Active polyphenolic compounds, nutrient contents and antioxidant capacity of extruded fish feed containing purple coneflower (Echinacea purpurea (L.) Moench.)

Tomasz Oniszczuk, Anna Oniszczuk, Ewa Gondek, Leszek Guz, Krzysztof Puk, Anna Kocira, Andrzej Kusz, Kamila Kasprzak, Agnieszka Wójtowicz

Department of Food Process Engineering, University of Life Sciences, Doswiadczenialna 44, 20-280 Lublin, Poland
Department of Inorganic Chemistry, Medical University in Lublin, Chodzki 4A, 20-093 Lublin, Poland
Department of Food Engineering and Process Management, Faculty of Food Sciences, Warsaw University of Life Sciences (WULS-SGGW), Nowoursynowska 159C, 02-776 Warsaw, Poland
Department of Fish Diseases and Biology, University of Life Sciences, Akademicka 13, 20-950 Lublin, Poland
Institute of Agricultural Sciences, State School of Higher Education in Chełm, Pocztowa 34, 22-100 Chełm, Poland
Department of Technology Fundamentals, University of Life Sciences, Doswiadczenialna 50A, 20-680 Lublin, Poland

Received 12 February 2016; revised 11 May 2016; accepted 15 November 2016
Available online 22 November 2016

KEYWORDS
Fish feed; Echinacea purpurea; Extrusion-cooking; Antioxidants; Polyphenols

Abstract The growth of fish is directly dependent on feed composition and quality. Medicinal plants can be added to fish feed as adjuvant therapy for the prevention of fish diseases. The purple coneflower (Echinacea purpurea (L.) Moench.) has been reported to have multiple biological effects, including immunomodulatory and antioxidant activity. The most active compounds of E. purpurea are polyphenols - caffeic acid derivatives: caffic acid, chlorogenic acid, cynamar, echinacoside and cichoric acid.

Due to a relatively limited number of studies on the use of the purple coneflower as a nutritional supplement for fish feeding, extruded fish feed with addition of Echinacea roots was produced. In the feed total phenolic content, selected polyphenol contents, the energetic value, nutrient contents and antioxidant capacity were examined.

* Corresponding author. Fax: +48 81535 7378.
E-mail address: anoniszczuk@o2.pl (A. Oniszczuk).
Peer review under responsibility of King Saud University.
1. Introduction

The growth of fish is directly dependent on feed composition and quality. Medicinal plants can be added to fish feed as adjuvant therapy for the prevention of fish diseases. In our previous studies we have shown that Echinacea purpurea plays a role in the control of diseases of ornamental fish (Guz et al., 2011). This plant acts as both an immunostimulant and a disease control agent in fish. It may be recommended as a dietary supplement in order to improve aquaculture production (Aly et al., 2007; Aly and Mohamed, 2010). The enhancement of non-specific immune parameters by an Echinacea supplemented diet is possibly an important factor in protecting fish against bacterial challenge and in reducing their percentage mortality (Guz et al., 2011).

Echinacea (purple coneflower), originated in the North America was brought to Europe in the late 19th century (Dall’Acqua et al., 2010; Zolgharnein et al., 2010). Extracts and dietary supplements from this plant showed antioxidative, antibacterial, antiviral, antifungal properties and are used in treatment of inflammatory and viral diseases (Hu and Kitts, 2000; Nematalia et al., 2011). The immunotropic action of Echinacea extracts consists in their influence on the metabolism of granulocytes, lymphocytes and monocytes/macrophages (Luettig et al., 1989; Roesler et al., 1991). The results of many research works have shown that Echinacea extracts stimulate the secretion of nitric oxide and cytokines, such as II-1, II-6, II-8, II-12, TNF-α and a heightened activation of macrophages (Burger et al., 1997; Mishima et al., 2004). The most active compounds of E. purpurea are polyphenols - caffeic acid derivatives: caftaric acid, chlorogenic acid, cynarin, echinacoside and cichoric acid (Pellati et al., 2004; Thygesen et al., 2007; Yanli et al., 2011). All investigated Echinacea species did possess radical scavenging activity, but E. purpurea being the most efficient (Pellati et al., 2004). Cichoric acid, the main compound of Echinacea root, is an appropriate marker of the quality of E. purpurea containing product, because it has strong antioxidant, immunostimulatory and antiviral, properties, and it is susceptible to degradation (Zolgharnein et al., 2010).

The aim of this work was production of directly expanded fish feed enriched with E. purpurea using extrusion-cooking, determination of the active phenolic compounds content and antioxidant activity of the product. Extrusion-cooking is one of the most interesting techniques of feed production. This method seems to be one of the most appropriate for obtaining the maximum nutritive value of products. Versatility of technical and technological solutions allows applying various raw materials and additives (Mościcki et al., 2012).

Apart from the presence of phenolic compounds and antioxidant properties in the feed the energetic value and nutrient contents were also examined. Reliable data on nutrient content are crucial in the evaluation of the potential inclusion of feed ingredients in diets, to develop cheaper feed formulations. Dietary proteins, microelements and vitamins, due to its impact on animal growth and high cost, have received priority in fish nutrition studies.

2. Materials and methods

2.1. Materials, chemicals and equipment

The basic raw materials for the preparation of feed were obtained from ANIMEX Group S.A., Zamosc Branch. Dried Echinacea root was obtained from “A Herbal Farm Waldemar Lupa”, Poland.

Standards of rutin, gallic, cichoric, caftaric and caffeic acids, echinacoside as well as 2,2-diphenyl-1-picrylhydrazyl (DPPH), were purchased from Sigma–Aldrich Fine Chemicals (St. Louis, MO, USA).

Solvents used for HPLC were HPLC-grade, purchased from J.T. Baker (the Netherlands), and was water purified using a Millipore laboratory ultra pure water system (Simplicity™ system, Millipore, Molsheim, France). Methanol used for preparation of the extracts was of analytical grade and obtained from the Polish Reagents (POCH, Gliwice, Poland).

The TLC–DPPH test was performed on the HPTLC silica gel 60 F254 plates (Merck, Darmstadt, Germany). The extracts were prepared using ultrasonic bath (Bandelin Electronic, Heidelberg, Germany). Absorbance was measured by a GENESYS™ 20 UV–Vis spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) in a 1 cm quartz cell.

2.2. Feed processing

The feed was produced by extrusion-cooking according to a standard developed for carp breeding (Oniszczuk et al., 2012) with the addition of Echinacea. Extrusion-cooking is the main process in the manufacturing fish feed compounds (Barrows et al., 2007). The balancing of feed recipes and the production technology were based on broadly available food tables and a set of devices supplied by the Department of Food Process Engineering, Lublin University of Life Sciences (Oniszczuk et al., 2012). The main components used in the production of feed were as follows: maize, wheat, soybean, fishmeal, fodder yeast, rapeseed oil, chalk fodder,
microelements, vitamins and Echinacea root. The feed was produced by extrusion-cooking using a modified version of the TS-45 single-screw extruder, equipped with a plasticizing unit modified to L/D = 16/1 (Metalchem, Gliwice, Poland). Extruder parameters during feed processing were as follows: feeding rate 21.25 kg h\(^{-1}\), screw speed 100 rpm, mass pressure 0.08 MPa, specific mechanical energy 0.147 kWh kg\(^{-1}\) and fat addition of 10%.

The marking of basic nutrients was carried out in the Central Equipment Laboratory of Lublin University of Life Sciences.

2.3. Extraction procedure - ultrasound assisted extraction (UAE)

Dried, pulverized and sieved Echinacea root and dried feed with addition of Echinacea root (0, 10, 30 and 60 g kg\(^{-1}\) of feed) were extracted with 40 mL of methanol for each sample, in ultrasonic bath (Bandelin Electronic, Sonorex RK 100H, Germany) for 30 min at 60°C. The same procedure was repeated two more times for each sample (Oniszczuk et al., 2014; Oniszczuk and Podgórski, 2015). Extracts were combined and evaporated to dryness. The residues were dissolved in 10 mL of methanol and filtered through a 0.45 µm filter. The whole procedure was repeated three times for each sample.

2.4. Colorimetric analyses of total phenolic content

The content of total phenolic compounds was determined using the Folin–Ciocalteu method (Burns et al., 2000). Folin–Ciocalteu reagent (3 mL) was added to either 50 µL of extract or 50 µL of standard solutions and diluted with distilled water. After 8 min reaction time saturated sodium carbonate solution (9 mL) was added and the test solution was made up to 100 mL with H\(_2\)O and mixed. After 2 h the absorbance was measured at 765 nm. Gallic acid was used as standard in concentrations 0.01–2.0 mg mL\(^{-1}\). The content of total phenolic compounds in the extracts was calculated using linear regression.

2.5. Chromatographic analysis

HPLC determination of cichoric, caftaric, caffeic acids and echinacoside in samples was performed with a Knauer liquid chromatograph equipped with a 200 mm × 4.6 mm, d\(_p\) 5 µm, Hypersil ODS column, a UV–visible detector, and a Rheodyne injector with 20 µL loop. Isocratic elution was carried out with 25% aqueous methanol containing 1% acetic acid. Identification and measurement of the peaks were performed at 320 nm. The contents of analyzed substances in the extracts were determined by use of calibration plot. Calibration plots for all compounds were linear in range 0.01–3 mg mL\(^{-1}\) (\(R^2 > 0.9970\)).

2.6. Antioxidant activity of feed

The radical-scavenging activity of the analyzed compounds was determined spectrophotometrically by the DPPH (2,2-diphenyl-1-picrylhydrazyl radical) assay (Kedare and Singh, 2011; Marteau et al., 2013; Oniszczuk et al., 2015). DPPH solution was prepared by mixing 4 mg (10\(^{-5}\) moles) DPPH with 100 mL of methanol. The concentration of the resulting solution was 0.1 mM. Fresh reagent was always prepared prior to analysis. A reference sample was prepared by mixing 2.0 mL DPPH (0.1 mM) with 1.0 mL of methanol. Afterward each of the examined extracts was mixed with DPPH solution. For each sample, the same amount (1 mL) of the extract was added to 2.0 mL of 0.1 mM DPPH solution. The loss of DPPH absorbance was measured in the excess of the extracts. Each measurement was repeated three times at 517 nm at room temperature. The final result is the average of three replicates performed. Free radical scavenging activity was calculated as a percentage of DPPH decoloration according to the following formula:

\[
\text{Scavenging capacity (\%)} = \frac{100(A_0 - A_1)}{A_0}
\]

Here, A\(_0\) is the absorbance of the sample except tested extracts and A\(_1\) is the absorbance of the sample with tested extracts.

2.7. TLC–DPPH test

The analyzed extracts and solution of rutin standard (concentration 0.5 mg mL\(^{-1}\)) were applied to chromatographic plates with the applicator Desaga AS-30. The samples were applied spot-wise, with a distance of 7 mm between them, and a 5 mm distance from both the left and low edge of the plate. The plates were developed in vertical chambers pre-saturated for 15 min with the optimized mobile phase: acetonitrile:water:chloroform:formic acid (60:15:10:5, v/v/v/v). The 10 µL aliquots of extracts were applied onto the plates and developed. Then the plates were dried for 10 min before derivatization. The TLC plates were immersed for 5 s in freshly prepared 0.1% (w/v) methanolic DPPH solution. After removing DPPH excess, plates were kept in the dark for 5 min and then scanned by using a flat-bed scanner, every 5 min for an hour. The test was performed in triplicate.

2.8. Image processing using Sorbfil TLC Videodensitometer program

The results of TLC–DPPH test were documented by flat-bed scanning, saved in the form of jpg documents. The JPG image was saved at a resolution of 40 pixels cm\(^{-1}\) for image analysis by Sorbfil TLC Videodensitometer software (Sorbpolymer, Russia). The image file was opened with the software. Suitable width of each track line was set and the evaluation of the chosen track to measure peak area was performed by Process Track command. The software evaluated a band in each track on a TLC image on the assumption that the size and the intensity of a bright spots depended on the quantity of a substance in the band. In order to change the videoscan images into chromatograms, resembling those obtained in high-performance liquid chromatography (HPLC), a rectangular selection tool was used to outline the tracks. RF and peak area were determined. The total areas under the peaks in one track (one extract) were measured and compared with the area obtained for rutin, a compound with a recognized free radical scavenging potential.
Active polyphenolic compounds, nutrients contents and antioxidant capacity of extruded fish feed

3. Results and discussion

In the present work, antioxidant properties of methanolic extract from feed with addition of *Echinacea* roots were evaluated by means of the DPPH radical scavenging test. The scavenging activity of the investigated samples against DPPH radical was expressed as scavenging %.

In the study four samples of fish feed with a varied content of the *E. purpurea* root (0, 10, 30 and 60 g kg\(^{-1}\)) were used. The feed was prepared by using extrusion-cooking, one of the most interesting techniques of feed production. The extraction of dried plants was performed using methanol and sonication, because results of recent study confirm that ultrasound-assisted extraction is an effective, easy in operation, reliable and feasible method for extraction of polyphenols from plant material (González-Centeno et al., 2015; Wianowska et al., 2009; Oniszczuk and Olech, 2016; Oniszczuk and Podgórska, 2015). The active antioxidants are well extracted with alcoholic solutions, due to their relatively high polarity. In order to determine total phenolic compounds colorimetric and analysis of all extracts were performed.

Experiment clearly confirmed that content of phenolic compounds and antioxidant activity increased with the addition of *Echinacea* roots in feed. The following samples gave positive results: extracts of coneflower and feed with 30 g kg\(^{-1}\) (K-2) and 60 g kg\(^{-1}\) (K-3) addition of this plant. Feed without any additives (K-0) and with 10 g kg\(^{-1}\) (K-1) content of *Echinacea* roots did not exhibit free radical scavenging properties.

The strongest free radical scavenging properties were observed for coneflower extract. However feed with addition of 60 g kg\(^{-1}\) of *Echinacea* roots possessed high antioxidant activity. High radical scavenging properties of active samples were observed immediately after first minutes of the experiment. Besides extracts, antioxidant activity of rutin (0.5 mg mL\(^{-1}\)) was examined, as a reference point. It is clear, that coneflower extract possesses higher radical scavenging properties as solution of rutin in concentration 0.5 mg mL\(^{-1}\) (Fig. 1, Table 1).

In order to confirm the results obtained by spectrophotometric method, TLC–DPPH test was performed. The results of TLC–DPPH test indicated, that antioxidant potential of analyzed samples was positively correlated with the content of *Echinacea* roots in feed. This finding was based on comparison of the activity of the separated spots in relation to rutin antioxidant properties. The highest radical scavenging activity was observed for crude *Echinacea* roots extract followed by feed with addition of 60 g kg\(^{-1}\) of *Echinacea* roots. The highest radical scavenging activity, demonstrated using TLC–DPPH test, for all samples was observed after 30 min (Table 2, Fig. 2).

The values of standard deviation (SD), as a measure of repeatability of TLC–DPPH test, were in good agreement with requirements for a developed method. The above results explicitly demonstrate that antioxidant activity of examined samples depends largely on the compounds present in raw material.

Chicoric acid is the main compound of *Echinacea* root and marker of the quality of product containing *E. purpurea*; therefore, determination of this compound in samples was performed. Moreover, in the extracts of coneflower and in samples of feed, contents of following active polyphenols were confirmed: caftaric and caffeic acids (samples: K-1, K-2, K-3), echinacoside (samples: K-2, K-3). Findings of HPLC analysis demonstrated, that chicoric acid and other polyphenols (susceptible to degradation) do not deactivate during extrusion process.

Unprocessed herbs, fruits and vegetables can be a source of many compounds, for example polyphenols, having biological activity (Nowak et al., 2016; Swieca, 2015). These substances are often thermolabile and may decompose during the processing of plant materials. For that reason important is to develop the technology, that has no negative influence on antioxidant activity of compounds present in the raw material. Farmed fish are exposed to a continuous antigenic pressure by microbial and environmental agents, which may lead to a condition of chronic inflammation. Recently, a positive correlation has been established between dietary supplementation with antioxidants and the reduction of detrimental effects such as reduced growth rates, alterations in the physical condition and health of fish, and the activation of stress responses in fish under stocking density (Guz et al., 2011).

In view of the notion that polyphenols are endowed with antioxidant and anti-inflammatory activities, farmed sea bass (*Dicentrarchus labrax* L.) have been administered with red grape polyphenol-enriched feed (Magrone et al., 2016). Polyphenols were extracted from the seeds of Canosina Nero di Troia Vitis vinifera and mixed with conventional feed at two different concentrations (100 and 200 mg/kg, resp.). Data suggest that polyphenol-administered sea bass generate lower

![Figure 1](image-url)  Free radical scavenging activity of analyzed samples. K 0–3 – extracts of feed with addition of 0, 10, 30, 60 g kg\(^{-1}\) of *Echinacea* root respectively, E – extract of *Echinacea* root, CA – rutin (0.5 mg mL\(^{-1}\)).
levels of intestinal proinflammatory cytokines, while producing larger amounts of spleen IFN-\(\gamma\), as an expression of a robust and protective adaptive immune response. Increase of MMCs corroborates the evidence for a protective spleen response induced by feed enriched with polyphenols.

Li et al. (2016) have demonstrated, that rich in polyphenols extracts of *Ginkgo biloba* leaves decreased the generation of reactive oxygen species (ROS), inhibited the oxidation of cellular components and restored the activities of enzymatic antioxidants in OH-treated carp erythrocytes. 

Rattanachaikunsopon and Phumkhachorn (Rattanachaikunsopon and Phumkhachorn, 2007) evaluated the antimicrobial activity against fish bacterial pathogens of flavonoids (morin, morin-3-O-lyxoside, morin-3-O-arabinoside, quercetin, and quercetin-3-O-arabinoside) isolated from the leaves of *Psidium guajava*. All analyzed flavonoids were shown to have bacteriostatic effect on the tested bacteria, including *Aeromonas hydrophila*, *Aeromonas salmonicida*, *Flavobacterium columnare*, *Lactococcus garvieae*, *Streptococcus agalactiae*, *Vibrio salmonicida*.

Results of the present study indicate that high-temperature extrusion-cooking used to produce feed does not have effect on the inactivate antioxidant compounds, which are presented in raw *E. purpurea*.

Extrusion-cooking is a convenient method of feed processing due to the fact that it produces stable products with all nutritive compounds preserved or enhanced by the addition of natural, biologically active components (Wojtowicz and Mosicki, 2009). Universality and variety of technological solutions allow applying various raw materials and additives, and its composition will influence on quality, dietary and pro-health properties, sensory, and nutritional characteristics of extruded products (Stojesk\'a et al., 2010).

The research results from many authors have shown that *Echinacea* extracts take an active part in the destruction of peroxidation products and their toxic metabolites, and in the stabilization of the body’s antioxidant (Magginia et al., 2012; Nemattala et al., 2011; Thygesen et al., 2007). Own research conducted for the first time has proven antioxidant potential of the fish feed with addition of the purple coneflower, produced by extrusion-cooking.

### Table 1 Content of selected polyphenols and total phenolic compounds in analyzed extracts.

| Sample        | Content (mg g\(^{-1}\) dry weight) |
|---------------|-----------------------------------|
|               | Total phenolic | Cichoric acid | Caftaric acid | Caffeic acid | Echinacoside |
| Ekstrakt of *Echinacea* | 30.541 ± 0.952 | 16.835 ± 0.28 | 2.518 ± 0.147 | 0.324 ± 0.021 | 0.342 ± 0.016 |
| K\(^{-}0\)    | 1.024 ± 0.041 | -             | -             | -            | -            |
| K\(^{-}1\)    | 1.058 ± 0.044 | 0.037 ± 0.002 | 0.071 ± 0.002 | 0.005 ± 0.000 | -            |
| K\(^{-}2\)    | 2.621 ± 0.123 | 0.582 ± 0.022 | 0.156 ± 0.007 | 0.015 ± 0.000 | 0.012 ± 0.000 |
| K\(^{-}3\)    | 4.213 ± 0.201 | 1.134 ± 0.056 | 0.361 ± 0.014 | 0.094 ± 0.001 | 0.018 ± 0.003 |

\(^a\) SD – standard deviation; 
\(^b\) K\(^{-}0\)–3 – samples with addition of 0, 10, 30, 60 g kg\(^{-1}\) of *Echinacea* root respectively.

### Table 2 Activity of investigated samples in relation to rutin’s activity (concentration 0.5 mg mL\(^{-1}\)).

| Time (min) | Activity in relation to rutin (area under the common peak/area under rutin peak) |
|-----------|---------------------------------------------------------------------------------|
|           | Extract of *Echinacea* root | Fish feed with 10 g kg\(^{-1}\) addition of *Echinacea* root | Fish feed with 30 g kg\(^{-1}\) addition of *Echinacea* root | Fish feed with 60 g kg\(^{-1}\) addition of *Echinacea* root |
| 15        | 3.458 ± 0.050                                  | 0.060 ± 0.001                                  | 0.250 ± 0.011                                  | 0.770 ± 0.023                                 |
| 30        | 4.00 ± 0.120                                  | 0.120 ± 0.003                                  | 0.430 ± 0.018                                  | 1.71 ± 0.042                                  |
| 60        | 3.91 ± 0.090                                  | 0.110 ± 0.004                                  | 0.390 ± 0.013                                  | 1.23 ± 0.031                                  |

\(^a\) SD – standard deviation (\(n = 3\)).

Figure 2 HPTLC, the analyzed extracts scanned after 30 min. System: silica/acetonitrile:water:chloroform:formic acid (60:15:10:5, v/v/v/v). Plates after development dried (10 min) and derivatized by immersion in 0.1% (w/v) DPPH solution in methanol, kept in the dark (5 min) and then scanned. 1 – sample K-2, 2 – sample K-1, 3 – sample K-3, 4 – extract of *Echinacea* root, 5 – rutin (0.5 mg mL\(^{-1}\)).
Active polyphenolic compounds, nutrients contents and antioxidant capacity of extruded fish feed

Table 3 The content of feed components in prepared blends.

| Sample | Echinacea addition (g kg⁻¹) | Metabolic energy (MJ kg⁻¹) | Protein general (g kg⁻¹) | Raw fiber (g kg⁻¹) | Raw fat (g kg⁻¹) | Ca (g kg⁻¹) | P (g kg⁻¹) | N (g kg⁻¹) |
|--------|-----------------------------|---------------------------|-------------------------|-------------------|----------------|------------|-----------|------------|
| K₀⁻₀   | 0                           | 15.16 ± 0.39              | 386.70 ± 4.71           | 66.70 ± 1.79      | 95.20 ± 3.32  | 14.90 ± 0.31| 7.20 ± 0.06| 61.80 ± 1.92|
| K⁻₁    | 10                          | 15.26 ± 0.43              | 385.50 ± 2.25           | 62.10 ± 0.38      | 94.80 ± 2.55  | 13.90 ± 0.07| 7.00 ± 0.12| 61.70 ± 2.25|
| K⁻₂    | 30                          | 14.72 ± 0.08              | 379.80 ± 8.67           | 75.40 ± 1.12      | 98.20 ± 0.78  | 14.20 ± 0.61| 7.00 ± 0.21| 60.30 ± 0.45|
| K⁻₃    | 60                          | 14.93 ± 0.52              | 389.50 ± 0.98           | 75.10 ± 0.08      | 93.50 ± 3.11  | 14.20 ± 0.27| 6.90 ± 0.08| 62.30 ± 0.76|

a SD – standard deviation (n = 3).

K 0–3 – samples with addition of 0, 10, 30, 60 g kg⁻¹ of Echinacea root respectively.

An important thing in our study was to confirm that fish feed containing purple coneflower has balanced composition, with high content of essential nutrients - protein, fat and microelements such as calcium, nitrogen, and phosphorus (Table 3).

The main ingredients of fish feeds are fish meal and fish oil. These two constituents supply essential fatty and amino acids necessary for the fish for normal growth (Al Mahmud et al., 2012). Issues of economic and ecological sustainability are creating a significant pressure to reduce levels of fish meal in fish feeds. The addition of *Echinacea* root to feed caused a decrease in the level of fish meals and an increase in the levels of plant protein and oil sources, avoiding significant modification of the nutritive value of the diet. Plant proteins can be less costly and they are free of potential contaminants such as dioxin and mercury. However, fishmeal is an important constituent of fish feed and can be substituted only in a limited range by vegetable proteins, without reducing feed efficiency and growth (Al Mahmud et al., 2012). Extrusion-cooking process, used in the study reduces the level of thermolabile antinutrients present in plant material, improves the digestibility of dietary components and eliminates microbial load. Generally, *E. purpurea* has enormous potential to act as fish feed ingredients.

In conclusion, fish feed with addition of *E. purpurea* can be used as a nutritional supplement in the prevention of fish diseases caused by oxidative stress.

4. Conclusions

The results of the present study indicate that fish feed with addition of the root of *E. purpurea*, prepared using extrusion-cooking, has a potential to be a good source of natural radical scavengers and nutritive ingredients. Antioxidant properties of feed correlated well with the coneflower content. The study findings confirmed that high-temperature extrusion-cooking process does not deactivate phenolic antioxidant compounds, which are present both in the *Echinacea* roots and in the final product. An important thing in our study was to indicate, that fish feed produced by using extrusion-cooking, has balanced composition, with high content of essential nutrients - protein, fat and microelements such as calcium, nitrogen, and phosphorus.

In conclusion, fish feed with addition of *E. purpurea* can be used as a nutritional supplement in the prevention of fish diseases caused by oxidative stress.

References

Al Mahmud, N., Hasan, R.M.D., Hossain, B.M., Minar, M.F., 2012. Proximate composition of fish feed ingredients available in Lakhimpur Region, Bangladesh. Am.-Eur. J. Agric. Environ. Sci. 12, 556–560.

Aly, S.M., Mohamed, M.F., 2010. *Echinacea purpurea* and *Allium sativum* as immunostimulants in fish culture using Nile tilapia (*Oreochromis niloticus*). J. Anim. Physiol. Anim. Nutr. 94, 31–39.

Aly, S., John, G., El-Naggar, G., Mohamed, F., 2007. Effect of *Echinacea* on body growth, and survival and some hematological and immunological parameters of *Oreochromis niloticus* and their response to challenge infection. Egypt J. Aquat. Biol. Fish. 113, 435–445.

Barrows, F.T., Stone, D.A.J., Hardy, R.W., 2007. The effects of extrusion conditions on the nutritional value of soybean meal for rainbow trout (*Oncorhynchus mykiss*). Aquaculture 265, 244–252.

Burger, R.A., Torres, A.R., Warren, R.P., Caldwell, V.D., Hughes, B.G., 1997. *Echinacea* induced cytokine production by human macrophages. Int. J. Immunopharmacol. 19, 371–379.

Burns, J., Gardner, P.T., O’Neill, J., Crawford, S., Morecroft, I., McPhail, D.B., Lister, C., Matthews, D., MacLean, M.R., Lean, M.E.J., Duthie, G.G., Crozier, A., 2000. Relationship among antioxidant activity, vasodilation capacity, and phenolic content of red wines. J. Agric. Food Chem. 48, 220–230.

Dall’Acqua, S., Aiello, N., Scarrettzini, F., Albertini, V., Innocent, G., 2010. Analysis of highly secondary-metabolite producing roots and flowers of two *Echinacea* species. DC. var. *angustifolia* accesses. Ind. Crops Prod. 31, 466–468.

González-Centeno, M.R., Comas-Serra, F., Femenia, A., Rosselló, C., Simal, S., 2015. Effect of power ultrasound application on aqueous extraction of phenolic compounds and antioxidant capacity from grape pomace (*Vitis vinifera* L.): experimental kinetics and modelling. Ultrason. Sonochem. 22, 506–514.

Guz, L., Sopinska, A., Oniszczuk, T., 2011. Effect of *Echinacea purpurea* on growth and survival of guppy (*Poecilia reticulata*) challenged with *Aeromonas hydrophila*. Aquac. Nutr. 17, 595–700.

Hu, C., Kitts, D.D., 2000. Studies on the antioxidant activity of *Echinacea* root extract. J. Agric. Food Chem. 48, 1466–1472.

Kedare, S.B., Singh, R.P., 2011. Genesis and development of DPPH method of antioxidant assay. J. Food Sci. Technol. 48, 412–422.

Li, H., Zhou, X., Gao, P., Li, Q., Li, H., Huang, R., Wu, M., 2016. Inhibition of lipid oxidation in food and feed materials and hydroxyl radical-induced fish erythrocytes: a comparative study of *Ginkgo biloba* leaves extracts and synthetic antioxidants. Anim. Nutr. J. http://dx.doi.org/10.1016/j.aninu.2016.04.007.

Luettig, B., Steinmüller, C., Gilford, G.E., Wagner, H., Lohmann-Matthes, M.L., 1989. Macrophage activation by the polysaccharide arabinogalactan isolated from plant cell cultures of *Echinacea purpurea*. J. Natl Cancer Inst. 81, 669–675.

Magginia, R., Tozzinia, L., Pacificia, S., Raffaelli, A., Pardossia, A., 2012. Growth and accumulation of caffeic acid derivatives in *Echinacea angustifolia* DC. var. *angustifolia* grown in hydroponic culture. Ind. Crops Prod. 35, 269–273.

Magrone, T., Fontana, S., Laforgia, F., Dragone, T., Jirillo, E., Passantino, L., 2016. Administration of a polyphenol-enriched feed to farmed sea bass (*Dicentrarchus labrax* L.) modulates intestinal
and spleen immune responses. Oxid. Med. Cell. Longev. http://dx.doi.org/10.1155/2016/2827567.

Marteau, C., Nardello-Rataj, V., Favier, D., Aubry, J.M., 2013. Dual role of phenols as fragrances and antioxidants: mechanism, kinetics and drastic solvent effect. Flavour Fragr. J. 28, 30–38.

Mościcki, L., Mitrus, M., Wójtowicz, A., Oniszczuk, T., Rejak, A., Jansen, L., 2012. Application of extrusion-cooking for processing of thermoplastic starch (TPS). Food Res. Int. 47, 291–299.

Mishima, S., Saito, K., Maruyama, H., Inoue, M., Yamashita, T., Ishida, T., Gu, Y., 2004. Antioxidant and immuno-enhancing effects of Echinacea purpurea. Biol. Pharm. Bull. 27, 1004–1009.

Nematalla, K.M., Ghada, S.A.A., Yousef, M., Shabib, Z.A., 2011. Effect of Echinacea as antioxidant on markers of aging. Aust. J. Basic Appl. Sci. 5, 18–26.

Nowak, R., Szewczyk, K., Gawlik-Dziki, U., Rzymowska, J., Komsta, Ł., 2016. Antioxidative and cytotoxic potential of some Chenopodium L. species growing in Poland. Saudi J. Biol. Sci. 23, 15–23.

Oniszczuk, A., Olech, M., 2016. Optimization of ultrasound-assisted extraction and LC-ESI-MS/MS analysis of phenolic acids from Brassica oleracea L. var. sabellica. Ind. Crops Prod. 83, 359–363.

Oniszczuk, A., Podgórska, R., Oniszczuk, T., Żukiewicz-Sobczak, W., Nowak, R., Waksmundzka-Hajnos, M., 2014. Extraction methods for the determination of phenolic compounds from Equisetum arvense L. herb. Ind. Crops Prod. 61, 377–381.

Oniszczuk, A., Podgórski, R., 2015. Influence of different extraction method on quantification of selected flavonoids and phenolic acids from Tilia cordata inflorescence. Ind. Crops Prod. 76, 509–514.

Oniszczuk, A., Wojtunik, K., Oniszczuk, T., Wójtowicz, A., Mościcki, L., Waksmundzka-Hajnos, M., 2015. Radical scavenging activity of instant grits with addition of chamomile flowers determined by TLC-DPPH test and by spectrophotometric method. J. Liq. Chromatogr. Relat. Technol. 38, 1142–1146.

Oniszczuk, T., Mościcki, L., Wójtowicz, A., Mitrus, M., Rejak, A., Zawislak, K., Sobczak, P., Mazur, J., Oniszczuk, A., 2012. Influence of process conditions on quality and energy consumption during extrusion-cooking of carp feed. TEKA 2, 185–190.

Pellati, F., Benvenuti, S., Magro, L., Melegari, M., Soraghi, F., 2004. Analysis of phenolic compounds and radical scavenging activity of Echinacea spp. J. Pharm. Biochem. Anal. 35, 289–301.

Rattanachaikunsopon, P., Plunkachorn, P., 2007. Bacteriostatic effect of flavonoids isolated from leaves of Psidium guajava on fish pathogens. Fitoterapia 78, 434–436.

Roesler, J., Emmendörffer, A., Steimmelüer, C., Luetting, B., Wagner, H., Lohmann-Matthes, M.L., 1991. Application of purified polysaccharides from cell cultures of the plant Echinacea purpurea to test subjects mediates activation of the phagocyte system. Int. J. Immunopharmacol. 13, 931–941.

Stojeczka, V., Ainsworth, P., Plunkett, A., İbanoglu, S., 2010. The advantage of using extrusion processing for increasing dietary fibre level in gluten-free products. Food Chem. 121, 156–164.

Świeca, M., 2015. Elicitation with abiotic stresses improves pro-health constituents, antioxidant potential and nutritional quality of lentil sprouts. Saudi J. Biol. Sci. 22, 409–416.

Thygesen, L., Thulin, J., Mortensen, A., Skibsted, L.H., Molgaard, P., 2007. Antioxidant activity of eichoric acid and alkamides from Echinacea purpurea, alone and in combination. Food Chem. 101, 74–81.

Wianowska, D., Hajnos, M.L., Dawidowicz, A.L., Oniszczuk, A., Waksmundzka-Hajnos, M., Głowiak, K., 2009. Extraction methods of 10-deacetylbaccatin III, paclitaxel, and cephalomannine from Taxus baccata L. twigs: a comparison. J. Liq. Chromatogr. Relat. Technol. 32, 589–601.

Wójtowicz, A., Mościcki, L., 2009. Influence of extrusion-cooking parameters on some quality aspects of precooked pasta-like products. J. Food Sci. 74, 226–233.

Yanli, Z., Tiexin, T., Hanjun, H., Hong, W., Zhuoyan, H., 2011. Influence of several postharvest processing methods on polyphenol oxidase activity and eichoric acid content of Echinacea purpurea roots. Ind. Crops Prod. 34, 873–881.

Zolgharniein, N., Niazi, A., Afuni-Zadeh, S., Zamani, K., 2010. Determination of eichoric acid as a biomarker in echinacea purpurea cultivated in Iran using high performance liquid chromatography. Chin. Med. I, 23–27.