerides in the blood and lowering postprandial response were connected with its ability to create highly viscous solutions - the key issue will be such a pursuit of extraction process, that the mass of the polymer chain remained as the largest.

A major role in the degradation of β-glucan play endo-β-glucanases - β-1-4-glucanase is present in the grain, and β-1-3-glucanase is synthesized in the beginning of germination, which is initiated by the appropriate humidity and temperature. These enzymes can reduce the molecular weight of β-1-3-1-4-D-glucan, and even degrade it completely depending on the existence of a favourable enzymatic catalysis conditions. Laboratory methods in which β-glucanase is inactivated in ground grain by heating at a temperature of 85 ° C under reflux column in solution of 82% ethanol by weight prior to extraction [23] are effective only in small scale, since the cost of so constructed technology exceeds the potential gains from the product.

In turn, the solution of potential industrial application, presented in the patents, use the inactivation of enzymes by raising the temperature of the supernatant obtained after centrifugation of the intermediate stage of water extraction of β-D-glucan (that is de facto after the proceeded process of hydrolysis of the polymer) [24] or for economic reasons, provide methods for isolation of β-D-glucan by extraction with water without inactivation of endogenous enzymes [25]. Along with the prolongation of the time of the extraction the molar mass of β-D-glucans significantly lowers, and the precipitation of β-D-glucan is obtained with alternating freezing and thawing of solution.

During research on inactivation of endo-β-D-glucanase I focused on their inactivation directly in the grain. Closed volume of grain has been treated as a reactor, where the undesirable enzymatic activity is reduced because of the high temperature that came in during the appropriate heat treatment under conditions of initial humidity. The essence of the invention consists of subjecting the seeds before grinding to high temperature from 60 °C to 130 °C, preferably 90 °C, then it became part of extraction in alkaline solution with a pH of 8 to 11, preferably from 9 to 10, at a temperature between 10 °C to 50 °C, preferably from 20 °C to 30 °C. After neutralization of the alkaline solution filtrate is cooled to temperatures ranging from 10 °C to 40 °C, preferably from 25 °C to 30 °C and β-D-glucan is precipitated with alcohol, preferably ethyl alcohol chilled to a temperature of -20 °C to 0 °C, preferably -10 °C, titrating to its final concentration in the suspension amounted to 25% by weight. Initial treatment may also be subjected to such parts of grain as flour, bran, oatmeal or their mixture.

The advantage of the technology is the fact that the initial inactivation of enzymes from the glycosidase group (EC 3.2.1) to which belongs β-D-glucanase, allows to eliminate the activity of these hydrolytic enzymes, which is one of the factors that reduce the size of the β-D-glucan polymer. Thus, by the maintenance of the weight of the polymer and resulting high-viscosity - it becomes possible to use lower concentrations of alcohol to precipitate the β-D-glucan without significantly reducing the molecular weight of the polymer produced. The effectiveness of the methods of isolation of β-D-glucans by invention is allowed by earlier inactivation of endo-β-D-glucanase enzymes, present in the grain - what is achieved by heat treatment carried out prior to extraction. Heating should be carried out under conditions of aeration, for example by the air, spray, fluidic or microwave oven with forced convection. Then the raw material is subjected to wet or dry milling with typical for state of the art methods and devices. Fragmented material is suspended in a solution of pH of 8 to 11, preferably from 9 to 10. Chemicals to achieve such pH can be any strong alkaline such as: NH_4OH, KOH, NaOH, Ca $(OH)_2$, preferably to the use of NaOH. Extraction of β-D-glucans is proceeded in the temperature range from 10 °C to 50 °C, preferably from 20 °C to 30 °C under static conditions, shaking or blending, preferably with constant stirring.

After the extraction, the solid parts must be separated on the way of sedimentation, coagulation, filtration or centrifugation, preferably centrifugation, the starch should be digested with amylolytic enzyme in conditions relevant to its activities, the protein precipitated by lowering the pH with any strong or weak mineral or organic acid, preferably with the participation of the HCl and separate settlements on the road of sedimentation, coagulation, filtration or centrifugation, filtration. The filtrate is cooled in the range of 10 °C to 40 °C, preferably from 25 °C to 30 °C and chilled alcohol is added in the range from -20 °C to 0 °C, preferably -10 °C, from a group of methanol, ethanol, isopropanol, 2-propanol, preferably ethanol. A final concentration of ethyl alcohol in the suspension was from 20% to 30% by weight, preferably 25% by weight. Precipitated material was separated in the way of sedimentation, filtration, or centrifugation.

Summary and the possibility of using the results

Based on the results obtained, I created a technology that was granted with patent (co-ownership of the economic operator and the Wrocław University of Economics) in 2014, the technology has been implemented in 2014 at an innovative pilot line of my co-authorship in the Wroclaw Technology Park (II. E. 16) by the business operator and featured in 2015 in the competition NOT "For outstanding achievements in the field of the art" made in 2014.

The processes of wet extraction of β-D-glucan in industrial scale are usually limited by high-viscosity of aqueous extracts, even at low concentration of β-D-glucan, which leads to the emergence of large volume and high costs associated with drying and solvent recovery in stages. The high content of water is also a challenge in terms of microbiological quality, stability, oat lipids and the use of residual streams. Therefore, when developing components for traditional and cheap food products, it would be more economical to use the method of dry fractionation, which eliminates the need for energy-intensive stages of drying and at the same time, allows get fractions enriched in β-D-glucan with higher performance [26].

As mentioned above, the fractions enriched in β-D- glucan have a very limited range of applications even in the food industry, not to mention the possible use of biological activity of the compound in pharmaceutical applications. Technological problems related to the stage of drying and using the residual side streams have been resolved by

me in the form of the development of biorefining technology of concentrated oat bran of and designing the process line, on which the industrial production it is possible to (Publication n°2).

2. Harasym J., Brach J., Czarnota J. L., Stechman M., Slabisz A., Kowalska A., Chorowski M., Winkowski M., Madera A., *A method of production of beta-glucan, insoluble food fibre and oat protein preparation*, European Patent – EP 2515672 B1– 9 pages, Application date – 2010-12-22 nr PCT/PL2010/050063, 10809339.4, Patent granting publication date Bulletin EPO – 2016-07-06,

The process of multistage wasteless extraction, called biorefining, of oat as raw material is characterized by the fact that the raw material is separated into three product streams without creating waste. Products are: beta-glucan, a preparation of insoluble dietary fibre and protein preparation, while raw material is a concentrate of β-D-glucan derived from oat bran. The subject of the invention is also a technological line (fig. 2)

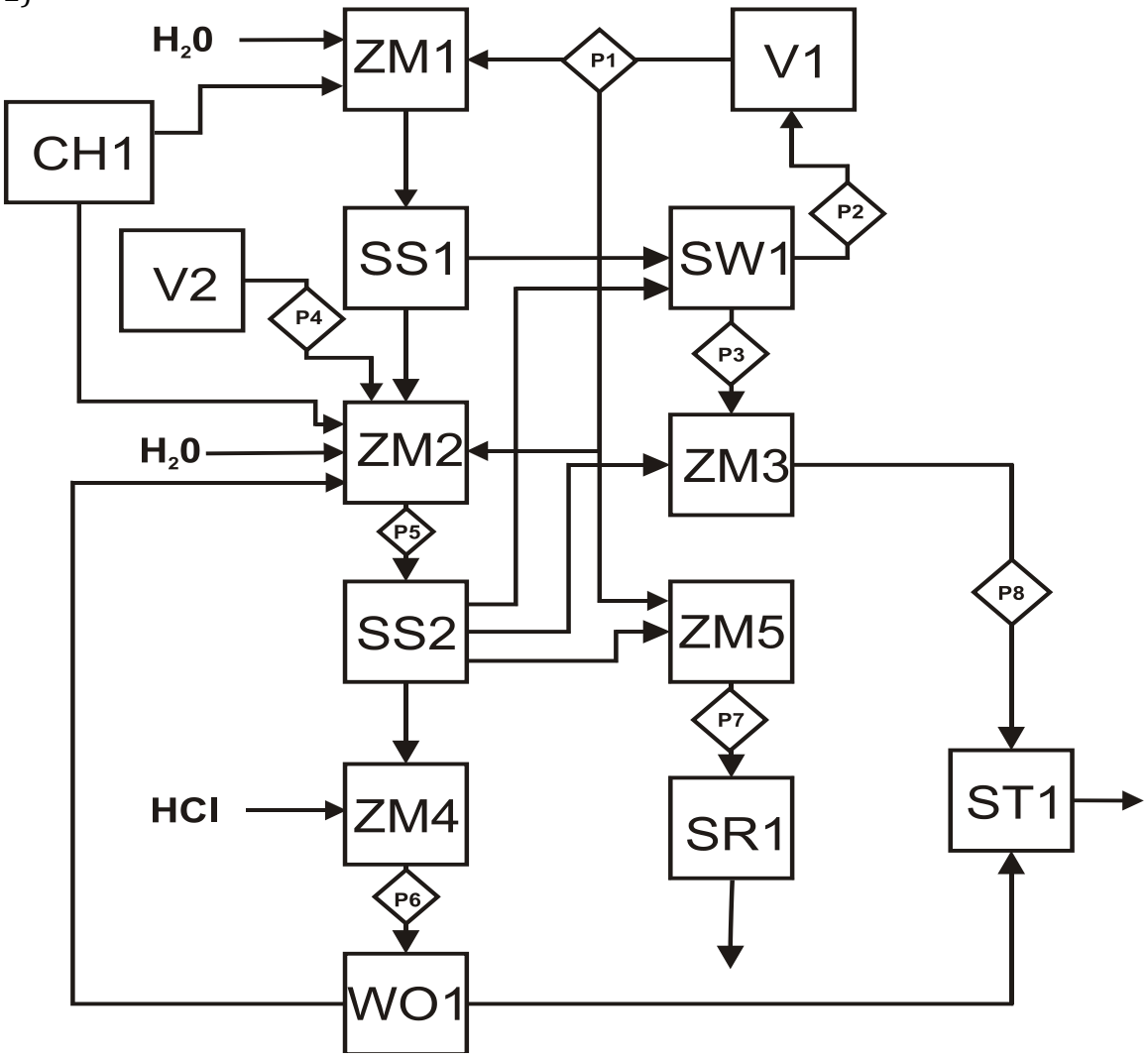


Fig.2. Pilot plant line flowchart

characterised by the fact that includes tanks and equipment presented in the Fig.2. combined in line of running pumps and piping, to which the raw material is delivered and comprising of: receiving raw material tank (ZM1) combined with storage tank of

ethanol (V1), pure ethanol pump (P1), cooling unit (CH1), vibrating sieve (SS1), ethanol recovery station (SW1), distillate transmission pump (P8), brew transmission pump (P7), receiver tank (ZM3), raw materials tank (ZM2), lye pumps (P13), 20% lye tank (V3), vibrating sieve (SS2), transfer pumps (P2), receiver (ZM5), transmission pump (P5), centrifugal separators (WA1), trash dryer (ST1), the receiver tank (ZM4), transmission pump (P04), a spray dryer (SR1) and transmission pump (P3).

The invention is associated also with the way of the production of β-glucan, an insoluble dietary fibre and oat bran protein, characterized by following processes:
the raw material is transported to the tank (ZM1), where water is added and the ethanol is added from the tank (V1) with the help of the pump (P1), then the suspension obtained is mixed at a temperature of ~ 80 ° c; then cooled with water from the cooling unit (CH1) then the suspension is delivered to vibrating sieve (SS1); containing ethanol filtrate is delivered to ethanol recovery station (SW1), where ethanol is recovered and returned with the pump (P8) to the tank (V1), and a decoction of the ethanol recovery is concentrated by evaporation of excess water, and so the resulting solution is delivered by the pump (P7) to the tank (ZM3), while the concentrated bran fraction remaining on the sieve is delivered to the tank (ZM2), where it is mixed in the water and a solution of NaOH, administered with the pump (P13) from the tank (V3) and then the suspension is mixed at a temperature of ~ 70 °C.

After cooling with water from the cooling unit (CH1), the suspension is delivered to the vibrating sieve (SS2) using pumps (P2) and the separated solids to the tank (ZM3), where they are mixed with decoction; then obtained from the sieve (SS2) filtrate is delivered to the tank (ZM5), where of HCI is added followed by precipitated protein fraction. The liquid from the tank (ZM5) is pumped (P5) to the centrifuge (WA1), where fraction of protein is separated, which is then transferred to the dryer(ST1) and after drying to packaging. Obtained in the stage of centrifugation the effluent is delivered to the tank (ZM2), where is neutralized by the addition of NaOH solution, administered with the pump (P13) from the tank (V3) and following the neutralization the addition of ethanol from the tank (V1) using pumps (P1) causes the precipitation of β-D-glucan. The suspension is delivered to vibrating sieve (SS2) using pumps (P2), where separated in solid form the β-D-glucan is delivered to the tank (ZM4), where it is mixed in a small amount of ethanol given from the tank (V1) using pumps (P1) and then with the pump (P4) it delivered to the spray dryer (SR1), from where further is delivered to packaging. Th obtained on the sieve (SS2) filtrate containing ethanol is delivered to alcohol recovery station (SW1), where recovers ethanol, which returns by pump (P5) to the tank (V1), then a decoction of ethanol recovery concentrated by evaporation of excess water and the resulting liquid is guided by the pump (P7) to the tank (ZM3), where it is mixed with other ingredients, and the suspension obtained is pumped (P3) to the dryer (ST1) , and dried preparation of insoluble fiber to packaging.

Summary and the possibility of using the results

Based on the results obtained, I created a technology that was granted with European Patent in 2016. The technology became the basis for the construction of an innovative line of my co-authorship (II. E. 16) in the Wroclaw Technology Park and was implemented in 2010 by the business operator (III. Q. 9) and featured in the 2011 contest of NOT with First Prize "For outstanding achievements in the field of the art" made in 2010 (III. D.3). β-D-glucan produced by this technology by the operator was delivered to the research planned and carried out in the framework of the grant being carried out in cooperation with the departments of WULS (SGGW) (WNOŻCZiK and WMW, Project Manager - Prof. J. Gromadzka-Ostrowska) and funded by the National

Science Centre NN312427440 pt. I*mpact of beta-glucans from oats on the inflammatory process in the digestive tract* (2011-2014).

β-D-glucan from oat of 1-3, 1-4 linkages is a substance with the impact on the health documented by many research, and therefore gained the authorization of the European Food Safety Authority to use "health claims" on the products containig it manufactured and marketed in the European Union. Also on the international market is recognized and accepted eg. by the US Food and Drug Administration. This shows the significant market potential and warrants further exploration and development of isolation methods from primary raw materials. At the same time, it is advisable to develop such a process, which is characterized by a low media consumption while yield of the active substance of raw material is highly-performed, which decides in present economic environment about the profitability of its industrial use.

Previously developed methods allow for the obtaining of up to 70% of β-D-glucan contained in the raw material. Unexpectedly turned out to be very effective is the use of the phenomenon of cavitation, caused by the spreading the ultrasonic wave in water, to raise the efficiency of extraction of active substance from raw oat, while reducing the value of process parameters and, in particular, substantially lowering the temperature of process stages. The results obtained were presented in the patent publication (n°3).

3. J. Harasym: *Sposób wyodrębniania beta-glukanu ze zbóż*, RP Patent-224430. Date of issue of the decision-2016-06-13

A cavitation is a physical phenomenon involving the rapid transformation phase of the liquid in gas phase influenced by lowering pressure. When the liquid rapidly accelerates in accordance with the principle of conservation of energy, the static pressure of the liquid decreases. Gaseous solvent penetrates the extracted substance with greater effectiveness than in the liquid state. This allows to increase the efficiency of extraction and shorten the duration of the process.

The essence of the invention involves the use of the phenomenon of cavitation during extraction of β-1-3,1-4-D-glucan from grain preferably oat. Raw material as grains, milled grain or its fractions, such as flour or bran are defatted in the conditions for the emergence of the phenomenon of sonic cavitation. Intense mixing due to the phenomenon of cavitation causes the effective penetration of plant tissue matrix by variable solvent of variable phase state, and thus removal of fat and mass transfer of lipids to 50% solution of water: an organic solvent, preferably ethyl alcohol that is mixed in the mass ratio relative to the raw material respectively from 1:1 to 1:5, 1:3.

Defatting process using the mixing of cavitation is run at 0°C to 60 °C, preferably 25 °C. The low-fat raw material is subjected to a water extraction using cavitation, preferably sonic, in the mass ratio of the solvent water relative to the mass of the original raw material in process ranging from 5:1 to 20:1, preferably 10:1. The pH of the extraction process is from 6.0 to 8.0 preferably 7.0 and is achieved by the relevant addition of chemical rising the alkalinity of solution, preferably metal hydroxide. The temperature of the extraction process in the cavitation reactor ranges from 1 °C to 60 °C, preferably 30 °C, the water extract is separated from slurries of insoluble with methods typical of filtration, centrifugation, sedimentation or flocculation. Separation of the extract from the solids is provided by typical methods and effluent containing water extract of defatted raw material is subjected to a treatment in cavitation reactor,

preferably sonic cavitation for 10-60 min, preferably for 30 min to denature proteins, and proteins are separated from the solution with methods typical of filtration, centrifugation, sedimentation or flocculation. Then deproteinated effluent containing water extract is subjected to cavitation treatment, preferably sonic cavitation in the presence of organic solvent, preferably ethyl alcohol in ratio of 1:0.1 to 1:0.3, 1:0.2, at 0°C to 30 °C, preferably from 15°C, leading to precipitation of beta-glucan, which after the separation of common methods is dried.

Summary and the possibility of using the results

As a result of the studies conducted using cavitation results obtained have been sold to the business operator that was granted with patent on the technology based on this study in 2016, also in 2016 was done PCT application. This technology allows to manufacture economically β-D-glucan of high purity, which enables the examination of its uses in the pharmaceutical industry. The operator further acts in the field of industrial and scientific use of the obtained preparation (the secret of the company).

Most of the methods of β-D-glucan isolation focuses on maintaining high molecular weight of polymer combining the proven health benefits with viscosity depending on the molecular mass of fraction [26]. As viscosity depending on the high molar mass of polymer creates certain constraints [27] the specific action were taken for food applications in order to improve the functional properties and widening the application of oat β-D-glucan by depolymerization. It has been shown that depolymerization using high pressure homogenization [28], sonication [29], ascorbic acid [30], the enzymatic hydrolysis [31], thermo-degradation during extrusion [32], enzymes or acid catalysis in a reduced moisture content [33] and the gamma radiation [34] is used to improve the physical and functional properties of β-D-glucan. Although these methods are effective in reducing the molar mass of used fraction, have some disadvantages as the high cost, poor performance or a long processing time. As far as the purpose of depolymerization of β-D-glucan is only to improve the rheological properties - the used method is considered only for performance and cost effectiveness.

However, when the goal is to maintain the metabolic activity, which depends on the structure of the chain - the polymerization process must be approached with great caution. The problem above has been developed within the framework of Task 1 (Development of technologies of β-D-glucan preparations receiving) of research grant NN312427440, for which I was responsible as a main contractor. The results have been presented in a scientific publication (n°4).

4. Harasym, J., Suchecka, D., Gromadzka-Ostrowska, J.: 2015. *Effect of raw material size reduction to freeze-milling on beta-glucan recovery process from oat bran*, Journal of Cereal Sciences, 61, p. 119-125.

There is a lack of widely designed human diet intervention studies, comparing the health benefits (lipid metabolism, sugar metabolism, protein metabolism, immunomodulatory effects etc.) of oat beta-glucan in relation to its molecular weight. Immerstrand et al. [35] in their study, evaluated cholesterol-lowering effects of various oat bran preparations differing in molecular weight (MW) of β-D- glucans (2348, 1311, 241, 56, 21 or <10 kDa in C57BL/6NCrl mice.) The results suggest that the molecular weights and viscous properties of beta-glucan in oat products may not be crucial parameters for their cholesterol-lowering effects. The evaluation was made for bran products, not for isolated β-D- glucan preparations and some interaction connected with

insoluble fiber presence and bioactive components of plant cell walls were neglected. Concerns about actual impact on metabolic eg. for reducing postprandial glycemia were recently confirmed by He and coworkers, who performed a meta-analysis of clinical studies showing significant differences in the effects of oat preparations vs purified β-D-glucan in the regulation of blood sugar after meal [36].

But the conclusion which was made can also be important for isolated β-D-glucan products, as it allows the hypothesis that properties connected with viscosity ie. cholesterol level lowering effects might be maintained with immuno-properties enhanced also for low molecular weight beta-glucan.

Mechanical treatment of polysaccharide raw material has been validated as a good method for reduction of particle size and several milling devices were studied for further processing facilitation [37, 38]. Some more opportunities appeared with freeze milling unit development and freeze-milling has been recognized as very efficient for cellulose homogenization [39]. Wet-milling is a most often used technology in the food industry, resulting in very fine particle size, but for beta-glucan recovery is not so applicable due to its gelling properties in water.

Some attempts has been made in replacing wet-milling in cereal processing with freeze-milling, Ngamnikom and Songsermpong [40] tested three different types of grinders (hammer mill, roller mill, and pin mill) both the freeze and the dry grinding processes for rice flour. Achieved results show that, freeze grinding with the hammer mill, significantly reduced both the average particle size and the damaged starch content and produce a higher yield after sieving in comparison with dry grinding using an identical grinder. The comparison with wet the grinding process demonstrated significantly higher specific energy consumption (13,868 kJ/kg) due to the large consumption of electrical energy by many machines in the process, while the energy consumption of freeze grinding was similar to dry grinding. Hence, the aim of the present study is to evaluate the effect of freeze-milling in a cross beater mill applied as a pre-treatment of raw material in the extraction of oat bran for beta-glucan recovery in pilot plant scale.

In order to compare the impact of the milling process in a frozen state of the characteristics of the process of β-D-glucan isolation the extraction process was carried out according to the method described in publication n° 2. (Fig. 3).

Oat fiber for low molecular weight beta-glucan extraction was prepared by freezing oat bran for 24 h at _20 _C in a freezer (M 410, New Brunswick Scientific), then milling frozen material in a cross beater mill (SK 100, Retsch) with 200 mm mesh size, collected and freezed again. Freeze-milling in a hammer mill was evaluated as the most efficient for particle size reduction. Grinding takes place in the cross beater mill using a hammering, impact and shearing action. The feed material passes through the hopper directly into the centre of the grinding chamber. It is then caught by the cross beater and crushed between the impact plates of the cross beater and the toothed grinding insert.

Reduction the size of the raw material meant that it was necessary to modify the process in which turned out to be unsuitable to use methods of filtration, which was replaced by centrifuging. As a result, the obtained preparation of β-D glucan of low molar mass was manufactured by a modified procedure carried out in accordance with the flow chart diagram in Fig. 4.

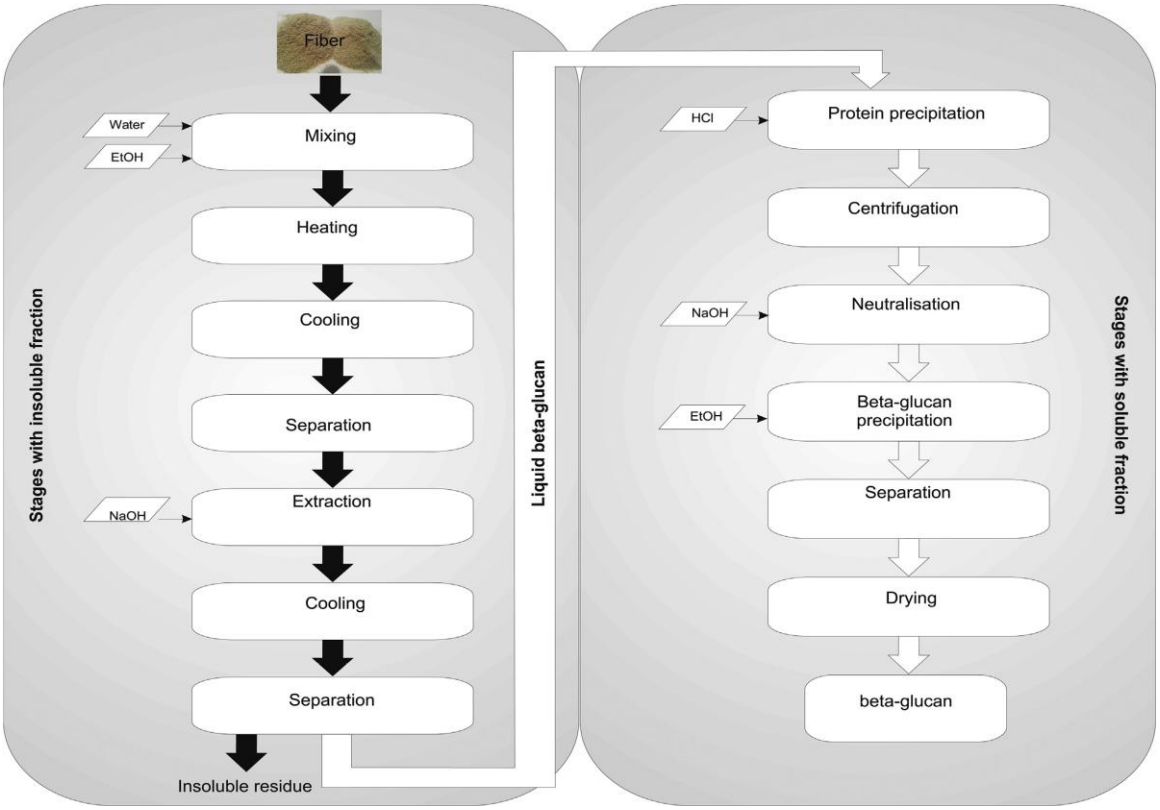


Fig. 3.Diagram of the extraction process of β-D-glucan of high molar mass

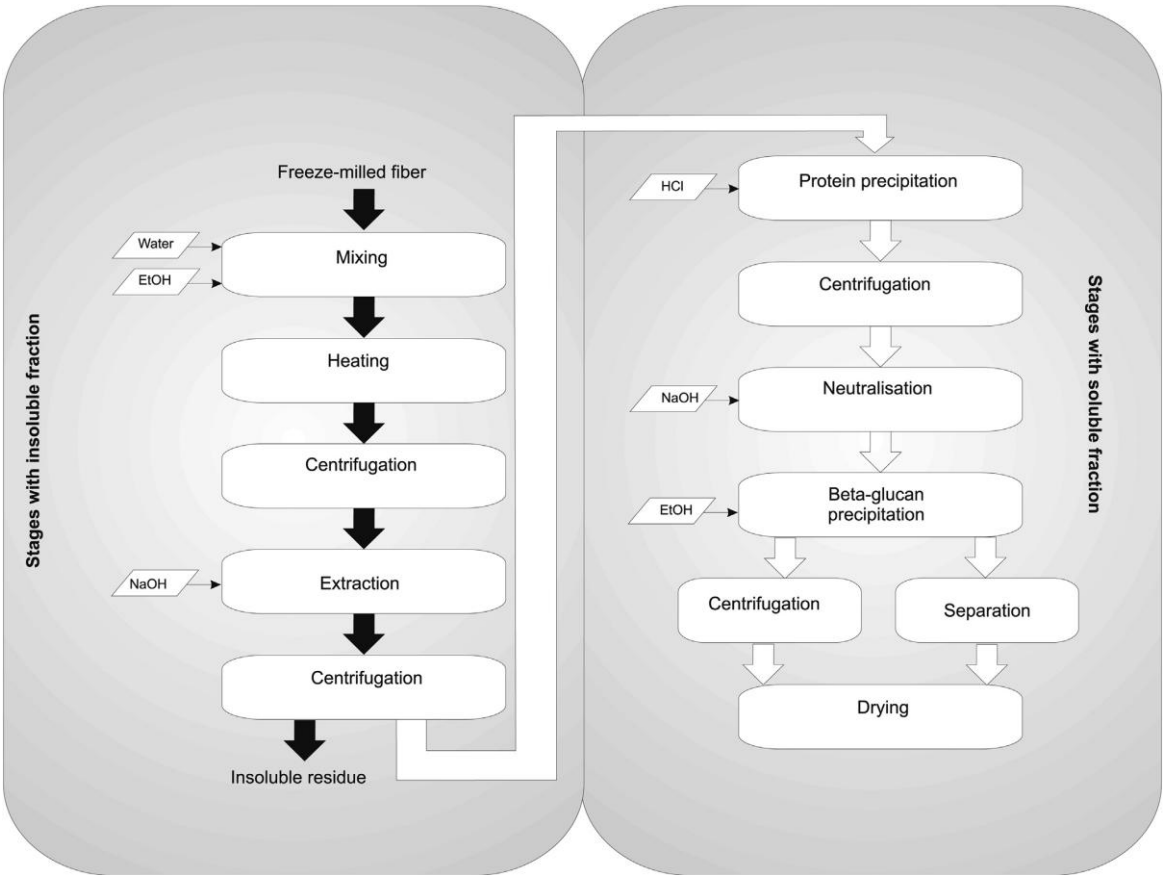


Fig 4. Diagram of the extraction process of β-D-glucan of low molar mass

The characteristics of the process parameters and the resulting β-D-glucan preparations are presented in tables 1 and 2.

Table 1. Yield and process parameters

	Process yield [%]	Beta-glucan yield [%]	Oat oil yield [%]	Moisture of beta-glucan preparation [%]	Insoluble residue [%]
CP	18.75	54.89	62%	9.5	29.8
MP	19.25	64.03	81%	5.2	20.8

CP – control process.
MP – modified process.

Table 2. Physicochemical properties of obtained preparations of β-D-glucan

	Purity [%]	Molecular weight g/mol]	Viscosity [mm^2/s]	Solubility [%]
BG	84.4 ± 1.87	69,652.7 ± 11,935.77	0.56 ± 0.04	88.1 ± 1.21
BGC	74.1 ± 1.44	2,179,701 ± 16,3012.6	4.96 ± 0.25	69.3 ± 0.72

BG – low-molecular beta-glucan isolate.
BGC – control beta-glucan isolate.

Summary and the possibility of using the results

The results were publish in important journal in the field with IF = 2.402. The results obtained made it possible to manufacture the low molar mass fraction of β-D-glucan which has been used in studies carried out in the framework of the research grant funded from the National Science Center. At the same time, the results obtained have enabled the execution of patent application protecting developed solution (III. Q. 5).

A lot of research confirms the positive impact of the β-D-glucan from oats on the digestive tract; however, the query activity was evaluated using very different sources of β-D-glucan as oat bran, concentrates of oat bran or food products containing a certain amount of β-D-glucan (3-30%). Hence the conclusions concerning the activity of β-D-glucan can be biased by the effects of other compounds. In addition, it should be noted that the application of oat bran or oat bran concentrate in dietary interventions especially in inflammatory bowel disease is limited due to the mechanic irritation caused by cellulose fraction.

In present study we used intragastric treatment of deoxycholate solution simulating the effect of bile, which causes acute inflammation of gastric tissue. To investigate β-D-glucans effect on acute inflammation of gastric tissue, which may in long term lead to chronic gastritis and gastric tumors, we examined the parameters of oxidative stress and defense as well as levels of cytokines engaged in inflammatory response of gastric mucosa. As the activity of β-D-glucans strictly depends on its structure and molecular weight the study used two molar mass of oat β-D-glucan preparations. This issue was examined and the results are presented in the following publications (n° 5).

5. Suchecka, D., Błaszczyk, K., Harasym, J., Gudej, S., Wilczak, J., Gromadzka-Ostrowska, J., 2017. *Impact of purified oat 1-3, 1-4- β -D-glucan of different molecular weight on alleviation of inflammation parameters during gastritis*, Journal of Functional Foods, 28, p. 11-18.

The aim of the study was to investigate the effects of dietary supplementation of β-D-glucan from oats with different molar masses on the acute inflammation of the stomach lining in animal model. Rats were observed daily during the experiment period and appeared to have good general state of health. Administration of sodium deoxycholate in experimental animals resulted in gastritis development visible in histopathological analysis (Fig.5), but there were no macroscopic organ abnormalities revealed by the autopsy.

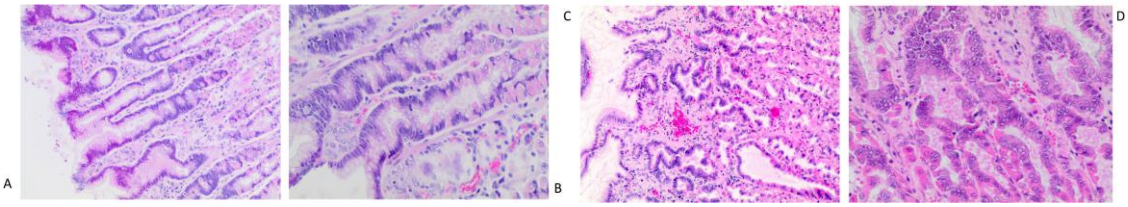


Fig.5. Stomach histological states from control (Ax100, Bx200) and deoxycholate treated (Cx100, Dx200) rats (hematoxylin-eosin stain): Microscopic picture of upper part of fundic mucosa of rat with normal morphology: A – Few intraepithelial lymphocytes present in superficial epithelium, B – Two mitotic figures present in isthmus of gastric glands; Microscopic picture of upper part of fundic mucosa of rat with mild foveolar hyperplasia: C – faveoli are slightly dilated and tortuous, one markedly dilated gland is present on the right side, D – faveoli are slightly dilated and tortuous and contain proteinaceous material.

In the present study we observed an increased concentration of lipid hydroperoxides and TBARS substances of the stomach of rats with gastritis, which suggests that sodium deoxycholate caused augmented ROS production and higher oxidative stress level in this tissue. Our results also demonstrated that both high and low molecular weight oat b-glucan fractions effectively lowered the level of lipid peroxidation and increased TAS in the stomach tissues of rats with induced gastritis (Fig.6). Our study showed that oat β-(1.3/1.4)-glucan also possesses bioactivity in the digestive tract and may protect gastric mucosa from oxidative damage occurring in gastritis by reducing the oxidative stress.

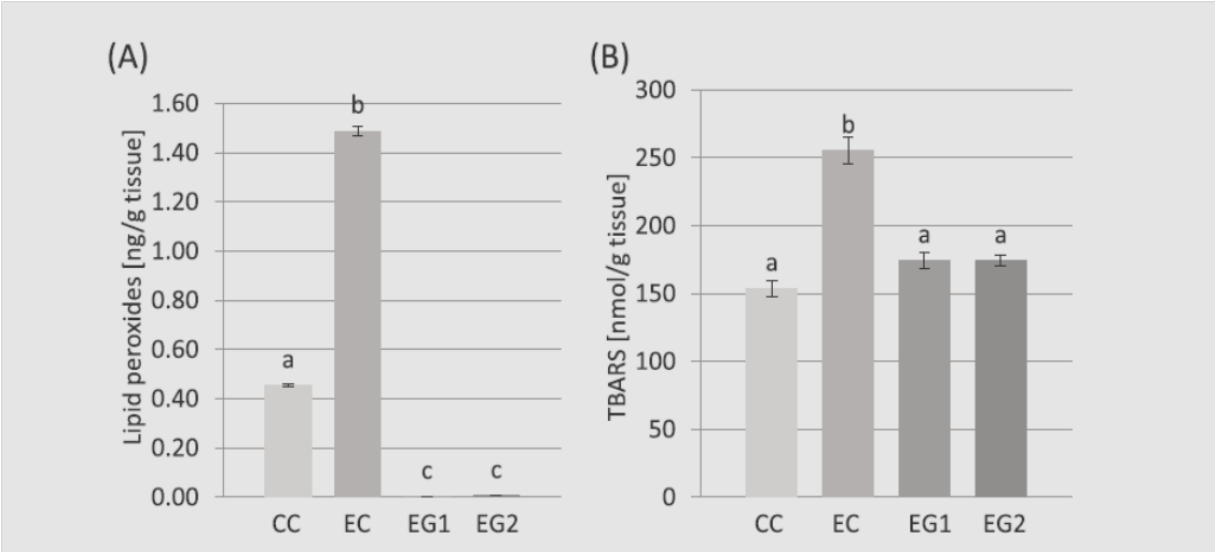


Fig.6. Oxidative stress parameters in stomach tissue. Data are shown as means ± SE. A – lipid peroxides concentration; B – thiobarbituric acid reactive substances concentration. Different letters indicate statistically significant differences between groups. (CC – control group, EC – experimental control group, EG1 – experimental group fed with high molecular weight b-glucan, EG2 – experimental group fed with low molecular weight b-glucan).

In the present study we also investigated the changes in the concentration of glutathione, which is one of the most important intracellular antioxidants. Glutathione is converted to its oxidized form: glutathione disulfide (GSSG) in the process of ROS scavenging catalyzed by glutathione peroxidase enzyme family. High GSH:GSSG ratio in cells is crucial in maintaining antioxidative defense and protecting the tissue from oxidative damage. In our study we observed that gastritis significantly lowered stomach GSH:GSSG ratio. Simultaneously, diet supplementation with high molecular weight β-D-glucan partially prevented the GSH:GSSG ratio from decreasing in rats with gastritis, suggesting its strong antioxidative effect and important role in maintaining redox balance (Fig.7).

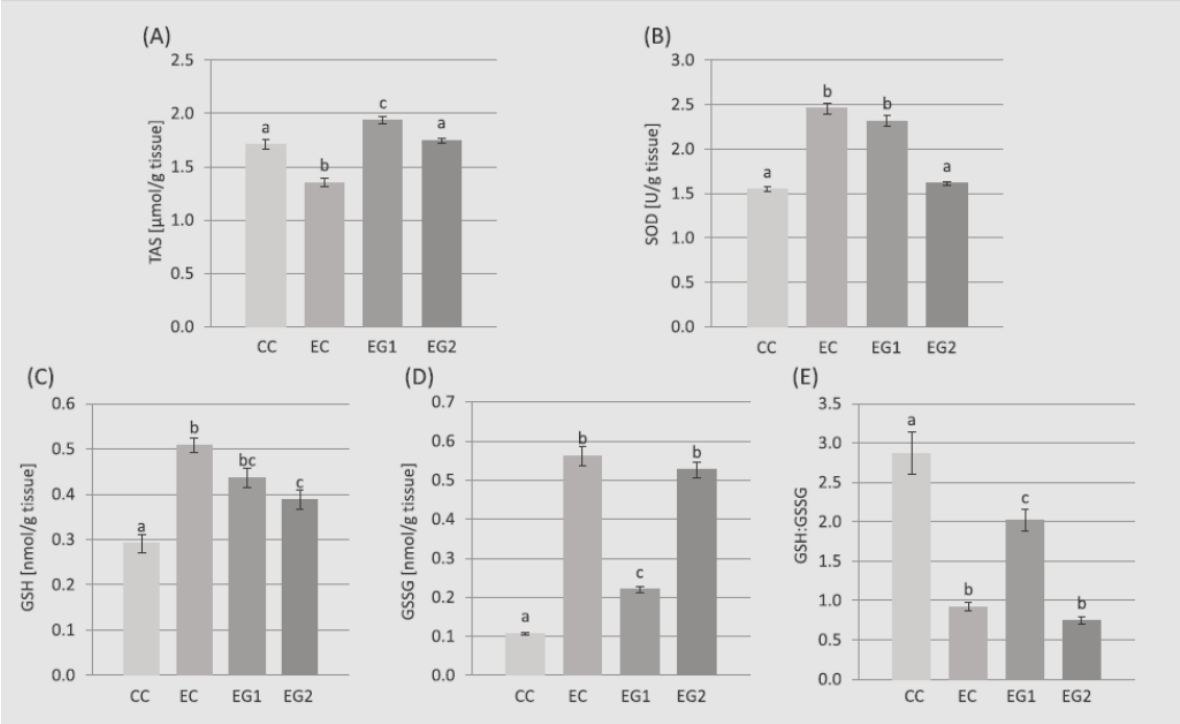
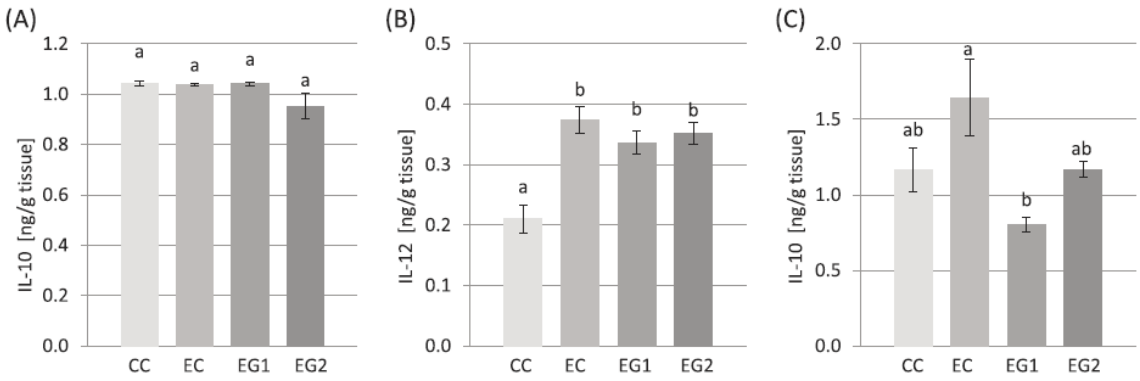


Fig. 7. Antioxidative potential and antioxidative status of stomach tissue. Data are shown as means ± SE. A – TAS; B – superoxide dismutase concentration; C – glutathione concentration; D – glutathione disulfide concentration; F – glutathione to glutathione disulfide concentrations ratio. Different letters indicate statistically significant differences between groups. (CC – control group, EC – experimental control group, EG1 –experimental group fed with high molecular weight b-glucan, EG2 – experimental group fed with low molecular weight b-glucan).

In our present study we noted a significant increase in the concentration of proinflammatory cytokine IL-12 in the stomach of rats with induced gastritis, as well as slight but not statistically significant increase in TNF-α. This partially corresponds with the findings of Diakowska and coworkers [41] who observed increased level of IL-12 in patients with stomach cancer. At the same time our data did not show any changes in the concentration of anti-inflammatory cytokine IL-10 in the stomach wall of rats with deoxycholate-induced gastritis. Diet supplementation with β-D-glucan regardless of its molecular weight did not affect significantly the concentration of IL-12, which indicates that β-D-glucan is not able to inhibit activation of macrophages in the epithelium of the stomach wall. However, we observed a significant decrease in the level of TNF-α in EG1

group caused by high-molecular weight β-D-glucan in comparison to both CC and EC groups. These results reveal anti-inflammatory and immunomodulatory action of this oat b-glucan preparation in both physiological and pathological states. Furthermore, low-molecular weight β-D-glucan (EG2) decreased the concentration of IL-10 in rats with gastritis, suggesting the anti-inflammatory properties (Fig.8)



Rys.8 Cytokines concentration in stomach tissue. Data are shown as means ± SE. A – IL-10 concentration; B – IL-12 concentration; C – TNF-a. Different letters indicate statistically significant differences between groups. (CC – control group, EC – experimental control group, EG1 – experimental group fed with high molecular weight b-glucan, EG2 – experimental group fed with low molecular weight b-glucan).

It has been shown that induction of gastritis with deoxycholate leads to activation of TLR receptors and further signaling cascades that stimulate IL-10 synthesis (42). It is possible that in the presence of low-molecular weight β-D-glucan activation of TLRs is attenuated, causing decreased production of IL-10 cytokine.

Summary and the possibility of using the results

The obtained results were published in the journal of high impact factor. Together with the other results obtained as a result of the implementation of the research grant enabled the staking of new research hypotheses, which was formed as the scientific basis of the new research project entitled: Impact of the fraction of oat beta-glucans on non-specific inflammation of the colon, which has received funding from Opus National Science Centre (UMO 2015/17/B/NZ9/01740-leader Prof. J. Gromadzka-Ostrowska), and in which I am the principal contractor (Task 1- Obtaining and characteristics of the deproteinated fraction of oat β-D-glucans) (II. I. 10).

Conclusions

The developed methods of extraction of β-D-glucan from oats allow selectively receive of fractions with different molar masses. Extracting β-D-glucan with high molar mass is subject to the control of enzymes that can decompose this compound, which is endogenous 1-3, 1-4, β-D-glucanohydrolase present in cereals. This enzyme after inactivation with the use of microwaves irradiation of raw material no longer affects the shortening of glucose polymer chain of β-D-glucan, which using alkaline extraction allows isolation of β-D-glucan of molar mass in the range of 2000-3000 g/mol. In turn, receiving β-D-glucan of low molar mass is often carried out using enzymatic decomposition of the chain, which gives a result in the form of a mixture of oligomers which does not have a counterpart in the actual frequency of linkages present in the β-D-glucan. In our studies, we have developed a non enzymatic method for decomposition of β-D-glucan, which allows obtain a pure preparation of low molar mass 100 g/mol..

Whereas the overall efficiency of the process, account should be taken of the fact that an important factor for the price is, if a large part of the faction of oats (usually only concentrate of β-glucan, and in fact its isolate) can be used in food products with high added value. For example, with the dry processes all fractions of oats are easy to store and provide for other types of applications, but these are the application are limited by specific physico-chemical properties of these fractions. In such a process, a first (dry type) would yield from the concentration step, in economically way, the partially enriched in β-D-glucan raw material.

This approach has been applied by me in the study, in which biorefining technology of the oat fiber with a high content of β-D-glucan was developed, which allows to obtain in a waste-free way three fractions: highly purified preparation of β-D-glucan, oat protein formulation and insoluble fiber.

β-D-glucan of oats administered in the diet revealed the activity supporting the maintenance of the natural antioxidant defenses of the body at the right level. Despite the recognized healthy impact of oat bran on the diseases of the stomach due to its high content of insoluble fraction-cellulose can cause symptoms of irritation. Purified form of β-D-glucan from oats, does not result in additional negative symptoms. The study showed significant differences in activity between formulations of different molar masses, and the results can then be used to create schemes of dietary intervention, as diseases of the gastrointestinal tract are particularly prone to this type of treatment.

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5. Overview of the other scientific achievements

My research interests are located between microbiology, biotechnology and food technology since my Master study at Biotechnology, Faculty of Fundamental Problems of Technology, Wrocław University of Technology. I graduated Master in 1994 with very good rating, based on passing very well masters exam and defending the same The Master Thesis work under the title "Microbial degradation of modeled petroleum wastewater" by the same obtaining the master's and engineer degree in biotechnology. In the same year I was matriculated for doctoral studies at the Faculty of Fundamental Problems of Technology of the Wrocław University of Technology, which is didn't due to adverse financial regulations. Also in the same year I hired in Food Biotechnology Department of Faculty of Engineering and Economics of the University of Economics in Wrocław as an research assistant. My scientific work is related to the use of physical factors for extraction of active substances or modification of plat raw materials for use in the prevention of health. From the very beginning, I work closely with the industry, solving existing problems and create innovative solutions and products.

My research interests includes the following thematic groups:

1. Extraction of organic acids and their salts from the broth after biosynthesis with microorganisms

Just after hiring at Food Biotechnology Department of Faculty of Engineering and Economics I was enabled to work with the industry by starting work on the assignment of the sugar factory in Racibórz, which intended to implement the technology of citric acid biosynthesis and recovery (II. E. 2, III. M. 1). The subject of my research work became a technology of recovery of citric acid from broth after biosynthesis involving mold *Aspergillus niger.* In view of the target value economy of the process technology from the beginning aimed at use of physical factors, such as temperature, pH or clearing compounds addition for initial purification of solution after the biosynthesis, to then get a citric acid or soluble citrate salts by crystallization directly from the solution. Such a method would represent an important alternative for the existing process of extracting citric acid, which consisted of the neutralization the solution with calcium oxide following by obtaining of insoluble calcium citrate, physical separation of it from the solution, and then decomposition of the salt obtained with the addition of sulphuric acid, as a result pure aqueous solution of citric acid is received headed next to the crystallization and resulting in great quantities of waste which was calcium sulphate named gypsum. Method of direct isolation of citric acid from broth after biosynthesis, especially on substrates containing molasses, represented the actual need for industry and pro-ecological, low waste alternative to the existing process.

For the production of citric acid from pure substrates such as white sugar, glucose, glucose syrup and starch, it is possible to apply the simplified ie. non citrate methods of isolation of citric acid. To obtain pure crystals of citric acid with high performance it is necessary preliminary removal of proteins, mineral salts and substances. Clarification

processes were investigated using bentonites, diatomaceous earth, silicic acid zol (Klar-Sol Super), as well as the effect of temperature was estimated. Studies have shown that after the separation of the mycelium is advisable the use of heat treatment of broth at temperature of 70 ° C during 30 min. The heat treatment lowers the color of purified solutions, reduced protein content by about 80% and turbidity of 70%. The best results from the studied substances were obtained for bentonites. Also good purifying performance was obtained with silicic acid zol (Klar-Sol Super, Erbsloh, Germany). The add-in of 0.25% of this compound to post fermentation reduced in 90% turbidity and more than of 60% reduced the content of proteins (II. D. 5, II. D. 8, III. B. 1, III. B. 3, III. B. 9, III. B. 12). In order to obtain sodium and potassium citrates the experiments conducted used sugars and molasses post fermentation broths obtained by submerged fermentation with mold *Aspergillus niger.* During the study were measured: acidity, citric acid content, the pH of the solution, the amount of protein and yield of sodium citrate synthesis. The post fermentation broth made on sugar substrates were led to a pH of 3.5; 4; 5; 5.2; 6; 7; 8. 9; 10. 12. 13 and 13.5, and then were further processed at 60, 70, 80 and 90 °C. The post fermentation broth made molasses due to the different initial conditions were treated firstly with calcium and then from calcium citrate obtained the calcium were removed with sodium from sodium carbonate addition resulting in sodium citrate solution and precipitates of calcium carbonate. Studies have confirmed the possibility of receiving sodium citrates directly from the post fermentation broth using the right combination of pH and temperature of the solution to achieve the maximum precipitation of protein substances and obtaining clear colourless crystals of citrates. The method is also characterized by a negligible amount of waste and a little negative impact for the environment (II. D. 4, II. E. 6, III. B.4, III. B.7, III. B.8). The physical agents used for the cleaning of solution after the biosynthesis of citric acid was also used polieterosulfic ultrafiltration membranes which resulted in obtaining a high degree of solution purification (II. D. 6, III. B. 10, III. B. 13). Study on citric acid recovery from postbiosynthesis broth obtained with different micro-organisms have been done in cooperation with the Department of Biotechnology and Microbiology of Foods, Faculty of Food Sciences, Agricultural Academy in Wrocław, with a team of Prof. dr hab. inż. Waldemar Rymowicz, from who I received a broth after the biosynthesis of citric acid with yeast *Yarrowia lipolytica* (II. D. 7, III. B. 6, III. B. 11). Received preliminary results became the basis to write, and the settlement of the PhD grant from the Ministry of Science and Informatics (II. I.2, II. E. 8), to write and defend a doctoral thesis (II.E.7) and the development of it-based 3 patent applications that received patents (II. B. 1, II. B. 2, II. B.3). After PhD defense I continued research in the field of mastered methodologies of extraction of fluids after biosynthesis involving *Aspergillus oryzae* for obtaining another organic acid with increasing industrial concern kojic acid (II. D. 9, II. D. 10, II. D. 11, II. E. 9, III. B.14, III.B.15, III. B.16, III.B.17, III.B.18).

2. The use of biotechnology in production processes

The use of biotechnological methods in creating technology was a natural step in my development, ranging from the Wroclaw University of Technology where I defended the Master thesis about the degradation of model petroleum wastewater using mixed bacterial culture isolated from the petrol station (II. E.1). Following the next request from the industry - I conducted research on the development of the analytical control methods of the process of biosynthesis of L-lysine (II. E.3), obtaining of pure cultures of *Bacillus flavus* (II. E. 4) and establishing the fermentation conditions and the composition of the fermentation medium (II. E 5) and participated in an International

Conference in the Netherlands entitled International Workshop for Cleaner Production (III. Q. 1), which allowed me to gain current knowledge in this area and resulted in writing the review paper on the use of biotechnological methods in developing clean technologies (II. D. 1). This topics is still active for the entire duration of my scientific work and carry out tasks within the framework of cooperation with other employees of the Food Biotechnology Department (now Department of Biotechnology and Food Analysis) for the feed yeast cultivation on stillage (II.I.4) or beta carotene biosynthesis in yeast *Rhodothorula rubra* (II.D.38, III.B.53, III.B.54, III.D.41, III.D.43, III.B.59). I have also cooperated with the business entity for extraction of astaxanthin from breeding algae *Haematococcus pluvialis* (III.A.11) and the process of stimulation of the micro-organisms in the biosynthesis of carotenoids (II. I. 9).

3. Cereal biorefinery - concept, resources and perspectives

Appropriate management of vegetable raw materials and the reusage of waste from the food industry is complementary to the other interests of my research (II. D. 2, II.D.3, III.B.2). In 2005, I started working with the economic operator concerned the development of the concept of cereal biorefinery technology producing products with high added value from cereal grain raw material. As a result of the three projects conducted in cooperation with the business operator, in two of which I was the head (III. F.1, III.F.2) and I received 2 awards of the Rector of the Wroclaw University of Economics for working with industry practice (II.J.2, II.J.3) have been developed: studies on the state of the art in the field of oats *Avena sativa* biorefining (II. E. 10), the theoretical process of isolation of β-D-glucan from oats (II. E 11), was carried out the assessment of suitability of the oat varieties available on the Polish market to biorefining process (II. E 12) and I designed and verified the technological process of isolation of β-D-glucan from oats in the laboratory scale (II. E. 13), and as a result of this the work was made a patent application, which in 2014 received a patent - part of my achievement (n°1). In the third project with the same economic entity, in which I was the main contractor, I elaborated the technological process of barley *Hordeum vulgare* biorefining in order to produce high-value compounds (II. E 14). Continuing the cooperation with the industry, I became a member of the Nutribiomed cluster, first as a representative of the business entity, and then as representative of Wrocław University of Economics, that due to my recommendation join the Cluster, and where I work as a WUE representative in the Cluster Council and I am member of the Cluster Steering Committee. This activity has resulted in cooperation with other entities of Cluster, with who I participated in the preparation of the project proposal submitted by the Wrocław Technology Park sp o.o. on behalf of the Cluster Nutribiomed, for which I drew up technical and technological assumptions for biorefining process of oat and derivatives, and a processing line (II.E.15), and after receiving funds from the Polish Agency for Enterprise Development in 2008, within the program POIG 5.1. (II.I.5) I co-created with dr. Łukasz Bobak of the Department of Technology Resources of Animal and Quality Management of prof. Tadeusz Trziszka from the Department of Food Science of the University of Life Sciences and MSc. Julian Rozwadowski from the company Chemiprojekt sp o.o. the process design system for the production of dietary supplements for the Wrocław Technology Park (II. E 16, III. M. 3). On the basis of the work carried out with business entity which was one of the founding members of the Cluster Nutribiomed was developed and transferred, whithin this project, the oats biorefining technology and the production line, which was covered by Polish patent application (III. Q. 5), and then in the procedure of the EURO-PCT has received a European patent that is part of achievement (n°2). At the same time I

did further work on the development of the concept of use cereal grains as raw material in cereals biorefinery (II. D. 23, II. (D). 24, II. D. 31, II. D. 33, II. D. 34) and, in particular, oats (II. (D) 18, II. D. 25, II. D. 26, II. K. 4, II. K 8), barley (II. E 17), rye (II. D. 32), teffu (II. (D) 37) or other cereals (II. (D). 36), as well as their by-products as wheat bran (II. D. 16, II. D. 32, II. D 35). Developing the concept of cereal biorefinery I also used pseudocereals, mainly buckwheat (II. (D) 12).

4. The metabolic activity of oat beta-glucans of varying molar mass

The possibility of receiving highly purified β-D-glucan preparations with different molar mass has made it possible to plan and carry out a series of research study using the pure substance. In cooperation with Prof. Małgorzata Kozlowska-Wojciechowska from the Warsaw Medical University the impact of beta-glucan with high molar mass obtained by patented technology (n°2) on cell apoptosis of melanoma skin HTB-140 was examined (II.A.8), and the results obtained led to the creation of a patent application (III.Q.5) protecting the use of the purified fraction for this purpose. Also β-D-glucan of low molar mass obtained by technology that is described in the publications from my achievement (n°4) showed the ability to induce apoptosis of malignant melanoma cells Me45 and skin cancer A431 (II.A.7, III.B.42, III.B.43, III.B.44), which has been established as a result of cooperation with the team of Prof. dr. hab. Jolanta Saczko and team of Medical Biochemistry Department at the Medical University of Piastów Śląskich in Wrocław.
Highly purified preparations with varying molar mass have been tested thoroughly in cooperation with Prof. dr. hab. Joanna Gromadzka-Ostrowska (Project Manager) from the Chair of Nutritional Physiology, Department of Dietetics, Faculty of Human Nutrition and Consumer Sciences within research grant funded by the National Center for Science NN 312427440 entitled *Impact of beta-glucans on the inflammatory process in the digestive tract.* The aim of the research project was to evaluate the anti-inflammatory, immunomodulating and metabolic activity *in vivo* in the gastrointestinal tract of two purified with different molar mass fraction of β-D-glucan from oat. The assessment was made in both people with chronic inflammation layer lining of the stomach, and in the animal model, where inflammation of the lining of the stomach or intestinal layer was induced experimentally. A wide range of research carried out during the project allowed for credible verification of the hypothesis of an important pro healthy activity of preparations, and the results obtained are significant and well documented rationale for the application of oat β-D-glucan, especially of low molecular mass, in practice. In the first stage of the project has been developed the technology of controlled methods of obtaining of β-1-3, 1-4-D-glucan with molecular mass fixed in two ranges. The method to receive a fraction of β-D-glucan oat was published [poz.4], and is the subject of patent application. Results from studies in humans have shown inter alia reduction in characteristic parameters of inflammation (including the concentration of C-reactive protein), and were presented at a scientific conference organized as part of United European Gastroenterology Week held in Vienna in October 2016. The (III.B.55, III.B.56), and the rest are currently being prepared for publication. An important part of the project base accounted from *in vivo* studies using animal models (rats adult males Spraque Dowley), part of which was experimentally induced inflammation of the stomach wall (application of sodium deoxycholate), part - inflammation of the intestines (intravenous LPS), a part constituted a control group. The study showed that the addition of 1% (w/w) of β-D-glucan has anti-inflammatory activity and relieve oxidative

stress in the blood and tissues of rats with colonic induced enteritis ((II.A.4, II. K.16, II.K.17, II.K.19, II.K.20, III.B.26, III. B.28, III.B.36, III.B.37, III. B.38, III.B.40, III.B.41), since the bacterial lipopolysaccharide injection resulted in systemic blood inflammatory response that was alleviated by supplementing with food β-D-glucan. The administration of β-D-glucan resulted in a reduction in blood cell populations involved in the inflammatory response, as well as reducing blood lipid peroxidation and increasing blood antioxidant defense. It was also observed anti-inflammatory and anti-oxidant activity of, β-D-glucan from oats in colonic tissue in rats with induced enteritis (II. A.6). Antioxidant effects of oats β-D-glucan in animals with intestinal inflammation has also been in other organs, m.in. in liver and stomach (II. A. 1, II. K. 18, III. B 23, III. B.24, III. B 25, III.B.27, III. B. 35, III.B.39) and spleen (II. A. 5, III.B.29), and especially the important antioxidant activity was revealed by low molar mass β-D-glucan preparation. Obtained as the results of the project have inspired to bring new research hypotheses on the effects of β-D-glucan of varying molar mass on inflammation in the large intestine, which resulted in the submission and reception of the next research grant from the National Science Centre under the program Opus (II. J.10).

5. Cereals and pseudocereals in functional foods

The interest in functional foods constitute a natural stage in the development of my research interests (II. A. 9, II. D.14, II. D. 15, II. D. 19, II. D 39, II. D.41, II.D.42, II. D.43, II. K. 2, II. K. 3, II. K. 5, III. B 60). To develop functional products I used the cereal and pseudocereal raw materials heading towards the food for people suffering from celiac disease. Because of this, I used the oats (II. (D) 20), and in fact oat bran to produce dry wafers (II. A. 3, III. B 22, III. B.31), as well as the oat cookies (II. K. 14, II. D. 30, II. K. 14) in cooperation with the economic operator (III. A. 6, III. A. 9, III. A. 10), or oat bran concentrate as a component of extruded bread (II. E 18, III. F. 3), but also oat grains as a raw material for the production of malt (II. D. 17). Also oat beta-glucan was used by me in the study of formulating food products as fat-free mayonnaise-in cooperation with the economic operator (II.E.19, III.F.4), beer (III.B.33, III.Q.5), gluten-free bread in cooperation with Prof. dr hab. inż. Halina Gambuś from the Carbohydrate Technology Department of Agricultural University in Krakow (II.K.10) or innovative fruit snacks in cooperation with a team of Porf. Marco Dalla Rosa of the University of Bologna, Italy (III. B.56). Other material suitable for the development of food products for people with celiac disease is buckwheat (II.B.52), which the nutritional characteristics (II.D.13, II.K. 1), the use in the manufacture of brewing malts (III.B.32) and the market potential of gluten-free beers (II.A.2, II.D.17, II.D.28, II.K.24, III.B.34) supervise a the scientific tutoring for research assistant that conducts his PhD thesis at the Department of Biotechnology and Food Analysis (III.K.1). The development of functional products with a high share of fiber is also suitable a byproduct of its typical processing - buckwheat hulls, on the basis of which I have developed a number of food products (II. D.29, II.K.9, II.K.13), covered by patent declarations (III.Q.5), where the manufacturing technologies have been transferred with license agreements (III.Q.7).

6. The physical agents as assistance factors

Physical agents as parameters supporting technological processes constitute the significant part of my research interests from the very beginning (II.D.27, II.K.11). Were firstly used by me in the course of research on extracting organic acids, then in the process of isolation of β-D-glucan. Using ultrasonic cavitation I have developed an improved economic isolation technology of very pure β-D-glucan from oats as part of my

achievement (n°3). I have studied the cold plasma treatment impact on wine (III.B.20) on germination of buckwheat (II.K.21). I studied static magnetic field impact on amylolutic activity of sprouting buckwheat (III.B.50). In 2011, I began research on using microwave radiation as a means of supporting the extraction of β-D-glucan and substances having an antioxidant activity from oat seed of *Avena sativa*. To test these I received research grant funded by the National Science Centre number NN312 506640 (II. I. 7). As a result, the realized research project examined and described the use of microwave radiation as a factor supporting the extraction of active ingredients from raw material of oat. For experiment the raw materials were selected with appropriate content and mutual relationship of the desired active compounds (polysaccharides, β-D-glucan, antioxidant compounds), and were characterized in terms of suitability for the extraction the various milling fractions of oats grains. Microwave radiation was applied at the stage of preparation of the material for the extraction and during extraction using a dedicated device microwave extractor. The effect of microwave radiation on selected parameters of the extraction process and the characteristics of the extracted material - polysaccharides, and, in particular, 1-3, 1-4-β-glucan and compounds with antioxidant were specified. The results of the analysis of literature and the experiment results were presented in the form of scientific reports [II.D.21, II.D.22, II.E.20, II.K.6, II.K.7, II.K.12, II. K.15, II.K.22, III.B.19, III.B.21, III.B.45, II.B.46, III.B.47, III.B.48, III.B. 49] and manuscripts are under the reviews process. Very promising application of microwave radiation to induce changes in gluten-free flours I use currently in the framework of the implementation of the Individual Fellowship of Marie Skłodowska-Curie from the Horizon 2020 (II.I.11, III.B.59) at University of Valladolid, Spain.

6. Synthetic characteristics of professional achievements

A detailed list of the published scientific works is presented in annex 3. My legacy includes 196 works, including:

-53 the original papers, not included in the scientific achievement,
-16 works of popular science
-84 scientific presentations at international (30) and national (54) conferences
-5 granted patents and 8 proceeded patent applications
-30 substantive reports and expert opinions

The total number of points is 741, IF 35.08, Hirsch index 4, the number of citations according to WoS 41

I had a total of 10 internships, including 2 stays at national science centers, 3 in international science centers and 5 in business entity. I have completed the 8 training schools about the protection of intellectual property, as well as I received a grant under the program Patent Plus, under which I completed a 3 year patent attorney application (2012-2015), and in 2016 I positively pass the national examination for patent attorney. Additionally, in 2013, I graduated from postgraduate study for R&D Project Manager.

I actively participated part in 25 international conferences taking place in Poland, Slovakia, Italy, Greece, Germany, Austria and Ireland. Two times I have presented lectures within the framework of the Erasmus + program at foreign universities- University of Bologna and University of Sassari, Italy.

I attended and I participate in 5 national research projects (MNiI, NCN and NCBiR) in one as a Manager, the others as a coworker. I also managed 3 grants within the special-purpose grants of Wroclaw University of Economics awarded within the framework of the development of researchers in the years 2000-2010. I am working on three research projects in international programs – one as a Manager, two as a member of the working groups.

I am a member of the national (PTTŻ, NOT SITSpoż) and international (IFA, AACCI, ACS) scientific societies, the reviewer in the national programs (NCBiR), foreign programs reviewed in Poland (NBCiR) and international programs (COST) and actively participate in the national (cluster Nutribiomed) and international (POSITIVe, EUROCAROTEN) research networks.

So far I've made a total of 136 reviews for international journals such as: Food and Bioprocess Technology, Food Research International, Journal of Cleaner Production, Bioactive Carbohydrates and Dietary Fiber, Journal of Nutrition Science, and Behaviors, Journal of Cereal Science, Journal of Food Science and Technology, Journal of Food Processing and Preservation, Nutrition Journal, Journal of Food Protection, Carbohydrate Polymers, Food & Function, Journal of Food Composition and Analysis , Food Chemistry, European Food Research and Technology, the European Journal of Nutrition (a detailed list in annex 4. III. P.). In 2016 I received from Elsevier Publisher – the Certificate Outstanding Rewiever and two times (2015, 2016) Certificate Recognized Rewiever for quality of made reviews.

I am the scientific tutor of the reasearch Assistant pursuing his doctoral thesis in the Department of Biotechnology and Food Analysis, at WUE, secondary promoter of PhD thesis of PhD candidate at Chair of Nutritional Physiology, Department of Dietetics, Faculty of Human Nutrition and Consumer Sciences, Warsaw University of Life Sciences (SGGW), and the external reviewer in the doctoral proceedings on the title Doctor Europeus of PhD candidate of the University of Sassari.

For the research and scientific activity I was awarded 9 times of the Individual Award of the Rector of the UEW for scientific and teaching, 1 time – First Award for Team of the Rector of SGGW in Warsaw for the publication series, together with the employees of the two other SGGW departments, 2 - times an individual Prize of the Rector of the UEW for scientific cooperation with industry, 2 - times the prize of the Council of Scientific and Technical Associations Federation of Wroclaw NOT in the contest named"The most outstanding achievements in the field of technology, in 2008 I received the Bronze Medal for Long Service from President of Poland, and in 2011 the Silver Badge of Honour of NOT.

I actively participate from the beginning at the administrative work at the University of Economics in Wrocław: as a delegate of the Rector to Organization of the 50th anniversary of the University; a member of the team hosting the inauguration of the academic year (for which I received the prize of the Rector); Secretary, and later President of the selection board for studies admittion; a member of the Electoral Commission at the Faculty of Engineering and Economic; a member of the Council of the Faculty of Engineering and Economic, Member of the Scientific Council of the Institute of Chemistry and Food Technology at Faculty of Engineering and Economic, President of

the mobility team in the field of food technology, Secretary of the Faculty team for the development of doctoral studies at the Faculty of Engineering and Economic, Doctors Representative in the team for modify the detailed procedure for performing the doctorate at Faculty of Engineering and Economic.

Since the beginning of the work in the department I teach of core and specialization courses as well as courses for doctoral studies. I was the tutor of students scientific trips, mentor of students' scientific association, tutor of students' practical stays, I have reviewed 29 of Master and Bachelor theses and have promoted 7 Bachelor and 32 Master theses, of which 3 were awarded at the contest. I was also the tutor of Master practice of the student from the University of Sassari under Erasmus+ program.

Tabulation of published scientific papers mentioning the number of points from the list of Impact Factor and the Ministery list for journals.

A quantitative list of the most important achievements of scientific research			
List of achievements	**Pre-doctoral**	**After PhD**	**A total of**
Peer-reviewed publications total	**3**	**50**	**43**
Publications in magazines in *Journal Citation Reports* ® indexed by *Thomson Reuters* ® *Web of Science* ®	**0**	**11**	**11**
Scoring for the publications by list MNiSW along with scores for the granted patents and applications	**7**	**734**	**741**
Thomson Reuters ® *Web of Science* ® Hirsch's Index	**0**	**41/4**	**41/4**
Elsevier ® *Scopus* ® Hirsch's Index	**0**	**52/4**	**52/4**
Patent for an invention granted by RP/granted abroad for the unit, where an employee is the creator of an invention/implementation w	**0**	**4/1/2**	**4/1/2**
Patent for an invention granted by POLAND or abroad for an entity other than the scientific unit, where an employee is the creator of an invention	**0**	**1**	**1**
Patent application at UP RP or abroad by a scientific body, which employee is the creator of an invention w	**0**	**4/3**	**4/3**
Participation in national conferences / oral presentations/posters presented	**1/0/5**	**12/19/25**	**13/19/30**
Participation in international conferences oral presentations/posters presented	**1/0/3**	**1/5/27**	**2/5/30**
Works for industry	**6**	**7**	**13**
Scientific documents in the form of reports with the implementation of research projects (in this reports from grants)	**7**	**13**	**20**
Research projects MNiSW/NCN/innovative discovery	**1**	**3/1**	**1/3/1**
EU-funded projects	**0**	**3**	**3**

Foreign internships scientific research	**0**	**3**	**3**
National scientific-research internships	**0**	**7**	**7**
Awards and recognitions	**0**	**12**	**12**

Scores of achievements by MNiSW list and Ministry regulation for evaluation of scientific body

Publications in peer-reviewed scientific journals in *Journal Citation Reports* ® indexed by *Thomson Reuters* ® *Web of Science* ®

Journal title	Number of publications	Points from MNiSW list	The sum	The IF
Journal of Cereal Science	**1**	**35**	**35**	**2.402 2,699** *2015 5 year*
Journal of Functional Foods	**2**	**45**	**90**	**4.269 3.973** *2015 5 year* **7,946 8.538**
International Journal of Biological Macromolecules	**3**	**25**	**75**	**3,138 3.22** *2015 5 year* **9.414 9.44**
Food Hydrocolloids	**1**	**45**	**45**	**3.858 4,703** *2015 5 year*
Food & Function	**1**	**30**	**30**	**2.686 3,178** *2015 5 year*
Journal of Cleaner Production	**1**	**40**	**40**	**4.959 5,315** *2015 5 year*
Cereal Foods World	**1**	**20**	**20**	**0.889 0.658** *2015 5 year*
Nutrition	**1**	**30**	**30**	**2.926 3.026** *2014 5 year*
A total of	**11**		**365**	**35.08 37.557**

Publications in peer-reviewed journals listed on list B MNiSW

Journal Title	The number of the publication	Points list Ministery	The sum of the points
Przegląd Zbożowo-Młynarski	**10 (2015 – 7, 2011-3)**	**2015 –6**	**42**
Nauki Inżynierskie i Technologie	**5 (2009-6, 2010-18, 2010-18)**	**2009 – 6, 2010 – 9, 2011 – 9)**	**42**
Ekologia i Technika	**1**	**1**	**1**
Przemysł Fermentacyjny i Owocowo-Warzywny	**1**	**5**	**5**
Nasza Rola	**1**	**2**	**2**
A total of	**18**		**92**

Chapters in monographs

The title of the monograph	The number of chapters	Points from MNiSW list	The sum
Choroby cywilizacyjne i społeczne XXI w. – przegląd	**2**	**4**	**8**

i badania			
Postęp cywilizacyjny – stan obecny i perspektywy	**2**	**4**	**8**
Rośliny w medycynie, farmacji i przemyśle	**3**	**4**	**12**
A total of	**7**		**28**

Other compact, peer-reviewed publications in the English language of annual International Conference			
The title of the Conference	The number of full-text publications	Points from MNiSW list	The sum
International Conference of Slovak Society of Chemistry	**12**	**3**	**36**
A total of	**12**		**36**

Patents granted by the Patent Office of the Republic of Poland, foreign offices, patent applications and implementations

Patents granted by the Polish Patent Office and foreign offices

Patent number/year grant	**According to MNiSW points[x, y]**
PL 203171 /2008	**25**
PL203172/2008	**25**
PL203173/2008	**25**
PL217750/2014	**25**
EP2515672B1/2016	**40**
PL/224430/2016	**15**
A total of	**155**

Patent applications

Application number/year of filing	**According to MNiSW points[x, y]**
384594/2008	**3**
389995 /2009	**2**
393523 /2010	**2**
EP 10903993.4/2014	**2**
393733 /2011	**2**
WO/2012/091581	**2**
WO/2012/102630	**2**
A total of	**15**

The implementation of inventions from patents and patent applications		
The number of the deployed patent/application	**Implementing company**	**Points from MNiSW[x]**
PL217750	**PW Futurum Ltd**	**25**
EP2515672B1	**PW Futurum Ltd**	**25**
A total of		**50**

A total of	**POINTS = 741**	**If = 35.08** **If (5) = 37.557**
In this the scientific achievement, as referred to in article 16 paragraphs 1 and 2. 2. The Act of 14 March 2003.	**160**	**6,375**

[in]- according to the Regulation of the Ministry of 17 October 2007.
[x]- according to the Regulation of the Ministry of 13 July 2012.
[y]- according to the Regulation of the Ministry of 27 October 2015.