



## Review

## Texture methods for evaluating meat and meat analogue structures: A review



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## ABSTRACT

Meat analogue products are considered to help consumers reducing their meat consumption. Their key success factor is their high similarity in sensory properties compared to meat. Even though the structure and texture characteristics of meat are well documented, dedicated methods used to analyse meat analogues are limited still. This review summarises texture and structure analysis methods of meat and meat analogues: mechanical testing; for example Texture Profile Analysis, spectroscopy; for example NMR and imaging techniques; for example hyperspectral imaging. Furthermore, the advantages and limitations of each texture and structure method are described. Finally, characterizations aspects specific to meat analogues are discussed. Promising methods for future research are described that have potential to get more insight into the fibers of meat analogues and the structure development during thermomechanical processing of meat analogues.

*Industrial relevance:* To be commercially successful for large groups of consumers, alternatives for meat should be highly similar to meat. That is why meat analogues should resemble existing meat in their texture. It is thus important to understand the texture properties with the help of relevant techniques, such as mechanical, spectroscopy and imaging techniques. In this manuscript, we describe promising texture methods for characterization of properties specific to meat analogues. The development of novel techniques to quantify meat analogue properties will stimulate the development of meat analogues that satisfy the values and wishes of consumers.

### 1. Introduction

Plant protein-based meat analogues that mimic the sensory properties of meat could be a route to help consumers to reduce their meat consumption (Elzerman, Hoek, van Boekel, & Luning, 2011; Hoek et al., 2011; Michel, Hartmann, & Siegrist, 2021). A reduction of meat consumption might lead to a lower environmental footprint of the diet because meat production leads to intensive use of land, water and energy (Tilman & Clark, 2014; Weinrich, 2019). However, the different nature of plant materials compared to those of meat, renders the imitation of meat texture a challenge. For example, plant proteins do not naturally occur in fibrillar orientation (Fuhrmeister & Meuser, 2003; Sun & Arntfield, 2010; Taherian et al., 2011). Although meat products are widely different in their properties, they do share many characteristics that they do not share with plant proteins. For example, the very small length scale of meat muscle structure consists of myofibrillar

protein and myoglobin positioned into a hierarchical fibrillar structure that is not easily replicated in plant-based meat analogues. The unique juiciness of meat is also a result of this hierarchical structure (Frank, Oytam, & Hughes, 2017). Besides, many of the unique meat properties are strongly dependent on the internal structure of the meat, which ranges from 100 nm to 100  $\mu\text{m}$ .

To be commercially successful in the short term, and for large groups of consumers, alternatives should not deviate too much from their current meal and thus resemble existing meat in their texture (Elzerman et al., 2011; Hoek et al., 2011; Michel et al., 2021). As described by Dekkers, Boom, and van der Goot (2018), two approaches exist to make meat analogues: top-down and bottom-up. The latter approach aims at mimicking the full hierarchical structure of meat, but these methods are laborious and require more resources than the top-down approach. Examples of the top-down approach are the shear cell technology and extrusion. Extrusion is widely used industrially to make currently

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available meat analogues. However, the fibrousness of meat analogues from plant proteins created via a top-down approach is typically less hierarchical. An important question though is whether similarities on a larger length scale are sufficient for similarities in sensory properties already. The first step towards insights is a characterization of the structures at different length scales for both meat and meat analogues.

The texture of meat has been widely studied. Many analytical techniques and methods are established for meat and fish, including sensory evaluation and mechanical methods. Therefore, while the existing methods are quite adequate for meat, it is not clear whether these would also be sufficient to characterize the differences between meat and the plant-based matrices. The objective of this paper is therefore to understand the potential of those analytical methods developed for meat to be used for meat analogues as well. To investigate this, we will review the available methods on their suitability for analysing plant-based meat analogues. We will then assess whether they cover the complete parameter space and describe the need for new techniques specifically for those properties of plant materials that are different from meat products.

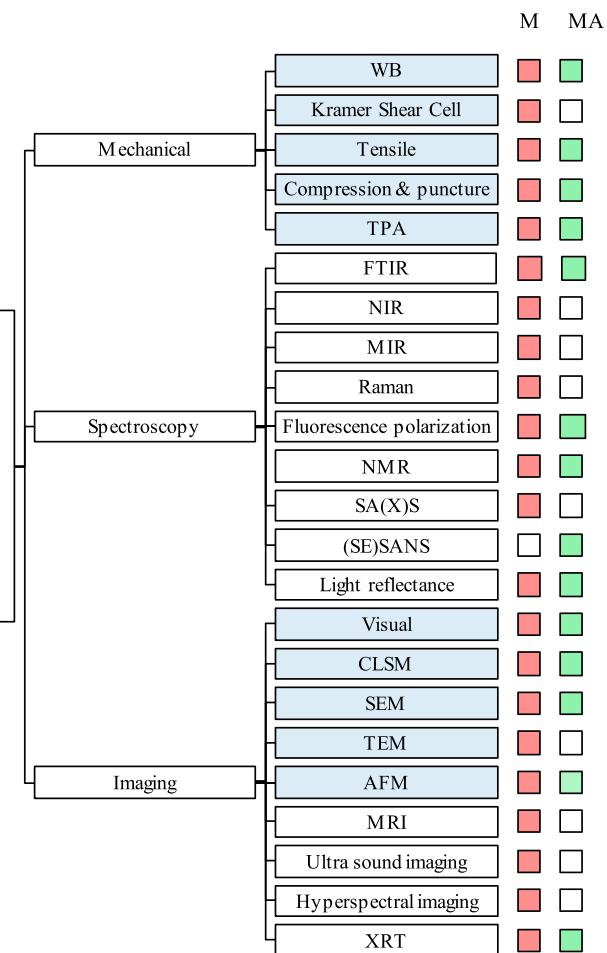
## 2. Instrumental techniques for texture of meat and meat analogues

Although texture is 'the combination of the rheological and structure (geometrical and surface) attributes of a food product perceptible by means of mechanical, tactile, and where appropriate, visual and auditory receptors' as defined in 2008 by the International Standards Organization (ISO, 2008), most techniques are focused on instrumental testing. Instrumental techniques to measure the texture of meat and meat analogues are often used instead of sensory experiments, as the latter is expensive, time-consuming and difficult to make quantitative. Instrumental techniques provide objective information on different structural parameters. Meat texture is characterized by different methods. Each method analyses meat products at a certain length scale. Typical approaches to study the texture and structure of meat and meat analogues include mechanical, spectroscopy and imaging characterization methods. This paper summarizes the basic technologies and the most recent advances of those technologies for processing different types of meat (i.e. beef, pork, and poultry) and meat analogues (i.e. shear cell structures and extruded products) (Fig. 1).

### 2.1. Mechanical techniques

Traditionally, texture is evaluated with mechanical methods. Such methods are used to analyse the mechanical properties of a product through compressing, shearing and/or pulling. Mechanical methods are applied to all kinds of food products, such as cheese, candy, pasta, but also meat and meat analogues. A limitation of the mechanical methods is that they are destructive, hence tested products cannot be used for other applications. A folding test is often performed as the first mechanical test. The test assesses the structural failure of both meat and meat analogue products based on a five-point grading system (Herrero et al., 2008; Kamani, Meera, Bhaskar, & Modi, 2019). It is an easy and fast method to obtain basic information about the texture of a product, but it is not fully quantitative.

After performing the folding test, one or more of the following tests are done. The Warner-Bratzler test measures the maximum shear force as a function of knife cutting movement through a meat product (Novakovi & Tomaševi, 2017). It is difficult to give a precise physical meaning to the Warner-Bratzler shear force because it measures a combination of shearing, compression and tensile stress, making it more a measurement of overall quality attributes (Voisey, 1976). Nevertheless, the Warner-Bratzler test is used to analyse the texture of different types of meat products, in particular whole muscle products and sausages (Table 1). The probe of the Warner-Bratzler test consists of a single blade with a V-shaped notch (Morey & Owens, 2017). This blade is used



**Figure 1.** Destructive (■) and non-destructive (□) texture and structure methods used for meat (M, ■) and meat analogues (MA, ■). Abbreviations: WB, Warner-Bratzler; TPA, Texture Profile Analysis; NIR, Near-infrared; MIR, Mid-infrared; SA(X)S, Small-angle (X-ray) scattering; (SE)SANS, (Spin-echo) Small-angle neutron scattering; CLSM, Confocal laser scanning microscopy; SEM, Scanning electron microscopy; TEM, Transmission electron microscopy; AFM, Atomic force microscopy; MRI, Magnetic resonance imaging; XRT, X-ray tomography.

to cut through the meat product, usually perpendicular to the longitudinal positioning of the muscle fibers, but some studies additionally measure the parallel direction (Cierach & Majewska, 1997). Furthermore, previous studies suggested that differences in the device, blade, product diameter, or settings used, influence the results (Novakovi & Tomaševi, 2017; Pool & Klose, 1969; Voisey & Larmond, 1974; Wheeler, Shackelford, & Koohmaraie, 1996). Thus, standardization will be important to obtain results with the Warner-Bratzler method that allows comparison between studies.

A few studies use the Kramer Shear Cell test to measure meat texture in addition to the Warner-Bratzler test (Table 1). This test simulates a single bite into a piece of food. The principle is similar to the Warner-Bratzler test, but it has multiple, blunt blades arranged in parallel that correspond to specific slots in the base of the cell (Barbut, 2015; Morey & Owens, 2017). Products, often multiple at once, are placed in the cell; the products are compressed and sheared when the blades push the products through the slots. The resulting parameters are averages of the forces required to shear the full product (Morey & Owens, 2017). This makes it possible to measure products with an uneven surface for example. Similar to the Warner-Bratzler test, the Kramer Shear Cell test does not evaluate a single mechanical property. Instead, it measures a

**Table 1**

Overview of mechanical techniques used in studies on meat and meat analogues from 2005 onwards. The colours in red and green indicate that the method is used for meat and meat analogues, respectively.

Technique	Properties	Product (m/ma)	Reference
Warner-Bratzler	Warner-Bratzler shear force, slope at yield, shear energy	Steak (m)	(Destefanis, Brugia, Barge, & Dal Molin, 2008; Peña-Gonzalez, Alarcon-Rojo, García-Galicia, Carrillo-Lopez, & Huerta-Jimenez, 2019; Ruiz De Huidobro, Miguel, Blázquez, & Onega, 2005)
		Chicken breast (m)	(Cavitt, Xiong, & Owens, 2005; U-Chupaj et al., 2017; Xiong, Cavitt, Meullenet, & Owens, 2006)
		Ham (m)	(Bermúdez, Franco, Carballo, & Lorenzo, 2014; Rizo, Peña, Alarcon-Rojo, Fiszman, & Tarrega, 2019)
		Meat patty (m)	(Naveena, Sen, Muthukumar, Vaithianathan, & Babji, 2006)
		Sausage (m)	(Barbut, Wood, & Marangoni, 2016; Cáceres, García, & Selgas, 2006; Del Nobile et al., 2009; Purohit, Reed, & Mohan, 2016; Szerman et al., 2015)
		High moisture extruded product (ma)	(Caporgno et al., 2020; Osen, Toelstede, Wild, Eisner, & Schweiggert-Weisz, 2014; Palanisamy, Töpfl, Aganovic, & Berger, 2018)
		Low moisture extruded product (ma)	(Samard & Ryu, 2019b)
		Patty (ma)	(Forghani, Eskandari, Aminlari, & Shekarforoush, 2017)
		Sausage (ma)	(Kamani, Meera, Bhaskar, & Modi, 2019)
		Chicken breast (m)	(Cavitt, Meullenet, Gandhapuneni, Youm, & Owens, 2005; Del Olmo, Morales, Ávila, Calzada, & Nuñez, 2010; Xiong et al., 2006)
Kramer Shear Cell	Shear force, maximum slope, total energy	Rabbit meat (m)	(Bianchi, Petracci, Pascual, & Cavani, 2007)
		Meat patty (m)	(Holliday, Sandlin, Schott, Malekian, & Finley, 2011)
		Steak (m)	(Peña-Gonzalez et al., 2019; Ruiz De Huidobro et al., 2005)
		Chicken breast (m)	(Dolores Romero de Ávila et al., 2014; U-Chupaj et al., 2017)
		Ham (m)	(Dolores Romero de Ávila et al., 2014; Rizo et al., 2019)
		Meat patty (m)	(Das, Prabhakaran, Tanwar, & Biswas, 2015)
		Sausage (m)	(Herrero et al., 2007, 2008; Laranjo et al., 2015; Purohit et al., 2016)
		Sausage (ma)	(Arora, Kamal, & Sharma, 2017; Kamani et al., 2019; Majzoobi, Talebanfar, Eskandari, & Farahnaky, 2017; Stephan, Ahlborn, Zajul, & Zorn, 2018)
		Patty (ma)	(Forghani et al., 2017; Kim et al., 2011; Lee & Hong, 2019)
		High moisture extruded product (ma)	(Chiang et al., 2019)
Texture Profile Analysis	Hardness, chewiness, springiness, adhesiveness, gumminess, resilience, etc.	Low moisture extruded product (ma)	(De Angelis et al., 2020; Samard, Gu, & Ryu, 2019; Samard & Ryu, 2019a, 2019b)
		Patty (ma)	(Christensen et al., 2011; Panea et al., 2018)
		High moisture extruded product (ma)	(Alirezalu, Hesari, Eskandari, Valizadeh, & Sorousazar, 2017)
		Rabbit meat (m)	(Cavitt, Meullenet, et al., 2005)
		Meat patty (m)	(Braeckman, Ronsse, Hidalgo, & Pieters, 2009; Naveena et al., 2006)
		Sausage (ma)	(Arora et al., 2017; Kamani et al., 2019)
		Steak (m)	(Zhang et al., 2019b)
		Chicken breast (m)	(Dolores Romero de Ávila et al., 2014)
		Ham (m)	(Dolores Romero de Ávila et al., 2014)
		Sausage (m)	(Darus, Masson, & Amico, 2005; Herrero et al., 2007, 2008)
Single compression	Stress, maximum compression load	Shear cell structures (ma)	(Dekkers, Nikiforidis, et al., 2016; Krintiras et al., 2015; Schreuders et al., 2019; Wang, Tian, Boom, & Goot, 2019)
		High moisture extruded product (ma)	(Pietsch, Werner, Karbstein, & Emin, 2019)
		Steak (m)	
		Sausage (m)	
Puncture test	Puncture force, puncture shear force	Chicken breast (m)	
		Meat patty (m)	
		Sausage (ma)	
		Steak (m)	
		Chicken breast (m)	
Tensile test	Tensile force, breaking strength	Ham (m)	
		Sausage (m)	
		Shear cell structures (ma)	
		High moisture extruded product (ma)	

combination of the effects of compression and shear, which could be seen as a limitation of the method. Xiong, Cavitt, Meullenet, and Owens (2006) compared the potential of the Kramer Shear Cell and the Warner-Bratzler method for the prediction of sensory tenderness of chicken breast, and found that the shear values correlated well with descriptive sensory attributes as well as consumer sensory attributes. Another study also indicated that both methods were successful in evaluating rabbit meat tenderness and presented similar levels of correlation with sensory scores (Bianchi, Petracci, Pascual, & Cavani, 2007). For both methods, the products need to have a specific thickness. This means that these methods can only be used on meat and meat analogues (extruded products, sheared, patties, sausages, etc.) that fulfil these requirements. While the methods are therefore suitable within a pre-defined range of similar products with a limited variation of parameter values, it is not clear yet whether these methods would also allow the comparison with plant-based meat analogues, which can have quite different properties. The Kramer Shear Cell is not yet used to

measure textural properties of meat analogues as far as the authors are aware.

Another mechanical test is the tensile test, which measures the resistance of a product against tearing. A product is mounted between two grips and extended in the tensile direction at a fixed speed until failure. Tensile parameters such as maximum rupture force, breaking strength and energy to fracture can be calculated from obtained stress and strain values. In general, tensile products have a dumbbell or dog-bone shape to conduct the stress towards the middle of the product and induce failure at the intended location. Tensile tests are used with a wide product range such as sausages, frankfurters, ham, whole muscle products (Table 1) and in the past also meat patties (Beilken, Eadie, Griffiths, Jones, & Harris, 1991; Spadaro & Keeton, 1996). Tensile tests have also been applied to meat analogues (Dekkers, Nikiforidis, & van der Goot, 2016; Schreuders et al., 2019). The ratio between the tensile strengths parallel and perpendicular to the (muscle-) fiber orientation provides insight into the anisotropy of the product (Barbut, 2015; Dekkers,

Nikiforidis, & van der Goot, 2016). For both meat and meat analogues a few studies calculate the anisotropic index (Dekkers, Hamoen, Boom, & van der Goot, 2018; Krintiras, Göbel, Van Der Goot, & Stefanidis, 2015; Schreuders et al., 2019). Christensen, Purslow, and Larsen (2000) studied the tensile properties of whole beef meat as well as single muscle fibers and perimysial connective tissue. The use of a mechanical testing method for single muscle fibers is unique and is not realistic with other mechanical testing methods. Therefore, the tensile test might be able to study the texture of products at a smaller length scale than other mentioned mechanical testing methods. This would allow for measuring the tensile strength of single meat analogue fibers from for example calcium-caseinate materials (Wang, Tian, Boom, & Goot, 2019).

Another mechanical method to quantify food texture is a single compression test. The single compression test is often performed as an axial compression test between two flat plates (Barbut, 2015). The products have to be smaller than the contact area of the probe in use. Products can be compressed until failure, or to a certain level of deformation. Single compression tests are not used often (Table 1) as a double compression test, often called Texture Profile Analysis (TPA), can provide more information within a single experiment. Similar considerations regarding reliability for single compression tests have to be taken as for TPA tests (Lepetit & Culoli, 1994). TPA is a compression technique that combines multiple textural parameters such as hardness, chewiness, adhesiveness, cohesiveness and springiness in a single measurement. The TPA parameters can be divided into primary parameters (hardness, springiness, adhesiveness and cohesiveness) and secondary parameters (gumminess, chewiness, resilience) (Novakovi & Tomaševi, 2017). Primary parameters can be directly determined from the obtained force/time graph, while secondary parameters are derived from the primary parameters. The test is based on simulating the biting action of the mouth by a two-cycle compression series (Barbut, 2015). TPA tests are widely applied on meat analogues and meat products ranging from whole muscle products to emulsified sausage products (Table 1). A puncture test is similar to a compression test, but the probe contact area is now much smaller than the size of the product, for example through use of a needle-shaped probe. During a puncture test, the material is compressed to a certain strain by a probe to quantify properties such as maximum force, breaking strength, and the penetration depth. According to Barbut (2015), it is commonly used for restructured products and emulsified meat products. However, literature only showed the use of a puncture test on chicken breast, meat patties and meat analogue sausages (Table 1). Penetration force, as measured with the puncture test, was found to be lower in sausages based on plant proteins than those based on poultry (Kamani et al., 2019). This indicated that the breaking force required to penetrate the outer skin of plant protein sausages is lower than in chicken sausages. In addition, penetration depth of plant based sausages was used as a measure for the strength of binding agents (Arora, Kamal, & Sharma, 2017). As meat and meat analogues are often heterogenous in structure, it can be hard to obtain compression type measurements that is representative for the whole product. A recent technique of multi-point indentation characterizes the local mechanical texture of meat and meat analogues by mapping the elastic modules as measured with a spherical probe of radius 1 mm (Boots et al., 2021).

Dolores Romero deÁvila, Isabel Cambero, Ordóñez, de la Hoz, and Herrero (2014) studied the mechanical properties of commercial cooked meat products by both TPA and tensile tests. They showed that the parameters from the TPA could be used to construct models to predict tensile test parameters such as breaking strength and energy to fracture, removing the need for tensile tests. Furthermore, Ruiz De Huidobro, Miguel, Blázquez, and Onega (2005) recommended the TPA method over the Warner-Bratzler method to predict meat texture on basis of a better correlation with sensory data and a higher accuracy. Similar conclusions were drawn by Caine, Aalhus, Best, Dugan, and Jeremiah (2003) who showed that TPA parameters correlated better with variations in sensory results of beef tenderness than the Warner-Bratzler test. Similar to the Warner-Bratzler test, the TPA test requires standardized

testing methods for trustworthy comparison between studies, and they can probably only make reliable correlations in limited parameter space.

For both meat and meat analogues textural elements can be studied with the Warner-Bratzler test, the tensile test, the TPA test and other compression techniques. The Kramer Shear Cell has only been used to quantify the texture of meat products but offers several benefits, such as the possibility to measure uneven products. Therefore, it might be a future direction for texture analysis of meat analogues. Furthermore, the recently developed multi-point indentation technique shows high potential to characterize heterogeneous meat and meat analogue structures. All described mechanical techniques analyse texture at a macroscale, except for the tensile test which can be used to analyse single muscle fibers at a smaller length scale. Therefore, we believe that the tensile test may also be used to analyse single fibers from meat analogues in the future. Furthermore, there is great importance for standardized testing methods of all mechanical tests described in this review to be able to compare different products (meat and meat analogues) and translate the quantitative analysis in sensory properties.

## 2.2. Spectroscopy

Spectroscopy (infrared, Raman, fluorescence polarization, NMR and light scattering) provides insight into the local composition (mostly surface of the product), intermolecular interaction as well as anisotropy of meat and meat analogues (Table 2). Proteins, lipids, water and other substances may be localised and quantified simultaneously. Spectroscopy is direct and non-invasive and requires only small products usually.

Infrared (IR) spectroscopy provides information on the chemical composition by measuring infrared absorption spectra. The spectrum can be used to characterize specific chemical bonds in products and can yield information about the composition, but also about the state of individual substances. In meat products, Fourier Transform IR spectroscopy (FTIR) was used to monitor conformational changes of myofibrillar proteins and connective tissue (Kohler et al., 2007; Perisic, Afseth, Ofstad, & Kohler, 2011). In meat analogues, FTIR was used to identify structural changes after processing in zein, pea and spirulina/lupin protein (like  $\alpha$ -helix and  $\beta$ -sheet) (Beck, Knoerzer, & Arcot, 2017; Mattice & Marangoni, 2020; Palanisamy, Töpfl, Berger, & Hertel, 2019).

A near-infrared (NIR) spectrum is often divided into two sections, namely, short wave near-infrared spectral region (SW-NIR) of 780–1100 nm and long wave near-infrared spectral region (LW-NIR) of 1100–2526 nm (Cheng et al., 2013). The spectrum shows broad overlapping peaks and large baseline variations, which requires mathematical processing to extract compositional information (Subramanian & Rodriguez-Saona, 2009). In meat products, NIR-spectra were used to subsequently predict the chemical composition (such as crude protein, intramuscular fat, moisture/dry matter, ash, gross energy, myoglobin and collagen), technological parameters (water holding capacity, Warner-Bratzler and slice shear force) and sensory attributes (juiciness, tenderness or firmness) (Prieto, Roehe, Lavin, Batten, & Andrés, 2009). This would fully eliminate the use of destructive analysis methods like mechanical measurements. However, its prediction is limited to a small range of products and was further hindered by the heterogeneity of intact meat products, and inconsistent product preparation.

The mid-infrared (MIR) spectrum is divided into four sections, namely, the X–H stretching region ( $4000\text{--}2500\text{ cm}^{-1}$ ), the triple bond region ( $2500\text{--}2000\text{ cm}^{-1}$ ), the double bond region ( $2000\text{--}1500\text{ cm}^{-1}$ ), and the fingerprint region ( $1500\text{--}400\text{ cm}^{-1}$ ) (Cheng et al., 2013). MIR spectroscopy was used to obtain information on the conformation of proteins (such as  $\alpha$ -helix or  $\beta$ -sheet) (Carbonaro & Nucara, 2010). Another study showed the analysis of food raw materials (such as skimmed milk powder, chicken meat powder, soy protein isolate, pea protein isolate and wheat flour) on the presence of several potential food adulterants (nitrogen-rich compounds, foreign protein and bulking agent) (da Costa Filho, Cobuccio, Mainali, Rault, & Cavin, 2020).

**Table 2**

Overview of spectroscopy techniques used in studies on meat and meat analogues from 2005 onwards. The colours in red and green indicate that the method is used for meat and meat analogues, respectively.

Technique	Properties	Product (m/ma)	Reference
FTIR	Composition and secondary protein conformation	Beef muscle (m) High moisture extruded product (ma)	(Kohler et al., 2007; Perisic et al., 2011) (Beck, Knoerzer, & Arcot, 2017; Mattice & Marangoni, 2020; Palanisamy, Töpfl, Berger, & Hertel, 2019)
NIR	Composition	Pork (m) Chicken (m) Beef (m)	(Balage, Silva, Bonin, Mazon, & Figueira, 2013; Fulladosa, Santos-Garcés, Picouet, & Gou, 2010; Gou et al., 2013; Mabood et al., 2020; Rady & Adedeji, 2018) (Jia, Wang, Yoon, Zhuang, & Li, 2018; Krepper et al., 2018; Nolasco Perez et al., 2018; Wold, Mage, Løvland, Sanden, & Ofstad, 2019) (Bonin et al., 2020; Cafferky et al., 2020; Cozzolino & Murray, 2004; Rady & Adedeji, 2018; Ripoll, Albert, Panea, Olleta, & Sanudo, 2008; Weng et al., 2020) (Carbonaro & Nucara, 2010).
MIR	Composition and secondary protein conformation	Beef (m)	
Raman	Secondary protein conformation & amino acid composition	Pork (m) Beef (m) Chicken (m) Sheep (m) Beef (m) High moisture extruded product (ma)	(Chen & Han, 2011; Olsen, Rukke, Flätten, & Isaksson, 2007; Pérez-Santaescolástica et al., 2019; Scheier, Scheeder, & Schmidt, 2015; Wang, Lonergan, & Yu, 2012) (Chen et al., 2020) (Phongpa-Ngan, Aggrey, Mulligan, & Wicker, 2014) (Schmidt, Scheier, & Hopkins, 2013) (Luc et al., 2008) (Ranasinghesagara, Hsieh, & Yao, 2005)
Fluorescence polarization spectroscopy	Muscle fiber direction		
NMR	Intermolecular interaction	Pork (m) Beef (m) Chicken (m) Shear cell structures (ma) Low and high moisture extruded product (ma)	(García-García, Cambero, Castejón, Escudero, & Fernández-Valle, 2019) (Graham et al., 2010; Jung et al., 2010) (Shaarani, Nott, & Hall, 2006) (Dekkers, de Kort, et al., 2016; Schreuders et al., 2020) (Chen, Wei, & Zhang, 2010)
SA(X)S	Insight into repetitive structure	Sheep (m) Goat (m) Beef (m)	(Goh et al., 2005; Hoban et al., 2016) (Hoban et al., 2016) (Hughes, Clarke, Li, Purslow, & Warner, 2019)
(SE)SANS	Fiber orientation	Shear cell structures (ma)	(Krintiras et al., 2014; Tian et al., 2020, 2018)
Light reflectance	Internal structure and fiber orientation	Beef (m) High moisture extruded product (ma)	(Ranasinghesagara & Yao, 2007) (Ranasinghesagara, Hsieh, Huff, & Yao, 2009; Ranasinghesagara, Hsieh, & Yao, 2006)

Raman spectroscopy provides information on secondary protein conformation (i.e.  $\alpha$ -helix and  $\beta$ -sheets) as well as on the amino acid composition (Overman & Thomas, 1999). In meat products, Raman spectroscopy has been successfully correlated with quality parameters such as protein solubility, apparent viscosity, water holding capacity, instrumental texture methods, and fatty acid composition (Herrero, 2008). Furthermore, Raman spectra could be correlated with sensory attributes (i.e. juiciness and chewiness) of pork loins (Wang, Lonergan, & Yu, 2012) and identify structural changes of muscle food components (proteins, lipids and water) due to handling, processing and storage (Pérez-Santaescolástica et al., 2019).

Fluorescence polarization spectroscopy analyses the natural fluorescence from a product. In meat, tryptophan is the major intrinsic fluorophore. It is a constituent of the proteins that have two preferential directions of alignment both parallel and perpendicular to the muscle fiber direction. Fluorescence polarization was used to characterize the structural organization and modifications related to sarcomere length in meat caused by processing (Luc, Clerjon, Peyrin, Lepetit, & Culioni, 2008) and in-line detecting of cold shortening in the bovine muscle (Luc et al., 2008). In meat analogues, fluorescence polarization can be used to characterize the anisotropy in high moisture extruded soy protein (Yao, Liu, & Hsieh, 2004). This method is based on the theory that polarization states of fluorescence light are affected by the structure of a product. It was found that products with a higher degree of fiber formation showed a higher polarization degree (Ranasinghesagara, Hsieh, & Yao, 2005).

Nuclear magnetic resonance spectroscopy (NMR) provides insights into the interaction between molecules (for example water-protein interactions) and thus provides insight into the structural features of meat and meat analogues. Several studies reviewed the application of ( $^1\text{H}$ ,  $^{13}\text{C}$  and  $^{31}\text{P}$ ) NMR in meat (Bertram & Ersten, 2004; Renou, Bielicki, Bonny, Donnat, & Foucat, 2003). NMR is also used to study water-protein interaction and correlate this with macroscopic properties such as water holding capacity, cooking loss, water and fat content and distribution, and changes associated during processing and storage (such as slaughtering, salting, frozen storage) (Marcone et al., 2013; Micklander, Peshlov, Purslow, & Engelsen, 2002). In plant-based materials, Time Domain (TD)-NMR gives an indication of the water-binding capacity of different proteins (gluten, soy protein isolate, pea protein isolate and lupin protein concentrate) (Peters, Vergeldt, Boom, & van der Goot, 2017). In addition, the water distribution was studied in a soy protein-gluten blend (Dekkers, de Kort et al., 2016) and pea protein-gluten blend (Schreuders, Bodnár, Erni, Boom, & der Goot, 2020).

Small-angle scattering (SAS) methods provide structural information over a size range from nanometer-to-micron length scale, being 0.2–100 nm using light, 1–100 nm using X-rays and 1–20 nm using neutrons (Larson, 1999, p. 150). In small-angle X-ray scattering (SAXS), an X-ray beam passes through a product and encounters structural obstructions (like collagen or myofibrils). SAXS provides insight into the repetitive structure in a product, such as the structure of the fibrils of actin, myosin

and collagen, and potentially provides estimates of the intramuscular fat (Goh et al., 2005; Hoban et al., 2016; Hughes, Clarke, Li, Purslow, & Warner, 2019). Small-angle neutron scattering (SANS) is used to investigate the structure on smaller scales and was used to study the internal structure of a fibrous calcium caseinate material (Tian et al., 2020). Spin-echo small-angle neutron scattering (SESANS) based on neutron diffraction can distinguish structures over three orders of magnitude – from 10 nm up to 10  $\mu\text{m}$ . SESANS quantified the thickness ( $\pm 138 \mu\text{m}$ ) and the number of fiber layers ( $\pm 36$ ) and the orientation of fibers in soy protein–gluten blends that were subjected to heat and shear deformation in a Couette Cell (Krintiras, Göbel, Bouwman, van der Goot, & Stefanidis, 2014). SESANS was also used to study the size and shape of the air bubbles in meat analogues of calcium caseinate (Tian, Wang, van der Goot, & Bouwman, 2018).

The continuous-time random walk (CTRW) theory of light transport has been used to study the spatial distribution of light reflectance on the surface of a (fibrous) product (Weiss, Porrà, & Masoliver, 1998). According to this theory, optical scattering depends on the transitional properties of scattering. The pattern of the scatter recorded by transmission or backscatter contains information on the internal structure of a material, such as meat (Ranasinghesagara & Yao, 2007) and meat analogues (Ranasinghesagara, Hsieh, Huff, & Yao, 2009; Ranasinghesagara, Hsieh, & Yao, 2006). In meat analogues, this method visualizes the degree of fiber formation and fiber orientation which shows potential as a fast, non-destructive method to monitor fiber formation in meat analogues (Ranasinghesagara et al., 2009; Ranasinghesagara et al., 2006). An extension of light scattering is diffusing wave spectroscopy (DWS), in which products with strong multiple scattering can be measured. In this novel DWS technique, the transport of photons through turbid products is treated as a diffusion process (Niu et al., 2019). In meat, DWS has been used to study the gelation process of myofibrillar protein extracted from squid (Niu et al., 2019).

In summary, spectroscopy can yield important information about the overall resolved composition of both meat and meat analogue, as well as intermolecular interactions and even about conformational changes of substances like proteins. It can be expected that the spectra of meat and meat analogues will be quite different because the spectra contain information about molecular properties. This limits its use for the actual comparison of the two types of materials. However, prediction models could be built from the correlation between the spectra and mechanical properties to make indirect comparisons between the materials. Spectroscopy can also give some information on the anisotropy. Light reflectance and SAS are promising methods to explore further for meat analogues to quantify fiber formation as it is relatively simple and easily incorporated into processing equipment, which will help to investigate the formation of the mesoscopic structure. SAXS and (SE)SANS methods typically yield information on smaller scales, but can also help in understanding the way the anisotropy is created from smaller-scale associations. These techniques require however very large infrastructure, and will thus be limited to research purposes.

### 2.3. Imaging

Imaging techniques can be used to reveal the structure of meat and meat analogues (Table 3). Visual inspection through splitting a meat or meat analogue is commonly used by product developers (Ranasinghesagara, 2008). Visual inspection is fast but destructive, not quantitative and prone to subjectivity. Microscopic (SEM, TEM, CLSM, AFM) characterization is used to construct images on different length scales ranging from macro to nano structure. The main drawback of those techniques is that they are destructive. Imaging using spectroscopic methods, such as MRI, ultrasound, hyperspectral and X-ray imaging does not require sample destruction. Image processing can be used to quantify the colour, shape, size, porosity and surface texture features of meat (Chmiel, Słowiński, & Dasiewicz, 2011; Du & Sun, 2006a, 2006b; Jackman, Sun, & Allen, 2011; Li, Kutsanedzie, Zhao, & Chen, 2016; Li,

Tan, & Shatadal, 2001; Ruedt, Gibis, & Weiss, 2020; Taheri-Garavand, Fatahi, Omid, & Makino, 2019). In meat analogues, edge detection, Hough transformation and region of interest analysis are used to quantify the fiber index value, which is shown to be strongly correlated with the polarization index (Ranasinghesagara et al., 2005).

Confocal laser scanning microscopy (CLSM) is a fluorescence technique to acquire 2D and limited 3D images of meat and meat analogue products. In meat, CLSM was used to visualize the connective tissue, myofibers and myofilaments and to monitor differences in structure between fresh and cooked meat of pork muscle, comminuted meat gels and beef (Du & Sun, 2009; Liu & Lanier, 2015; Straadt, Rasmussen, Andersen, & Bertram, 2007). A combination of CLSM and NMR yielded information about microstructural changes and water distribution in meat (Straadt et al., 2007). In meat analogues, CLSM has been used to visualize the effect of deformation on proteinaceous domains by comparing a sheared and a non-sheared pea protein-gluten blend (Schreuders et al., 2019). The domains were aligned along the shear direction in these blends (Schreuders et al., 2019), in soy protein concentrate (Grabowska et al., 2016) and soy protein-gluten (Dekkers, Emin, Boom, & van der Goot, 2018) after staining with Rhodamine B. Both the soy, pea and gluten showed fluorescence; the difference in intensity was used to indicate differences in protein concentration in different parts of the products.

Scanning electron microscopy (SEM) produces a surface image with resolution down to  $\sim 0.5 \text{ nm}$ . In meat products, SEM has been used to reveal process-related changes in meat structure (Cheng & Parrish, 1976; Hearne, Penfield, & Goertz, 1978; Wu, Dutson, & Smith, 1985). However, extensive sample preparation is needed for materials containing water or fat. These preparations can significantly change the original structure and may cause artefacts. Several techniques have been developed to overcome the disadvantages of high-vacuum SEM, in most cases at the cost of resolution. In cryo-SEM, water is frozen and may remain in that state in the product. Cryofixation is used to observe changes in the microstructure of beef steaks versus several cooking methods like temperature, time and treatments (García-Segovia, Andrés-Bello, & Martínez-Monzo, 2007) and of pork versus freezing rate and frozen storage time (Ngapo, Babare, Reynolds, & Mawson, 1999). Variable pressure scanning electron microscopy (VP-SEM) is used to examine the microstructure of meat products like the distribution of protein and fat phases in meat products (Liu & Lanier, 2015). Environmental scanning electron microscopy (ESEM) observes wet products at normal vapour pressures. This technique has been successfully used to investigate the microstructural changes of muscle meat in various meat types by heat treatment (Yarmand & Baumgartner, 2000; Yarmand & Homayouni, 2010). The shrinkage of pressure-treated and cooked pork meat structure was observed by ESEM. These ESEM observations were used to provide evidence for a higher shear force as measured with the Warner-Bratzler test (Duranton, Simonin, Chéret, Guillou, & de Lamballerie, 2012). SEM analysis combined with Energy-Dispersive X-ray spectroscopy (EDX) may identify the spatially resolved elemental composition of a surface and therefore identify the distribution of different components over the material surface (Ozuna, Puig, García-Pérez, Mulet, & Cárcel, 2013).

SEM has been used to study the microstructure of meat analogues. High moisture extruded soy protein isolate-wheat starch revealed a fine and tightly connected network structure (Lin, Huff, & Hsieh, 2002). In soy protein isolate - pectin blends, alignment along the shear direction was observed (Dekkers, Nikiforidis, & van der Goot, 2016). Soy protein with increasing levels of iota carrageenan showed a more compact network correlated with changes in cooking yield and expressible moisture (Palanisamy, Töpfl, Aganovic, & Berger, 2018). SEM of high moisture extruded lupin protein concentrate and isolate showed that a denser microstructure and higher number of fibrous layers were created by increasing temperature and screw speed along with decreasing water feed (Palanisamy, Franke, Berger, Heinz, & Töpfl, 2019).

Like SEM, transmission electron microscopy (TEM) requires

**Table 3**

Overview of imaging techniques used in studies on meat and meat analogues from 2005 onwards. The colours in red and green indicate that the method is used for meat and meat analogues, respectively.

Technique	Properties	Product (m/ma)	Reference
CLSM	2D and 3D visualization	Beef (m) Chicken (m) Pork (m) Shear cell structures (ma)	(Du & Sun, 2009) (Liu & Lanier, 2015) (Liu & Lanier, 2015; Straadt et al., 2007) (Dekkers, Emin, Boom, & van der Goot, 2018; Grabowska et al., 2016; Schreuders et al., 2019)
SEM	Surface image	Beef (m) Pork (m) Chicken (m) Goat (m) Shear cell structures (ma) High moisture extruded product (ma)	(García-Segovia et al., 2007; Mulot, Fatou-Toutie, Benkhelifa, Pathier, & Flick, 2019) (Duranton et al., 2012; García-García et al., 2019; Larrea et al., 2007; Ozuna et al., 2013) (Liu & Lanier, 2015) (Yarmand & Homayouni, 2010). (Dekkers, Nikiforidis, et al., 2016)
TEM	Inner structure	Beef (m) Pork (m)	(Listrat et al., 2015; Zhu et al., 2018) (Larrea et al., 2007)
AFM	Local 3D structure of a surface on nanometric scale	Goat (m) Beef (m) Chicken (m) High moisture extruded product (ma)	(Gao et al., 2016) (Wan, Wang, Wang, Zan, & Zhu, 2018) (Chen, Xu, & Zhou, 2016) (Zhang et al., 2019a)
MRI	Spatial map of the concentration and relaxation times	Chicken (m) Pork (m)	(Shaarani et al., 2006) (Antequera, Caro, Rodríguez, & Pérez, 2007; Fantazzini, Gombia, Schembri, Simoncini, & Virgili, 2009; Herrero, Cambero, et al., 2007)
Ultrasound imaging	Composition, viscoelastic properties	Beef (m) Pork (m) Dry cured meat products (m)	(Bouhrara et al., 2011) (Ayuso et al., 2013) (Corona et al., 2013)
Hyperspectral imaging	Spatially compositional analysis, fiber orientation	Beef (m) Pork (m) Lamb (m) Chicken (m)	(Cluff et al., 2008; ElMasry, Sun, & Allen, 2011; Rady & Adedeji, 2018, 2020; Van Beers, Aernouts, Reis, & Saeyns, 2017) (Barbin, ElMasry, Sun, & Allen, 2020; Cheng, Sun, Pu, & Wei, 2018; Huang, Liu, & Ngadi, 2017; Kucha, Liu, & Ngadi, 2018; Rady & Adedeji, 2018, 2020; Yang, Sun, & Cheng, 2017) (Kamruzzaman, ElMasry, Sun, & Allen, 2012) (Jia et al., 2018; Rady & Adedeji, 2018, 2020)
X-ray Tomography	Structure with a resolution from mm to $\mu\text{m}$	Beef (m) Pork (m) Chicken (m) High moisture extruded product (ma) Shear cell structures (ma)	(Einarsdóttir et al., 2014; Frisullo et al., 2010; Kröger et al., 2006; Mathankar et al., 2013; Miklos et al., 2015; Schoeman et al., 2016) (Brienne et al., 2001; Einarsdóttir et al., 2014; Frisullo et al., 2010; Kröger et al., 2006; Mathankar et al., 2013; Miklos et al., 2015; Schoeman et al., 2016) (Adedeji & Ngadi, 2011) (Philipp et al., 2017)
			(Dekkers, Hamoen, et al., 2018; Schreuders et al., 2019; Tian, Wang, van der Goot, & Bouwman, 2018; Wang, Tian, Boom, & van der Goot, 2019)

extensive sample preparation. As samples are created by microtoming, TEM provides information about the inner structure of meat, such as changes in the myofibrillar structure of beef upon cooking (Zhu, Kaur, Staincliffe, & Boland, 2018), the degradation of myofibrillar structure of lean meat by proteolytic action (Gerelt, Ikeuchi, & Suzuki, 2000) and calcium chloride addition (Gerelt, Ikeuchi, Nishiumi, & Suzuki, 2002).

Atomic force microscopy (AFM) explores the local 3D structure of a surface on a nanometer scale. AFM has been widely used to analyse the morphology and mechanical properties of meat proteins for understanding the structure and tenderness/toughness (Soltanizadeh & Kadivar, 2014) and investigates the effects of processing and preservation conditions (ultrasound,  $\text{CaCl}_2$  and sodium tripolyphosphate) on meat proteins (goat muscle fiber) (Gao et al., 2016). AFM-based infrared spectroscopy (AFM-IR) combines the spatial resolution of atomic force microscopy (AFM) with chemical analysis using infrared (IR) spectroscopy (Dazzi & Prater, 2017). For meat analogues, AFM-IR was used to determine the phase distribution of protein and lipids during high moisture extrusion of peanut protein at a nanoscale resolution (10 nm) (Zhang et al., 2019a).

Magnetic resonance imaging (MRI) is a non-invasive and non-destructive imaging technique that produces a spatial map of the concentration and relaxation times to give insight into the structural

features of meat and meat analogues (Duce, Ablett, Guiheneuf, Horsfield, & Hall, 1994; Mitchell, Scholz, Wang, & Song, 2001). Several studies on meat employed MRI to study the chemical composition, muscle structure as well as carcass compositions, adipose tissue distribution, connective tissue, and muscle fiber type (Marcone et al., 2013). MRI can also visualize the water distribution in meat products and the effect of processing such as freeze-thawing (Guiheneuf, Parker, Tessier, & Hall, 1997) or drying (Fantazzini, Gombia, Schembri, Simoncini, & Virgili, 2009; Ruiz-Cabrera, Gou, Foucat, Renou, & Daudin, 2004), moisture loss during processing (Antequera, Caro, Rodríguez, & Pérez, 2007), to quantify changes in the moisture and structure of cooked chicken meat (Shaarani, Nott, & Hall, 2006) and allows imaging of the connective network during the cooking of meat (Bouhrara et al., 2011). Magnetic resonance elastography (MRE) is a phase-contrast-based MRI imaging technique that can directly visualize and quantitatively measure localised viscoelastic properties like elasticity and stiffness (Gruwel, Latta, Matwy, & Tomanek, 2010; Manduca et al., 2001). MRE provides estimates of the mechanical properties such as shear modulus or Young's modulus of tissues (Gruwel et al., 2010; Sapin-De Brosses, Gennisson, Pernot, Fink, & Tanter, 2010). The analysis of strongly anisotropic beef muscle shows that MRE can distinguish between isotropic (viscous properties) and anisotropic (elastic properties) materials (Sinkus et al.,

2005).

Ultrasound imaging can be divided into low power ultrasound (LPU) and high power ultrasound (HPU) (Awad, Moharram, Shaltout, Asker, & Youssef, 2012). The latter uses frequencies that are disruptive for the physical, mechanical, or chemical properties of food products and are therefore promising in food preservation. LPU has been used as a non-invasive analysis method for monitoring food materials during processing or storage. In LPU, sound waves propagate through food materials, which leads to absorption and/or scattering of the waves. Different components will have specific local, acoustic impedance, which is the basis for image production. In the meat industry, LPU is used most often for compositional analysis as quality control of carcasses or live animals (Awad et al., 2012; Silva & Cadavez, 2012). Ultrasound imaging has also been successfully used for measuring the composition of chicken meat (Chanamai & McClements, 1999), carcass composition of pigs (Ayuso, González, Hernández, Corral, & Izquierdo, 2013) and dry-cured meat products (Corona et al., 2013). Ultrasound imaging of meat and meat products has even been mentioned to provide estimates of localised viscoelastic properties of meat tissues (Biswas & Mandal, 2020, pp. 3–17). Ultrasound imaging has been used to follow the ripening kinetics of tofu (Ting, Kuo, Lien, & Sheng, 2009).

Hyperspectral imaging (HSI) is the combination of multiple wavelengths together with other localised information. Infrared spectroscopy can be combined with microscopy providing spatially resolved compositional analysis (Dazzi & Prater, 2017; Zhang, Liu, et al., 2019). NIR combined with HSI provides both spectral (NIR spectrum) and localised (for each pixel) details together in the scanned region. This was reviewed for meat and fish to predict quantitatively and qualitative chemical, textural and structural characteristics of meat such as tenderness, water, water holding capacity, fat and protein content (Reis et al., 2018; Wu & Sun, 2013a,b)). By combining direct identification of different components and their spatial distribution in the tested product, hyperspectral imaging has the potential for objective quality evaluation of both meat and meat analogues. NIR HSI is already used for the detection and quantification of plant (texturized vegetable protein and gluten) and animal (chicken) based adulterants in minced beef and pork (Rady & Adedjeji, 2018, 2020).

Scattering techniques such as X-ray tomography, SAS, or light reflectance provide 3D structural insight. X-ray tomography (XRT) is based on variations in the attenuation of penetrating X-rays. The difference in the degree of X-ray attenuation is determined by the local density and compositional differences, which provides the locally resolved density with a spatial resolution down to 1  $\mu\text{m}$  and at a time scale of minutes. Micro-computed tomography ( $\mu\text{CT}$ ) is used to study the structure of small products with a resolution from mm to  $\mu\text{m}$ . In meat products, XRT is used for microstructural characterization, prediction of salt, water, (intramuscular) fat content and distribution, and the relationship with hardness (Schoeman, Williams, du Plessis, & Manley, 2016). Micro-computed tomography (Mathanker, Weckler, & Wang, 2013) was used to characterize microstructure, as well as the quantification and prediction of the composition in meat and fish hardness (Schoeman et al., 2016), intramuscular fat level and distribution in beef muscles (Frisullo, Marino, Laverse, Albenzio, & Del Nobile, 2010). In meat analogue products, XRT reveals the porosity in the structure. Air pockets that could be elongated and entrapped were studied in soy protein-pectin blends (Dekkers et al., 2018), soy protein-gluten blends and pea protein-gluten blends (Schreuders et al., 2019). In extrusion products, expansion (due to water evaporation) of the materials was visualized in extruded rice starch-pea protein in two directions (Philipp, Oey, Silcock, Beck, & Buckow, 2017). Air bubbles in a composite meat analogue made of calcium caseinate may contribute to fibrous properties (Tian et al., 2018; Wang et al., 2019). In general, XRT depends on differences in density and therefore is not well suited for finding information on the distribution of components that have similar densities. Advanced contrast modalities such as phase-contrast X-ray tomography describes both the meat structure and the different meat components (i.

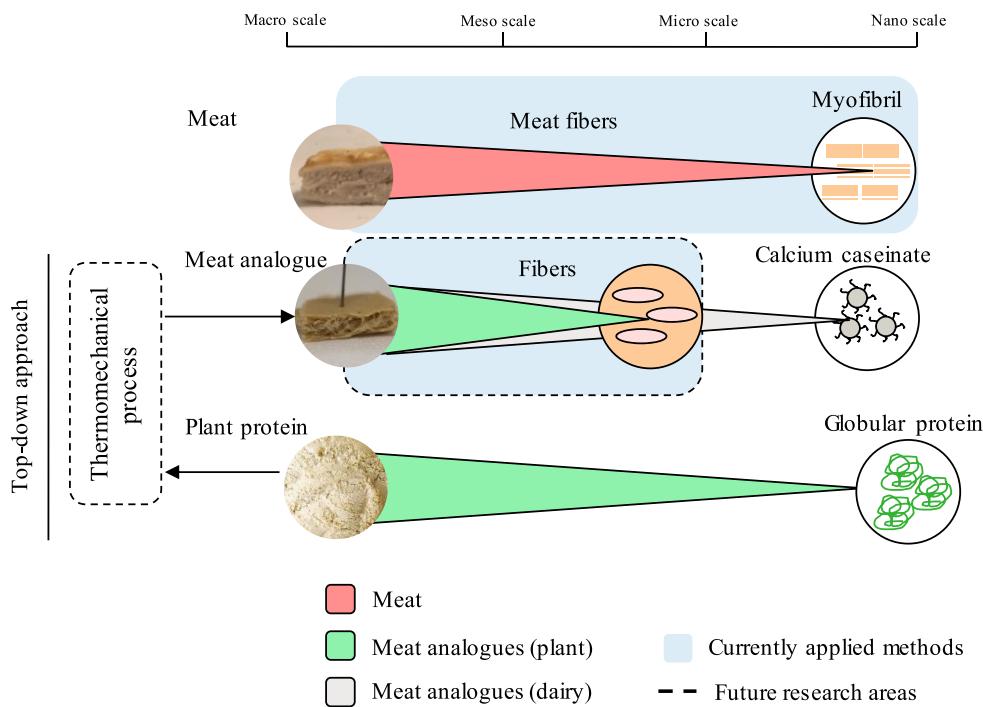
e. water, fat, connective tissue and myofibrils) qualitatively and quantitatively (Miklos, Nielsen, Einarsdóttir, Feidenhans'l, & Lametsch, 2015). Dual X-ray absorptiometry shows a moderate good correlation with meat tenderness and fat content in pork and beef meat (Brienne, Denoyelle, Baussart, & Daudin, 2001; Kröger, Bartle, West, Purchas, & Devine, 2006). The grating-based multimodal X-ray tomography method (including absorption, phase contrast and dark-field tomograms) was used to quantify the composition (i.e. meat matrix, fat, salt, oil droplets) and visualizes the microstructural changes of meat emulsion induced by heat treatment (Einarsdóttir et al., 2014).

As can be concluded from the information described above, imaging reveals important information about the intermolecular interaction, anisotropy and nano to macro structure of meat and meat analogues. While SEM and CLSM are used to reveal the structure of both meat and meat analogues, TEM and AFM have only been used to analyse meat, but not yet meat analogues. The fibrousness of meat analogues from plant proteins created via a top-down approach is typically less hierarchical than meat (Dekkers, Boom, & van der Goot, 2018). This implies that the meat analogues are structured on larger scales than is explored with TEM and AFM. Nevertheless, fibrous proteinaceous materials, such as those based on calcium caseinate may have a finer structure, which could justify further analysis. Given the ubiquity of water in meat analogues, we expect that ESEM and CLSM will be major methods for further structural analysis. CLSM can lead to 3D information through combining a stack of 2D pictures and also yields information on differences in composition, which could provide better insight into the orientation and the length of the structural elements in meat analogues. An important limitation of the microscopy methods is that they require extensive sample preparation, making them less suitable for further analysis. Non-destructive imaging methods like MRI and HSI used for meat provide information on the intermolecular interaction and spatially resolved compositional analysis for meat analogues simultaneously. For both meat and meat analogue products, structural changes have been analysed with XRT. For meat analogue products, XRT was used to quantify and visualize air, while for meat more structural aspects were studied with different types of XRT (like phase-contrast X-ray tomography or grating-based multimodal X-ray tomography).

### 3. Characterization aspects specific to meat analogues

This review focused on the texture and structure of meat and meat analogues as finished products (Fig. 2). But contradictory to meat, the fibrous structure of meat analogues has to be created in a production process. Therefore we are not just interested in the final structure of meat analogues, but also in the mechanism behind the creation of the fibrous structure. As the fibrous structure of meat analogues is often created during thermomechanical processing, it is important to understand the behaviour of different components during processing. The high temperature and pressure, often used during the production of meat analogues, limit the methods of analysis. However, a combination of different methods could be a route to gain information about the structure formation process. Mechanical methods cannot be used during processing, but spectroscopy and imaging techniques show potential. ESEM and XRT could be promising to study the changes in the structural elements during thermomechanical processing of meat analogues. The application of in-line light reflectance, SAS, NIR, or Raman spectroscopy during the processing of meat analogues could provide insight into structural elements. In-line ultrasound imaging is expected to be a promising method for studying air bubbles and mechanical properties of meat analogues during processing, as this was previously used for the analysis of dough (Koksel, Scanlon, & Page, 2016).

Another challenge to characterize meat analogues is the fibrous structure itself. To understand how to create a fibrous structure, knowledge of the fibers in meat analogues is required. So far, it is not completely clear what the geometry, size, binding pattern and adhesion or cohesion of the fibers in meat analogues will look like. The



**Figure 2.** Available texture and structure methods (○) on their suitability for meat (M, ■) and meat analogues (MA, ▲) and future potential research areas for meat analogues.

simultaneous use of different mechanical and imaging methods can provide a more holistic view on the fiber properties in meat analogues products. It can also be interesting to refer to meat and also non-food products containing fibers. Meat from different origin such as poultry or beef, consists of fibers with very different shapes and physical characteristics as was revealed with multi-point indentation, which is was used to spatially measure the local elastic modulus (Boots et al., 2021). In non-food products, such as thermoplastic, adhesion and cohesion of fibers in a matrix is studied. A combination of fiber-matrix wetting analysis and interfacial adhesion analysis was found to give a good understanding of the fiber-matrix interface (Tran et al., 2015). Such methods could also be promising in understanding the fibrous structure of meat analogues.

#### 4. Conclusion

An important step towards the development of next-generation meat analogues is a better insight into the texture properties of those products. To quantify those, analytical techniques are necessary. This review summarizes and discusses methods typically used to characterize the properties and quality of meat products and discusses the feasibility to apply those for meat analogues. At this moment the range of methods used for meat analogues is smaller compared to the methods available for meat. However, we conclude that a broad range of methods could be readily employed to analyse meat analogues or slightly modified to make those methods suitable to analyse meat analogues.

Several techniques elucidate structural features. Mechanical methods allow a direct comparison of the texture attributes between meat and meat analogues, tensile analysis, Warner-Bratzler test and compression techniques provide information about the strength of the product and can be applied to both meat and meat analogue products. Spectroscopy methods are non-destructive and fast but more expensive. Most imaging techniques are interesting to compare the structure of both meat and meat analogues. CLSM and XRT reveal 3D information. NIR, MIR, NMR and MIR provide both quantitative information about

the structural elements and information of the composition. Tensile analysis, image analysis, fluorescence spectroscopy, SAS and light reflectance, showed to be promising methods to quantify properties of the individual fibers and their formation process. TEM and AFM are interesting for nanoscale structure analysis, but have been applied to meat only so far.

Specifically for meat analogues there is a need to study the texture and structure at processing conditions as well. In-line NIR or ultrasound imaging could be promising to study the changes in the structural elements during thermomechanical processing of meat analogues. Furthermore, future research should focus on characterizing the fibers present in meat analogues with regards to geometry, size and adhesion and cohesion. This approach could optimize the conditions used during the processing of meat analogues process with the final purpose of resembling meat products in terms of texture and structure.

#### CRediT authorship contribution statement

**Floor K.G. Schreuders:** Conceptualization, Writing - original draft, Writing - review & editing. **Miek Schlangen:** Conceptualization, Writing - original draft, Writing - review & editing. **Konstantina Kyriakopoulou:** Supervision, Writing - review & editing. **Remko M. Boom:** Supervision, Writing - review & editing. **Atze Jan van der Goot:** Supervision, Writing - review & editing.

#### Declaration of competing interest

The authors report no conflicts of interest relevant to this article.

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