Innovative approaches for identifying a mechanically separated meat: evaluation of radiostrontium levels and development of a new tool of investigation

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Revised: 25 June 2019 / Accepted: 29 August 2019 / Published online: 3 September 2019
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Abstract Due to food safety concerns, the European Food Safety Authority (EFSA) asked to identify useful parameters for identifying the mechanically separated meat (MSM). In this work, the ^{90}Sr levels of MSM products were assessed for evaluating the suitability of this parameter for MSM identification. Indeed, this contaminant may accumulate in animal bones and bone fragments may be present in MSM. One hundred samples of fresh meats and meat products composed of different MSM percentages and without MSM were analyzed by ultra-low-level liquid scintillation counting. The ^{90}Sr activity concentrations detected in meat products with high percentage of MSM were significantly higher ($p \geq 0.05$) (0.099 Bq kg^{-1}) than those of fresh meats (0.024 Bq kg^{-1}) and meat products containing low percentage of MSM (0.011 Bq kg^{-1}). The percentage of correct MSM identification was 61%. In order to increase this percentage and to develop a new tool of investigation, the ^{90}Sr was elaborated in combination with other three parameters: ^{88}Sr , Ca and ash percentage, through a multivariate approach. The precision of this tool of investigation was significantly increased (87%), higher than the reference method (Ca level) (76%), representing a

valid novel approach in the identification of MSM products.

Keywords Bone fragments · Food safety · Liquid scintillation counting · Mechanically separated meat · Principal component analysis · Radiostrontium

Introduction

The European Regulation No. 853/2004/EC reports the following definition for mechanically separated meat (MSM): “the product obtained by removing meat from flesh-bearing bones after boning or from poultry carcasses, using mechanical means resulting in the loss or modification of the muscle fibre structure” (European Commission 2004). A MSM may be obtained through high pressure (HP-MSM, $p > 10^4 \text{ kPa}$, up to $4 \times 10^4 \text{ kPa}$ or more) or low pressure (LP-MSM, $p < 10^4 \text{ kPa}$) technology (EFSA 2013). The first type of meat is characterized by higher degree of muscle fibre destruction, if compared to the second one. However, the identification of both types of meat is important for evaluating their overall quality (nutritional, organoleptic, etc.) and for food safety. Indeed, MSM is considered more perishable than traditional minced meat (Viuda-Martos et al. 2012), since it may be contaminated by microorganisms, also pathogens, especially when the hygienic conditions of processing (environment, handlers, carcass, etc.) are poor. Moreover, the release of cellular fluids rich in nutrients, the cross-contamination and inappropriate storage temperatures may cause the bacterial growth increase (Yuste et al. 1999). Thus, the Panel on Biological Hazards (BIOHAZ) of the European Food Safety Authority recommended the Organisms in charge of food inspections (veterinary

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s13197-019-04076-y>) contains supplementary material, which is available to authorized users.

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services, reference laboratories, etc.) to develop new techniques for identifying mechanically separated meat, since the actual available tools may result not fully reliable. In particular, the rheological/textural parameters were classified as unuseful for this discrimination as well as several chemical parameters (protein, ash, cholesterol, iron, etc.). Only calcium content was indicated as reliable parameter for discriminating MSM from fresh and minced meat (calcium concentrations $> 1000 \text{ mg kg}^{-1}$ are attributed to MSM (European Commission 2005). Indeed, as reported by several authors (Tremlová and Štarha 2003; Branscheid et al. 2009) and confirmed in the European Regulation No 999/2001/EC (European Commission 2001), MSM may contain bone parts and the periosteum (bone skin). However, this maximum level was established only for high pressure MSM, since calcium amounts of low pressure MSM may be lower than this limit. Moreover, calcium concentrations higher than 1000 mg kg^{-1} could be verified also in non-MSM products containing ingredients rich in calcium (i.e. concentrated plant extract, cheese, etc.) and/or food additives admitted in fresh meat preparations such as calcium ascorbate (E302) or calcium citrate (E333). All these considerations suggest that the calcium content maybe questionable as discrimination parameter between MSM and non-MSM products. So, an analytical gap subsists.

In order to redress this analytical gap, the EFSA Panel mentioned above asked “to identify and rank the parameters that may be used to distinguish between the different types of MSM and compare them as well with fresh meat, minced meat and meat preparations, as defined in EU legislation”.

Radiostrontium (^{90}Sr) is a fission product of plutonium and uranium in nuclear weapons and reactors. It may be found in the environment, especially after fallout from atmospheric testing of nuclear weapons, while in Europe and Asia it was largely dispersed in the Chernobyl disaster of 1986 (Yablokov and Nesterenko 2009). It decays emitting beta particles and forming yttrium-90 (^{90}Y) which is also a beta emitter. ^{90}Sr and ^{90}Y are characterized by half-life equal to 29.1 years and 64 h, respectively (Wilken and Joshi 1991; Stamoulis et al. 2007). The main health effects caused by the ingestion of this radionuclide are bone tumors and tumors of the blood-cell forming organs. When ^{90}Sr is present in animal feeds, it may be taken into animal body, and then about 15% of what enters is deposited in bone. This is due to high chemical affinity of ^{90}Sr with calcium. Taking into account that ^{90}Sr contamination of animal feeds may result significant, this accumulation may be verified representing a food safety/animal wellness concern (Iammarino et al. 2015). Iammarino et al. (2018) reported ^{90}Sr mean activity concentration of 1.4 Bq kg^{-1} in chicken bones. Consequently, the MSM addition could

lead to significant ^{90}Sr levels in meat products. Moreover, this radionuclide could be tested as “marker” of meat products composed of MSM.

In this work, the ^{90}Sr levels quantified in MSM and non-MSM products were elaborated for evaluating the suitability of this parameter as tool for identifying the MSM presence in meat products.

For the same purpose, this parameter was also evaluated in combination with other chemical parameters obtained during the radiochemical procedure (^{88}Sr and ash percentage) and with calcium levels, through a multivariate approach. These approaches were then compared, and the most accurate was suggested as new tool of investigation for MSM identification in meat products.

Materials and methods

Chemicals

All chemicals used in this study together with their suppliers and purity grades are described in supplementary material.

Instrumentation

The sample preparation for bone fragments optical observation was carried out using a fully enclosed tissue processor (ASP300S, Leica Biosystems s.r.l., Buccinasco (MI), Italy), an embedding module, a cold plates module and a microtome (Canova, Diapath s.p.a., Martinengo (BG), Italy), a water bath and a histology oven (Bio-Optica s.p.a., Milan (Italy)). An Eclipse 50i Upright Microscope ($100\times$ magnification) and a DS-Fi1 High-Definition Color Camera Head, together with Imaging Software NIS-Elements F 3.2 (Nikon Instrument s.p.a., Campi Bisenzio (FI), Italy) were used for image acquisition/elaboration.

The ^{90}Sr activity concentration was determined using a liquid scintillation counter (LSC, Quantulus Wallac 1200, PerkinElmer, Turku, Finland). The WinQ Wallac Software and EASY view Spectrum Analysis Software (PerkinElmer, Waltham, MA, USA) were used for instrumental control and data elaboration.

The radiochemical separations require several analytical steps that may compromise the method reproducibility. As a consequence, the chemical yields of the elements of interest (Sr and Y) have to be assessed. In this procedure, the chemical yields were determined by Inductively Coupled Mass Spectrometry (ICP/MS). An inductively coupled plasma mass spectrometer (ELAN DRC II, PerkinElmer, Turku, Finland) was used. The calibration procedures and acquisition parameters of both instruments (LSC and ICP/MS) were described elsewhere (Iammarino et al. 2016a).

This method was defined “ultra-sensible” since it reaches sensitivity higher than other available techniques (Minimum Detectable Activity (M.D.A.): 8 mBq kg^{-1}). This peculiarity is useful, since the ^{90}Sr levels may result very low in meat samples.

In order to verify the calcium levels, with the purpose to evaluate the weaknesses of this parameter in MSM identification, this element was quantified by ICP/MS.

Sampling

Overall, 100 meat samples were collected from local stores and then stored in laboratory until analysis. 1 kg of each sample was homogenized by blade homogenizer. 2.0 g of the sample were used for the determination of calcium in duplicate (*see par. Calcium determination by ICP/MS spectrometry*), and the remaining part was ashed for the determination of ^{90}Sr , as described below. Two types of samples were investigated: (1) NO-MSM (50 samples): 24 samples of fresh meat (8 chicken breast, 6 turkey breast, 4 pork belly, 4 pork loin and 2 beef tenderloin), 16 samples of meat products (6 salami, 2 mortadella, 4 ham and 4 wurstel composed of pork meat not mechanically separated), and 10 samples of wurstel composed of chicken/turkey meat not mechanically separated; (2) MSM (50 samples): 40 samples of wurstel (HP-MSM) composed by MSM of chicken and chicken/turkey origin in the percentage range 57–94%, 10 samples of breaded meat (LP-MSM) composed by MSM of chicken and chicken/turkey origin in the percentage range 20–40%. The samples were produced in 10 Italian regions, Holland and Poland. More precisely, the HP-MSM samples were produced in Emilia Romagna-Italy (12 samples), Veneto-Italy (10 samples), Lombardy-Italy (8 samples), Poland (4 samples), Abruzzo-Italy (2 samples), Friuli-Venezia Giulia-Italy (2 samples), Trentino-Alto Adige-Italy (2 samples); the LP-MSM samples were produced in Veneto-Italy (6 samples), Marche-Italy (2 samples), Apulia-Italy (2 samples); the NO-MSM were produced in Apulia-Italy (25 samples), Basilicata-Italy (15 samples), Emilia Romagna-Italy (6 samples), Tuscany-Italy (2 samples), Holland (2 samples). The storage temperature has no significant effect on the chemical parameters evaluated in this study. However, in order to avoid any chemical-physical change of product, once arrived in laboratory the samples were stored at $-18 \text{ }^{\circ}\text{C} \pm 2 \text{ }^{\circ}\text{C}$ until analysis.

Bone fragments observation

The bone fragments presence was verified by light microscopy observation in different samples composed of different MSM percentages. The hematoxylin/eosin staining was used for this purpose, since this technique is widely

used for recognizing various tissue types (Fisher et al. 2008). The samples were fixed in 10% neutral buffered formalin and placed in cassettes that were loaded in tissue processor. After dehydration with ethanol and diaphanization with o-xylene, the samples were treated with paraffin for inclusion. After cooling, at least 5 sections ($3 \text{ }\mu\text{m}$ -thick) for each sample were prepared and dried at $37 \text{ }^{\circ}\text{C} \pm 3 \text{ }^{\circ}\text{C}$. The sections were then subjected to hematoxylin/eosin staining.

Sample preparation for ^{90}Sr determinations

The radiochemical procedure for determining the ^{90}Sr activity concentration was described elsewhere (Iammarino et al. 2016a). Due to long procedure that requires about 17 days, the determinations were carried out once (no replicates). Briefly, 1 kg of homogenized sample was ashed. The ashes were dissolved in HNO_3/HF mixture and, after leaching, the ^{90}Sr (when present) was precipitated as oxalate salt adding oxalic acid dehydrate, sodium acetate and adjusting pH to 4.5 by addition of ammonium hydroxide. The precipitate was dissolved in a $\text{H}_2\text{O}_2/\text{HNO}_3$ mixture and evaporated to dryness. The residue was dissolved in 0.1 M HCl. After removing by precipitation of two radiometric interfering of ^{90}Sr (^{210}Pb and ^{210}Bi) (Pan et al. 2017), the ^{90}Y that may be already present in the sample was removed by using 20% Bis-(2-ethylhexyl) phosphate (HDEHP) in toluene. The acid phase was recovered. After reaching of $^{90}\text{Sr}/^{90}\text{Y}$ secular equilibrium, the ^{90}Y was separated using 5% HDEHP in toluene. The organic phase containing ^{90}Y was purified using 0.1 M HCl and then the ^{90}Y was extracted using 3 M HNO_3 . Oxalic acid anhydrous and ammonium hydroxide were added for reaching the pH 2.5. In these conditions, the ^{90}Y was precipitated as oxalate. The precipitate was dissolved in a $\text{H}_2\text{O}_2/\text{HNO}_3$ mixture and evaporated to dryness. The residue was re-dissolved in HCl and the solution was evaporated to dryness again. 8 mL of 0.1 M HCl were used to dissolve the residue, and the sample was transferred into a scintillation vial, together with 12 mL of scintillation cocktail. This mixture was analyzed by ultra-low-level liquid scintillation counting. The final quantification of ^{90}Sr activity (A) was obtained using the following equation:

$$A = \frac{(R_c - R_f) \lambda_Y \cdot \Delta T}{(1 - e^{-\lambda_Y \Delta T}) \cdot W \cdot \varepsilon \cdot Y_{\text{Sr}} \cdot Y_Y} \cdot e^{\lambda_Y \Delta T_1} \cdot e^{\lambda_{\text{Sr}} \Delta T_2}$$

where R_c is the count rate; R_f is the background counting rating; λ_Y and λ_{Sr} are the ^{90}Y and ^{90}Sr decaying constants, respectively; ΔT is the time counting; Y_{Sr} and Y_Y are the strontium and yttrium chemical yields, respectively, W is the sample weight; ε is the counting efficiency; ΔT_1 is the time range between ^{90}Y separation and counting start; ΔT_2

is the time range between ^{90}Sr separation and ^{90}Sr solution reference data.

The method was validated, according to the reference European regulations and guidelines (Eurachem 2014; European Parliament/Council of the European Union 2017), while the decision threshold and detection limit were calculated in compliance with ISO reference protocol (11929:2010). The method, accredited by Italian Organism for accreditation ACCREDIA, is characterized by precision (as CV%, $n = 6$) lower than 15%, mean recovery percentage of 90.5% ($n = 12$), counting efficiency of 89%, measurement uncertainty of 16%, and it is applied successfully per the routinely analysis both of fresh meats and meat products at the Italian National reference Centre for the Detection of Radioactivity in Feed and Foodstuff, since 2011.

During this radiochemical procedure, the amount of ashes obtained after sample ashing (measured by XS205 DualRange Analytical Balance, Mettler Toledo, Woburn, MA, USA), and the concentration of stable Sr (^{88}Sr), quantified by ICP/MS, (*see par. Calcium determination by ICP/MS spectrometry*) were determined, in order to develop a multi-parameter tool of investigation for the identification of MSM. The determinations of these two parameters did not require additional time, since they are included in the validated procedure for the determination of ^{90}Sr .

As stated above, in Europe the environmental contamination by ^{90}Sr is mainly due to the Chernobyl disaster of 1986. 200,000 km² of Europe were involved, and the extent of radionuclides deposition on the soil was dependent on the air contamination and on the rainfall intensity (World Health Organization 2005). Consequently, the radiocontamination level may vary depending on the geographical area, considerably. The area of production of each sample containing MSM was considered for evaluating the extent to which the geographical area can affect the ^{90}Sr contamination of MSM samples.

Calcium determination by ICP/MS spectrometry

The analytical determinations of calcium (and ^{88}Sr) were carried out by using an improved version of the UNI EN reference method (15763:2009). This procedure is used for the determination of trace elements in food at Chemistry Department of Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Foggia, Italy, since it was accredited in 2010 by the Italian Organization for laboratory accreditation ACCREDIA. The analytical procedure was already fully described elsewhere, together with all validation parameters (Miedico et al. 2017). Briefly, 1.0 g of homogenized sample was mineralized by microwave-assisted acid digestion. This step was obtained by using an

Ethos-One Microwave Reaction System (Milestone s.r.l. Sorisole, Bergamo, Italy), according to the UNI EN reference procedure (13805:2014). After mineralization, the solution was transferred into 50 mL polypropylene disposable tubes and filled to mark by adding ultrapure water. The ^{44}Ca isotope was used and the instrumental calibration was performed by standard addition into the mineralized and diluted solution. Four addition levels (40–80–200–1000 ng mL⁻¹) and a not added level were used. The limits of quantification (LOQs) of calcium and strontium correspond to 1500 and 9.2 ng g⁻¹, respectively.

Two replicates of each sample were analysed. Each batch analysis was composed of five samples. The calcium and ^{88}Sr concentrations were evaluated as the mean of two measurements.

Statistical analysis

Despite the fact that this radiochemical method adopted in this study is characterized by high sensitivity, concentration activities lower than M.D.A. or the total absence of the radionuclide under investigation may be often verified. In these cases, a concentration equal to the M.D.A. of the method (0.008 Bq kg⁻¹) was assigned. This approach, named “upper-bound”, was indicated by Italian Institute of Health in the document “Rapporti ISTISAN 04/15”, as a protective measure, relating to human health and environment (Italian National Institute of Health 2004).

For statistical analysis purpose, the samples were subdivided into three categories: HP-MSM (MSM percentage in the range 57–94%); LP-MSM (MSM percentage in the range 20–40%) (HP: high percentage; LP: low percentage); NO MSM (without MSM). The ^{90}Sr levels registered for these three types of meat samples were compared by ANOVA one-way test ($p \geq 0.05$) and F-test, where applicable. The same statistical indexes were used to compare the ^{88}Sr and Ca concentrations and the ashes percentages. The Pearson's r correlation coefficient was elaborated in order to evaluate the correlation between the ^{90}Sr levels detected in MSM samples and the percentages of MSM declared on the product labels. Finally, the principal component analysis (PCA) was elaborated, for developing a novel tool of investigation for identifying a product containing MSM. PCA was performed by using the “Statistical Analysis” tool of MetaboAnalyst—statistical, functional and integrative of metabolomics data (Xia and Wishart 2016). It was used as first step for data reduction and visualization, with the aim to highlight any variation in the obtained dataset. The principal components were rank ordered by the variability that they represent in the data set, with the first principal component accounting for the greatest variability in the data and so on (Trygg et al. 2007). The ^{90}Sr activity concentration, together with ^{88}Sr

Fig. 1 Bone fragments observation by light microscopy (100x magnification). Wurstel sample containing 91% of mechanically separated meat of chicken origin

concentration and ashes percentage registered in each sample were taken into account for this purpose. The calcium concentrations were also included as final step.

Results and discussion

Bone fragments observation

Five meat samples (3 wurstel with MSM percentage in the range 91–94%, and 2 breaded meat, with MSM percentage of 40%), obtained from mechanical separation of meat at high and low pressure, respectively, were observed by light microscopy, in order to verify the presence of bone fragments. Five slides were prepared and observed for each sample. What is interesting is that bone fragments were observed only in wurstel samples, and not all slides showed this presence. This means that the simple observation of a MSM sample by light microscopy may lead to a wrong interpretation of results, since the probability to obtain a “false negative” response is high. This is due to the small amount of sample that is treated and observed. Through this test, the presence of bone fragments in MSM products was confirmed. In Fig. 1 some examples related to these tests are shown.

Determination of ^{90}Sr activity concentration

In Table 1, the activity concentrations of ^{90}Sr quantified during this survey, subdivided for sample type are reported. As a first important comment, the activity concentrations detected during this study do not represent a food safety concern, since the levels resulted well lower than the maximum permitted level for this type of food (in the case of a nuclear accident or any other case of radiological emergency) defined in the Council Regulation (Euratom) 2016/52 (750 Bq kg^{-1}) (European Council 2016). Moreover, no correlation was verified between ^{90}Sr levels detected and geographic origin of sample. This is largely justifiable taking into account the high variability of both

bone fragments amount in the samples and the contamination levels of these fragments.

In Fig. 2, some spectra obtained by ultra-low-level liquid scintillation counting (LSC) are shown. In this figure, a significant difference of the ^{90}Y signal, obtained by analyzing meat samples composed by different percentages of MSM, may be appreciated. This is probably due to the presence of not negligible amounts of bone fragments, contaminated by low levels of ^{90}Sr , identified and quantified through the elaboration of the characteristic ^{90}Y LSC spectra. However, this type of contamination was not verified in all samples composed by high percentages of MSM. Indeed, the ^{90}Sr levels are highly variable, depending in the first instance on the amounts of bone fragments in the MSM, and on the bone contamination (linked to the geographical area of breeding and to the animal feeding). Indeed, the ^{90}Sr activity concentrations detected in MSM samples may result variable also when the geographical area of production is the same [i.e. from 0.008 (M.D.A.) to 0.412 Bq kg^{-1}].

Regarding NO-MSM samples, the presence of quantifiable levels of ^{90}Sr was registered only in 21 samples (42%). The ^{90}Sr activity concentrations detected in these samples were in the range $0.010\text{--}0.107 \text{ Bq kg}^{-1}$, with a mean activity concentration equal to 0.024 Bq kg^{-1} (Table 1). According to the actual knowledge about ^{90}Sr accumulation in foodstuffs, these low levels confirmed that fresh meats and meat products not containing MSM cannot be considered particularly interesting as indicators of this type of environmental contamination. The mean level of contamination registered during this study resulted lower than that reported in a recent monitoring developed in Italy (0.083 Bq kg^{-1}), related to fresh meats and meat products not containing MSM. The quantifiable activity concentrations of ^{90}Sr in these samples were probably due to the presence of ingredients containing low levels of the radionuclide, in the product formulation [i.e. concentrated plant extract, cheese, etc., as reported by Iammarino et al. (2016b)].

Table 1 ^{90}Sr Concentration activity, ^{88}Sr and calcium concentration, and ashes percentage determined by analyzing 100 samples of fresh meats and meat products composed of different percentages of mechanically separated meat (MSM)

Sample type	MSM%	Number of samples analyzed	Number of activity concentration	^{90}Sr activity concentrations < M.D.A.	^{90}Sr activity concentrations > M.D.A. [min–max]	^{90}Sr mean activity concentration (Bq kg^{-1})*	^{88}Sr mean concentration (mg kg^{-1})* $\pm$ [SD]	Ca mean concentration (mg kg^{-1})* $\pm$ [SD]	Ashes mean%* $\pm$ [SD]
HP-MSM	57–94	40	5	0.032–0.412		0.099 ^a	0.66 ^{ab} [0.36]	1028.9 ^a [374.8]	3.1 ^a [0.5]
LP-MSM	20–40	10	7	0.010–0.029		0.011 ^b	1.04 ^{bc} [0.27]	544.4 ^a [207.7]	1.9 ^b [0.2]
NO-MSM	–	50	29	0.011–0.107		0.024 ^b	0.53 ^c [0.59]	93.6 ^b [45.6]	1.9 ^{ab} [1.0]

SD standard deviation

M.D.A. = minimal detectable activity = 0.008 Bq kg^{-1} *Means within column with different superscripts differ ($p < 0.05$)

No quantifiable activity concentrations of ^{90}Sr were detected in 7 LP-MSM samples, out of 10. This result is largely justifiable, taking into account both the low percentage of MSM in the formulations of these products (lower than 40%), and the technology used for obtaining this type of MSM, mainly by low pressure. Moreover, no ingredients that may contain quantifiable levels of ^{90}Sr (Iammarino et al. 2016b) were declared on the product labels of LP-MSM samples. Quantifiable activity concentrations of ^{90}Sr ($> \text{M.D.A.}$) were detected in 3 LP-MSM samples. This activity concentrations resulted equal to 0.010 ± 0.002 , 0.011 ± 0.002 and $0.029 \pm 0.005 \text{ Bq kg}^{-1}$.

The most interesting results obtained during this study were registered, as expected, for HP-MSM products. Indeed, only 5 samples (10%) resulted as not contaminated by ^{90}Sr . The ^{90}Sr activity concentrations resulted in the range 0.032–0.412 Bq kg^{-1} . The level of the radionuclide was coupled to the percentage of MSM declared on the label, by using the Pearson's r correlation coefficient, for evaluating a direct proportionality between two variables. This proportionality was not verified, since the r value resulted equal to 0.26. This missing result may be justified considering the variability of both bone contamination (linked to the geographical area of breeding and to the animal feeding) and bone fragments percentage in the MSM.

Taking as reference the mean ^{90}Sr level detected in meat products by the Italian National reference centre for the detection of radioactivity in feed and foodstuff (Iammarino et al. 2016b), (0.083 Bq kg^{-1}), the mean activity concentration detected in HP-MSM samples (0.099 Bq kg^{-1}) resulted slightly higher. This difference confirmed that the presence of bone fragments may lead to a slight increase in the ^{90}Sr levels in the final product. This is an important finding of this study, since the HP-MSM samples may be considered significant matrices of investigation, within the radiocontamination surveillance programs.

^{90}Sr “cut-off” for MSM identification

The radiocontamination of meat products by ^{90}Sr was tested as a chemical indicator for identifying a MSM product, following the recommendation recently raised by EFSA (2013). According to actual knowledge about ^{90}Sr accumulation in foodstuffs, fresh meats and meat products do not accumulate significant amounts of ^{90}Sr (Semioshkina et al. 2006; Kenesariyev et al. 2007). Consequently, the contamination levels of these products are very low, close to the method M.D.A. It is therefore reasonable to statistically elaborate the ^{90}Sr activity concentrations detected in NO-MSM samples for identifying a

Fig. 2 Ultra-low-level liquid scintillation counting spectra examples. **A** ^{90}Y source with activity concentration of 1.0 Bq L^{-1} . **B** Wurstel sample of chicken/turkey origin with ^{90}Sr activity concentration of 0.412 Bq kg^{-1} (a); Wurstel sample of chicken origin with ^{90}Sr activity concentration lower than minimal detectable activity (0.008 Bq kg^{-1}) (b)

level (“cut-off”) that may be useful for discriminating MSM from NO-MSM samples. This elaboration was conducted by using an approach similar to that reported in recently published studies (Iammarino et al. 2014, 2017). A possible maximum level to suggest for this purpose was elaborated as follows: the mean value related to this group of samples (0.024 Bq kg^{-1}) was added to 3σ ($\sigma = 0.028 \text{ Bq kg}^{-1}$), obtaining an activity concentration equal to 0.108 Bq kg^{-1} . This concentration was then increased, considering the measurement uncertainty of the method (16.0%), obtaining a final value equal to 0.125 Bq kg^{-1} .

This value was used as “cut-off” for discriminating two types of samples, MSM and NO-MSM, among 100 samples analyzed during this study. Taking into account only the MSM samples (50, both HP and LP), as appreciable in Fig. 3, only 11 samples (22%) can be classified as MSM, by this way. Remaining 39 samples (78%) would be classified as NO-MSM, wrongly. Considering other 50 samples analyzed, that were obviously classified as NO-MSM, correctly, this approach gave a percentage of correct classified samples of 61% (50 NO-MSM and 11 MSM). Given these results, this approach may be useful for identifying HP-MSM products, if the activity concentration is higher than 0.125 Bq kg^{-1} . Below this value, the discrimination

Fig. 3 Distribution of ^{90}Sr activity concentrations detected by analyzing 100 samples of fresh meats and meat products

MSM/NO-MSM is not possible, since the contamination may be due to the presence of bone fragments but also to the presence of particular ingredients that may be slightly contaminated by ^{90}Sr (i.e. vegetable plant extract, spices, cheese, etc.). This situation is not rare, considering that the concentrated plant extract and spices are widely used in fresh meat preparations (i.e. fresh sausage, hamburger, etc.), and that the ^{90}Sr contamination levels of these ingredients may reach 0.37 Bq kg^{-1} (Iammarino et al. 2016b).

MSM identification by multivariate approach

In order to develop a complete tool of investigation, useful for classifying all types of meat products, the principal component analysis (PCA) was elaborated, by adding the ^{88}Sr concentrations and the ash percentages. Indeed, the determinations of these two indexes do not weigh on times and costs of analysis, since they are accomplished during the ^{90}Sr radiochemical determination. The multivariate approaches represent a new frontier in food analysis, especially regarding food authenticity and traceability. Indeed, food authenticity is addressed using several laboratory methods that lead to large data sets. Multivariate approaches are then required to process these data and to give an answer about the classification of the analyzed samples (Oliveri and Downey 2012; Oliveri and Simonetti 2016).

As shown in Fig. 4a, we observed that the different types of samples clustered separately along the first principal component (PC1) that accounts for 46.9% of the total variance. In particular, the separation of HP-MSM samples from other types was almost complete, whereas LP-MSM and NO-MSM samples clustered together. This result confirmed that NO-MSM samples and meat samples composed by low percentages of MSM (LP-MSM), obtained by low pressure technology, are very similar from a chemical point of view. An analogous result was recently obtained by Dalipi et al. (2018) and by Mello et al. (2017). The majority of MSM samples were correctly identified.

However, 16 samples out of 50 analyzed clustered together with NO-MSM samples. From these results, we obtained a correct identification in 73% of samples analyzed (98% of NO-MSM, 0% of LP-MSM and 60% of HP-MSM samples). This result is comparable to that obtained by using only the calcium concentration for the same purpose (*see below*).

This multivariate approach was also studied by adding the calcium concentration as evaluation parameter. Although the mean levels of this element quantified in HP-MSM, LP-MSM and NO-MSM products were different (Table 1), the concentrations distribution of HP-MSM and LP-MSM resulted comparable ($p < 0.05$). Taking as reference the maximum concentrations of 1000 mg kg^{-1} , used for discriminating MSM from NO-MSM, all LP-MSM samples (10) resulted lower than this level, in the range $358.0\text{--}880.5 \text{ mg kg}^{-1}$. As stated above, relating to ^{90}Sr activity concentrations, this missing identification as MSM samples may be due to the low percentage of MSM (in the range 20–40%) and/or to the low pressure used for obtaining this type of MSM. Likewise, 14 HP-MSM samples, out of 40, resulted characterized by calcium concentration lower than 1000 mg kg^{-1} (in the range $129.3\text{--}981.0 \text{ mg kg}^{-1}$). The percentages of MSM of these products, as declared on the labels, were 57% for 10 samples and 70% for remaining 4. The low levels of calcium quantified were presumably due to these not high percentages of MSM. Overall, the identification of MSM by using the calcium parameter resulted correct in 76% of analyzed samples (100% of NO-MSM, 0% of LP-MSM and 65% of HP-MSM samples). This result confirmed the need of new tools of investigation, highlighted by EFSA (2013), for the identification of MSM products, since 24% of identifications were missed.

The results obtained by adding the calcium concentrations to other three parameters available (^{90}Sr , ^{88}Sr and ash) for multivariate analysis, are shown in Fig. 4b. The result obtained was similar to that commented above without calcium levels. Indeed, also in this case, the different types of samples clustered separately along the first

Fig. 4 Comparison of meat products with different percentages of mechanically separated meat—principal component analysis. Three parameters: ^{90}Sr activity concentration, ^{88}Sr concentration, ash

principal component (PC1) that accounts for 46.7% of the total variance. However, a better identification of HP-MSM samples was obtained. Only 4 samples out of 40 analyzed clustered together with NO-MSM samples. One LP-MSM and all NO-MSM samples were correctly identified. As a preliminary result, from these data, we obtained a correct identification in 87% of analyzed samples (100% of NO-

percentage (a); four parameters: ^{90}Sr activity concentration, ^{88}Sr and Ca concentrations, ash percentage (b)

MSM, 10% of LP-MSM and 90% of HP-MSM samples). A precision increase of 14% and 11% was registered comparing this approach to multivariate analysis (three parameters) and calcium content, respectively. Future studies will be focused on the statistical improvement of this multivariate approach and to its validation.

Conclusion

In this study, 100 samples of fresh meats and meat products composed of different percentages of mechanically separated meat (MSM) and without MSM were analyzed with the aim to quantify the activity concentrations of radiostrontium (^{90}Sr). Due to the possible presence of bone fragments in MSM, this radionuclide was investigated because it may accumulate in animal bones. Hence, the suitability of this parameter for discriminating MSM products was evaluated. The radiochemical determinations were carried out by using a validated ultra-sensible method by ultra-low-level liquid scintillation counting able to detect trace levels (up to 8 mBq kg^{-1}) of ^{90}Sr .

The ^{90}Sr mean activity concentration detected in meat products with high percentage of MSM (in the range 57–94%) (HP-MSM) was significantly higher ($p < 0.05$) (0.099 Bq kg^{-1}) than that detected in fresh meats (NO-MSM) (0.024 Bq kg^{-1}) and in meat products containing low percentage of MSM ($< 40\%$) (LP-MSM) (0.011 Bq kg^{-1}). There is no concern for food safety, but this finding allows the inclusion of the HP-MSM among the representative food matrices to take into account within the radiocontamination surveillance programs.

The ^{90}Sr levels registered during this survey were statistically elaborated for evaluating a “cut-off” value useful for discriminating MSM from NO-MSM products. A ^{90}Sr activity concentration higher than 0.125 Bq kg^{-1} may be considered significant for identifying a HP-MSM product. Above this level the radiocontamination is presumably due to the presence of bone fragments slightly contaminated by ^{90}Sr . Below this value the discrimination MSM/NO-MSM is not possible, since the contamination may be due to the presence of both bone fragments and particular ingredients (vegetable plant extract, spices, cheese, etc.) that may be slightly contaminated by ^{90}Sr . By using this method of discrimination, only 61% of samples were classified correctly (11 MSM and 50 NO-MSM samples).

Other two parameters, ^{88}Sr and ash percentage, available from this radiochemical procedure, were used together with ^{90}Sr activity concentration for developing a novel tool of investigation for identifying a MSM through a multivariate approach. The precision of this tool (73%) was comparable to the reference method (determination of calcium concentration, also evaluated in this study, 76%) for the discrimination MSM/NO-MSM products.

A significant improvement was obtained by adding the calcium concentration, determined by a validated ICP-MS method, as a parameter for the multivariate approach. By using four parameters, ^{90}Sr activity concentration, ^{88}Sr and Ca concentrations and ash percentage, the precision of this tool of investigation was increased to 87%, representing an

improvement in this field and a valid novel approach in the identification of MSM products.

Acknowledgements This work was supported by the Italian Ministry of Health who funded the Project code GR-2013-02358862. Dr. Nicola Bortone, Dr. Rita Damiano, Dr. Esther De Simone, Mr. Michele Nicolini and Mr. Rosario Accettulli, (Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Foggia, Italy) are gratefully acknowledged for technical assistance.

Funding This work was funded by the Italian Ministry of Health (Grant Number GR-2013-02358862).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics requirements This article does not contain any studies with human participants or animals performed by any of the authors.

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