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Scientific Research

Feasibility study of the stereological method to determine the percentage of meat used in raw (Hamburger) and heat-treated (Sausage) meat products

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ABSTRACT

Accurate food labelling is of utmost importance for fair trade and empowering consumers to make knowledgeable choices. Quantitative assessment of the meats is one of the most important factors in authentication of this meat product. Therefore, the purpose of this research was the efficiency of the stereological method for detection of the percentage of meat used in raw (Hamburger) and heat-treated (Sausage) meat products. In this study, three samples of beef burger (containing 30, 60 and 90 % meat) and sausage (containing 40, 60 and 90 % meat) were prepared. Each sample was flattened, and then fractionator sampling using a perforated plate as a cutting guide was used for getting systematic uniform random blocks. Totally, 12 blocks were taken from each sample. Each block was fixed in 10 % neutral formalin and embedded in paraffin. The obtained samples, after preparation of tissue sections and staining using haematoxylin and eosin (H&E), were studied by a light microscope. The data were analyzed using one-way ANOVA statistical method and t-test was used to compare significance difference between groups. Stereological analysis showed that the estimated meat percentages in beef burger in different percentages (30, 60 and 90%) did not have a significant difference with the real percentages of meat. In the case of sausage, there was significant difference between the estimated meat percentages and the real percentages of meat in different samples of sausage. Stereology method can be a suitable complementary method to detect the amount of meat used in raw meat products.

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1- Introduction

Meat is a balanced diet containing 20 to 35% protein, which is able to provide all essential amino acids [1]. In addition, it contains good levels of minerals, fatty acids and vitamins (B_6 ،B_{12} and D) [2] and therefore, it is a valuable source of various micronutrients that are necessary for growth and development [1]. The average per capita consumption of meat and the total amount of meat consumed have increased worldwide, especially in developing countries, due to population growth [1], increased income, and socio-cultural traditions that place a high value on meat consumption [3]. [1]. Although a diet without meat and meat products may have a number of benefits for human health, some important micronutrients are not easily available through non-meat foods [4].

In general, protein sources of animal origin such as eggs, milk and beef are considered as a complete source in terms of providing essential amino acids for human growth and development. However, plant proteins may contain insufficient levels of one or more essential amino acids, such that the amount of sulfur-containing amino acids methionine and cysteine in legumes and lysine in cereals is at a low level [4]. Because the diet of people in developing countries mainly includes crops, they often suffer from specific deficiencies in the essential amino acids lysine and myoteen. These two amino acids are especially important to the aspartate family pathway[1] are responsible for the synthesis of two other essential amino acids (threonine and isoleucine) [5]. The low amount of lysine and methionine in these plants has caused the nutritional value of these crops to decrease to 50-75% compared to a diet with balanced levels of essential amino acids. This limitation of essential amino acids can lead to non-specific symptoms of protein deficiency in humans, such as decreased resistance to diseases, decreased blood proteins, and mental and physical retardation in young children [6].

As people's awareness of food safety, traceability, and authenticity is increasing, accurate food labeling and description, especially in more processed foods, is very important [7]. For processed food products, the easiest source of information; Label and its ingredients [8]. Correct labeling is essential to support and promote fair trade, and the unfortunate mismatch between the actual ingredients used and those listed on the labels will lead to increased consumer concern. In this regard, validating the amount of meat used in the preparation of meat products is one of the most important factors in the evaluation of these products. Many studies have focused particularly on meat products such as ground beef or sausages, as the opportunity for mislabeling is greater in these products [9]. Therefore, identifying ingredients declared on labels should be the main priority of regulatory bodies to combat food fraud at the supply level. Also, food label control is very important in controlling food quality, ensuring safety and protecting consumers [10]. Nowadays, these frauds are considered as a health and economic problem in the food industry, and therefore it is necessary to use and design an accurate, sensitive and reliable method to determine the percentage of meat used in these products [1, 11].

Based on the equipment, techniques used, as well as the cost and method of sample preparation; Various methods have been proposed to identify the components of meat products [12, 13]. Until now, in many studies, histology has been used to identify and diagnose the amount of meat and to determine the quality and nutritional value of meat products [14]. These methods enable the direct detection of a tissue in meat products. Therefore, in most studies, it has been used to detect tissue fraud in food [14, 15] and past studies have shown histological methods as an effective method in detecting unauthorized tissues in meat products [16]. Mokhtar et al also reported using histological and chemical analysis in 2018 that non-permissive tissues such as lung, heart, bone, cartilage, fascia (dense fibrous connective tissue), intestine, thyroid gland, tendon and suspicious tissues of fetus and plants It is added to minced meat and sausage. The presence of fascia and cartilage in excess of the allowed amount in meat product samples reduces the quality and nutritional value of meat [17]. In addition, the presence of brain tissue can also cause the risk of carrying infectious agents that can be transferred to humans, such as prions [18]. Other studies have been conducted on different meat products using histological techniques such as hamburger [19] and hot dog [20] and the presence of plant material, fat tissue, blood vessels, peripheral nerves, bone and cartilage has been reported.

Stereology technique (three-dimensional microscopic analysis) is a branch of applied mathematics [21] that obtains three-dimensional (3D) information from two-dimensional (2D) images of tissue components with the help of mathematical, statistical and geometrical principles. Some have confused stereology with morphometry, while morphometry is the measurement of the form or structure of tissue using calipers [22], so stereology is a subset of morphometry [23]. Others confuse stereology with the concept of stereoscopy.

[1]-Aspartate Family Pathways

Stereoscopy uses a pair of two-dimensional images of the same section obtained at slightly different angles (stereo pairs) and combines the two images into one three-dimensional image [23].

In the technique of stereology, to measure quantities, from uniform random sampling without bias[2] and preparing slices with the same thickness from the sections in each tissue block is used [24]. The biggest advantage of using stereology methods is non-bias and efficiency, which cannot be found in other morphometric studies [25]. In stereology, various aspects such as tissue volume determination, determination of the number of specific particles in the sample, surface calculation, length calculation and various other parameters can be selected depending on the subject being studied [26]. In a study that was conducted on 105 different meat sandwiches from different restaurants, the analysis of images prepared from optical and electron microscopes and histological methods, the presence of numerous illegal tissues such as lung, rumen, large blood vessels, cartilage, heart muscle, tissue showed lymph (spleen) and plant material [16]. Although the study on determining the percentage of illegal tissues in meat products by histological method It has been done, but the information on determining the percentage of meat by stereological method is limited.

Due to the non-compliance of the label of meat products with the product formulation and the inability of chemical quality control tests in accordance with the national standard of Iran to determine the amount of meat used in these products, this research aims to investigate the control of the amount of meat used in meat products with a new technique and Strict stereology was performed and the efficiency of stereology test in determining the percentage of meat used in raw (hamburger) and heated (sausage) meat products was investigated, so that if it is effective, it will be presented to the relevant policy makers.

2- Materials and methods

1-2- sample preparation

In this research, three hamburger samples (containing 30, 60 and 90% meat) were used as raw meat products and three sausage samples (containing 40, 60 and 90% meat) were used as heated meat products. Samples of meat products were prepared in the production unit of Gostar Sina Company with the coordination of Iran Meat Products Industry Association. Sausage and hamburger samples were prepared for each product with different percentage of meat (in accordance with the division stated in the relevant national standards used in the production unit) in the amount required for three repetitions of the test.

The ingredients used in the preparation of hamburgers were prepared according to the Iranian national standard number 2304 in the percentages of 30, 60 and 90 meat and according to the formulation stated in Table 1, in the production unit for each group. The obtained mixture was mixed with additives to create a one-hand dough, and then it was put into a freezer bag and, while being labeled, it was kept in a freezer at -18°C for quarantine procedures.

[2]-Unbiased

Table 1 Hamburger formulation with different percentages of red meat

	Hamburger; g (%)		
Compounds	**30%**	**60%**	**90%**
Beef	300 (30)	600 (60)	900 (90)
Fat	-	100 (10)	-
Vegetable oil	100 (10)	-	-
onion	250 (25)	200 (20)	27 (2.7)
Soy	120 (12)	-	-
Toasted flour	80 (8)	80 (8)	50 (5)
Bell pepper	30 (3)	-	-
Refined salt	10 (1)	10 (1)	13 (1.3)
Spices	10 (1)	10 (1)	10 (1)
Water	100 (10)	-	-
Total	1000 (100)	1000 (100)	1000 (100)

High-quality raw materials with a birth certificate according to the health permit and according to the national standard of Iran No. 2303 were selected for the preparation of sausages. First, pour all the required ingredients according to the sausage formulation in Table 2 into the cutter until the meat dough is obtained[3] homogenized, catheterization was continued. Meat paste obtained using a filling machine[4] It entered the special polyamide coating, which entered the baking room after labeling. In the baking room, the temperature was applied at 60-80 degrees Celsius for 45 minutes to 1.5 hours. Finally, in order to cool the desired sausage, it was first passed under the cold water shower and then entered the cold room at 2 to 6 degrees Celsius until the temperature of the center of the product reaches the optimal level.

[3]- Farsh
[4]- Filler

Table 2 Sausage formulations with different percentages of red meat.

	Sausage; g (%)		
Compounds	**40%**	**60%**	**90%**
Beef	2751 (39.3)	4200 (60)	5400 (90)
Wheat flour	594 (8.49)	210 (3)	-
Potato starch	110 (1.57)	140 (2)	60 (1)
Oil	1099 (15.7)	350 (5)	60 (1)
Pasteurized liquid eggs	220 (3.14)	490 (7)	60 (1)
Refined salt	88 (1.26)	94.5 (1.35)	60 (1)
Gluten	175 (2.5)	210 (3)	-
Soy	66 (0.94)	-	-
Spices	88 (1.26)	84 (1.2)	60 (1)
garlic	112 (1.6)	-	30 (0.5)
milk powder	-	70 (1)	60 (1)
Sugar	22 (0.314)	-	-
Sodium phosphate	20 (0.28)	28 (0.4)	24 (0.4)
Ascorbic acid solution	3 (0.04)	2.8 (0.04)	3 (0.05)
Sodium nitrite	0.7 (0.01)	0.84 (0.012)	0.72 (0.012)
Ice	1652 (23.6)	1120 (16)	186 (3.1)
Total	7000 (100)	7000 (100)	6000 (100)

2-2- **Stereology**

Sausage and hamburger samples were transferred to the histology laboratory of the Faculty of Veterinary Medicine of Tehran University in the cold chain, and after coding the samples, each sample was flattened according to the Iranian national standard number 6103. Sampling direction of grid plate as cutting guide to obtain systematic uniform random blocks[5] used and a total of 12 blocks were obtained from each sample. Each block in formalin[6] Neutral 10% fixed and then the samples for examination under light microscope[7] They were prepared and blocked in paraffin. Each block covered with paraffin was cut into 6 micrometer thicknesses and a slide was taken from each block and stained with hematoxylin and eosin.[8] (H&E) were stained. To estimate the volume, an optical microscope equipped with a digital camera[9] Was used. Microscopic fields were selected by moving the microscope plate in the x and y directions for a fixed distance using a motorized stage. 36 cross-sections were prepared from each sample and at least 8 images from each section were prepared and analyzed with the lens of 4 optical microscopes. Fractional volume calculation (V_{IN}) meat was estimated using the test point system (pictures 1 and 2) and the following formula:

$$\text{(sample)} \sum P \,/\, \text{(meat)} \sum P = \text{(meat)}\ V_{IN}$$

where (meat) ΣP is the number of points that hit the striated muscle fibers and (sample) ΣP is the number of points that hit the whole sample. probes

[5]- Systematic uniform random sampling

[6]- Merck; Darmstadt, Germany

[7]- CX40; Olympus, Tokyo, Japan

[8]- Hematoxylin and Eosin, Merck; Darmstadt, Germany

[9]- MB-225, Olympus, Tokyo, Japan

Geometry that is necessary for stereological analysis using dedicated software[10] were produced

2-3- Statistical analysis

Using the one-way ANOVA statistical test, the observed percentages of the meat amounts with the actual percentage added to the comparison sample and the ratio of the observed tissue surface and the total surface area for each photo were reported as the mean ± standard deviation and to determine the difference in meaning You have between the actual and the estimated percentage oft-test used. the amount of*P* value (percentage of the measured data in the confidence range of 95% of the total uncertainty) was considered less than 0.05.

3- Results and discussion

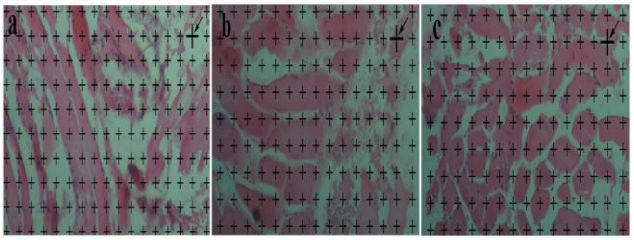
Today, consumers are demanding more than ever for accurate and transparent information about food products, and meat products are no exception. Considering that it is not possible to evaluate the quality of the meat used in the production of meat products by using the usual quality control methods, therefore the structure of the components can be determined by complementary methods such as histological methods. The donor observed the meat product and evaluated it in terms of quality. In addition, its use in completing standards and implementing laws related to meat products is very effective and beneficial. In this regard, stereology is a science that, although it has a long history, has opened its way well in the field of various branches of experimental sciences in recent years, and has been widely used, especially in the field of histology and toxicology. In this study, tissue image analysis was used to evaluate the estimated percentages of meat in meat products.

In the present study, quantitative detection of the amount of meat used in the sampleRaw (hamburger) and heated (sausage) meat products) was investigated. The results of stereology in hamburgers are shown in Table 3 and Figure 1. There is no significant difference between the percentage of estimated meat and the actual amount of meat in different samples of hamburgers (P>0.05). Therefore, the estimated percentages of meat in hamburgers in different samples show the actual amount of meat content in this product.

Table 3 The estimated percentages of meat with different percentages in hamburger.

	Sample 1	Sample 2	Sample 3	Mean± SD	*P*-value
Hamburger 30%	28.3	29.1	26.4	27.9±1.3	0.12
Hamburger 60%	59.4	58.5	59.8	59.2±0.66	0.18
Hamburger 90%	88.7	89.1	85.6	87.8±1.9	0.18

Significance level (*P*-value) calculated for the comparison of the estimated percentages with the real percentages.



[10] - ImageJ; https://imagej.nih.gov

Fig 1 Point system for estimation of volume in different meat percentages of hamburger including 30 % (a), 60% (b) and 90% (c).

Unlike hamburger samples, there is a significant difference between the estimated percentage of meat and the actual percentage of meat in different sausage samples ($P<0.05$) (Table 4 and Figure 2). Therefore, the estimated percentage of meat content in different sausage samples cannot show the actual amount of meat content in this product.

Table 4 The estimated percentages of meat with different percentages in sausage.

	Sample 1	**Sample 2**	**Sample 3**	**Mean± SD**	***P*-value**
Sausage 40 %	14.8	17.3	15.4	15.8±1.3	**0.001**
Sausage 60 %	35.2	30.7	36.1	34±2.8	**0.004**
Sausage 90 %	60.3	64.2	58.4	60.9±2.9	**0.003**

Significance level (*P*-value) calculated for the comparison of the estimated percentages with the real percentages.

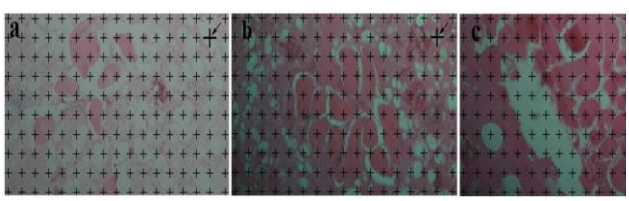


Fig 2 Point system for estimation of volume in different meat percentages of sausage including 40 % (a), 60% (b) and 90% (c).

So far, studies have been conducted in the field of quantitative detection of illegal tissues in meat products using histological tests. The investigation of the effect of the histology method in the quantitative determination of illegal plant and animal content in minced meat by Sadeghinezhad et al. (2016) showed that the estimated percentages of minced meat mixed with 5, 10, 15 and 20 percent soy and sand was, it corresponded with the real values and therefore they suggested the histology method as a suitable method for quantitative and qualitative examination of meat products [27]. Also, the results show that the addition of plant materials to meat products reduces the quality of meat and affects food safety due to the allergenicity of these compounds.

In Tremlova and Starha's study, stereological technique was used to investigate the size and number of chicken bone pieces in 26 samples of poultry meat in products such as sausage, salami, and minced meat [28]. In another study, these two researchers used the histological method to measure bone fragments in 29 samples of different meat products and finally confirmed the obtained results with the help of chemical analyzes [29]. In the study of Lukaskova et al., the efficiency of using the stereological technique in meat products to detect the percentage of wheat protein in the sausage model has been confirmed [30].

In another study, stereological methods were used to identify illegal tissues in meat products and determine the percentage of meat used in minced meat, hamburgers, and sausages collected from brands in the market. Maghami et al. reported that there was a clear difference in the percentage of real meat compared to the percentage on the label, and tissues such as skeletal and smooth muscles, blood vessels, nerve, bone, adipose tissue, glandular tissue, cartilage, bone, tendon, skin , lymphatic tissues and plant materials were observed in these products and also confirmed that stereology is a reliable method to determine and confirm the percentage of meat used in meat products [31].

In general, based on the results, the determined percentage of meat in hamburger in different percentages (30, 60 and 90%) has no significant difference with the actual percentage.$P>0.05$) and the stereological test has been able to accurately detect the amount of meat added in this meat product, but in the case of sausage, a significant difference was observed between the estimated percentage of meat and the actual percentage of meat consumed.$P<0.05$). In a study, the percentage of meat used in the model of raw meat products (barbecue) and heated (sausage) was investigated, and as in the present study, there was a significant difference between different percentages of sausage and the actual percentage. that in the case of kebab bites, the determined amount was not significantly different from the actual amount, so histological methods are able to detect the percentage of added meat [32], which can be the result of tissue damage caused by heat application during product processing and from loss of its structural details [32]. However, unlike the present study, in Maghami et al.'s research, the stereological results of a standard sample with 65% meat showed a very slight difference with the estimated percentage of meat (63-

64%), although this study was only conducted on a 65% sausage sample [31].

In addition to these studies, it has been shown that histological tests are an effective method for evaluating the percentage of unauthorized tissues (bones and skin) in raw and heated meat products (sausages and kebabs), however, quantitative evaluation Additive tissues in raw products have been easier than heated products, and due to the resistance of bone compared to tissue preparation stages, it has been more possible to evaluate the percentage of added bone [33].

4- General conclusion

The results of this study showed that the stereological method can be used to estimate the amount of meat used in raw meat products, but it does not have the necessary accuracy in the case of heated meat products. Therefore, the stereological method can be used as a complementary and reliable method to determine the percentage of meat added in raw meat products (hamburgers) and compare it with labeling.

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6- Ethical considerations of the plan

The researcher's declaration form and ethics assessment in the research was completed and announced, and based on this, the ethics committee of the Institute of Nutritional Research and Food Industries of Iran issued the ethics code number IR.SBMU.nnftri.Rec.1399.022 on 05/05/2019. did

7- Resources

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چکیده

برچسب‌گذاری دقیق مواد غذایی برای تجارتی منصفانه و توانمندسازی مصرف کنندگان جهت داشتن انتخابی آگاهانه از اهمیت بالایی برخوردار است. صحه‌گذاری میزان گوشت به‌کار رفته در تهیه فرآورده‌های گوشتی از مهم‌ترین فاکتورهای ارزیابی این فرآورده‌ها می‌باشد. لذا هدف از این تحقیق، کارایی آزمون استریولوژی در تشخیص مقدار گوشت بکار رفته در فرآورده‌های گوشتی خام (همبرگر) و حرارت دیده (سوسیس) بود. سه نمونه همبرگر (حاوی ۳۰، ۶۰ و ۹۰ درصد گوشت) به عنوان فرآورده گوشتی خام و سوسیس (حاوی ۴۰، ۶۰ و ۹۰ درصد گوشت) به عنوان فرآورده گوشتی حرارت دیده تهیه شدند. نمونه‌برداری Fractionator برای به دست آوردن بلوک‌های تصادفی یکنواخت سیستماتیک[11] استفاده شد. در مجموع از هر نمونه ۱۲ بلوک به دست آمد و هر بلوک در فرمالین خنثی ۱۰ درصد تثبیت و در پارافین بلوک‌گیری شد. نمونه‌های به دست آمده، پس از تهیه مقاطع بافتی و رنگ‌آمیزی با هماتوکسیلین و ائوزین (H&E)، توسط میکروسکوپ نوری مورد مطالعه قرار گرفتند. داده‌ها با روش آماری one-way ANOVA آنالیز و جهت مقایسه تفاوت‌های آماری بین گروه‌ها، از t-test استفاده شد. آنالیز استریولوژی نشان داد که درصد تعیین شده گوشت در همبرگر در درصدهای مختلف با درصد واقعی دارای اختلاف معنی‌داری نبوده، اما در مورد سوسیس بین درصد تخمین زده و درصد واقعی گوشت اختلاف معنی‌دار بوده است. روش استریولوژی می‌تواند روش مکمل مناسبی برای تشخیص مقدار گوشت اضافه شده به فرآورده‌های گوشتی خام باشد.

[11]- Systematic uniform random sampling