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Raw poultry meatballs with soya flour: Shelf life and nutritional value

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Abstract: Poultry meat is a valuable source of protein for human consumption. It plays an important role in countries with poor ungulate meat production, including the Republic of Kazakhstan. The intake of fibre by the Kazakh population also remains low, while the intake of saturated fatty acids is excessive. Therefore, it is recommended to combine meat with plant products, e.g. soya flour. In the present research, we developed and evaluated a new meatball product containing different amounts of soya flour. The meatballs proved to be a semi-finished high-protein product. They also demonstrated a good fatty acid and mineral profile. The product with 30% of soya flour showed the best results: 27% of protein, low content of saturated fatty acid, and shelf life of 48 h. To extend the shelf life of the meatballs under refrigerator conditions, new disinfection methods should be developed.

Keywords: Soya flour, chicken, meatballs, nutritional value, predictive microbiology, shelf life

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INTRODUCTION

People living in developing countries, such as Kazakhstan, need high nutritive products. In this context, meat is considered one of the main sources of protein for consumers due to its high nutritive value. However, meat is expensive. Moreover, Kazakhstan has a specific lack of food-producing animals, and, thus, a low production of meat obtained from domestic and wild ungulates [1]. As a result, the food security of meat products there remains unsatisfactory [2]. Hence, Kazakhstan needs to develop a more competitive meat industry to improve meat production and market [3].

A relatively low production of beef in Kazakhstan is becoming an urgent problem, considering that Muslims represent a large group of Kazakh population, and they do not eat pork. Poultry meat could also improve protein intake by Kazakh people. Combining meat with products of plant origin is highly convenient for several nutritional purposes. A recent study by Shakiyeva *et al.* of the nutritional status of Kazakh people aged over 40 y.o. demonstrated a low fibre intake and excessive levels of saturated fatty acids [4]. Most plant proteins have a good fatty acid profile, which makes them preferable for human consumption. In addition, vegetables are an important source of fibre. Therefore, the nutritional composition of vegetables has several benefits for human health.

Soya is one of the plant products that could be combined with meat to formulate a new product. Although soya has lower levels of lysine or sulphur amino acids compared with meat, this food product is an important source of protein and fibre [5]. Soya also possesses isoflavones, which have been implicated as substances with important health benefits for more than a decade [6]. A recent research conducted by Ferguson *et al.* demonstrated the positive effect of moderate consumption of isoflavones on metabolic response [7]. This property makes soya beneficial for consumers suffering from obesity or insulin resistance [8]. All these aspects justify the formulation of meat products that combine ordinary or germinated soya flour with poultry meat.

The introduction of innovative flour-based functional foods into the market demonstrated a positive economic effect [9]. However, the high initial bacteria load in raw soya, raw germinated soya, and poultry meat is one of the main problems associated with this type of product. Moreover, meat is an excellent nutritional source for several types of bacteria, even taking into consideration that this type of product requires thermal treatment. Therefore, producers of this type of food should be careful when defining the shelf life and storage conditions.

The present paper introduces meatballs containing minced poultry meat with different percentages of germinated and non-germinated soya flour. Direct and indirect methods, e.g. predictive microbiology, were employed to determine the nutritional composition, minerals, heavy metals, and the shelf life of the new product.

STUDY OBJECTS AND METHODS

Formulation and elaboration. The meatballs were made of chicken breast. The minced chicken meat was combined with different concentrations and types of germinated and non-germinated soya flour to produce four different meatball samples. Sample A contained 70% of minced chicken and 30% of dry germinated soya flour. Sample B included 85% of minced chicken and 15% of dry germinated soya flour. Sample C contained 70% of minced chicken and 30% of dry non-germinated soya flour. Sample D consisted of 85% of minced chicken and 15% of dry non-germinated soya flour. The meatballs were stored at 4–6°C during the research process.

Microbiological analysis. The microbiological analysis was performed on days 0, 3, and 7. The samples were tested for *Salmonella spp.* and *Listeria monocytogenes* and proved to contain neither. 25 g of meatballs were homogenised with 225 mL of buffered peptone water (Merck, Germany) in a stomacher (MIX2, AES-Laboratory, France) for 2 min. The total viable counts were evaluated in plate count agar (Liofilchem, Teramo, Italy) incubated at 32ºC for 72 h. *Enterobacteriaceae* were counted in violet red bile glucose agar (Liofilchem) incubated at 32ºC for 24 h. Coliforms were detected in violet red bile lactose agar. *Escherichia coli* were detected in Fluorocult® (Merck) incubated at 42ºC for 24 h. The presence of presumptive *Staphylococcus aureus* was evaluated in Baird–Parker agar (bioMérieux, Marcy l´Étoile, France) incubated at 37ºC for 48 h. The presence of *Salmonella* was detected according to ISO 6579-1:2017 [10]. The meatball homogenate prepared as described above was incubated at 37ºC for 24 h. A 100-µL aliquot of the incubated peptone water was transferred to 10 mL of Rappaport–Vassiliadis (RV) enrichment broth and incubated at 42ºC for 24 h. Next, one RV broth loopful was streaked on xylose-lysine-deoxycholate agar (Oxoid) and SM-ID2 (bioMérieux) and incubated at 37ºC for 24 h. *Listeria monocytogenes* was determined according to ISO 11290-1:2018 [11]. Twenty five grams of meatballs were incubated in half-strength Fraser broth (Oxoid) at 30ºC for 24 h. Then, 100 µL was transferred to a tube containing 10 mL of Fraser broth and incubated at 37ºC for 48 h. Finally, the half- and full-strength Fraser broths were plated out on Aloa® agar (bioMérieux), and the plates were incubated at 37ºC for 48 h. All analyses were performed in duplicate.

Predictive microbiology. The data obtained for the microbiological analysis were compared with the data and scenarios obtained from ComBase, www.combase.cc (University of Tasmania, Tasmania, Australia; and the USDA Agricultural Research Service, Beltsville, MA, USA), which is a free on-line modelling database for predictive microbiology. The parameters used were those obtained from the initial analysis of the meatballs. Several conditions for bacterial growth were tested to assure the results obtained for the shelf life of the product.

pH measurement. The pH level was measured using a Crison PH 25+ pH meter with a penetration electrode (Crison Instruments, Barcelona, Spain) by introducing the electrode into the meatballs. Determinations for each treated meatball were performed in triplicate every three days.

Nutritional analysis. All analyses for the proximate composition were performed using standard AOAC methods [12]. The moisture content was determined by drying samples in a laboratory drying oven (Selecta, Barcelona, Spain) until the weight became constant. The total protein was determined by the Kjeldahl method. A factor of 6.25 was used to convert total nitrogen into crude protein. The samples were digested using a Kjeltec 1007 digester (Tecator, Höganäs, Sweden) and distilled using a Kjeltec 1026 distilling unit (Tecator). The lipid content was assayed by extraction with diethyl ether/petroleum benzene (1/1, v/v) in a Soxhlet system (Soxtec HT 1043, Tecator). The ash content was assessed by incineration in a muffle furnace (Utena, Lithuania). The carbohydrate quantity and energy content were obtained by calculations.

Minerals and heavy metals. Minerals and heavy metals were analysed by the method of inductively coupled plasma-optical emission spectrometry (ICP-OES). One gram of sample and 4 mL of 69% HNO_3 (Hiperpur, Panreac, Spain) were homogenised in glass tubes and incubated at room temperature for 1 h. Afterwards, 1.5 mL of 33% (w/v) H_2O_2 (Panreac) was added, and the mixture was heated first at 120ºC for 10 min in a heater block (Selecta) and then at 130ºC for 3 h. After the samples were cooled down to room temperature, Milli-Q water was added until the volume reached 25 mL. The samples were analysed in an Optima 4300 DV ICP-OES (PerkinElmer, MA, USA) under the following conditions: plasma flow, 15 L/min; auxiliary flow, 0.2 L/min; nebuliser flow, 0.8 L/min; sample flow, 1.5 mL/min.

Urease activity. The urease test was conducted as follows: 10 mL of a buffered urea solution (pH = 7.0) was added to 0.2 g of finely ground soya (test sample), and 10 mL of a phosphate-buffered solution was added to 0.2 g of the same sample (blank sample). The two solutions were incubated at 30ºC for 30 min under stirring. In the presence of significant urease activity, the pH of the test solution increased due to the ammonia released from the urea. Immediately after the incubation, the pH of the solutions was determined, and the difference between the pH of the test and the blank samples was calculated as the urease activity index. The pH was measured as described in section 2.4.

RESULTS AND DISCUSSION

Table 1 shows the microbiological analysis results of the four samples.

The total bacterial count, as well as coliform and enterobacteria counts were higher for the sample with germinated soya flour compared with that with non-germinated soya flour. The values reached one logarithmic cycle or above. This result was expected, since germinated soya contains more sugars than oligosaccharides, which should be a good advantage for bacterial growth [13]. However, the *E. coli* counts were very similar in all the samples.

L. monocytogenes and *Salmonella* were not isolated from any of the analysed samples. Variations in *Salmonella* prevalence depend on the origin of the poultry meat, as shown in [14]. Despite the importance of *Salmonella* tests for poultry production, the incidence of this foodborne pathogen has decreased in recent years [15]. In the case of *L. monocytogenes*, the past century saw a substantial improvement in quality regarding the prevalence of this microorganism in food [16]. For instance, the prevalence of *L. monocytogenes* in poultry breast was reported to reach 20% in 1990s. However, it has dropped to 8.6% in the last few years [17].

According to the ComBase results obtained for *Salmonella*-positive poultry samples, a value of 3.68 log CFU/g could be reached after one week under the following conditions of storage: temperature, 7ºC; pH, 6.4; a physical state for bacteria, 1; no lag phase. Comparatively higher values of *L. monocytogenes* could be reached, even at temperatures below 7ºC, for the same storage time. The total aerobic count was a good indicator of the shelf life of the product, and values above 7 log CFU/g indicated a marked alteration in the meatballs. The product needs to be stored at refrigeration values. Thus, *Pseudomonads* or *Brochothrix thermosphacta* can be selected in ComBase to predict the storage stability of the meatballs, as these bacteria are frequently related to meat spoilage [18, 19].

For *Pseudomonads* and *B. thermosphacta,* values above 7.5 log CFU/g could be achieved at 48 h of storage under the following conditions: temperature, 5ºC; pH, 6.4; water activity, 0.99; initial value, 5 log CFU/g; physical state for bacteria, 1; no lag phase. However, the meatballs developed in this study showed values higher than 7.5 log CFU/g (total aerobic count) after just one week of storage. The fact that a physical state of 1 implies no lag phase presupposes an extreme case that could rarely occur in real situations. In any case, a semi-manufactured product, such as the meatballs under study, could only have a maximum shelf life of 48 h at refrigeration temperature. The obtained data and the fact that the meatballs contained only raw ingredients proved that an adequate shelf life could be achieved by packaging or disinfection methods, e.g. ionising radiation, or refrigeration [20].

The values obtained for the urease activity confirmed the absence of thermal treatment in soya flour. The pH value ranged from 7.14 for the product with only 15% of germinated soya flour to 8.30 for the product with 30% of germinated soya flour. In the control samples and the samples with the cooked soya flour, the values were always ≤ 7 due to the absence of urease in the treated product. For the products with 15 and 30% of germinated soya flour, pH was 6.63 and 6.82, respectively According to Craven and Mercuri [21], several commercial texturised soya proteins were used in meat products with no increase in bacterial counts relative to the control samples. In the present study, the less processed soya flour caused higher bacterial counts.

Table 1 Microbial counts and pH on day 0, 3, and 7 in meatball samples with soya flour

Samples (minced meat/soya proportion)	Day	pH	TAC*	*Enterobacteriaceae*	Coliforms	*Escherichia coli*	*Staphylococcus aureus*	*Listeria monocytogenes*	*Salmonella*
70/30 germinated soya	0	6.44	8.8×10^6	6.2×10^5	1.8×10^4	2.0×10^2	< 50	nd	nd
	3	6.89	4.3×10^7	3.2×10^6	6.4×10^5	2.6×10^2	< 50	nd	nd
	7	7.12	9.1×10^8	6.8×10^6	1.2×10^6	3.3×10^3	< 50	nd	nd
85/15 germinated soya	0	6.33	5.0×10^6	2.4×10^5	1.0×10^4	3.1×10^2	< 50	nd	nd
	3	6.78	6.3×10^7	9.2×10^5	2.4×10^5	8.6×10^2	< 50	nd	nd
	7	6.99	9.8×10^8	3.8×10^6	5.9×10^5	3.6×10^3	< 50	nd	nd
70/30 non-germinated soya	0	6.31	3.5×10^5	4.5×10^4	2.0×10^3	1.2×10^2	< 50	nd	nd
	3	6.84	4.8×10^6	2.2×10^5	2.4×10^5	4.5×10^2	< 50	nd	nd
	7	6.92	4.2×10^7	2.8×10^6	5.9×10^5	9.2×10^2	< 50	nd	nd
85/15 non-germinated soya	0	6.39	1.7×10^6	6.2×10^5	9.0×10^3	1.5×10^2	< 50	nd	nd
	3	6.90	5.2×10^7	1.5×10^6	3.3×10^4	1.8×10^2	< 50	nd	nd
	7	7.21	2.2×10^8	9.3×10^6	8.5×10^5	4.1×10^2	< 50	nd	nd

*TAC: total aerobic coun
nd: not detected

Table 2 Nutritional composition of meatball samples with soya flour

Parameter	Samples (minced meat/soya proportion)			
	70/30 germinated soya	85/15 germinated soya	70/30 non-germinated soya	85/15 non-germinated soya
Dry weight	43.45	33.40	45.31	35.03
Protein	27.18	24.37	29.49	25.69
Fat	2.85	2.52	2.17	3.20
Saturated fat	0.73	0.65	0.50	0.82
Monounsaturated fat	0.57	0.54	0.44	0.66
Polyunsaturated fat	1.55	1.33	1.23	1.72
Ash	2.23	1.79	2.53	1.66
Carbohydrate	11.19	4.79	11.12	4.48
Energy, kcal/100 g	179.13	139.04	181.97	149.48
Sodium, mg/100 g	40.38	48.97	45.18	53.57

As for nutritional properties, the idea of using soya protein is more than 30 years old [21]. Chicken breast was found to achieve a maximum protein content of 34.5%, although values of 24% are most frequently reported [22, 23]. For the products developed and studied in the present research, similar values to those described by Lonergan *et al.* [23] were obtained in the meatballs prepared with 15% of soya flour. However, an important increase in protein content was observed for the meatballs with 30% of germinated soya flour. Their protein level was ≥ 27% (Table 2).

The primary objective of the present study was to obtain a high-protein product. The research proved that could only be achieved by adding 30% of soya flour to the meatballs. Of the two samples with 30% of soya flour, the meatballs with non-geminated soya flour showed better results, with 2% more protein than in the samples that contained germinated soya flour.

The meatballs with 30% of soya flour proved to have nutritional advantages. In addition, combination of protein and fibre can promote satiety [24]. The products with 30% of soya flour had a higher dry weight values than those with 15% of soya flour. This fact could trigger a higher water intake and, hence, an increase in satiety. Satiety is an important aspect to consider both for diabetes and/or dietetic treatment of obesity. The effect of high protein intake on satiety is so strong that a remission of pre-diabetes to normal glucose tolerance was observed in patients fed with a 30% dietetic protein for 6 months [25]. As revealed above, isoflavones in soya could also help to improve insulin tolerance [7, 8].

Serdaroglu *et al.* and Ikhlas *et al.* studied the quality of low-fat beef meatballs with 10% of various legumes, excluding soya flour [26, 27]. They reported lower protein values (≤ 24%) than those obtained for the products developed in the present study. Increased amounts of legume flour were suggested to be used as extenders for meatballs [26]. The meatball samples used in the present research showed no differences in total, saturated, and unsaturated fat (Table 2). Their values were always ≤ 4% and sometimes even ≤ 3%. Judging from these fat contents, the proposed meatballs had lower energy value and fat content than beef, pork, or even some turkey parts or duck meat [22]. Likewise, the low amount of saturated fat together with the high polyunsaturated fat content can improve traditional Kazakh diet.

As stated in [28], saturated fats should provide about 7% of dietary energy. The content of saturated fats in the

Table 3 Minerals and heavy metals in meatballs with soya flour

Minerals and heavy metals, mg/kg	Samples (minced meat/soya proportion)			
	70/30 germinated soya	85/15 germinated soya	70/30 non-germinated soya	85/15 non-germinated soya
Mg	656.22	393.57	476.01	421.97
P	1872.63	1520.86	1636.46	1629.99
K	5652.08	3930.54	4987.42	4360.88
Ca	802.88	386.48	476.07	318.47
Fe	16.14	9.20	14.27	10.97
Ni	0.50	0.21	0.35	0.6
Cu	3.58	1.33	2.51	1.51
Zn	11.09	8.02	9.36	8.66
As	0.0065	0.0055	0.0056	0.0033
Se	0.0956	0.1097	0.1067	0.1255
Cd	0.0064	0.0033	0.0047	0.0031
Hg	0.0104	0.0063	0.0055	0.0046
Pb	0.0065	0.0034	0.0032	0.0013

meatballs was ≤ 1%. Therefore, 200 g of the meatballs contained 60 g of protein and about 2 g of saturated fats, i.e. 240 and 18 kcal, respectively. It complies with the general recommendations for saturated fat intake.

As for minerals and heavy metals, the four meatball samples were tested for a total of 13 elements (Table 3).

Remarkably, the meatballs with 70% and 30% of germinated soya flour demonstrated higher contents of Mg, P, K, Ca, Fe, and Zn and a double or more of Cd, Hg, and Pb than the other samples. These results were probably due to the higher mineral content found in the soya flour, as the content increased with soya flour concentration in the produced meatballs. Therefore, plant food products can be expected to have higher amounts of minerals than animal food products.

The study conducted by a Chinese research team showed high levels of As, Cu, and Zn in poultry tissues, which were mainly attributed to feed supplements [29]. The Chinese study proved that the amounts of As found in inorganic poultry meat in Lianzhou and Guangzhou pose a significant public health risk, considering the high level of bladder or lung cancer in these cities. In the products designed in this study, the As level was an order of magnitude lower than that obtained by Hu *et al.* [29]. The levels of Cd and Pb in the present study were also lower. However, we detected higher levels of Cu and Ni. In any case, the soya flour used in this study was poorly processed and did not undergo any thermal treatment, as verified by the urease test.

Soya has important anti-nutritive agents, so this product has to be treated to avoid the effect of these compounds. These anti-nutritive factors are phytic acid, phytates, and protease trypsin inhibitors. The presence of natural phytates, for instance, significantly increases the calcium requirements. In soybeans, the phytic acid content is 1.00–1.47% of dry weight, which means more than 50% of phosphorous [30]. The treatments to eliminate trypsin inhibitors from soybean flour were recently reviewed by Vagadia *et al.* [31]. The cooking of soya flour in an alkaline system at 90ºC for 15 min is sufficient to inactivate the protease trypsin inhibitors.

CONCLUSION

The present research introduced, developed, and described a new poultry meat product: meatballs formulated with germinated or non-germinated soya flour. The use of 30% of soya flour resulted in a semi-finished high-protein product. The soya flour used in the formulation produced a number of other positive effects, e.g. low and well-balanced fat content and increased amounts of fibre and isoflavones. The increase in mineral content could depend on the specific plant origin of the soya flour, and additional treatments are necessary to avoid the negative effect of anti-nutritive compounds. Direct microbiological analyses and predictive microbiology showed that the mixture of minced poultry meat and soya flour produced a product with a shelf life of 48 h. In order to extend the shelf life of the product, specific packaging procedures or disinfection techniques should be applied.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest related to this article.

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