

Encapsulation of Meat Product Ingredients and Influence on Product Quality

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1. Introduction

For centuries meat has been considered important for optimal human growth and development (Pereira and Vicente, 2013). In the narrow sense, meat is defined as muscle tissue including interstitial fatty and connective tissues, blood and lymph vessels of animals (pig, poultry, ruminants, game). Generally speaking, meat is an important source of protein, fat, energy, essential amino acids, as well as micronutrients, such as iron, selenium, zinc and vitamin B₁₂ (Zhang *et al.*, 2010; Pereira and Vicente, 2013).

In addition, meat also contains high water content (approximately 75 per cent in the muscle), and is therefore susceptible to various changes. These changes are induced by various internal and external factors, such as enzymes, microorganisms, oxygen, light, temperature. Processes that occur in meat over time include protein degradation, lipid oxidation and microflora development. As a consequence of these changes, meat deteriorates quickly, its quality degrades and meat becomes unsuitable or even unsafe for consumption.

Since meat deteriorated quickly and as it was not readily available in the early human communities, different preservation principles were developed to extend its sustainability. Drying, smoking, heat treatment and salting were the earliest treatments used in meat preservation. Combining muscle tissue with other edible animal tissues (fatty tissue, offals, skin tissue, etc.), with the addition of spices and application of one or more preservation treatments, has led to the emergence of different meat products.

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For centuries meat products were used in nutrition as a significant source of meat proteins, energy and other nutrients. Nowadays, the availability of fresh meat has increased significantly, new preservation procedures have been developed and conventional ones have been improved. Hence, in addition to good nutritional qualities, meat products are now more highly appreciated because of their sensory characteristics which depend on the protein/fat/water ratio, usage of non-meat ingredients (salt, nitrates, phosphates, etc.), preservation procedures (smoking, drying, fermentation, etc.) and their interactions. However, in the last decades of the 20th century, new scientific insights found that certain components of meat/meat products have an adverse effect on health (salt, nitrites, saturated fatty acids (SFA), etc.), while increase in the intake of some other components can facilitate the prevention and treatment of certain illnesses (antioxidants, n-3 polyunsaturated fatty acids (n-3 PUFA), minerals, vitamins, etc.). Concurrently with scientific discoveries, consumer awareness has also changed; therefore, nowadays people are showing greater interest in food that contains bioactive or functional components which will give additional benefits to their health status (Hygreeva *et al.*, 2014). Accordingly, demand for natural, organic and/or clean label meat products has also increased (Cho *et al.*, 2017). This creates room for the use of different ingredients containing components which do not originate from meat or are not present in sufficient amounts, and are not traditionally used, with the aim to suppress the deterioration of meat/meat products and/or provide physiological benefits to health. In line with that, numerous research was conducted with the aim of prolonging shelf-life and/or enhancing the functional value of meat and meat products by using natural or synthetic ingredients with antioxidant, antibacterial, antiviral and probiotic activity. To this end, different ingredients containing bioactive components, such as phytosterols, polyphenols, carotenoids, dietary lipids, probiotics, prebiotics and botanicals were used (Zhang *et al.*, 2010; Olmedilla-Alonso *et al.*, 2013; Hygreeva *et al.*, 2014).

These active and bioactive compounds undergo different reactions in meat systems, and therefore degrade during processing and storage. To prevent rapid degradation and undesirable reactions and maintain full functionality, these compounds should be stabilized or immobilized in meat systems (Table 1).

Emulsions, double emulsions and gel-like matrixes were used in stabilization and immobilization of oils rich with PUFA (Jimenez-Colmenero *et al.*, 2015; Serdaroglu *et al.*, 2016; Stajić *et al.*, 2018a). Furthermore, double emulsions were used with the purpose of enclosing various aromas, bioactive compounds or sensitive food components (Serdaroglu *et al.*, 2016). Different encapsulation techniques were used for the stabilization of synthetic or natural active ingredients which were added in animal feed to enhance the functional value of meat (Dunne *et al.*, 2011), extend meat's shelf-life (de Oliveira Monteschio *et al.*, 2017), and prolong raw and thermally processed ground meat deterioration (Kılıç *et al.* 2014; Pabast *et al.*, 2018) while in meat products, they act as replacements of additives and/or improve functional value (Muthukumarasamy and Holley, 2006; Lorenzo *et al.*, 2016).

2. Encapsulation

In general, as presented in Table 1, activities aimed at improving the quality of meat/meat products can be divided into direct and indirect ones. Animal diet represents an indirect activity which is a longer-lasting and not fully controlled process through

Table 1: An Overview of Immobilization Techniques for the Improvement of Meat/Meat Products Quality and Achieved Goals

Approach	Application	Active Component	Achieved Goals	Immobilization Technique
Indirect	Animal feed	Lipids and essential oils	Protection from ruminal bihydrogenation; FA profile meat improvement, prolong the shelf-life of meat	Spray-drying
Direct – meat and meat products	Row, freeze and thermal treated ground meats: patty-type, fresh sausages, etc.	Phosphates Essential oils	Lipid oxidation reduction during storage Antioxidant and antimicrobial activity	Spray-drying, fluid bed coating, spray-cooling Double emulsions, spray-drying, extrusion
		Fish oil, plant oils	Improvement of FA profile	Organogels, oleogels, spray-drying, external gelation Spray-drying
	Meat products: fermented sausages, emulsified sausages (e.g. frankfurters), etc.	Essential oils Fish oil, plant oils	Antioxidant and antimicrobial activity Improvement of FA profile	
				Organogels, oleogels, oil-bulking, double emulsions; spray-drying, extrusion, coacervation
		NaCl, ascorbic acid, acidulants Probiotics Volatile essential oils	Delaying lipid rancidity and pH drop Protection sensitive bacteria; antimicrobial activity Antimicrobial effect of active packaging systems	Spray-drying, spray-cooling fluid bed coating, coacervation Extrusion, emulsification, freeze-drying Nanoliposomes

FA: Fatty Acids

which meat quality can be improved (Živković *et al.*, 2013). However, direct addition of functional components into meat/meat batters (meat products) is a simpler and more controlled approach (Živković *et al.*, 2013).

3. Improving Meat Quality by Animal Diet – Indirect Approach

Many meat components, such as proteins, minerals, vitamins, conjugated linoleic acid, carnitine, carnosine, etc., have a positive effect on human health. However, meat lipids, due to the high content of SFA, have poorer nutritional qualities. Animal diet is the major factor influencing the composition and quality of muscle and fatty tissues, especially in monogastric animals (pigs, poultry and fish) since their organism absorbs fatty acids in their intact form (Živković *et al.*, 2013).

3.1 Improvement of the Fatty Acid Profile

In ruminants, due to ruminal biohydrogenation, compounds (such as unsaturated fatty acids (UFA)) are altered. Therefore, rumen protection technologies have emerged over the years and these include the encapsulation of UFA inside a microbial-resistant shell (Jenkins and Bridges Jr., 2007). The procedure implies the encapsulation of active compounds (e.g. oils rich in PUFA) in a pH sensitive matrix which remains intact at rumen pH, but gets broken down at the lower pH in the abomasum, thereby constituents were released for absorption in the small intestine (Dunne *et al.*, 2011; Scollan *et al.*, 2014).

According to literature data, ruminal protection of fatty acids by encapsulation has been achieved in several ways. One way implies the incorporation of fatty acids (or oils) within proteins (e.g. casein) cross-linked with formaldehyde which provides resistance to microbial degradation in rumen (Jenkins and Bridges Jr., 2007). Modification of this encapsulation procedure without the use of formaldehyde was also reported (Dunne *et al.*, 2011). Furthermore, encasing oils rich in PUFA within a sphere of high-melting-point SFA is another encapsulation procedure used for rumen-protected products (Jenkins and Bridges Jr., 2007).

Lipids encapsulated in formaldehyde-treated protein matrix were applied in the diet of ruminants in several research studies. Garrett *et al.* (1976) used encapsulated lipids supplement (70 per cent sunflower seed and 30 per cent soybean protected lipid – containing 21 per cent linoleic acid) in the feed of lambs and steers. The major changes were increases in the contents of linoleic and α -linolenic acids and decreases in the contents of myristic, palmitic, palmitoleic and oleic acids, in both fatty tissue and muscle lipids. Furthermore, they reported that approximately 18-25 per cent of the consumed linoleic acid (as encapsulated lipids) was stored in the body tissues of cattle.

Scollan *et al.* (2007) examined the effects of feeding an encapsulated lipids supplement (70 per cent soybean, 22 per cent linseed and 8 per cent sunflower-seed oils) on the fatty acid composition of beef *Longissimus thoracis* muscle and the associated subcutaneous adipose tissue. The results were similar to Garrett *et al.* (1976) – encapsulation provides a high level of protection from ruminal biohydrogenation, the meat had a lower content of total fat, and increases of linoleic and α -linolenic acids, and decreases of SFA contents were observed in the subcutaneous adipose tissue

and muscle lipids. This increase of the PUFA/SFA ratio to 0.28 was also recorded, resulting in beef meat with healthier lipids content.

The limitation of the previous research was that it had no influence on long chain n-3 PUFA content, especially on the content of eicosapentaenoic (EPA) and docosahexaenoic acids (DHA), which are highly associated with numerous beneficial effects on human health (Zhang *et al.*, 2010). This can be overcome by using encapsulated fish oils, as reported by Dunne *et al.* (2011). In this research, fish oil was encapsulated by technology that was modified and formaldehyde is not used. Beef heifers were offered a ration, containing three different proportions of encapsulated fish oil fortified with vitamin E, and the fatty acid profile of neck muscle intramuscular lipids (neutral and polar lipids) were examined. The authors also stated that encapsulation provides considerable ruminal protection of dietary PUFA since the EPA and DHA content in meat increased three and two times, respectively. The authors concluded that by applying this technology of rumen protection, it is possible to achieve EPA and DHA concentrations which would allow muscle to be labeled as a 'source' of these fatty acids in human nutrition. Kitessa *et al.* (2001) also reported three times higher content of both EPA and DHA and twice higher content of linolenic acid in the *Longissimus* muscle of lambs fed with the addition of encapsulated (protein/formaldehyde technology) tuna fish oil. Furthermore, significant incorporations of these fatty acids in omental and perirenal fat were observed.

In general, the results indicate a great potential of encapsulation as a technique for ruminal protection of dietary lipids, resulting in numerous commercial supplements which were used in some research. The reported differences were due to different sources of fatty acids (linseed, canola, fish oils), different muscles (neck, leg, loin) and fatty tissues (subcutaneous, omental, perirenal), which should be taken into account when considering the effects of the use of encapsulated dietary lipids.

3.2 Improvement of Oxidative Stability

A very important fact that should not be neglected is that the improvement in the fatty acid profile should not lead to intensive lipid oxidation and thus influence meat aroma and color, especially during storage and retail display (Dunne *et al.*, 2011).

The use of oil enriched with natural antioxidants (e.g. tocopherols) can suppress lipid oxidation in meat. In their research, Dunne *et al.* (2011) used encapsulated fish oil fortified with vitamin E and found that ground beef with the highest EPA and DHA contents (which corresponds to the highest dietary encapsulated oil intake) reached critical lipid oxidation levels (TBARS value – thiobarbituric acid reactive substances) at five to six days of simulated retail display, indicating that more vitamin E is required. However, although lipid oxidation is associated with color stability, it did not affect color stability.

In the research of de Oliveira Monteschio *et al.* (2017), encapsulated eugenol, thymol and vanillin mixture (commercial blend) was used alone or in combination with clove and/or rosemary essential oils, in the feed of heifers. The use of essential oils and/or their encapsulated active principles did not affect some of the observed parameters which are very important for meat quality, such as pH, fat thickness, marbling, muscle area (*Longissimus thoracis* was examined), water loss by thawing and dripping, and cooking loss during the aging period. Color parameters were not affected, except for redness (at 14 days of aging), which was lower in control than in

other diets, indicating lower maintenance of red color during aging in the control feed. Meat from heifers fed with the use of a mixture of essential oils and their encapsulated active principles was more tender than control after 14 days of aging. Furthermore, the control feed showed the lowest antioxidant capacity and the highest lipid oxidation rate during 14 days of aging. The use of encapsulated eugenol, thymol and vanillin mixture and its mixture with clove essential oil in the feed of heifers resulted in meat with higher antioxidant activity and lower lipid oxidation rate during 14 days of aging. The results indicate a good potential for using encapsulated active compounds in animal feed to maintain and prolong the shelf-life of beef.

4. Improving Meat/Meat Products Quality – Direct Approach

Besides using different immobilized compounds in animal diet to improve meat quality and shelf-life, among which some can positively affect the immune and digestive system of animals and thus can be natural alternatives to antibiotics (de Oliveira Monteschio *et al.*, 2017), improving meat quality and prolonging meat stability during retail period can be achieved by direct application of immobilized compounds into meat systems.

4.1 Encapsulation of Commonly Used Non-meat Ingredients

Encapsulation technology can be used for the controlled release of commonly used compounds in meat systems (salt, phosphates, etc.) during the retail period, thus increasing and prolonging the stability (lipid, microbial...) of fresh meat, ground meats prepared for further thermal treatments (e.g. patties) or already thermal-treated ground meats.

Sodium chloride: Salt (sodium chloride) is one of the most common non-meat ingredients and probably the oldest non-meat ingredient used in meat processing. Apart from contributing to preservation and flavor, salt is very important for textural properties of meat products because it is critical to solubilization and extraction of salt-soluble proteins (Sebranek, 2009). On the other hand, salt lowers the freezing point and catalyzes lipid oxidation reactions in meat systems. One approach to overcoming this is the encapsulation of salt within layer(s) of hydrogenated vegetable oil, which melts at higher temperatures, prevents salt from interacting with proteins and water. This way, salt is released into the meat system slowly, or during the thermal processing, or when thermal processing is over. Various commercial products of encapsulated salt are offered for use in meat and fish processing, marinades, sauces and in seasoning mixtures. The use of encapsulated salts assumes that it is not required for protein extraction and preservation before thermal processing (Monahan and Troy, 1997).

Phosphates: Phosphates are commonly used ingredients in meat processing. They are used as blends of mono-, di- and poly-phosphates to improve the water-holding capacity (and thus the juiciness and tenderness of meat), but also to enhance color and lipid stability. Tripolyphosphates are more effective in prolonging lipid oxidation than di- or mono-phosphates, but they are hydrolyzed by phosphatases in the meat system before thermal processing (Sickler *et al.*, 2013). Thus, encapsulation protects phosphates from degradation by heat sensitive phosphatases active in raw meat or ground meat stored for some time before thermal processing (Sickler *et al.*, 2013).

In their research, Sickler *et al.* (2013) compared unencapsulated sodium tripolyphosphate (STP) and STP encapsulated with hydrogenated vegetable oil designed to release the phosphate once the temperature reaches 74°C, in ground turkey breast meat cold stored up to ten days. The cooking loss and pH were not influenced by encapsulation, which means that encapsulation would not alter the influence of STP on the water-holding capacity. The use of encapsulated STP significantly improved lipid stability during storage compared to unencapsulated STP, which probably increased pigment stability because encapsulated treatments were more red (higher a^* values). The authors concluded that the results indicate a potential use of encapsulated STP in meat products, such as: uncured precooked ground turkey and chicken sausages and nuggets, beef patties, restructured roast beef, meat loaves, which have a delayed thermal processing step after incorporating ingredients. Similar to Sickler *et al.* (2013), Kiliç *et al.* (2014) reported that in general, the use of encapsulated phosphates (in hydrogenated vegetable oil) instead of unencapsulated can significantly improve the lipid stability of cooked ground chicken meat and ground beef without any significant adverse effects on cooking loss, color and pH values.

Some different results regarding the effect of encapsulated STP on lipid oxidation in cooked ground beef during the seven-day storage were reported by Bilecen and Kiliç (2019), who examined the encapsulation effect of STP and sodium pyrophosphate (SPP) and their combination. They confirmed the results of previous research, and found no effect of encapsulation of STT, and reported similar finds for SPP on cooking loss, pH and color parameters, but reported that only encapsulated SPP significantly reduced lipid oxidation during storage. The combination (1:1 ratio) of encapsulated STP and SPP also significantly reduced lipid oxidation, but treatment with this combination shows lower color stability (decrease of redness and increase of yellowness) during storage as compared to the one with encapsulated STP. The authors selected this combination for potential use in ready-to-eat meat product formulations, but pointed out that it may reduce the beneficial effect of encapsulated STP on cooking loss.

Since phosphates were encapsulated with hydrogenated vegetable oil, it is important to mention the research of Du and Claus (2015) and Claus *et al.* (2016), who concluded that the melting properties of wall material should allow the release of phosphates at higher temperatures. At these temperatures, the inactivation of phosphatases is more complete, reducing the phosphate degradation (by the heat-sensitive phosphatases) and allows phosphates' maximum effect in prolonging lipid oxidation.

Ascorbic/Erythorbic acids: In processing cured meat products, reducing compounds, such as ascorbic acid, erythorbic acid and their sodium salts are commonly used. These compounds are very important in reactions of nitrite and myoglobin resulting in the formation of nitrosylmyoglobin, which is responsible for cured meat color (Sebranek, 2009). Acids are very reactive and can cause fast nitrite reduction and loss from curing mixture before nitrosylmyoglobin is formed, so that sodium salts are commonly used forms (Sebranek, 2009). Thus, the application of encapsulation techniques can be useful in providing protection and stability of ascorbic acid in cured meat systems. Comunian *et al.* (2014) encapsulated ascorbic acid by the coacervation technique (in gum arabic) and applied freeze-dried microcapsules in chicken frankfurter formulation. The results indicated that encapsulation provided the stability of ascorbic acid during processing and storage of frankfurters, and no significant differences in instrumental

color, oxidative stability and sensory properties were found compared to the treatment containing sodium erythorbate.

Acidulants: Decreasing pH values in meat products have a preservative effect because lower external pH disturbs the homeostasis of different pathogens and spoilage bacteria (Leistner, 2000). However, due to fast pH drop and/or long time between mixing/staffing and thermal processing, this results in massive protein denaturation, which leads to texture deficiency and fat separation and in turn, to an unacceptable product (Barbut, 2005). Therefore, a slow and targeted release of acidulants, immediately before or during thermal processing, is recommended. This can also be achieved by their encapsulation within a layer of hydrogenated vegetable oil designed to melt (and thus release acid) at higher temperatures. Barbut (2005) reported that the addition of liquid lactic acid led to fast protein denaturation, clumping and moisture release of meat batters after thermal processing, while the use of encapsulated lactic acid (with hydrogenated vegetable oil designed to release acid at 51-55°C) resolved these deficiencies. Similar results were reported for encapsulated citric and gluconic acids. Cordray and Huffman (1985) reported that the use of encapsulated lactic acid and glucono-delta-lactone led to a more intense flavor and more desirable color of restructured heat processed meat products without an adverse effect on physico-chemical and sensory properties. The use of acidulants encapsulated (by spray-drying) in water soluble coatings (i.e. maltodextrin) and designed to be released after meat emulsion is formed (before thermal processing), so as not to alter the emulsion stability, was confirmed by patents of Percel *et al.* (1985a, 1985b).

4.2 New/natural Antioxidants, Antimicrobials and Colorants

For several decades already, the meat processing industry has constantly been searching for natural alternatives to synthetic ingredients which were traditionally used, such as nitrates, pigments (i.e. cochineal carmine), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), etc.

Besides the encapsulation of commonly used ingredients (salt, phosphates, acidulants), which have been in use for several decades and has resulted in numerous commercial products, as of recently different encapsulation techniques have been used for immobilization of plant and herbal sourced bioactive compounds with antioxidant and antibacterial activity which exhibit a beneficial influence on human health. However, most of these bioactive compounds exhibit low water-solubility, strong off-flavors and are unstable in meat systems during processing and/or storage, which is why encapsulation is used to enable their protection as well as controlled and targeted release (Vinceković *et al.*, 2017).

In addition to spray-drying, other commonly used encapsulation techniques, applied to overcome restrictions of different compounds, are freeze drying, extrusion methods, molecular inclusion, coacervation, nanostructured lipid matrices or/and solvent evaporation (Gómez *et al.*, 2018).

In the research of Baldin *et al.* (2016), encapsulated (in maltodextrin by spray-drying) jaboticaba (*Myrciaria cauliflora*) extract was used in fresh pork sausage for providing red color pigment and antioxidant activity. The authors reported that encapsulated jaboticaba extract reduced lipid oxidation and provided pigment stability during 15 days of storage, but concentrations higher than 2 per cent in sausage formulation can impact sensory characteristics negatively. Moreover, the authors suggested the use of 2 per cent of encapsulated jaboticaba extract as a natural

replacement for cochineal carmine in fresh sausages. Spray-drying was also used for encapsulation of propolis co-product extract (capsul® as wall material) to provide oxidative stability of burger meat during 28 days of freeze storage (Reis *et al.*, 2017). Encapsulated propolis co-product extract exhibits antioxidant activity as effective as sodium erythorbate and has potential usage as natural replacement for synthetic ingredients.

Wu *et al.* (2019) used myofibrillar proteins as carriers to encapsulate curcumin (lipophilic antioxidant) by the pH shifting method. The authors reported positive effects of myofibrillar proteins as carriers on the improvement of solubility and stability of curcumin-myofibrillar proteins nanocomplexes, which possess strong antioxidant activity and thus can improve the stability of marinated chicken meat during 12 days of storage.

Clove (*Syzygium aromaticum*, L.) essential oil is another potential natural alternative to chemical preservatives in meat processing due to its antimicrobial (against several pathogenic bacteria) and antioxidative activity (Radünz *et al.*, 2019). However, strong odor limits its usage. Encapsulation is suggested as a possible solution. Radünz *et al.* (2019) reported that encapsulated clove essential oil (extrusion, with sodium alginate and emulsifiers) showed low antioxidant activity but also strong antimicrobial inhibition. In burger-like meat products, after seven-day storage, encapsulated clove essential oil exhibited antimicrobial effect against *Staphylococcus aureus* in a similar way as would the presence of nitrite.

Spray-dried micronized encapsulated essential oil blend (citrus, onion, and garlic mixed in a sodium tripolyphosphate and potassium lactate base) was suggested as a potential alternative to potassium lactate in growth control of *Listeria monocytogenes* and *Salmonella* serovars in some ready-to-eat (RTE) meat products during storage (Casco *et al.*, 2015). However, the authors reported limitations regarding bacteria type and types of RTE meat products; namely, the encapsulated essential oil blend did not exhibit *Salmonella* reduction in chunked and formed cured ham and formed roast beef, but was as efficient as potassium lactate in emulsified poultry bologna. Furthermore, a potential alternative to potassium lactate in growth control of *Listeria monocytogenes* was reported in ham and roast beef products only. Also, sensory analysis was not performed, so the authors suggested consumer acceptability testing.

Healthier products can be obtained by replacing fatty tissue in meat products with oils with favorable fatty acid profile. However, due to high PUFA contents, these oils are susceptible to lipid oxidation. Different encapsulation techniques (described below) can be applied to delay or prevent lipid oxidation. Furthermore, incorporation of natural compounds with antioxidative activity in oils before encapsulation can provide more oxidative stability. While the replacement of 50 per cent of fatty tissue in burgers with encapsulated chia oil (by external ion gelation technique) decreases oxidative stability, the addition of rosemary in chia oil before encapsulation provides necessary oxidative stability during 120 days of storage, without impairing the sensory quality (Heck *et al.*, 2018).

4.3 Improvement of Fatty Acid Profile of Processed Meats

Given that scientists have established a link between the lower rates of heart disease among Greenland's Eskimos and their diet rich in n-3 PUFA (Nichols *et al.*, 2010), numerous research pointed out the benefit of these fatty acids, not only in the prevention of coronary heart disease, but also for other illnesses, such as cancer,

inflammatory diseases, depression, diabetes type 2 (McAfee *et al.*, 2010; Zhang *et al.*, 2010). Furthermore, since the 1960s, SFA were marked for increasing risk of coronary heart disease and medical societies recommended lowering their intake, while new research indicated that the content of individual fatty acids, such as palmitic acid, is even greater than the total SFA (Kleber *et al.*, 2018). The amount of the fatty tissue and its fatty acid profile are of importance for sensory quality and stability of meat, especially for meat products (processed meat). Namely, color, odor, taste and texture are strongly dependent of total fat (fatty tissue) content, while SFAs affect the hardness of the fatty tissue and reduce its susceptibility to oxidation (Stajić *et al.*, 2018b). In general (with differences between the most commonly used animal species), oleic (C18:1) and palmitic (C16:0) are the most dominant in the fatty acids profile of meat and fatty tissue, while the n-3 PUFA content is generally low (Wood *et al.*, 2008). Furthermore, the contents of myristic acid (C14:0) and stearic acid (C18:0), which some research studies also correlated with coronary heart disease risk (McAfee *et al.*, 2010), are also important (Wood *et al.*, 2008). The total fat content in meat products can be very high, even exceeding 40 per cent, and which can be found in dry-fermented sausages. Meat and meat products are among the main sources of dietary fats, therefore the introduction of oils with a favorable fatty acids profile, as a replacement for fatty tissue, can help improve their nutritional properties. However, the created products must have the same or imperceptibly-altered sensory qualities. Because of their susceptibility to oxidation, oils rich in monounsaturated fatty acids (MUFA) and PUFA can be stabilized by emulsification and encapsulation before their implementation in meat systems (Delgado-Pando *et al.*, 2010; Josquin *et al.*, 2012). Literature data pointed at the oil-in-water emulsion system with soy protein isolates (SPI) as the earliest and the prevalent used method, while gel-like systems were noted in some new research (Stajić *et al.*, 2018b). Furthermore, different encapsulation techniques were used to increase the stability of oils, while further stabilization of encapsulated oils in gel-like matrix was reported in different types of meat products (e.g. fermented sausages, emulsified sausages, fresh sausage, etc.).

The effect of the use of immobilized oils on the quality of meat products depends on oil type and amount, immobilization technique and the production process and ingredients, which can, more or less, result in favorable lipids changes. Fermented sausages (dry and semi-dry) undergo the fermentation/drying/ripening processes and can be stored for several months in cold or room temperatures, either packed or unpacked. Emulsified sausages (e.g. frankfurters) after heat processing (70-75°C in thermal centre), are usually packed (vacuum or MAP) and cold-stored for several weeks. Ground meats (e.g. burgers, fresh sausages) can be frozen and stored for several months before heat processing.

Among meat products, fermented sausages have a very long tradition and the high fat content is very important for their sensory properties. Stajić *et al.* (2014) and Stajić *et al.* (2018b) used grapeseed and flaxseed oil respectively, encapsulated by electrostatic extrusion in calcium alginate, to partially replace pork backfat in dry-fermented sausage formulation. The oils were also added (to the same extent) as emulsion (with SPI) and alginate gel. The authors replaced 20 per cent of backfat with encapsulated grapeseed and flaxseed oil, with the average fat content of 40.3 per cent and 40.1 per cent (to gain about 5 per cent oil content in initially batch) respectively and with the average particle size of 714 and 1161 µm respectively (Fig. 1). In both studies, these encapsulated oils did not alter the production process to a

large extent (weight loss, pH change, basic chemical composition), but they did lead to significantly lower hardness and chewiness compared to control and to the treatments with oils added as emulsion and alginate gel. The authors indicated the possibility that the large number of microspheres prevent meat pieces from binding firmly during fermentation and ripening. The influence on color was different and was attributed to the different color characteristics of oils. Regarding sensory characteristics, in both studies, treatments with encapsulated oils were, in general, perceived as acceptable. However, lower hardness and chewiness were perceived as less desirable by consumers/panelists. Encapsulation of oils (as other two treatments) provided stability to dry-fermented sausages during cold storage in vacuum.

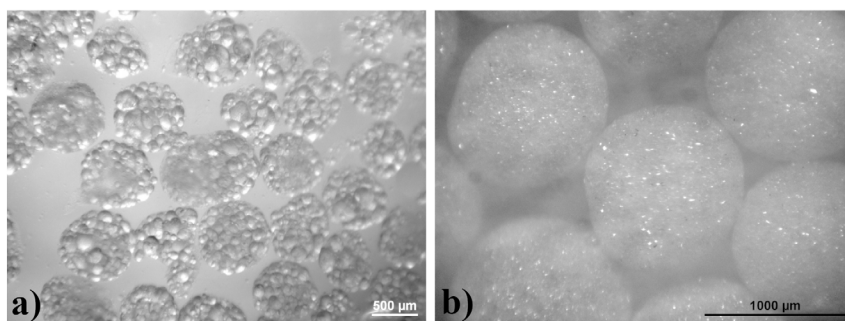


Fig. 1: Grapeseed oil (a) and flaxseed oil (b) encapsulated by electrostatic extrusion

Fish and flaxseed oils encapsulated by spray-drying (added as commercially-available ingredients) were used for partial replacement up to 30 per cent of pork backfat in fermented sausages formulation (Pelser *et al.*, 2007; Josquin *et al.*, 2012). Thus the nutritional properties improved significantly, with higher PUFA and lower SFA contents and lower n-6/n-3 ratios. The use of encapsulated oils, despite higher PUFA contents, did not increase lipid oxidation during cold storage even in a modified atmosphere containing oxygen (Josquin *et al.*, 2012). Regarding lipid oxidation, fermented sausages with encapsulated fish oil (containing ascorbic acid) were found to be more stable than the ones with pre-emulsified (with SPI) fish oil (Josquin *et al.*, 2012). Contrary to Stajić *et al.* (2014) and Stajić *et al.* (2018b), the use of oils encapsulated by spray-drying led to firmer sausages without altering their sensory quality. Encapsulation masks the impact of sensory properties of fish oils (e.g. fishy taste), hence the sensory quality of fermented sausages with encapsulated fish oil was closest to the control treatment, unlike the sausages with liquid and pre-emulsified oil (with SPI). The results of this research pointed at encapsulation of fish oil as the best way to introduce this oil into fermented sausage formulation. The fish oil encapsulated by spray-drying (in maltodextrin, gum arabic and caseinate) and further stabilized in konjac glucomannan matrix was introduced in Spanish salchichón formulations to partially replace (up to 75 per cent) pork backfat (Lorenzo *et al.*, 2016). The increment of encapsulated fish oil (in konjac matrix) content led to harder and darker sausages, and also to a progressive increase in lipid oxidation, so that contrary to the previously-mentioned researches, encapsulation did not provide lipids protection. However, in the following research (Munekata *et al.*, 2017), oxidative stability was obtained by using natural antioxidants.

Frankfurters are worldwide popular emulsion-type and ready-to-serve meat products, made from pork and beef, as well as poultry meat. In the research of Salcedo-Sandoval *et al.* (2015), fish oil encapsulated in filled hydrogel particles (casein and pectin) was used as a replacement for backfat in low-fat pork frankfurters. The results indicated that the introduction of encapsulated fish oil into low-fat frankfurters will not alter technological properties – processing loss, purge loss, instrumental color and texture were similar (or even improved) to control treatment and moreover remain stable during the storage period. Encapsulation provides better oxidative stability as compared to oil-in-water emulsion. The possible explanation could be that encapsulated particles are more stable during thermal processing than conventional emulsions, and that casein and pectin are more effective at scavenging free radicals and chelating metals (Salcedo-Sandoval *et al.*, 2015). However, frankfurters with encapsulated fish oil were less oxidatively stable than the control treatment (only backfat), especially after 30 days of cold storage. Sensory properties were close to control and significantly better as compared to the treatment with emulsified fish oil. In the study of Domínguez *et al.* (2017), encapsulation (spray-drying; in maltodextrin, gum arabic and caseinate) of fish oil did not ensure its oxidative stability in pork frankfurters with partially replacement backfat, despite the use of BHT as antioxidant. Moreover, these frankfurters showed higher TBARs values, not only when compared to the control ones (only backfat), but also to frankfurters with pre-emulsified (with sodium caseinate) oil. The authors indicate that this may be due to high temperatures during the spray-drying process (and its length) which degrades BHT, or that antioxidant activity of BHT was limited by the encapsulation process.

Ground-type meat products, such as burgers (ground beef patty) and chicken nuggets, are widely consumed. Burgers and other ground beef patty-type products contain about 20-30 per cent (Heck *et al.*, 2019) of animal fat and the content of 20±2 per cent of fat was generally recommended for optimum palatability (Rust and Knipe, 2014). They can be sold fresh or frozen. The fat content was also important for the sensory quality (appearance, texture, aroma) and technological properties (cooking yield and shrinkage), and products with lower fat content had generally lower consumer acceptance (Rust and Knipe, 2014; Heck *et al.*, 2019). The encapsulated chia oil (by external ion gelation technique and 25 per cent% of oil content) was used for partial replacement of 50 per cent of backfat in beef burgers freeze-stored for 120 days. The modified burgers, besides having an improved fatty acid content, also had lower total fat contents, while instrumental color parameters were not affected, except lightness after cooking (higher values were measured). While 50 per cent replacement of backfat with encapsulated chia oil decreases oxidative stability, the addition of rosemary in chia oil before encapsulation provides the necessary oxidative stability during 120 days of storage, without impairing sensory quality, thus representing a new strategy to the introduction of oils with favorable fatty acid contents into cooked/ready-to-cook meat products (Heck *et al.*, 2018; Heck *et al.*, 2019).

The demand for frozen breaded pre-fried ready-to-cook meat products, such as chicken nuggets, has increased in recent years (Pérez-Palacios *et al.*, 2018). Hence, these meat products have become interesting in the sense of creating functional food by changing the fatty acid profile. To improve the functional properties of chicken nuggets, spray-dried multilayered (lecithin, maltodextrin) microencapsulated fish oil (10 per cent oil content) was added at the level of 5 per cent into their formulation

(Pérez-Palacios *et al.*, 2018). The introduction of microencapsulated fish oil into chicken nuggets did not alter their sensory properties – consumers were not able to differentiate between the control treatment (without fish oil) and nuggets with microencapsulated fish oil, as shown in the results of the triangle test. Furthermore, as in the previously mentioned research, the encapsulation provides protection against lipid and protein oxidation, contrary to the nuggets with unencapsulated fish oil.

4.4 Probiotics

Improving the nutritional properties of meat products by using probiotics has been studied in the past several decades. Many fermented meat products, which do not require heat treatment during processing, such as dry-fermented sausages with probiotics, have been developed. The main requirements for the use of probiotics include their non-pathogenic nature, good resistance to conditions in the gastrointestinal tract, health benefits and a non-negative effect on sensory quality (Toldrá and Reig, 2011). Also, good adaptability to the conditions of dry-fermented sausages (low pH, high salt content, high nitrite content and low a_w values) is of great importance (Toldrá and Reig, 2011) because it is assumed that at least 6 log cfu/g of probiotics in the final product is required for them to have beneficial effects on human health (Muthukumarasamy and Holley, 2006). Again, encapsulation techniques could have the potential to provide protection to probiotics against such environmental and gastrointestinal conditions.

In the research of Muthukumarasamy and Holley (2006), the probiotic bacterium *Lactobacillus reuteri* was encapsulated by the extrusion and two-phase emulsion method with sodium alginate as a wall material and was added (as co-culture) in the amount of 7 cfu/g of meat batter (containing starter cultures *Pediococcus pentosaceus* and *Staphylococcus carnosus*), stuffed into fibrous casings and subjected to the fermentation/drying process. Both encapsulation techniques provide survival of *Lactobacillus reuteri* in sufficient numbers (> 6 log/g) until the end of the production process. The addition of encapsulated *Lactobacillus reuteri* did not influence pH and a_w values during production and in the final product. Furthermore, regarding sensory properties, no significant differences were found when comparing dry-fermented sausages containing encapsulated *Lactobacillus reuteri* with control (only starter cultures). According to the authors, this is probably the first study investigating the use of encapsulated probiotics in dry-fermented sausages, indicating that encapsulation techniques have great potential to provide a sufficient number of probiotics in this type of meat products.

In the next study, the same authors (Muthukumarasamy and Holley, 2007) encapsulated *Lactobacillus reuteri* and *Bifidobacterium longum*, separately or combined, by extrusion with sodium alginate as a wall material and added encapsulates to meat batter of dry-fermented sausages as a co-culture in the amount of 7 cfu/g. Regarding technological properties (pH and a_w values), the results confirmed those from the previous research. Encapsulation, as in the previous research, provided protection of probiotics during fermentation/drying process for both probiotics, but reduced their inhibitory activity against *E. coli* O157:H7. Therefore, the authors recommended a combination of unencapsulated and encapsulated forms to gain both viability and inhibitory activity of *Lactobacillus reuteri* against *E. coli* O157:H7 in dry-fermented sausages.

4.5 Active Packaging Systems

The packaging of fresh and processed meats (meat products) extends their shelf-life by reducing the influence of internal and external factors (oxygen, light, temperature, activity of enzymes and microorganisms) that cause meat/processed meat deterioration. Active packaging has become of interest in recent years because, in addition to decreasing meat/processed meats' decay, the developed active packaging technologies also reduce environmental pollution related to packaging (Wrona *et al.*, 2017). These technologies enable the incorporation of different active compounds into the packaging materials which, during the storage of meat/meat products, adsorb substances from the food or the environment (that cause deterioration) or are released into package environment to reduce the influence of factors that cause decay (McMillin, 2017). Thus, encapsulation could provide the protection of active compounds during their incorporation into packaging materials and also slow down and target their release during meat storage (Wrona *et al.*, 2017).

Several researches successfully applied encapsulated active compounds for developing active packaging for meat/meat products. Wrona *et al.* (2017) incorporated encapsulated green tea extract (crystalline microporous aluminosilicates as carrier) into polyethylene in two concentrations and packed minced pork meat in vacuum bags, covered by these materials under normal atmosphere. The encapsulation provides protection of green tea extract versus processing temperature during package material extrusion. Furthermore, active packaging increases the stability of minced meat up to nine days of storage. Minced meat packaged in active materials had lower met-myoglobin content (causes brown color), higher instrumental redness compared to control sample (no active packaging) at day nine of storage. The results of sensory evaluation correlated with the results of chemical and instrumental color analysis, while the aroma of experimental samples was assessed as fresh meat vs. vinegar in the control sample. However, active packaging with encapsulated green tea extract could not decrease the deterioration of minced meat after day nine and a higher concentration of the extract did not have a more pronounced impact during the entire storage period.

In the research of Pabast *et al.* (2018), nano-encapsulated *Satureja khuzestanica* essential oil (nanoliposomes, sonication method) was incorporated into chitosan-based material and used for coating the lamb meat that was cold stored for 20 days. Encapsulation enabled the controlled release of active compounds on meat surface during storage, while the coating with encapsulated *Satureja khuzestanica* essential oil significantly reduced microbial growth and provided stability during the entire storage period – the critical value of total viable counts (7 log CFU/g flesh) was not reached by the end of storage while in the control sample, it was reached after nine days. Moreover, significant reduction of lipid oxidation was also observed by the end of storage. Sensory quality (color and odor) was very stable throughout the whole storage period, which leads to the conclusion that chitosan coatings containing encapsulated *Satureja* essential oil have a potential for usage in active package development for the meat industry.

Another encapsulation technique was successfully developed and applied by Rajaei *et al.* (2017), to enhance meat's shelf-life. As in the previously mentioned research, in this research encapsulation also improved the effectiveness of essential oils. Clove essential oils (CEO) encapsulated by chitosan-myristic acid nanogel were used for coating beef meat cold-stored for 12 days. Nanogel-encapsulated CEO

exhibited high antibacterial activity against *Salmonella enteric* and did not impair the color of beef meat during the storage period.

5. Other Techniques for Non-meat Ingredient Stabilization

As mentioned above, emulsions and double emulsions are able to stabilize some functional and bioactive compounds added to meat products with the aim to improve its nutritive value and provide functional properties. Emulsion type systems are mostly used as animal fat replacers, carrying oils rich in PUFA in order to improve the fatty acid profile of meat products and provide a health-promoting (functional) potential. Such an approach could be very challenging because of a possible adverse effect on sensory properties of products and their proneness to enhanced oxidation changes (Muguerza *et al.*, 2004; Vasilev *et al.*, 2010; Vasilev *et al.*, 2011; Grasso *et al.*, 2014).

Edible oils could be stabilized in emulsion-type systems by means of different strategies, including organogelation, oil-bulking, structured emulsions (hydrogelled and organogelled structured emulsions) (Jimenez-Colmenero *et al.*, 2015) and double emulsions (Serdaroğlu *et al.*, 2016).

5.1 Organogels

Organogels are bi-continuous systems which consist of gelators and non-polar solvents. The gelators tend to form fibrillary network structures that prevent the flow of non-polar phase. The most commonly used gelators could be divided into two groups: gelators that bind hydrogen, such as amino acids, amide and urea moieties, and carbohydrates, as well as gelators that do not bind hydrogen, including anthracene, anthraquinone and steroid-based molecules (Sahoo *et al.*, 2011). According to Esposito *et al.* (2018), organogelators could be divided into polymeric organic gelators (POGs) and low-molecular weight organogelators (LMOGs), and according to their origin, into synthetic molecules and biomolecules. Synthetic LMOGs include sorbitan monostearat, monopalmitate as well as glyceril fatty acid esters, while synthetic POGs are based on acrylic acid, sodium allyl or styrene sulfonate, polyethylene and polymethyl methacrylate, etc. As for biomolecules, lecithin is widely used, representing the group of phospholipids originating from soybean or egg yolk. Other biomolecules are phytosterols, sugars, carbohydrates, peptides and their derivatives, as well as waxes.

The potential use of organogels in meat products has been studied by several authors. Barbut *et al.* (2016a) designed organogels consisting of canola oil as the solvent and ethylcellulose and sorbitan monostearate as gelators, in order to replace beef fat in meat batter. The organogel was prepared by mixing canola oil, ethylcellulose and sorbitan monostearate by means of overhead mechanical stirrer in a gravity convection oven, heated to 140°C. The whole process lasted 60 min (50 min to reach the target temperature, and 10 min of holding period), followed by chilling to the final temperature of 5°C. The organogel prepared in this manner, containing ethylcellulose in the concentration of 8 per cent, could be used as a beef-fat replacer in frankfurters without significantly affecting sensory properties. In higher concentrations of organogels, such as 10, 12 and 15 per cent in sausages, it resulted in higher hardness and less juiciness of the product. The same authors (Barbut *et al.*, 2016b) examined the potential use of organogel as fat replacer in breakfast sausage. The organogel was

prepared by mixing canola oil with powdered gelators (10 per cent ethyl cellulose and 1.5 per cent sorbitan monostearate) at room temperature, followed by the same procedure described for frankfurters. In order to reduce lipid oxidation, the authors used butylated hydroxyl toluene (50 ppm) and rosemary oleoresin (0.6 per cent). As breakfast sausages belong to meat preparations intended for use after heat treatment, cooking loss is one of the important technological properties of such products. The use of organogels contributed to the retention of fat and water during heating, as well as to the enhancement of the texture, giving promising results in such type of meat products.

Soy proteins are widely used in the meat industry as a cheaper source of proteins, but they also show good gelling properties in organogels containing vegetable oils. Utrilla *et al.* (2014) used organogel consisting of olive oil, soy protein concentrate and water in the ratio of 10:1:8, respectively. The preparation process included mixing soy protein concentrate with hot water for two minutes followed by olive oil emulsification for another three minutes. Such obtained organogel was used as a substitute of high-fat pork meat (50 per cent fat) in a venison+pork Salchichon sausages. Salchichon is a type of fermented sausage, whose production includes the drying and ripening processes. From the technological point of view, it was possible to replace 15-55 per cent of fat pork in these sausages, but research into consumer acceptance showed that the sausages, where more than 25 per cent of pork meat was replaced, were not acceptable due to poor flavor and texture.

5.2 Oleogels

Oleogel is a type of organogel, which consists of two components, including liquid phase on the one hand, and the gelling agent on the other. The liquid phase could be polar, which is a characteristic of water (hydrogel), as well as non-polar, which includes oils (oleogel). The gelling agent forms a three-dimensional network which accounts for the immobilization of the liquid phase, giving stability to the whole system (Jimenez-Colmenero *et al.*, 2015; Singh *et al.*, 2017). The greatest advantage of oleogels in food processing is the possibility to obtain a stable system containing more than 90 per cent of oil by means of highly effective gelling agents. Such gelators include waxes, ethylcellulose, polymers, ceramides, phytosterol-based and carbohydrate-based ones (Patel *et al.*, 2014; Singh *et al.*; 2017). Even more, oleogels could be relatively easy to obtain through a one-step process by mixing a gelling agent with oil under specific temperature and sharing conditions, or through a two-step process by drying (dehydration) of previously prepared oil-in-water emulsions (Jimenez-Colmenero *et al.*, 2015).

The use of oleogels in meat products has recently been studied by several authors in order to replace animal fat in Bologna and Frankfurter-type sausages and patties, which are known as high-fat meat products.

Frankfurter-type sausages are high-fat emulsion-type cooked sausages, which often served as a model for the production of low-fat or PUFA-enriched meat products. But there are not many studies concerning the use of oleogels as fat replacers. Wolfer *et al.* (2018) used rice bran wax as the gelling agent and soybean oil as the liquid phase to replace the total amount of pork back-fat in frankfurter-type sausage, obtaining promising results. Oleogel-containing frankfurters showed a similar texture and aroma as the control, with the exception of lighter color and reduced flavor. The authors used two variations of soybean oil oleogels, where the first one contained 2.5

per cent rice bran wax, and the second 10 per cent rice bran wax, prepared in a steel bowl by mixing and heating to the temperature of 90°C for 2h. These oleogels were added to meat batter after reaching 4.4°C in the bowl chopper, followed by chopping to 13°C. There was also a third option, where oleogel with 2.5 per cent rice bran wax was later added to the batter in a bowl chopper, after reaching 10°C, followed by chopping to 13°C, in order to reduce the shear force acting on the oleogel during production. Such an approach helped to keep oleogel more intact, preventing it from association with the hydrophilic protein phase and being more similar to the control sausage, especially in color and texture. Even more, it showed that oleogels should not be treated exactly the same way as fatty tissue during production, but that they require a different approach in order to obtain optimal results. Another study concerning the use of oleogels in frankfurter-type sausages was conducted by Kouzounis *et al.* (2017), where monoglycerides and phytosterols served as gelators. These oleogels were prepared by addition of 20 per cent w/w monoglycerides and phytosterols in 1:1 and 3:1 ratio, in preheated sunflower oil (80°C), followed by stirring at 90°C for 60 min, homogenization in Ultra Turrax for 1 min and finally chilling at about 20°C. The 3:1 ratio was assessed as the optimal monoglycerides/phytosterols ratio for oleogel structuring, giving a stronger gel network in sunflower oil. This oleogel was used to replace 50 per cent of pork back-fat in frankfurters, where the product with acceptable sensory properties and improved fatty acid profile was obtained.

Bologna-type sausage is a finely comminuted cooked sausage from pork, usually containing up to 35 per cent animal fat. In order to produce a PUFA-enriched Bologna sausage, da Silva *et al.*, (2019) tried to replace 25, 50, 75 and 100 per cent animal fat by means of oleogel obtained by pork skin, water and sunflower oil. The pork skin served as the source for the gelling agent as the skin collagen was transformed into gelatin by previous cooking at 80°C for 40 min, followed by mixing with water and sunflower oil in the 1.5:1.5:1 ratio, respectively. The authors concluded that oleogel obtained in such a manner could replace up to 50 per cent of pork back-fat, without adverse effects on the sensory properties of Bologna sausage. The products where 75 and 100 per cent fat were replaced showed significantly lower redness and higher yellowness, as well as fewer defects in texture, aroma and overall acceptability.

The oleogel structuring is a promising solution for stabilization of PUFA-enriched patty-type products which can contain about 20-30 per cent of fat. Oh *et al.*, (2019) obtained oleogel by stabilizing canola oil with hydroxypropyl methylcellulose (HPMC), using it to replace 50 and 100 per cent of animal fat in meat patties. The oleogel preparation is somewhat more complex than the preceding ones and has several steps. The HPMC should first be dissolved in distilled water in the concentration of 1 per cent w/w and left overnight. The solution should be homogenized in a centrifuge at 11,000 rpm for 15 min, followed by freeze-drying, thus obtaining HPMC foams which should be ground and consequently added into the oil and shared by means of overhead stirrer at 400 rpm for 3 min. The mixture should be placed overnight in order to provide oil absorption which finally results in the formation of HPMC oleogel. The authors used canola oil as a PUFA source and obtained products with good sensory properties and oxidative stability, where products with a 50 per cent fat replacement with HPMC oleogel showed the best results.

5.3 Oil-bulking

Oil-bulking represents a process of incorporation of oils rich in polyunsaturated fatty acids into a gel-like matrix. Such oil-bulking systems could serve as a fatty tissue

replacer in meat products. Polysaccharides possess the ability to form stable gels which could stabilize incorporated oil droplets. Konjac gum, alginate, inulin and dextrin are reported as good oil-bulking agents. The stability of such systems is based on the interactions between the oil molecules by means of hydrogen bonding of oil carbonyl groups and water, as well as carbohydrate molecules (Herrero *et al.*, 2014). The same authors reported that a stable oil-bulking system containing 55 per cent olive oil could be obtained through the following steps: first, 1 per cent sodium alginate, 1 per cent calcium sulfate, 0.75 per cent sodium pyrophosphate, 2.25 per cent dextrin and 40 per cent water were mixed in a homogenizer at 1500 rpm for 20s. The second step was the incorporation of olive oil in the matrix by slow adding and simultaneous mixing at the same speed. The other combination included the same process, but with 2.25 per cent inulin instead of dextrin as a bulking agent. Such oil-bulking systems possess good textural properties and could be used as fat replacers and at the same time for PUFA enrichment of meat products. Interestingly, the purpose of calcium sulphate and sodium pyrophosphate that were used in this study was to make the gelling process slower, which was particularly attributed to calcium salt. Some other studies (Vasilev *et al.*, 2016) indicate that calcium and potassium salts could also be successfully used as sodium chloride substitutes in functional fermented sausages enriched with inulin suspension as a fat replacer; so the overall approach should take into account, not only lowering saturated fat, but also lowering sodium in the design of healthier meat products.

Triki *et al.* (2013) investigated the influence of konjac-based olive oil-bulking system as a beef fat replacer in a Merguez sausage (fresh sausage from beef and lamb meat, spiced with chili pepper). Konjac gel was obtained by mixing 5 per cent konjac powder with 64.8 per cent water, including 3 min of mixing, 5 min of pause and another 3 min of mixing. Then 1 per cent of i-carrageenan was added followed by the addition of 20 per cent of olive oil and another 3 min of mixing. Along with this process, a starch gel was prepared by mixing 3 per cent corn starch powder with 16.2 per cent water. The starch gel was mixed with the above described konjac+carrageenan gel for another 3 min. After chilling to 10°C, 10 per cent calcium hydroxide was added to the mixture by gentle stirring. The oil-bulking system prepared in this manner was used to replace 75 and 100 per cent of beef fat in sausages (21.75 and 29 per cent in sausages). The sausages showed good sensory properties, oxidative stability and appropriate shelf-life.

Alginate was also successfully applied as a gelling agent to incorporate different plant oils into a gel-like matrix used as a fat replacer in different meat systems. Stajić *et al.* (2018a) used a commercial alginate mixture to prepare a gel-like matrix for linseed oil immobilization with alginate/oil/water ratio of 1/7/14 as a fat replacer in all-chicken frankfurters. They replaced 25 per cent and 50 per cent of chicken skin emulsion to provide approximately 2g and 4g of linseed oil in 100g of frankfurters which is about 50 and 100 per cent of the recommended daily intake of alpha-linolenic acid (ALA). Beside fatty acid profile improvement and an increase in yellowness (due to linseed oil characteristics), the modified frankfurters had similar physico-chemical characteristics as the control treatment, and were stable during the six-week cold storage and moreover, they had acceptable sensory properties. Alginate gel-like matrixes for plant oil (grapeseed and flaxseed oil) immobilization were also used (though in the modified alginate/oil/water ratio of 1/10/15) as back-fat replacement (at the level of 20 per cent) in dryfermented sausage formulation, as reported by Stajić *et al.* (2014)

and Stajić *et al.* (2018b). Moreover, in the research where grapeseed oil was used (Stajić *et al.*, 2014), the authors pointed out alginate gel matrix as the most promising immobilization technique among all other techniques that were experimented with (emulsion with soy protein isolate and encapsulation by electrostatic extrusion).

Inulin is a very promising gelator on the one hand because of its prebiotic and health-promoting properties, especially having in mind that some studies indicate a certain harmful potential of gums and carrageenans and on the other, because of its technological and sensory properties which make inulin a good fat replacer in meat products (Vasilev *et al.*, 2013; Vasilev *et al.*, 2017). An inulin-based oil-bulking system was designed by Glisic *et al.* (2019) in order to replace pork backfat in fermented sausages and thus enrich the product with a prebiotic (inulin) as well as PUFAs originated from linseed oil. The oil-bulking system consisted of two phases – the first included linseed pre-emulsion which was obtained with 15 per cent water, 20 per cent linseed oil and 3 per cent soybean lecithin mixed in a homogenizer until a thick paste consistency was obtained; the second phase was made of 35 per cent water, 25 per cent inulin powder and 2 per cent pork gelatine dissolved by heating (60°C) and homogenized for 2 min at medium speed. Then, the oil pre-emulsion was gradually added into the inulin suspension at room temperature, using a homogenizer at medium speed for about 3 min. The oil-bulking system obtained in this manner was chilled and consequently frozen before being used for sausage production. Such a system served to replace 64 per cent of pork back-fat (16 per cent in the sausage stuffing) in fermented sausages. The sausages were of good quality, but with reduced hardness to a certain degree, higher yellowness and they were also more susceptible to oxidation than the conventional sausages, which could be attributed to the PUFA-rich linseed oil.

5.4 Structured Emulsions

Structured emulsions consist of two or more phases, where one is dispersed into another in the form of small droplets and additionally structured – stabilized mainly by hydrogels and organogels. Because of the structuring process, the emulsions get a solid structure and could be successfully used as a fatty-tissue replacer in meat products (Jimenez-Colmenero *et al.*, 2015).

The use of hydrogels for emulsion structuring is described by Alejandre *et al.* (2019). The authors first prepared two phases: the oil phase consisted of 40 per cent canola oil, 0.05 per cent polysorbate 80 and 0.01 per cent BHT as antioxidant; the aqueous phase consisted of distilled water and 1.5 per cent and 3 per cent kappa carrageenan. These two phases were separately heated at 80°C and subsequently mixed and thoroughly homogenized. The authors also investigated the use of organogels for emulsion structuring, where a structured emulsion was prepared from 12 per cent ethylcellulose, 1.5 per cent or 3 per cent glycerol monostearate, 0.01 per cent BHT and canola oil, by heating in an oven to 140°C. The hydrogels and organogels prepared in this manner were used for beef-fat replacement in the emulsion type sausage. The modified sausages showed no significant difference in color and texture; moreover, they showed a lower oxidation level than the control, because of the presence of antioxidants. However, organogelled emulsions showed a better ability to stabilize meat batter than the hydrogelled emulsions which showed a bit less uniform microstructure and higher fat loss during heat treatment.

A study concerning the use of organogels for emulsion structuring in frankfurter sausages was conducted by Panagiotopoulou *et al.* (2016). The authors used a two-step approach: first, the preparation of an organogel, which was tested as a fat replacer itself, and second, the preparation of an organogel-in-water structured emulsion which also served as a fat substitute in frankfurters. The organogel was prepared from sunflower oil as a liquid phase, and phytosterol and γ -Oryzanol as a structuring agent. The structurants were used in two ratios – 30:70 and 60:40, in the amount of 10 per cent and 20 per cent (w/w) in sunflower oil. In order to obtain the organogel-in-water emulsion, such organogels were mixed with water at 80°C in a high shear dispenser Ultra Turrax for one minute at 14000 rpm. Tween 20 (1.7 per cent w/w) and xanthan gum (0.15 per cent w/w) served as emulsifying agents. As a pork back-fat substitute in frankfurters, the organogel containing phytosterol and γ -Oryzanol in the 60:40 ratio showed the best results, without affecting the sensory properties of the product, while the organogel with the 30:70 ratio was more appropriate for organogel-in-water emulsion stabilization.

5.5 Double Emulsions

Double emulsions represent multi-layered systems, where one layer represents oil-in-water emulsion and the other, water-in-oil emulsion. Such systems could serve as a carrier of functional ingredients in food, especially as animal fatty-tissue replacers in meat products. Serdaroğlu *et al.* (2016) described a two-step procedure: the inner water phase was obtained by distilled water and 0.6 per cent sodium chloride. Olive oil was prepared by mixing with 6.4 per cent emulsifier (polyglycerol polyricinoleate) at 50°C for 20 min. The emulsion was prepared by gradually introducing the water phase in the oil phase, and simultaneously mixing by high speed mixer (4400 rpm). The outer water phase was prepared by mixing 10 per cent sodium caseinate and 0.6 per cent sodium chloride at 50°C and 500 rpm for five minutes, followed by a one-hour stay in the water bath at 50°C. The phases prepared in this way were kept at 4°C for another 12 h in order to complete their fixation process before the final merging into a double emulsion. The final step included warming the phases at 50°C until they reached room temperature, followed by gradually pipetting the inner water-in-oil emulsion into the outer water phase and simultaneously mixing by 5200 rpm. The final double emulsion was stabilized by further emulsification for 10 min. This double emulsion was used as a fat replacer in a model system meat emulsion. The results showed appropriate technological properties, as well as oxidative stability, while the texture profile analysis showed significant changes, such as lower hardness, cohesiveness, gumminess and chewiness.

6. Conclusion and Future Perspectives

The stability of meat systems (raw meat, raw ground meat, processed meat) during storage and retail display are of great importance for the meat industry. Furthermore, with new scientific discoveries about the influence of meat/meat products on health, consumer awareness also changed and the design of natural, organic, clean label and meat products with a reduced negative impact or with bioactive or functional components also came in the focus of the meat industry. Immobilization techniques hence became a step in meat processing, enabling the preparation of different, primarily non-meat components (with bioactive compounds) for their optimal use. As

these components are often in liquid form and given that powder is quite suitable for application in meat processing, different encapsulation techniques based on drying are becoming more interesting.

Taking into account all physico-chemical properties of different meat systems, active components, with proper selection of the encapsulation material and encapsulation techniques, new and/or natural ingredients for the meat industry can be produced in order to meet the ever complex demands of markets.

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