Acta Agroph., 2018, 25(4), 421-435
doi: 10.31545/

A STUDY ON THE TWIN SCREW EXTRUSION-COOKING OF PLANT-MEAT PET FOOD MIXTURES

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Abstract. A study was conducted on the possibility of application of "dry" extrusion-cooking technology for the processing of plant-meat mixtures. The effect of the concentration of the meat material (meat-bone pulp), leguminous material (faba bean), wheat grain meal and extrusion temperature on the process run, physicochemical properties and on the microstructure of the extrudates was investigated. It was demonstrated that extrusion-cooking with a twin-screw extruder permits the processing of blends with up to 30% meat-bone pulp content. Increase in the content of the meat material caused a decrease of specific density and water solubility index (WSI) of the extrudates. At the same time, a significant increase in the content of proteins, fat, and ash was observed. Extrusion temperature increase from 130 to 250°C caused an increase in the degree of expansion ratio and impact strength of the extrudates and a decrease in specific density of the products. At the same time, the microstructure of the products was changed from cohesive and compacted to more expanded and porous.

Keywords: extrusion-cooking, faba been, meat-bone pulp, pet food, wheat

INTRODUCTION

Extrusion processing has become very popular in the feed and food industries due to its high versatility, productivity, and product quality (Guz *et al.* 2011, Ayadi *et al.* 2012, Samuelsen *et al.* 2013, Guz *et al.* 2014, Oniszczuk *et al.* 2017). During the extrusion process, as a result of high temperature, pressure and shear forces, the material processed is intensively mixed, compressed and plasticised, inclusive of the liquefaction of the mass due to phase transition. At the time of liquefaction the material is cooked and forced under pressure through the die of the extruder (Castells *et al.* 2005). Such treatment permits physicochemical transformation of the material; gelatinisation of starch, denaturation of protein, inactivation of some anti-nutritional factors, and the obtainment of a product with very high microbiological purity. It has been demonstrated that the process of extrusion leads to a reduction of the total number of microorganisms (Okelo *et al.* 2006) and spores of bacteria (Likimani and Sofos 1990). Van de Velde *et al.* (1984), using the most thermo-resistant strains of bacteria (*Bacillus stearothermophilus FS 1518*), demonstrated that twin-screw extrusion guarantees sterility of the extrudate if – for a given temperature – a minimum time of the material retention in the extruder is guaranteed. It needs to be noted, however, that only strict control of the time of material retention in the extruder and assurance of appropriate extrusion temperature provides a guarantee of sterility of the product. Fulfilment of those conditions is possible only in the case of counter-rotating twin-screw extrusion.

The physical properties of the extrudate, e.g. porosity structure, texture, expansion, specific density, water solubility index (WSI), play a significant role in the case of pet food. These properties can be created through the composition of the blend material as well as the parameters of the extrusion process, such as the profile of barrel temperature, pressure distribution, configuration and speed of the extruder screws, size and shape of the die (Sobota and Rzedzicki 2009, Ayadi *et al*. 2012, Samuelsen *et al.* 2013, Wójtowicz *et al.* 2015).

The aim of the study was to investigate the applicability of meat-bone pulp for the production of pet food using the counter-rotating twin-screw extrusion process. The influence of the raw material composition (concentration of meat-bone pulp and faba bean meal) and of the process temperature on the physical properties, chemical composition and microstructure of the products was analysed.

MATERIALS AND METHODS

Meat-bone pulp (MBP) from mechanical de-boning of poultry carcasses (Indykpol S.A., Lublin, Poland), faba bean meal (FBM) (*Vicia Faba L.* cv. Nadwiślański) (Agropol, Motycz, Poland) and wheat grain meal (WGM) (*Triticum aestivum L.* cv. Henika) (Agropol, Motycz, Poland) were used in this study. The chemical composition of these materials is presented in Table 1.

The plant materials were fragmented in an impact grinder type H-111/3 (Agromet, Jawor, Poland), using a sieve with a hole diameter of 3 mm. The meat component was mixed with the plant materials in a periodic mixer type H-095 (Agro-Wikt, Opoczno, Poland) with capacity of 80 dm^3.

The study was conducted using a counter-rotating twin-screw extruder (Metalchem, Gliwice, Poland) with conical screws, with L:D ratio of 12:1 and with 3 open forming dies, each 6 mm in diameter. The screw speed applied was 75 rpm.

Table 1. Chemical composition of raw materials

Nutrient	Meat-bone pulp	Wheat grain meal	Faba bean meal
Dry matter (%)	43.96	87.17	89.97
Crude protein (% d.w.)	43.25	17.21	29.87
Crude fat (% d.w.)	19.98	1.45	0.90
Ash (% d.w.)	33.15	2.33	4.51
TDF (% d.w.)	–	21.31	35.00
IDF (% d.w.)	–	17.54	29.22
SDF (% d.w.)	–	3.77	5.78

TDF – total dietary fibre, IDF – insoluble dietary fibre; SDF – soluble dietary fibre; d.w. – dry weight

The model of the experiment is presented in Table 2. In the first part of the experiment, the share of the meat-bone pulp varied in the range of 5 to 30% (samples 1-6). At the same time, a constant ratio (1:4) between faba bean meal and wheat grain meal was used. In the second part of the experiment, the share of faba bean was variable (between 0 and 30%, samples 7-13) and a constant ratio of meat-bone pulp (20%) was used. In the third part of the work, the blends composition was constant while the temperature of extrusion was varied between 130 and 250°C (samples 14-20). The parameter ranges applied in the study and the composition of the blend were determined on the basis of pilot experiments. Only such parameters were adopted that guaranteed correct and stable run of the process.

Table 2. Model of experiment

Sample	Components (%)			Profile of barrel temperature (°C)
	Meat-bone pulp	Faba bean meal	Wheat grain meal	
1	5	19	76	120/140/180/180/130
2	10	18	72	
3	15	17	68	
4	20	16	64	
5	25	15	60	
6	30	14	56	
7	20	0	80	120/140/180/180/130
8	20	5	75	
9	20	10	70	
10	20	15	65	
11	20	20	60	
12	20	25	55	
13	20	30	50	
14	20	20	60	90/110/130/130/130
15				110/130/150/150/130
16				120/140/170/170/130
17				125/150/190/190/130
18				130/160/210/210/130
19				140/170/230/230/130
20				150/180/250/250/130

To maintain constant and comparable conditions of extrusion, samples with a lower content of the meat component were moistened to the moisture content of 28%. That moisture level permitted the process of “dry” extrusion, and at the same time contributed to the maintenance of high nutritional value of the extrudates.

Expansion ratio was calculated as the ratio of the cross section area of the extrudates to the cross section area of the die. Specific density was calculated from the ratio of the mass to the volume of the samples tested (Rzedzicki *et al.* 2000). Measurements of impact strength were made with the use of the Charpy impact test. Charpy hammer (Wolfgang Ohst, Rathenow, Germany) with supports spacing of 40 mm and hammer of 0.5 J was used. The water solubility index (WSI) was assayed according to the centrifuge method (AACC, 88-04) modified by the authors. Ground sample (2 g) was placed in centrifuge tubes, 30 ml of distilled water was added, then the tubes were stopped and shaken vigorously. The suspension was left to rest for 5 minutes, then it was centrifuged for 15 min at 2200 g. 10 ml of the supernatant was dried to solid mass.

$$WSI(\%) = (weight\ of\ dried\ supernatant \frac{30\,ml}{10\,ml} / dry\ weight\ of\ sample)100\%$$

Moisture content (MC) was determined with the method AACC 44-15A. Dry matter (DM) content was calculated from the formula: 100% – MC (%) = DM (%). Ash content was assayed according to the method AACC 08-01, protein content with the method AACC 46-08, using nitrogen to protein conversion factor of N x 6.25. For protein content analysis the Kjeltec^{TM} 2300 Automatic Analyzer (Foss, Hoganas, Sweden) was used. Free fat was determined in accordance with the method AACC 30-26 (AACC, 2000) by means of Soxtec^{TM} 2050 (Foss, Hoganas, Sweden). Enzymatic method was applied to determine the content of total dietary fibre (TDF), insoluble dietary fibre (IDF) and soluble dietary fibre (SDF). In the enzymatic fibre determinations, Megazyme enzymes and procedures were employed (AOAC 991.43; AACC 32-07; AACC 32-21; AOAC 985.29; AACC 32-05). The content of minerals – calcium, magnesium, sodium, potassium, manganese, copper, iron, zinc, chromium and nickel – in the extrudates was assayed with the method AOAC 975.03 (AOAC, 1990).

Changes in the microstructure of the extrudates were analysed as a function of the increasing proportion of faba bean and process temperature. Samples were used to slice off fragments of extrudates that were then glued with silver paste onto specimen circles, and sprayed with carbon and gold in a vacuum sprayer type JEE 4X (JEOL, Tokyo, Japan). Microscope analyses were made with the help of electron microscope type JSM 5200 (JEOL, Tokyo, Japan).

Chemical analyses were made in three replicates. Physical properties were analysed in five replicates. Mean values, standard deviations, and significance of differences between mean values (Duncan test, $P \leq 0.05$) were determined. Backward stepwise regression procedure, in which the choice of predictive variables was carried out by an automatic procedure, was used to build regression models (α = 0.05). The dependent variables for which regression models were built were expansion ratio, specific density, impact strength, WSI, crude protein, crude fat, ash and TDF. The predictor variables were extrusion temperature, meat-bone pulp and faba bean meal content. Statistical analysis of the results was performed with the use of the program SAS 9.1.3 (SAS Institute Inc., Cary, USA).

RESULTS

Effect of the concentration of meat-bone pulp (MBP)

Extrudates with a content of MBP had a compact and hard structure, resistant to crumbling. Increase of the content of MBP in the extrudates caused an increase of radial expansion and impact strength. At the same time, a decrease in the specific density of the products was observed (Tab. 3). The index of dry matter solubility (WSI) was low and fell within the range from 5.51 to 8.92% (Tab. 3). Increase in the content of the meat component caused a significant drop (Duncan test, $P \leq 0.05$) in the values of WSI.

Table 3. Physical properties of the extrudates

Sample	Expansion ratio	Specific density (kg m^{-3})	Impact strength (J cm^{-2})	WSI (% d.w.)
1	$1.79^{g} \pm 0.03$	$988.78^{a} \pm 3.12$	$0.058^{j} \pm 0.001$	$8.92^{a} \pm 0.38$
2	$1.85^{f} \pm 0.04$	$982.26^{b} \pm 2.02$	$0.061^{j} \pm 0.001$	$7.16^{b} \pm 0.02$
3	$1.98^{e} \pm 0.02$	$937.53^{e} \pm 1.03$	$0.072^{g} \pm 0.002$	$7.05^{bc} \pm 0.04$
4	$1.98^{e} \pm 0.02$	$934.34^{f} \pm 1.31$	$0.067^{ih} \pm 0.001$	$6.14^{h} \pm 0.07$
5	$2.00^{ed} \pm 0.03$	$883.51^{m} \pm 1.93$	$0.077^{f} \pm 0.003$	$5.80^{i} \pm 0.39$
6	$1.99^{ed} \pm 0.03$	$900.42^{k} \pm 2.12$	$0.08^{fe} \pm 0.001$	$5.51^{j} \pm 0.26$
7	$2.07^{cba} \pm 0.03$	$848.28^{n} \pm 1.51$	$0.052^{k} \pm 0.004$	$6.41^{gf} \pm 0.01$
8	$2.05^{dcba} \pm 0.04$	$889.27^{l} \pm 1.58$	$0.06^{j} \pm 0.001$	$6.53^{fe} \pm 0.02$
9	$2.04^{edcb} \pm 0.02$	$915.48^{i} \pm 0.97$	$0.07^{hg} \pm 0.002$	$6.43^{gf} \pm 0.18$
10	$2.03^{edc} \pm 0.02$	$924.59^{h} \pm 1.17$	$0.065^{i} \pm 0.001$	$6.31^{hgf} \pm 0.02$
11	$2.04^{edcb} \pm 0.03$	$916.41^{i} \pm 0.85$	$0.085^{cb} \pm 0.002$	$6.33^{hgf} \pm 0.05$
12	$2.04^{edcb} \pm 0.02$	$915.84^{i} \pm 1.58$	$0.084^{dcb} \pm 0.001$	$6.85^{dc} \pm 0.04$
13	$2.04^{edcb} \pm 0.02$	$908.78^{j} \pm 1.05$	$0.096^{a} \pm 0.003$	$6.34^{hgf} \pm 0.05$
14	$1.52^{h} \pm 0.01$	$987.57^{a} \pm 0.72$	$0.053^{k} \pm 0.002$	$6.40^{hgf} \pm 0.05$
15	$1.78^{g} \pm 0.02$	$950.48^{d} \pm 1.23$	$0.061^{j} \pm 0.003$	$6.77^{ed} \pm 0.01$
16	$1.90^{f} \pm 0.03$	$953.9^{c} \pm 2.01$	$0.081^{de} \pm 0.004$	$6.56^{fe} \pm 0.1$
17	$2.04^{edcb} \pm 0.03$	$949.28^{d} \pm 0.97$	$0.087^{b} \pm 0.001$	$6.58^{fe} \pm 0.09$
18	$2.08^{cba} \pm 0.04$	$931.43^{g} \pm 1.56$	$0.082^{edc} \pm 0.001$	$6.55^{fe} \pm 0.06$
19	$2.11^{a} \pm 0.02$	$899.97^{k} \pm 1.02$	$0.077^{f} \pm 0.002$	$6.21^{hg} \pm 0.03$
20	$2.10^{ba} \pm 0.04$	$901.93^{k} \pm 0.91$	$0.073^{g} \pm 0.001$	$6.43^{gf} \pm 0.04$

WSI – water solubility index; a–n – means within in a column with different letters differ at $P \leq 0.05$; d.w. – dry weight

The addition of MBP to the wheat-faba bean blend caused also significant changes in the chemical composition of the extrudates. A significant increase was noted in the content of protein, fat and ash. Sample with 30% addition of the meat-bone pulp was characterised by 21.96% d.w. content of protein, 4.02% d.w. content of crude fat and 4.57% d.w. content of ash. The dietary fibre content (TDF), including soluble (SDF) and insoluble fractions (IDF), decreased with the addition of MBP (Tab. 4).

Table 4. Chemical composition of the extrudates

Sample	Dry matter	Crude protein	Crude fat	Ash	TDF	SDF	IDF
	(%)	(% d.w.)					
1	$91.16^{gh} \pm 0.03$	$18.79^{j} \pm 0.04$	$1.57^{i} \pm 0.02$	$3.26^{n} \pm 0.01$	$21.46^{e} \pm 0.13$	$5.16^{d} \pm 0.06$	$16.3^{i} \pm 0.09$
2	$91.13^{h} \pm 0.06$	$19.47^{h} \pm 0.05$	$1.96^{h} \pm 0.04$	$4.07^{m} \pm 0.02$	$21.64^{e} \pm 0.12$	$5.05^{ef} \pm 0.05$	$16.59^{h} \pm 0.07$
3	$91.73^{d} \pm 0.05$	$20.38^{f} \pm 0.49$	$2.33^{fg} \pm 0.09$	$5.01^{l} \pm 0.03$	$20.92^{g} \pm 0.04$	$4.88^{g} \pm 0.02$	$16.04^{j} \pm 0.06$
4	$92.36^{a} \pm 0.04$	$20.61^{e} \pm 0.07$	$2.81^{bcd} \pm 0.06$	$5.84^{ij} \pm 0.04$	$20.05^{i} \pm 0.1$	$4.73^{hij} \pm 0.03$	$15.32^{k} \pm 0.07$
5	$91.18^{fgh} \pm 0.16$	$21.34^{cd} \pm 0.06$	$3.07^{b} \pm 0.67$	$6.63^{b} \pm 0.07$	$19.12^{k} \pm 0.14$	$4.48^{k} \pm 0.04$	$14.64^{l} \pm 0.1$
6	$91.21^{fgh} \pm 0.06$	$21.96^{b} \pm 0.07$	$4.02^{a} \pm 0.1$	$7.57^{a} \pm 0.06$	$17.52^{m} \pm 0.03$	$3.84^{l} \pm 0.03$	$13.68^{o} \pm 0.06$
7	$91.26^{fgh} \pm 0.07$	$19.12^{i} \pm 0.07$	$2.96^{bc} \pm 0.08$	$5.67^{k} \pm 0.02$	$18.58^{l} \pm 0.1$	$4.65^{ij} \pm 0.07$	$13.93^{n} \pm 0.03$
8	$92.31^{a} \pm 0.06$	$19.57^{h} \pm 0.07$	$2.84^{bcd} \pm 0.09$	$5.79^{j} \pm 0.01$	$19.12^{k} \pm 0.12$	$4.81^{gh} \pm 0.08$	$14.31^{m} \pm 0.04$
9	$91.18^{fgh} \pm 0.05$	$20.13^{g} \pm 0.07$	$2.84^{bcd} \pm 0.05$	$5.88^{i} \pm 0.03$	$19.69^{j} \pm 0.11$	$4.98^{f} \pm 0.03$	$14.71^{l} \pm 0.08$
10	$91.52^{e} \pm 0.07$	$20.53^{fe} \pm 0.09$	$2.81^{bcd} \pm 0.07$	$5.96^{h} \pm 0.05$	$20.62^{h} \pm 0.18$	$5.38^{c} \pm 0.08$	$15.24^{k} \pm 0.1$
11	$91.34^{efg} \pm 0.04$	$21.17^{d} \pm 0.01$	$2.66^{cde} \pm 0.1$	$6.12^{efg} \pm 0.02$	$21.57^{e} \pm 0.03$	$5.09^{de} \pm 0.03$	$16.48^{h} \pm 0.042$
12	$91.38^{ef} \pm 0.02$	$21.84^{b} \pm 0.04$	$2.39^{efg} \pm 0.09$	$6.23^{d} \pm 0.03$	$22.54^{b} \pm 0.18$	$5.42^{c} \pm 0.07$	$17.12^{b} \pm 0.11$
13	$91.21^{fgh} \pm 0.05$	$22.38^{a} \pm 0.11$	$2.11^{gh} \pm 0.04$	$6.31^{c} \pm 0.04$	$22.9^{a} \pm 0.05$	$5.53^{b} \pm 0.03$	$17.37^{a} \pm 0.02$
14	$91.84^{cd} \pm 0.08$	$21.39^{cd} \pm 0.04$	$2.9^{bc} \pm 0.04$	$6.14^{ef} \pm 0.01$	$21.24^{f} \pm 0.06$	$4.64^{j} \pm 0.01$	$16.6^{gh} \pm 0.05$
15	$91.94^{cd} \pm 0.05$	$21.41^{c} \pm 0.01$	$2.86^{bcd} \pm 0.02$	$6.15^{e} \pm 0.03$	$21.58^{e} \pm 0.11$	$4.87^{g} \pm 0.07$	$16.71f^{g} \pm 0.04$
16	$92.46^{a} \pm 0.06$	$21.25^{cd} \pm 0.05$	$2.66^{cde} \pm 0.13$	$6.07^{g} \pm 0.05$	$21.56^{e} \pm 0.12$	$4.74^{ih} \pm 0.1$	$16.82^{def} \pm 0.02$
17	$92.04^{b} \pm 0.04$	$21.25^{cd} \pm 0.02$	$2.57^{def} \pm 0.02$	$6.11^{efg} \pm 0.04$	$22.13^{c} \pm 0.08$	$5.17^{d} \pm 0.02$	$16.96^{c} \pm 0.06$
18	$91.51^{e} \pm 0.03$	$21.2^{cd} \pm 0.03$	$2.4^{efg} \pm 0.06$	$6.12^{efg} \pm 0.02$	$21.92^{d} \pm 0.11$	$5.04^{fe} \pm 0.04$	$16.88^{cd} \pm 0.07$
19	$91.27^{fgh} \pm 0.07$	$21.23^{cd} \pm 0.02$	$2.21^{gh} \pm 0.02$	$6.08^{fg} \pm 0.02$	$22.62^{b} \pm 0.08$	$5.78^{a} \pm 0.06$	$16.84^{de} \pm 0.02$
20	$91.49^{e} \pm 0.04$	$21.18^{cd} \pm 0.02$	$2.16^{gh} \pm 0.11$	$6.1^{efg} \pm 0.03$	$22.12^{c} \pm 0.18$	$5.38^{c} \pm 0.08$	$16.74^{ef} \pm 0.1$

a-o – means within in a column with different letters differ at $P \leq 0.05$; TDF – total dietary fibre; SDF – soluble dietary fibre; IDF – insoluble dietary fibre; d.w. – dry weight

Increasing shares of MBP (from 5 to 30%) led to a distinct increase in the content of Ca, Fe, Zn, Cu, Cr, Ni and a slight increase in the level of Na and Mg (Tab. 5). At the same time, the content of K did not change significantly. Moreover, a notable drop in the content of Mn was noted, probably as a result of increase in the content of the meat component, at the expense of reduced levels of faba bean and wheat grain meals.

The extruded samples were characterised by a big diversity of form and structure. That diversity resulted both from the varied raw material composition and from the variable process temperature. The sample containing 20% of meat-bone pulp and 80% of wheat grain meal, presented in Figure 1, was characterised by a low specific density and relatively high degree of expansion. The product had an irregular, porous macrostructure (Fig. 1A). The size of air pores was highly various and ranged from several to few hundreds micrometres. There is an observable absence of a distinct cellular structure of the extrudates, characteristic for expanded products. The product appears to be insufficiently processed. However, analysis of the microstructure (Figs 1B-C) revealed that during the process of extrusion the material processed was completely liquefied. The microstructure has the form of a liquefied and set mass, with small pores with the size of less than twenty micrometres. At magnification of x1000 (Fig. 1C) one can see melted starch granules, encased in a protein matrix.

Table 5. Composition of macro- and microelements in the extrudates (mg kg^{-1} d.w.)

Sample	Macroelements				Microelements					
	K	Ca	Na	Mg	Fe	Cu	Mn	Ni	Cr	Zn
1	1735^{fedc}	1140^{m}	421^{j}	1554^{k}	47.953^{f}	6.69^{j}	40.686^{ed}	0.639^{h}	0.528^{b}	46.917^{ji}
2	1727^{fedc}	1290^{l}	421^{j}	1576^{j}	50.08^{e}	6.72^{j}	38.851^{f}	0.761^{g}	0.26^{f}	48.03^{ihg}
3	1715^{fed}	1410^{k}	432^{ji}	1680^{hg}	52.775^{c}	6.67^{j}	35.08^{g}	0.88^{f}	0.44^{dc}	49.26^{gfe}
4	1715^{fed}	1600^{j}	452^{hgf}	1702^{edc}	53.848^{c}	7.0^{ihg}	32.79^{h}	0.96^{fe}	0.41^{ed}	50.443^{edcb}
5	1700^{fe}	1780^{i}	482^{b}	1705^{edc}	55.843^{ba}	7.18^{gfe}	30.189^{i}	1.001^{ed}	0.421^{edc}	51.734^{ba}
6	1670^{f}	2450^{h}	522^{a}	1777^{a}	56.594^{a}	7.55^{b}	29.603^{i}	1.11^{cb}	0.645^{a}	52.53^{a}
7	1221^{i}	2440^{h}	441^{hg}	1668^{h}	50.43^{e}	6.047^{f}	38.872^{f}	0.603^{h}	0.38^{e}	45.905^{j}
8	1428^{h}	2660^{g}	450^{hg}	1653^{i}	51.03^{ed}	6.918^{ih}	39.406^{f}	0.94^{fe}	0.465^{dc}	47.42^{hi}
9	1553^{g}	2820^{f}	458^{gfe}	1699^{ed}	52.96^{c}	7.367^{edcb}	40.59^{ed}	1.007^{edc}	0.47^{c}	48.5^{hgf}
10	1753^{fedc}	3160^{e}	461^{gfed}	1683^{gf}	53.204^{c}	7.471^{dcb}	40.623^{ed}	0.95^{fe}	0.42^{edc}	49.917^{fed}
11	1795^{edc}	3190^{e}	469^{fedcb}	1694^{gfed}	53.652^{c}	7.51^{cb}	42.982^{b}	1.08^{dcb}	0.312^{f}	50.937^{dcb}
12	1966^{b}	3290^{cb}	477^{dcb}	1728^{b}	54.295^{cb}	8.144^{a}	44.19^{a}	1.18^{ba}	0.42^{edc}	51.747^{ba}
13	2078^{a}	3710^{a}	479^{cb}	1774^{a}	55.803^{ba}	8.247^{a}	44.24^{a}	1.25^{a}	0.274^{f}	51.62^{cba}
14	1798^{edc}	3220^{ed}	459^{gfe}	1696^{fed}	53.086^{c}	7.25^{fe}	40.948^{d}	0.941^{fe}	0.3^{f}	49.58^{fed}
15	1829^{c}	3280^{dc}	463^{gfedc}	1692^{gfe}	52.58^{dc}	7.31^{ed}	42.988^{b}	0.972^{fe}	0.28^{f}	50.003^{fedc}
16	1826^{c}	3280^{dc}	471^{edcb}	1707^{edc}	53.534^{c}	7.027^{hg}	43.07^{b}	0.95^{fe}	0.29^{f}	50.23^{edcb}
17	1819^{dc}	3300^{cb}	469^{fedcb}	1704^{edc}	53.527^{c}	6.82^{ji}	40.109^{e}	0.99^{ed}	0.3^{f}	50.763^{edcb}
18	1817^{dc}	3320^{cb}	473^{edcb}	1709^{dc}	53.52^{c}	7.087^{hgf}	42.00^{c}	0.97^{fe}	0.32^{f}	50.323^{edcb}
19	1820^{dc}	3300^{cb}	472^{edcb}	1715^{cb}	53.52^{c}	7.29^{fed}	43.52^{ba}	1.08^{dcb}	0.27^{f}	50.07^{fedc}
20	1817^{dc}	3360^{b}	465^{fedcb}	1704^{edc}	53.521^{c}	7.25^{fe}	43.83^{a}	1.11^{cb}	0.28^{f}	50.69^{edcb}

a-m – means within in a column with different letters differ at $P \leq 0.05$

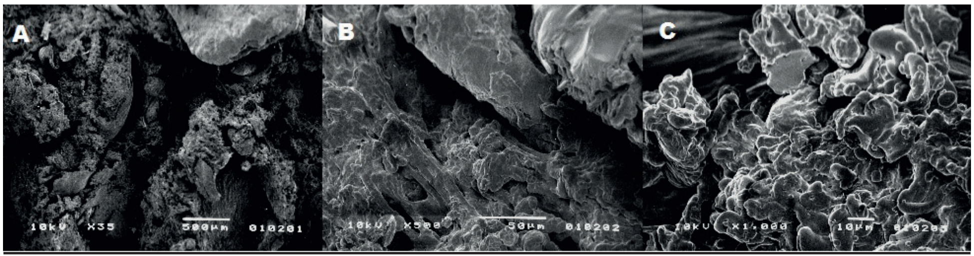

Fig. 1. Scanning electron macro- and micrographs of extruded products. raw composition: 20% meat-bone pulp and 80% wheat; profile of barrel temperature: 120/140/180/180/130°C. magnification: a) x35; b) x500; c) x1000

Effect of the concentration of faba bean meal (FBM)

Increase in the content of FBM at constant extrusion conditions did not significantly affect the degree of radial expansion and WSI, but it did cause an increase in the specific density and impact strength of the products (Tab. 3). The highest impact strength (0.096 J cm^{-2}) was characteristic of extrduates composed of 20% MBP, 30% FBM and 50% WGM (sample 13) (Tab. 3).

The introduction of FBM to the wheat–meat blends led to an increase of protein content in the extrudates. Products without an addition of FBM (sample 7) contained 19.12% d.w. of protein, while those with 30% share of faba bean – 22.38% d.w. (Tab. 4). The introduction of a greater share of FBM increased also the content of TDF, IDF and SDF. At the same time, a decrease in the fat content was observed (Tab. 4).

As a result of the introduction of FBM, a significant increase (Duncan test, $P \leq 0.05$) was noted in the contents of K, Ca, Na, Mg, Fe, Mn, Zn, Cu and Ni. Only in the case of Cr content no clear tendency was observed (Tab. 5).

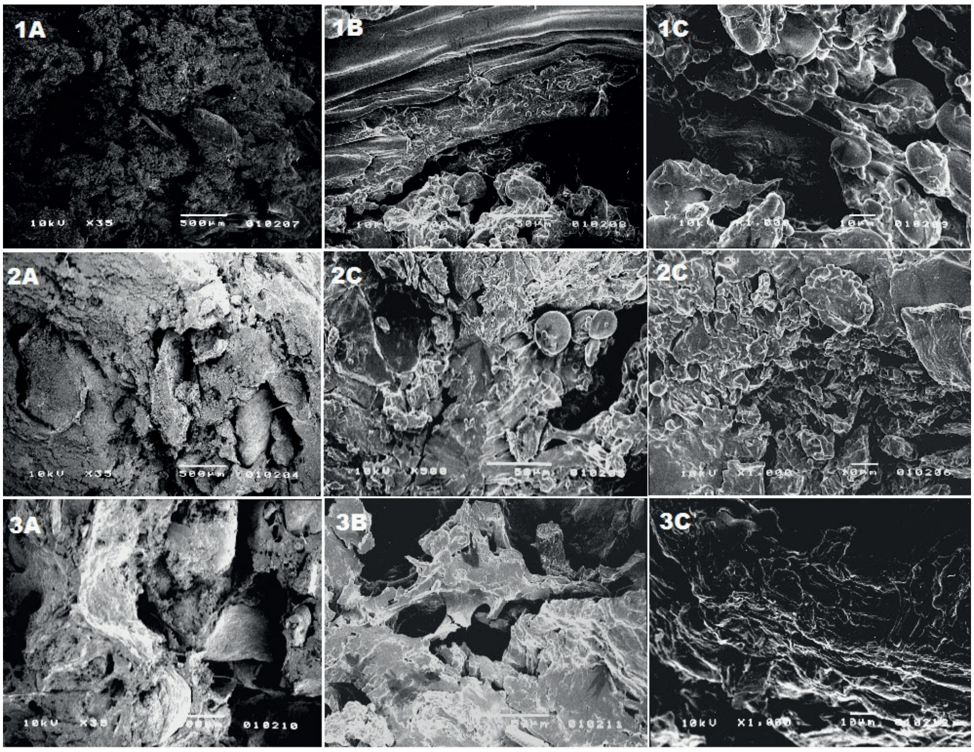

Fig. 2. Scanning electron macro- and micrographs of extruded products. raw composition: 20% meat-bone pulp, 20% faba bean and 60% wheat; profile of barrel temperature: 1) 90/110/130/130/130°C; 2) 120/140/180/180/130°C; 3)150/180/250/250/130°C. magnification: a) x35; b) x500; c) x1000

The addition of faba bean at the dose of 20%, with simultaneous reduction of the share of wheat grain meal to 60% (sample 11), caused an increase of specific density of the extrudate. The microstructure of the products become densely-packed, compact, with hard texture (Figs 2-2A). The cell walls were thick, with characteristic fraying. There were air pores with sizes from about one micrometre to over ten micrometres (Figs 2-2B and 2-2C). The structure of the cell walls had the form of small melted fragments of the material, encased in protein mass.

Effect of the process temperature

Increase of the extrusion temperature of blends with a constant material composition (20% MBP, 20% FBM, 60% WGM) caused an increase in the degree of radial expansion and impact strength of the products (Tab. 3). At the same time, a decrease of specific density was observed. No significant changes were noted in the values of WSI.

The study showed no significant changes in protein and ash content as a function of the process temperature. Whereas, with increase in the process temperature from 130°C to 250°C, determinability of fat decreased from 2.9 to 2.16% d.w. The use of high extrusion temperatures caused an increase in the contents of TDF as well as SDF (Tab. 4).

Process temperature did not have any significant effect on the content of macroelements (K, Ca, Na and Mg) and microelements (Fe, Cu, Cr and Zn) in the extrudates (Tab. 5).

The study demonstrated a very strong effect of extrusion temperature on the internal structure of the product. Application of low process temperatures (130°C) resulted in the appearance of cohesive and compacted, gritty-like structure (Figs 2-1A). This product was characterised by low expansion ratio and low impact strength. Photographs of microstructure (Figs 2-1B and 1C) reveal non-liquefied starch granules, encased in and delicately bonded with thin protein bridges. Most probably, under conditions of low extrusion temperature and the protective action of fat, starch was not completely gelatinised. Increase of extrusion temperature caused a clearly visible change in the microstructure of the products (Figs 2-2A and 2-3A). The gritty-like macrostructure observed earlier, disappeared. At a magnification x500 (Figs 2-3B) one can clearly see homogeneous cell walls and air spaces with sizes of as much as tens of micrometres. The confirmation of complete liquefaction of the material is the image of microstructure visible in Figures 2-3C. Cell walls have the form of multi-layered structures, built of flat and well-liquefied fragments of material.

DISCUSSION

The application of twin-screw counter-rotating extrusion permitted the processing of plant-meat blends with the content of the meat component of up to 30%. Any higher content of the meat-bone pulp caused an excessive moisture of the material, which led to the material sticking to the feeder screws and the extruder screws. The disturbances observed in material dosage caused non-uniform flow of the mass in the extruder barrel. That resulted in the material caking on the barrel walls and on the screws, and blocking the extruder.

Products with MBP were characterised by a relatively low degree of expansion and a thick and compact structure. Colona *et al.* (1989) report that the diameter of products of pet food type should be about 200-300% relative to the die diameter. The resulting products were characterised by slightly lower expansion. The content of MBP was positively correlated with radial expansion and negatively correlated with specific density (Tab. 6). The tendency noted could have been a result of increase in the content of fat in the extruded blend. According to Colona *et al.* (1989), fat content increase to 5% resulted in an increase in the degree of radial expansion. Many researchers have found an opposite effect in the case of increase of protein content. Allen *et al.* (2007) observed a decrease in radial expansion and an increase in specific density of extrudates, with an increase in the content of protein in maize extrudates. Seker (2005) claims that the formation of a strong protein matrix can inhibit the liberation of water vapour from the product exiting the die of the extruder, and contribute to the formation of a compact and dense structure of extrudates. The presented study, however, did not confirm aforementioned results. The increase in the share of high-protein materials such as MBP and FBM had no impact on the decrease in the degree of expansion ratio.

Table 6. Regression models for the physical properties

	Expansion ratio			Specific density			Impact strength			WSI		
	B_W	SE	P- value	B_W	SE	P- value	B_W	SE	P- value	B_W	SE	P- value
Intercept	1.043	0.177	<0.0001	1069.61	44.33	<0.0001	0.004	0.016	0.8000	8.910	0.270	<0.0001
Meat-bone pulp	0.009	0.004	0.06	–3.91	1.03	0.0016	0.001	0.000	0.0081	0.120	0.010	<0.0001
Faba bean meal	–	–	–	2.39	0.78	0.0076	0.001	0.000	0.0005	–	–	–
Extrusion temperature	0.004	0.001	0.0001	–0.59	0.20	0.0100	0.001	0.000	0.0660	–	–	–
R^2	0.62			0.69			0.66			0.81		

WSI – Water solubility index; B_w – Regression coefficients ; SE – Standard error; P-value – only significant variables shown. Backward elimination of insignificant variables

An increase of radial expansion was noted as a result of increase in the process temperature (Tab. 6). At the same time, there was a visible decrease in the specific density of extrudates. The tendency observed resulted from better liquefaction of the mass and lowering of its viscosity under conditions of higher extrusion temperatures. Well liquefied mass passed more easily through the die apertures, causing more intensive expansion of the products, and hence a drop in the specific density of extrudates. Similar trends were noted by Rzedzicki *at al.* (2004) and by Balandran-Quintana *et al.* (1998).

In the case of extrudates for animal feed, the texture and hardness of the products are of high importance. Especially in the diet of carnivores (e.g. dogs, cats), a compact and hard structure is desirable. Increase the contents of both MBP and FBM in the extrudates caused an increase in the impact strength of the products (Tab. 6). According to Faubion and Hoseney (1982), the tendency observed may be a result of increase in protein content. The impact strength of the products was also significantly affected by the process temperature (Tab. 6). The highest impact strength (0.087 J cm^{-2}, sample 17) was characteristic of products extruded at temperature of 190ºC. Temperature increase above that value caused increased degradation of the product and loosening of its structure, manifested in lowered hardness of products (0.073 J cm^{-2}, sample 20). A similar relationship was noted by Colona *et al*. (1989).

Increase in the content of the meat component in the extruded blend led to a decrease of WSI of extrudates (Tab. 6). Fat contained in the meat component could have caused a lubricating effect and thus decrease the shear rate gradient. Reduced friction slowed down the depolymerisation of starch, the consequence of which was a decrease of WSI. It should be mentioned that increase in fat content intensifies the formation of insoluble starch-lipid complexes, which in the opinion of Colona *et al*. (1989) can also be a cause of decrease in WSI values of products. This is supported by the research of Van Hoan *et al.* (2010) who, extruding rice-soybean blends enriched with an addition of soybean oil, noted a decrease of WSI with increase of fat content in the material extruded. In addition, increasing share of meat component was related with a lower level of high-starch plant components: wheat and faba bean. According to Colona *et al*. (1989) and Ding *et al.* (2005), it is mainly the content of starch and the degree of its depolymerisation that determine the value of WSI of extrudates. Decrease in the content of starch in the extruded material could also have been a cause of the decrease of WSI. Pet food should be characterised by low values of WSI. Such products are digested slowly and will give a feeling of satiety for a long of time after ingestion (Brennan *et al*. 2012).

Literature data indicate that an increase of extrusion temperature leads to an increase in the value of WSI (Ding *et al*. 2006, Singh *et al*. 2007). This study showed that the extrusion temperature did not cause any significant changes in the values of WSI (Tab. 6). The important role in this case was that of a high moisture content of the extruded material, inhibiting the depolymerisation of starch. Increase of extrusion temperature was also conducive to increased complexation of lipids. The formation of insoluble starch-lipid complexes could also have had an effect on relatively low WSI values of products obtained at high temperature of extrusion.

Increase in the levels of the meat component and the leguminous component caused an increase in the contents of protein and ash in the extrudates (Tab. 7). Whereas, an increase in fat content in the products was noted only as a result of addition of the fat-rich meat-bone pulp. The concentrate of the meat component in the extrudates was negatively correlated with the content of dietary fibre (Tab. 7). Extrusion-cooking causes partial degradation of molecular mass of fibre components and lowering of their determinability on the one hand, while on the other hand extrusion may be conductive to the formation of new fibre components e.g. resistant starch or products of the Maillard reaction (Singh *et al*. 2007, Sobota and Rzedzicki 2009). Therefore, the content of dietary fibre fractions determined in the extrudates is the result of the above processes. Increase of extrusion temperature from 130 to 250°C caused a significant increase in the contents of TDF and SDF fractions. No significant changes in the content of IDF were observed.

Table 7. Regression models for crude protein, crude fat, ash and TDF content

	Crude protein			Crude fat			Ash			TDF		
	B_W	SE	P- value	B_W	SE	P- value	B_W	SE	P- value	B_W	SE	P- value
Intercept	160.49	1.31	<0.0001	27.16	3.12	<0.0001	23.71	0.77	<0.0001	122.19	8.65	<0.0001
Meat-bone pulp	1.48	0.05	<0.0001	0.82	0.07	<0.0001	1.63	0.03	<0.0001	–2.04	0.20	<0.0001
Faba bean meal	1.11	0.04	<0.0001	–0.26	0.05	<0.0003	0.23	0.02	<0.0001	1.65	0.15	<0.0001
Extrusion temperature	–	–	–	–0.07	0.01	<0.0002	–	–		0.10	0.04	<0.0170
R^2	0.99			0.92			0.99			0.93		

TDF – total dietary fibre, WSI – Water solubility index; B_w – Regression coefficients ; SE– Standard error; P-value – only significant variables shown. Backward elimination of insignificant variables

Increase of the temperature of extrusion may lead to an increase in the content of certain microelements (Fe, Ni). This is related with the wear of the working parts of the extruder and with migration of elements to the product. However, in the presented study, extrusion temperature had no significant effect on the levels of most macro- and microelements (Duncan test, $P \leq 0.05$). The decisive role in this case could have been that of high moisture of the material and high content of fat which were factors reducing the intensity of friction of the material against the working elements of the extruder. Therefore, the results obtained do not support the data obtained by Camire *et al*. (1993). Those authors concluded that the process temperature could have a significant effect on the rate of wear of the working elements of the extruder and on the migration of alloy elements of steel (mainly Fe) to the products. Also Alonso *et al*. (2001) noted a higher content of Fe in extruded pea and beans compared to the raw material.

CONCLUSION

The "dry" extrusion of blends with a content of MBP proceeded correctly within a broad range of parameters. The products obtained were characterised by diverse physical properties and chemical composition. The recommended parameters of the process of "dry" extrusion of blends of MBP with plant materials (FBM and WGM) are as follows: meat bone-pulp content of up to 30%, raw material moisture: 28%, profile of temperature distribution in the extruder cylinder: 120/140/180/180/130°C; die diameter 3 x 6 mm, screw speed 75 rpm. The application of wheat and faba bean as the plant materials permitted the obtainment of products with cohesive structure and high impact strength, and resistant to crushing. After supplementation of the material blend with taste and flavour components, they can be excellent dry food for dogs and cats.

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BADANIA PROCESU EKSTRUZJI DWUŚLIMAKOWEJ MIESZANEK ROŚLINNO-MIĘSNYCH PRZEZNACZONYCH DLA ZWIERZĄT DOMOWYCH

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Streszczenie. Przeprowadzono badania nad możliwością wykorzystania technologii ekstruzji „suchej” do przetwarzania mieszanek roślinno-mięsnych. Określono wpływ udziału komponentów mięsnych (miazga mięsno-kostna), roślin strączkowych (nasiona bobiku), rozdrobnionego ziarna pszenicy oraz temperatury wytłaczania na przebieg procesu, właściwości fizyko-chemiczne oraz mikrostrukturę ekstrudatów. Wykazano, że zastosowanie ekstrudera dwuślimakowego umożliwia przetwarzanie mieszanek z udziałem miazgi mięsno-kostnej dochodzącej do 30%. Zwiększenie udziału komponentu mięsnego powoduje obniżenie gęstości właściwej i rozpuszczalności suchej masy (WSI) ekstrudatów. Odnotowano także, wzrost zawartości białka, tłuszczu i popiołu w ekstrudatach. Podniesienie temperatury wytłaczania z 130 do 250°C wpłynęło na wzrost stopnia ekspandowania i udarności oraz obniżenie gęstości właściwej ekstrudatów. Jednocześnie mikrostruktura ekstrudatów z formy zwięzłej i zbitej przeszła w formę bardziej wyekspandowaną i porowatą.

Słowa kluczowe: ekstruzja, bobik, miazga mięsno-kostna, karma, pszenica