Comparison of products made from meat batter with various types and quantities of blood products

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Summary
The objective of this study was to investigate the effect of blood product enrichment on techno-functional and instrumentally measured sensory properties of meat batter products. Types and quantities of enrichment were determined according to the literature taking into account the usability. The factors of the research were the followings: type of blood product enrichment (blood plasma, hemoglobin and whole blood powder), quantity of blood product enrichment (control; 10 g·kg^{-1}, 30 g·kg^{-1} and 50 g·kg^{-1} in case of hemoglobin and whole blood powder; 10 g·kg^{-1}, 30 g·kg^{-1}, 50 g·kg^{-1}, 100 g·kg^{-1} and 150 g·kg^{-1} in case of blood plasma powder) and storage time (0 days, 30 days, 60 days and 90 days). Effects of the factors on the measured attributes were evaluated by general linear models by multivariate analysis of variance (MANOVA) and Tukey's honestly significant difference (HSD) post-hoc test for statistical significance of $p < 0.05$. A clearly positive effect of blood products containing albumen was found on textural properties, which was observed by scanning electron microscopy. Besides, type as well as quantity of the blood product enrichment affected colour and techno-functional properties.

Keywords
animal blood; by-product; food technology; food texture; product development; scanning electron microscopy

Utilization of by-products of animal origin, in particular blood, is a good tool of sustainability, especially regarding the protein aspect of the global nutrition issues. In addition, it can beneficially contribute to solving the serious health problem of iron deficiency anemia, which is important not only in developing countries but in developed countries as well [1]. Use of animal blood in food industry may considerably alleviate these current and future problems as well as blood could be a good raw material for food production not only because of its high protein content with great biological value but of its high hem-iron content, too [2–6]. Functional foods can be developed based on enrichment with blood products. However, effects of blood enrichment on organoleptic properties characteristics have to be investigated before industrial utilization as they are important for consumers. Darkening, which is caused by heat-denaturation of hemoglobin [7], is undesirable for consumers but, in the case of meat products, a certain level of blood or hemin content may be acceptable to consumers [8, 9]. The darker and more intensive colour may mean stronger and/or more desirable taste for consumers [10]. Besides, modern consumers also prefer foods from sustainable systems and utilization of by-products make meat industry more sustainable. Majority of modern consumers do not eat blood and other offal but most of them are open-minded and positive [11]. In order to make the valuable resource well utilizable for the industry, effects of various types and quantities of blood product enrichment on food products have to be investigated.

It is hard to find clear relations between measured dependents and factors in very complex systems. Thus, a homogenous food matrix was required to investigate the effects of various types

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and quantities of blood product enrichment. Meat batter products offer an adequate solution, the use of blood in which use is acceptable. Meat batters can transform into a visco-elastic gel structure by heat treatment. This structure can be 1. a filled gel, if one macromolecule is forming the gel matrix while other molecules fill the interstitial spaces, 2. a complex gel, if the gel matrix is produced by interactions among more than one component and 3. a mixed gel, if macromolecules independently develop two or more dimensional networks without interactions among the polymers. These structures can be observed by scanning electron microscope (SEM) [12, 13]. In the following, it is described which properties are relevant to be studied in a heat-treated meat matrix.

Earlier investigations classified the techno-functional properties of proteins to five categories: 1. hydration properties (water-protein interactions, such as solubility, hydration, dispersibility, swelling, water-holding capacity), 2. rheological properties (protein-protein interactions, such as precipitation, gelation, texturization), 3. surface properties (such as emulsification and foaming capacity), 4. sensory properties (such as taste, first of all umami, colour and texture) and 5. other properties (like adhesion, cohesion or film formation [14].

From the point of view of consumers, the three most important sensory attributes are appearance, texture and being free from foreign taste and foreign odour. Besides the protein level, quality and quantity of fats and oils also affect the texture and colour of meat products [15]. A common meat batter recipe was the basic recipe in this investigation and only the appropriate amount of blood product was added to samples.

Blood consists of several fractions. However, there are only a few types of blood products, which can be separated easily. Because of the cheap and easy separation, these main fractions are the most important blood products for the industry. Blood can be separated into two main fractions: plasma and red blood cell (RBC) fraction. Plasma is the intercellular fluid and RBC fraction, or also known as hemoglobin fraction, is suspended in the plasma. Red blood cells make the most part of RBC fraction but it contains the other blood cells as well. Effects of plasma and RBC enrichment in meat products were investigated in this study.

Blood plasma is a material that excellently enhances functional properties of heat-treated meat products, in particular gel formation [16–18]. Plasma proteins contain globular proteins (approximately 60 % albumins and 40 % globulins) and approximately 3–4 % fibrinogen [19]. These plasma proteins can develop a three-dimensional network forming a consistent gel as a result of heat treatment [20]. Adding blood plasma into pork meat batter favourably affects thermal stability, yields lower cooking loss but increases lightness and decreases redness of the product [17, 18]. Moreover, blood plasma, as a cold-set binding agent, increases the hardness and springiness of meat products [21]. Salt content has a significant effect on techno-functional properties of products made from meat batter. Thus, a high salt content of blood plasma powder (BPP, 150 $g \cdot kg^{-1}$) must be considered when setting the plasma powder levels of recipes. Most producers try to keep the salt content of products made from meat batter below 22 $g \cdot kg^{-1}$. Therefore, the upper limit of BPP, which can be added into the meat batter, is approximately 150 $g \cdot kg^{-1}$ and at this plasma powder level salt should not be added into it additionally.

Addition of whole blood, or better still RBC fraction, is the best way to increase the well-absorbable iron content of meat products. However, this greatly decreases lightness of the product because of denaturation of the hem pigment. The darkening caused by hemoglobin addition is not linear. Contents of 20–50 $g \cdot kg^{-1}$ of raw whole blood may be acceptable in meat products and this quantity leads to the total iron content of 220–350 $mg \cdot kg^{-1}$ (wet weight basis) [22]. According to a recent study, 20 $g \cdot kg^{-1}$ raw blood addition can develop a desirable colour for products made from meat batter, while no off-flavour is perceived at this content. Off-flavour appears above 40 $g \cdot kg^{-1}$, while products made from meat batter with a lower blood content are not significantly different from the control products regarding their colour, flavour and texture measured by sensory evaluation [23]. Thus, the maximum limit of added whole blood powder (WBP) and hemoglobin powder (HGP) is around 40 $g \cdot kg^{-1}$. Glycosylated nitrosyl-hemoglobin of RBC origin was used successfully for substituting sodium nitrite to produce pink colour and reduce nitrite content in meat batter [24].

In this study, various types of blood products were added in different amounts to pork meat batters: WBP at 10 $g \cdot kg^{-1}$, 30 $g \cdot kg^{-1}$ and 50 $g \cdot kg^{-1}$, HGP at 10 $g \cdot kg^{-1}$, 30 $g \cdot kg^{-1}$ and 50 $g \cdot kg^{-1}$, BPP at 10 $g \cdot kg^{-1}$, 30 $g \cdot kg^{-1}$, 50 $g \cdot kg^{-1}$, 100 $g \cdot kg^{-1}$ and 150 $g \cdot kg^{-1}$. Then the filled and heat-treated meat products were investigated by various methods. Powdered blood products were chosen to be used because these are easy to handle and have a long shelf-life compared to liquid blood. For instance, fresh blood can be used within one or two days

only. The aim of the study was to promote the use of animal blood for human consumption by investigating the texture-developing effect of powdered blood products in foods made from meat batter. Besides making high-quality electron microscopic images, understanding the effect of adding powdered blood products on properties of food products made from meat batter was an aim of this study. Effects of the enrichment on sensory (colour, texture) and techno-functional attributes (pH, water activity, water holding capacity, cooking loss), which were measured by instrumental methods, was also investigated. Since texture of products made from meat batter usually changes during the refrigerated storage [25], storage time, as a second factor, was also considered.

MATERIALS AND METHODS

Materials and sample preparation

Commercially available minced pork meat of *Sus scrofa domestica* with visually established 70 % lean meat content and 30 % fat content was used in the investigation. Ice flakes were produced by an ice flake maker (Brema Group, Villa Cortese, Italy) in the laboratory. Salt (edible iodized salt NaCl obtained in a grocery shop in Budapest, Hungary), tetrasodium pyrophosphate (Solvent Kereskedőház, Budapest, Hungary) and sodium ascorbate (Reanal Finomvegyszergyár, Budapest, Hungary), which are important in developing a good quality meat batter product with a long shelf-life, were used according to the recipes presented in Tab. 1.

Special ingredients, which were used for enrichment during this investigation, were hemoglobin powder 92B (Sonac Burgum, Sumar, Netherlands), plasma powder 70B (Sonac) and whole blood powder Vepro 95 phf (Solvent Kereskedőház, Budapest, Hungary). These blood products contain bovine and porcine blood in a guaranteed proportion. The basic recipe remained unchanged and the proportions of the ingredients did not change. In Tab. 1, the proportion of the base recipe decreased with the proportion of enrichment.

Meat batter was prepared in a cutter (Rewebo, Piazzola sul Brenta, Italy), which provided shredding and homogenity without a rise in temperature. Common salt (NaCl) was used instead of nitrite salt because the colour change caused by hem-iron enrichment could be investigated only by eliminating the effect of nitroso-myochromogen formation. Then, meat batter samples were filled into water vapour barrier casing. In this way, cooking loss could be determined based on the remaining liquid in the casing after removal of the meat product. Samples filled in casing were heat-treated in steam and hot air mixture at 80 °C core temperature for 20 min with average 10 min heating and 10 min cooling times. Samples were packaged in plastic vacuum packaging after measurement of the cooking loss. Vacuum packaging preserved the samples like the original water vapour barrier casing, which was removed.

Tab. 1. Sample codes and mass of ingredients in recipes of meat batter enriched with various blood products.

Sample code	Ingredients [g kg^{-1}]							
	Porcine minced meat	Ice flakes	Salt	Tetrasodium pyrophosphate	Sodium ascorbate	BPP	WBP	HGP
CON	578.4	404.9	11.6	2.3	2.9		–	–
Samples with blood plasma powder								
BPP10	576.9	403.8	4.2	2.3	2.9	10	–	–
BPP30	565.3	395.7	4.1	2.2	2.9	30	–	–
BPP50	553.6	387.5	4.0	2.2	2.8	50	–	–
BPP100	524.4	367.1	3.8	2.1	2.6	100	–	–
BPP150	495.3	346.7	3.6	2.0	2.5	150	–	–
Samples with whole blood powder								
WBP10	572.6	400.8	11.5	2.3	2.9	–	10	–
WBP30	561.0	392.7	11.2	2.2	2.8	–	30	–
WBP50	549.4	384.6	11.0	2.2	2.7	–	50	–
Samples with hemoglobin powder								
HGP10	572.6	400.8	11.5	2.3	2.9	–	–	10
HGP30	561.0	392.7	11.2	2.2	2.8	–	–	30
HGP50	549.4	384.6	11.0	2.2	2.7	–	–	50

CON – control, BPP – blood plasma powder., WBP – whole blood powder, HGP – hemoglobin powder

Tab. 2. Calculated protein, fat and water content in meat batter enriched with various blood products.

Sample	Protein content [g·kg^{-1}]	Fat content [g·kg^{-1}]	Water content [g·kg^{-1}]
CON	86.0	161.9	734.8
BPP10	92.2	161.4	729.6
BPP30	104.1	159.5	715.2
BPP50	117.3	156.6	719.4
BPP100	147.5	149.3	695.7
BPP150	177.6	142.1	672.1
WBP10	94.1	160.3	728.4
WBP30	110.4	157.1	715.7
WBP50	126.7	153.8	702.9
HGP10	94.3	160.3	727.9
HGP30	111.0	157.1	714.3
HGP50	127.6	153.9	700.5

Designation of samples is explained in Tab. 1.

Effects of the enrichment on proteins, fat and water content of raw meat batters were calculated and are shown in Tab. 2. Nutrition data were calculated according to the specification and ratio of ingredients.

Refrigerated storage was the second/third factor beside the type and/or quantity of various blood powder enrichments. Samples were stored for 1 day at 2 ± 1 °C and then the first measurement was carried out. Then, samples were stored for 90 days, which is a normal time of minimum durability in case of this type of commercial products, at the same temperature. Samples were analysed in 30 days intervals.

Experimental design

In this paper, the results of two experimental settings are presented according to the following research plans:

1. Comparison of the effect of enrichment with three different blood products (WBP, BPP and HGP) in three different quantities (10 g·kg^{-1}, 30 g·kg^{-1} and 50 g·kg^{-1}) in sample groups with four different storage times (0, 30, 60, 90 days) based on a 3 × 3 × 4 full factorial experimental design plus a control sample group without any enrichment.
2. Comparison of the effect of enrichment with only BPP but in six different quantities (0, 10, 30, 50, 100, 150 g·kg^{-1}) in sample groups with four different storage times (0, 30, 60, 90 days) based on a 1 × 6 × 4 full factorial experimental design.

Differences between samples with high blood plasma content and with other types of enrichment were also evaluated. The aim of the research plans was to detect the effect of different factors (type of added blood product; quantity of added blood product; storage time) on texture (hardness, cohesivity, springiness, chewiness, shear force) and other techno-functional attributes (water activity (a_w), dry matter content (*DMC*), water holding capacity (*WHC*), colour (redness-greenness a^*, yellowness-blueness b^*, lightness L^*, chroma C^*).

Texture measurement

Texture of samples was measured by TA.XT Plus Texture Analyser (Stable Micro Systems, Godalming, United Kingdom). Texture profile analysis was carried out. Type of the used probe was a p/75v. steel cylinder plate with samples positioned in the centre. Test speed and post-test speed was 1 mm·s^{-1}. Maximal compression was 50 % of sample height (10 mm) and it lasted 1 s. The following attributes were measured:

- Hardness – resistance (expressed in newtons) at maximum compression during the first compression. It is the force necessary to attain a given deformation, representing the hardness of the sample at first bite [26].
- Cohesiveness – the ratio (dimensionless) of positive force during the second compression cycle to that of the first one (downward strokes only) [26]. It indicates the strength of the internal bonds making up the body of the sample.
- Springiness – mechanical textural attribute relating to the rapidity and degree of recovery from a deforming force. It is the ratio (dimensioneless) between the height of the sample relaxed up to the moment of the second compression and the original height of the sample [27]
- Chewiness – the force (expressed in newtons) required to chew a solid sample to a steady state of swallowing (hardness × cohesiveness × springiness) [26, 27].

Temperature of samples was 20 °C during the measurements. Each sample group was measured six times.

Warner-Bratzler (W-B) test was carried out as well, which investigates the force (expressed in newtons) needed for shear the sample [26]. Straight-edged W-B sharp was used. Pre-test speed and test speed were 2 mm·s^{-1} and post-test speed was 10 mm·s^{-1}. The sharp completely passed through the samples, going 25 mm from the top of the sample with a force of 0.049 N.

All data were evaluated using Texture Exponent 32 software (Stable Micro Systems).

Colour measurement

Minolta CR-400 (Konica Minolta, Tokyo, Japan) colorimeter was used for the reflectional colour measurement. The theory of the measurement is based on the fact that any colour can be generated by the mixture of three ones defined by wavelength. The ratio of these three lights of different wavelength were plotted in a coordinate system named CIELAB colour space. The colour coordinates can be numbered making colours analysable. The instrument was calibrated with a standard white plate. Each sample group was measured three times. Measured attributes were redness-greenness (a^*), yellowness-blueness (b^*), and lightness (L^*).

Chroma (C^*) was calculated as Eq. 1 [28].

$$C^* = \sqrt{a^{*2} + b^{*2}} \tag{1}$$

Total colour difference (ΔE) was calculated according to the Eq. 2 [28]:

$$\Delta E^*_{ab} = \sqrt[2]{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \tag{2}$$

where differences are calculated between means of different sample groups according to Eq. 3 [28]:

$$\Delta L^* = \Delta L^*_1 - \Delta L^*_2 \tag{3}$$

$$\Delta a^* = \Delta a^*_1 - \Delta a^*_2 \tag{4}$$

$$\Delta b^* = \Delta b^*_1 - \Delta b^*_2 \tag{5}$$

Water holding capacity

A pressing test was carried out to measure the water holding capacity (*WHC*) by a modified method of Grau and Hamm [29]. The result of this test (in squared millimetres per milligram) defines what surface of filter paper can be moisturized (what the amount of the sample's water content cannot be held) by 1 mg sample under mechanical stress (pressure). Smaller nominal value means better *WHC*. An amount of 1.5–2.0 mg of the sample was pressed on a filter paper with 0.5 kg mass between glass layers for 5 min during the test. The liquid, which was released from the meat product, made a blot on the filter paper. The weight of the whole filter paper and the non-wetted part of the filter paper were measured by an analytical balance ABJ-NM/ABS-N (Kern and Sohn, Balingen, Germany). The moisturized filter paper surface was calculated based on weight difference. The result of the test was calculated from the quotient of the moisturized filter paper surface and meat product whole mass. Each sample group was measured three times.

Dry matter content

A weighed amount of 3–5 g of the sample was put into an open Petri dish. Then samples were put into a laboratory drying oven (Labor Műszeripari Művek, Budapest, Hungary) and dried at 105 °C until constant weight. Samples were cooled in a desiccator and then weighed again. Each sample group was measured three times.

Water activity

Water activity (a_w) was measured using LabMaster-a_w neo type water activity measurement device (Novasina, Lachen, Switzerland). This instrument requires very small sample amount and can fully control the temperature between 0 °C and 60 °C during the measurement. Measurements were performed at room temperature (20 °C) to control the integrity of samples for relevant data detection. Each sample group was measured twice.

pH measurement

Testo 206 pH stick (Testo, Vienna, Austria) pH meter was used for pH measurement. The device was calibrated before each measurement series with two standard liquid samples. Each sample group was measured three times.

Statistical analysis

Results were evaluated by SPSS Statistic v25 (IBM, Armonk, New York, USA) and Microsoft Excel 365 version 2010 (Microsoft, Redmond, Washington, USA) software to detect the effects of the type and/or amount of enrichment and storage time on texture parameters and other techno-functional properties. Multivariate analysis of variance (MANOVA) was carried out, which can compare the means of several sample groups of related variables. The value of the unexplained variance rate (Wilks's lambda) was evaluated. Homogenous groups were separated by Tukey's post hoc test. Texture attributes and techno-functional attributes were evaluated separately by two different MANOVA runs.

Homogenity of variances was checked by Levene's test. Normality of residuals was checked by Kolmogorov-Smirnov test in case of colour attributes and by Shapiro-Wilk test in case of texture attributes. Obtained values are given in Tab. 3.

In case of *DMC*, *WHC*, a_w and pH, normality of residuals and linear correlation could not be significantly validated. Other, more complex correlations may be presumed, so results of these attributes are considered to be trend-like.

Scanning electron microscopy

Sample preparation was based on recent research [12, 13]. First, microstructure of the samples had to be fixed and they were dehydrated.

Tab. 3. Results of statistical tests to check the requirements of MANOVA.

	Homogenity of variances		Normality of residuals	
Texture attributes				
	F(41,59)	*p*	*W*(101)	*p*
Hardness	5.315	< 0.01	0.975	0.05
Cohesiveness	3.672	< 0.01	0.945	< 0.001
Springiness	1.844	0.015	0.572	< 0.001
Chewiness	3.039	< 0.01	0.898	< 0.001
Shredding force	2.731	< 0.01	0.968	0.015
Colour attributes				
	F(41,59)	*p*	*D*(141)	*p*
*L**	1.627	0.026	0.066	0.009
*a**	37.946	< 0.01	0.241	< 0.001
*b**	7.149	< 0.01	0.142	0.001
*C**	12.976	< 0.01	0.180	< 0.001

*L** – lightness, *a** – redness-greenness, *b** – yellowness-blueness, *C** – chroma.
F(41,59) – Levene's statistics (in brackets number of groups, degree of freedom), D(141) – Kolmogorov-Smirnov's statistics (degree of freedom in brackets), W(101) – Shapiro-Wilk's statistics (defree of freedom in brackets).

Then, pieces of 0.5 mm × 1 mm × 3 mm were cut from the samples. These pieces were soaked in glutaraldehyde (25 g·kg^{-1}; Sigma-Aldrich, St. Louis, Missouri, USA) in a 0.1 mol·l^{-1} phosphate buffer solution (pH 7.0) for 24 h and then in ethanol (Lach-ner, Neratovice, Czech Republic) for 24 h. The pieces were freeze-dried in an individually designed equipment based on a vacuum pump by Leybold (Cologne, Germany) at 10 Pa maximum vacuum and –19 °C initial sample temperature. Before the examinations, samples were stored in a desiccator. Analytical samples were observed by Quanta 3D two-beam scanning electron microscope (FEI, Hillsboro, Oregon, USA) at a temperature of 20 °C, vacuum of 130 Pa and 100% relative humidity.

RESULTS AND DISCUSSION

Texture attributes

Meat batter products were enriched with various blood products. Texture was similar to that of common meat batter products. Results of hardness, cohesiveness, springiness, chewiness and shear force were similar in all types of blood products. Only in case of cohesiveness, a significant difference ($p < 0.05$) was determined between control samples and other samples, cohesiveness of control samples being lower. Regarding shear force, a part of enriched samples from all sample types could be separated from the control sample ($p < 0.05$), namely, a part of sample groups enriched with HGP and WBP, which were different, and a part of these, which were not different from the control sample group ($p < 0.05$). A sample group enriched with BPP was also significantly different ($p < 0.05$). Sample groups, which were enriched with 100 g·kg^{-1} and 150 g·kg^{-1} BPP, were significantly different ($p < 0.05$) from all the other samples regarding hardness, chewiness and shear force. Hardness and chewiness of sample groups enriched with 100 g·kg^{-1} and 150 g·kg^{-1} BPP were nearly two times greater than hardness of other sample groups. Shear force of sample groups enriched with 100 g·kg^{-1} and 150 g·kg^{-1} was nearly 25 % higher than mean shear force of other sample groups. Albumin proteins of blood plasma have a proven strong effect on texture of products [21]. These are similar to egg white albumins. However, main protein of RBC fraction, hemoglobin, does not have a similar effect. According to the present research, effect of various blood proteins was similar below 50 g·kg^{-1}, because meat proteins develop a structure, in which added proteins in this quantity cannot cause expressive changes. However, proteins added at 100 g·kg^{-1} and 150 g·kg^{-1} BPP were already able to affect the microstructure.

When the content of enrichment alone was examined, nominal differences between sample groups were quite small. These differences could be measured by the used instrumental texture measurement methods but organoleptically could not be perceived except hardness and chewiness. Regarding hardness and chewiness, sample groups with 100 g·kg^{-1} and 150 g·kg^{-1} BPP enrichment had significantly higher values ($p < 0.05$) than other sample groups but were not significantly different from each other. Regarding cohesiveness, control samples and samples with 10 g·kg^{-1} enrichment of each blood product were significantly different from other sample groups (lower; $p < 0.05$) but were not significantly different from each other, too. There was no significant difference in cohesiveness ($p < 0.05$) between sample groups made with different blood product contents. Regarding shear force, three groups could be separated ($p < 0.05$): 1. a group of samples with 0 g·kg^{-1}, 10 g·kg^{-1}, 30 g·kg^{-1} and 50 g·kg^{-1} of each blood product, 2. a group of samples with 30 g·kg^{-1}, 50 g·kg^{-1} of each blood product and 100 g·kg^{-1} of BPP and 3. a group of samples with 100 g·kg^{-1} and 150 g·kg^{-1} of BPP.

Storage time had a great effect on texture of samples, hardness and chewiness increasing from 22.49 N to 45.30 N and from 80.88 N to an average

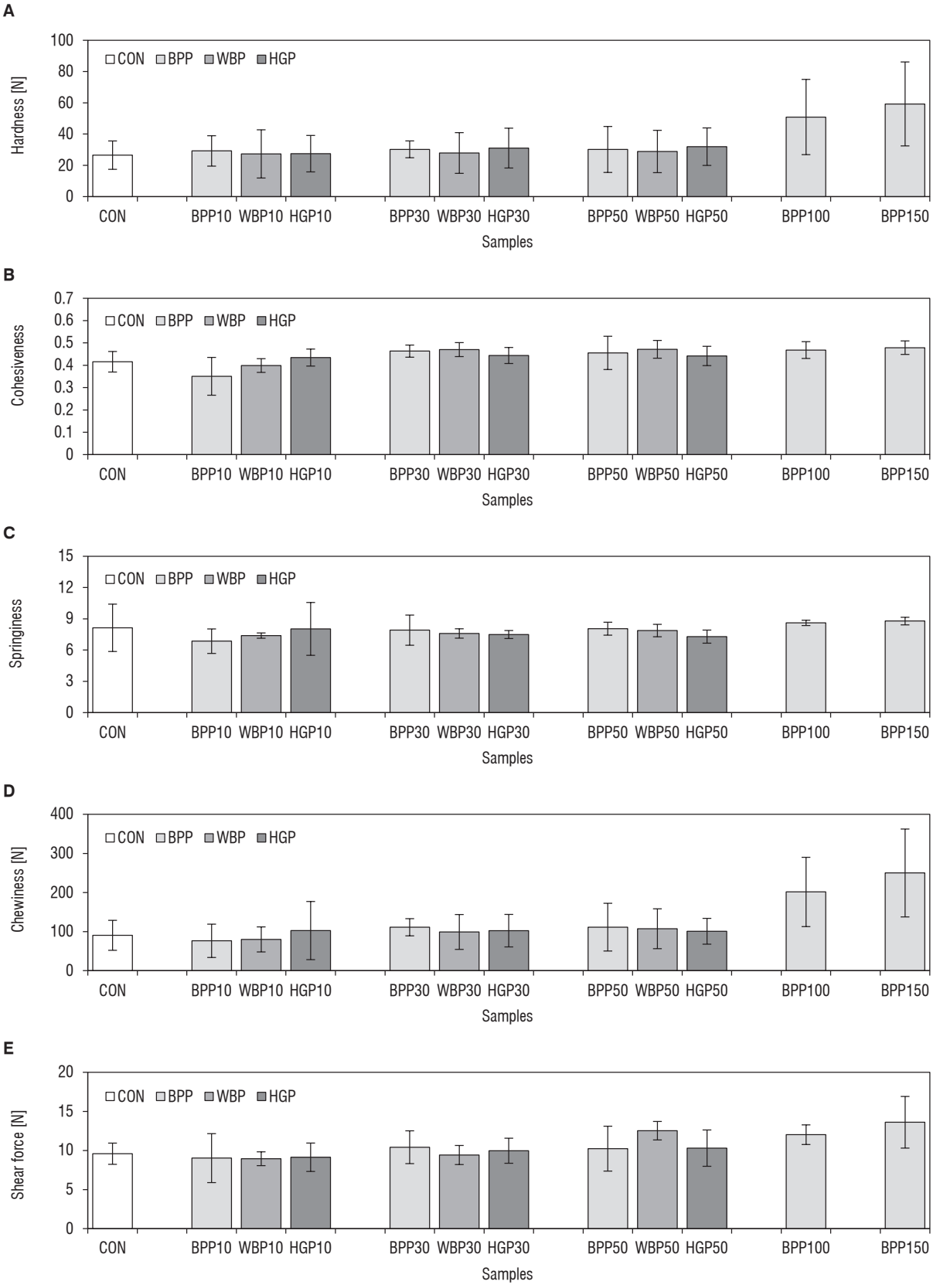


Fig. 1. Texture parameters of meat batter samples enriched with various blood products.

A – hardness, B – cohesiveness, C – springiness, D – chewiness, E – shear force.
CON – control, BPP – blood plasma powder, WBP – whole blood powder, HGP – hemoglobin powder.
Designation of samples is explained in Tab. 1.

of 142.70 N. Cohesiveness decreased during the cold storage by 11 %. Regarding hardness, the group of control samples, the group of samples stored for 30 days and the group of samples with 60 days and 90 days storage time were significantly different ($p < 0.05$). Regarding chewiness, the group of control samples, the group of samples with 30 days and 90 days storage time and the group of samples stored for 60 days and 90 days were significantly different ($p < 0.05$). So some samples stored for 90 days could be associated with the 30 days storage group and some with the 60 days storage group. The increasing hardness can be explained on the one hand by binding compounds being stabilized over time, developing continuously their bindings and, on the other hand, by oxidative damage to proteins through the formation of protein carbonyls and cross-links between proteins [30]. Similar changes were observed in recent studies [31, 32]. No change was detected in springiness and shear force.

The overall MANOVA result was significant for raw material of the enrichment, content of blood products as well as storage time (Wilks' lambda: 0.718; 0.225; 0.166; $p < 0.01$). Content × raw material interaction and content × storage time interaction also had a significant effect on dependent variables (Wilks' lambda: 0.458; 0.152; $p < 0.01$). Other two-way and three-way interactions were not significant. Content had a stronger effect on dependent variables than the type of the blood products in the investigated quantity. Texture attributes are shown in Fig. 1, which represents the changes resulting from the use of various type and quantities of enrichment. Changes of texture attributes were similar as a result of storage in each sample group with different levels of the two factors.

Colour attributes

Based on lightness, all sample groups with different quantity and type of blood products could be separated significantly from each other ($p < 0.05$) except sample groups that contained 10 g·kg^{-1} and 150 g·kg^{-1} BPP. The red hem pigment turns black as a result of heat treatment and this caused the difference between different sample types. Sample groups enriched with HGP were the darkest with an average L^* value of 37.74. Sample groups enriched with WBP had an average L^* value of 38.95. Average L^* value of sample groups enriched with BPP was 66.33 and sample groups without any enrichment were the brightest with L^* value of 72.12. It could be explained not only by the heat-coagulated hem-iron content of the darkest two enrichment types, but also by the higher water content of sample groups with BPP enrichment.

Sample groups enriched with WBP and HGP were similar based on redness-greenness and yellowness-blueness values. These sample groups were the reddest and the bluest. Sample groups enriched with BPP were significantly different ($p < 0.05$) from the other sample groups based on a^* and b^*. These samples had the most yellowish colour. Control samples were significantly different from enriched sample groups as well ($p < 0.05$) and they were the least red. The greatest difference could be observed in redness-greenness, as control samples had an average a^* value of 2.38, samples with BPP enrichment had an average a^* value of 3.98. Samples with WBP had an average a^* value of 10.13 and samples with HGP had an average a^* value of 10.98. In case of chroma, there was no significant difference ($p < 0.05$) between the different sample groups but trend-like results could be very well observed. Content of various types of enrichment affected the redness-greenness and yellowness-blueness of sample groups as expected.

The significant colour change caused by storage had a clear trend as sample groups with 30 days and 60 days storage were similar but different ($p < 0.05$) from sample groups with 0 days and 90 days storage time in all colour parameters. Brightening of the colour of the samples could be observed. For instance, lightness of the control samples and samples from the first 60 days was similar, but the lightness started to increase (L^* of control samples was 70.48 at the beginning of storage, 71.46 after the first 30 days and 71.52 after 60 days) and then lightness was clearly higher after the 90 days (76.68). A similar trend could be observed for redness-greenness. These results were statistically significant ($p < 0.05$), but nominally not significant. So, the difference could be explained by the expanded uncertainty of the colour measurement and by heterogeneity of the sample, which was characterized by air bubbles in the texture. Two colour parameters, which best illustrated the effects of enrichment, are shown in Fig. 2, which presents the effects of type and quantity of enrichment.

The overall MANOVA result was significant for quantity of enrichment, type of enrichment, for storage time as well as for quantity × type of enrichment interaction, quantity of enrichment × storage time interaction, type of enrichment × storage time interaction and quantity of enrichment × type of enrichment × storage time interaction (Wilks' lambda of 0.019; 0.013; 0.184; 0.381; 0.383; 0.386 and 0.658, respectively;

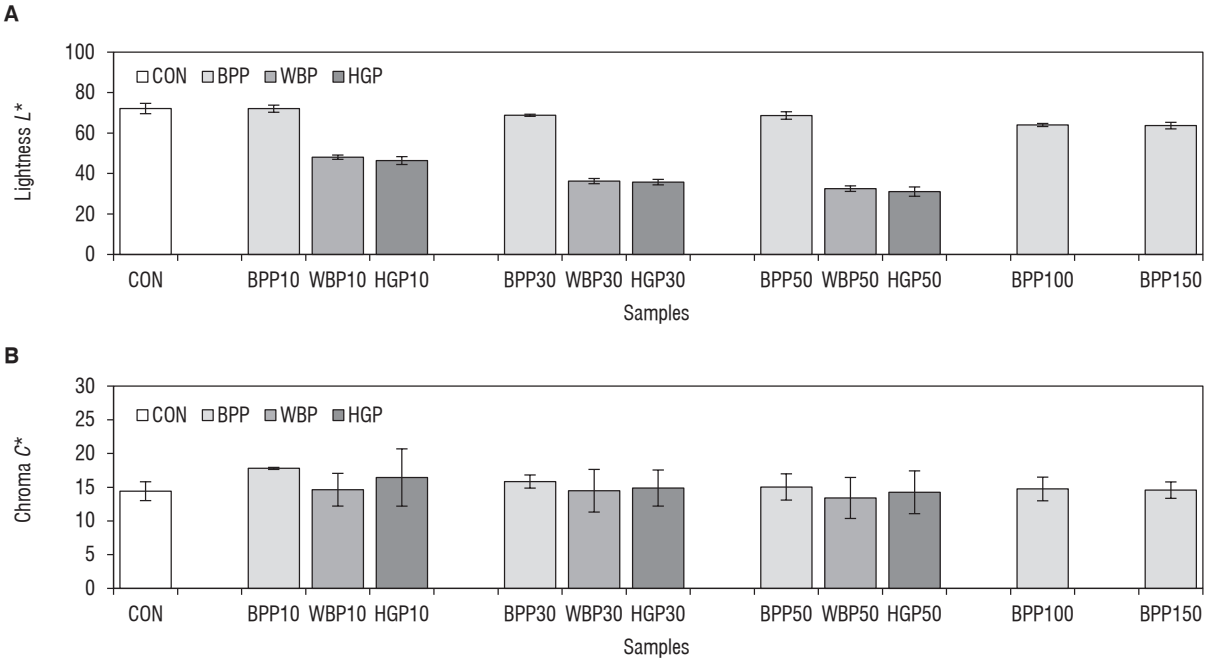


Fig. 2. Two colour parameters of meat batter samples, which best illustrate the effects of enrichment.

A – lightness *L**, B – chroma *C**.
CON – control, BPP – blood plasma powder, WBP – whole blood powder, HGP – hemoglobin powder.
Designation of samples is explained in Tab. 1.

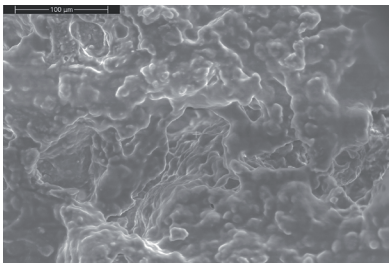
$p < 0.01$ for factors and two-way interactions, while $p = 0.014$ for three-way interaction). It can be stated that type and quantity of enrichment were the most decisive, effect of storage time was less pronounced.

Dry matter content, water holding capacity, water activity and pH

For *DMC*, *WHC*, a_w and pH, trend-like results were observed but a linear model could not be generated for most of the measured attributes. Globally, *DMC* was 28.31 ± 8.74 g·kg^{-1}, *WHC* was 2.14 ± 2.05 and a_w was 0.940 ± 0.013. Expanded uncertainty of *DMC* and *WHC* measurements were relatively high and this might have been the reason that significant results were difficult to observe. Cooking loss was very high. In case of control samples and samples enriched with 10 g·kg^{-1} of blood products, cooking loss was 250 g·kg^{-1}. In case of samples enriched with 30 g·kg^{-1} of blood products, cooking loss was 70–110 g·kg^{-1}. In case of other sample groups, cooking loss was below 10 g·kg^{-1}. This well proved the *WHC* of blood proteins. Cooking loss can be reduced or eliminated by using blood products, without any additives, if it is an important aspect in a certain product development. Results of *WHC* were proportionally similar to the preceding results and can be similarly explained. *DMC* had a relatively great standard deviation but results followed the amount of bound water. Various types of blood proteins did not affect the pH value of the final product besides the ascorbic acid and phosphate. So, pH of different sample groups, including samples with different enrichment type and different storage time, was not significantly different.

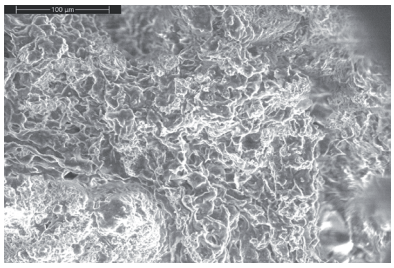
Nutritional aspects

The increase in proteins content was significant. The added iron content in case of porcine WBP was 1.5 mg and in case of bovine WBP was 2.9 mg in 100 g sample in case of 10 g·kg^{-1} enrichment [5, 33]. Iron added with 10 g·kg^{-1} porcine WBP would cover 18.8 % and with 10 g·kg^{-1} bovine WBP would cover 36.3 % of the iron need of an average man, or in case of 10 g·kg^{-1} porcine blood powder, would cover 8.3 %, in case of bovine blood powder would cover 16.1 % of the iron need of an average woman [34]. So, increase in the iron content was also significant in case of WBP and HGP enrichment. The values presented here were calculated from openly available databases (FoodData Central, USDA Agriculture Research Service, Washington, D.C., USA; Ingredients, Sonac Burgum, Sumar, Netherlands). No specific data were available on the iron con-

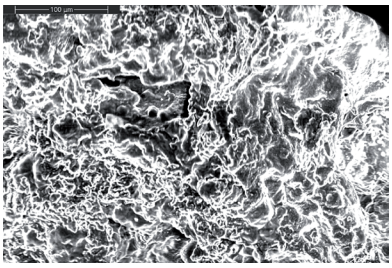


CON (Control)

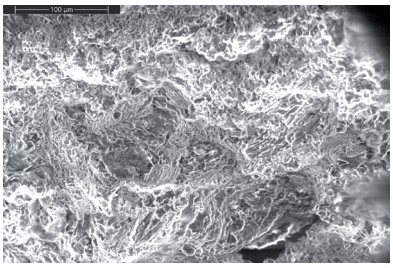
Samples with blood plasma powder



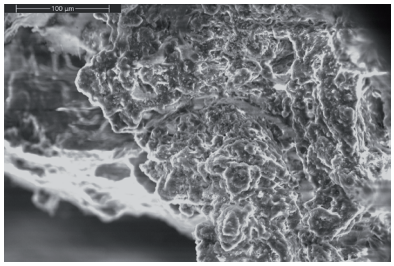
BPP10



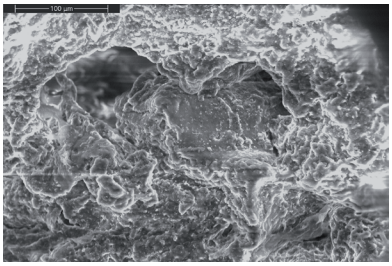
BPP30



BPP50

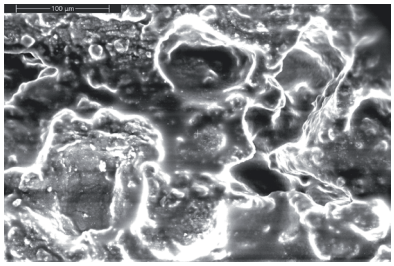


BPP100

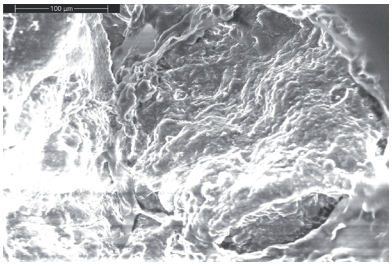


BPP150

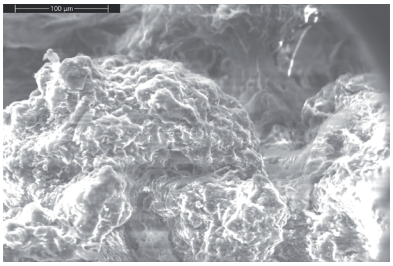
Samples with whole blood powder



WBP10

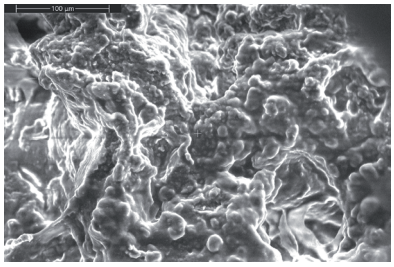


WBP30

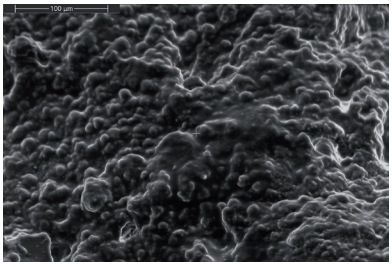


WBP50

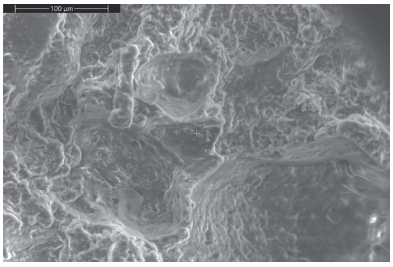
Samples with hemoglobin powder



HGP10



HGP30



HGP50

Fig. 3. Scanning electron microscopy pictures of heat-treated, prepared meat batter product samples enriched with various type and quantity of blood products.

Designation of samples is explained in Tab. 1.

tent of the samples. It is important to mention that excessive iron consumption can cause iron poisoning. However, iron poisoning is caused by elemental iron and not by hem-iron. In addition, an intake of 20 $mg \cdot kg^{-1}$ can be considered iron poisoning.

Quality of the final product can be enhanced by the effect of albumin proteins on texture properties [35]. Albumins can develop a harder, better chewable and easier to slice meat product with more protein. This change in the microstructure can be observed and presented by SEM.

Microstructure

Results of SEM, which can be seen in Fig. 3, showed the structure of a usual heat-treated meat-emulsion system, which is characterized by a three-dimensional protein network [13]. Cavities in the sponge-like structure were developed by the expanding water, fat and air inside the heat-treated meat batter. Size and distribution of cavities were similar in all sample groups. The size of cavities was random but these may have been caused by filling non-compliance. Fat globules, which come from the finely cut pork meat and fat, could not be observed, so the emulsion of meat batter was stable. SEM revealed significant differences in the microstructure of meat batter products enriched with different type and quantity of blood products. The more albumin-type protein was added into the meat batter, the more crumb-like, more filamentous, more porous network was observed, being richer in small aggregates. Hemoglobin enrichment could not make a change in the microstructure because the main protein fraction of HGP is obviously hemoglobin and it does not contain relevant quantity of albumin proteins. Whole blood powder, which contains the albumin fraction, could cause a difference in the higher content. Blood plasma powder, which contains the most albumin as the main protein fraction, developed a protein network with tines as structure components and aggregates, which were embedded in the microstructure. When the quantity of albumin was increased, more crumb-like microstructure could be observed.

CONCLUSIONS

Each goal of the present investigation had been achieved. Different types and quantities of blood products were found to cause significant effects on the techno-functional and instrumentally measured sensory properties of meat batter products. Based on this research blood, which is a neglected animal by-product, a good non-allergenic protein source and the best absorbable iron source, can be utilized in meat batter products. However, it is important to consider the salt content of BPP because high salt content can cause undesired effect in nutritional and sensory properties. Hemoglobin can help in developing a functional meat product, which may have a role in prevention and treatment of iron deficiency anemia. Different colour changes caused by enrichment with various types and amounts of blood product may be important for consumers but, at this content, may cause development of deeper and desirably darker colour. Sensory attributes changed leading to a harder and darker product, which may be desirable if consumer's attention is drawn to it and it can be possibly used for marketing purposes as an indicator of the high nutrient content of the product. Beside sustainability and quality aspects, a decrease in the cooking loss and an increase in *WHC* give economic benefit to utilizing blood in meat batter products.

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