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To cite this article: A Tasić et al 2017 IOP Conf. Ser.: Earth Environ. Sci. 85 012056

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Determination of calcium content in mechanically separated meat

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Abstract. One of the most important control parameters for mechanically separated meat (MSM) is the calcium content, which indicates the presence of bone. The aim of this study was to examine the calcium content in three types of locally-produced MSM from differing animal species. Calcium was quantified in the examined samples of MSM, using indirect titration, and three differing napthol indicators (eriochrome black T, hydroxy naphthol blue and hydroxy naphthol blue disodium salt) were used to track the end point of titration. Each of the three different indicators showed a recovery of more than 90% when examining a certified reference material, and so all three could be successfully used to determine the amount of calcium in MSM. The calcium levels in the MSM samples examined were in the range of 0.050 to 0.100%, with that of beef MSM being the highest. Using a previously published binary logistic regression model for classifying meats as MSM, this corresponded to probabilities of between 0.69 and 0.94, confirming that the three types of MSM examined could indeed be properly classified as MSM.

1. Introduction

Mechanically separated meat (MSM) or mechanically recovered meat are generic terms used to describe residual meat that is separated using machinery from animal bones or carcasses from which most of the meat has previously been removed manually. Mechanical separation involves grinding meat and bones together, as well as passing the mixture through micro sieves or slits to remove bone particles [1,2]. This process enables the use of most of the meat remaining on the bones, which would otherwise be difficult or unprofitable to use. The resulting MSM has the appearance of finely chopped meat. Lipid oxidation is a major factor in reducing the quality and acceptability of meat products, those MSM with a high degree of unsaturation and fat content are more susceptible to lipid oxidation [3]. Meat quality changes can be also perceived by changes in taste, color, texture, nutritional value and the production of potentially toxic compounds [4]. While the amount of calcium is a quality parameter that does not change over time, but the amount above the permissible limit is forbidden in MSM. The chemical composition of MSM is variable due to the natural variation within and between animal species, diet regimens, age of slaughtered animals, meat pieces, bones, pre-treatment of bone (freezing), and machine type in the production process [5]. Poor installation of equipment can also lead to the production of an unacceptable particle size, which affects the quality of products that contain MSM. During the mechanical separation of the bones from the meat, it is inevitable that some bone particles pass into the MSM. These particles contain high levels of calcium, which is reflected in

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higher calcium levels in MSM than in the meat [6]. Calcium increases during calcification processes, it varies with bone type, as well as with species, feeding or age of the slaughtered animals.

Determination of calcium content in MSM is an indicator of the amount of bone in the product, and can be used to control the yield of mechanical separation processes [7]. Calcium makes up about 37% of the content of bone ash, so that the content of calcium and ash can be used equally to estimate bone content. The calcium content is frequently used as one of the criteria to identify MSM, although the starting material can also affect the amount of calcium in MSM [8]. Testing and control of MSM is important because the product is used in the composition of some sausages, salami and dry soups, in amounts up to about 24% [11]. With increasing use of MSM in such products, besides standard determination of nutritional value, it is also important to control the amount of calcium present.

Chemical methods for the determination of mineral matter or ash in MSM are indirect methods for determining the percentage of bone in the product, but at the same time they are used as a parameter for recognizing different types of MSM. Different methods are used to determine calcium. Calcium is determined using the atomic absorption spectrophotometry method (ISO 6869: 2000) and the AOAC method, using inductively coupled plasma-optic emission spectrometry [9]. Determination of calcium in a food matrix can also be performed using a standard titration method. The method especially standardised for the determination of calcium in MSM is the AOAC Official Method [10]. The advantages of this method are that it is simple and quick, but also it does not require the use of expensive instrumentation.

The aim of this study was to determine the amount of calcium present in different types of locallyproduced MSM using the AOAC titration method [10] and compare the results with a previouslypublished regression analysis for classifying product as MSM [7]. In addition, three different napthol indicators were used to determine the calcium content of a reference MSM.

2. Materials and Methods

The amount of calcium was determined in MSMs (beef, chicken and turkey meat) which had been produced for further use in food products in Serbia. The certified reference material used was beef MSM (DRRR, Germany). All chemicals used were p.a. purities. Indirect titration with the disodium salt of ethylenediaminetetraacetic acid was used, and the titrations were conducted using a calcium carbonate solution. Amounts of MSM (10 g) were digested in a solution of hydrochloric acid, the solution cooled and filtered through filter paper. The aliquoted digests were diluted volumetrically with distilled water and potassium hydroxide and potassium cyanide were added; the final pH was 12.5 ± 0.2 . The solutions were titrated, with the titration end point being the appearance of violet color for the indicator hydroxyinatol blue, but two other indicators were used in parallel: disodium hydroxyphenol blue (end point a purple colour) and eriochrome black T (end point a red colour).

The limit of detection (LOD) and limit of quantification (LOQ) were calculated from measurements using blank samples. Standard deviations were calculated using ten independent measurements of blank samples. LOD was defined as $3\times$ the standard deviation. The LOQ was defined as $10\times$ the standard deviation.

3. Results and Discussion

Table 1 shows the mean calcium content of different MSM matrices after titration with different indicators.

Table 1. Calcium content (mg/kg) measured in different types of MSM (10 replications)					
Replication	Beef	Chicken	Turkey		
1					
1	890	750	680		
2	995	780	620		
3	895	680	690		
4	890	700	700		

59th International Meat Industry Conference MEATCON2017

IOP Conf. Series: Earth and Environmental Science 85 (2017) 012056

5	1000	760	850	
6	990	900	860	
7	1000	790	800	
8	980	500	780	
9	990	550	760	
10	900	800	770	
Mean value (mg/kg)	953	721	790	

The mean calcium content for beef MSM was 953 mg/kg, or in the range from 0.089 to 0.100%, and this was the highest calcium level detected in the MSMs examined. The quantities of calcium measured in chicken and turkey MSM ranged from 0.05 to 0.09%; and 0.062 to 0.086%, respectively.

In order to validate the method and determine the recovery, the calcium content in the certified reference material was determined using the three different indicators, and the results obtained are shown in Table 2. The certified reference material had a calcium content of 830 mg/kg (ppm), as stated by the manufacturer. Among the three indicators, Eriochrome black T indicator produced the greatest accuracy when used as a titration indicator (Table 2). However, it should be noted the pH range of this indicator is 7.5 to 10.5, which is not entirely suitable for this analysis. The disodium salt of hydroxy naphthol blue, on the other hand, more satisfies the pH range for the determination, i.e. the pH range of this indicator is from 12 to 13. In spite of that, this indicator recovered slightly lower calcium levels in the reference material (Table 2).

DIASES						
Replication	Eriochrome black T	Hydroxy naphthol	Hydroxy naphthol blue			
		blue	disodium salt			
1	850	850	820			
2	860	870	855			
3	820	830	890			
4	845	880	845			
5	865	900	865			
6	870	840	865			
7	865	880	870			
8	885	910	890			
9	835	860	855			
10	830	820	845			
Mean value	853	864	860			
(mg/kg)						
Standard deviation	20.17	29.51	21.21			
(mg/kg)						
RSD, %	2.37	3.42	2.47			
Bias, %	2.77	4.10	3.61			

Table 2. Calcium levels (mg/kg) in certified reference material detected by titration using three different indicators, and calculated means, standard deviations, relative standard deviations (RSD) and

The indicators used in the study are all naphthols, and showed good agreement and high accuracy in the determination of calcium (Table 2). Recoveries were greater than 90%, which confirms that all three tested indicators could be successfully used to determine the amount of calcium in MSM.

It is important to emphasize that pH has a high impact on the titration reaction and the formation of a complex compound. Also, cyanide ions that mask the presence of other ions in solution and enable better determination are of great importance in determining calcium levels.

The LOD for any analytical procedure, the point at which analysis is just feasible, is determined by a statistical approach based on measuring replicate blank (negative) samples or by an empirical approach, consisting of measuring progressively more dilute concentrations of analyte. The LOQ, or concentration at which quantitative results can be reported with a high degree of confidence, is likewise determined by either approach. Table 3 shows the values obtained for the detection limit and limit of quantification using the blank sample and all three types of indicators.

Replication	Eriochrome black T	Hydroxy naphthol blue	Hydroxy naphthol blue disodium salt
1	8	24	12
2	16	35	15
3	0	12	30
4	24	16	25
5	20	18	36
6	10	20	24
7	12	22	8
8	22	30	16
9	23	35	26
10	28	10	14
Mean value (mg/kg)	16.3	22.2	20.6
Standard deviation (mg/kg)	8.69	8.85	8.91
LOD (mg/kg)	26.07	26.55	26.73
LOQ (mg/kg)	86.90	88.50	89.10

Table 3. Calcium levels (mg/kg) in blank material detected by titration using three different indicators, and calculated means, standard deviations, LODs and LOQs

In the EU, the maximum calcium level for the so-called low pressure MSM is 100 mg/100 g (1000 ppm). Therefore, machinery should be adjusted so as not to exceed this limit. In addition to the percentage of calcium, bone particles and their size are also of great importance, because large particles might cause a gritty texture and potential dental problems. Therefore, bone particle size is regulated in places like the United States, where 90% of the bone particles cannot exceed 0.5 mm and no particle should be larger than 0.85 mm. A high bone content, such as we detected in our beef MSM, means that the pressure used in the deboning process was too high or that the meat-to-bone ratio was too low.

The European Food Safety Authority (EFSA, Italy) conducted a binary logistic regression analysis to identify the probability of a product being classified as MSM based on calcium content, among other things. This analysis showed calcium was the most appropriate indicator for classifying a product as MSM [7]. According to that model, a calcium content corresponding to probabilities from 0.05 to 0.99 qualify a product to be classified as MSM [7]. Our results for calcium content in the examined MSM were in the range of 50 to 100 mg/100 g, and from the binary logistic regression model, this corresponds to probabilities of between 0.69 to 0.94, indicating that the products should be classified as MSM. This confirms that the three types of MSM examined can indeed be classified as MSM, because they were in the given range of probability of the model.

4. Conclusion

This work showed that all three types of tested indicators can be used during the examination of the presence of calcium in MSM. The highest accuracy was obtained using the Eriochrome black T indicator, and it achieved the smallest LOD and LOQ among the three indicators. It is important to note that the detection limits and quantification limits for calcium testing obtained by using the different indicators did not differ much. From the presented results obtained by determining the presence of calcium in commercial MSM, it can be concluded that all examined MSM products had a calcium content below the limit prescribed by Serbian legislation.

Acknowledgment

This paper was supported by Ministry of Education, Science and Technological Development, Republic of Serbia, through the funding of Project No III 46009.

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