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Determination of the Content of Bone Inclusions in Multicomponent Meat Products

Çok Bileşenli Et Ürünlerinde Kemik Kalıntı İçeriğinin Belirlenmesi

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ABSTRACT

A specific criterion for the use of mechanically deboned poultry meat in sausages and other meat products is the presence of bone inclusions. Known methods for determining bone inclusions in mechanically deboned meat do not provide the required accuracy for meat products as they contain spices, starch, dietary fiber, and other ingredients. These methods are too complicated and expensive. The purpose of this study is to develop a simple and accurate method for the gravimetric determination of bone inclusions in meat products. To reach the purpose the known methods of quantitative analysis of bone inclusions in meat products and the repeatability and reproducibility of the said methods were improved. The results of the research consist in the developed gravimetric method for determining bone inclusions in meat products and its metrological acceptance duly proven. Samples of meat products are treated with 2% alkali solution (KOH) for complete dissolution of proteins and fat. The samples are then treated with a concentrated solution of zinc chloride ($ZnCl_2$). This reagent suspends food moisture retaining additives, such as starch and fibers. As a result, their density is higher than that of spices, but lower than that of bone inclusions. When the resulting suspension is allowed to stand, the spices float to the surface, and the bone inclusions precipitate. The precipitate is washed, dried, and the mass of bone inclusions is determined. Experiments showed that the relative total error in measurements of bone inclusions increases with a decrease in their content in meat products and does not exceed 45% for the official norms of Ukraine ranging from 0.1% to 0.2% of bone inclusions. The conclusion can be drawn that the method for determining the mass fraction of bone inclusions in multicomponent meat products, developed and approved at the official level for analytical practice, expands the possibility of determining the degree of falsification of these products at the level of qualitative and quantitative expertise in accordance with the normative documents in force.

Keywords: Bone inclusions, chemical reagent, gravimetric analysis, mechanically deboned poultry meat

ÖZ

Sosislerde ve diğer et ürünlerinde mekanik olarak kemiği çıkarılmış kanatlı etinin kullanımı için özel bir kriter, kemik kalıntılarının varlığıdır. Mekanik olarak kemiği çıkarılmış ette kemik kalıntılarını belirlemek için bilinen yöntemler, baharat, nişasta, diyet lifi ve diğer bileşenleri içerdiklerinden et ürünleri için gerekli doğruluğu sağlama ve bu yöntemler çok karmaşık ve pahalıdır. Bu çalışmanın amacı, et ürünlerindeki kemik kapanımlarının gravimetrik tayini için basit ve doğru bir yöntem geliştirmektir. Amaca ulaşmak için et ürünlerindeki kemik kapanımlarının bilinen kantitatif analiz yöntemleri ve söz konusu yöntemlerin tekrarlanabilirliği ve yeniden üretilebilirliği geliştirildi. Araştırmanın sonuçları, et ürünlerinde kemik kapanımlarını belirlemek için geliştirilmiş gravimetrik yöntem ve bunun metrolojik kabulü usulüne uygun olarak kanıtlanmıştır. Et ürünleri örnekleri, proteinlerin ve yağın tamamen çözünmesi için %2 alkali solüsyon (KOH) ile işlenir. Numuneler daha sonra konsantre bir çinko klorür ($ZnCl_2$) çözeltisi ile işlendi. Bu reaktif, nişasta ve lifler gibi gıda nemi tutucu katkı maddelerini askıya alınır. Sonuç olarak, yoğunlukları baharatlardan daha yüksek, ancak kemik kapanımlarından daha düşüktür. Ortaya çıkan süspansiyonun beklemesine izin verildiğinde, baharatlar yüzeye çıkar ve kemik kalıntıları çökelir. Çökelti yıkanır, kurutulur ve kemik kapanımlarının kütlesi belirlendi. Deneyler, kemik kapanımlarının ölçümlerindeki nispi toplam hatanın et ürünlerindeki içeriklerinde bir azalma ile arttığını ve Ukrayna'nın resmi normları için kemik kapanımlarının %0,1 ila %0,2'si için %45'i geçmediğini gösterdi. Analitik uygulama için resmi düzeyde geliştirilen ve onaylanan çok bileşenli et ürünlerinde kemik kapanımlarının kütle fraksiyonunu belirleme yönteminin, bu ürünlerin tahrif derecesini niteliksel düzeyde belirleme olasılığını genişlettiği sonucuna varılabilir.

Anahtar Kelimeler: Kemik kalıntıları, kimyasal reaktif, gravimetrik analiz, mekanik olarak kemiği çıkarılmış kanatlı eti

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INTRODUCTION

Globally, multiple methods for analyzing bone inclusions in meat mechanically separated from bones (chemical, biochemical, and others) are used[1-6], which do not cover the field of application with such a complex multicomponent system as meat products. Literature data on a number of the claimed methods (classical and innovative)[7-12] used for this purpose are limited and inaccessible for a number of laboratories; therefore, the said publications were not taken for discussion here-

after. However, on the basis of our own research results regarding the direct application of the classical method for determining the mass fraction of bone inclusions in mechanically deboned meat[1] found that this method does not provide the required measurement accuracy.[13] The quantitative analysis of these inclusions in multicomponent meat products (sausages, canned foods, convenience foods), which are often falsified by the unauthorized replacement of expensive raw meats of hand deboning with cheap mechanically deboned meat was not accurate. Yet, considering the availability and simplicity of its implementation, as well as the importance of and urgency in identifying falsification of meat products, it was decided to conduct a set of theoretical and experimental studies aimed at improving the gravimetric sedimentation method with the aim of using it to analyze bone inclusions in multicomponent meat products. Therefore, we believe our study can have important regulatory and legal significance for protecting the interests of consumers of meat products in addition to scientific and practical applications.

MATERIAL AND METHODS

Considering the multifactorial nature of the research, in the context of the general experimental plan, a certain sequence of implementation of its main stages was envisaged: conducting research to clarify the significant physical and chemical characteristics of the food ingredients and chemical reagents involved in the research → production of control and prototypes of meat products according to an individual recipe → separation of fine mincing samples → treatment of each sample with an alkaline solution → sedimentation and decantation of the resulting solutions → treatment of sediments with a chemical reagent that dissolves starch and fibers, having a specific gravity less than the specific gravity of the bone residue and greater than that of spices → settling solutions → decantation of the resulting suspensions → drying the precipitate to constant weight and calculation of the content of bone inclusions in each sample.

Methods

Isolation of bone inclusions from mechanically deboned poultry meat and determination of their mass was carried out according to.[1]

To improve the test integrity, the main chemical reagents for research were prepared manually as below:

- To prepare a solution of potassium hydroxide with a mass fraction of 2%, a weighed portion of potassium hydroxide weighing (20.0 ± 0.5) g was dissolved in 980 cm^3 of distilled water, cooled, and stored in a closed glass or polyethylene container. Shelf-life of potassium hydroxide solution at room temperature is no more than 60 days.
- The preparation of a concentrated solution of zinc chloride ($ZnCl_2$) was carried out as follows: a sample of $ZnCl_2$ of 280.0 g to 300.0 g (depending on its water content) was dissolved in 100.0 cm^3 of distilled water, with continuous cooling of the dishes under cold running water. The solution was allowed to stand for a day (a saturated solution is characterized by the presence of a precipitate of undissolved $ZnCl_2$) and decanted. The decant was diluted with distilled water sequentially as follows: first, a saturated solution of $ZnCl_2$ was poured into a measuring cylinder, then a hydrometer of the appropriate measurement range was placed there; and with continuous stirring and gradually adding distilled water, the solution was brought to a concentration γ_p from 1.65 g/cm^3 to 1.8 g/cm^3. The resulting solution was filtered through funnels for filtering – glass porous filters POR160 or POR250, or through gauze folded four times. The finished filtered concentrated solution was stored at room temperature in a closed glass container for no more than 90 days.

The determination of the mass fraction of bone inclusions in meat products was carried out in the following sequence: a portion of each of the samples of meat products weighing 50 g was placed in a beaker of volume 250 cm^3, then 100 cm^3 of a 2% KOH solution was added, thoroughly mixed, and heated in a water bath to a temperature of about 100 °C repeating the procedure multiple times, until the muscle, connective, and fat fractions were completely dissolved. The decant was removed, and the resulting amorphous gelatinous precipitate, washed with distilled water, was treated with a concentrated solution of $ZnCl_2$, stirring continuously, to dissolve starch and/or fiber and to separate the dispersed phase: spices (the light fraction floated to the surface of the $ZnCl_2$ solution, whereas heavier bone inclusions precipitated). After washing with distilled water (until the $ZnCl_2$ solution was completely removed), the bone residue was filtered through a paper filter, dried to constant weight, weighed, and the mass fraction of bone inclusions calculated.

Measurements of the weights of the samples were carried out using a balance AXIS AD 50 with a measurement error of 0.001 g.

The concentration of solutions was determined using a general-purpose hydrometer AON-1, length 170.0 mm, diameter 20.0 mm, error ± 1.0 kg/m^3, measurement range from 1.60 g/cm^3 to 1.66 g/cm^3; from 1.66 g/cm^3 to 1.72 g/cm^3; from 1.72 g/cm^3 to 1.78 g/cm^3; and from 1.78 g/cm^3 to 1.84 g/cm^3.

Calculations of the metrological characteristics of the method for analyzing bone inclusions in multicomponent meat products were carried out according to DSTU-N RMG 61:2006[14] using a specially created software. The evaluation factors for the correctness of the analysis method were:

Qualitative characteristics of the analysis method - accuracy, correctness, and precision (repeatability and reproducibility) of the analysis

Quantitative characteristics of the analysis method - indicators of accuracy, correctness, and precision (repeatability and reproducibility) of the analysis.

The experiments were carried out under the following conditions: room air temperature 20°C ± 5°C, atmospheric pressure 84.0–106.7 kPa, relative air humidity 30.0%–80.0%; AC frequency 50 Hz ± 1 Hz; voltage in the electrical network 220 V ± 10 V.

The procedure for reproducibility determination of indicators of accuracy, correctness, and precision of measurements was carried out in 5 laboratories of Ukraine, duly accredited for technical competence and independence.

Materials

Based on the analysis of the data set forth in[15,16], in the industry standard of Ukraine[17], in the US Code of Federal Regulations[18], as well as on the results of research[19]; a limitation of the range of measurements of the mass fraction of bone inclusions in meat products was substantiated when carrying out experiments in the range of 0.02%–1.5%. The correctness of the decision is confirmed by the fact that the above range covers the limitation of the content of bone inclusions in sausages, depending on their grade, in the range of 0.1%–0.2%, provided for by the National Standards of Ukraine.[20,21] To improve the measurement accuracy and estimate

the boundaries of possible errors in this case, the total measurement range was divided into a number of smaller ranges.

According to an individual formulation, 92 samples of meat products were taken from the same batch of hand deboned finely chopped meat. The said samples differed in their formulations (Figure 1). The order of performing technological operations is set out in Figure 1.

The program for calculating measurement errors provided for 2 parallel options:

- processing of the results of measurements of bone inclusions in samples of meat products containing only bone inclusions (Series 1 – control);
- processing of the results of measurements of bone inclusions in samples of meat products containing spices (Series 2), spices and starch (Series 3), and spices and fibers (Series 4) along with bone inclusions.

Statistical Analysis

The study was replicated three times and statistical analysis was performed using Microsoft Excel 2010 and Statistica-6.0 software. Means and standard deviations were calculated. In parallel, a Student test was performed to identify differences between the average values of the test parameters. The differences between the values of the said parameters were found to be reliable if the probability value was greater or equal to 95% ($P \leq .5$). Metrological characteristics of the method for analyzing bone inclusions in multicomponent meat products were carried out according to DSTU-N RMG 61:2006[14] using specially designed software. The evaluation factors for the accuracy of the analysis method were characteristics of the analysis method - trueness and precision (repeatability and reproducibility).

Adding ingredients to the chopped meat samples 1 – 4, % by mass

Sample 1:	Sample 2:	Sample 3:	Sample 4:
- salt – 0 - spices – 0	- salt – 2.0 - spices – 0.2	- salt – 2.0 - spices – 0.2 - starch – 1.0	- salt – 2.0 - spices – 0.2 - fibers – 1.0

↓ ↓ ↓ ↓

Partition of the samples 1–4 into 23 sub-samples 1-1 to 4-23, each of them being added a specified quantity of bone inclusions, % by mass:

↓ ↓ ↓ ↓

1	%	2	%	3	%	4	%
1-1	0.02	2-1	0.02	3-1	0.02	4-1	0.02
1-2	0.05	2-2	0.05	3-2	0.05	4-2	0.05
1-3	0.08	2-3	0.08	3-3	0.08	4-3	0.08
1-4	0.10	2-4	0.10	3-4	0.10	4-4	0.10
1-5	0.15	2-5	0.15	3-5	0.15	4-5	0.15
1-6	0.20	2-6	0.20	3-6	0.20	4-6	0.20
1-7	0.25	2-7	0.25	3-7	0.25	4-7	0.25
1-8	0.30	2-8	0.30	3-8	0.30	4-8	0.30
1-9	0.35	2-9	0.35	3-9	0.35	4-9	0.35
1-10	0.40	2-10	0.40	3-10	0.40	4-10	0.40
1-11	0.45	2-11	0.45	3-11	0.45	4-11	0.45
1-12	0.50	2-12	0.50	3-12	0.50	4-12	0.50
1-13	0.55	2-13	0.55	3-13	0.55	4-13	0.55
1-14	0.60	2-14	0.60	3-14	0.60	4-14	0.60
1-15	0.70	2-15	0.70	3-15	0.70	4-15	0.70
1-16	0.80	2-16	0.80	3-16	0.80	4-16	0.80
1-17	0.90	2-17	0.90	3-17	0.90	4-17	0.90
1-18	1.0	2-18	1.0	3-18	1.0	4-18	1.0
1-19	1.1	2-19	1.1	3-19	1.1	4-19	1.1
1-20	1.2	2-20	1.2	3-20	1.2	4-20	1.2
1-21	1.3	2-21	1.3	3-21	1.3	4-21	1.3
1-22	1.4	2-22	1.4	3-22	1.4	4-22	1.4
1-23	1.5	2-23	1.5	3-23	1.5	4-23	1.5

Figure 1. The procedure for performing technological operations when manufacturing the samples of meat products.

RESULTS

According to the experimental plan, significant physical and chemical characteristics of the ingredients used in research as bone inclusions and spices were analytically and experimentally determined, as well as the effectiveness of the chemical reagents used was established:

- The range of changes in the specific gravity (γ_b) of bone inclusions isolated from the meat of mechanical deboning of carcasses and/or parts of carcasses of broilers 1.75 g/cm^3 ≤ γ_b ≤ 2.0 g/cm^3
- The range of variation in the specific gravity (γ_{sp}) of spices used in the technology of meat products 0.5 g/cm^3 ≤ γ_{sp} ≤ 0.62 g/cm^3
- One of the safest and most effective chemical reagents dissolving starch and fiber is a concentrated solution of $ZnCl_2$, the concentration of which determined its specific gravity in the range of 1.62 g/cm^3 ≤ (γ_r) ≤ 1.7 g/cm^3, which is more than the specific gravity of spices, but less than that of the bone residue
- For a more complete dissolution of protein and fat in samples of meat products, it is advisable to use a solution KOH with a concentration of 2%.

It was also determined that approximately 70 cm^3 of a concentrated solution of $ZnCl_2$ is consumed per 1 parallel portion of the analyzed sample.

The results of comparative measurements obtained in each of 5 participating independent laboratories under all conditions stipulated by the developed gravimetric analysis of bone inclusions in samples of meat products of similar composition and identical measurement ranges were in agreement with each other at a fairly acceptable level – the discrepancy between the accuracy indicators was no more than 0.2%.[19]

The averaged results of the carried out intra- and interlaboratory measurements and calculations of metrological indicators within the boundaries of each accepted range at a confidence level of $P = .95$ are presented in Table 1.

For admission of the method for measuring the mass fraction of bone inclusions in multicomponent meat products to analytical practice, in accordance with the requirements of GOST 8.010-99[22], procedures were carried out to generalize, systematize, and mathematically process the corresponding metrological indicators presented in Table 1.

As a result of this work, the metrological characteristics of this method were obtained depending on the measurement range at a confidence level of $P = .95$, set forth in[23] and presented in Table 2.

From the analysis of the data given in Table 2, it follows that the relative total error in measurements of bone inclusions increases with a decrease in their content in meat products and does not exceed 45% for conditions that in Ukraine provide for limiting their content in these products within 0.1% up to 0.2%.[20,21]

The measurement results served as the basis for certification and official admission of this gravimetric method to analytical practice, which makes it possible to separate bone inclusions from accompanying impurities and fairly reliably measure the mass fraction of bone inclusions in multicomponent meat products.[24]

Table 1. The results of evaluating the metrological indicators of measuring the content of bone inclusions in samples of meat products depending on the measurement range at a confidence level of $P = .95$.

Ranges with limit values mass fraction of bone inclusions, %	Metrological indices, %					
	Repeatability index as standard deviation, σrm	Repeatability limit, rnm	Reproducibility index as standard deviation, σRm	Reproducibility limit, Rm	Correctness index, ± Δcm	Accuracy index, ± Δm Samples 1 (control) and 2
(0.02 ≤ X ≤ 0.05)	0.0026	0.0087	0.0020	0.0055	0.0246	0.025
(0.05 ≤ X ≤ 0.1)	0.0045	0.0150	0.0039	0.0109	0.0557	0.056
(0.1 ≤ X ≤ 0.15)	0.0046	0.0153	0.0040	00111	0.0885	0.089
(0.15 ≤ X ≤ 0.2)	0.0042	0.0139	0.0141	0.0390	0.1028	0.106
(0.2 ≤ X ≤ 0.3)	0.0167	0.0551	0.0260	0.0721	0.1236	0.134
(0.3 ≤ X ≤ 0.6)	0.0072	0.0237	0.0066	0.0182	0.1578	0.264
(0.6 ≤ X ≤ 1.5)	0.0104	0.0344	0.0143	0.0395	0.2629	0.251
Samples 3 and 4						
(0.02 ≤ X ≤ 0.05)	0.0053	0.0175	0.0042	0.0117	0.0251	0.026
(0.05 ≤ X ≤ 0.1)	0.0067	0.0222	0.0088	0.0243	0.0564	0.059
(0.1 ≤ X ≤ 0.15)	0.0084	0.0279	0.0136	0.0377	0.0900	0.094
(0.15 ≤ X ≤ 0.2)	0.0132	0.0436	0.0150	0.0415	0.1027	0.107
(0.2 ≤ X ≤ 0.3)	0.0080	0.0265	0.0353	0.0976	0.1272	0.145
(0.3 ≤ X ≤ 0.6)	0.0101	0.0333	0.0465	0.1289	0.1539	0.179
(0.6 ≤ X ≤ 1.5)	0.0325	0.1075	0.0762	0.2111	0.2825	0.320

Table 2. Metrological characteristics of the method for measuring bone inclusions in meat products depending on the measurement range at a confidence level of P = .95.

Mass fraction of bone inclusions: measurement range, %	Limit of the relative total error, ±δ, % ($P = .95$)	Operative control criteria:		
		repeatability r, % ($P = .95$; n = 2)	reproducibility, R, % ($P = .95$; n = 2)	accuracy, K, % ($P = .95$)
From 0.05 to 0.30 inclusive	45	18	23	45
Over 0.30 to 1.0 inclusive	30	12	15	30
Over 1.0 to 1.5 inclusive	24	10	12	24

DISCUSSION

On the basis of results of this study, we believe that the gravimetric method for determining the mass fraction of bone inclusions in multicomponent meat products developed by us is quite accurate, simple, and accessible for use by a wide range of research laboratories with various levels of equipment. Such an estimate is substantiated not only in the works of the authors of this article[5,19,23,25]; but also the works of other authors published in different years describe the use of a fairly common chemical method with the determination of calcium (Ca^{2+}) ions, which showed sufficient accuracy.[3,12,26,27] However, this method is associated with the need to use atomic absorption spectroscopy, which limits its implementation in practical conditions.[26,27] In[26], there are concerns about the limited use of the chemical method with the determination of Ca^{2+} ions in relation to meat products with dairy ingredients in the formulations (powdered milk, whey, etc.), which are rich sources of calcium. These concerns are justified as dairy ingredients are used in dietary meat products[28,29]; however, for cheap mince products containing mechanically deboned meat, the use of dairy ingredients is not typical.

In general, the error of the known indirect method for the quantitative analysis of bone inclusions in meat products by the content of Ca in them[12] is primarily owing to the quality of the meat desinewing, that is, it directly depends on the content of connective tissue in the meat. In addition, based on the results of studies[1] of the relationship "Ca content - content of bone inclusions," in the mechanically deboned meat by plasma atomic absorption spectrometry and confirmed by gravimetric analysis of bone inclusions, significant differences were revealed between the obtained conversion formula for these components and the conversion formula given in the method used by the International Association of Official Analytical Chemists AOAC 983.19.[30] The result of comparative calculations using these formulae for the mass fraction of bone inclusions at the same Ca content was 2 times less than that of.[1] The same researchers found that in the range of Ca content in mechanically deboned meat from 0.2% to 0.4%, the relative error in determining Ca by plasma atomic absorption spectrometry is 28%–29%. Hypothetically, the measurement error in the mass fraction of bone inclusions in meat products by this method can increase because of their complex composition. Thus, caution is required in both the accuracy of the quantitative analysis of bone inclusions in meat products using the "Ca content - bone inclusion content" relationship and the norms of bone inclusions content in official documents.[5,19]

Another known and well-established histological method for the quantitative calculation of bone inclusions in a mince product is based on a detailed study of histological preparations and morphometric analysis of the bones. This method, although using an image analysis system, is a time-consuming and complex process; and identifying and accurately determining the boundaries of the measured bone particles is practically impossible without manual correction of the computer actions by a highly experienced histologist.[2,26,31-33] Researchers[10] have shown that the accuracy of measuring the amount of ingredients in meat products using this method is negatively affected by the nonlinear correlation between the volumetric and mass content data of various inclusions.

We are fully aware of the possibility and expediency of using other, high-tech methods for determining the content of mechanically deboned meat in meat products under laboratory conditions. In particular, the constant technical improvement of the production of this type of meat raw materials already often limits the use of traditional methods for determining the quality parameters of meat products.[26,34] High-tech methods for determining the content of mechanically deboned meat in meat products include methods based on irradiation coupled with electronic spin resonance[35,36] and the evaluation of radiostrontium levels[37], laser-induced breakdown spectroscopy[38], X-ray application[4,7], Raman spectroscopy[39,40], innovative ultrasound method[41], computed tomography[26], and so on. In connection with the rapid development of food science and the corresponding innovative technologies of meat products, it is possible to foresee the introduction of novelty formulations using mechanically deboned meat, control of which requires both new instrumental methods and traditional methods, in particular the improved gravimetric method proposed by us for determining bone inclusions in meat products.

In conclusion, from the analysis of the data obtained, it follows that the method for determining the mass fraction of bone inclusions in multicomponent meat products, developed and approved at the official level for analytical practice, deserves attention as it expands the possibility of determining the degree of falsification of these products at the level of qualitative and quantitative expertise in accordance with the requirements normative documentation and legislation of Ukraine.[42]

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