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Collagen use for co-extruded sausage casings – A review

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ABSTRACT

Background: In today's sausage production, various types of casings are employed including: natural, manufactured collagen, cellulose and plastic, as well as the new type of co-extruded casings made of collagen, alginate or alginate-collagen hybrids. Casings play important functional roles in sausage production from stuffing right up until the consumer eats the product. The selection of the right casing is critical as it influences the integrity, size and shape of the sausage while converting the soft, flowable raw meat batter into the desired semi-rigid sausage. The two most important casings' physical properties are their barrier properties and mechanical strength; both strongly affect consumer's perception of bite/snap and flavor.

Scope and approach: Currently edible casings include natural sheep and pig intestines as well as collagen originating from bovine skins. This review focuses on the various aspects of latter collagen used for co-extrusion production, of high quality sausages in an economical way.

Key finding and conclusions: Currently these relatively new co-extrusion gels come only with basic information about pH, protein content, and microbial counts, but nothing about physical characteristics such as viscosity, work to extrude and shear thinning properties. This is important as variations in collagen structure and functionality can be the results of environmental factors such as nutrition, housing, as well as age and genetics. Overall, the rising costs and shortage of intestines and increasing need for Kosher and Halal products, is putting pressure on collagen manufacturers to look for alternative sources with best performance, as described in the review.

1. Introduction

1.1. History of sausage making

Sausages are believed to be one of the oldest form of meat processing. While it is unknown when exactly the first sausage was made, there is evidence they existed for at least several thousand years. The origin of sausage making probably began when people learned that salt is an effective preservative. Sausage making evolved as an effort to economize and preserve meat that could not be consumed fresh at slaughter (Marchello & Garden-Robinson, 2017). A record of a cooked meat product stuffed into a goat stomach was found in the ancient city of Babylon (Anonymous, 1990). There is also mention of sausage in the oldest Greek cook book which dates back to 228 BC (Tauber, 1976). In ancient Rome, 'farcimina' (sausages) were made and flavored with oriental spices, such as, pepper, cinnamon, ginger, and nutmeg (Jensen, 1953). By the late medieval period, the demand for sausages had outgrown the supply of casings, and salted casings were priced as delicacies in Europe (Fröhner, 1921). The Industrial Revolution led to advancements in new sausage production processes, but the custom of using natural casings did not change (Harper, 2013; Schutz, 1921a, 1921b).

In the early 1900's, after the publication of the book named 'The Jungle', the United States introduced tougher inspection laws for the meat industry as well as the 'national pure-food law' (later named 'The Food and Drug Act'), which marked the beginning of the modern era of the American meat industry. Even prior to that, the 1871 German regulations, forced many German cities to construct public slaughterhouses, which helped Germans to reach high hygienic and technological standards. This was also a start for European cities to centralize animal slaughtering in their municipalities. This enabled an efficient utilization of various by-products, including the processing of intestines into sausage casings (Savic & Savic, 2016, pp. 47 – 87, 141 – 207, 212–258).

As the meat packing industry continued to evolve, sausages became increasingly popular and hog casings became an important commodity. This led to the start of the modern day casing suppliers. Until the 1960's, animal and cellulose casings were the only type of casing used.

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However, the 1960's saw the introduction of the first artificial sausage casings made from regenerated collagen. The fusion of traditional sausage making art together with advances in man-made casing manufacturing started what could be called the modern Sausage Age; i.e., a period in which sausages stuffed into attractive and functional man-made or natural casings became popular all around the world. This was the time when sausages reached great international popularity, with various regions developing their own unique sausage in natural or man-made casings (Savic & Savic, 2016, pp. 47 – 87, 141 – 207, 212–258).

Today, many types of sausage casings are used, including: natural, manufactured collagen, cellulose, and plastic as well as co-extruded casings made of collagen, alginate or alginate-collagen hybrid casings (Harper, 2013).

1.2. Types of sausage casings

1.2.1. Natural casings

Sausages have always had an important place in the human diet due to their long shelf life (e.g. dry sausage), unique texture, flavor, nutritional density and price. Sausages can be relatively inexpensive processed meat products resulting in many different varieties based on preference, culture and climate.

Traditionally, sausage meat has been stuffed into intestines derived from pigs, sheep, and cattle (Wijnker, 2009, pp. 1–114). Sheep casings are obtained from the small intestine; note: the large intestines are not used for casings. The collagen content in the intestines of young sheep is higher than in the same intestinal section of a mature animal, however the intestines of the mature animal is always tougher. This is due to a higher proportion of stable crosslinks within collagen fibers of a mature animal (Savic & Savic, 2002, pp. 3–354). Sheep runners are fermented prior to the cleaning process, in contrary to hog casings. After the manure is removed/stripped from the intestines, the runners are placed in cold storage overnight to allow the mucosa to degrade for easier removal, while the submucosa becomes more hydrated; the latter permits manual or machine sliming without damage. Sheep casings are not reversed (turned inside out) during processing, while beef casings are (Savic & Savic, 2002, pp. 3–354; Wijnker, 2009, pp. 1–114). Runners are never stored more than 2 days because prolonged storage will weaken the resulting casings. By no means should the casings be frozen during storage as they would lose their elasticity and firmness (Savic & Savic, 2002, pp. 3–354). Koolmees et al. (2004) described that after storage the runners (about 25–27 m long) were placed in water at 15–20 °C in order to facilitate the removal of the mucosa. Later the cleaned casings are stored in salt. Sheep casings, as well as other natural casings are soaked in water prior to stuffing the sausage mix. This treatment removes part of the salt and the casing wall becomes more elastic, as the collagen fibers absorb water (Heinz & Hautzinger, 2007). Cleaned casings containing only submucosa, support stresses in all directions, which allows them to adapt to the extension and retraction of the sausage mass during processing and storage.

In the case of pig intestines several parts can be processed into casings; e.g., stomach, bungs, small and large intestines. The most important are the small intestines. The cleaning and preservation are similar to the procedure employed for sheep casings. Small runners are first pressed successive between rollers, which removes epithelial layer and usually both basement membrane and muscularis mucosae, as well as the muscle layers and the serosa; i.e. leaving only the collagen rich submucosa. The submucosa of pig casing contains a network of collagen and elastic fibers, blood vessels and is less brittle than beef casing, which contain both the muscularis and mucosa layers. During cleaning of the intestines, the slime can be harvested for the pharmaceutical industry and used for the manufacturing medicines like heparin (anti-coagulant). Small diameter casings, free of holes, are of “A” quality and are used for fine emulsion type sausages. The “B” quality casings should be of good strength and of other physical properties, however they may have some small holes. They are used for coarse ground products such as fresh pork sausages (Escoubas et al. 2010, pp. 1–30; Heinz & Hautzinger, 2007; Savic & Savic, 2002, pp. 3–354; Smits & Keizer, 2003).

Beef casings are also used for sausage production. However, due to several cases of Bovine Spongiform Encephalopathy (BSE), European casings have been designated as specified risk material and therefore banned from human consumption completely in 1997. Overall, BSE infectivity has so far only been confirmed in the distal ileum and not in any part of the intestinal tract used for the production of beef casings. Although BSE has not been identified in any other part of the intestinal tract, the production of beef casings in Europe was terminated and currently only imports from countries with a negligible BSE risk are allowed. While sheep and hog casings are typically eaten with the sausage, beef casings are usually removed prior to consumption as they are thicker and tougher/hard to chew (Wijnker, 2009, pp. 1–114; Wijnker et al., 2008).

After cleaning, the casings are also stored in salt solution to lower the water activity, which inhibits microbial growth and preserves the casing (Heinz & Hautzinger, 2007; Ockerman & Hansen, 1988; Savic & Savic, 2002, pp. 3–354; Smits & Keizer, 2003).

Natural casings are perceived to have the highest quality due to special characteristics; such as bite, knack, curvature, snap. Currently, not all these characteristics (e.g., permeability, pressure resistance, smoke absorption, and flexibility) can be reproduced by artificial casings. Because of that, natural casings are often considered as the gold standard for sausage casings and are expected to remain the preferred casing for traditional and some premium sausages (Anonymous, 2018; Savic & Savic, 2016, pp. 47 – 87, 141 – 207, 212–258).

1.2.2. Manufactured collagen casings

The limited supply of animal casings and their relatively high price encouraged the development of alternative type of casings (Kramlich et al., 1973). Development of manufactured collagen casings began in 1925 and production started in 1930. In 1960, more edible collagen casing came into the market (Savic & Savic, 2016, pp. 47 – 87, 141 – 207, 212–258).

Manufactured collagen casings are composed of both fibrous and solubilized material that is extracted from hides, bones and connective tissue (Ratanavaraporn et al., 2008). Generally speaking, the production of these casings is a proprietary process (Barbut, 2010). However, several steps are typically used across the board. In the case of beef collagen casing, the first step involves separating the corium layer from the rest of the hide, followed by decalcification and grinding. Acid is then added to induce swelling of the collagen and the so called collagen dough is extruded using either a ‘wet’ or ‘dry’ extrusion process (Savic & Savic, 2016, pp. 47 – 87, 141 – 207, 212–258). The ‘wet’ extrusion (‘American’ process) typically uses H_2SO_4 while the ‘dry’ process (‘German’ process) typically uses HCl (Savic & Savic, 2016, pp. 47 – 87, 141 – 207, 212–258). Crosslinking agents, such as glutaraldehyde or other aldehydes (e.g., from liquid smoke) may be added to control the extent of crosslinking and improve the strength of the casing. Cellulose fibers can also be added to improve the casing's strength while plasticizing agents, such as glycerol and sorbitol, are often added to reduce the casing's brittleness (Savic & Savic, 2016, pp. 47 – 87, 141 – 207, 212–258; Ustunol, 2009). Overall, the latter molecules work by decreasing the intermolecular forces along the polymer chains allowing for more chain mobility and flexibility.

Manufactured collagen casings have gained popularity because they possess many similarities to traditional animal casings, including the fact that they are edible. The cooking characteristics and tenderness of sausages stuffed in manufactured collagen casings are quite similar to those in natural casings (Savic & Savic, 2016, pp. 47 – 87, 141 – 207, 212–258). In addition, they show improved uniformity, strength, flexibility, hygiene and are easy to use during filling, portioning and slicing (Kutas, 1987; Ockerman & Hansen, 1988; Savic & Savic, 2016, pp. 47 – 87, 141 – 207, 212–258). Manufactured collagen casings do not have

size variations or weak spots like natural casings. Overall, they are easier to work with compared to natural casings as they are very uniform (important for portion control), allow using higher filling pressure within fast stuffing machines, less expensive to buy, and require less labor (i.e. arrive as a tube that can be put directly onto the stuffing horn) (Savic & Savic, 2016, pp. 47 – 87, 141 – 207, 212–258). Manufactured collagen casings also result in a cleaner, more sanitary product than natural casings (i.e., no/minimal microbial load due to exposure to the high pH during manufacturing). They require no soaking prior to stuffing and can be stored longer than natural casings (Savic & Savic, 2016, pp. 47 – 87, 141 – 207, 212–258). However, both manufactured collagen, natural casings are of a finite length, and thus sausage stuffing remains a batch operation (Harper, 2013).

1.2.3. Cellulose casings

In 1870, German army sausage suppliers first used parchment paper casings for their sausages. These casings were the first known type of artificial casing used. Although they are rarely used today (except for some ethnic specialty sausages), other types of cellulose casings are common. Manufactured cellulose casing are very popular for high volume products such as frankfurter type sausages, bologna and salami. These casings are very strong and can be used on highly automated equipment. Because of their uniformity and the ability to control the degree of stretching, portion control is easy. Cellulose casings are non-digestible and have to be peeled off prior to consumption. In the case of small diameter products (hot dogs), the casing is peeled off at the plant by an automated high-speed peeler. In case of larger diameter products, like salami, the casing is usually peeled off by the consumer. Some cellulose type casings are coated with a protein layer to improve the adherence to the product. This is especially important in case shrinkage of the meat batter is expected (e.g., semi/fully dried sausages). Cellulose casings are water and smoke permeable unless they are coated with plastic (Barbut, 2015). Cellulose casings can be divided into three groups; non-fibrous cellulose casings, fibrous cellulose casings, and plastic (e.g., Polyvinylidene Chloride; PVDC)-coated fibrous cellulose casings (Savic & Savic, 2016, pp. 47 – 87, 141 – 207, 212–258). Small diameter non-fibrous cellulose casings are designed to give maximum uniformity in diameter and are mainly used for the production of skinless wieners, hot dogs and smoked sausages (casings removed at the plant after heat processing). Fibrous casings consist of a cellulose hydrate (produced from a cellulose xanthate derivative) that has been reinforced with regenerated cellulose fibers (Savic & Savic, 2016, pp. 47 – 87, 141 – 207, 212–258). Fibrous casings come in three basic types; regular, easy peel, and moisture proof (PVDC coated). Easy peel and PVDC coated fibrous casings are used for cooked cold cut sausages, as well as other large diameter products (Harper, 2013; Thode, 2011).

1.2.4. Synthetic polymer casings

Today, sausage casings are also made from synthetic polymers; such as PVDC, polyester, polyethylene, polyamide, polypropylene or combinations of these materials (Thode, 2011). Synthetic casings are a good choice for large-diameter sausages since they are known for their relative strength and uniformity. Another advantage of plastic films is that they offer protection against oxidation since they are impermeable to oxygen and this can significantly help to enhance products' shelf life. However, the properties of these casings vary depending on the type of polymer, thickness, additives used, as well as post-processing treatments. Like cellulose casings, synthetic polymer casings are indigestible and must be removed prior to consumption. Typically, these casings cannot be smoked, although recently smokeable synthetic casings, with small holes, have been developed (Barbut, 2015; Savic & Savic, 2016, pp. 47 – 87, 141 – 207, 212–258).

1.2.5. Co-extruded collagen casing

Co-extrusion is a production method, which avoids the intermediate stages of preparing and storing pre-made casings. Unlike traditional sausage production, where casing is stuffed with meat, co-extrusion introduces liquid/semi liquid material (to the sausage surface) that is later gelled in place. Initial reports and patents regarding the technology started to appear about 40 years ago. However due to various technical challenges, co-extrusion has only started to be used, on a large scale, about 10–15 years ago. It was in 1982, that Unilever Ltd. London-Rotterdam developed co-extrusion technology, which allowed forming the casings directly on the sausage surface. However, problems with casing stability as well as the high investment cost and complexity of the process deterred sausage manufacturers from implementing the technology (Savic & Savic, 2016, pp. 47 – 87, 141 – 207, 212–258). During the late 1990's Townsend Engineering significantly improved the co-extrusion process and within the last 15 years several major North American and European companies adopted the technology on a large scale (Savic & Savic, 2016, pp. 47 – 87, 141 – 207, 212–258). Today, co-extruded casings can be made of collagen, alginate, or an alginate-collagen hybrid material. Additives, such as, cellulose or potato starch, may also be added to the casing to improve certain functionalities.

2. Co-extruded collagen casings

2.1. Main source of collagen and structure

For (co-extruded) collagen casings bovine skin is the main source of collagen and why this technology will be reviewed in more detail below. Collagen is a by-product of the meat packing industry. It is obtained from skin of slaughtered beef cattle and makes an intermediate stop at the leather tannery, where the hair and dermal layer are separated from the collagen-rich corium layer. Wide varieties of skins go to tanneries for further processing and most are graded for leather use. The dermal or the grain section receives separate chemical and tanning treatments, which permanently stabilizes it and results in leather. The corium continues for conversion into either second-grade leather or edible products, such as collagen casings, food grade gelatin or pet snacks (Covington, 2009a, pp. 29–71).

The dermis/corium is the layer under the epidermis, providing physical strength and flexibility to skin. The dermis/corium is divided into two regions: the papillary dermis, composed of loose connective tissue containing hair follicles and glands. The reticular dermis containing large bundles of collagen interwoven in many directions. In general, 100 kg of skin contains about 40 kg of collagen and 60 kg of water (Covington, 2009a, pp. 29–71). The dermis/corium thickness varies in different anatomical regions of the animal (Falanga et al., 2004; Savic & Savic, 2002, pp. 3–354). Covington (2009a, pp. 29–71) described the skin as anisotropic, i.e., its structure and properties vary over the surface. The parts of the skin can be defined in terms of the "butt", the "belly" and the "neck". The butt is defined as the region up to half way from the backbone to the belly edge, and two-thirds of the way from the root of the tail to the neck edge. Within this region, the fibre structure is relatively consistent and hence the physical properties of the skin are consistent. The remaining regions to the side of the butts are called the bellies and the region beyond the butt, towards the head, is the neck. The butt has a tight fibre structure, making the skin relatively firm and stiff. It is thick compared to the belly, but thinner compared to the neck. For gel production, only the bellies or the belly and the butt of beef cattle are used in one piece (Kempers, 2019). The bellies of all skins are the thinnest parts, with an open structure, making them relatively weak. The neck is the thickest part, but also has relatively more open structure. Due to the differences in collagen fibre structure, it is a challenge to make a constant quality collagen gel (Covington, 2009a, pp. 29–71).

Collagens can be divided into two major groups: fibrillar and non-fibrillar (Maxwell, 2007; Oechsle, 2016). This review focuses on the fibril forming collagens, as they are predominantly found in the skin, intestine, tendon and bone. A fibre is composed of tightly packed fibrils.

Collagen types, which are capable of forming fibrils are types I, II, III, V and XI. The most abundant fibrillary collagen is type I, present in bovine skin and commonly used for making collagen casings (Oechsle, 2016).

The basic structural unit of collagen is composed of three polypeptide chains, also referred to as tropocollagen. Tropocollagen is the basic molecular unit of collagen with a general molecular weight of about 300 kDa, length of 280 nm and width of 1.5 nm (Brodsky et al., 2003). The polypeptides could have the same sequence, hence forming homotrimer molecules, or have different sequences forming heterotrimer molecules, depending on the collagen type (Maxwell, 2007; Oechsle, 2016). In collagen type I, the polypeptides are arranged in the form of a triple helix with two identical chains (α1) and the third, which differs to some extent in its amino acid composition (α2). The triple helix structure is stabilized by hydrogen bonds. All collagen types contain the sequence $(glycine\text{-}X\text{-}Y)_n$ giving rise to α-chains. The X and Y can be any amino acid, but the positions are frequently occupied by proline and hydroxyproline, respectively. Hydroxyproline is in fact not a naturally occurring free amino acid, but is formed from proline. The chemical change takes place after the protein is synthesized. Hydroxyproline is formed by an enzymatic post-translational modification of proline by the enzyme 4-prolyl hydroxylase. The hydroxyproline and proline provide rigidity in the triple helix structure, and force the peptide chain into a left-handed helix. The smallest amino acid glycine allows tight packing of the three α-chains and provides flexibility to the peptide backbone (Savic & Savic, 2002, pp. 3–354; Chambers, 2004; Oechsle, 2016).

Areas within the collagen fibres, characterized by a lack of hydroxyproline and proline and subsequently a lack of triple helix structure are referred to as the telopeptide regions (at both the N- and C-terminal ends). The telopeptide region is rich in lysine and hydroxylysine residues and has three main functions; 1) stabilization of the molecule via intra-molecular and inter-molecular crosslinking, 2) crosslinking with other extracellular matrix proteins, and 3) formation of "sticky ends" by disulphide bonds to aid self-assembly. Tropocollagen molecules overlap approximately one-fourth with its neighbouring molecule (67 nm) due to assembling in a quarter-staggered fibril array (; Savic & Savic, 2002, pp 3-354; Chambers, 2004). This characteristic banding pattern has a periodicity of 65–70 nm and is also known as the D-period (Hoogenkamp, 2015; Versteegden, 2017). Fig. 1 shows the construction of a collagen fibre starting with the structural formula of glycine, proline and hydroxyproline. Followed by collagen triple helix forming microfibrils, which in turn assemble to collagen fibrils and fibres (Oechsle, 2016).

2.2. Production of collagen from bovine skin

2.2.1. Dehairing process at the tanneries

The dehairing process of hides is carried out in tanneries and is discussed here as the chemicals used also affect the collagen later harvested from bovine skins. The aim of the dehairing and liming processes is to remove the hair, epidermis and to some degree the interfibrillary proteins, and thus to prepare the hide for the removal of loose flesh and fat, by the fleshing process. The most frequently used dehairing technique is based upon alkaline treatment of the hides. There are two version of the process, namely hair dissolving (burning) and hair saving (Covington, 2009a, pp. 29–71; Savic & Savic, 2002, pp. 3–354). In the hair dissolving process, a strong reducing agent such as 2% Na_2S is immediately added to the treatment vessel. The sodium sulphide rapidly dissolves in water to yield caustic soda and sodium sulfhydrate (NaHS): $Na_2S + H_2O - > NaOH + NaHS$. This results in a direct attack on the disulphide bonds, which breaks down the keratin molecules in the hair. Due to the strong alkaline conditions, the disulphide bonds of the keratin molecule are quickly hydrolyzed and the hair is dissolved, beginning at the tip and then proceeding to the follicle. Softened hair stubble remains temporarily attached but the stubble is subsequently being removed as the soft follicular keratins are destroyed during the lime application that follows the sulphide addition. It is a rather simple technology with minimum process control required (Covington, 2009a, pp. 29–71; Hood, 1987).

The best-known method for hair saving in the leather industry has been described (Covington, 2009a, pp. 29–71; Hood, 1987) as follows: The skin is impregnated with sodium hydrosulphide for 2 h, to drop the pH from 11.2 to 8.5. After 2 h, the solution is drained and calcium hypochlorite is added for 5 min. Then lime is added to raise the pH to 12.5. A rubbing action on the skin is necessary to remove the hair, which can be recovered as a valuable by-product. A relime step, with sulphide, is carried out to dissolve residual hair. It is a more complicated technology than hair dissolving, which requires a more precise, process control especially pH control.

2.2.2. Liming and its effect on collagen structure

Liming results in swelling of the skin due to the high pH. The skin can be swollen by three mechanisms:

- Charge effects, based on breaking salt links and creating more charges within the protein structure
- Osmotic swelling, caused by the imbalance between the ionic concentration outside and inside the skin
- Lyotropic swelling, caused by disruption to the collagen structure by molecules such as lithium bromide, that can be inserted among the hydrogen bonding

In the case of alkali swelling, the mechanisms are charge effects and lyotropy. The net effect of alkali on collagen is breaking the natural salt links and making the protein anionic. The repelling effect of the anionic centres causes the collagen structure to open up, allowing water in, which is observed as swelling. The opening of the collagen structure occurs in response to the deamidation by hydrolysis of the side-chain glutamine and the asparagine residues. This results in a sharp decrease of the isoelectric point (pI) of the native collagen fibers (Covington, 2009b, pp. 134–153; Hood, 1987; Lischuk et al., 2006; Maxwell et al., 2006). Long alkaline treatment yields collagen with pI values closer to 5.0, whereas mildly treated collagen records a value of 7.8, and acid treated collagen a value of 9.0–9.2 (Hood, 1987). It seems that the prolonged lime treatment, at pH above 11, results in fully anionic structures. In such a case, the fibrils will absorb water, swell and open up. At the end, the fibers never regain their native associations even

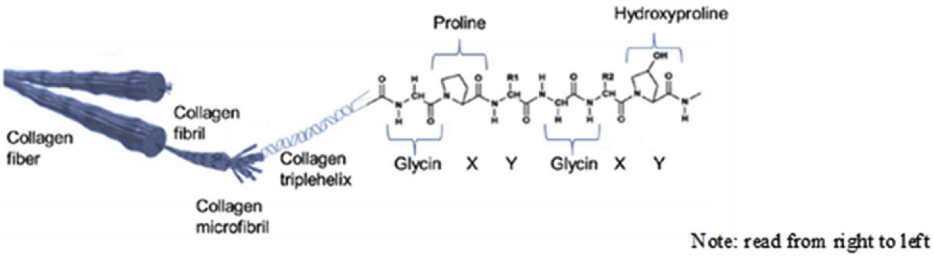

Fig. 1. Systematic illustration of collagen triple helices forming micro fibrils, which in turn assemble to collagen fibrils and fibres (From: Oechsle, 2016).

though collagen is later passed through a series of different pH and solvent changes. The collagen suitable for producing casings no longer possesses the native molecular properties after the hair removal process. The structure is permanently changed and the fibers have an increased contact area that can later be used for cross-link formation. Overall, cross-linking is the chemical basis for the permanent stabilization of collagen casing after drying (Hood, 1987; Maxwell et al., 2006; Savic & Savic, 2002, pp. 3–354; Varnali, 2002).

The consequences of lime treatment are:

- Separation of collagen from its associations with non-collagenous proteins and carbohydrates
- Altering collagen ionic nature and solute-binding properties
- Destroying the native cross-linked and hydrogen bond structure, by the calcium ions.

This does not say that the lime treatment has a negative effect; in fact, there is a need for disruption of some of the native structures and biochemical associations in order to make an acceptable casing. Overall, this means that physicochemical data derived from isolated native collagen is of limited value when trying to interpret performance properties of collagen casings (Covington, 2009b, pp. 134–153; Hood, 1987) and that is one of the industry's main challenges today.

2.2.3. Swelling of collagen fibers due to liming

Fig. 2 illustrates how the standard tannery lime treatment can affect the swelling of bovine hide collagen. The graph shows the percentage swelling against different pH values for purified collagen and for alkaline treated collagen (lime treatment). As can be seen, the water absorption properties are pH dependent and the liming process intensifies the swelling maxima experienced under alkaline and acid conditions. Of special interest for the manufacturers of collagen casings is the swelling behaviour at pH 3. At this particular pH the collagen shows maximal gel viscosity, at any given solids level and this encourages uniform fibre dispersion and flow properties of the collagen dough used to create the casings during the extrusion process. Besides the pH effect on viscosity, other physical changes also occur; e.g., collagen is more firm and translucent above pH 9 and below pH 4.7. In the middle pH range (pH 5–9) where swelling is minimal, the collagen exhibits white opaque color, since water absorption is minimal in this region. Overall, liming effects may be enhanced by time, temperature and pH increase (Loders Croklaan, 2004; Hood, 1987; Varnali, 2002; Covington, 2009b, pp. 134–153).

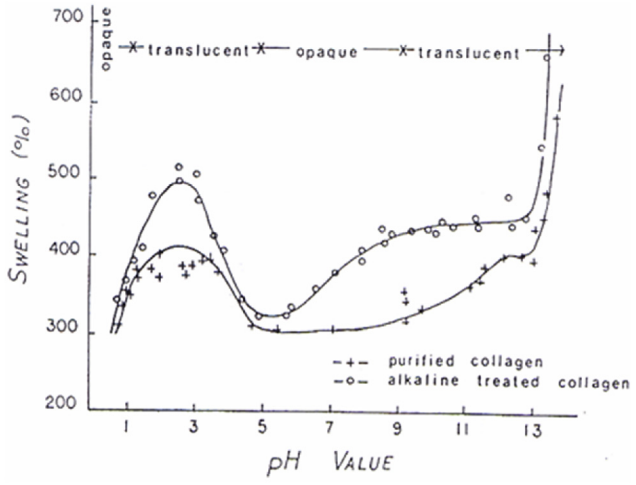

Fig. 2. Effect of pH on extent of swelling in purified and alkali-treated bovine collagen (From: Hood, 1987).

2.2.4. Further processing of beef hides by collagen casing manufacturers

The liming process should be executed as long as necessary (few h to several days) to achieve the required characteristics for the final application. Prolonged treatment under high alkaline conditions eventually destroys the triple helical structure and fibrillar integrity, thereby yielding low molecular weight polypeptides, which are useless for collagen extrusion (Hood, 1987). Properly delimed corium has a final pH of 3.5–5.0, which can be achieved by careful chemical treatment with $(NH_4)_2SO_4$, NH_4Cl and/or acidic buffers (lactic acid). Deliming process is essential to achieve the desired balance between elasticity and strength of the collagen fibers (Hood, 1987). Lime treated corium may contain 0.3–1.5% Ca^{2+}, while properly delimed hide contains 0.2% or less. The deliming process is ultimately dependent upon the swelling phenomena of collagen, which was previously explained. Calcium removal, within edible corium destined for extrusion, is essential, so that residual $Ca(OH)_2$ does not interfere with uniform swelling of the fibrils (Hood, 1987). After deliming, the hide is homogenized and deaerated, followed by the addition of acetic/lactic acid and packaging of the gel (Loders Croklaan, 2004).

2.3. Setting of pre-made and co-extruded collagen casing

The resulting collagen gel has to be formed into pre-made or co-extruded casing. The first is produced in specialized facilities where the gel is forced into an extrusion die which is creating the casing (at the desired diameter), initially “suspended” in a stream of warm air inside a closed environment/tunnel. The air is saturated with a cross-linking agent such as glutaraldehyde, which induces bonding among adjacent collagen molecules. The tubular casing then travels through a hot air zone to help dry it out. Later it is washed with water to remove excess of the cross-linking agents used (i.e., no glutaraldehyde can be incorporated into food). The casings can then be colored, and coated with a plasticizer, such as glycerol, to make them easier to handle at the meat processing plant. The casing is then shirred into sticks that can measure up to 20 m per stick.

With co-extruded casings, the collagen gel (usually 3–6% protein) is sent to the meat plant where it is co-extruded on top of the sausage meat coming out of the extruder. In the co-extrusion process, a thin layer of casing material is extruded onto the meat batter as it is coming out of the extrusion nozzle. Fig. 3 shows an overview of the extrusion technology and the system setup is provided. The co-extrusion head is equipped with two counter rotating cones (Fig. 3). The resulting alignment depends on the gap between the two cones and their relative speeds. Fig. 4 shows the resulting alignment with a setting of both cones rotating at 200 rpm. It demonstrates that the fibers deposited within the outer layer are positioned at about 40° angle to the product's long axis mostly. The middle layer shows random orientation, while the inner layer shows −30° orientation (i.e., because of its proximity to the inner rotating cone). Orienting the fibers improves the mechanical strength of casings by reducing the probability of casing splitting later on during the cooking process (Hoogenkamp et al., 2015; Ustunol, 2009).

Producing co-extruded casings with hydrocolloid gums, such as alginate, requires a different extrusion system. As alginate is composed of two monosaccharides, guluronic acid (G) and mannuronic acid (M) and able to form a gel in the presence of polyvalent metal cations (Harper, 2013), there is no need to provide orientation during extrusion. Today hybrid casings made of, for example, collagen and alginate are also available, and they require the counter-rotating extrusion to align the collagen fibers. Other polysaccharides that have been tried, as additives to collagen and/or alginate include gums, such as carrageenan, which can provide some stability to the final casing (Bontjer et al., 2011; Ioi, 2013; Visser, 2012).

Once the casings have been extruded, subsequent treatments are required to stabilize them onto the product. In the case of collagen, the first step is de-watering, which is done by exposing the collagen coated product to a brine solution (e.g., 20–30% NaCl), which removes some of

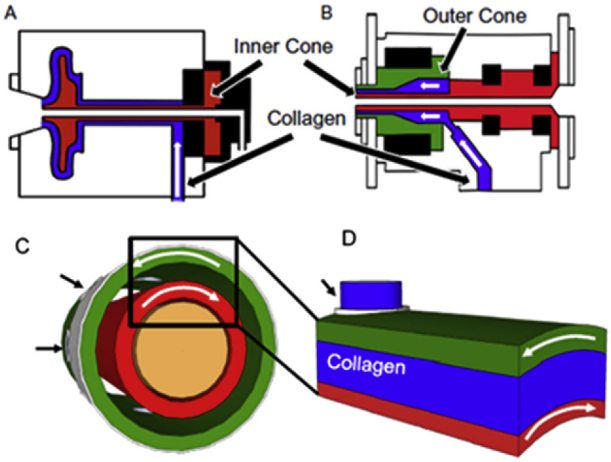

Fig. 3. Overview of the extrusion technology and system setup. (A) Single cone extruder design and (B) counter-rotating cone extruder design, that can be used for collagen extrusion. Either one or two rotating cones can be used to influence the fiber direction. The inner cone (red) and the outer cone (green) are the rotating parts between which the collagen gel (blue color) is extruded. White arrows show the direction of collagen flow. (C and D) Cross-section of the extrusion head, with counter-rotating inner (red) and outer (green) cones molding the collagen film during extrusion (From: Hoogenkamp et al., 2015). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

the water from the casing (to get the collagen molecules closer together). The sausages can be either drenched or sprayed with saturated NaCl solution to dehydrate and harden the casing (Kobussen et al., 2000; 2012). This also allows the collagen to conform to the shape of the meat batter. Further stabilization is performed by air-drying as well as cross-linking with smoke condensate and/or other agents. Smoke is used because of the presence of aldehydes, which are capable to crosslink the proteins. In addition, the smoke adds flavor, color and preservation (i.e. wood smoke has over 400 chemical compounds); (Toledo, 2007). As for sausages with alginate casings, the sausages are drenched in a calcium chloride solution (10–15%) to quickly form a casing. Hybrid alginate/collagen casings are first gelled with calcium chloride solution followed by air-drying and crosslinking with liquid smoke. After the dehydration step the casings are of sufficient strength to undergo crimping or linking. Linking is accomplished by squeezing the sausage rope to define the individual sausages' length and weight. The crimper consists of an upper and lower v-formed guides. The crimping process involves the v-cutouts slowly closing on the sausage. The guides displace the meat within the coating until there is only a thin neck of casing, which can then be cut (Bradshaw & Taylor, 1971; Ioi, 2013).

Overall, there are a few different co-extrusion collagen gels/dispersions on the market, with different size fibers and adjusted to different pH values by different acids. The pH values of the commercial samples range between 2.04 and 2.67. For example, gels can be prepared with HCl (note: this is at the last stage after washing off the alkaline solution; see previous text), while other gels can be prepared with lactic and acetic acid. These collagen gels also show different viscosities; measured as work required to extrude them through a 7 mm opening die (3.19–5.16 Nm; Barbut & Ioi, 2019).

Today most co-extrusion gels come with very basic data about pH, percent protein, and microbial counts, but no data concerning physical properties such as viscosity, work to extrude, and shear thinning. Such data can definitely help meat processors make more informed decisions, regarding a product and/or batch they would like to use. (Note: the batch issue is mentioned here because variations among batches can occur, due to the nature of this product, which is affected by animal age, breed, and preparation procedure. See next section for more details).

Overall, there is limited information published regarding co-extruded collagen casing's characteristics. Table 1 shows the mechanical properties of collagen films prepared by co-extrusion and compares cross-linking with smoke condensate (SC), and glutaraldehyde (GA). A smoke condensate (a 15 concentration) (Charsol Select 24P Liquid Smoke, Red Arrow Products, Manitowoc, WI, USA) dissolved in deionized water, and glutaraldehyde (EM Grade, Canemco, Canton de Gore, QC, CAN) solutions of 0.1, 0.5 and 1.0 vol% GA in 1M HEPES buffer at pH 7.4 were used. As can be seen, there are quite some differences in the tensile strength and percent elongation among the commercial gels, regardless of the cross-linking agent used (Barbut & Ioi, 2019).

It is also important to understand that the onset and denaturation temperatures of the raw collagen doughs after being treated with salt and the smoke condensate changes (Table 2). This is a clear indication that the collagen is significantly modified by the preparation process of the gel (see Sections 2.2.2 and 2.2.3 about liming and acidification). Overall, native collagen samples show a denaturation transition at about 60 °C (Bernal & Stanley, 1986). After the liming, and acidification of the collagen, the denaturation point goes down to about 34 °C (Table 2). Then the collagen goes through a second modification as it is exposed to the co-extrusion treatments (brining in a saturated salt solution, and cross-linking with aldehydes). This results in the denaturation point basically doubling and reaching about 62 °C (Table 2).

Also, acid type and pH have an effect on final collagen structure and film functionality. Oechsle et al. (2014) studied rheological properties of collagen suspended in phosphoric, sulfuric, hydrochloric and perchloric acid at pH 1, 2 and 3. The results showed that collagen entanglement increases with increasing pH values below the isoelectric point. This knowledge could be used to effectively modulate collagen structure and film functionality. For instance highly entangled collagen matrices are more likely to be fabricated into co-extruded collagen casings with high elasticity and tensile strength. These findings emphasize the importance of the collagen raw materials preparation steps for subsequent processing in either pre-made or co-extruded casings.

Oechsle et al. (2015) found that collagen can be modified by adding chaotropic or kosmotropic salts of the reversed Hofmeister series. The study demonstrated that collagen entanglement and microstructure strongly depend on the ionic strength and type of salt. Whereby chaotropes form fine precipitates and kosmotropes leading to elastic three-dimensional networks. Higher salt concentrations increase the collagen–collagen interactions due to ions withdrawing the water from the collagen molecules. Therefore, salt addition is a convenient tool to modify collagen structure, rheology, and functionality for various applications. Nevertheless, modification of the collagen matrix by salt is a physical process, thus structures need to be later fixed by chemical reactions in order to preserve the changes in the conformation.

2.4. Advantages of co-extrusion process

The major advantage of the co-extrusion process is that it is a continuous operation as opposed to the traditional batch process, and thus capable of large production volumes (Smits, 1985). While the initial equipment costs can be high, the increased output and decreased labour costs are economically advantageous. The speed and uniformity of the co-extrusion process also make it appealing to meat processors (Bontjer et al., 2011). With fewer people handling the product, there is a lower risk of microbial contamination compared to traditional batch operations. Additionally, the amount of waste (i.e., casings ends; casings that break cannot be used for re-stuffing), and re-work are significantly reduced when using the co-extrusion principle (Anonymous, 2012). Since co-extruded casings are edible, they can easily be used for cook-in-bag processing. This prevents the risk of post-cooking microbial contamination (e.g., Listeria) from machines such as peelers, collators and slicers (Barbut, 2015; Harper, 2013). Today, it is estimated that one third of the small diameter sausage production in the US is employing

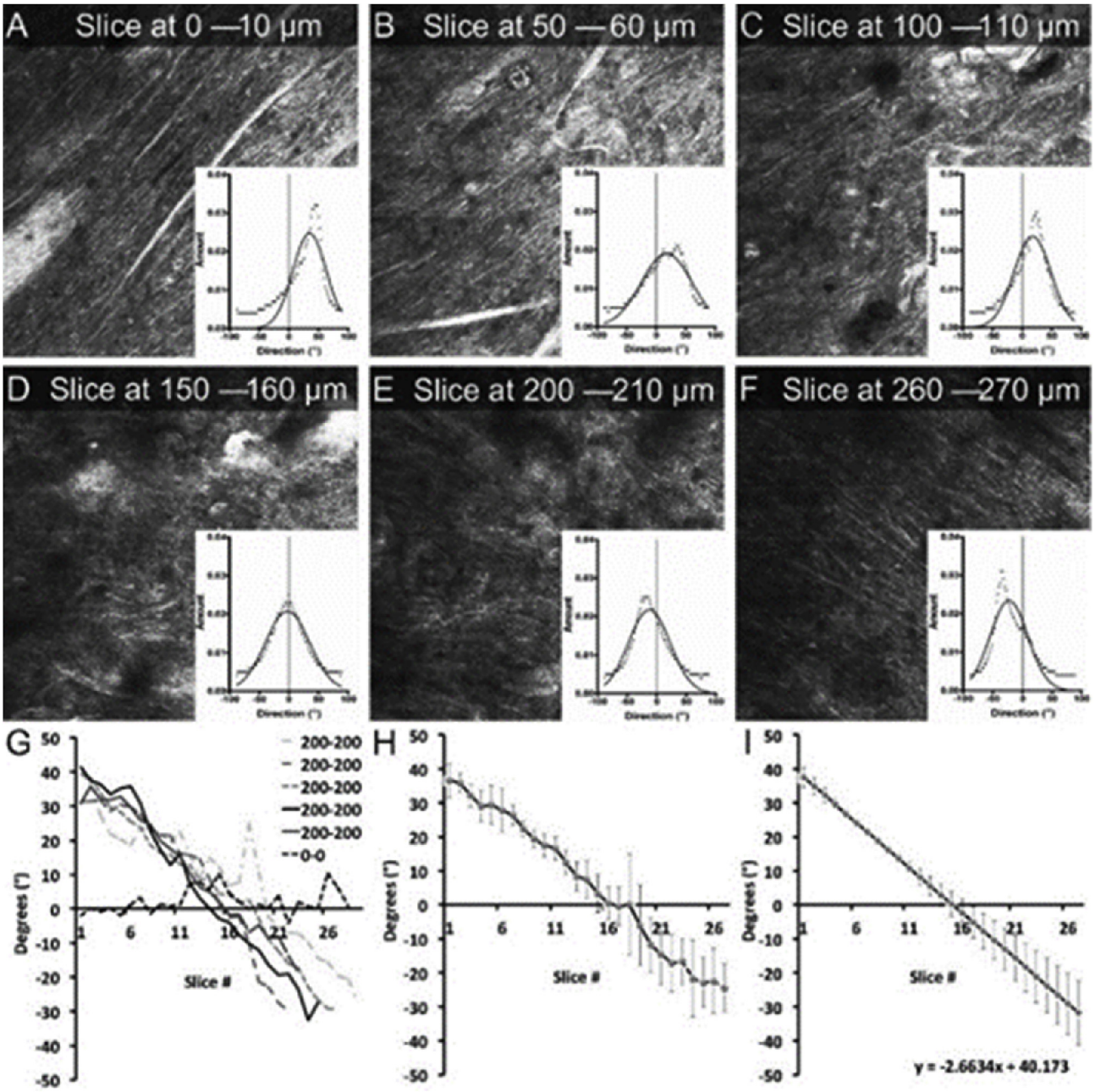

Fig. 4. Directional organization of extrusion-generated 3-D collagen films. (A–F) Second harmonic signal images and quantification of fiber orientation (graphs, inset) from individual slices obtained at the indicated depth position of the image stack from films generated at 200–200 rpm cone speeds. Along the *x*-axis, the fiber orientation is shown (in degrees) where the collagen fiber orientation in the slices is measured and subsequently fitted with a Gaussian curve (solid lines). The peak of the curve represents the dominant fiber direction; (G) dominant fiber directions from speed 200–200 plotted against a direction for 0–0 control cone speed; (H) average dominant fiber direction profile of all 200–200 speeds combined. Error bars represent the standard deviation. The graph's solid black line changes to a dotted line from slice 21 to slice 27, which represents a deviating number of available data points (lower than $n = 5$); (I) the trendline average from combined 200–200 cone speeds. (From: Hoogenkamp et al., 2015).

this technology.

2.5. Functional properties of co-extruded casings

Casings play important functional roles in sausage production from the moment of stuffing right up until the consumer eats the product. The selection of the right casing is critical as it not only influences the integrity, size and shape of the sausage but also assists in the conversion of raw flowable meat batter into the desired semi-solid sausage product (Savic & Savic, 2016, pp. 47 – 87, 141 – 207, 212–258). Casings are designed to accommodate a sausage manufacturer's quality and processing needs. The ideal collagen casing should provide sufficient strength to undergo processing, while remaining tender enough during consumption (Miller, Karmas, & Lu, 1983). It is not possible to create an all-purpose sausage casing suited for all types of sausages, as processing requirements can vary greatly. However, every sausage casing must fulfill some basic functional properties. The two most important are: barrier properties (i.e., permeability to water vapor, smoke and gases) and mechanical strength. A number of methods have been employed to objectively test the properties of casings (Barbut, 2010; Harper et al., 2012, 2013; Hoogenkamp et al., 2015; Miller et al. 1983). Properties of interest also include caliber uniformity, light transparency, shrink

Table 1
Mechanical properties of cross-linked films: C1 (Collagen 1), C2 (Collagen 2), C3 (Collagen 3), C4 (Collagen 4) and C5 (Collagen 5). (From: Barbut & Ioi, 2019).

Collagen	Crosslinker[1]	**Tensile strength[2] MPa**	Percent Elongation[2] %	**Distance at Break[3] mm**	Work to Break[3] Nmm	**Thickness mm**
C1	SC	0.67 ± 0.04^{a}	24.80 ± 1.23^{ab}	3.85 ± 0.22^{a}	2.75 ± 0.48^{a}	0.35 ± 0.01^{ab}
C2	SC	0.53 ± 0.02^{ab}	26.32 ± 1.18^{a}	3.39 ± 0.18^{a}	1.52 ± 0.14^{a}	0.30 ± 0.01^{b}
C3	SC	0.38 ± 0.05^{b}	22.41 ± 0.78^{abc}	3.21 ± 0.30^{a}	1.24 ± 0.34^{a}	0.34 ± 0.01^{ab}
C4	SC	0.32 ± 0.07^{b}	21.37 ± 0.99^{bc}	3.27 ± 0.35^{a}	1.23 ± 0.31^{a}	0.36 ± 0.02^{a}
C5	SC	0.39 ± 0.16^{b}	18.81 ± 2.08^{c}	2.59 ± 0.04^{a}	0.85 ± 0.10^{a}	0.38 ± 0.03^{a}
C1	GA	0.91 ± 0.17^{d}	26.26 ± 4.65^{d}	2.77 ± 0.52^{d}	1.79 ± 0.57^{d}	0.36 ± 0.03^{d}
C2	GA	0.66 ± 0.02^{e}	20.38 ± 1.43^{d}	2.34 ± 0.44^{d}	1.06 ± 0.37^{d}	0.45 ± 0.01^{d}
C3	GA	0.41 ± 0.13^{f}	18.95 ± 2.56^{d}	2.66 ± 0.39^{d}	1.35 ± 0.27^{d}	0.38 ± 0.05^{d}
C4	GA	0.61 ± 0.20^{ef}	24.18 ± 3.72^{d}	2.74 ± 0.29^{d}	1.52 ± 0.11^{d}	0.38 ± 0.04^{d}
C5	GA	0.60 ± 0.18^{ef}	22.04 ± 2.60^{d}	2.87 ± 0.60^{d}	1.91 ± 0.76^{d}	0.39 ± 0.05^{d}

[1]Smoke condensate (SC), Glutaraldehyde (GA). [2]Tensile test. [3]Puncture test.
[2]Means in columns with same letter are not significantly different $p > 0.05$: letters^{a-c} refer to Smoke Condensate treated films; $^{d-e}$ Glutaraldehyde treated films.

Table 2
Analysis of endothermic peaks from differential scanning calorimetry (DSC) thermograms. Five commercial collagen samples were tested as collagen dispersions and partially dehydrated/brined films: C1 (Collagen 1), C2 (Collagen 2), C3 (Collagen 3), C4 (Collagen 4) and C5 (Collagen 5) (From: Barbut et al., 2020).

Collagen	Treatment	Onset temperature (°C)	**Temperature of denaturation (°C)**	**Enthalpy ΔH (J/g)**
C1	Dispersion	33.54 ± 0.21	36.71 ± 0.51	5.33 ± 0.61
C2	Dispersion	34.59 ± 0.15	38.44 ± 0.06	3.05 ± 0.31
C3	Dispersion	34.26 ± 0.01	38.09 ± 0.08	4.12 ± 0.10
C4	Dispersion	35.41 ± 0.11	38.94 ± 0.02	3.93 ± 0.26
C5	Dispersion	33.45 ± 0.10	37.30 ± 0.21	4.45 ± 0.03
C1	Film	59.90 ± 0.23	64.87 ± 0.12	3.07 ± 0.55
C2	Film	58.40 ± 0.21	63.88 ± 0.57	1.76 ± 0.38
C3	Film	60.32 ± 1.61	65.00 ± 0.68	3.05 ± 0.21
C4	Film	58.22 ± 0.24	63.94 ± 0.61	3.06 ± 0.82
C5	Film	58.30 ± 0.40	65.34 ± 0.37	4.19 ± 0.37

ability, temperature resistance, color, peel ability, printability, texture and potential use to carry functional ingredients (Harper, 2013; Ioi, 2013; Savic & Savic, 2016, pp. 47 – 87, 141 – 207, 212–258).

Permeability depends on the casings type (e.g., composition, thickness), degree of extension, degree of water saturation, pH, and moisture content of the meat batter. Permeability describes the extent to which a permeating substance dissolves and then the rate at which it diffuses through the film. This migration of permeant is ultimately driven by concentration gradients. Polarity of a given material is obviously an important factor affecting permeability. In general, protein films have high permeability to polar substances such as water vapor, and low permeability to nonpolar substances such as oxygen, aroma compounds and oils (Krochta, 2002). Collagen films, for example, have an excellent oxygen barrier at 0% relative humidity, but the oxygen permeability increases rapidly with increasing relative humidity (Lieberman & Gilbert, 1973). The migration of water and other substances into and out of the sausage is an important phenomenon, which produces complex effects on the texture, nutritive and marketing value of the sausage (Savic & Savic, 2016, pp. 47 – 87, 141 – 207, 212–258).

Variations in water content and the resulting water activity (Aw) strongly influence sensory quality and storage stability of the sausage. Changes in moisture content of the sausage are governed by the casing permeability properties. Water vapor permeability of the casing depends on aspects such as the level of dry and wet heat used during cooking, degree of casing extension, water saturation of the casing wall and pH of the meat batter. The casing permeability ultimately determines the weight loss from the product (Savic & Savic, 2016, pp. 47 – 87, 141 – 207, 212–258). In case of dry fermented products, a high degree of water permeability is preferred and co-extruded as well as manufactured collagen casings are often used.

With respect to shelf life, oxygen permeability of the casing is of crucial importance for the sausage. Oxygen in its ground state is relatively non-reactive, however it can be turned into reactive species, referred to as active oxygen forms or free radicals, which can accelerate lipid oxidation (Savic & Savic, 2016, pp. 47 – 87, 141 – 207, 212–258). Casing type and thickness will influence the rate of oxygen permeability, as well as the temperature and humidity. Microbial and biochemical spoilage can also be the result of oxygen, which is dissolved or entrained in the sausage mass. In addition, oxygen can cause protein oxidation, flavor oxidation, changes in color and loss of nutritional value. Consequently, reducing oxygen in packages (commonly done by vacuum packing) can help in retarding deteriorative reactions and extending shelf life of sausages. In a co-extrusion process, which uses “cook in the bag” technology, sausages are vacuum-packed, partially cooked (55 °C), vacuum packed and then further cooked until core temperature of 74 °C. The chance of any contamination is minimal and product shelf life is longer than for items produced using the more traditional cellulose casing method (Savic & Savic, 2016, pp. 47 – 87, 141 – 207, 212–258).

With respect to permeability, certain casings are readily permeable to smoke. Components in the smoke improve the flavor, can help peelability and extend the shelf life of the final product by lowering microbial growth (e.g. acid components in the smoke). Smoke components also produce a brownish/golden color on the peripheral layer of the sausage; partially due to the Maillard reaction. The penetration of the smoke components is limited and ranges from 2 to 6 mm in depth depending on the intensity of the smoking process, application time, casing type, etc. It should be noted that the smoking process also depends on the humidity of the air and surface dryness of the sausage. If relative humidity is too high, the sausage will not be uniform in color as some components will be washed off and result in an uneven colouring. If humidity is too low the surface will be unable to absorb enough smoke and the surface will have insufficient color and lower flavor profile (Savic & Savic, 2016, pp. 47 – 87, 141 – 207, 212–258).

Next to permeability, the mechanical strength of the casing is a very important property. During processing the casings must be strong enough to hold the meat, but also be able to expand (elastic properties) during cooking (Bakker et al., 1999; Simelane & Ustunol, 2005). The casing also helps control the gelling process of the meat batter inside, which is a prerequisite for structuring the sausage matrix. The tensile strength of a casing is defined as the maximum stress (force/area) that a casing can withstand while being stretched before it breaks (Olivas & Barbosa-Cánovas, 2008). Tensile strength of a casing is determined by the collagen fibers, which are inter-molecularly, linked to each other via crosslinks. High tensile strength and elasticity are very important in certain type of sausages. These properties are also affected by factors, such as molecular weight, structure of the material, the amount of plasticizer/other additives and thickness of the casing (Savic & Savic, 2016, pp. 47 – 87, 141 – 207, 212–258). The presence of low level of

cellulose fibers in manufactured collagen casing can make them stronger compared to casings made only from regenerated collagen fibers.

The mechanical strength of a casing is important as it influences consumer's perception of the bite/snap of the sausage (Bakker et al., 1999). Harper et al. (2012) studied the texture and microstructure of four commercially available edible collagen casings and a natural sheep casing. Mechanical (textural) characteristics of the casings were evaluated by using shear, puncture and burst measurements. Shear force values were significantly higher for uncooked and cooked sausages prepared from natural sheep casing and one of the manufactured collagen casings tested, compared to those of the so called 'tender breakfast' and 'European wiener' manufactured collagen casings. Overall, it took more force to shear uncooked sausages width wise than length wise regardless of the type of casing. This phenomenon was not seen to the same extent in cooked sausages.

The elasticity of the casing represents the maximum force of casing extensibility. The elasticity of the casing (E modulus) can be measured with a texture analyser. The percentage elongation, at break, is calculated by dividing the change in length of the casings/film, during stretching, by the initial length of the casings/film, giving an indication of the elasticity (Wang et al., 2007). Harper et al. (2012) also determined the elasticity, shear force and distance to break of these four commercially manufactured collagen casings and a natural sheep casing, and reported that the distance to shear the casing of cooked sausages was lower than those for the uncooked sausages. A possible explanation is the fact that when the meat is cooked it gels, expands, and becomes a more solid like structure. This structure is exerting more force on the casing, thus limiting the ability of the casing to stretch and resulting in a lower distance at break. Contradictory results by Amin and Ustunol (2007), reported that uncooked natural casings show a significantly lower percent elongation compared to uncooked manufactured collagen casings. However, the study by Harper et al. (2012) demonstrated that there is wide variety of manufactured casings on the market with different mechanical properties, and that can potentially explain the differences in the results between the two studies. The challenge to match as many sensory properties of natural casings as possible remains a major priority of manufacturers of collagen casings (Savic & Savic, 2016, pp. 47 – 87, 141 – 207, 212–258).

2.6. Factors affecting collagen characteristics related to casing properties

2.6.1. Microbiology

Microorganisms can initiate collagenase activity, which could potentially destroy collagen fibre structure (Ponsen, 2010; Savic & Savic, 2016, pp. 47 – 87, 141 – 207, 212–258). Not all skins suitable for leather production are an appropriate source of edible collagen; e.g., in the US only skins originating from USDA inspected meat producing plants are acceptable; skins from animals with serious dermatological problems are ineligible.

2.6.2. Species

Every animal species (e.g. bovine, pig, chicken) has its own specific amino acid composition, which determines the final properties of the collagen. Hydroxyproline content is extremely important, because it affects the properties of any preparation made from collagen (Gómez-Guillén et al., 2002). Animal species is important due to the differences in fibre density as well as weave pattern and therefore the type of possible application (Maxwell, 2007). Angele et al. (2004) evaluated the physico-chemical properties of equine- and bovine-collagen-based scaffold and found a highly significant effect of collagen type and crosslinking on degradation of the collagen samples by collagenase treatment. Crosslinked equine samples showed a significantly lower swelling ratio compared to bovine collagen samples. The amino acid composition of equine collagen revealed a higher amount of hydroxylysine and lysine. Shrinkage temperatures of non-crosslinked samples also showed a significant difference between equine (60 °C) and bovine collagen (57 °C). These properties are obviously of importance when producing collagen casings.

2.6.3. Age

Animal age is also important for casing production. Very young animals (fetal to 6 months) contain collagen with weak fibre strength, which is easily solubilized prior to extrusion, making it unsuitable for use in casings. Burke (1980) reported that heavy steers, aged approximately 2 years, produced acceptable material. Joseph (2003) stated that the average age for good beef collagen quality is 18–36 months. Age is also important when looking at collagen obtained from broilers. Collagen from young broilers is less cross-linked, as the degree of covalent crosslinking increases with advancing age; this in turn is related to lysyl oxidase initiated cross-linking. As fast growing broilers are commonly processed at 5–6 weeks, these cross-links have not been fully developed yet (Oechsle, 2016). With increasing age, the formation of covalent bonds and crosslinks, between adjacent tropocollagen molecules, in the microfibrils and between adjacent microfibrils increases. This means that the soluble "young" collagen progressively changes into "mature" collagen. Collagen and elastin undergo these continuous advancing molecular changes during life. The young collagen is capable of rapidly re-assembling to form a collagen triple helix. In contrast, mature collagen will only re-assemble over a period of days (Savic & Savic, 2016, pp. 47 – 87, 141 – 207, 212–258). The alterations of the physical and chemical properties of collagen fibers, due to aging, are very distinct. The fibers become increasingly insoluble with age, their ability to swell in acid solutions decreases due to cross-link formation, and both the mechanical strength and stiffness increases (Varnali, 2002).

2.6.4. Environment

Schwanz and Schnäckel (2007) and Harper et al. (2012) observed that animal casings can vary considerably in their quality. The difference in quality is possibly caused by the environment the animals are raised in. Schwanz and Schnäckel (2007) compared natural casings from Mongolia, Turkey, New Zealand, and Iran for their textural properties, such as tensile strength and elasticity. Differences in tear strength and elasticity were found and according to Savic and Savic (2016, pp 47-87, 141-207, 212-258) Savic & Savic, 2016 they could be related to differences in climate. For example, New Zealand has a mild climate, where the animals have more fresh feed throughout the year and therefore sheep casings from that country are less strong compared to casing originating from Iranian animals (hot climate). Casings from Iran are stronger and according to Savic and Savic (2016, pp 47-87, 141-207, 212-258)Savic & Savic, 2016 not suitable for all operations on sophisticated filling/linking equipment.

The suitability of a collagen gel for a certain sausage product is determined by the degree of natural cross-links present in the collagen gel, which depends on the animal species, age and climate and is determined by the extent of the liming process/amount of cross-links broken down during the liming process (Ponsen, 2010).

2.7. Other collagen sources for co-extruded casings

The use of co-extruded sausage casings has increased due to the rising costs of natural intestinal-derived casings (Barbut, 2010). Moreover, hygienic production and high volume sausage production requiring consistent quality characteristics, are becoming more important today. In addition, the increased demand for Kosher and Halal products puts pressure on non-pork collagen supply. Currently, beef collagen is the primary source for co-extruded casings. Chicken collagen could be used to supply the need (Cliche et al., 2003; Munasinghe et al., 2015; Oechsle et al., 2016), since it is a common by product. While some chicken skin is incorporated into meat emulsion, or used as a source of fat for soup preparation, not all is currently utilized by the industry;

only some is used for animal feed. However, the large amount of collagen contained in chicken skin offers greater potential if diverted to casing production. Other potential chicken collagen sources are cartilages, bones and feet (Osburn, 2002, pp. 445–466). It should be noted that currently chicken collagen is not used on a large scale for co-extrusion sausage production and is now mainly undergoing testing and improvement (Oechsle et al., 2017). In any case, the reason that chicken collagen could be an alternative for bovine collagen is that they both contain the fibril-forming collagen types I and III.

Chicken collagen from commercial broilers is less crosslinked compared to bovine collagen. As indicated before, i.e. lower degree of covalent crosslinking as broilers chicken are processed at approximately 5–6 weeks, while the average age of beef cattle is 18–36 months. Oechsle et al. (2016) characterized chicken bone and skin collagen in order to determine their suitability for preparing sausage casings applying the co-extrusion technology. Chicken skin exhibited a fibrous microstructure with thin fibers and a lower ability to swell. Extraction of collagen from chicken bones was not successful, as large and firm fragments led to an inhomogeneous particulate suspension. Although the extraction did not succeed completely, the collagen fibers obtained from the bones were short. This observation was the reason for them to conclude that collagen from chicken bone is not suitable for making casings. Because of the short fibers, gel viscosity was found to be insufficient, mainly due to a low entanglement between the fibers. On the other hand, chicken skin collagen yielded more entangled long collagen fibers and a gel with higher viscosity, therefore providing the desired properties for casing extrusion and subsequent crosslinking. The researchers concluded that chicken skin collagen displayed the most suitable source of collagen for the co-extrusion process compared to the well-established bovine hide split collagen. The same was reported by Munasinghe et al. (2015) who indicated that opportunities exist for further development of chicken collagen film as an alternative to beef collagen. Overall, they investigated possible use of underutilizing chicken by-products, as an alternative collagen source, for industries such as pharmaceutical, cosmetics, biomedical materials and the food industry.

In a later study, Oechsle et al. (2017) reported on the possible modification of extruded chicken skin collagen films and telopeptide-poor collagen from bovine hide by the addition of NaCl (0.05 mol/kg) and/or partial substitution of collagen by 1.25% soy protein isolate. Salt addition to the initial gel allowed forming beef and chicken collagen films with high tensile strength and elasticity. In contrast, a substitution with soy proteins decreased gel and film strength. This weakening of the collagen networks could be compensated by adding NaCl, leading to more homogeneous gels yielding films with higher storage moduli upon extrusion. The compensating NaCl effect was more pronounced for chicken skin than for the beef (telopeptide-poor collagen), suggesting differences in molecular interactions and network formation between the two different collagen types. The authors concluded by saying that the modulation of chicken collagen by NaCl and soy proteins enabled them to produce functional chicken collagen films. Currently beef collagen is the primary source for co-extruded casings. However, one of the major suppliers has recently introduced chicken collagen and it is yet to be seen how it will be accepted by the market.

3. Conclusions and future prospects

This review compiles currently available information regarding aspects of co-extrusion collagen casing production and manufacturing, which are important in producing high quality sausages. Furthermore, information regarding factors affecting collagen characteristics and possible use of other collagen sources for co-extrusion purposes are discussed. The latter is especially important today as the cost of natural casings is rapidly rising and the demand for Kosher and Halal products puts pressure on non-pork collagen supply. In addition, sustainable animal production is of utmost importance today and valuating meat plant by-products, with no/low value; can increase sustainability of the whole meat production chain.

Declaration of competing interest

None.

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