FOODINTEGRITY HANDBOOK

A GUIDE TO FOOD AUTHENTICITY ISSUES AND ANALYTICAL SOLUTIONS

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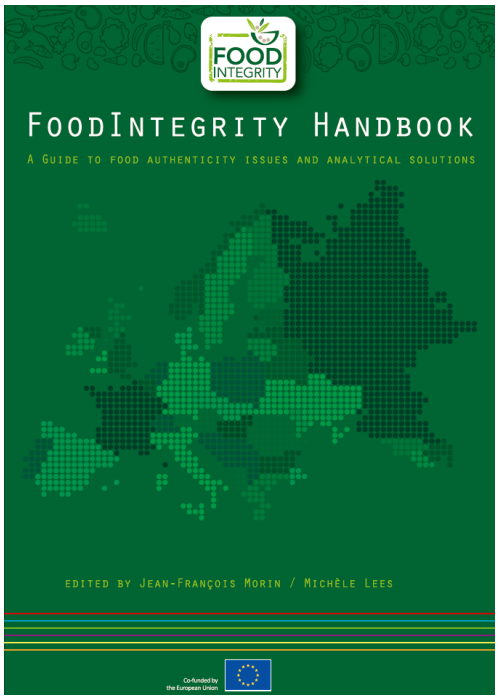

ISBN *print version* *978-2-9566303-0-2*
electronic version *978-2-9566303-1-9*

https://doi.org/10.32741/fihb

Determination of species origin of gelatine in foods

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General overview of the product

Gelatine is widely used in the food industry. With excellent gelling properties, it is used in a broad range of food products, including confectioneries, desserts and pies, often as a binder or to enhance texture. Gelatine is also an important product in the pharmaceutical, medical, cosmetic, adhesive and photographic industries. It is prepared from the partial hydrolysis of skin and bone material, usually of bovine or porcine source, and the correct labelling of foods regarding species origin is therefore important for those with religious or ethical preferences to avoid these species.

After starch, gelatine has the second greatest proportion of the global market share in terms of value, representing over a quarter of the market share for food hydrocolloids (Gelatine Manufacturers of Europe[1]). In 2008, approximately 326 000 tons of gelatine were produced. In a growing market, it is believed that around 400 000 tons of gelatine were consumed in 2017, according to Global Industry Analysts, in a market worth USD 1.77 billion [1]. Gelatine appeals to the food industry since it is often (depending on species and tissue source) free of colour, odour and taste and thus can be added to products without affecting perceived quality. As a by-product of the meat and fish industries, gelatine is often sold as a natural ingredient and may appeal to customers since it is sold as a sustainable by-product[2].

Most gelatine manufactured globally is of bovine or porcine origin although piscine and poultry gelatines are also available. Many consumers abstain from the consumption of bovine products (e.g. those adhering to Hinduism) or porcine products (e.g. those adhering to Islamic law seek Halal products and followers of Judaism seek Kosher products), or indeed any animal tissues (e.g. vegetarian and vegan consumers). Since porcine gelatine is cheaper than bovine gelatine, many producers prefer using this gelatine in their products for profit gain [2]. There have been instances of products being incorrectly labelled, either due to deliberate fraud or contamination with an alternative species of gelatine (e.g. [2] and UK Food Standards Agency, 2009). In terms of food authenticity and food integrity, it is therefore important that analytical methods are available to determine and verify the species origin(s) of any gelatine present in foods. The high levels of sequence homology between collagens of different species, particularly of bovine and porcine origins, present a significant challenge in distinguishing gelatine species origin.

[1] https://www.gelatine.org/gelatine/comparison-hydrocolloids.html

[2] https://www.gelatine.org/ and https://www.gelatine.org/gme/sustainability.html

https://doi.org/10.32741/fihb.21.gelatine

1. Product Identity

1.1. Definition of the product and manufacturing process

1.1.1. Gelatine composition and properties

Collagen is an abundant structural protein in animals. Prepared from the collagen of bone, skin and other connective tissue, collagen consists of three amino acid chains wound together as a triple helix, stabilised by interlinking bridges between adjacent collagen chains. It is a robust protein which survives high levels of processing, albeit in an altered state. Concerning collagen and gelatine composition, proline constitutes around 18 % of the amino acid composition of collagen and is often subject to hydroxylation during collagen synthesis. Asparaginyl and glutaminyl residues within collagen undergo deamidation during gelatine manufacture. Approximately one third of the amino acid composition of bovine and porcine collagen is glycine. In addition to collagen protein, gelatines also contain low levels of minerals and water.

Gelatine is often labelled on foods according to the animal of origin (usually beef, pork, fish or chicken). As discussed below, gelatine is a highly processed product prepared by the aggressive processing of collagen causing partial hydrolysis of the protein and degradation of the DNA. The final product, especially for bovine and porcine gelatines, is a powder or granules with no apparent indicator of animal origin. When gelatine is manufactured, the animal origin of each batch is denoted by a paper trail. Certificates are issued and used to distinguish bovine gelatine from porcine or indeed from any animal origin of gelatine. However, gelatine can potentially be prepared from the collagen of any animal species. This is especially true for bone material but less so for hide material, since hide cutting instruments are animal-specific due to the variations in hide thickness between species. There is therefore potential for unscrupulous manufacturers to adulterate such highly processed products leaving no visible trace of animal origin for purchasers to consider. There is also the chance of accidental contamination of one gelatine species with another with no visible indication.

Gelatines can differ in terms of their strength. The Bloom test (1925) determines the weight in grams needed by a specified plunger to depress the surface of the gel at a specified temperature 4 mm without breaking it [3]. The result is expressed in Bloom (grades), e.g. Bloom 50 or Bloom 325. The higher a Bloom value, the higher the melting and gelling points of a gel, and the shorter its gelling times. Bloom strength depends on a number of factors including the age of the starting material and the processing method used. The Bloom can therefore not be predicted accurately from the starting material at the factory but can be predicted with in a range. Once manufacture is complete, the final gelatine product requires testing in order to accurately determine the Bloom. The higher the Bloom, the higher the financial value of a gelatine. In general, the lower the extraction temperature, the higher the Bloom, although this is also influenced by other factors including pH and processing time.

1.1.2. Gelatine Manufacturing Process

Gelatines which are commercially available globally tend to be prepared from bone and hide of cows older than 18 months, calf hide, pig hide, chicken skin or feet and fish skin or swim bladder. The raw materials used to produce gelatines in Europe are shown in Figure 1. The process for the industrial preparation of each gelatine depends on the starting material as is summarised below. Gelatines are often prepared by incubation in an acid or alkali followed by high temperature extraction and sterilisation and many gelatine manufacturing plants handle only one type of

gelatine, although some plants handle more than one species. Since gelatine is prepared from bone or hide material, there is opportunity for unscrupulous manufacturers to prepare a batch of gelatine from any animal species if the raw materials were available and label it with an alternative species origin. This batch could then enter the food chain.

Approximately 80 % of the gelatine prepared in Europe is derived from porcine skins and 15 % from cattle split. The remaining 5 % is prepared from porcine and bovine bones and fish[3]. Globally, 46 % is prepared from porcine hide, 29.4 % from bovine split, 23.1 % from bones and 1.5 % from other sources including chicken [4]. There is also interest in increasing the amount of fish gelatine which is manufactured, due partly to its abundance and biodegradability [1,5].

The processes used to prepare gelatine in industry differ depending on the starting material and examples of the acid, alkali and enzymatic processes used to prepare gelatines are discussed below. The gelatine yield from the raw starting materials tends to be approximately 10 %.

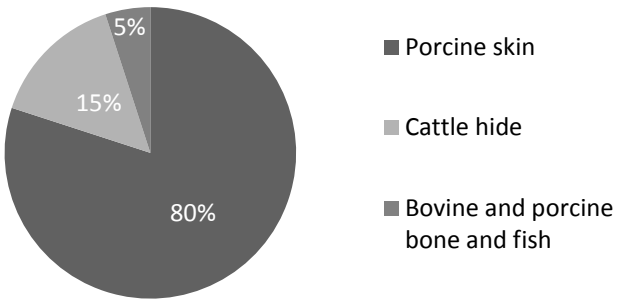

Figure 1. Production of edible gelatines in Europe, by raw material. Information taken from http://www.gelatine.org/gelatine/manufacturing.html

1.1.2.1. Acid process

Pork hide, fish, poultry (chicken) and calf hide gelatines are produced by an acid incubation process which is quicker and less aggressive than alkali processes. Younger collagens (pig and calf) which have softer cross links to stabilise the helical collagen structure respond better to acid treatment which is a relatively gentle treatment of the cross links. The acid cleaves the protein and results in branched chains of protein. Gelatine prepared from hide is cheap and often used in food. Amongst other effects caused during this aggressive manufacturing process, incubation in acid is known to cause depurination degradation of DNA [6]. The manufacture of calf hide uses an acid process or acid followed by the alkali process.

Chicken skin and feet are used for gelatine preparation. Fish gelatine is prepared from the skin and swim bladders of farmed, warm-water fish since cold-water fish gelatine has poor quality with a very low melting temperature. Chicken and fish gelatines are prepared by incubation in acid at 5°C. Citric, lactic, acetic or phosphoric, or a blend of some or all of these acids, are used. The process then uses a filtration treatment to remove the oil before drying gently in drying tunnels.

[3] https://www.gelatine.org/gelatine/manufacturing.html

1.1.2.2. Alkali process

Bovine bone and hide of cows older than 18 months tend to be prepared by an alkali incubation process. The alkali process is a more time-intensive process than the acid process. The only bovine bone from which gelatine is prepared is the thigh bone as it has the correct sinew level required for gelatine preparation. The bone is demineralised prior to further treatment.

Cows tend to be 18 months old at slaughter and thus have more established cross links in the hide compared to pigs which are slaughtered at a younger age. Cow hide gelatine is prepared by the alkali process which is a relatively more aggressive process and results in longer, straighter chains. Material is soaked in lime, sometimes along with sodium hydroxide, calcium chloride and/or sodium sulphide over an incubation period of several months with an aim of yielding only pure gelatine which is used by the pharmaceutical, plasma replacement and photographic film industries.

1.1.2.3. Enzymatic production

Enzymatic production of gelatine is cheaper than acid or alkali treatments. Enzymes such as Alcalase® and Neutrase® are used followed by incubation in lime which is used as a preservative. Enzymatic methods involve reduced levels of processing and tend to result in gelatines of a darker colour which command a lower financial value.

1.2. Current standards of identity or related legislation

Processed foods and other consumer products require accurate labelling according to the species they contain to enable consumers to make informed decisions about the food they buy. The commercial pressure for suppliers to provide gelatines of known species is driven largely by due diligence. Issues surrounding the discovery of horse meat in beef products in Europe in 2013 highlighted consumer interest in food labelling and authenticity and the subsequent Elliott Review (2014) recommends that managing the food supply chain must involve more than maintaining a paper trail [7]. Given the above, there are benefits in developing a reliable test method to aid with labelling in terms of the species origin of added gelatine.

Gelatine falls under the European Commission's scope of 'other products of animal origin.' Therefore the rules governing both imports and intra-community trade of other products of animal origin for human consumption, laid down in Council Directive 2002/99/EC, apply to gelatine. This Directive, which has been amended several times, harmonises the rules and establishes the animal and public health rules for the import and trade in the Community for animal products where specific Community rules have not been laid down elsewhere. The EU Health and Consumer Protection Directorate publish opinions regarding the use of gelatine in food, feed, cosmetics, pharmaceutical and medical products with respect to risks from Transmissible Spongiform Encephalopathies (TSEs).

The Gelatine Manufacturers of Europe (GME) is an organization involving gelatine and collagen peptide manufacturers in Europe since 1974. According to its mission and objectives, GME's primary focus is on setting the highest standards for quality, safety and sustainability amongst its member companies[4].

[4] https://www.gelatine.org/gme/mission-and-objectives.html

There is no legislation requiring that the animal origin of gelatines is included on food labels, but many suppliers choose to include this information to better-inform consumers. A paper trail is the method used to determine the origin of batches of gelatine. Supplier premises may be inspected by accreditation and certification bodies, including Halal food certification bodies.

2. Authenticity issues

2.1. Identification of current authenticity issues

Regarding authenticity issues, as previously mentioned, due to the high levels of processing involved in the preparation of gelatines, resulting in gelatine granules or powders with little or no apparent indicators as to origin, there is potential for deliberate adulteration or accidental contamination to occur using raw material from animals other than those on the product label. Since many consumers choose to abstain from certain species due to religious rules or ethical preferences it is important that methods are available to ensure correct labelling.

Since the physical appearance of gelatines provides little or no significant indicator of animal origin, it is perceivable that batches of gelatine can be mislabelled or mixed, either fraudulently or accidentally. Also, certain gelatine manufacturers use the same factory establishments to process gelatine of different origins and therefore mixing of species can occur at the point of manufacture.

The widespread adulteration of processed beef products with horse in 2013 highlighted that species-related fraud is present in the food chain. The subsequent Elliott Review (2014) into the integrity and assurance of food supply networks investigated, amongst other aspects, the ‘causes of the systemic failure that enabled the horsemeat fraud’. Further highlighting the issue in terms of gelatine adulteration, highly processed gelatine (hydrolysed collagen) of bovine origin has been found by the UK Food Standards Agency as a plumping agent in chicken breasts labelled as containing chicken only (UK Food Standards Agency, 2009). Therefore, methods to determine species origin of processed products such as gelatine would support the food chain and consumers by aiding policing against known potential threats. Further, given the religious and ethical sensitivities regarding the species origin of gelatine, it is important that analytical methods are available to authenticate the animal origin of gelatines in foods and capsules. While gelatine manufacturers are audited to support the species authenticity of gelatine, there is still opportunity for the accidental and deliberate mislabelling, particularly since porcine gelatine is cheaper in terms of cost than bovine gelatine [2]. Analytical methods which can determine the presence of an adulterating gelatine present at low levels when mixed with an alternative gelatine are required with a high level of sensitivity to support food integrity.

2.2. Potential threat to public health

Following the incidence of Bovine Spongiform Encephalopathy (BSE) in cattle in the EU in 1986, strict restrictions were imposed regarding the food use of certain animal tissues where the BSE-inducing prions can be present at high levels. These regulations are slowly being relaxed in terms of which tissues can enter the animal and human food chains based on on-going risk assessments in the light of other controls that are now in place.

Due to concerns linked to BSE in 1997, the TSE Advisory Body, in collaboration with the US Food and Drug Administration, began monitoring the potential risk of transmitting BSE. The disease was mainly associated with consumption of tissue of the nervous system including skull, brain and vertebrae. It was recognized that the heat, alkali and filtration treatments used during gelatine manufacture could be effective in reducing the level of contaminating transmissible spongiform encephalopathies. In 2002, the Scientific Steering Committee of the European Union (SSC) stated that the risk associated with bovine gelatine is very low or zero and, in 2004, a team determined that the acid and alkali processes of gelatine manufacture from bovine bone reduce infectivity to undetectable levels [8].

Regarding alternative threats to health, from a nutritional point of view, although gelatine is composed of around 98 % protein (dry weight), it does not contain all essential amino acids and therefore must be consumed only as part of a balanced diet.

3. Analytical methods used to test for authenticity

While there are no officially recognised methods, researchers in the past have investigated technologies to determine the species origin of gelatine. As discussed above, due to the high levels of homology in structure, properties and amino acid sequence between bovine and porcine gelatines, conventional physicochemical methods cannot be applied.

Gelatines are manufactured by an aggressive processing causing partial hydrolysis of the collagen. As detailed above, the raw material is incubated either in acid or in alkali, followed by extraction at high temperature, filtration and further high temperature sterilization. Under such conditions, most of the DNA is denatured and some protein molecules show signs of denaturation. Conventional methods used in species determination of foods such as Polymerase Chain (PCR) and Enzyme-Linked Immunosorbent Assay (ELISA), which rely on high quality DNA and protein respectively, are not generally applicable. Indeed, ELISA assays designed for species determination of processed meat, such as the Biokits Cooked Species Identification Test Kit (Neogen Europe Limited) carry notices confirming that they are not necessarily applicable to gelatine determination.

Since bovine and porcine gelatines share around 95 % amino acid sequence homology [9], and much homology in structural and physicochemical properties, they are difficult to differentiate by conventional physicochemical methods such as calcium phosphate precipitation [10] and HPLC [11]. Further, methods such as Fourier-transform infrared spectroscopy (FTIR), one-dimensional and two-dimensional gel profiling have shown low sensitivity and cannot be used to determine gelatines or products containing a mixture of species [12,13].

Similarly, conventional PCR can often not be applied due to high levels of denaturation of the DNA [14]. In a study investigating the gelatine origin of 36 foods, in only twelve of the foods was any DNA detected [15]. There has been some success regarding the ability to apply PCR to determine gelatine species origin with one team reporting the ability of PCR targeting the Mitochondrial Cytochrome b gene to differentiate bovine and porcine DNA at the 0.1 % level [12] while others gained false negative results when evaluating their Real-Time PCR methods [16]. Recent work comparing real-time PCR with liquid chromatography mass spectrometry methods demonstrated that the PCR technique could not always be applied to correctly assign gelatine species of origin in all samples due to no traces of DNA remaining in some products. Therefore, while a small amount of success has been reported for the DNA-based methods investigated by the scientific

community, alternative methodology capable of accurate, specific and precise determination with high sensitivity across a wide range of food types, which also addresses false positive and false negative issues in gelatine species determination, is required.

There are emerging technologies which focus on the screening of collagen peptides present in a food sample. Peptides are often more robust to degradation compared to DNA and whole proteins which become fragmented and denatured during manufacture. The peptide complement tends to be comparatively intact for the food material. Peptide mass spectrometry can be used to determine species-specific peptides. Matrix Assisted Laser Desorption Ionisation Time-of-flight Mass spectrometry (MALDI-ToF MS) has been used to compare and contrast the collagen peptide fingerprint of species. Indeed, this technology has been used in research to determine species markers in ancient archaeological collagen samples, so robust is the collagen protein [17].

In terms of using MALDI-ToF technology to support food integrity, issues regarding sensitivity have been reported. Pork gelatine could only be determined in bovine gelatine when present at 20 % (w/w) [18] whereas significantly greater sensitivity is required in the food chain with the UK Food Standards Agency requiring sensitivity at the 1 % (w/w) level. The early stages of work have also been carried out using low resolution liquid chromatography mass spectrometry (LC-MS) instruments using multivariate analysis to compare the mass spectral data for bovine, porcine and fish gelatine with success on a limited number of samples and sample types tested to date [2]. However, development of methods with integrated confirmatory techniques, such as tandem mass spectrometry (MS/MS) would prove valuable as a single test to determine origin.

One successful emerging technology, in the form of high-resolution accurate mass liquid chromatography mass spectrometry (HR LC-MS/MS), can be applied to compare and contrast samples of high sequence homology to determine species differences. The peptides in a food sample are separated by nano-flow liquid chromatography. Each peptide is fragmented and then further fragmented in the mass spectrometer and the accurate mass of each fragment ion is measured. Algorithms are used to determine the amino acid composition of each peptide and to ultimately determine species origin, screening for marker peptide.

A full scan (untargeted) high resolution, high accuracy mass spectrometry (HR LC-MS/MS) method is available for the qualitative determination of the animal origin of gelatine extracted from foods [19]. Despite the high levels of collagen amino acid sequence homology between bovine and porcine gelatine, the method can differentiate a wide range of species using a suite of peptides in a proprietary database which contains species-marker peptides to differentiate not only bovine and porcine, but also species including equine, ovine, piscine and poultry gelatines amongst others [20]. In this work, a library of collagen sequences was prepared using molecular mining of Expressed Sequence Tags (EST) and other databases, coupled with *de novo* sequencing from thirty-two different mammalian species, identified peptides which can be used as species markers in gelatine. Species identity of these peptides was verified by mapping the phylogeny of the peptides [20]. The quality and verification of the database is critical: unlike for other proteins, so aggressive is the gelatine manufacturing process, the modifications caused to the collagen protein during processing cannot necessarily be correctly predicted by conventional proteomics software and database packages in order to build a database. It is critical that the species specificity of marker peptides is independently verified by testing a wide range of same-species collagens so that potentially incorrect sequences are not attributed to species specificity [20]. This HR LC-MS/MS method has been evaluated on a range of foods. Based on the threshold applied by the UK Food Standards Agency to the adulteration of processed meats during the horse meat issues of 2013 which required detection of adulterant at 1 % (w/w), this method was evaluated on a range of

gelatine-rich foods containing an adulterating gelatine at levels of 0.5 % (w/w). Both the accuracy and the precision of the method were 100 % and the maximum specificity was also demonstrated ([19], unpublished data). The tissue origin (bone or skin) of the gelatine can also be determined by this method. Another benefit of untargeted methods is that all the peptide data from a sample can be archived and interrogated at a later date should the need arise in the future to investigate the presence of a new species of interest once the peptide sequence data for that species is available. HR LC-MS/MS methods benefit from confirmatory techniques also, by analysing the fragmentation patterns of the peptides to verify correct marker peptide, and thus species, assignment. This way, matrix interferences can be ruled out meaning the false positive rate of the method is not an issue which is another benefit over techniques such as ELISA or PCR.

Some progress towards developing a quantitative HR LC-MS/MS method has been made by developing internal standards by oxygen-18 labelling of gelatine marker peptides. The incorporation of stable isotopes into peptides results in a fixed mass shift with no effect on the chemical properties of the peptides. Therefore, the relative abundances of the labelled peptides from different samples can be accurately quantified using HR LC-MS/MS [9]. The method was developed on pure gelatines and its future application in the food industry relies on developing an extraction method and accurate measuring method prior to the analysis by HR LC-MS/MS.

Although showing excellent capability to determine the species origin of gelatines, high resolution accurate mass spectrometry methods require very high initial investment, highly trained personnel and elevated instrument upkeep costs. These instruments tend to be used more for research discovery purposes than for the routine analyses which tend to be required to support the food chain in terms of screening for adulteration. In the future, it is likely that more and more targeted methods will be developed from the data generated by these HR LC-MS/MS research instruments to screen for a pre-selected target list of species-specific marker peptides in gelatine food extracts. Such targeted methods, by Selected Reaction Monitoring mass spectrometry (SRM) are relatively low cost and are already used routinely to screen for other contaminants in the food chain including veterinary drugs, pesticide residues, mycotoxins, natural toxins, processing contaminants including acrylamide and materials which migrate into food from containers and packaging materials.

A recent evaluation of a targeted SRM mass spectrometry method tested forty-eight food samples simulating commercial food products and food supplement capsules containing bovine and porcine gelatine mixtures, alongside relevant positive and negative quality control samples. The foods were analysed in two ways: to determine the origin of the adulterating gelatine (a) when present at 1 % of the total mass of the food matrix and (b) when present at 1 % of the total mass of gelatine within the food matrix. The adulterating gelatine was present at as little as 0.07 % of the total food sample mass, depending on the food matrix type. The method showed 100 % accuracy and precision across all samples and the specificity of the method was also of the highest level, screening for fourteen bovine- and eight porcine-specific markers (Project FA0165, Department for Environment, Food and Rural Affairs, 2018[5]). Given that the EU Commissioning Body advised that the threshold for 'deliberate adulteration' of an undeclared meat product is 1 % w/w, this method is well within this tolerance. A further benefit of this form of technology is that there is also evidence that targeted mass spectrometry methods such as this one could offer a greater dynamic range than HR LC-MS/MS methods for quantification of peptides [21]. This is an aspect worthy of future investigation in relation to gelatine peptides in order to inform as to whether deliberate adulteration or accidental low concentration contamination may have taken

[5] Pending publication, https://www.gov.uk/government/organisations/department-for-environment-food-rural-affairs

place. SRM methods offer the benefits of screening for a wide range of known species peptide markers, screening for the precursor ion of each peptide and, critically, for each of its four confirmatory product ions, which must all be identified in a product to provide consumers and producers alike the confidence that the results are correct and not due for example to matrix interferences.

4. Overview of methods for authenticity testing

The following table provides a summary of the methods and the authenticity issues they address.

Analytical technique	Indicative data or analyte	Authenticity issue / information
Polymerase Chain Reaction (PCR)	e.g. Mitochondrial cytochrome b gene target	High levels of DNA degradation contribute to the lack of methods to determine the species origin of gelatine in foods.
High Resolution Accurate Mass Spectrometry	Determination of suite of marker peptides for a wide range of species by untargeted analysis.	Relevant to single species or mixtures of gelatines extracted from foods, sensitive to 0.5 % or lower, can screen for a wide range of species in a single analysis. Potential to develop quantitative analysis of food extracts. High financial investment required.
Selected Reaction Monitoring (SRM) Mass Spectrometry	Targeted analysis for a suite of species marker peptides.	Determination of species from a target list of marker peptides. Relevant to single species or mixtures of gelatine extracted from foods, sensitive to 0.07 % depending on matrix. Potential to develop quantitative methods. Suitable for routine, high throughput screening of foods.

5. Conclusion

As markets fluctuate regarding livestock in the food chain, the possibility exists for the carcasses of any animal species, for example horse, to be used to produce gelatine for financial gain.

There is evidence that adulteration of gelatine is occurring in the food chain and indeed the UK Food Standards Agency discovered fraudulent use of hydrolysed collagen in plumping agents added to chicken fillets in 2009. Research work has also discovered mislabelling of gelatines in food products [2]. Methods are required to distinguish gelatines from different species to support authenticity and integrity in the food chain and also to inform consumer choice to support ethical and religious preferences. Gelatine is a highly processed product, manufactured under conditions of high temperature and long-term exposure to acid or alkali. These conditions cause denaturation of DNA and protein structure and therefore conventional animal origin determination methods, such as PCR techniques and ELISA, cannot be applied.

Mass spectrometry methods are emerging for the determination of species origin of gelatine. Full scan (untargeted) technologies, coupled to strictly curated and independently verified databases, offer the capability to screen for a range of species in a single qualitative analysis and therefore the opportunity to uncover unexpected issues in the food chain during routine analysis. The potential now also exists to develop these methods to allow quantitation. The importance of such food

screening capabilities was highlighted by the unexpected discovery of fraudulently-added horse meat in processed foods in 2013. Furthermore, untargeted methods allow data to be archived and re-interrogated should the need arise in the future to investigate the presence of a new species of interest.

While HR LC-MS/MS methods require high initial investment and expert data interpretation, the alternative routine and higher throughput technology of SRM mass spectrometry can be applied to determine species in a targeted method to determine species from a pre-determined list of marker peptides. This technology has been tested both on samples when the adulterating gelatine was present at 1 % of the total mass of the food and when the adulterating gelatine was present at 1 % of the total gelatine content of the food. The method has also been shown to be sensitive to an adulterating gelatine present at less than 0.1 % (less than 0.1 % mass of the total mass of the food sample). The method offers excellent potential for quantitative analysis in the future to further support the food chain in terms of product adulteration. Finally, MS/MS methods offer confirmatory data to ensure correct species identification and to overcome false positive results caused by matrix interferences.

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