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Mineral Composition of Deer Meat Pate

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Abstract: In this study, the mineral composition of a pate made with red deer meat (maral meat), beans and a protein enriching additive was determined. To do so, three formulations of the pate (with different meat, additive and bean ratios) were produced. For each formulation, the content of mineral elements was then determined using inductively coupled plasma mass-spectrometry (ICP-MS). Increased level of minerals, such as K, P, Na, Ca and Mg were obtained in formulations where the additive and the beans were added. The addition of beans significantly increased Ca and P content. The mineral content of the overall better balanced formulation of the maral meat pate was compared to that of other pates.

Key words: Pate, mineral, ICP-MS, protein fortifier, deer meat

INTRODUCTION

Pate is a widely consumed meat product. It is considered a savory food and it is available in terrines, jars, tins, casings or vacuum packs (Echarte *et al.*, 2004). Pate often consists of ground liver and fat to which additives are added (Vossen *et al.*, 2012). There are two main methods of pate production. The first consists of using pork, beef or poultry by-products to which salt and spices are added. The second approach is more complex as it includes meat, vegetables, cereals, as well as various other ingredients (Bezuglova *et al.*, 2004; Ryazanova *et al.*, 2015).

Over the past decades, the food industry has been using food additives widely. These include protein agents of various origins, polysaccharides, fibers and probiotics. In addition to additives, protein-fat emulsions (PFE) obtained from meat by-products and vegetables have been used.

Consumers have included the meat of game animals, like deer meat, in their diet. The maral-(*Cervus elaphus sibiricus*, Severzov) is one of the largest representative of red deer. It inhabits areas located in the east and south-east of Kazakhstan (Korzhikenova *et al.*, 2014). Maral meat is currently used as a main component in the production of meat products because of its numerous health benefits compared to other meat types. Maral meat is rich in minerals, essential amino acids, vitamins (5-10 times more than in beef), protein (it has a protein content of 19-20%) and it is low in fat (its fat content varies between 1.1 and 3.9%) (Assenova *et al.*, 2016). Maral meat also contains bioactive substances, ferments and hormones which are considered beneficial. The caloric value of maral meat is 944 to 1154 kilocalories per 100 g. Kaimbayeva (2014) determined that, while for the most part, the mineral composition of maral meat is equivalent to beef, some elements are found in greater amounts. Maral meat is richer in calcium, fluorine, iron, copper, zinc and chromium compared to beef (Uzakov and Kaimbayeva, 2015). Enrichment of maral meat pates with nutrients of plant origin has the potential to further enhance its mineral content. By doing this, the pates would also gain fibers, carbohydrate and vitamin content. Macro and micro elements can also be gained using this strategy. Bean is a plant raw material that is used in the fabrication of meat products. Bean (*Phaseolus vulgaris* L.) is tasty, it has high protein content, it contains vitamin A, C and B's group as well as mineral elements. In bean's mature seeds, the protein content ranges from 17 to 33%, the fat content varies from 0.8 to 3.6%, the starch content is between 50 and 60% and fiber content represents 5 to 8%. The essential amino-acids composition is as follows (%): arginine, 8.1-9.9; histidine, 2.3-3.6; lysine, 3.4-5.7; methionine, 1.7-1.9; tyrosine, 2.4-3.0; tryptophan, 0.8-1.8 and cystine, 1.2-1.6. It should also be noted that the bean proteins are better digested by the human body compared to pea and lentil proteins (Shpaar, 2000).

During the development of new meat food formulations, special consideration should be given to the mineral composition of the final food product. Minerals required for adequate nutrition, are divided into two groups: major minerals (Ca, Mg, K, P, S, Cl, Na) that food contains in large amount and trace elements (Fe, I, Zn, Cu, Mn, Co, Cr, Mb, Ni, Va, Se, F, Si, Ge, B) found in low concentrations. Some of these trace elements are

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known to be toxic at large doses but they are nevertheless essential at low doses (Szefer and Nriagu, 2006; WHO, 1996).

The purpose of this study was to determine the mineral composition of different compositions of pates containing maral meat, a protein fortifier and beans. A second objective was to evaluate the influence of the protein enriching additive and the bean fraction on the mineral composition of the pate.

MATERIALS AND METHODS

Preparation of the protein fortifier: Cattle rumen (after preliminary technological treatment), horse rendered fat and blood, salt, garlic, nitrite, black powdered pepper and bacterial ferment (BF) were used to produce the protein enriching additive. The protein additive was prepared in two stages. The first stage consisted in preparing the protein-fat emulsion (PFE). To do so, some cattle rumen was boiled for 1 h. Then, the broth obtained was mixed to horse rendered fat and blood in the following proportions: 75, 15 and 10%, respectively. The mixing of these ingredients lasted 10 to 12 min. The chemical composition of the emulsion was as follows: protein-10.26%, fat-13.68%; total solids-14.07%, ash-0.95%. The second stage consisted in boiling the cattle rumen for 1.5 to 2 h using a rumen/water ratio of 1:3. The cooked rumen was grinded and mixed with PFE at a ratio of 2:1. The addition of 3% of salt followed. Small amounts of garlic, nitrite and pepper were also added. Everything was mixed for 5 to 7 min. At the end of the mixing process, 5 to 10% of bacterial starter (including Str. Lactis, Str. Diacetilactis, Str. Cremoris) was added. Mixing continued for 2 to 3 min. A stable protein fortifier's color was obtained because bacterial starter and blood were used in its preparation. The protein enriching additive was stored for 6-12 h at 4°C before being used to prepare the pates.

Preparation of the pate: The pate recipe calls for ground maral meat, protein fortifier, boiled ground beans, rumen broth, boiled carrot, onion, wheat flour, salt, black powdered pepper and spices. The pates were manufactured using three formulations with different proportions of protein enriching additive and beans. The first formulation included 85% of maral's meat without protein enriching additive and beans. The second formulation consisted of 50% maral's meat, 15% of protein enriching additive and 20% of grinded boiled bean. The third formulation included 50% maral's meat, 25% of protein additive and 10% of grinded boiled beans. The formulations are shown in the Table 1.

The maral meat was washed and cut into small pieces (100-150 g each), then it was blanched in water for 30 min (90-95°C) using a meat/water ratio of 1:3. Concurrently, beans were washed and boiled in water for 45-60 min. The boiled beans were then processed to obtained ground beans. Fresh carrots were cleaned and boiled until softened. The carrots were then cut into small pieces. The onions were peeled, cut and sauteed in vegetable oil for 10-15 min. Onions were then weighted and cut into small pieces. The patties were made in a cutter machine. The grinded maral meat, protein fortifier, pieces of onions, wheat flour, salt, pepper and spices (to taste) were gradually added. Then, at the mixing stage, beans, carrots and rumen broth were added, a small quantity at a time.

Determination of the mineral elements: One to two grams of the sample was placed in a high-pressure Teflon container. Each sample was combusted at 400°C for 4 h and then to 600°C for 2 h using a muffle furnace. A representative 1 g (dry weight) of ashes was digested by adding 3 cm^3 HNO_3 and 2 cm^3 of HF. This was placed in a microwave at 200°C for 20 min (Berghof Speed Wave microwave system, Germany). After microwave digestion, the samples were diluted with 1% HNO_3 in a 10 cm^3 vessel.

The content of elements in muscle samples was determined with an inductively coupled plasma-mass spectrometric method (ICP-MS, Varian-820 MS, Varian Company, Australia). The method was validated with certified reference materials. Calibration standards Var-TS-MS, IV-ICPMS-71A (Inorganic Ventures Company, USA) were used for calibrating the mass-spectrometer. The sensitivity of the mass-spectrometer was tuned up using a diluted calibration solution Var-TS-MS with concentration of Ba, Be, Ce, Co, B, Pb, Mg, Tl, Th of 10 µg/L. Three calibration solutions were used for the detector calibration. They were IV-ICPMS-71A of Cd, Pb, Cu, Zn elements diluted to 10, 50 and 100 µg/L. Discrepancies between the certified values and concentrations quantified were below 10%. The operating parameters of the inductively coupled plasma mass spectrometer Varian ICP 820-MS were as follows: plasma flow 17.5 L/min; auxiliary flow 1.7 L/min; sheath gas 0.2 L/min; nebulizer flow 1.0 L/min; sampling depth 6.5 mm; RF power 1.4 kW; pump rate 5.0 rpm; stabilization delay 10.0 s.

All analyses were performed in triplicates and the results, given in mg/kg wet weight, are expressed as mean±(SE). The results are presented in Table 2.

Statistical analysis: Differences between samples were evaluated using the t-test. The differences were considered to be statistically significant at $p \leq 0.05$. The statistical analysis was performed using the free software R 3.02 (R Core Team, 2013).

RESULTS AND DISCUSSION

Differences in mineral contents in pates can be explained by the different formulations. For this reason, the proportion of each ingredient (maral meat, protein enriching additive and beans) in each recipe was expected to influence the mineral content. Thus, with protein enriching additive and beans, recipes 2 and 3 were expected to yield an overall higher mineral concentration compared to recipe 1, prepared with only maral meat. This was confirmed by our analysis.

Comparing recipe 2 and recipe 3 (Table 2), it was observed that the concentration of Ca and P in recipe 2 were higher than in recipe 3. This can be explained by the amount of beans added in recipe 2 (20%) compared to recipe 3 (10%). Beans are rich in calcium (up to 1200 mg/kg) and in phosphorous (up to 4000 mg/kg).

As the concentration of Ca in recipe 1 was 93.38 mg/kg, adding the protein enriching additive and the beans translated in an increase in Ca content. In recipe 2, the Ca content was 277.30 mg/kg and in the recipe 3, it was 195.88 mg/kg (because of the lower proportion of beans added to the pate). As expected, beans also contributed to the increase in phosphorous level in recipes 2 (1521.91 mg/kg) and 3 (1326.37 mg/kg) compared to recipe 1 (763.92 mg/kg). These differences were verified statistically.

Differences in mineral content of pates were also affected by the mineral composition of the protein enriching additive. As is known, the rumen, which is a constituent of the protein enriching additive, contains Ca (150 mg/kg), Mg (140 mg/kg), Na (650 mg/kg), K (3250 mg/kg), P (840 mg/kg), S (1480 mg/kg) and Fe (30 mg/kg) (Assenova, 1996). The addition of rumen in the protein fortifier is likely the primary reason for the increase in potassium level in recipes 2 (2225.23 mg/kg) and 3 (2283.79 mg/kg) compared to recipe 1 (2067.45 mg/kg). The concentrations were statistically different in both recipes 2 and 3 compared to recipe 1. The modest increase in Mg (although not significant) was also likely due to the protein fortifier.

Another key ingredient of the protein enriching additive was cattle blood. The blood is rich in easily digestible forms of iron. Inorganic substances in the blood are represented with more than 30 mineral elements in the form of salts and organic matters. The iron content in blood varies from 30 to 55 mg/100 g, while in beef meat it represents 2.6 mg/100 g and in pork meat it represents 1.9 mg/100 g (Yudina, 2008). Despite its blood content, the fortifier did not contribute a significant increase in iron content. The Fe content in pate did not vary significantly between recipes. A slight increase was noted from 23.76 mg/kg in recipe 1 to 24.12 mg/kg in recipe 2 and 24.78 mg/kg in recipe 3, respectively.

The content of aluminum in all recipes was found to be about 10 mg/kg, selenium levels were also constant at about 0.06 mg/kg. Copper and zinc concentrations did not vary significantly either.

Recent studies on chemical and amino acid composition of pates (Okuskhanova *et al*., 2015) have shown that recipe 3 appeared to be better balanced and offers higher biological and nutritive value compared to

Table 1: Recipe for maral patties

	Mass part (%)		
Ingredient	Recipe 1	Recipe 2	Recipe 3
Ground maral meat	85	50	50
Protein fortifier	-	15	25
Boiled ground beans	-	20	10
Rumen broth	7	7	7
Boiled carrots	4	4	4
Onions (sautéed)	1	1	1
Wheat flour	2	2	2
Salt	1	1	1
Black pepper	0.1	0.1	0.1
Spices	<0.1	<0.1	<0.1

Table 2: Mineral element concentration in pate, mg/kg

	Recipe		
Minerals	Recipe 1	Recipe 2	Recipe 3
K	2067.45±95.71	2225.23±71.44*	2283.79±93.96*
P	763.92±94.89	1521.91±86.71*	1326.37±69.74*
Na	521.16±29.85	581.06±27.98*	591.47±37.60*
Mg	156.98±18.14	177.48±20.30*	185.21±19.28*
Ca	93.38±12.13	277.30±15.53*	195.88±14.76*
Fe	23.76±1.89	24.12±2.47	24.78±1.59
Zn	21.91±2.06	23.75±2.29	25.77±1.57
Al	10.88±0.73	10.29±0.84	10.47±0.85
Cu	1.77±0.24	1.78±0.15	1.81±0.26
Se	0.051±0.005	0.062±0.004	0.064±0.003

*Statistically different from recipe 1 ($p<0.05$)

the other recipes. Thus, recipe 3 was chosen as a reference point to compare our data with data found in the literature.

The results of this study were compared with the findings of several other research papers on the development of pates. The first is a deer liver pate, developed by Loboda (2013). This pate includes liver of domesticated deer, unsalted butter, prune, carrot, onion, pepper and salt. The second is a beef liver pate to which an extract of algae containing 0.1% of iodine (Tolkunov and Bolshakova, 2014) was added. The third is a goat pate, developed by Dalmas *et al*. (2011) that is prepared with “variety meat”, blood, liver, lard and other ingredients. The fourth is a pate with horse liver, horse meat and meat of broiler chickens that has been developed by Khvorostova (2013). Finally, the fifth is a Spanish canning pate (Zurero-Cosano *et al*., 1989).

According to these comparisons, the calcium level of recipe 3 is higher (195.88 mg/kg) than in the level reported for the goat pate (108.0 mg/kg) (Dalmas *et al*., 2011) and lower than in the levels reported in studies of Loboda (2013) (deer liver pate, 266.5 mg/kg) and Tolkunov and Bolshakova (2014) (pate to which an extract of algae was added, 417.0 mg/kg). Calcium is essential for the maintenance of skeletal growth and structure, muscular function, nerve conduction and normal blood clotting. The total amount of calcium found in an adult body can reach 1.5 kg. The daily requirement is 800-1500 mg. Calcium is considered an anti-stress, anti-allergenic and antioxidant nutrient. Lack of calcium

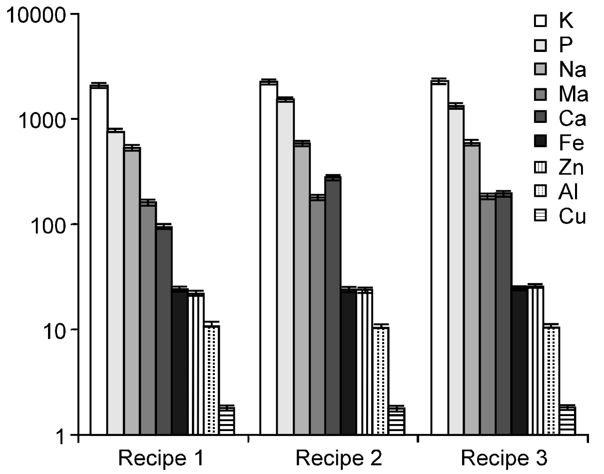

Fig. 1: Chart presenting the mineral element concentrations of each recipe

leads to osteoporosis and rickets, thyroid gland dysfunction, hypocalcemia, lethargy and poor appetite (Zand *et al*., 2015). The enrichment of meat products with calcium is considered important. The reason for this is linked to the low calcium concentration in meat creating an imbalance in the calcium/phosphorous ratio. Recipe 3 is enriched in calcium and compares well with other enriched pate's formulations.

When comparing the level of potassium in recipe 3 to other pates, it was found that the level of potassium (2283.79 mg/kg) was similar to the goat pate (2490.0 mg/kg), reported by Dalmas *et al*., 2011, but much higher than what was reported for the pate supplemented with an algae extract studied by Tolkunov and Bolshakova (2014) (1338.0 mg/kg) and the deer liver pate studied by Loboda (2013) (1430.30 mg/kg). The daily requirement for potassium is 3000-5000 mg. Potassium is an essential element for pH regulation in the human body. Potassium is crucial to heart function and plays a key role in skeletal and smooth muscle contraction, making it important for normal digestive and muscular function. Along with sodium, it regulates the water balance and the acid-base balance in the blood and tissues. Some common problems that have been associated with low potassium levels include hypertension, congestive heart failure, cardiac arrhythmia, fatigue, depression and other mood changes (Adrogue *et al*., 2014). Recipe 3 provides a good source of potassium.

The Na concentration in recipe 3 is very low (591.47 mg/kg) compare to those found in the goat pate studied by Dalmas *et al*., (2011) (9560 mg/kg) and the deer liver pate studied by Loboda (2013) (4515.1 mg/kg). The daily requirement for Na is 4000-6000 mg. The body uses sodium to control blood pressure and blood volume, it is used by muscles and nerves to work properly, it helps our body retain water and pH. It enables our cell walls to draw in nutrients. The lack of sodium leads to dizziness, spasms in abdominal cavity, headache, low blood pressure and disorientation (http://www.cdc.gov/salt/) The low sodium content in recipe 3 is due to the use of maral meat (540-720 mg/kg) instead of other meat types like beef (730 mg/kg), for instance. Although sodium is an important element, reduced sodium content is actually desirable. This is because, each day, humans consume enough sodium. In fact, a large variety of foods, including bread, sausage and canned food are also sources of sodium.

In recipe 3, the concentration of magnesium (185.21 mg/kg) was found to be similar to those reported for goat pate (197 mg/kg) by Dalmas *et al*. (2011). The concentration in recipe 3 was, however, lower than the values reported for the deer liver pate (258.1 mg/kg) developed by Loboda (2013) as well as the pate made with beef and pork liver developed by Tolkunov and Bolshakova (2014) (213 mg/kg). It is known that magnesium helps to maintain normal nerve and muscle function, supports a healthy immune system, keeps the heart beat steady and helps bones remain strong. Magnesium also helps regulate blood glucose levels and aid in the production of energy and protein. Magnesium is needed by the body to activate numerous enzymes that control the metabolism of carbohydrates, fats and electrolyte (Watson *et al*., 2013). The daily requirement is 400-750 mg.

The concentration of zinc in the pate of recipe 3 (25.77 mg/kg) is quite similar to those results obtained by Loboda (2013), for deer liver pate (24.06 mg/kg). This was also the case with Spanish liver pate (23.5 mg/kg) and with the goat pate (22.7 mg/kg) that Dalmas *et al*. (2011) worked on. Zinc is known to be involved in most metabolic pathways in humans, important for cell and organ structure and integrity, involved in the synthesis and metabolism of carbohydrates, lipids, proteins, nucleic acids and other micro-nutrients. Deficiency can lead to loss of appetite, growth retardation, skin changes and immunological abnormalities, gastrointestinal diseases, diabetes (Zhu *et al*., 2011). The daily requirement is 12-50 mg. Zinc is an essential constituent of various enzymes that are needed for the normal bio-physiological processes in the body. It also plays an important role in the regulation of gene expression (Sitalakshmi and Sai Kumar, 2014).

The level of copper (1.81 mg/kg) found is much lower than those obtained by Dalmas (16.3 mg/kg), Loboda (2013) (16.45 mg/kg) and Zurero-Cosano (1989) (8.63 mg/kg), but is similar to Khvorostova's result (1.82 mg/kg). Copper is an essential trace metal for normal muscle contraction and metabolic processes. Copper is required for various functions including the formation of the pigment melanin in the skin. It also helps in the transport of electrons, in the synthesis of phospholipids, and the formation of collagen. It is necessary for the formation of hemoglobin in the blood along with iron. It is a constituent of enzymes involved in the oxidation of fatty acids. It is necessary for healthy hair. Very high intakes can cause health problems such as liver and kidney damage and the deficiency leads to heart disease (Karpel and Peden, 1972).

The concentration of iron in recipe 3 (24.78 mg/kg) is lower by a factor of 2-3 compared to the pates developed by Loboda (2013) (70.94 mg/kg), Dalmas (64.8 mg/kg) and to the canned Spanish liver pate (56.5 mg/kg), determined by Zurero-Cosano (1989). The pate, developed by Tolkunov and Bolshakova (2014) (20.0 mg/kg) had similar Fe concentration to recipe 3. Iron is an essential nutrient for oxygen transport and cellular energy generation, it is needed to form hemoglobin. The daily requirement is 1-2 mg, but since only 10-20% is absorbed by the body, the daily uptake of iron should be 10-30 mg (Yudina, 2008). Iron deficit leads to anemia, pale skin, motor and mental problems and damage of the central nervous system function (Cabrera *et al.*, 2010).

The level of selenium (0.064 mg/kg) in recipe 3 was much lower than the levels measured by Loboda (2013) (0.42 mg/kg) and Khvorostova (2013) (0.24 mg/kg). Selenium is an essential trace element which protect against a number of cancers. It works as an antioxidant, especially when combined with vitamin E. Lack of selenium in the human body leads to Keshan's disease, hypothyroidism, slow functioning of the immune system, degeneration, necrosis, or atrophy in the cartilage tissue (Cabrera *et al.*, 2010). The daily requirement is 55-220 mg.

To sum up, pate made following recipe 3 provides a good source of calcium, phosphorous, potassium, magnesium and zinc. The pate is, however, lower in sodium, copper and selenium. Compared with other types of pates, its mineral composition is well balanced.

Conclusion: Human nutritional requirements demand at least 49 nutrients to meet organic metabolic needs. Of these, 23 mineral elements are involved in physiological and biochemical activities. The use of non-traditional meat (maral meat) in combination with protein fortifier and beans in the formulations of pates allows for key mineral element enrichment of this meat food. Using this strategy, we have noted significant increases in the levels of calcium and phosphorous (due to bean addition) as well as potassium, sodium and magnesium (due to the protein fortifier).

Pates made of maral meat, a protein fortifier and beans provide a nutritious, low fat and low calorie product. Furthermore, it is an adequate source of minerals. This type of pate would represent a good food choice, especially for elderly people and people with obesity.

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