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Animal husbandry

IMPROVING OF THE OXIDATIVE STABILITY AND
QUALITY OF NEW FUNCTIONAL HORSE MEAT
DELICACY ENRICHED WITH SEA BUCKTHORN
(*HIPPOPHAE RHAMNOIDES*) FRUIT POWDER
EXTRACTS OR SEED KERNEL PUMPKIN
(*CUCURBITA PERO L.*) FLOUR

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Abstract

The antioxidant and antimicrobial activity of powder fruit extract of sea buckthorn (FESB) (*Hippophae rhamnoides*) or seed kernel pumpkin (SKPF) (*Cucurbita pero L.*) flour can be used for producing new functional cooked and smoked horse meat product since many of these natural biological active substances have the potential to improve the oxidative stability of pigments, lipids and proteins of whole muscle delicacies. The objective of this study was to establish the potential of two concentrations 0.5% and 1.0% of FESB or SKPF as additives for processing of functional cooked and smoked horse "Jaya" delicacy with improved quality and oxidative stability. The sensory analysed flavour, odour and colour, colour characteristics (L^* , a^* , b^*), pH value, free amino nitrogen, total protein carbonyls, acid value, peroxide value and TBARS were determined in this experiment. The addition of 1.0% of SKPF and especially 1.0% of FESB improve the oxidative stability and quality of the new functional horse meat delicacy and save its sensory and colour characteristics. The applied technology allows new functional cooked and smoked hole muscle horse meat products to be produced, enriched with 9.81 ± 0.26 mg/g sea buckthorn (FESB) (*Hippophae rhamnoides*) or with 9.75 ± 0.20 mg/g seed kernel pumpkin (SKPF) (*Cucurbita pero L.*) flour.

Key words: sensory properties, colour characteristics, hydrolysis, oxidation, functionality, horse meat product

Introduction. Horse meat has a high nutritional value. It contains all the essential amino acids, more than 20 minerals and vitamins of groups A and B. In addition, the fatty acid composition of its fat is unique [1]. One of the strategies for designing novel functional meat products is the addition of functional ingredients, such as natural antioxidants [2]. A number of meat-based functional foods has been designed based on this strategy [3]. The possibilities for production of whole muscle cured horse meat [4] and boiled and smoked horse products after injecting with multicomponent brine [5] were discussed. Independently of this, in literature no information can be found about processing of functional horse meat products [6, 7].

Simultaneously, a significant number of natural extracts and flours are available to be used in functional foods. One of the most discussed plants with similar properties in the last years has been sea buckthorn (*Hippophae rhamnoides L.*) [8]. In the sea buckthorn fruit juice seven flavonols were identified. The highest quantity among them have the isorhamnetin 3-O-glycosides [8] but they are poor radical scavengers. Quercetin 3-O-glycosides, catechins, and hydroxybenzoic acids with a catechol structure are strong antioxidants presented in the juice but their concentrations were small [8]. Conversely, the ascorbic acid was shown to be the major antioxidant in sea buckthorn juice [8]. The remainder is due to flavan-3-ols (proanthocyanidins) [8]. Sea buckthorn (*Hippophae rhamnoides L.*) seeds crude extracts have antioxidant and antibacterial activities for *Bacillus cereus*, *Bacillus coagulans*, *Bacillus subtilis*, *Listeria monocytogenes*, *Yersinia enterocolitica*, were found [9]. Except antioxidant activity, the fruits sea buckthorn (*Hippophae rhamnoides*) extract also manifested immunomodulatory properties and can inhibit chromium-induced free radical production, apoptosis, DNA fragmentation. In addition, it had marked cytoprotective properties and may restore the cells antioxidant status [10].

Another interesting component with possible application in functional meat products formulation is pumpkin seeds. The pumpkin seed kernel flour is rich in oil and crude protein and contains considerable amounts of P, K, Mg, Mn and Ca, and total essential amino acids. This flour could be potentially added as a functional agent to ground meat formulations [11]. It is rich in vitamins A, K, B₃ and folate too [12] and is also a good source of ω -3 and ω -6 fatty acids as well as linoleic acid [12]. Pumpkin seeds or pumpkin seed powder could be a good addition to the diets of older people, especially those suffering from osteoporosis [13]. The pumpkin seeds zinc content helps keep bone mineral density high [13] and can also be helpful in treating arthritis by reducing inflammation. It can promote good prostate health and reduce the risk of getting kidney stones and is capable of balancing the hormones of both men and women. It has an ability to boost metabolism and help burn fat and promotes sleeping [13].

The aim of the study was to establish the potential of two concentrations 0.5% and 1.0% of sea buckthorn (FESB) (*Hippophae rhamnoides*) or seed kernel

pumpkin (SKPF) (*Cucurbita pepo* L.) flour as additives for processing of functional cooked and smoked horse “Jaya” delicacy with improved quality and oxidative stability.

Material and methods. The horse meat was supplied by the AF Kainar Ltd., Almaty, Kazakhstan. The Jaya was made in the Meat Training and Production Center of the Almaty Technological University, Almaty, Kazakhstan. A chilled horse meat first category of fatness was used. The upper muscle tissue layer together with the surface fat layer was removed from the pelvis-thigh part of the horse carcass. The Jaya was shaped of semi-circular pieces not heavier than 0.4 kg with a thickness of about 10 cm. The meat pieces were injected with brine containing 2.5 kg salt and 150 g sugar per 100 kg of raw material with a density of 1.0923–1.1065 g/cm³ and then were flooded with the rest brine and were refrigerated. The salted meat was massaged in tenderizer for 40 min. The Jaya was boiled in cooking-smoking chambers to a temperature in the centre of 74–75 °C for 2–2.5 h until the temperature at the centre of the product had reached 72 °C. The boiled product was chilled and smoked for 30 min at a smoke temperature of 40 °C. After cooking Jaya was cooled down to 10–12 °C and was vacuum-packaged before sampling. The vacuum-packed samples were stored for 21 d at 0–4 °C.

The five samples were studied: the control sample – the pieces of horse meat were injected with 20% brine, described above; sample FESB1 – injected with 20% brine containing 2.5 kg fruit extract of sea buckthorn/100 kg (equivalent to 0.5% concentration in the finished product); sample FESB2 – injected with 20% brine containing 5.0 kg fruit extract of sea buckthorn/100 kg (equivalent to 1.0% concentration in the finished product); sample SKPF1 – injected with 20% brine containing 2.5 kg seed kernel pumpkin flour/100kg (equivalent to 0.5% SKPF concentration in the finished product) and sample SKPF2 – injected with 20% brine containing 5.0 kg seed kernel pumpkin flour/100kg (equivalent to 1.0% SKPF concentration in the finished product).

The sea buckthorn (*Hippophae rhamnoides* L.) fruit powder extract was supplied by Dannie Chen Shaanxi Jintai Biological Engineering Co., Ltd. (Xi’an, Shaanxi, China).

The seed kernel pumpkin (*Cucurbita pepo* L.) flour was purchased from Europa-Biofarm ZAO NPO (Volgograd, Russia).

The salt and sugar were bought from the local market. All other reagents used were of analytical grade and were purchased from Merck (Germany). The sensory characteristics of the samples were determined by a panel consisting of five members with proven tasting abilities. The panellists had passed the triangular test for differentiation of fresh and rancid sausage flavour, odour and colour. The samples were scored using 1 to 5 scales [14].

Colourimeter Konica Minolta model CR-410 (Konica Minolta Holding, Inc., Ewing, New Jersey, USA) was used to evaluate the lightness (L* value), red

component (a^* value) and yellow component (b^* value) of the colour [14].

The modified titration method of Sørensen was used for determination of free amino nitrogen [14].

A protein oxidation was measured by estimation of carbonyl groups formed [14].

As a standard of the rate of lipolysis, the acid value (AV) of the extracted lipids was measured following EN ISO 660:2001 procedure [15].

The IDF standard method was used to determine the peroxide values (PV) of meat products. The total lipids extracted from samples were used [16].

2-Thiobarbituric acid reactive substances: TBARS were determined by the method described by BOTSOGLOU et al. [17]. The double beam UV-VIS spectrophotometer Camspec model M550 (Camspec Ltd, Cambridge, UK) was used.

pH of the samples was determined by pH-meter Microsyst MS 2004 (Microsyst, Plovdiv, Bulgaria), equipped by combined pH electrode Sensorex combination recorder S450CD (Sensorex pH Electrode Station, Garden Grove, CA, USA) [18].

The high performance liquid chromatography (HPLC) method with coulometric electrochemical detector was used to analyze the oil soluble antioxidants extracted from FESB and SKPF and their concentrations in Jaya horse meat [19].

The preparation of samples for microbiological analysis and the total plate count of facultative anaerobic mesophilic microorganisms were done by ISO 4833:2003 [20] method.

The data of different samples were analysed independently by SAS software [14]. The Student-Newman-Keuls multiple range test was used to compare differences among means. Mean values and standard errors of the mean were reported. Significance of differences was defined at $p \leq 0.05$.

T a b l e 1

Sensory evaluations of surface cross-sectional colour, flavour and odour of the vacuum-packed samples after 21 d of storage at 0–4 °C

Samples	Sensory evaluations		
	Colour of the cross-sectional surface view	Odour	Flavour
C	2.65±0.09 ^e	2.90±0.03 ^e	2.75±0.10 ^d
FESB1	4.30±0.07 ^c	4.90±0.05 ^b	4.90±0.01 ^a
FESB2	4.85±0.02 ^a	5.00±0.02 ^a	4.50±0.05 ^b
SKPF1	4.70±0.03 ^b	4.80±0.03 ^c	4.55±0.04 ^b
SKPF2	3.50±0.08 ^d	4.70±0.04 ^d	4.35±0.08 ^c

Means ± standard deviations. The different superscripted suffixes ^{a,b,c,d,e} after the standard deviations denote statistical differences amongst samples in every one column ($p \leq 0.05$)

Results and discussion. The concentrations of oil soluble antioxidants extracted from FESB and SKPF enriched Jaya horse meat after 21 d of the samples storage at 0–4 °C were determined as following: in sample FESB1 – 4.78±0.21 mg/g, in sample FESB2 – 9.81±0.26 mg/g, in sample SKPF1 – 4.73±0.19 mg/g and in sample SKPF2 – 9.75±0.20 mg/g.

Sensory evaluations. On the 21d of storage at 0–4 °C the highest sensory scores of flavour, odour and colour were awarded in the samples FESB2 (Table 1). Very close to those results were the sensory scores assessed for samples SKPF1 and FESB1 (Table 1). The control sample C had the worst sensory properties. It was found that its scores were significantly ($p \leq 0.05$) lower than the others. Those results allow concluding that addition of 2.5% FESB in the pickle at most significant degree preserves the fresh colour and flavour, and especially the odour of 21d stored vacuum-packed cooked and smoked horse meat product. Similarly, SERIKKAISAI et al. [14] also reported a good effect of mixture of dry goji berry and pumpkin powder on the quality and the storage stability of cooked and smoked beef strip loin.

T a b l e 2

Changes of the colour characteristics (L^* , a^* , b^*) of the cross-sectional surface view of the vacuum-packed samples during 21 d of storage at 0–4 °C

Charac- teristics	Samples	Time of storage		
		1 d	11 d	21 d
L^*	C	49.77±0.10 ^e	52.68±0.20 ^j	53.40±0.15 ⁱ
	FESB1	48.34±0.11 ^d	49.94±0.12 ^f	52.62±0.16 ^j
	FESB2	50.51±0.16 ^g	51.44±0.19 ^h	52.33±0.18 ⁱ
	SKPF1	47.67±0.12 ^{a,b}	47.75±0.14 ^b	48.89±0.15 ^{e,f}
	SKPF2	47.43±0.15 ^a	47.91±0.13 ^{b,c}	48.28±0.11 ^d
a^*	C	17.38±0.19 ^d	18.72±0.13 ^h	19.45±0.18 ⁱ
	FESB1	15.76±0.14 ^b	16.77±0.17 ^c	17.67±0.16 ^e
	FESB2	19.21±0.19 ⁱ	19.48±0.20 ⁱ	19.52±0.17 ^j
	SKPF1	15.73±0.21 ^b	18.63±0.17 ^g	19.21±0.20 ⁱ
	SKPF2	12.23±0.15 ^a	18.01±0.12 ^f	18.32±0.19 ^g
b^*	C	7.05±0.14 ^a	7.54±0.13 ^c	7.87±0.21 ^d
	FESB1	7.60±0.10 ^c	7.99±0.12 ^{d,e}	8.03±0.16 ^{d,e}
	FESB2	7.71±0.14 ^{c,d}	8.17±0.13 ^e	8.85±0.11 ^j
	SKPF1	7.33±0.18 ^b	7.67±0.17 ^d	8.08±0.10 ^{d,e}
	SKPF2	7.46±0.15 ^b	7.58±0.19 ^c	8.29±0.11 ^f

Means ± standard deviations. The various superscripted suffixes a,b,c,d,e,f,g,h,i,j after standard deviations mean statistical differences between the samples for each of the color characteristics ($p \leq 0.05$), both by rows and by columns

Table 3

Changes of the pH values, free amino nitrogen, total protein carbonyls, acid value, peroxide value and TBARS of the vacuum packed samples after 21 d of storage at 0 – –4 °C

Studied parameter	Samples				
	C	FESB1	FESB2	SKPF1	SKPF2
Injected brine, %	20	20	20	20	20
Yield, %	84	85	86	84	85
pH of the pickle	8.18±0.03 ^c	6.90±0.04 ^b	6.81±0.02 ^a	7.00±0.03 ^d	6.99±0.03 ^e
pH of raw material	5.62±0.02 ^a	5.59±0.04 ^a	5.60±0.02 ^a	5.61±0.02 ^a	5.61±0.03 ^a
pH finished product 1d	6.34±0.04 ^b	6.27±0.02 ^a	6.21±0.04 ^a	6.45±0.01 ^c	6.66±0.02 ^d
pH after 21d of storage	5.59±0.03 ^a	6.44±0.05 ^c	6.33±0.03 ^b	6.57±0.03 ^d	6.75±0.04 ^e
Free amino nitrogen, mg/100g 1d	6.42±0.19 ^a	7.25±0.13 ^{b,c}	7.07±0.20 ^b	7.30±0.10 ^{b,c}	7.04±0.19 ^b
Free amino nitrogen, mg/100g 21d	18.81±0.21 ^c	13.76±0.18 ^b	13.68±0.10 ^b	13.37±0.15 ^a	13.45±0.10 ^a
Protein carbonyls, nmol/mg proteins 1d	0.58±0.17 ^a	0.62±0.18 ^a	0.59±0.16 ^a	0.62±0.16 ^a	0.63±0.13 ^a
Protein carbonyls, nmol/mg proteins 21d	4.12±0.23 ^e	3.03±0.27 ^c	2.01±0.24 ^a	3.28±0.22 ^d	2.63±0.23 ^b
Acid value, mg KOH/g fats 1d	0.49±0.08 ^a	0.50±0.09 ^a	0.47±0.07 ^a	0.49±0.09 ^a	0.52±0.06 ^a
Acid value, mg KOH/g fats 21d	2.17±0.11 ^c	1.65±0.13 ^b	1.39±0.11 ^a	1.47±0.10 ^{a,b}	1.30±0.14 ^a
Peroxide value, meqv O ₂ /kg fats 1d	0.40±0.05 ^{a,b}	0.35±0.04 ^a	0.30±0.05 ^a	0.38±0.06 ^{a,b}	0.33±0.07 ^a
Peroxide value, meqv O ₂ /kg fats 21d	1.78±0.07 ^c	1.44±0.06 ^b	1.33±0.07 ^a	1.50±0.05 ^b	1.39±0.08 ^a
TBARS, mg MDA/kg 1d	0.27±0.04 ^a	0.24±0.03 ^a	0.23±0.01 ^a	0.26±0.02 ^a	0.25±0.04 ^a
TBARS, mg MDA/kg 21d	1.94±0.11 ^c	1.08±0.07 ^b	0.89±0.08 ^a	1.10±0.05 ^b	0.93±0.06 ^a

Means ± standard deviations. The different superscripted suffixes ^{a,b,c,d,e} after the standard deviations denote statistical differences amongst samples in every one row ($p \leq 0.05$)

Colour characteristics. Changes of the lightness (L* value), redness (a* value) and yellowness (b* value) were presented in Table 2. The samples FESB1 and FESB2 were again characterized by the most significant changes. The results obtained are in good agreement with the data from the sensory analysis and show the better effect of FESB enrichment on colour characteristics of Jaya horse meat in comparison with SKPF enrichment.

Oxidative stability and quality. After 21 days of storage the following changes in horse meat Jaya samples were determined. The contents of free amino nitrogen in all samples were significantly ($p \leq 0.05$) lower in comparison with control samples C, especially samples SKPF1 and SKPF2. An increase of protein carbonyls in all samples during 21 d storage at 0–4 °C was established. This process was significantly ($p \leq 0.05$) the slowest in samples FESB2 and SKPF2

T a b l e 4

Count of facultative anaerobic mesophilic microorganisms of the vacuum packed samples during 21 d of storage at 0–4 °C

Samples	Facultative anaerobic mesophilic microorganisms, log cfu/g		
	1 d	11 d	21 d
C	2.04 ^a	5.14 ^c	6.47 ^d
FESB1	2.01 ^a	3.97 ^b	4.95 ^c
FESB2	2.00 ^a	3.21 ^a	4.36 ^a
SKPF1	2.03 ^a	4.05 ^b	5.00 ^c
SKPF2	2.02 ^a	5 3.33 ^a	4.52 ^b

Means \pm standard deviations. The different superscripted suffixes ^{a,b,c,d} after the standard deviations denote statistical differences amongst samples in each column ($p \leq 0.05$)

where the reduction in the total protein carbonyls of 51% and 36% was determined (Table 3). A significant ($p \leq 0.05$) increase of acid values in all samples during 21 d refrigeration was established (Table 3). In comparison with the controls, the lipolytic changes of samples FESB2 and SKPF2 were lower with 38%, and in samples FESB1 and SKPF1 with 28%. Similar changes were found considering the changes in peroxide value and TBARS (Table 3). The significant ($p \leq 0.05$) reduction of primary lipid oxidation products (lipid hydroperoxides) of 24% was determined in samples FESB2 and SKPF2 and of 17% – in samples FESB1 and SKPF1, resp. of 28% while the reduction of secondary lipid oxidation products (TBARS) was of 53% in samples FESB2 and SKPF2 and of 44% in samples FESB1 and SKPF1 resp. A comparison of the pH of samples after 21 d of storage showed a slight (1.3–2.6%) but significant ($p \leq 0.05$) increase in samples SKPF2, FESB2, SKPF1 and FESB1 (Table 3). Contrary to control samples C, a statistically significant reduction of pH of 11.8% was found. The results obtained for the number of facultative anaerobic mesophilic microorganisms of vacuum-packed samples after 21 days of refrigerated storage (Table 4) confirm the above conclusions.

Conclusions. The addition of 5.0% seed kernel pumpkin (*Cucurbita pepo* L.) flour or especially 5.0% sea buckthorn (*Hippophae rhamnoides*) fruit powder extract to the pickle for injection of whole muscle horse meat is appropriate for production of new functional cooked and smoked horse meat Jaya product containing approximately 1% of biologically active substances. Application of similar concentrations of SKPF and especially of FESB guarantee effective inhibition of lipolytic changes, protein and lipid oxidation and improve the oxidative stability and quality of new functional horse meat delicacies saving their sensory and colour characteristics.

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