ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)
Food Science and Technology

DOI: https://doi.org/10.1590/fst.03018

The effect of bioactive components of plant origin on the physicochemical and sensory characteristics of functional sausages

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Abstract

The aim of this study was to determine the effect of bioactive compounds of natural origin on the quality of sausages. Four variants of sausages were manufactured: a control variant (C) and three variants with the addition of catuaba, galangal, roseroot, maca, guarana and polyfloral honey (E_1, E_2, E_3). The pH values, colour and chemical parameters (total phenolic content, total antioxidant activity and anti-inflamantory activity) of the meat batters and finished products were determined. Additionally, texture and sensory analyses of the sausages were performed. It was found that regardless of slightly lower acceptability, sausages with bioactive components were characterised by increased antioxidant properties, higher total phenol values and higher anti-inflamantory activity. The best results were obtained when the highest level of bioactive compounds was used (1.734% of catuaba bark, 0.022% of ground great galangal root, 0.458% of ground roseroot, 0.614% of maca root extract (4:1), 0.600% of ground guarana and 1.146% of polyfloral honey).

Keywords: functional foods; sausages; natural components; bioactive component.

Practical Application: Functional sausages for consumption.

1 Introduction

Due to the natural aging process, the production of androgens decreases with age. The fall in testosterone production in men ranges from 1% to 2% per year (Feldman et al., 2002). This process affects the reduction of bone mineral density, structure composition, syndrome stiffness, metabolism, diabetes and cardiovascular diseases (Gomuła & Rabijewski, 2010; Huhtaniemi, 2014; Mouser et al., 2016). Studies have shown that low levels of androgens are associated with symptoms of fatigue, irritability, dyspnea, vigorous decline, low vigour, lack of physical strength and sexual dysfunction in men (Barrett-Connor et al., 1999; Giltay et al., 2012; Rhoden & Morgentaler, 2004; Wang et al., 2009). Low levels of androgens also increase the risk of symptoms of depression (Ford et al., 2016; McHenry et al., 2014; Westley et al., 2015). The elimination of the negative symptoms associated with a decrease in androgen production may be addressed by components naturally occurring in foods and herbs with proven health-promoting and stimulating properties.

A plant with documented pro-health characteristics, particularly suitable for the elderly struggling with the decline of androgen production is Catuaba (*Trichilia catigua*). Extracts from the catuaba cortex are used in diseases related to oxidative stress, and also as a neurostimulant, anti-neuritic and aphrodisiac agent (Campos et al., 2005; Chassot et al., 2011; Kamdem et al., 2013). Additionally, catuaba is characterised by its analgesic (Viana et al., 2011), antimicrobial (Pizzolatti et al., 2002), antioxidant (Brighente et al., 2007; Chassot et al., 2011; Tang et al., 2007) and anti-inflammatory (Barbosa et al., 2004) properties.

Another well-known plant with adaptogenic properties that increase the body's immunity and allow it to cope with all sorts of stressful situations (Wal & Wal, 2013) is roseroot (*Rhodiola rosea*). Its most active ingredient, which has the chemical structure of phenolic glycosides is salicylic acid (Cao et al., 2006; Chen et al., 2008). Roseroot contains, among others, proanthocyanidins, flavonoids, glycosides, organic acids and essential oils (Majewska et al., 2006; Wal & Wal, 2013).

The health properties of the maca root (*Lepidium meyenii*) have also been documented. For example, maca improves sexual function, counteracts the symptoms of menopause (Lee et al., 2016) and has a beneficial effect on spermatogenesis (Melnikovova et al., 2015). Studies have also demonstrated its neuroprotective effect (Pino-Figueroa et al., 2010). Tang et al. (2017) indicate that polysaccharides isolated from the maca root may be considered in the future as a functional food ingredient limiting fatigue.

Also galangal (*Alpinia galanga*) contains many bioactive compounds such essential oils, tannins, glycosides, phenolic compounds and diterpenes. There are 387 different activities of this plant (Ravindran et al., 2012). A lot of physiological function of this plant have been reported (Ravindran et al.,

Received 29 Jan., 2018
Accepted 18 July, 2018
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2012). For example galangin (3,5,7-trihydroxyflavone), a flavonoid contained in the galgan, has an antineoplastic effect (Quadri et al., 2000). A very important function of galangin is also the aromatase inhibition that prevents the conversion of testosterone to estrogens (Heo et al., 2001).

Further, guarana (*Paullinia cupana, Sapindaceae*) is not only a source of caffeine, but also contains many other active compounds such as catechine, choline, adenine, hypoxanthine, theophylline, saponins, theobromine and essential oils. It affects positively the wellbeing of people in depression, strengthens the body's resistance to stress, and also soothes migraines. In addition, guarana has strong antioxidant and antimicrobial properties; thus, it can protect against the growth of food-spoiling bacteria and fungi (Basile et al., 2005; Majhenič et al., 2007).

A food ingredient of proven bioactive action is honey. It exhibits antioxidant, anti-inflammatory, anti-cancer, antihyperlipidemic, cardioprotective activity and is useful in the treatment of eye diseases, the digestive system, neurological disorders, fertility disorders and wound healing (Meo et al., 2017; Oryan et al., 2016; Rao et al., 2016; Rashad et al., 2009). It has also been shown that honey has a beneficial effect on the levels of follicle-stimulating hormone (FSH) and luteinising hormone (LH) responsible for testosterone production (Kenani et al., 2015; Rajabzadeh et al., 2015).

Recently, there has been an increased interest in functional food, exhibiting pro-health properties among different consumer groups. Sausages with natural bioactive components fall within this trend (Bhat & Bhat, 2011). The purpose of the research was to examine the influence of bioactive components, such as catuaba, galangal, roseroot, maca root, guarana and polyfloral honey on the quality and sensory characteristics of functional sausages.

2 Materials and methods

2.1 Material and reagents

Pork ham, pork shoulder, pork chuck, pork back fat and beef shin shank were obtained from a regional distributor (ŁMEAT JSC, Łuków, Poland), while all non-meat ingredients were delivered from the local market: ground black pepper, dried marjoram, garlic, ground lovage root and ground allspice from PRYMAT Ltd (Poland), bear's garlic from Dary Natury Co. (Poland), catuaba bark and ground grater galangal root from Nanga Co. (Poland), ground roseroot, maca root extract 4:1 and ground guarana from Młyn Oliwski Ltd, (Poland) and polyfloral honey was collected from the village of Kaborno (Poland). All chemicals and reagents used were of analytical grade and were procured from Sigma-Aldrich (St. Louis, MO, USA).

2.2 Product formulation and processing

At first, boneless meat and fat was cut into cubes measuring 3 cm x 3 cm x 3 cm and dry cured with a mixture of salt and nitrine (2%) for 4 days at 3-4 °C. The raw material composition of the analysed sausages is indicated in Table 1. The standard spice blend contained 25.18% of ground black pepper, 7.32% of dried marjoram, 26.35% of fresh garlic, 7.47% of bear's garlic, 20.50% of dried garlic, 5.86% of ground lovage root and 7.32% of ground allspice.

Cured meat and fat were ground in a grinder (ZELMER 687.5, Poland) with a 16 mm (pork ham), 8 mm (pork shoulder and pork chuck), 2 mm (pork back fat and beef shin shank) diameter of mincing plate. Then beef shin shank was mixed in a Multi-Function All-in-One Cooker (Speedcook, RPOL, Mielec, Poland) with standard spice blend and ice (control group), or in the case of other groups with standard spice blend, polyfloral honey, ground greater galangal root, ground roseroot, maca root extract, ground guarana and frozen catuaba decoction (prior to the process, catuaba bark was cooked for 10 minutes, strained, cooled and frozen) in the proportions indicated in Table 1. Subsequently, the remaining meat and back fat were added and mixed manually. The resulting batters were stuffed into natural casings from sheep (22-24 mm) using a batch-wise stuffing machine (MR13, Oscar Cooks, Austria). Then, the sausages were set for 5 hours at 8-10 °C, air dried for 1.5 hours at 25-30 °C, warm smoked (60% alder, 20% beech, 20% cherry of wood chips) for 2 hours (1 hour at 40-50 °C and 1 hour at 50-60 °C) in a traditional smokehouse with a

Table 1. Raw material composition of analyzed sausages.

INGREDIENTS		GROUP			
		C[1]	E_1	E_2	E_3
CURED MEAT AND FAT MIXTURE (Σ100%)	pork ham	42.850	42.850	42.850	42.850
	pork shoulder	12.790	12.790	12.790	12.790
	pork chuck	18.410	18.410	18.410	18.410
	pork backfat	11.650	11.650	11.650	11.650
	beef shin-shank	14.300	14.300	14.300	14.300
OTHERS (%)[2]	standard spice blend	0.683	0.683	0.683	0.683
	ice (C) or frozen decoction of catuaba bark (E_1, E_2, E_3)	20.000	20.000	20.000	20.000
BIOACTIVE COMPONENTS (%)[2]	catuaba bark used for decoction	-	0.867	1.301	1.734
	ground greater galangal root	-	0.011	0.017	0.022
	ground roseroot	-	0.229	0.344	0.458
	maca root extract (4:1)	-	0.307	0.461	0.614
	ground guarana	-	0.300	0.450	0.600
	polyfloral honey	-	0.573	0.860	1.146

[1]C – control group; E_1, E_2, E_3 – groups, where bioactive components were used (E_2=1.5 x E_1, E_3=2 x E_1); [2]Quantity in relation to cured meat and fat mixture (100%).

generator (Dymbox, Poland). Following that, the sausages were steamed in a convection oven at 71-73 °C to obtain an internal temperature of 68 °C (CPE 110, Küppersbuch, Germany) and cooled in air for 1 hour at about 10 °C. Ultimately, the obtained meat products were vacuum-packed in LD–PE bags, using a vacuum-packaging machine (VAC-20 SL 2A, Edesa, Spain). The physicochemical and consumer evaluation were carried out 24 hours after production. Each sausages group was produced in three separate trials.

2.3 Physico-chemical analysis of sausages

pH determination

The pH value of sausages was measured according to the ISO 2917:2001/Ap1:2002 standard (ISO, 2002), using a pH-meter equipped with a glass electrode (Testo 205, Testo Inc., Lenzkirch, Germany). After calibration against buffers of pH 4.0 and 7.0, measurement was conducted (an electrode was inserted to a depth of 2 cm directly into the samples, the temperature of the samples during measurements was 2 ± 1 °C). Analysis of each group was carried out in triplicate.

Instrumental colour measurement

The instrumental measurement of the colour parameters of sausages was determined using a Minolta chromameter (CR-400, Konica Minolta Inc., Tokyo, Japan), with a spot diameter of 8 mm, a D_{65} illuminant and an observer angle of 2°. Lightness (L*), redness (a*) and yellowness (b*) were recorded on the CIE LAB colour space system. Before measurement, the chromameter was calibrated using the white standard calibration plate late (L* = 98.45, a* = -0.10, b* = -0.13). Measurements of the samples were taken from five locations including the centre and every quarter of the sample. At least 10 measurements were recorded from each sample. Additionally, the hue angle (tonality) and saturation index (vivacity) were estimated and determined according to the following formulas (Artés et al., 2002) (Equations 1 and 2):

$$hue\ angle = arctg\left(b^*/a^*\right) \quad (1)$$

$$saturation\ index = \left(a^{*2} + b^{*2}\right)^{1/2} \quad (2)$$

Texture Profile Analysis (TPA)

Texture profile analysis (TPA) was carried out using the Instron 5965 Universal Testing Machine (Instron, Norwood, MA, USA). For the compression of uniform disks of sausages' slices, a flat, 4 cm diameter cylinder probe was used. Samples measuring 18 mm in height and 18 mm in diameter were double compressed to the point of 50% reduction of their initial height with a relaxation time of 5 s (cell capacity – 500 N; head speed – 2 mm/min.). Based on the force versus time curve, hardness (N/cm^2), springiness (-), cohesiveness (J/cm^2) and chewiness (N) were determined. The measurement was conducted in three repetitions for each sample.

Proximate Composition Analysis

The determination of moisture, fat and protein content was performed in the spectral range of 780–2500 nm, using a near-infrared spectrometer (NIR Flex N-500, Büchi Labortechnik AG, Flawil, Switzerland). Portions of meat batters or homogenized sausages weighing about 150g were placed on a glass Petri dish at a depth of 0.5 cm. The measurement was conducted in three repetitions for each sample using a NIRFlex Solids module of spectral range 12.500–4.000 cm^{-1} in reflectance mode with a resolution of 8 cm^{-1} (Wyrwisz et al., 2012).

Determination of phenolic compounds

The total phenolic content (TPC) of batters and sausages was measured by the method described by Singleton & Rossi (1965) with some modifications. For this purpose 2.5 g of sausage from each group was homogenized with 7.5 ml of ethanol for 120 s at 12 rpm x 1000 (Ultra Turrax homogeniser, T18 basic, IKA Werke, Staufen, Germany). Then to 0.1 mL of obtained extracts 6.0 ml of distilled water and 0.5 mL of Folin–Ciocalteu reagent were added and mixed thoroughly. After 3 min., 1.5 ml of sodium carbonate of 200 mg/mL concentration was added to reactive mixtures, which were finally adjusted with water to 10 mL and stored for 30 min in a water bath (WNB 7, Memmert, Schwabach, Germany) in the temperature of 40 °C. Absorbance was measured spectrophotometrically at a wavelength of 765 nm (SparkTM 10M, Tecan Group Ltd, Männedorf, Switzerland). Gallic acid solutions (0 mg/mL, 0.2 mg/mL, 0.3 mg/mL, 0.4 mg/mL, 0.5 mg/mL) were used to prepare a calibration curve. The concentration of total phenolic content in the sausages was expressed as gallic acid equivalents (GAE) in mg per 100 g of sample (mg of GAE/100 g). All measurements were performed in three replications.

DPPH radical scavenging activity

DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging activity of batters and sausages was determined spectrophotometrically according to a modified method described by Sánchez-Moreno et al. (1998). For this purpose, 2.5 g of samples from each group were homogenized with 7.5 ml of ethanol for 120 s at 12 rpm x 1000 (Ultra Turrax homogeniser, T18 basic, IKA Werke, Staufen, Germany), extracted at room temperature for 10 min on a rotary shaker (MyLab SLRM-3, NanoEnTek Inc., Korea) and then centrifuged at 1800 rpm for 10 min (MPW Med. Instruments, Poland). Ultimately, the ethanolic extracts located above the sediments were collected. Next, 3.5 mL of 0.1 mM DPPH ethanolic solution was added to 0.5 mL of obtained extracts, vortexed thoroughly and left in the dark at 25 °C for 20 min. Absorbance was measured spectrophotometrically at a wavelength of 517 nm (SparkTM 10M, Tecan Group Ltd, Männedorf, Switzerland). Ethanol was used as blank and all measurements were performed in three replications. The total antioxidant activity (TAA) expressed as % reduction of DPPH was calculated as (Sánchez-Moreno et al., 1998) (Equation 3):

$$TAA\ (\%) = \left(1 - A_s / A_c\right) \times 100 \quad (3)$$

where A_s is the absorbance of the test sample and A_c is the absorbance of the control (containing all the reagents except extract).

Anti-inflammatory compounds

The anti-inflammatory activity of batters and sausages based in the mechanism of the Morgan–Elson reaction was evaluated by modification of the method described by Osés et al. (2016). 5 g of samples from each group were homogenized (Ultra Turrax homogeniser, T18 basic, IKA Werke, Staufen, Germany) with 10 ml 50% ethanol solution in water. Then, 70 µl of 5 mg/ml hyaluronic acid sodium salt from *Streptococcus equi* and 100 µl of buffer (0.2 M sodium formate, 0.1M sodium chloride and 0.2 mg/mL bovine serum albumin, pH adjusted to 3.68 with formic acid) were added to 200 µl of samples. Then, mixtures were mixed gently and heated at 37 °C for 10 min (water bath, WNB 7, Memmert, Schwabach, Germany). Next, 50 µl of hyaluronidase from bovine testes type IV-S (600 U/mL) prepared in 0.9% sodium chloride was added and incubated 1 hour at the same conditions. 100 µl of 0.8 M potassium tetraborate was used to stop the enzymatic reaction. After a 3 min incubation in a water bath (WNB 7, Memmert, Schwabach, Germany), samples were cooled to room temperature. Ultimately, 750 µl of dimethylaminobenzaldehyde (DMAB) solution were added (2 g of DMAB were added to 2.5 mL of 10 N hydrochloric acid and 17.5 ml of glacial acetic acid, and then a 50% solution with glacial acetic acid was prepared). Absorbance of samples was measured spectrophotometrically at a wavelength of 586 nm (SparkTM 10M, Tecan Group Ltd, Männedorf, Switzerland) after a 20 min incubation (water bath, 37 °C). Buffer was used as blank and N-acetyl-d-glucosamine standard solutions were used as standard for calibration curves. All measurements were performed in three replications. 1 unit (U) of hyaluronidase activity catalyses the release of 1 µmol N-acetyl-d-glucosamine (NAG) per min under specified conditions. The percentage of enzyme inhibition was calculated as (Osés, et al., 2016):

$$I = (1 - Y / X) \times 100 \quad (4)$$

where I (%) – percentage of inhibition, X – µmol of NAG in the control sample and Y – µmol of NAG of each sample reaction.

2.4 Consumer evaluation of sausages

The acceptability of the sausages was evaluated by 45 consumers (45 men ranging in age from 25 to 69 years) in standard conditions, including room temperature, individual booths and light of approximately 500 lx. Samples with dimensions 18 × 50 mm were presented to the consumers randomly and codified with four random numbers. Consumer were asked to express their opinion by placing a vertical line on the 10 cm unstructured line scales with defined border values. Nine sensory attributes were evaluated: colour (undesirable – desirable), odour (undesirable – desirable), taste (undesirable – desirable), foreign odour (imperceptible – perceptible), foreign taste (imperceptible – perceptible), juiciness (dry – juicy), consistency (undesirable – desirable), fat perceptibility (imperceptible – perceptible) and overall acceptability (undesirable – desirable). Tea without sugar was served between tastings to purge the palate of sample residues. Consumer evaluation was undertaken in three sessions and a complete block design was used, where each consumer assessed all groups in each session.

2.5 Statistical analysis

All data were analysed statistically by analysis of variance (One-Way ANOVA) using the Statistica software version 12.5 (StatSoft Inc., Tulsa, USA). The differences between groups were tested according to Tukey's test, performed at the significant level of $p<0.05$. The results in the tables are presented as mean values and standard deviations (SD). Furthermore, the relation between total phenolic content (TPC) and total antioxidant activity (TAA) was evaluated using Pearson's linear correlation at $p<0.05$.

3. Results and discussion

3.1 Physicochemical properties

The addition of different levels of bioactive components of plant origin did not have any significant effect ($p\geq0.05$) on pH values between all treatments (Table 2). These were in the range of 6.15-6.20 for meat batters and 5.82-5.88 for finished products.

Nevertheless, added ingredients significantly affected ($p<0.05$) colour parameters among experimental groups (Table 2). Considering meat batters, as well as finished products, a decrease in L* values was observed along an increase in the addition of experimental components. Additionally, sausages batters from group E_3 were characterised by significantly higher values of a* and b* parameters, as well as higher saturation index than other groups. All samples had a hue angle index around 1° indicating their red colour. In the case of sausages, results demonstrated that only a* values did not changed when experimental components were added (Table 1). Additionally, in the case of other color parameters, no significant ($p\geq0.05$) differences between sausages from E_2 and E_3 groups were observed. In general, fortified sausages were statistically darker ($p<0.05$) and their b* values, as well hue angle and saturation index, were more intense than those of the control group. Change in the colour parameters of products before and after thermal processing may be attributed to the colour of the used components and the amount in which they were added. The increase of sausage yellowness, however, is usually caused by lower antioxidative efficiency (Sebranek et al., 2005). In this case, it could be triggered by the presence of catechins in roseroot for example (Adamczyk et al., 2016; Wagh, et al., 2015).

The statistical analysis did not show significant differences ($p>0.05$) in the textural properties of sausages with various levels of bioactive ingredients compared to the control group (Table 3).

Hardness, springiness, cohesiveness, adhesiveness, as well as, the chewiness values of samples were relatively constant, which may be related to the composition of the sausages. Additionally, no differences were observed ($p\geq0.05$) for moisture, fat or protein among experimental groups, both in meat batters (67.22% ± 0.59, 13.33% ± 0.28 and 15.44% ± 0.35, respectively) and final products (59.02% ± 0.62, 17.63% ± 0.24 and 18.86% ± 0.44, respectively). The obtained results for basic composition were similar to the results generally reported for this kind of products and should not affect their physicochemical quality (Thomas et al., 2008).

As shown in Table 4, it was found that the total phenolic content, total antioxidant activity and anti-inflamantory activity of examined groups showed considerable variations. All three analysed parameters were influenced by the level of bioactive ingredients addition ($p<0.05$).

It was observed that the total phenolic content (mg/100 g of f.w.) of the meat batters increased with bioactive components content. According to the control group, the amount of phenols doubled when the highest dose of components was used. Similar observations were noted for the finished products. This could be a result of the presence of polyphenols from galangal (Cheah & Abu-Hasim, 2000), maca (Sandoval et al., 2002), guarana (Basile et al., 2005), roseroot (Adamczyk et al., 2016) and honey (Pyrzynska & Biesaga, 2009).

In this study, groups with bioactive components of plant origin, both before and after thermal processing, provided relatively high total antioxidant activity (above 76.85% DPPH and 86.58% DPPH respectively). The control group was characterised by the lowest total antioxidant activity (32.53% DPPH for sausage batter and 46.88% DPPH for the finished product). It is known that polyphenols in products of plant origin contribute to their potent antioxidant capacity (Rice-Evans et al., 1996). For example, the

Table 2. pH and color parameters of analyzed meat batters and sausages.

PARAMETERS	GROUP			
	C[1]	**E_1**	**E_2**	**E_3**
SAUSAGE MEAT BATTERS				
pH [-]	6.19±0.02	6.15±0.02	6.17±0.03	6.20±0.02
L* [-]	58.21^{c}±01.2	57.90^{b}±1.26	56.41^{ab}±1.90	55.15^{a}±1.12
a* [-]	10.86^{a}±1.99	10.53^{a}±1.15	11.06^{a}±0.33	12.63^{b}±0.60
b* [-]	15.91^{a}±1.81	16.71^{a}±1.33	16.39^{a}±0.83	19.31^{b}±0.93
Hue angle [°]	0.98±0.05	1.01±0.03	0.98±0.03	0.99±0.02
Saturation index [%]	19.28^{a}±2.5	19.75^{a}±1.68	19.77^{a}±0.72	23.08^{b}±1.01
SAUSAGES				
H [-]	5.82±0.03	5.87±0.06	5.88±0.07	5.85±0.02
L* [-]	61.22^{b}±0.98	58.85^{b}±0.48	57.53^{a}±0.80	57.23^{a}±0.65
a* [-]	12.06±0.59	11.71±0.39	11.87±0.44	12.03±1.13
b* [-]	7.69^{a}±1.07	10.11^{b}±0.73	12.00^{c}±0.66	11.94^{c}±0.68
Hue angle [°]	0.57^{a}±0.07	0.71^{b}±0.04	0.79^{c}±0.03	0.78^{c}±0.05
Saturation index [%]	14.34^{a}±0.68	15.48^{b}±0.61	16.89^{c}±0.62	16.96^{c}±1.06

[1]C – control group; E_1, E_2, E_3 – groups, where bioactive components were used (E_2=1.5 x E_1, E_3=2 x E_1); different letters within a row are significantly different ($p<0.05$).

Table 3. Textural properties of analyzed sausages.

PARAMETERS	GROUP			
	C[1]	**E_1**	**E_2**	**E_3**
Hardness [N]	89.26±6.57	81.58±6.37	88.13±7.51	91.29±10.05
Springiness [-]	0.719±0.061	0.705±0.055	0.685±0.039	0.674±0.057
Cohesiveness [-]	0.498±0.058	0.442±0.031	0.439±0.055	0.456±0.511
Adhesiveness [J/cm^2]	-0.157±0.020	-0.122±0.014	-0.125±0.022	-0.135±0.028
Chewiness [N]	0.498±0.058	0.442±0.031	0.439±0.055	0.456±0.051

[1]C – control group; E_1, E_2, E_3 – groups, where bioactive components were used (E_2=1.5 x E_1, E_3=2 x E_1).

Table 4. Chemical parameters of analyzed meat batters and sausages.

PARAMETERS	GROUP			
	C[1]	E_1	E_2	E_3
SAUSAGE MEAT BATTERS				
Total phenolic content [mg GAE/100 g]	12.40^{a1}±0.99	22.19^{b}±0.88	23.93^{bc}±0.61	25.69^{c}±0.84
Total antioxidant activity [%]	32.53^{a}±0.27	76.85^{b}±0.13	85.14^{c}±0.12	85.70^{d}±0.19
Ant-inflamantory activity [%]	0.27^{a}±0.03	0.91^{b}±0.07	2.42^{c}±0.19	2.63^{c}±0.16
SAUSAGES				
Total phenolic content [mg GAE/100 g]	15.69^{a}±0.78	27.51^{b}±1.58	29.68^{bc}±0.93	31.84^{c}±0.51
Total antioxidant activity [%]	46.88^{a}±0.11	86.58^{b}±0.57	86.98^{bc}±0.38	87.57^{c}±0.09
Ant-inflamantory activity [%]	0.09^{a}±0.02	0.77^{b}±0.15	0.99^{bc}±0.14	1.30^{c}±0.13

[1]C – control group; E_1, E_2, E_3 – groups, where bioactive components were used (E_2=1.5 x E_1, E_3=2 x E_1); different letters within a row are significantly different ($p < 0.05$).

Table 5. The semi-consumer scaling method of sensory evaluation of analyzed sausages.

SENSORY ATRIBUTES	GROUP			
	C[1]	E_1	E_2	E_3
COLOR	6.73±2.07	6.26±1.91	5.68±2.24	5.71±2.29
ODOR	7.34±1.87	7.23±1.76	6.63±2.36	6.26±2.08
FOREIGN ODOR	1.90±2.10	2.12±2.69	2.78±2.71	2.64±2.53
TASTE	6.45^{b2}±2.24	5.95^{a}±2.41	4.68^{a}±2.66	5.09^{a}±2.14
FOREIGN TASTE	1.65^{a}±1.91	2.78^{ab}±2.52	3.46^{b}±2.71	3.56^{b}±2.85
JUICINES	6.03±2.41	5.80±2.17	5.51±2.22	4.90±2.00
CONSISTENCY	6.76±2.36	6.83±2.15	6.00±2.32	6.01±2.42
FAT PERCEPTIBILITY	6.46^{b}±2.48	4.83^{a}±2.52	4.33^{a}±2.39	4.35^{a}±2.29
OVERALL ACCEPTABILITY	6.86^{b}±2.08	6.23^{ab}±1.92	5.37^{a}±2.43	5.70^{ab}±2.25

[1]C – control group; E_1, E_2, E_3 – groups, where bioactive components were used (E_2=1.5 x E_1, E_3=2 x E_1); different letters within a row are significantly different ($p<0.05$).

isothiocyanates present in maca (Sandoval et al., 2002) or the monophenolics and vitamin C present in honey (Schramm et al., 2003) have been shown to have antioxidant activities. Also in our study, the relationships between phenolic content (Y) and total antioxidant activity (X) of meat batters (r^2 = 0.98, Y = 0.23273X + 4.7501), as well as finished products (r^2 = 0.96, Y = 0.34935X − 0.7209) showed a positive high correlation. The obtained data indicate that the TAA in examined groups were contributed mainly by phenolic compounds contained in the used components, and their redox properties (Zheng & Wang, 2001). Additionally, the data are in agreement with the report of Tangkanakul et al. (2009), who showed that natural antioxidants retained their activity after the thermal processing of food.

According to Table 4, the used ingredients improved the anti-inflammatory activity of meat batters and finished products ($p<0.05$). When the highest levels of bioactive components were used, the increases compared to the control groups were almost tenfold and fifteenfold respectively. It is suggested that the antioxidant content of products can determine their anti-inflammatory activity, since the formation of free radicals and destruction can be inhibited by antioxidants. It is also believed that, among others, honey owes its anti-inflammatory properties to the ability to inhibit feedback leading to inflammation caused by hydrogen peroxide (Bashkaran et al., 2011; Kassim et al., 2010).

3.2 Consumer evaluation

The sensory traits of experimental sausages are presented in Table 5. The addition of experimental components up to 4.60% did not affect consumers' assessment of sausage colour, odour, juiciness and consistency. There were also no significant differences ($p\geq0.05$) in taste, fat perceptibility and overall acceptability between groups with different levels of bioactive components (E_1, E_2, E_3); however, some differences in relation to the control group (C) were identified.

Although fat was the most noW celu ticeable in control sausages ($p<0.05$), they were characterized by better taste and overall acceptability. This is probably due to the fact that fat in meat products is thought to be a flavour-carrier, which delivers taste and odour compounds in certain foods (Mendoza et al., 2001). Additionally, the slightly lower acceptability of experimental sausages could be related to the taste of additives that are not specific to this type of product, although Mohammed (2006) reported that the addition of up to 7.5% bee honey to beef sausages had a positive effect on their sensory acceptability. The application of other ingredients such as catuaba, galangal, roseroot, maca root and guarana at higher levels might be limited. Despite this fact, the assessments of the groups where the experimental components mixture was used up to 4.60% in relation to the cured meat were at a relatively high level, oscillating around 6.

4. Conclusion

Based on the obtained results, sausages with 1.734% of catuaba bark, 0.022% of ground great galangal root, 0.458% of ground roseroot, 0.614% of maca root extract (4:1), 0.600% of ground guarana and 1.146% of polyfloral honey were characterized not only by good sensorial acceptability, but also by the best antioxidant and anti-inflamantory properties. Additionally, their regular intake could prevent other maladies typical in men; however, this requires further research.

Acknowledgements

Research financed by Polish Ministry of Science and Higher Education within funds of Faculty of Human Nutrition and Consumer Sciences, Warsaw University of Life Science (WULS), for scientific research.

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