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Determining the content of polyphenolic compounds and antioxidant activity of new products which constitute potential functional food

Introduction

Scientific research on food that has a beneficial effect on health is one of the strategies for preventing and alleviating the course of chronic diseases and cancer since a great number of health-related problems is strictly connected with improper diet. Functional food is a food claimed to have a nutritional function and a positive effect on human body. It improves health and well-being moreover, reduces the risk of many diseases. It is important to mention that functional food should match the conventional form of food. What is more, functional food incorporates traditional food with drugs. It could be designed to meet the requirements of specific consumer groups. One of the applied methods of producing functional food is the addition of plants, which often contain biologically active polyphenolic compounds. Polyphenols belong to a group of secondary plant metabolites. They have a number of beneficial pro-health properties, which are mainly determined by their antioxidant activity. Polyphenol-rich diet contributes to counteracting or soothing the course of cardiovascular diseases, cancer, metabolic and autoimmune diseases or neurodegenerative processes. In the era of civilization diseases, better known as lifestyle diseases it is reasonable and highly recommended to search for a new range of scientifically tested functional food.

The aim of the research

The aim of this study was to design, produce and analyse new products that may constitute potential functional food as well as animal feed. By analysing their extracts I tried to determine the qualitative and quantitative content of natural polyphenolic compounds and antioxidant potential. Interdisciplinary work was carried out in cooperation with, among others the Department of Process Engineering and Department of Fish Diseases and Biology at the University of Life Sciences in Lublin and Laboratoire de Nutrition et Technologie Alimentaire Université des Frères Mentouri in Algeria.

Materials and methods

The new functional range of food discussed in this dissertation has consisted of:

- extruded gluten-free buckwheat (*Fagopyrum esculentum* M., *tataricum* G.) pasta produced using various technological parameters
- extruded instant pasta based on refined and wholegrain wheat (*Triticum aestivum* L.) and (*Triticum aestivum* var. *spelta*) spelt flours
- gluten-free pasta with a different percentage of pomegranate seed powder (*Punica granatum* L.)
- extruded snacks enriched with various contents of kale (*Brassica oleacea* var. *sabellica*)
- extruded feed for fish of the species Danio rerio, enriched with a different quantitative addition of purple coneflower root (*Echinacea purpurea* (L.) Moench.)

In order to isolate polyphenolic compounds from the products, I performed ultrasound-assisted extraction (UAE). The obtained extracts were analysed for the content of polyphenols by high-performance liquid chromatography HPLC-ESI-MS/MS (Agilent 1200 Series chromatograph, QTRAP 3200) and HPLC-UV/VIS (Knauer chromatograph). With the object of determining antioxidant activity of samples I used spectrophotometric methods (Thermo Scientific UV-Vis Genesys20 spectrophotometer) applying a solution of the stable DPPH radical and Folin-Ciocalteu reagent. Furthermore, I used thin layer chromatography (TLC-DPPH test) to confirm the antioxidant activity of the extracts. The plates were developed in horizontal chambers (DS-II Chromdes) anteriorly using an automatic applicator (Desaga AS-30) for applying extracts. For the quantitative interpretation of the obtained data I used the Sorbfil TLC Videodensitometer computer program. I performed each analysis three times. I used the Statistica 13.0 program (Statsoft, Poland) for statistical data processing.

Moreover, the interdisciplinary cooperation allowed for the enrichment of the research methodology concerning a new range of functional food. As a supplement to the delineated research (going beyond the topic of the dissertation), a number of nutritional, technological and functional tests of products were carried out.

Results

I determined chromatographically 15 free phenolic acids in all extracts from extruded precooked pasta with the addition of buckwheat flour differing in the used technological parameters (i.e. the rotational speeds of the extruder screw - 60, 80, 100 and 120 revolutions per minute and moisture levels in the raw material - 30, 32 and 34%). The experiment has proved that the level of moisture in the raw material had an impact on the content of phenolic acids. The richest phenolic acids composition was present in samples produced at flour moisture of 32%, in particular 100 rpm. In the next stage I determined the antioxidant activity of buckwheat flour and pasta made from flour moistened up to 32% content using the TLC-DPPH test. The highest free radical scavenging activity was observed in flour from roasted buckwheat. I noted the highest free radical scavenging activity among the analysed pasta for samples obtained at the extruder screw speed of 100 rpm. This experiment has shown that samples with the previously determined highest content of free phenolic acids were simultaneously characterized by the most significant antioxidant activity.

Precooked pasta products based on refined and wholegrain wheat and spelt flours were produced by extrusion method using optimal technological conditions (moisture content of the raw material equals to 32%, rotational speed of the extruder screw 100 rpm). I chromatographically detected 8 free phenolic acids in all samples. Their content was higher in pasta made from wholegrain rather than refined flour. The results of the free radical scavenging measurements of the extracts using the spectrophotometric analysis against DPPH radical and those obtained from planar chromatography (TLC-DPPH) were similar. Wholegrain spelt pasta showed the highest radical scavenging activity, wholegrain wheat - slightly lower. Bioactive polyphenolic compounds are mainly located in the outer layer of the seeds. Therefore, in order to obtain health-beneficial pasta from the relevant cereals, the advantageous solution is to minimize the purification of the flours used in its production.

The TLC-DPPH test of extracts of gluten-free pastas enriched with 5, 7, 10 and 12.5% pomegranate seed powder indicated that the percentage content of enriching supplement had a significant effect on the capacity of scavenging the DPPH free radical. Only 5% addition shows such properties, while antioxidant capacity increases with the greater addition of pomegranate. Pasta with 12.5% pomegranate seed content extract had the highest scavenging ability. On the basis of a compromise between improving health-related values and obtaining a finished

product with a technologically acceptable quality of 7.5%, the addition of pomegranate seed powder was recognized as the most optimal.

Chromatographic analysis of extruded corn snacks with the addition of kale has shown a significant concentration of phenolic acids. I have quantified 13 phenolic acids in snacks with 8% of kale. I determined 12 phenolic acids in products with 6% enriching addition, whereas in the case of 4 and 2% of kale - 10. Both qualitative and quantitative content of polyphenols increased with the growth in the percentage of kale in the extruded snacks. I noted high DPPH free scavenging potential for snacks enriched with 8, 6 and 4% of kale. The antioxidant activity of the samples was positively correlated with the percentage of plant addition.

Moreover, I analysed extruded fish feed with a different enriching additions of purple coneflower root (10, 30 and 60 g / kg of finished product). I determined quantitatively 4 polyphenolic compounds contained in the food with the Echinacea content of 30 and 60 g / kg. All the methods used to determine the antioxidant activity of the samples (TLC-DPPH test, spectrophotometrically by the DPPH and Folin-Ciocalteu) has shown that it increases with augmenting content of purple coneflower root in the produced extruded feed. Due to methods with the use of DPPH radical, the significant free radical scavenging properties were observed for samples with Echinacea purpurea L. content of 30 and 60 g / kg.

Conclusions

The research work consisted in designing, manufacturing (using modern extrusion techniques) and analysing a new range of functional food and animal feed in order to determine polyphenolic profile and antioxidant potential. Chromatographic analysis has shown a wide variety of phenolic acids contain in those innovative products. Increasing the amount of enriching addition of polyphenolic plants in food and feed for animals is positively correlated with the qualitative and quantitative content of phenolic acids and the level of antioxidant activity. It also depends on the applied technological parameters and the intensity of purification and type of flour used in the production process.

According to the obtained results, the applied production technique did not cause the degradation of thermolabile polyphenolic compounds. It impacts on the presence of pro-health properties of the relevant food. They resulted from the wide activity of polyphenols, which many times constitute a specific therapeutic potential. The food discussed in this dissertation can constitute a new range of functional food, aiming at satisfying the special needs of people suffering from celiac disease and gluten intolerance as well as being one of the methods of preventing chronic civilization diseases.