Institute of Soil Science
and Plant Cultivation
State Research Institute

Summary of Professional Accomplishments

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Pulawy, 2020

1. Name

Iwona Kowalska

2. Diplomas, degrees conferred in specific areas of science or arts, including the name of the institution which conferred the degree, year of degree conferment, title of the PhD dissertation.

15.06.2004 - Master's degree in food technology and human nutrition.
Academy of Agriculture (currently University of Life Sciences) in Lublin, Faculty of Agriculture, major: food technology and human nutrition, specialties: food analysis. Diploma thesis entitled: ,,Changes in vitamin C content, phenolic compounds, their antioxidant and L-phenylalanine ammoniacolysis activity during lettuce storage, in control plants and treated with calcium ions'' under the direction of prof. Irena Perucka.

30.06.2009 - Doctor of Agricultural Sciences in agronomy.
Given by the resolution of the Scientific Council of IUNG-PIB in Puławy, dated 30.06.2009. Doctoral thesis titled ,,The title of doctor of agronomy". The dissertation entitled "Composition and content of phenolic compounds in above-ground parts of *Medicago truncatula* Gaertner''.
Promoter: dr. Anna Stochmal
Reviewers: prof. Irena Perucka,
prof. Wiesław Oleszek.

3. Information on employment in research institutes or faculties/departments or school of arts

01.11.2004 - 30.04.2005 - <u>trainee</u> at the Department of Biochemistry and Crop Quality, IUNG-PIB in Pulawy,

01.05.2005 - 30.04.2007 - <u>technician</u> in the Department of Biochemistry and Crop Quality, IUNG-PIB in Pulawy,

01.05.2007 - 31.03.2009 - <u>specialist</u> at the Department of Biochemistry and Crop Quality, IUNG-PIB in Pulawy,

01.04.2009 - 30.06.2009 - <u>assistant</u> at the Department of Biochemistry and Crop Quality, IUNG-PIB in Pulawy,

01.07.2009 - 30.06.2013 - <u>senior research and technical specialist</u> at the Department of Biochemistry and Crop Quality, IUNG-PIB in Pulawy, 01.07.2013 - until now - assistant professor at the Department of Biochemistry and Crop Quality, IUNG-PIB in Pulawy,

01.06.2020 - until now - head of the Department of Biochemistry and Crop Quality, IUNG-PIB in Pulawy.

4. Description of the achievements, set out in art. 219 para 1 point 2 of the Act

a) a title of scientific achievement:

,,Phytochemical characterization and biological activity
of wheat (*Triticum* L.)''

b) List of publications constituting a scientific achievement

The basis of the scientific achievement is a series of five single-issue, original scientific publications, whose total *Impact Factor* (IF) according to the year of publication is **13.365**, and the number of points according to the list of the Ministry of Science and Higher Education (MNiSW) is **325** (according to the year of publication) and **420** (according to the list of 18.12.2019).

1. **Kowalska I**.* **(65%),** Pecio L., Cieśla L., Oleszek W., Stochmal A.: Isolation, chemical characterization, and free radical scavenging activity of phenolics from *Triticum aestivum L*. aerial parts. Journal of Agricultural and Food Chemistry, 62 (46), 11200–11208, **2014.**
   IF_{2014} = 2.912, IF_{2019} = 4.192
   Points $MNiSW_{2014}$ = 45, $MNiSW_{2019}$ = 140
2. **Kowalska I**.* **(60%),** Jędrejek D., Jończyk K., Stochmal A.: UPLC–PDA–ESI–MS analysis and TLC–DPPH· activity of wheat varieties. Acta Chromatographica, 31, 2, 151–156, **2019**.
   IF_{2019} = 1.418
   Points $MNiSW_{2019}$ = 20
3. **Kowalska I**.* **(70%),** Kowalczyk M.: Determination of benzoxazinoids in spring and winter varieties of wheat using ultra-performance liquid chromatography coupled with mass spectrometry. Acta Chromatographica, 31, 3, 179–182, **2019**.
   IF_{2019} = 1.418
   Points $MNiSW_{2019}$ = 20
4. **Kowalska I**. **(50%),** Adach W., Stochmal A., Olas B.: A comparison of the effects of apigenin and seven of its derivatives on selected biomarkers of oxidative stress and coagulation *in vitro*. Food and Chemical Toxicology, **2020**, 136, https://doi.org/10.1016/j.fct.2019.111016

IF_{2019} = 4.679

Points $MNiSW_{2019}$ = 100

5. **Kowalska I**.* **(65%),** Jędrejek D.: Benzoxazinoid and alkylresorcinol content, and their antioxidant potential, in a grain of spring and winter wheat cultivated under different production systems. Journal of Cereal Science, **2020**, https://doi.org/10.1016/j.jcs.2020.103063

   IF_{2019} = 2.938

   Points $MNiSW_{2019}$ = 140

* - publications in which I'm a corresponding author

Notwithstanding the above compilation, copies of the publications constituting the scientific work are set out in **Annex 3** and the co-authors' declarations stating their respective contributions to these publications are set out in **Annex 5**.
The above mentioned works constituting the habilitation achievements are discussed below (point 4c) according to their numbering (**1 - 5**). The supplementary literature quoted in the text is placed at the end of the description of the scientific achievement.

c) Discussion of the scientific objective of the above mentioned work and the results achieved, including discussion of their possible use.

As a head of project and main contractor of the project no. 2011/01/D/NZ9/04684 entitled ,,Characteristics of wheat secondary metabolites (phenolic compounds, saponins, hydroxamic acids) as an element of cereals' functional genomics'', I have carried out research work and conducted in-depth literature studies on the aerial parts and spring and winter wheat varieties, grown in different farming systems. I published the obtained results in the form of a series of publications (**1-5**), which I consider to be my greatest achievement in scientific activity to date and submit as a basis for applying for the postdoctoral degree.

Introduction and description of the purpose of the work

Wheat (*Triticum* L.) is the cereal of greatest economic importance both in the world and in Poland. This is due to the versatility of grain use as well as its high production potential. Among more than 20 known subspecies of wheat, common wheat (*Triticum aestivum* L.) dominates in cultivation. The grain is used mainly for consumption purposes, e.g. for production of bread, pasta, cakes, culinary products, breakfast cereals, dry gluten or malt. Epidemiological studies confirm that the consumption of whole cereal grains and whole-grain food products is associated with the reduction of the risk of civilization diseases (Liu et al., 2012). The by-products of milling, mainly bran, are a valuable concentrate feed for monogastric animals. Straw is often a raw material for energy purposes.

The contribution of natural substances (secondary metabolites) to the interaction of plants with the environment can be varied and often contradictory. On the one hand, they can serve as a defense against pathogens, herbivores or other plants, on the other hand, they can serve as attractants attracted by color or smell. Similarly, the production of natural substances may be the result of abiotic factors. In all cases of environmental changes, the plant reacts with changes in the dynamics of biosynthesis or degradation of natural substances. These changes can't always be clearly correlated and justified. It is probably most difficult to react with pathogens, because natural products do not act specifically, but show a wide spectrum of biological activity, have a long evolutionary history and thanks to the existence of appropriate enzymes in nature are quickly degradable. Nevertheless, learning about the mechanisms of plant resistance at the metabolic level is still a big challenge for science, despite many published scientific papers on the subject. Correlating the dynamics of the accumulation of certain metabolites with the expression of specific genes allows a better understanding of these mechanisms, as well as the molecular basis for the formation of qualitative traits, and perhaps in the future will allow genetic manipulations that will lead to full immunity and the use of plant protection products in plant production will become superfluous.

Wheat is of interest to geneticists in many scientific institutions. Molecular studies carried out on cereal plants are extremely difficult, due to their large genome (Bossolini et al., 2007). The research work aims at obtaining a complete picture of the organization of genetic material. Particular emphasis is placed on the identification of coding sequences and recording them in an international gene bank, as well as monitoring and identification of secondary metabolites (metabolomics) (Fiehn et. al., 2000; Sumner et. al., 2003). Wheat varieties are one of the largest groups in the gene bank, with about 800 000 records. Borner et al. (2010) demonstrated the usefulness of creating a gene bank in determining those genes that are responsible for the important agronomic characteristics of wheat. In order for this direction of research to develop, it is necessary to first characterize the species phytochemical characteristics and develop analytical methods. This is an important aspect in the relationship between the plant and its surroundings and also has an impact on the health of cereal products, mainly from whole grain. One of the groups of these compounds that are of wider interest are phenolic compounds. They belong to the group of antioxidants found in larger quantities in cereal products and from the point of view of their health-promoting properties and the proportion of cereals in the diet, they are of significant preventive and curative importance in various disease units. These compounds also have a protective function in plants against pathogenic infectious agents. Their insecticidal activity is associated with their effect as food detectors, digestive inhibitors and direct toxicants. Their content can be influenced by several factors, such as plant species, variety, growth phase, or physiological and environmental factors (soil, agronomy, climate) (Carbone et al., 2011).

The content of hydroxamic acids is also very important, as they play a very important role in shaping the natural resistance of plants to agrophages (Adhikari et al., 2015). Knowledge of this fact can be an important determinant in the actions of growers looking for sources of resistance in the starting materials for breeding. This can also be reflected in agrotechnical activities, taking into account the use in practice of crop afterbirds from plants containing large amounts of hydroxamic acids or their derivatives. Due to the fact that wheat grain is the basic cereal for consumption in Poland, and the food law allows for marking food products with nutrition and health claims, it encourages the search for wheat varieties characterized by a high content of bioactive components with health-promoting properties. Such substances include alkylresorcinol (resorcinol lipids), compounds considered as biological markers of healthy food (Ross et al., 2004). Epidemiological studies confirm that the consumption of whole cereal grains and whole-grain foods is associated with a reduction in the risk of diabetes, obesity, coronary heart disease, stroke and even certain types of cancer (Korycinska et al., 2009). A detailed knowledge of the protective mechanisms of components contained in cereal grains requires the presence of such a biomarker, which would enable to track the fate of wholesome cereal products after they are consumed and absorbed. On the other hand, they play the role of a specific barrier that ensures the effective survival of seeds during the resting period. Their phytoncidic activity protects the seeds from external threats during germination, harvest and storage (Konopka et al., 2017).

The primary objective of the conducted research was to get a thorough phytochemical knowledge of the most frequently cultivated grain in Poland, that is wheat (*Triticum* L.). The genus *Triticum* includes over 20 wheat species and many thousands of agricultural varieties. The largest number of types of this cereal is undoubtedly related to the age of wheat (the oldest next to barley) and the ease of crossing with other types of plants within the grass family.

Detailed research topics included:

- isolation, identification and determination of structures of isolated phenolic compounds from aerial parts of *Triticum aestivum* L. var. Legend,

- determination of biological (antioxidant) activity of phenolic compounds and whole wheat extracts (TLC-DPPH• test),
- development of methods for quantitative determination of phenolic compounds (UPLC-PDA-ESI-MS) and benzoxazinones (UPLC-MS/MS) in aerial parts; alkylresorcinols (UPLC-PDA-ESI-MS) and benzoxazinones (UPLC-MS/MS) in grain, winter and spring wheat varieties,
- qualitative analysis of the aerial parts of wheat for the content of phenolic compounds and benzoxazinones,
- qualitative analysis of wheat grain for alkylresorcinols and benzoxazinones,
- determination of the content of individual secondary metabolites in the aboveground and grain parts, in winter and spring wheat varieties, depending on the farming system (organic and conventional).

Research material

The research material described in the selected series of publications came from many years of research conducted in an experimental facility located in the experimental station IUNG-PIB in Osiny, Lublin Province (geographical location -51° 52'02"N, 22°05'23E, height - 155 m above sea level). Location of the experimental fields in one location allows for conducting observations and studies in identical habitat conditions limiting the influence of variability related to soil or meteorological conditions. An important element is also the fact that the object has been functioning since 1984. This fact results in the fact that, apart from the value of the site under comparable habitat conditions, the research was conducted under constant methodological assumptions concerning the functioning of various production systems.

The material for the study consisted of aerial parts of wheat in the BBCH 47 phase (open leaf vagina of the flag leaf) and grain, both winter and spring varieties, grown in the organic and conventional system. In the organic farming system (potato - spring wheat - red clover with grass grown for two years - winter wheat + catch crop) no seed dressing, mineral fertilization, herbicides, fungicides and growth regulators were applied. Fertilization included only manure application (30 t/ha) before potato cultivation. In the conventional farming system (winter rape - winter wheat - spring wheat), intensive plant production technology was conducted, characterized by, among others, high consumption of industrial means of production. These two production systems were located on the same soil type. Wheat was grown on experimental fields of 1 ha, for each farming system. Sowing and harvesting conditions were the same for all tested varieties.

Methodology and discussion of research results

The first aim of my research was phytochemical analysis of the aerial parts of wheat. As a result of the extraction of the aerial parts of *Triticum aestivum* L. var. Legend in the BBCH 47 phase and preliminary purification by extraction into the solid phase, I obtained the raw 40% MeOH (phenolic fraction), 80% MeOH and 100% MeOH fraction. UPLC-PDA-ESI-MS/MS analyses of the phenolic fraction showed that it contains numerous phenolic compounds, mainly phenolic acids and flavonoids. Preparatory separation of this fraction on the column filled with RP-18 medium allowed to obtain 41 fractions. They were analyzed using a liquid chromatograph (HPLC) with a 996 PDA detector by Waters. The analysis of the obtained spectra allowed to separate fractions containing one glycoside and fractions containing a mixture of two or more compounds. Some of the fractions were cleaned using a preparation column washed isocratically with methanol/1% H_3PO_4 in an appropriate ratio, while the fractions obtained in small quantities were cleaned using a semipreparative HPLC chromatograph from Gilson. The cleaning of the individual fractions allowed me to isolate 14 single patterns of phenolic compounds. In order to determine the chemical structure of the isolated compounds, mass spectroscopy (HRESI-MS), hydrogen and carbon nuclear magnetic resonance (^{1}H and ^{13}C NMR), as well as experiments such as COSY, TOCSY, HSQC, HSQC-TOCSY, HMBC were performed. Absolute configuration of sugars was also determined. The preliminary realization of the tasks including the determination of the structures of isolated compounds was done in Italy, in collaboration with the team of Prof. Cosimo Pizza and Prof. Sonia Piacente from the University of Salerno. Compounds isolated from *Triticum aestivum* L. var. Legend has classified into the following groups: phenolic acids (including caffeic and ferulic acid derivatives, esterified with quinic acid), flavonoid glycosides and acylated flavonoid glycosides. These were: 5-*O*-caffeoylquinic acid, 4-*O*-caffeoylquinic acid, *p*-coumaric acid, luteolin *C*-hexoside *O*-hexoside, luteolin *C*-(pentosyl-hexoside), isoorientin (luteoliny 6-*C*-*β*-glucopiranozyd), isoschaftoside (apigenin 6-*C*-*α*-arabinopyranozyd 8-*C*-*β*-glucopyranozyd), apigenin 6-*C*-*β*-xylopyranozyd 8-*C*-*β*-glucopyranozyd, luteolin *C*-hexoside *O*-deoxyhexoside, luteolin 6-*C*-[6$_{\mathrm{Glc}''}$-*O*-*E*-caffeoyl-*β*-D-glucopyranosyl(1''→2)-*β*-glucopyranoside], isoscoparin 2"-*O*-*α*-L-rhamnopyranoside, luteolin 6-*C*-[5$_{\mathrm{Rib}''}$-*O*-*E*-feruoyl-*β*-D-ribofuranosyl(1"→2)-*β*-glucopyranoside], tricin 7-*O*-rutinoside and 3',4',5'-*O*-trimethyltricetin 7-*O*-[*β*-D-glucuropyranosyl(1''→2)-*β*-D-glucopyranoside]. Three of the compounds that I isolated: luteolin 6-*C*-[6$_{\mathrm{Glc}''}$-*O*-*E*-caffeoyl-*β*-D-glucopyranosyl(1''→2)-*β*-

glucopyranoside], luteolin 6-*C*-[$5_{Rib''}$-*O*-*E*-feruoyl-*β*-D-ribofuranosyl(1”→2)-*β*-glucopyranoside] and 3’,4’,5’-*O*-trimethyltricetin 7-*O*-[*β*-D-glucuropyranosyl(1’’→2)-*β*-D-glucopyranoside] turned out to be **new compounds, not yet described in the scientific literature**. Apart from 14 isolated compounds, five other phenolic compounds were identified in the extract from aboveground parts of *T. aestivum* var. Legend: 3-*O*-caffeoylquinic acid, 3-*O*-feruoylquinic acid, 5-*O*-feruoylquinic acid, 4-*O*-feruoylquinic acid and isoorientin 6”-*O*-*β*-D-xylopyranoside.

The obtaining of single compounds as standards created the possibility to develop new UPLC methods for their quantitative determination. Quantitative analysis of 19 phenolic compounds in the extract from aboveground parts of *T. aestivum* var. Legenda was performed. It was shown that 86.6% of total content of phenolic compounds were flavonoids and their derivatives, while luteolin and its derivatives were the dominant compounds. The main compounds were: isoorientin (luteoliny 6-*C*-*β*-glucopiranozyd) determined at 39.41 ± 2.66 mg/g d.m. and luteolin *C*-hexoside *O*-deoxyhexoside (32.90 ± 2.21 mg/g d.m.).

The next stage of the study was to determine the antioxidant activity of *Triticum aestivum* L. var. Legend of 14 phenolic compounds isolated from aerial parts. In order to assess the free radical scavenging capacity of individual compounds, I used a thin layer chromatography technique (TLC-DPPH$^{\bullet}$ test). I applied standard solutions of isolated phenolic acids, flavonoids and rutin (group standard) on silica gel chromatographic plates and developed in a chromatographic chamber saturated with pairs of eluent: acetonitrile-chloroform-water-formic acid (60:10:15:5, v/v/v/v). After development and drying, I immersed them in 0.2% methanol solution of DPPH$^{\bullet}$. The first evaluation of the obtained results was performed by scanning the plates 30 minutes after the development of the DPPH$^{\bullet}$ solution. The next six scans of the plates were made every 10 minutes. Compounds with free radical scavenging ability appeared as yellow bands on purple background. For quantitative comparison of the test results graphical image processing using Sorbfil TLC computer program was used. The ability of phenolic compounds to capture free radicals strongly depended on their chemical structure, among other things, on the amount and location of free hydroxyl groups that influence the activity in question. The isolated phenolic acids proved to be strong scavengers of free radicals in relation to routine. Caffeic acid derivatives (5-*O*-caffeoylquinic acid, 4-*O*-caffeoylquinic acid) present in *Triticum aestivum* L. can be considered as the main free radical scavengers responsible for terminating the radical reaction chain. All isolated and active phenolic acids were present in esterified form and had free hydroxyl groups responsible for the ability to capture free radicals. Among newly isolated compounds, two of them: luteolin 6-*C*-[$6_{Glc''}$-*O*-*E*-

caffeoyl-*β*-D-glucopyranosyl(1''→2)-*β*-glucopyranoside] and luteolin 6-*C*-[$5_{Rib''}$-*O*-*E*-feruoyl-*β*-D-ribofuranosyl(1"→2)-*β*-glucopyranoside] showed high antioxidant activity and were acylated with caffeic or ferulic acid. *Triticum aestivum* L. var. Legend turned out to be rich in flavones, especially luteolin derivatives. All isolated luteolin derivatives were strong free radical scavengers, e.g., luteolin *C*-hexoside *O*-hexoside, luteolin *C*-(pentosyl-hexoside), isoorientin (luteoliny 6-*C*-*β*-glucopiranozyd) and luteolin *C*-hexoside *O*-deoxyhexoside. The relatively high ability of luteolin glycosides to capture free radicals can be attributed not only to the presence of a free hydroxyl group in the C7 position, but also to the catechol group in the B ring. All other flavonoids isolated were less active or their activity was not observed even in the presence of free hydroxyl group in C7 position, e.g: apigenin 6-*C*-*β*-xylopyranozyd 8-*C*-*β*-glucopyranozyd (inactive), isoschaftoside (apigenin 6-*C*-*α*-arabinopyranozyd 8-*C*-*β*-glucopyranozyd) (low activity) or isoscoparin 2"-*O*-*α*-L-rhamnopyranoside (low activity). In conclusion, it can be emphasized that coffee acid derivatives and luteolin glycosides, with a free hydroxyl group in the C7 position, were the main compounds of the phenolic fraction *Triticum aestivum* L., responsible for the ability to capture free radicals. These results are presented in **publication 1**.

Another aim of my research was to determine whether and to what extent the organic farming system affects the accumulation of secondary metabolites with antioxidant properties in the aerial parts of wheat (**publication 2**).

The development of a new method for the determination of phenolic compounds in the aerial parts (at the BBCH 47 stage) of wheat allowed to determine their content in 13 spring wheat varieties and 12 winter wheat varieties, grown in the organic system. In the methodological assumptions I took into account the comparison of selected varieties grown in the organic and conventional system (these tests were also performed for 4 selected varieties of winter wheat, grown in the conventional system). Quantitative determination of 7 phenolic acids and 12 flavonoids and their total content was performed using the UHPLC-PDA-ESI-MS system (Waters Corp., USA). Phenolic acids were quantified using chlorogenic acid as a group standard and UV detection at 320 nm. Flavonoids were quantified using rutin as a group standard and UV detection at 255 nm. A calibration curve method was used in the concentration range 1.69-270.00 μg/ml for chlorogenic acid and 1.60-256.00 μg/ml for rutin. Both curves were characterized by good linearity, with the value of the correlation coefficient r^2 = 0.999. Quantitative analyses were based on peak surface fields, corresponding to phenolic acids or flavonoids, on UV chromatograms for aerial parts of wheat. Peaks were identified based on their retention time, mass of ions [M-H]- and UV spectrum. The content of individual compounds differed significantly between varieties. The total content of phenolic compounds in wheat cultivars grown in the organic system was twice as high in spring cultivars as in winter. In the samples of plants from the organic system higher amounts of phenolic acids were recorded, and in the samples from the conventional system - flavonoids. In spring wheat cultivars, flavonoids were on average 10 times higher than phenolic acids. At the same time, significant differences were found in the concentration of phenolic acids and flavonoids depending on the form of wheat and cultivar. Average concentrations of the studied compounds were significantly higher in spring wheat cultivars than in winter cultivars. The content of all phenolic acids was highest in spring wheat cultivars, Trappe (1377.96 μg/g) and Kandela (1004.93 μg/g). Among the flavonoids studied it was found that isoorientin is the main compound in all investigated cultivars. Spring cultivars, Kandela and Ostka Smolicka showed the highest content of flavonoids (13753.7 and 12484.1 μg/g respectively).

Using the thin layer chromatography technique (TLC-DPPH$^\bullet$ test) I determined the antioxidant activity of purified phenolic fractions of 25 wheat varieties, grown in the organic system. As a group standard I used the rutin. For quantitative comparison of the test results I used graphic image processing using ImageJ computer program. The results of TLC-DPPH$^\bullet$ test showed that spring wheat cultivars were generally richer source of compounds with direct antioxidant properties than winter cultivars, whose average activity was 3.76 and 2.85, respectively. The average antioxidant activity of spring wheat cultivars ranged from 1.98 to 4.89; in relation to rutin (activity=1.00). I showed a significant correlation ($p \leq 0.05$) between the content of phenolic acids and the level of antioxidant activity. Comparing the antioxidant activity of four winter wheat cultivars (Arkadia, Bamberka, Jantarka, Sailor) grown in both farming systems, I showed that the analyzed cultivars had a higher average activity in conventional crops and it amounted to respectively: 2.86, 4.08, 4.99 i 3.50.

The results may be used in the selection of genotypes suitable for cultivation under organic farming conditions. Characteristics of phenolic profiles of qualitative wheat varieties is important in creating breeding programs aimed at shaping resistance of new varieties. Higher concentrations of antioxidant phenolic compounds in the vegetative parts of wheat may be important in shaping plant resistance and its defensive reactions against pathogenic factors and thus reduce the risk of yield loss and quality deterioration.

Among the 14 phenolic compounds isolated by me from the above-ground parts of wheat, two of them were apigenin derivatives: isoschaftoside (apigenin 6-*C*-*α*-arabinopyranozyd 8-*C*-*β*-glucopyranozyd) and apigenin 6-*C*-*β*-xylopyranozyd 8-*C*-*β*-glucopyranozyd, which differed in the type of sugar molecule in position C6.

In **publication 4** the influence of these compounds on clotting processes and oxidative stress in plasma treated with H_2O_2/Fe *in vitro* was compared. Plasma lipid peroxidation was quantified by thiobarbituric acid reactive substances (TBARS). TBARS are by-products of lipid peroxidation, the concentration of which is an indicator of the presence of ROS (reactive oxygen species). The most commonly used product of lipid peroxidation is malone dialdehyde (MDA). MDA combines with two thiobarbituric acid (TBA) molecules to form a pink light-absorbing complex in the 532-535 nm wavelength range. To form TBARS, the sample was exposed to a temperature of 100°C for 10 minutes. After cooling and centrifuging the sample, absorbance was measured by spectrophotometric measurement. In addition, the level of thiol groups (-SH), whose oxidation is also one of the biomarkers of oxidative stress, was determined. This study consisted in the reaction of -SH groups in proteins with Ellman's Reagent (DTNB), which led to disulphide bridges breaking up. As a result of the reaction anion of 5-tio-2-nitrobenzoic acid (TNB2-) was formed, intensely colored yellow. The concentration of TNB2- was measured colorimetrically at 412 nm. The level of carbonyl groups in plasma proteins was also determined. During the study of carbonyl groups, the protein was precipitated using trichloroacetic acid (TCA). The precipitate was washed three times with ethanol-ethyl acetate mixture, again dissolved with guanidine hydrochloride in 2M HCl and then precipitated. 2,4-dinitrophenylhydrazine (DNPH) was used to measure the final concentration of carbonyl groups. The compound of carbonyl with DNPH was detected by spectrophotometric measurement at 360 nm, taking into account the molar absorption coefficient of DNP. Since changes in coagulation are often correlated with oxidative stress, another aim of the study was to determine the effect of apigenin and its derivatives on selected plasma coagulation parameters such as thrombin time (TT), prothrombin time (PT) and activated partial thromboplastin time (APTT).

The results indicate that apigenin derivatives had different effects on clotting processes and biomarkers of oxidative stress in plasma treated with H_2O_2/Fe, but both compounds showed antioxidative effects. Isoschaftoside (apigenin 6-*C*-*α*-arabinopyranozyd 8-*C*-*β*-glucopyranozyd) showed a positive effect in inhibiting oxidation of thiol groups. Apigenin 6-*C*-*β*-xylopyranozyd 8-*C*-*β*-glucopyranozyd reduced plasma lipid peroxidation induced by H_2O_2/Fe at all tested concentrations (1-50 μg/ml). This compound at the highest concentration (50 μg/ml) significantly inhibited this process (by about 40%) compared to the positive control. Furthermore, apigenin 6-*C*-*β*-xylopyranozyd 8-*C*-*β*-glucopyranozyd significantly inhibited plasma protein carbonylation induced by H_2O_2/Fe at all concentrations used. The highest inhibitory effect, about 50% reduction compared to the positive control, was observed at its highest concentration (50 μg/ml). **Publication 4** is the first to characterize the effect of apigenin and its derivatives on clotting processes and *in vitro* oxidative stress.

I also determined the influence of the cultivar and management system on the content of benzoxazinones (including hydroxamic acids) in the aerial parts of 12 varieties of winter wheat and 13 varieties of spring wheat, in the BBCH 47 phase, grown in the organic system and four varieties (for comparison) in the conventional system (**publication 3**). After developing a new UPLC-MS/MS method for the determination of benzoxazinones (hydroxamic acids and their glycosides) in plant material, I carried an analysis of these compounds in the aerial parts of winter and spring wheat cultivated in two farming systems. For the analysis the Waters Acquity ultra performance liquid chromatography system, equipped with Waters TQ mass detector and Acquity PDA photodiode detector was used. Analyses were separated on the Waters BEH C18 column using a concentration gradient of acetonitrile containing 0.1% formic acid (mobile phase B) in distilled water containing 0.1% formic acid (mobile phase A) at a flow rate of 700 μl/min. The column outlet was ionized by electrospraying and the resulting negatively charged ions were studied. The analysis of hydroxamic acids and their glycosides was performed in MRM mode. Absorbance by outflow from the light column in the range of 210 to 400 nm was also recorded. The method was calibrated based on a series of calibration solutions with concentrations from 1.25 to 40.00 ng/μl, made from 1000 ng/μl standard solutions. In the aerial parts of the tested spring wheat cultivars 6-methoxy-2-benzoxazolinone (MBOA) and 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one glycoside (DIMBOA-Glc) were found, and in one of the winter wheat cultivars (Sailor) the presence of 2,4-dihydroxy-1,4-benzoxazin-3-one glycoside (DIBOA-Glc) in the amount of 0.89 μg/g was additionally demonstrated. Comparing the acid content of the same cultivars that were grown both in the organic and conventional system, a higher content was found in the cultivars grown in the organic system. The tested winter wheat cultivars contained less benzoxazinones than spring cultivars. The highest total content of the studied compounds was characteristic for the cultivars: Bravura (52.46 μg/g), Lagwa (34.67 μg/g) and Kandela (30.14 μg/g).

High concentration of benzoxazinones in the aerial parts of wheat plays a key role in forming plant resistance to pests and diseases, which may also affect grain quality in the future.

The next stage of my research was the analysis of wheat grain. The development of the UPLC method allowed to determine the composition and content of alkylresorcinols in the grain of 13 varieties of spring wheat grown in the organic system (including 4 varieties grown also in the conventional system) and 13 varieties of winter wheat grown in the organic system (including 4 varieties grown also in the conventional system) (**publication 5**). The determination of alkylresorcinols content was performed using UPLC-PDA-MS/MS system (Waters Corp., USA). For quantitative analysis 5-eicosylresorcinol (C20:0) was used as a group standard and UV detection at 280 nm. A calibration curve method was used in the concentration range 7.81-500.00 μg/ml and was characterized by good linearity, with the value of correlation coefficient $r^2 > 0.999$. Quantitation of individual ARs was based on the external standard method and peak area calculated from UV chromatogram for wheat grain. Peaks were identified based on UV, MS and MS/MS spectra. The grains of the examined wheat cultivars contained such alkylresorcinols as: 5-*n*-heptadecylresorcinol (C17:0, [M+H]+ 349), 5-*n*-nonadecanylresorcinol (C19:0, [M+H]+ 377), 5-*n*-heneicosylresorcinol (C21:0, [M+H]+ 405), 5-*n*-tricosylresorcinol (C23:0, [M+H]+ 433) and 5-*n*-pentacosylresorcinol (C25:0, [M+H]+ 461). These were mainly saturated homologues with side chains from 17 to 25 carbon atoms, with the highest content of C21:0 (about 50% of the total content) and C19:0, in all tested varieties. The total content of alkylresorcinols of 13 varieties of winter and spring wheat, grown in the organic system, was comparable. It was shown that the varieties grown in the conventional system contained lower amount of alkylresorcinols than the same varieties grown in the organic system. The results of these studies allowed to separate the varieties of winter and spring wheat with the highest content of these compounds. Among winter wheat cultivars, the best source of resorcinol lipids turned out to be the varieties: Julius (total content is 1272.4 μg/g), KWS Ozon (1045.8 μg/g) and Jantarka (1043.1 μg/g). Among the tested spring wheat cultivars the highest total content of the tested compounds was found in: Izera (995.0 μg/g), Kandela (865.2 μg/g) and Katoda (823.4 μg/g).

The detailed characteristics of these health beneficial natural products in wheat grain are important for the agricultural industry in breeding programs for the development of bread wheat varieties with added value in the form of health promoting compounds.

The next stage of my research was to study the antioxidant activity of alkylresorcinols (**publication 5**). The antioxidant properties are one of the most important biological properties. The results of recent years prove that reactive oxygen species (ROS) are involved in the pathomechanism of many diseases and disorders, including atherosclerosis, Alzheimer's disease, bronchial asthma, all kinds of inflammation, some cancers. Therefore, there is a need to develop analytical methods to assess the ability to scavenge free radicals and to identify compounds with such properties in complex samples of natural origin. **For the first time** for quantitative antioxidant analysis of alkylresorcinols I created and applied TLC-DPPH$^{\bullet}$ method with ImageJ program. The previously used TLC-DPPH$^{\bullet}$ method, combined with image processing, was used to quantitatively measure the direct antioxidant properties of phenolic compounds present in complex samples in aerial parts. My research has confirmed that, after modification, this method is suitable for determining the activity of resorcinol lipids present in cereal grain. I used α-tocopherol (vitamin E equivalent) as standard in these studies, as it is known as a natural lipophilic antioxidant. Compared to the previous method, I significantly modified both the composition of the mobile phase and the conditions of graphic processing of TLC plates. Alkylresorcinols are structurally similar to tocopherols, except that they have a simple aliphatic hydrocarbon side chain and a single phenolic ring. Due to their ability to give hydrogen and remove radicals, they can serve as a source of *in vitro* antioxidants.

The results of the TLC-DPPH$^{\bullet}$ test showed that spring wheat varieties are generally a richer source of compounds with direct anti-free radical properties than winter varieties, whose average activity is 0.166 and 0.129, respectively, compared to vitamin E equivalent (1.00). This is confirmed by the fact that the antioxidant properties of alkylresorcinols are significantly lower than α-tocopherol, which is consistent with previous literature data (Parikka et al., 2006; Korycińska et al., 2009). I demonstrated a relationship between the content of alkylresorcinols and free radical scavenging. Apart from the phenolic ring, the length of the lateral aliphatic chain plays an important role in the antioxidant activity of resorcinol lipids. I found a significant ($p < 0.05$) correlation between antioxidant activity and total resorcinol lipids. This is confirmed by the fact that Izera (spring variety) and Julius (winter variety), which showed the highest total alkylresorcinol content, mainly C21:0, also showed the highest DPPH radical scavenging activity. The results of my research confirmed the findings of Konopka et al. (2017), who showed that the antioxidant potential (DPPH and Rancimat tests) was mainly related to the variety. In this study I also found a significant ($p < 0.05$) correlation between antioxidant activity and sometimes after derivatization. The activity changed sinusoidally and showed the highest level 80 minutes after derivatization, both in spring and winter varieties. I also showed a statistically significant influence of different agricultural systems on the antioxidant activity of alkylresorcinols in winter and spring wheat. Comparing the free radical scavenging activity of four spring wheat cultivars (Izera, Kandela, Ostka Smolicka and Waluta) and winter wheat (Arkadia, Bamberka, Jantarka, Sailor) grown in both farming systems, I showed that the antioxidant activity was significantly ($p < 0.05$) higher in organic farming, except for Sailor cultivar, where no significant differences were found. The interactions of ,,variety'' x ,,cultivation system'' and ,,time after derivatization'' x ,,cultivation system'' were significant ($p < 0.05$).

The data obtained are important for national nutritional needs. The free radical scavenging properties of alkylresorcinol contained in wheat grain may be potentially important for the food industry, which is constantly looking for natural antioxidants to protect the grain during processing and storage. This research was also intended to contribute to the understanding of the nutraceutical properties of whole wheat grains and to promote progress in wheat varieties with a high level of health of natural products.

After the development of the UPLC-MS/MS method for the determination of benzoxazinones in plant material, I conducted the analysis of these compounds in the grain of 13 varieties of winter wheat and 13 varieties of spring wheat cultivated in the organic system and 4 varieties each of these wheat cultivated for comparison in the conventional system (**publication 5**). The analysis was based on the Waters Acquity ultra performance liquid chromatography system, equipped with the Waters TQ mass detector and Acquity PDA photodiode detector. The composition and content of hydroxamic acids and their glycosides were determined both in grain collected directly from the field (humidity up to 14%) and in grain moistened (hydroconditioned) in distilled water for 48 hours at room temperature, which was then dried to the initial moisture content. No benzoxazinones were found in grain collected directly from the field, while hydroconditioned grain showed the presence of hydroxamic acids and their glycosides: DIBOA (2,4-dihydroxy-1,4-benzoxazin-3-one), DIBOA-Glc, DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one), DIMBOA-Glc; lactam: HBOA (2-hydroxy-1,4-benzoxazin-3-one) and benzoxazolinone: MBOA (6-methoxy-2-benzoxazolinone). By determining the composition and content of these metabolites in the grain, it was shown that they contain much more of these compounds than the aerial parts of wheat. Similarly as in the aerial parts, the grain of winter wheat varieties contained much less of these compounds than the grain of spring wheat varieties. Among spring wheat cultivars grown in the organic system, Izera (858.7 μg/g), Ostka Smolicka (540.7 μg/g) and Koksa (364.8 μg/g) proved to be a good source of the tested compounds. Among 13 cultivars of winter wheat cultivated in the organic system the highest total content of benzoxazinones was characterized by Jantarka (546.2 μg/g), Bamberka (494.1 μg/g) and Muszelka (489.3 μg/g). Moreover, in the system of organic farming, winter and spring wheat grain contained more benzoxazinones than in the same varieties grown conventionally.

Summary and application of research results

- ✓ the aboveground parts of wheat contain phenolic acids, flavonoids and hydroxamic acids, while the grain is a source of alkylresorcinols (resorcinol lipids) and benzoxazines,
- ✓ three of the isolated flavonoids, proved to be new compounds, not yet identified in the plant world and not described in the scientific literature,

- ✓ the identification of compounds with antioxidant properties showed the presence of numerous natural metabolites in wheat, which have the ability to capture free radicals that are involved in the pathomechanism of many diseases,
- ✓ development of new methods of quantitative determination of phenolic compounds (UPLC-PDA-ESI-MS), alkylresorcinols (UPLC-PDA-ESI-MS) and benzoxazinones (UPLC-MS/MS) in aerial parts and alkylresorcinols (UPLC-PDA-ESI-MS) and benzoxazinones (UPLC-MS/MS) in grains of winter and spring wheat varieties, significantly enriched the existing scientific workshop by improving the qualitative and quantitative analyses of individual metabolites, so that the results obtained could be published in international literature,
- ✓ analysis of grain and aerial parts of a large number of Polish wheat varieties, coming from various farming systems (organic and conventional) in terms of composition and content of the examined metabolites, allowed to determine the partial influence of the variety and farming system on the composition of metabolic profile of the examined varieties of *Triticum* L. and to indicate the best source of these compounds. This is an important aspect in the relationship between plants and the environment, as well as it has an impact on the health of cereal products, mainly whole grain. Knowledge of this fact can be an important determinant in the actions of growers looking for sources of resistance in the starting materials for breeding. It can also be reflected in the agrotechnical activities taking into account the use in practice of crop afterbeds from plants containing large amounts of hydroxamic acids or their derivatives,
- ✓ the results of the research allowed to separate wheat varieties with the highest pro-healthy value of grain, which should, among others, be intensively promoted as a raw material for the production of functional food,
- ✓ due to the fact that wheat is one of the most commonly consumed cereals and contains a significant amount of alkylresorcinol, it is highly probable that it will be consumed in quantities that allow biological action,
- ✓ the results obtained have contributed to a better understanding of the detailed phytochemical characteristics of wheat, which can make a major contribution to the study of functional genomics of cereal plants.

Supplementary literature

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5. Presentation of significant scientific or artistic activity carried out at more than one university, scientific or cultural institution, especially at foreign institutions

Presentation of other scientific and research achievements

The scientific information of my scientific activity is presented in the list of scientific achievements - Annex 4. Below I present a description of my scientific development to date and a general outline of my scientific-research activity.

5.1 Research achievements before obtaining the degree of doctor of agricultural sciences

After graduation, I completed an internship at the Department of Biochemistry and Crop Quality, IUNG-PIB in Pulawy, which allowed me to learn in practice the research issues of the Institute, the technique and methodology of conducting field experiments and work in a biochemical laboratory. I was able to further develop my knowledge and scientific interests by employing me in this Department since May 1st 2005. I conducted scientific research under the direction of Professor Wiesław Oleszek. My research interests included isolation, identification, qualitative and quantitative analysis, and the study of polymorphism of secondary metabolites, mainly *Medicago truncatula* phenolic compounds (**Annex 4; 4.1.1, 4.1.2, 4.2.1, 4.2.5, 7.1.1, 7.2.1, 7.2.19-7.2.21, 7.2.42 - 7.2.44**). This work was carried out by the project ,,Saponins and flavonoids *Medicago truncatula* (chemical composition, determination, occurrence) as a contribution to the study of functional genomics (metabolomics) in the Fabaceae family" (**Annex 4; 9.1.2**). From the three-year (2005-2007) field experiments I obtained plant material for the study. From the aerial parts of *Medicago truncatula* of Jemalong A17 cultivar I isolated twenty-three flavonoid compounds, including twenty flavones and three flavonols, using preparation chromatography techniques: 3,7,5'-*O*-triglucoside laricitrin (**1**), 7-*O*-[*β*-D-glucuronopyranosyl(1→2)-*O*-*β*-D-glucuronopyranoside] luteolin (**2**), 7-*O*-[*β*-D-glucuronopyranosyl(1→2)-*O*-*β*-D-glucuronopyranoside] apigenin (**3**), 7-*O*-[*β*-D-glucuronopyranosyl(1→2)-*O*-*β*-D-glucuronopyranoside] chrysoeriol (**4**), 7-*O*-*β*-D-glucuronopyranoside luteolin (**5**), 7-*O*-[*β*-D-glucuronopyranosyl(1→2)-*O*-*β*-D-glucuronopyranoside] tricin (**6**), 7-*O*-{2-*O*-feruloyl-[*β*-D-glucuronopyranosyl(1→3)]-*O*-*β*-D-glucuronopyranosyl(1→2)-*O*-*β*-D-glucuronopyranoside} apigenin (**7**), rutin (**8**), 7-*O*-{2-*O*-*p*-coumaroyl-[*β*-D-glucuronopyranosyl(1→3)]-*O*-*β*-D-glucuronopyranosyl(1→2)-*O*-*β*-D-glucuronopyranoside} apigenin (**9**), 7-*O*-{2'-*O*-feruloyl-[*β*-D-glucuronopyranosyl(1→3)]-*O*-*β*-D-glucuronopyranosyl(1→2)-*O*-*β*-D-glucuronopyranoside} chrysoeriol (**10**), 7-*O*-[2'-*O*-

sinapoyl-*β*-D-glucuronopyranosyl(1→2)-*O*-*β*-D-glucuronopyranoside] apigenin (**11**), 7-*O*-{2'-*O*-feruloyl-[*β*-D-glucuronopyranosyl(1→3)]-*O*-*β*-D-glucuronopyranosyl(1→2)-*O*-*β*-D-glucuronopyranoside}tricin (**12**), 7-*O*-{2'-*O*-*p*-coumaroyl-[*β*-D-glucuronopyranosyl(1→3)]-*β*-D-glucuronopyranosyl(1→2)-*O*-*β*-D-glucuronopyranoside} chrysoeriol (**13**), 7-*O*-{2-*p*-coumaroyl-[*β*-D-glucuronopyranosyl(1→3)]-*O*-*β*-D- glucuronopyranosyl(1→2)-*O*-*β*-D-glucuronopyranoside}tricin (**14**), 7-*O*-{2'-*O*-feruloyl-[*β*-D-glucuronopyranosyl(1→3)]-*β*-D-glucuronopyranosyl(1→2)-*O*-*β*-D-glucopyranoside} apigenin (**15**), 7-*O*-[2'-*O*-sinapoyl-*β*-D-glucuronopyranosyl(1→2)-*O*-*β*-D-glucuronopyranoside] tricin (**16**), 7-*O*-*β*-D-glucuronopyranoside tricin (**17**), 7-*O*-[2'-*O*-feruloyl-*β*-D-glucuronopyranosyl(1→2)-*O*-*β*-D-glucuronopyranoside] tricin (**18**), 7-*O*-[2'-*p*-coumaroyl-*β*-D-glucuronopyranosyl(1→2)-*O*-*β*-D-glucuronopyranoside] tricin (**19**), 7-*O*-[2-*O*-feruloyl-*O*-*β*-D-glucuronopyranosyl(1→2)-*O*-*β*-D-glucopyranoside] tricin (**20**), 3,5'-digluko-3-metylomiricetin (**21**), 7-*O*-[*β*-D-glucuronopyranosyl (1→3)-*O*-*β*-D-glucuronopyranosyl(1→2)-*O*-*β*-D-glucuronopyranoside] apigenin (**22**) and 7-*O*-[*β*-D-glucuronopyranosyl-4'-*O*-glucopyranoside] tricin (**23**). Eight of the compounds I have isolated are **new substances** (**4, 11, 13-15, 20, 22** and **23**), not found in any other plant species and not described in the scientific literature. The structure of the isolated flavonoids has been determined using mass spectrometry and hydrogen and proton nuclear magnetic resonance (^{1}H and ^{13}C NMR), in collaboration with the University of Salerno, Italy (Prof. Cosimo Pizza, Prof. Sonia Piacente) (**Annex 4; 17.2.1**). The possession of 23 isolated patterns of flavonoid compounds from aerial parts of *M. truncatula* made it possible to develop a method of ultra-performance liquid chromatography (UPLC) for quantitative determination of these compounds in plant samples. I determined the content of flavonoids in leaves and stems of three varieties of *M. truncatula* (*M. truncatula* var *truncatula*, *M. truncatula* var *longispina* and *M. truncatula* Jemalong A17). In the leaves and stems of the tested cultivars of *M. truncatula* the dominant compound was 7-*O*-*β*-D-glucuronopyranoside tricin (**17**). The total content of flavonoid compounds in the leaves of three tested cultivars of *M. truncatula* was high and amounted on average to about 3.5% d.m. and in the stems to 1.5% d.m. The most numerous group of tricin glycosides constituted 76% of flavonoid content, which indicated that *M. truncatula* may be a good source of these compounds. However, no statistically significant differences in the content of flavonoids in different developmental phases of alfalfa plants were found.

In the framework of the NUTRA-SNACKS project (**Annex 4; 4.2.2, 4.2.3, 9.1.6, 14.1**) I carried out a preliminary extraction of the aboveground parts of mint (*Mentha longifolia* L.) to obtain an aqueous extract (**Annex 4; 4.2.2 to 4.2.4, 7.2.2, 7.2.3**).

For the ,,*Medicago truncatula* Phytochemical Research Cycle", I received the 2007 Team Prize of Director IUNG-PIB II (**Annex 4, 17.4.1**). In order to develop my analytical skills necessary for my doctoral thesis I participated in 2008 in a specialist course "Mass spectrometry and its application".

The above-described results of research on the aerial parts of *M. truncatula* are part of my doctoral thesis entitled ,,The composition and content of phenolic compounds in the aerial parts of *Medicago truncatula* Gaertner'' which was promoted by Dr. Anna Stochmal. I obtained my doctoral degree in agricultural sciences on 30.06.2009 on the basis of the resolution of the Scientific Council of IUNG-PIB in Puławy, after public defense of my doctoral dissertation, which, according to reviewers, deserved a distinction. My doctoral dissertation was awarded by the Director of IUNG-PIB Professor Seweryn Kukuła (**Annex 4; 17.4.2**).

Summary

My scientific achievements <u>before obtaining the degree of doctor of agricultural sciences</u> include: two peer-reviewed scientific publications and five publications published in journal supplements (**Annex 4; 4.1.1, 4.1.2, 4.2.1-4.2.5**) with a total IF=**11.16** and MNiSW=**114** (according to the year of issue) and **440** (according to the appendix of 18.12.2019). The results were presented in the form of a lecture, six abstracts and three posters at national and international conferences (**Annex 4; 7.1a, 7.2.a, 7.2.c**).

5.2 Research achievements after obtaining the degree of doctor of agricultural sciences.

After obtaining the degree of doctor of agricultural sciences in agronomy, I continued to work on the isolation of phenolic compounds from the roots of *M. truncatula*. Out of the seven isolated compounds, three phenolic glycosides turned out to be **new compounds**, not yet described in the scientific literature: 5-*O*-{[5''-*O*-E-(4'''-*O*-threo-guaiacylglycerol)-feruloyl]-β-apiofuranosyl-(1→2)-β-xylopyranosyl} gentisic acid (**1**), 5-*O*-[(5''-*O*-vanilloyl)-*β*-apiofuranosyl-(1→2)-*β*-xylopyranosyl] gentisic acid (**2**) and 1-*O*-[E-(4''-*O*-threo-guaiacylglycerol)-feruloyl]-3-*O*-C-galacturonopyranosyl glycerol (**3**). The chemical structures of the new compounds were defined in cooperation with the University of Salerno, Italy. The remaining isolated four known compounds are: 5-*O*-*β*-xylopyranosyl gentisic acid (**4**), vicenin-2 (**5**), hovetrichoside C (**6**) and pterosupin (**7**).

In cooperation with Dr Ireneusz Kapusta, from the University of Rzeszów (**Annex 4; 17.3.7**), a chapter in the monograph entitled ,,*Medicago truncatula* - a model plant for the metabolism of Fabaceae'' has been written (**Annex 4; 2.1**). In addition, I have completed and published the other research results contained in my doctoral thesis (**Annex 4; 4.1.3, 4.2.6- 4.2.8, 7.2.4-7.2.6**).

The directions of my research activities, conducted after obtaining the degree of doctor, are mainly focused on the following issues:

- phytochemical characteristics of cultivated plants,
- sources of compounds with antioxidant properties.

<u>Phytochemical characteristics of cultivated plants</u>

Due to the fact that wheat (*Triticum* L.) is the cereal of the greatest economic importance both in the world and in Poland, which results, among other things, from the versatility of the use of grain, as well as its high production potential, I have addressed this issue as part of the implementation of the National Science Centre project entitled ,,The characteristics of secondary metabolites (phenolic compounds, saponins, hydroxamic acids) of wheat as an element of the study of functional genomics of cereal plants'', of which I was the head of project (**Annex 4; 9.1.1**). For this purpose, I have established cooperation with the Department of Bioeconomy and Systems Analysis of IUNG-PIB, in the field of obtaining plant material for research, from various management systems. Moreover, the determination of the structures of isolated phenolic compounds from wheat was performed in Italy, in cooperation with the University of Salerno. This project has produced a series of publications constituting a scientific achievement, details of which can be found in point 4 (**Annex 4; I 1-5**). In addition, the research results were presented in the form of one publication in magazine supplements, a lecture at a national scientific conference, three abstracts and three posters (**Annex 4; 4.2.10, 7.2.8, 7.2.31, 7.2.37**).

I continued my research on wheat within the framework of cooperation with the Department of Bioeconomy and Systems Analysis of IUNG-PIB (**Annex 4; 17.3.8**), as a contractor in three national scientific projects awarded on the basis of the decision of the Minister of Agriculture and Rural Development (MRiRW) (**Annex 4; 9.1.3-9.1.5**). The scope of work included research on the selection of spring cereal varieties, as well as research on the evaluation and improvement of the yield quality of contemporary and former spring wheat varieties, their usefulness for the baking and pasta industry and their health potential.

He was also a contractor in the research project entitled ,,The research of wheat grain''. The next research project was carried out as a contractor in the research project ,,Relationship of winter wheat grain contamination with deoxynivalenol with plant morphology and phenolic acids content'' in cooperation with the Department of Cereal Crop Production of IUNG-PIB. A comparative analysis of selected primary and secondary metabolites of wheat grain, including phenolic acids, was performed. The effect of this cooperation is that I acted as an assistant promoter in the doctoral program of Edyta Aleksandrowicz, M.Sc. ,,The evaluation of accumulation of mycotoxins and bioactive components in the grain of winter wheat cultivars as a reaction to stress caused by *Fusarium* fungi infection''. The doctoral dissertation was initiated on 26.04.2019, in the field of agricultural, forest and veterinary sciences, in the discipline of agronomy, specialization: quality of plant raw materials (**Annex 4; 7.1.3, 7.2.16, 7.2.17, 7.2.41, 15.1.2, 17.3.9, 17.5.1**).

My research on the characteristics of secondary metabolites of wheat grain has been conducted so far, among others, in cooperation with the Department of Agroengineering, Faculty of Environmental Management and Agriculture, West Pomeranian University of Technology in Szczecin. They are aimed at determining the effect of sulphur preparations on the content of phenolic acids, alkylresorcinols and their antioxidant activity (**Annex 4; 17.3.6**). Moreover, thanks to this cooperation I took part in two trainings on statistical analyses, which improvement is necessary in scientific work (**Annex 4; 17.1.5, 17.1.10**).

Clover (*Trifolium* L.), a plant that is important from both an agricultural and a health-promoting point of view, has also become an object of my interest. Given the important role of the genus *Trifolium* in the prevention and treatment of various diseases, or in the nutrition of livestock, as well as the presence of anti-nutritional substances in their composition, it was advisable to conduct research towards a closer understanding of the chemical composition of various clover species. This issue was dealt with in the framework of the research project I was leading in the field of clovers. I dealt with this issue as part of my research project ,,Active compounds of selected plant species from the bean family'' including ,,Research on the potential use of biologically active and medicinal substances present in various species of the genus *Trifolium*'' (**Annex 4; 15.1.1**). I conducted the works in cooperation with the Department of General Biochemistry, Faculty of Biology and Environmental Protection, University of Lodz (**Annex 4; 17.3.5**), as well as the Department of Forage Crop Production of IUNG-PIB (**Annex 4; 17.3.12**). For the research I used 88 species of *Trifolium* L., the seeds of which I obtained in cooperation with the Leibniz-Institute of Plant Genetics and Crop Plant Research (IPK) – Department of Genebank., Germany (**Annex 4; 17.2.2**). The work included, among other things, the extraction and identification of individual secondary metabolites from clover species, determination of chemical structure of isolated compounds, determination of biological activity of selected *Trifolium* species as well as development of UPLC methods for determination of the content of tested compounds.

As part of the realization of the above topic, I conducted the separation and purification of single phenolic compounds of aerial parts of *Trifolium scabrum* L. Conducting the preparative separation of the phenolic fraction and purification of the resulting fractions allowed me to obtain sixteen single compounds whose structures were determined by mass spectroscopy (ESI-MS) and hydrogen and carbon, nuclear magnetic resonance (^{1}H and ^{13}C NMR) analyses. Among 16 isolated **for the first time** compounds from *T. scabrum*, 14 isoflavones (glycones and their glycosylated derivatives) and 2 flavonol glycosides were identified. They were: 3'-hydroxydaidzin (**1**), daidzin (**2**), isoquercitrin (**3**), calycosin-7-*O*-*β*-D-glucoside (**4**), genistin (**5**), astragalin (**6**), 6''-*O*-acetyl-daidzin (**7**), pratensein-7-*O*-*β*-D-glucoside (**8**), pseudobaptigenin-7-*O*-*β*-D-glucoside (**9**), daidzenin (**10**), ononin (**11**), genistein (**12**), formononetin-7-*O*-*β*-D-glucoside-6''-*O*-malonate (**13**), sissotrin (**14**), formononetin (**15**) and biochanin A (**16**). Qualitative analysis showed that *T. scabrum* L. contains a high amount of isoflavones. The availability of 16 standards allowed to develop a method of ultraperformance liquid chromatography coupled with mass spectrometry (UPLC-MS) of quantitative determination of these compounds in plant samples. The total content of *T. scabrum* phenolic compounds was 45.64 ± 3.57 mg/g dry matter (d.m.), including isoflavones 41.60 ± 3.41 mg/g d.m. Daidzein and its derivatives accounted for 44% of total polyphenols in the aerial parts of *T. scabrum*. The dominant isoflavones were: daidzin (10.93 mg/g d.m.), 6''-*O*-acetyl-daidzin (6.35 mg/g d.m.) and ononin (5.90 mg/g d.m.). It turned out that in comparison to soybean and soybean products, which contain about 0.2-1.6 mg of isoflavones/g d.m., as well as red clover (11.80-39.51 mg/g d.m.), the aerial parts of *T. scabrum* L., can be an alternative, new and natural source of isoflavones. Using the TLC-DPPH$^{\bullet}$ method I determined the antioxidant activity of 16 isolated compounds. Among the compounds tested, they showed antioxidant activity: 3'-hydroxydaidzin (**1**), isoquercitrin (**3**), calycosin-7-*O*-*β*-D-glucoside (**4**), astragalin (**6**), daidzein (**10**), genistein (**12**) and biochanin A (**16**). My results indicate that *T. scabrum* extract can be used as an ingredient in functional foods or as a food supplement due to its high isoflavone content, with documented antiradical activity. The study was conducted with my participation: 8 publications with IF (**Annex 4; 4.1.4, 4.1.5, 4.1.8-4.1.10, 4.1.14, 4.1.16, 4.1.17**), publication without IF (**Annex 4; 4.1.20**), lecture (**Annex 4; 7. 1.4**), 4 abstracts (**Annex 4; 7.2.11, 7.2.18, 7.2.22, 7.2.24**) and 5 posters (**Annex 4; 7.2.35, 7.2.38, 7.2.45, 7.2.48, 7.2.53**).

The result e.g. of this work was also the team **award of the Director** of IUNG-PIB I degree in 2014, for the overall publication achievements with a high IF coefficient.

The research on specific (secondary) metabolites of alfalfa (*Medicago* L.), which began at the beginning of my research work, even before I obtained my doctoral degree, I continued as a contractor in a research project within the framework of the Institute's statutory activity entitled ,,The content of active secondary metabolites in new varieties of alfalfa, grafted with *Sinorhizobium meliloti* root bacteria''. The project was implemented in cooperation with the Department of Agricultural Microbiology IUNG-PIB (**Annex 4; 15.1.3, 17.3.10**). Previous work has shown that *Medicago* plants contain a number of active compounds. Due to the emergence of new varieties of this plant and the use of vaccines containing symbiotic bacteria in the cultivation of the plant, it has become interesting to determine their impact on the chemical composition of alfalfa and the content of active compounds, as well as the effectiveness of this fertilization system (**Annex 4, 7.2.13, 7.2.15, 7.2.40**). I also took part in a scientific workshop on this issue, entitled ,,The effects of lucerne on the chemical composition and content of active compounds and the effectiveness of this fertilization system'' (**Annex 4, 7.2.13, 7.2.15, 7.2.40**). I also participated in a scientific workshop on this subject, ,,Symbiotic microorganisms in science and practice'' (**Annex 4; 17.1.6**).

I was also involved in research to determine the polyphenolic profile of roasted coffee, selected functional additives: cinnamon, ginger, cardamom and chilli; lettuce, onion and walnut. These studies were conducted in cooperation with the Department of Biochemistry and Food Chemistry, Faculty of Food Sciences and Biotechnology, University of Life Sciences in Lublin and the Department of Heat Technology and Process Engineering, Faculty of Production Engineering, University of Life Sciences in Lublin. The analysis of the polyphenolic profile was carried out using high-performance liquid chromatography techniques combined with mass spectrometry (UPLC/MS). Several phenolic compounds were identified in coffee brew before digestion, mainly substances from the group of hydroxycinamic acids. Concentrations of all phenolic compounds were higher before in vitro digestion than after the process. By analyzing the cinnamon extracts, 8 active compounds were identified in the infusion: four compounds from the proanthocyanidin group, cinnamic acid as a representative of the hydroxycinamic acid group and other polyphenols such as coumarin, cinnamon aldehyde *cis* and *trans*. Moreover, all these compounds were present in the cinnamon extract after digestion. Five volatile compounds were identified in the ginger infusion: four were gingerol derivatives and the fifth identified was shogail. Moreover, all these compounds were present in ginger extracts after digestion. As a result of the analysis, four phenolic compounds were identified in an aqueous infusion of cardamom: protocatechuic acid, vanillic acid, *p*-cumaric acid and ferulic acid. The qualitative-quantitative analysis of chilli phenolic profile showed the presence of eight biologically active compounds: capsaicin and quercetin, apigenin and luteolin derivatives. In onion extract, quercetin glucoside, quercetin diglucoside and isoramnetin glucoside were identified, while lettuce contained coffee acid derivatives, quercetin glucuronide and kaempferol. The results obtained in this collaboration formed the basis for 5 publications in the IF journal, 2 abstracts and 5 posters (**Annex 4; 4.1.12, 4.1.13, 4.1.15, 4.1.18, 4.1.19, 7.2.10, 7.2.25, 7.2.32, 7.2.34, 7.2.39, 7.2.49, 7.2.54, 17.3.2, 17.3.3**).

The aim of my next research was to analyze the phenolic compounds contained in the sprouts of selected edible plants, including lentils (*Lens* Mill.), vetches (*Vicia* L.), clovers (*Trifolium* L.) and alfalfa (*Medicago* L.), to determine their content and antioxidant activity. Sprouts are a group of plant products, which in recent years has become increasingly important among consumers who value a healthy lifestyle. They are characterized by higher nutritional value than dry seeds. In the sprouts of the tested bean plants I identified different groups of phenolic compounds. The highest content of total polyphenols was found in the extract of lentil sprouts (12.70±0.18 mg/g d.m.), followed by vetch (7.07±0.09 mg/g d.m.). The results were presented in the form of 2 lectures, 3 abstracts and poster. In my opinion it would be valuable to introduce sprouts to cereal products (extrudates). Several times I applied for funds for this research, within the framework of competitions of the National Science Centre, forming a scientific consortium with the Medical University of Lublin (Faculty of Pharmacy with the Division of Medical Analytics) and the University of Life Sciences in Lublin (Faculty of Food Sciences and Biotechnology and Faculty of Production Engineering), however, so far I have not obtained funding (**Annex 4; 7. 1.2, 7.1.6, 7.2.14, 7.2.27, 7.2.30, 7.2.51, 9.3.1-9.3.4, 17.3.1, 17.3.3**).

Part of my scientific work was a review of the literature on bioactive compounds contained in energy plants and wood biomass, their properties and applicability in pharmaceutical, cosmetic and food industries. The aim of the review was to show information about other possibilities of using biomass instead of and/or in combination with energy production. The review concerned: maize (*Zea mays* L.), sorghum (*Sorghum* spp), giant knotweed (*Fallopia sachalinensis*), japanese knotweed (*Fallopia japonica*), Jerusalem artichoke (*Helianthus tuberosus* L.), giant miscanthus (*Miscanthus giganteus*), reed canary grass (*Phalaris arundinacea*), switchgrass (*Panicum virgatum* L.), cup plant (*Silphium perfoliatum*), willow (*Salix viminalis* L.) and black locust (*Robinia pseudoacacia*). The available literature data proved that many of them have the potential to become a suitable source of valuable phytochemicals for industrial applications, due to significant concentrations of antioxidants and other biologically active compounds. In addition, some compounds used in the pharmaceutical, cosmetic and food industries can be produced and extracted during various types of pre-treatment of lignocellulose, the main component of many energy plant biomasses. The result of these works was a scientific publication in Phytochemistry Review, which was prepared in cooperation with the Department of Physical Properties of Plant Materials, Institute of Agrophysics Bohdan Dobrzanski of the Polish Academy of Sciences in Lublin (**Annex 4; 4.1.22, 7.3.4**).

Sources of compounds with antioxidant properties

Apart from the issue concerning mainly phenolic compounds, which I discussed above, my professional activity also included the evaluation of antioxidant properties of both pure compounds and plant extracts.

The results of recent years show that reactive oxygen species (ROS) are involved in the pathomechanism of many diseases and disorders, including atherosclerosis, Alzheimer's disease, bronchial asthma, all kinds of inflammation, some cancers. Therefore, there is a need to develop analytical methods to assess the ability to scavenge free radicals and to identify compounds with such properties in complex samples of natural origin. In my work, I used the technique of thin layer chromatography (TLC-DPPH$^•$ test) to assess free radical scavenging capacity, modifying it depending on the polarity of compounds present in phenolic fractions or extracts. For quantitative comparison of research results I used graphic image processing with ImageJ computer program. The usefulness of the proposed technique of testing direct antioxidant properties of plant secondary metabolites was confirmed by numerous examples of samples of natural origin. As part of the implementation of the above issue, I determined the antioxidant activity: secondary metabolites of alfalfa (*Medicago sativa* L. and *Medicago truncatula* L.), *in vitro* cultured *Medicago truncatula* phenolic compounds, biologically active substances of various species of the genus *Trifolium* (including isoflavones of the aerial parts of *Trifolium scabrum* L.), phenolic compounds of edible plant sprouts, flavonoids from the aerial parts of lentils (*Lens culinaris*), edible plant germ extracts, phenolic compounds isolated from the aerial parts of wheat (*Triticum aestivum* L.), alkylresorcinols and phenolic acids of winter and spring wheat grains, holoparasites of the genus *Orobanche*, active compounds of honeysuckle, hops, dandelion, knotweed, goldenrod, barley, buckwheat, cucurbits (pumpkin, zucchini, cucumber, white and yellow pattypan squash) and asteroids (chicory, green and red lettuce, jerusalem artichoke). She carried out this research in cooperation with the company:

University of Ankara (Turkey), University of Istanbul (Turkey), University of Alabama (USA), Medical University of Lublin, University of Lodz, Department of Cereal Crop Production IUNG-PIB, Department of Systems and Economics of Plant Production IUNG-PIB and Department of Weed Science and Soil Tillage Systems IUNG-PIB (based in Wroclaw). The research results obtained within the framework of this cooperation became the basis for writing a chapter in the monograph, preparing 7 publications in the IF journal, giving 4 lectures, presenting 13 abstracts and 9 posters (**Annex 4; 2. 2, 4.1.6, 4.1.7, 4.1.9, 4.1.11, 4.1.21, 4.1.23, 4.1.26, 7.1.2, 7.1.4-7.1.6, 7.2.7, 7.2.9, 7.2.12, 7.2. 14, 7.2.15, 7.2.18, 7.2.23, 7.2.24, 7.2.26-7.2.30, 7.2.33, 7.2.36, 7.2.46, 7.2.47, 7.2.50-7.2.53, 7.2.55**).

Summary

My scientific achievements after obtaining the degree of doctor of agricultural sciences include: 24 peer-reviewed scientific publications, 5 publications published in journal supplements and 2 chapters in the monograph (**Annex 4; 2.1, 2.2, 4.1.3- 4.1.26, 4.2.6-4.2.10**) with total IF=**50.92** and MNiSW=**635** (according to the year of publication) and **1850** (according to the appendix of 18.12.2019). The results were presented in the form of 5 lectures, 24 abstracts and 22 posters at national and international conferences (**Annex 4; 7.1. 2-7.1.6, 7.2.4-7.2.18, 7.2.22-7.2.30**).

6. Presentation of teaching and organizational achievements as well as achievements in popularization of science or art.

I was the scientific supervisor of five foreign PhD students (from Italy, Georgia, Egypt and Tunisia) during their stay in the Department of Biochemistry and Crop Quality, high school students, students and trainees of national research centers (8 people), including a group of students from the University of Life Sciences in Lublin. I'm an auxiliary supervisor in the doctoral thesis of Mrs. Edyta Aleksandrowicz from the Department of Cereal Crop Production, IUNG-PIB (**Annex 4; 17.5.1**). I improve my qualifications by participating in training courses in mass spectrometry, statistical methods, functional food, laboratory instrumental methods and rules of participation and application for scientific projects (**Annex 4; 17.1.1-17.1.10**).

Within the framework of scientific organization activities I was a member of the organizing committee of three national and two international scientific conferences, as well as a member of the scientific committee of one national scientific conference (**Annex 4; 8.1-8.6**). I actively cooperate with scientific teams from other IUNG-PIB scientific institutions, with domestic and foreign scientific institutions (**Annex 4; 17.2, 17.3**).

7. **Apart from information set out in 1-6 above, the applicant may include other information about his/her professional career, which he/she deems important.**

The list of all publications, other scientific and research achievements, including information about participation in the work of research teams implementing European projects and programs, internships, international and national cooperation, awards received for scientific activities, the function of an auxiliary promoter and manager of the Department of Biochemistry and Crop Quality are included in **Annex 4**.

Kowalska Iwona ....
(Applicant's signature)