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Assessment of total protein and soy protein contents in some heated meat products in Tehran, Iran

Masoomeh Fekri^{1,2}, Gholamreza Jahed Khaniki^{1*}, Mohaddeseh Pirhadi¹, Mahdieh Abbassi²

¹Division of Food Safety and Hygiene, Department of Environmental Health Engineering, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

²Food and Drug Control Reference Laboratories Center, Department of Food Animal Origin, Food and Drug Organization, Ministry of Health and Medical Education, Tehran, Iran

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ABSTRACT

Heated meat products are emulsion which have various nutrition materials such as meat as an animal protein and soya as a plant protein. The nutritional value of meat proteins is very high than soya protein but the meat is more expensive than soya which the producers substitute the meat with soya. This study was assessed the soy protein and the total protein contents in some heated meat products collected from food stores in Tehran, Iran. Twenty samples of heated meat products with 40%, 55% and 70% of meat were randomly collected from food stores. The heated meat products samples were transferred to the food analysis lab. The total protein was determined by the macro Kjeldahl method after sample preparation and homogenization. Also, the soy protein content in samples was measured by using the enzyme-linked immunosorbent assay (ELISA) method. Results showed that 4 samples of heated meat products had less total protein content than the standard limit and 16 samples were in accordance with Iranian national standard. Soy protein content in 3 from 8 samples of heated meat products with 40% meat was higher than the standard limit and the others placed in the standard limit (approximate with 4% soy protein). Also, Soy protein content in 6 from 8 samples of heated meat products with 55% meat was higher than the standard limit and only 2 samples were in accordance with the standard limit. All samples of heated meat products with 70% meat set in a standard limit. It was concluded that some heated meat products do not correspond with the Iranian national standard range. The food quality control lab requires doing attention and sensation for correct formulation according to national standard measures.

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1. Introduction

Soybean protein is one of the most important and predominant plant proteins that is added to meat products such as sausages and hamburgers (1). Since animal protein consumption alone is not enough to meet human nutritional needs and on the other hand, the production of these protein substances is also expensive, the use of cheap and affordable proteins, such as soy protein can solve this need. Among non-meat proteins, soy protein is the most commonly used in meat products (2).

Application of soy beans in meat products improves the absorption of water insausages, and provides better emulsion. For this reason, sausage producers are keen to use it in the preparation of such products (3). In many countries, the addition of soy protein to meat products is prohibited and fraudulent. But it is used in Iran, and it should not exceed the national standard, in the composition of meat products (4). In Iran, soy protein is commonly used as flour, which has a special flavor and, if used extensively in meat products, consumers tend to feel an unpleasant taste in the mouth. In some cases, producers use a large amount of animal fat to cover the taste of soy.

* Corresponding author. Tel.: +98 2188954914
E-mail address: ghjahed@sina.tums.ac.ir

Due to the presence of saturated fatty acids, animal fats can be considered as one of the most important factors in increasing blood cholesterol levels and causing cardiovascular disease. Therefore, the high use of saturated fats following the use of soy beans can also endanger the health of the consumer (5).

On the other hand, soy protein is considered as an allergenic food. Almost 8% of children and 2% of adults have food allergy (6). In 1999, the Codex Alimentary Commission established that all foods containing irritant substances, such as soy, should be clearly labeled so that consumers who have allergies do not use them (7). Considering the reasons mentioned above, the use of soya in meat products can be important in the health and safety of these products. Some people in Tehran city of Iran consume the heated meat products in their daily nutrition and the heated meat products must be controlled for adding the soya protein by food labs. Therefore, the aim of this study was to assess the total protein and the amount of soy protein in collected heated meat products from the food stores of Tehran city and to compare the levels with the national standard limit in order to control the quality of these products.

2. Materials and Methods

2.1. Sample collection and preparation

20 samples of heated meat products with different meat percentages (40%, 55% and 70% meat) were randomly purchased from food stores in Tehran city of Iran. Samples were transferred to food lab in safe conditions. The registration of the characteristics and related specifications (factory name, date of production of meat percentage and license number) of each sample were done. Then the samples cut to fine small by a meat grinder and entered to a homogenizer for uniformity and homogeneity. Then, the total protein and soy protein contents of each sample was measured using macro ecological method and based on AOAC method. The total protein and soy protein were determined by macro kjeldahl method and the enzyme-linked immunosorbent assay (ELISA) method, respectively.

2.2. Sample examination

2.2.1. Measurement of total protein

To measure total protein, 2 g of homogeneous sample was weighed with a precision scale of 0.001 g and entered into protein digestive tubes of Buchi 321 kjeldahl Distiller. One gram of copper sulfate and 10 g

of potassium sulfate and a number of glass pearls were added to the tube. Twenty five ml of concentrated sulfuric acid were then added and, until complete digestion, direct heating of the electric oven was used.

After cooling the contents, the sample tube is connected to the distillation system after adding 200 ml of distilled water and 25 ml of NaOH 40%, the contents of the tube were distilled for 10 min.

The ammonia gas obtained through a refrigerant was collected in a 500 ml Erlenmeyer container containing 50 ml of boric acid plus several drops of methyl ester. Then, Erlen Meyer content was titrated by 0.1% normal value and based on the amount of NaOH, the total protein content was calculated based on the following formula.

$$T_p = \frac{(V_1 - V_2)N \times 6.25}{M \times 100}$$

T_p = Total protein in grams percent

V_1 = Amount of NaOH 0.1 N for control titration

V_2 = Amount of NaOH 0.1 N for sample typing

N = Meq N is equal to 0.014.

M = Sample weight

2.2.2. Soy protein measurement

To measure soy protein in samples, the extraction and preparation of the samples was done according to the manufacturer's instructions Verotox quantitative soy allergen test made by Neogen USA, which is an ELISA-sandwich method (S-ELISA). To do the test, first 5 g of the homogenized sample was weighed with a precision scale of 0.0001 g and the extraction solution was add for 15 min in a Bain-marie at 60°C. It was then shaken at least once in a minute to completely break it up and mix. Then, it was straightened with plain paper and centrifuged 5 ml of filtered solution at 14000 rpm for 5 min.

The supernatant of the clear solution was prepared as a sample for use with a soy protein assay kit and added to the kit packs containing the antibody. Antibody bonding with soy protein was performed at this stage. Then, after washing the wells, the proteins that were not bonded were removed. Then the conjugated enzyme was used to mark binding proteins. The substrate solution used again after was washing.

In this case, the labeled antibodies appeared in color and at the end, a red stop solution was used. After 20 s, the sample wells were read on a micro well reader with a filter of 650 nm. After comparing the soybean standard curve, the amount of soybeans used in the samples was measured.

The contents of total protein and soy protein in heated meat products was compared according with the Iranian national standard No. 2303 (4).

3. Results

Results showed that out of 8 samples of heated meat products with 40% meat, the total protein content was the in the Iranian national standard limit in only 2 samples and the soybean percent was higher than the national standard limit in 3 samples. The remaining samples of heated meat products with 40% meat were in terms of total protein and soy protein contents with the corresponding standard (Table 1). In Table 2, the total protein content of 2 heated meat products samples with 55% meat was less than standard (minimum 12%). The soy protein was found in 2 out of 6 heated meat products samples for 55% meat. In Table 3, the results show that total protein and soy protein levels in 70% samples correspond to the total protein content (minimum 14%) and soybean protein content with the corresponding standard. The results of Table 4 show that total protein content in all samples as well as soy protein content in heated meat products samples of 40% meat have a statistically significant difference ($p < 0.05$).

Table 1. Percentage of total protein and soy protein in heated meat products with 40% meat

Sample No.	Total protein(%)	Soy protein(%)	Soy protein Standard(%)
1	7.92	3.006	≤ 4
2	12.74	4.257	≤ 4
3	11.44	0.389	≤ 4
4	12.71	0.949	≤ 4
5	10.93	4.005	≤ 4
6	8.33	0.216	≤ 4
7	12.56	2.825	≤ 4
8	12.91	4.89	≤ 4

Table 2. Percentage of total protein and soy protein in in heated meat products with 55% meat

Sample No.	Total protein (%)	Soy protein (%)	Soy protein Standard (%)
1	11.38	0.152	-
2	14.25	0.821	-
3	14.65	0.04	-
4	15.83	2.29	-
5	11.52	4.94	-
6	15.12	0.06	-

Table 3. Total protein and soy protein content in in heated meat products with 70% meat

Sample No.	Total protein (%)	Soy protein (%)	Soy protein Standard (%)
1	14.01	0.61	-
2	16.92	0.401	-
3	18.15	0.116	-
4	14.22	0.03	-
5	17.55	0.405	-
6	15.53	0.558	-

Table 4. Total mean and standard deviation of total protein and soy protein in samples (in percent)

Parameter/samples	N = 8	N = 6	N = 6
Total protein (%)	11.19 $\pm 2.01^*$	13.79 $\pm 1.88^*$	6.06 $\pm 1.74^*$
Soy protein	2.56 $\pm 1.83^*$	1.30 ± 1.94	0.35 ± 0.23

* It is statistically significant ($p < 0.05$).

4. Discussion

The use of soya is different in meat products such as sausages in different countries. In some countries, the presence or absence of soy beans in meat products is important, and in some others, the amount of soy used is important. Therefore, it is necessary to invent and apply quantitative and qualitative diagnostic methods of soy in these products. Different methods such as microscopy (8), immunochemical methods, immune diffusion and gel electrophoresis (3) are used to identify soybeans in meat products. But, none of these methods were completely satisfactory because they were not able to measure soy in most cases in meat products, and on the other hand, it took a lot of time to measure (9). By using the histological method and PCR

(Polymerase Chain Reaction), the presence of soy protein can be found in meat products. In this regard, in a study, Jahed Khaniki et al. (2003) managed to distinguish soybean texture from muscle tissue in 80 frozen hamburger samples using a histological method (10). An approach commonly used in food control laboratories to measure soy beans in heated of meat products by the Enzyme-Linked Immune Sorbent Assay (ELISA) method, which is indicated by AOAC (11). ELISA is also used to detect transgenic food products or genetic alterations that have a protein base (12). In the ELISA method, polyclonal antibodies are used to identify the protein of soya flour. Cota et al. (1998) managed to construct this antibody to combat fraud in the use of soy flour in meat products (13). In this study, the total protein and soy protein in heated meat products were measured using macro kjeldahl and ELISA methods, respectively. In the sample of heated meat products with meat 40%, the maximum amount of soy protein that can be present is 4%. Meanwhile, in the three samples tested in this study, the soy protein content was higher than this amount, and soy protein was detected in all samples. In a study, Medina (1988) reported on the extraction and measurement of soy protein in meat products including sausages by ELISA (14). Also, Macedo et al. (1999 analyzed quantitatively the levels of soy protein in 39 hamburgers from beef and chicken) using the ELISA method. It was concluded that the ELISA method is a fast and easy method for evaluating soy protein in food products (15). In 2004, Koppelman et al. analyzed the amount of soy in the foods by ELISA and they were able to detect the soy beans in the range of 1ppm (16). Also, Montserrat et al. (2010) succeeded in using the method Real-time PCRs to identify the soybeans in 35 types of foods including meat products, fish and cake. Some products were made from soy flour. They concluded that using the Real-time PCR method is easy method to identify soy DNA in samples (17). Usually, Soya protein assay biokits are used in the ELISA method based on the enzyme immune response. This method has a good susceptibility to soy protein in the presence of vegetables, meat and other proteins, and it is an indirectly competitive immune-enzyme assay to determine the amount of used soy protein (18). The results of this study showed that the average percentage of soy protein by using ELISA method in heated meat products samples with 40% meat was $2.68\% \pm 1.83\%$, which corresponds to the relevant standard to some extent and the average percentage of total protein was $11.19 \pm 2.01\%$ which is placed in within the standard limit. But in samples with 55%

meat, the soya protein content was $13.8 \pm 1.94\%$, which did not match the relevant standard. The average percentage of total protein was $13.79\% \pm 1.88\%$ which the standard protein limit must be at least 12%. The average percentage of soybean in samples of heated meat products with 70% meat is zero, which corresponds to the relevant standard limit and the average percentage of total protein was $6.06\% \pm 1.74\%$ while the standard protein range must be at least 14%. Also, soy protein was detected in two samples of heated meat products containing 55% meat and the total protein content measured by macro kjeldahl was also lower than standard limit (minimum 12%). Due to the fact that the heated meat products are emulsions in which the raw materials after mixing and uniformity are not recognizable in appearance and it can have the highest possible degree of fraud among different food groups. The addition of soymeal flour instead of meat in these products, in addition to the economic harm that the consumer is concerned, can, in some cases, affect the health of consumers who are susceptible to soy protein and allergy.

5. Conclusion

According to the findings of the present study, the cases of non-compliance with the Iranian national standard were observed in some samples of heated meat. In this regard, food quality control laboratories need to be more precise and more sensitive about determining the correct formulation and in accordance with national standards. The use of ELISA method can be used to identify the soybean protein and also it can determine its amount in meat products as an accurate, sensitive, fast and cost-effective method in food quality control laboratories.

Conflict of interest

The authors have no conflict of interest.

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