Fat and Organogel Structure Within Pâté and Their Influence on Texture and Sensory Attributes

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Abstract

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Five commercial brand pâtés were characterized by examining their texture, microstructure, fatty acid composition, melting profile, and polymorphism. Powder x-ray diffraction studies demonstrated that while the fat extracted from one of the pâtés crystallized in a β polymorphic form; while embedded in a pâté protein matrix, it was crystallized in a β’ polymorphic form. This implies an effect of the food matrix on fat crystallization and structure and an interaction between fat and other components present in the food matrix. Five different canola oil organogel formulations were used to replace pork fat in liver pâté to improve its polyunsaturated fat content and evaluate their effects on texture and sensory properties. Pâtés made using organogels showed comparable textural, physical, and sensory properties as the traditional pâté made with pork fat while reducing the saturated fat content by 62%. Organogel replaced pâtés were perceived to have similar hardness, oiliness, and juiciness in the sensory test compared to control pâté made with pork fat. Partial replacement of pork fats with canola oil organogels was done to improve oil retention, sensory and textural properties of liver pâté. Oil retention performance could be retained at 60% pork fat replacement while maintaining the textural properties and having minimal effect on the sensory properties and colour of the products.
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CHAPTER 1 Introduction

Different governmental institutions, especially those in North America and Europe, have suggested reducing the consumption of saturated and trans fatty acids in the diet, and have encouraged people to transition to a diet with greater amount of polyunsaturated fats (WHO, 2013). The challenge with polyunsaturated fats is the lack of the ability to form solid structures at room temperature. Consequently, the incorporation of polyunsaturated fats in foods can negatively affect textural and sensory properties of food products. The use of ethylcellulose to create organogels could help structure vegetable oils and has been shown to have a great potential in food industry (Rogers et al., 2014; Stortz & Marangoni, 2013; Stortz, Zetzl, Barbut, Cattaruzza, & Marangoni, 2012). By trapping the liquid oil in a gel network, the resulting products can be as firmed at room temperature to function similarly to saturated fat containing products (Stortz et al., 2012). As a result, organogel structures can be utilized to mimic the properties of solid fat while at the same time maintaining the nutritional properties of the healthier unsaturated fatty acids.

Replacement of saturated fats has been conducted in different comminuted meat products (i.e., frankfurters, sausages) with liquid unsaturated vegetable oils such as canola oil and olive oil with the aid of hydrocolloids, in an attempt to improve the fatty acid compositions of the product. It was reported that the textural properties of these meat products were significantly different than the original products (Barbut et al., 2016; Delgado-Pando et al., 2012). It was also reported that the straight replacement of beef fat with liquid canola oil in comminuted products results in a firmer and more rubbery product, which is undesirable (Barbut et al., 2016). In pâté type products, in order to reduce saturated fat content, Morales-Irigoyen, Severiano-Perez, Rodriguez-Huezo, & Totosaus (2012) employed liquid canola oil with the combination of xanthan gum and sodium caseinate to replace 50% of the lard in a pâté. The resulting pâté product was softer and showed
higher syneresis compared to the control. However, fat replacement in pâté using canola oil organogels have never been attempted before and showed promising results based on the previous experiment (i.e. frankfurter).

Overall, pâté is classified as an emulsified meat product made primarily from liver, fat, meat, and spices (Barbut, 2015). It is commonly consumed around the world due to its rich flavour and smooth texture. Pâté’s unique texture and taste can be attributed to the type of fat used and to its relatively high fat content. Various studies have indicated that saturated fats play an important role in texture, mouthfeel, moistness, and sensory acceptability of such emulsified meat products (Chin, Keeton, Miller, Longnecker, & Lamkey, 2000; Hughes, Mullen, & Troy, 1998). Unlike other emulsified meat products, pork meat is added precooked (i.e., denatured proteins) in the pâté making process. Therefore, liver proteins become the main emulsifying and binding agents. Temperature control during chopping is critical in the pâté making process; i.e., the meat and fat are processed at a relatively high temperature (50-55°C) to ensure proper emulsification of the fat globules. Furthermore, the pâté batter should be stuffed with sufficient pressure to avoid fat separation to the outer layer of the product. The lack of functional muscle proteins during chopping emphasizes the important contribution of the fat and liver proteins to the formation of an acceptable texture in the final product. These points highlight the importance of understanding the functionality of the fat substitute used to make the fat replace pâté.

1.1 Objectives

1. To study the effect of fat structure on the textural properties of pâté.
2. To find the best performing canola oil organogel formulation to replace saturated fat in pork liver pâté.
3. To find the optimum level of fat replacement while keeping the sensory and textural properties as close to traditional liver pâté.
1.2 Hypothesis

1. Fat structure has a significant effect on textural properties of the pâté.
2. Organogels made with a combination of surfactant and ethylcellulose (EC) can be used to replace fat.
3. Up to 50% fat replacement will yield pâté with the same sensory and textural properties as control.
1.3 References


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CHAPTER 2 Literature Review

2.1 Liver Pâté

Liver pâtés are widely consumed around the world both as everyday diet in some countries or in special occasions in others. It is mainly consumed due to its rich taste and smooth texture. Pâté’s unique texture and taste can be attributed to the type of fat used and to its relatively high fat content. The varieties of liver pâtés are limitless due to combinations of different fats (pork, duck, chicken), herbs, spices, as well as other ingredients such as wine, port, and brandy. Pâtés are generally filled into moulds and sold as is, while other are stuffed into casings. The four major ingredients in liver pâtés are liver, meat, salt, and fat. Most of the ingredients such as fat and meat are pre-cooked before chopping while the liver is mainly used as emulsifier and added raw. Pâtés are cured products, therefore nitrite is also added into the product (Feiner, 2006).

Sourcing ingredients for pâté is relatively easy as lower quality meat such as DFD (Dark, Firm, and Dry) and PSE (Pale, Soft, and Exudative) meats are acceptable since most of the meats would be precooked and therefore their proteins are denatured and non-functional. Liver is one of the most important ingredients in the pâté (up to 30% of the pâté) as the activated proteins in the liver contained both hydrophilic and lipophilic groups that help emulsify the fat in the products. Proteins that can be found in liver include albumin, globulin, glycoprotein, as well as collagen. Different kind of fats and oils can be used in pâté production depending on the type of pâté. In pork-based pâtés, fat from shoulder and leg cuts are often utilized as they are usually common by products during carcass processing. Fat is very important to the final texture of the pâté as it affects the spreadability and smoothness of the finished products. Also, in high fat liver pâté, the amount of raw liver used is important in preventing fat separation, especially in pâté packaged in retort punch (Feiner, 2006).
The amount of additives added to liver sausages can vary quite significantly. Salt is commonly added to processed meat products to extract the salt soluble protein fraction, improve shelf-life, and taste. As mentioned before, most of the myofibrillar proteins have been pre-cooked and are no longer functional. Other salts such as nitrite is very important in liver pâté production as it helps develop colour and flavour in the final products. Nitrite also contributes to anti-
botulinum effects and longer shelf-life and therefore is commonly used at the highest permitted level. Ascorbic acid or erythorbate should also be added to accelerate colour development (Feiner, 2006).

An emulsifier such as monoglyceride and diglyceride, can be added to lower the risk of fat separation during cooking. Natural emulsifiers such as caseinate, egg protein, and milk or blood plasma are also often added to improve stability and improve taste, however addition of an emulsifier is usually not needed unless the formula is high in fat (above 32%) (Feiner, 2006). Spices and herbs are added to improve taste and mask unwanted flavours. Lastly, phosphates are not usually added to liver pâté as the myofibrillar proteins in pâté are precooked and are no longer functional (Feiner, 2006).

### 2.2 Health Implications of Saturated Fats

Saturated fat is the main type of fat found in animal muscle food products, high-fat dairy foods and some plant kernel based oils such as palm oil. They are defined as fat molecules or fatty acids that do not possess a double bond (C=C). Saturated fat not only provides dietary calories, it also contributes to the functionality as well as organoleptic properties of food products including texture, mouthfeel and aftertaste (Bañón, Díaz, Nieto, Castillo, & Álvarez, 2008; Keeton, 1991). Substitution of solid fat by health-promoting unsaturated fat and reduction of fat without scarifying
the consumer acceptability and sensory qualities associated with the food product is a huge
formidable challenge in the food industry.

In the past two decades, due to an increase in public health consciousness, there is an
elevating urge in reducing the amount of saturated fat in food products associated with our diet.
World Health Organization and several Canadian and U.S. health agencies recommended a
reduction in overall saturated fat intake and an increase in monounsaturated (MUFA) and
polyunsaturated fat (PUFA) consumption as they were proven to have a couple health benefits
including anti-inflammation and blood cholesterol lowering (WHO, 2013). They reported a
substitution of saturated fat in our diet by MUPA and PUFA can reduce the risk associated by
approximately 30%. Moreover, according to the United States Department of Agriculture,
saturated fat remains a huge risk factor for the development of cardiovascular diseases and should
therefore not account for more than 10% of our daily dietary caloric intake (U.S. Department of
Health and Human Services & U.S. Department of Agriculture, 2005).

Negative health impacts associated with the consumption of saturated fat are myriad, with
increasing the risk associated with CVDs being the most pronounced and widely studied. The
relationship between occurrence of different CVDs and high blood saturated fat and cholesterol
are consistent and evident. Various studies have reported a reduction in saturated fat in our diet
can reduce the overall risk of having a cardiovascular event by 10 - 14 % (Hooper et al. 2012:
Micha et al. 2010). Both Mozaffarian (2010) and Harcombe (2015) reported partial replacement
of saturated fat by PUFA can strongly lower the risk and mortality associated with CVDs and
CHDs. Generally, it is believed that dietary saturated fat raises the serum Low Density
Lipoproteins (LDL) cholesterol level thereby increases the risk of developing coronary heart
diseases (CHD) and stroke.
A high intake of saturated fat can also be considered as a risk factor of several types of cancers. Lin (2009) reported a positive correlation between high saturated fat intake and the incidence of colorectal cancer, whereas Huncharek and Kupelnick (2001) reported a strong and evident relationship between consumption of animal fat and the development of ovarian cancer. An increasing risk of prostate cancer was also demonstrated by Yang et al. (2015). In a study conducted by Corwin et al. (2006), bone mineral density was also found to be negatively influenced by the level of saturated fat consumption. Overall, high consumption of saturated fats is not recommended and has a detrimental effect on health.

2.3 Fat Replacement/Reduction in Meat Products

There are numerous aspects that need to be considered in the development of fat-replaced products (Colmenero, 1996). The new meat products must have good technological, sensory, and nutritional properties, as well as be convenient for consumers. Generally there are three means of fat replacement/reduction in meat products: modification of carcass composition, manipulation of meat raw materials, and reformulation of meat products (Jiménez-Colmenero, Carballo, & Cofrades, 2001).

The first way to develop fat-replaced products is to modify/improve fatty acid composition of meat products. Jiménez-Colmenero (2007) reported that changes in lipid present in the meat can be achieved by means of animal production practices. Genetics and dietary approaches have been used to alter fatty acid profiles of the meat resulting in meat lower in saturated fats (Givens, Khem, & Gibbs, 2006). However, the easiest way to reduce fat in meat products is to manipulate the raw materials, mainly the fat trimmings being used. However, this is sometimes not feasible because of lower yields, costs, and other considerations (i.e. cannot be used in making processed meat with fixed formulations) (Jiménez-Colmenero et al., 2001). The most common method used for
production of fat-replaced products is by reformulation of meat products. Replacement of saturated fats has been reported in different comminuted meat products such as frankfurters, and sausages with the aid of different fillers and binders. It was reported that the textural properties of these meat products were quite different than original products (Chin, Keeton, Miller, Longnecker, & Lamkey, 2000; E. Hughes, Mullen, & Troy, 1998). It was reported that the straight replacement of beef fat with liquid canola oil in comminuted products results in a firmer and more rubbery product, which is undesirable (Barbut et al., 2016). Another study reported that animal fat plays a significant role in flavour intensity, juiciness, and tenderness of the product (Resurreccion, 2004). A study on meat sausages showed that replacement using fish oil and higher amount of carrageenan showed low cook loss and moderate hardness (Marchetti, Andres, & Califano, 2014).

Similar studies on fat reduction were conducted on pâté in order to reduce its trans and saturated fat content. Morales-Irigoyen, Severiano-Perez, Rodriguez-Huezo, & Totosaus (2012) employed liquid canola oil with the combination of xanthan gum and sodium caseinates to replace 50% of lard in liver pâté. The resulting product was softer as well as showed greater syneresis compared to the control. Addition of binders and fillers such as carrageenan, xanthan gums, sodium caseinates, and starch will result in a negative effect on the consumer willingness to purchase, as most food companies are starting to move to clean label products. It was also reported that the canola, sodium caseinates, and xanthan gum combination was only able to replace a maximum of 50% lard (Morales-Irigoyen et al., 2012). Other researchers attempted to replace pork fat in pork liver pâtés by replacing lard with a mixture of olive, linseed, and fish oils. They reported negative effects on the rheological properties of the meat systems. (Delgado-Pando et al., 2012). 207
2.4 Organogelation Using Ethylcellulose (EC)

Structuring of vegetable oils using edible ingredients has received more attention in the past decade, and was first researched by Gandolfo, Bot, & Flöter (2004). There are three basic methods to achieve organogelation; polymer gelation, self-assembled fibrillar network, and network formation of crystalline particles (Bot, Veldhuizen, den Adel, & Roijers, 2009; Pernetti, van Malssen, Flöter, & Bot, 2007; Rogers, 2009). In the current study, the method of polymer gelation to replace saturated fat in pâté with highly unsaturated canola oil was used. Therefore, polymer gelation would be discussed in a greater detail in this section.

One of the edible polymers that can be used as an organogelator is ethylcellulose (EC). EC is one of the most promising organogelators in the market, due to its lower price compared to other gelators and its availability (cellulose is one of the most common polymer). Moreover, EC is a food grade GRAS (Generally Recognized as Safe) ingredient in most countries and have been proven to work in various food systems such as comminuted meat products, confectionary products, cheeses, fat emulsions, etc. (Barbut et al., 2016; Stortz, Zetzl, Barbut, Cattaruzza, & Marangoni, 2012; Zetzl et al., 2014). EC is produced by etherification of cellulose and is not soluble in water due to high ethoxyl content (Gravelle, Barbut, & Marangoni, 2012). However, EC is soluble in various organic solvents such as aromatic hydrocarbons and aliphatic alcohols (Koch, 1937).

Gravelle, Barbut, Quinton, & Marangoni, (2014) summarized that there were several main factors having a significant effect on the mechanical strengths of vegetable oil organogels: type of oil, molecular weight of the EC, and incorporation of surfactant to the system. It was reported that more polar oil results in firmer organogels; higher EC molecular weight results in higher gel strength; addition of surfactants can increased the overall gel strength of the gels.
In order to be able to be incorporated into vegetable oil, EC first needs to be heated above its glass transition temperature of 140°C (Laredo, Barbut, & Marangoni, 2011). After cooling, the EC forms a gel network strengthened by van der Waals forces as well as hydrogen bonding, entrapping the liquid oil in place (Laredo et al., 2011). As previously mentioned, the hardness of the gels can be adjusted by adjusting the organogel formulation (concentration of gelators and surfactant).

### 2.5 Sensory Analysis of Meat Products

Sensory profile analysis is an objective way to describe the eating quality of the meat products. Basic attributes that can be tested using this method include appearance, odour, flavour as well as juiciness and tenderness (Bejerholm & Aaslyng, 2004; Wood, Nute, & Cuthbertson, 1993). A study in for European countries found that appearance attributes such as colour is very important in determining the quality of the product (Grunert, 1997). Sensory profile analysis is also important in determining consumer acceptance of new products (i.e. new flavour, low-sodium, low fat). A study by Stubenitsky, Aaron, Catt, & Mela (1999) reported that a long term acceptance study of low and reduced fat pork sausages in a 3-month period of blind tasting, showed similar acceptance between full fat versus sausages containing 65% of the original fat content. These studies have proven that sensory profile analysis can be utilized to determine consumers acceptance in new products. In the current study, the authors are using the Qualitative Descriptive Analysis (QDA) to determine the sensory qualities of fat replaced pâté with canola oil organogels. These studies emphasize the importance of what attributes, parameters, and factors to run a sensory analysis of meat products.

There are some parameters that need to be followed when running a sensory analysis for meat products. Firstly, the test must take place in a controlled testing environment. The facility has
to be an accessible location with sufficient space, temperature and humidity control, as well as free from noise and odours (Meilgaard, Carr, & Civille, 2006). Red light is often used in certain tests to avoid visual bias when colour is not one of the parameters being tested. Sample preparation and presentation are important factors in determining the success of the experiment. Samples have to be standardized by weight, dimensions, and also temperature (Meilgaard et al. 2006). Panelists have to receive an adequate amount of samples to be able to properly evaluate the products. Size of the cut is also important in determining the juiciness of the product (i.e. large enough to simulate a regular eating experience). Moreover, sample presentation ensures all panelists receive the samples at appropriate temperature, especially in product with high fat content (O’Mahony, 1986).

Temperature of the samples can influence the volatile aromatics being released at the time of serving. Samples should be covered during before serving to prevent discoloration and surface drying (Meilgaard et al., 2006).

There are many accepted methods to determine the sensory qualities of meat. In this section (Munoz & Civille, 1998). Quantitative Descriptive Analysis (QDA) can be used to evaluate sensory qualities accurately with more than 10 panelists. Using trained panelists in QDA would yield more accurate results than using untrained panelists. The panelists need to be trained with products with similar characteristics for optimum results (Meilgaard et al., 2006). Each characteristic (i.e. juiciness, hardness, oiliness) is rated on a 15-cm line scale with descriptors of “weak” and “strong” as endpoint anchors. It is important that the panelists are trained to use the entire line scale. To ensure quality of the test, no more than six or seven attributes should be evaluated at each setting to avoid panelist fatigue (Munoz, Civille, & Carr, 1992).
2.6 Conclusion

In order to successfully formulate a fat-replaced liver pâté, the production process of the pâté, sensory evaluation techniques, and various fat replacement methods in meat products warrants further study and evaluation. The demands for healthier foods are increasing as consumers are becoming more health conscious. Therefore, studying the importance of fat in the pâté system is important in order to come up with a fat replacement/reduction solution. The next three chapters will be discussing the study of fat structure on the textural characteristics of pâté, fat replacement in pâté using canola oil organogels, and partial fat replacement of pâté to improve sensory and textural properties.
2.7 References


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CHAPTER 3

Influence of Fat Structure on the Mechanical Properties of Commercial Pâté Products

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Abstract

Five commercial brand pâtés were characterized by examining their texture, microstructure, fatty acid composition, melting profile, and polymorphism. Pâtés evaluated at 4 °C showed much higher hardness values compared to when tested at 22 °C. Pâtés with higher fat content and higher saturated fatty acid and triacylglycerol contents were found to be harder. Smaller fat globules were found to be correlated with higher hardness values. Increases in solid fat content were correlated with an increased hardness at 4 °C vs. room temperature, but could not explain differences observed at a specific temperature. Powder x-ray diffraction studies demonstrated that while the fat extracted from one of the pâtés crystallized in a β polymorphic form while embedded in a pâté protein matrix, it was crystallized in a β’ polymorphic form. This implies an effect of the food matrix on fat crystallization and structure and an interaction between fat and other components present in the food matrix.

1. Introduction

Pâté is classified as an emulsified meat product made primarily from liver, fat, meat, and spices. It is commonly consumed around the world due to its rich and smooth texture. Pâté’s unique texture and taste can be attributed to the type of fat used and to its relatively high fat content.
Various studies have suggested that saturated fats play a big role in texture, mouthfeel, moistness, and sensory acceptability of emulsified meat products (Barbut, Wood, & Marangoni, 2016; Chin, Keeton, Miller, Longnecker, & Lamkey, 2000; Hughes, Mullen, & Troy, 1998).

Unlike other emulsified meat products, pork meat is added precooked during the pâté making process. Therefore, liver proteins become the main emulsifying and binding agents. Temperature control during chopping is also a very important part of the pâté making process. During the process, the meat and fat must be added at high temperature (>50°C) to ensure proper emulsification of the fat globules. Furthermore, the pâté batter has to be stuffed properly, with sufficient pressure to avoid fat separation to the outer layer of the product. The lack of functional muscle proteins during chopping, emphasizes the important contribution of liver proteins and fat to the texture of the final product. Milk protein concentrate is commonly added to pâté formulations to improve emulsification (Barbut, 2015a; Morales-Irigoyen, Severiano-Perez, Rodriguez-Huezo, & Totosaus, 2012). Overall, only limited information regarding the processing characteristics of pâtés are available in the literature.

The demand for low-fat meat products is continually increasing as the general population becomes more health conscious (Marchetti et al., 2014). To meet this demand, the meat industry has focused on lower fat formulations in their products. While fat content in traditional pâté can be as high as 50%, pâté with fat content of as low as 17% can now also be found on the market (Resurreccion, 2004). The understanding of factors influencing pâté texture and quality is generally lacking. In order to formulate low-fat pâté products, it is necessary to first understand the influence of fat and fat structure on pâté quality. In the present study, we focus on the characterization of the fat present in five commercial pâté products at different length scales. Molecular composition, solid state structure, and melting behaviours are characterized and their
2. Material and Methods

2.1 Materials

Five commercial pâté products were purchased from local grocery stores. The products were chosen based on their fat levels and their major meat ingredients. Products were bought at the same time for the entire experiment to avoid variation between batches. Three sausages were bought per product type as replications.

2.2 Back Extrusion

The samples were stuffed into polypropylene test tubes (27 mm diameter) and kept in the refrigerator (4 °C) overnight to allow them to set. The following day, half of the samples were tested directly out of the refrigerator while the other samples were allowed to equilibrate at room temperature (22 °C) for 2 hrs. Back extrusion tests were performed using a texture analyzer (Texture Technologies Corp., Model TA.XT2, Scarsdale, NY, USA). A stainless steel cylindrical probe (height: 9.8 cm, diameter: 1.8 cm) with enlarged spherical tip (height: 1.3 cm, diameter: 2.0 cm) was used to penetrate the product, in the test tubes, for 30 mm at a rate of 1.5 mm/s. Results were recorded as described by Gravelle et al. (2014).

2.3 Fatty Acid Composition

The fatty acid composition of extracted pâté fats was determined using gas chromatography (Ghazani, García-Llatas, & Marangoni, 2013). Samples were subjected to a transmethylation procedure in accordance with Christie (1982). Fatty acid methyl esters (FAME) were analyzed by using a capillary GC equipped with a BPX70 column, 60 m × 0.22 mm internal diameter and with 0.25 µm film thickness (SGE Inc., Austin, TX, USA). A gas chromatograph (Agilent 6890 series,
Agilent Technologies, Inc., Wilmington, DE, USA) with an auto sampler (7,683 series) was used. The oven temperature was programmed to increase from 110 to 230 °C at a rate of 4 °C/min and held at 230 °C for 10 min. The injector and detector temperatures were 240 and 280 °C, respectively. Hydrogen was used as the carrier gas at an average velocity of 25 cm/s. Peaks were identified via comparison to FAME standards. Samples were measured in triplicate.

2.4 Microstructure

Samples were prepared for light microscopy following the method used by Barbut et al. (2016). Samples (20 × 20 × 5 mm) were cut from the center part of the liver sausages and then fixed and stained using Masson stain. Slides were observed using a light microscope (Model BX60, Olympus Optical Ltd., Tokyo, Japan) and images were captured using a computerized image analysis system (Image-Pro Plus, Version 5.1, Media Cybernetics Inc., Silver Spring, MD, USA).

2.5 Fat Extraction

Samples were smeared onto the side of extraction thimbles, with the total amount not exceeding 4 g, to ensure maximum surface area for the extraction. The thimbles were dried at 60 °C to remove moisture until constant weight was obtained. The thimbles were then placed in the soxhlet extraction apparatus. Approximately 200 ml petroleum ether was poured into the soxhlet flask, heated gently to allow a continual reflux of petroleum ether, and samples were extracted for 3–4 hrs. The petroleum ether was then evaporated in the drying oven.

2.6 Differential Scanning Calorimetry (DSC)

The melting and crystallization of pâtés and pâtés fats were studied using a DSC unit (Q2000 TA instruments, New Castle, DE, USA) following the procedure outlined by Blake, Co, & Marangoni (2014). Samples were prepared by placing pâté or extracted fat (8–12 mg) in an
aluminum pan (prepared at 4°C inside a walk-in fridge). The thermal regimen used in the test was as follows: samples equilibrated at 4 °C for 10 min, heated from 4 to 80 °C at rate of 5 °C/min, equilibrated at 80 °C for 10 min, followed by cooling from 80 to -5 °C at 5 °C/min, equilibrated at -5 °C for 10 min, and re-heated to 80°C at 5 °C/min. The peak melting temperatures (T_m) and enthalpy of melting (ΔH_m) were determined from the DSC curves using the software supplied with the instrument (Universal Analysis Software, TA Instruments, New Castle, DE, USA). Results were obtained in triplicate.

2.7 Powder X-ray Diffraction

Samples were prepared by filling the well of a metal sample holder with pâté or extracted fat until the unit was even with the surrounding slide. All preparations were done at 4 °C. Diffraction patterns were obtained using an X-ray diffractometer (Rigaku Multiflex, TX, USA) Cu source with k = 1.5459 Å, wide angle X-ray scans (15°–25° at 0.2°/min). The measurements were collected in triplicate at 4 °C.

2.8 Solid Fat Content

Solid fat content of the pâté samples was measured at 4°C and at room temperature (22°C) following the AOCS Official Method Cd 16b-93. The extracted fat was inserted into NMR tubes and melted at 100°C for 60 min, incubated at 60°C for 15 min and then incubated at 4°C and room temperature (RT) (mimicking products served at refrigerated and room temperature respectively) for 30 min before testing. All measurements were done in triplicate using a pNMR analyzer (Bruker PC/20 Series Minispec, Bruker Optics Ltd., Milton, ON, Canada).

2.9 Triacylglycerole Composition

Triacylglycerol (TAG) analysis of each extracted fat sample was carried out using high performance liquid chromatography (HPLC model 110, Agilent Tech, Palo Alto, CA, USA).
equipped with a quaternary pump, auto sampler, refractive index detector, and software program (HP Chem Station version A.10, Hewlett-Packard, Palo Alto, CA, USA). 30 µL of each pâté fat sample was dissolved in 600 µL of chloroform and 1000 µL (60:40 v/v) acetone-acetonitrile solution. 10 µL of each sample was injected into a column (Econosil C18, 250 × 4.6 mm) in the isocratic mode at 1.0 mL/ min flow rate. The mobile phase was (60:40 v/v) acetone-acetonitrile. The peaks were compared to internal standards (Sigma Aldrich, Oakville, ON, Canada) and quantified by integration of the relative peak area. The measurements were taken in triplicate following the method of (Mottram, Crossman, & Evershed, 2001).

2.10 Fat Globules Size Analysis

Image analysis of fat globules size were carried out using image analysis software (Image-Pro, Media Cybernetics Inc., Rockville, MD, USA). The area of the fat globules was averaged and reported.

2.11 Statistical Analysis

Statistical analysis of correlation a table was completed using Graphpad Prism 5.0 (GraphPad, San Diego, CA, USA).

3. Results and Discussion

3.1 Texture analysis

The effect of temperature on the hardness of the five commercial pâté products can be observed in Figure 3.1. When tested at room temperature, the products were significantly softer, as compared to values obtained at 4°C. Lower hardness values in samples tested at 22°C can be mainly attributed to the difference in solid fat content (SFC) in the samples as lard starts melting at ~20°C (Campos, Narine, & Marangoni, 2002). This point will be further addressed in the
following section. Variations between products tested at the same temperature were also observed as pâtés with higher fat contents (pâtés B, C, and E; Table 3.1) showed higher hardness values at 4°C. The high hardness values of pâtés with a higher fat content were expected, as the increase in overall fat content combined with a higher solid fat content results in a greater amount of hard, crystalline fat in the product. Pâté D displayed a high hardness value at 4°C despite having the lowest overall fat content. This can perhaps be attributed to the presence of modified corn starch, as discussed by Carballo, Fernandez, Barreto, Solas, & Colmenero (1996) as well as the presence of egg white proteins that form a gel upon coagulation. At room temperature, pâté A, B, D, and E showed a gradual increase in hardness values as the fat content increases.

Fig. 3.1 Effects of temperature on the hardness values of commercial pâtés (Products A – E) tested at room temperature (RT) and 4°C. Bars indicate standard error of the mean. n = 2-10 per sample.

Table 3.1 Overall composition* of five commercial liver pâtés.

<table>
<thead>
<tr>
<th>Pâté</th>
<th>Carbohydrates (%)</th>
<th>Lipid (%)</th>
<th>Protein (%)</th>
<th>Sodium (mg/30g)</th>
<th>Main functional ingredients</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>6.7</td>
<td>20.0</td>
<td>13.3</td>
<td>230</td>
<td>Pork, chicken liver, pork fat, pork, modified milk</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>-----</td>
<td>-----</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td></td>
<td>14.0</td>
<td>10.0</td>
<td>220</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>3.3</td>
<td>26.7</td>
<td>10.0</td>
<td>220</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pork fat, pork liver, ham, liquid egg white, modified milk ingredients.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>6.7</td>
<td>36.7</td>
<td>6.7</td>
<td>140</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Duck (fat, liver, foie gras, skin), cream 10% M.F, modified milk ingredients, egg white.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>13.3</td>
<td>16.7</td>
<td>10.0</td>
<td>140</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chicken liver, chicken fat, chicken, modified corn starch, butter, egg white.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>6.7</td>
<td>40.0</td>
<td>10.0</td>
<td>140</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pork fat, pork liver, liquid egg white, pork rind, modified milk ingredients.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data obtained from the nutritional labels and ingredient lists appearing on each package.

### 3.2 Solid fat content (SFC), fatty acid, and triacylglycerol (TAG) analysis

The SFCs of the 5 commercial pâtés are presented in Figure 3.2. As expected, the SFC is significantly higher when tested at 4°C compared to room temperature. This is in line with the hardness results that show increased hardness when tested at 4 °C (Fig. 3.1).

Overall lipid contents varied between the pâtés. However, the fatty acid compositions of extracted fats from all 5 pâtés were relatively similar (Table 3.2). The majority of the fatty acids are C16:0, C18:0, C18:1 and C18:2. The total saturated fat in all 5 pâtés ranged from 32 to 37 %.

The unsaturated fatty acid content ranged from 59 to 66%. Pâté C (made from duck liver) contained a higher amount of C18:1 fatty acid which may contribute to the lower hardness value observed at room temperature. Overall, at room temperature some of the saturated fatty acids in the pâtés are in an oil form resulting in a softer texture.

The TAG compositions of the pâtés varied between samples (Table 3.3). The most noticeable difference was observed in pâté C, as the amount of lower melting point TAGs is noticeably higher compared to the other 4 pâtés. A higher content of lower melting TAGs (most...
notably OLO) would be responsible then for lower hardness values, as well as lower SFCs at room temperature for this particular sample. Pâté A, B, and D had similar TAG compositions with a greater content of high melting TAGs. Higher SFCs seen in these three samples, when tested at both 22 °C and 4 °C, are due to a relatively high amount of high melting TAGs in solid form even at room temperature. Pâté E has a comparatively low level of high melting TAGs (most notably POS) and a relatively higher amount of lower melting TAGs compared to pâté A, B, and D. This finding is consistent with the SFC determined in Pâté E, which showed the highest difference between SFC at the two temperature points. Figure 3.3 shows a strong correlation between hardness and SFC of pâtés at 22 °C and 4 °C. Higher hardness values at 4 °C are directly correlated with the higher solid fat content. On the other hand, lower hardness values at 22 °C can be correlated with lower SFC values at this temperature.

![Solid fat contents of commercial pâtés (Products A – E) tested at room temperature (RT) and at 4°C. Bars indicate standard error of the mean. n = 3 per sample.](image)

**Fig. 3.2**

Table 3.2 Fatty acid compositions of fat extracted from the five commercial pâtés (A – E). Values represent means and standard deviations. n = 3 per sample.

<table>
<thead>
<tr>
<th>Products</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatty acids composition (w/w%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C12:0</td>
<td>-</td>
<td>-</td>
<td>0.50 ± 0.0022</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
### Table 3.3

<table>
<thead>
<tr>
<th>Triacylglycerols (TAGs)</th>
<th>Melting point (°C)</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>PoLPo + LnLP</td>
<td>0.42 ± 0.05</td>
<td>1.09 ± 0.13</td>
<td>3.45 ± 0.17</td>
<td>0.75 ± 0.08</td>
<td>2.1 ± 0.23</td>
<td></td>
</tr>
<tr>
<td>LLL + LLnO</td>
<td>0.79 ± 0.01</td>
<td>2.64 ± 0.17</td>
<td>5.93 ± 0.03</td>
<td>2.96 ± 0.13</td>
<td>1.6 ± 0.18</td>
<td></td>
</tr>
<tr>
<td>LLO</td>
<td>0.9 ± 0.13</td>
<td>2.62 ± 0.07</td>
<td>3.13 ± 0.36</td>
<td>3.41 ± 0.14</td>
<td>2.5 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>OLO</td>
<td>3.49 ± 0.13</td>
<td>6.22 ± 0.15</td>
<td>11.18 ± 0.80</td>
<td>5.35 ± 0.61</td>
<td>6.3 ± 0.14</td>
<td></td>
</tr>
<tr>
<td>LLP</td>
<td>0.83 ± 0.04</td>
<td>1.86 ± 0.31</td>
<td>0.48 ± 0.05</td>
<td>0.76 ± 0.13</td>
<td>2 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>OOO</td>
<td>0.76 ± 0.04</td>
<td>-</td>
<td>1.28 ± 0.04</td>
<td>-</td>
<td>2.1 ± 0.43</td>
<td></td>
</tr>
<tr>
<td>OPL</td>
<td>8.04 ± 0.36</td>
<td>18.13 ± 0.46</td>
<td>14.4 ± 0.94</td>
<td>17.3 ± 0.37</td>
<td>14 ± 0.49</td>
<td></td>
</tr>
<tr>
<td>LLS + OPoP</td>
<td>3.5 ± 0.32</td>
<td>2.1 ± 0.06</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>SLO</td>
<td>6.69 ± 0.15</td>
<td>7.62 ± 0.23</td>
<td>11.5 ± 0.65</td>
<td>6.3 ± 0.21</td>
<td>7.8 ± 0.41</td>
<td></td>
</tr>
<tr>
<td>OPO</td>
<td>28.80 ± 0.65</td>
<td>27.4 ± 0.97</td>
<td>21.7 ± 0.34</td>
<td>24.6 ± 1.04</td>
<td>19.3 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>OSO</td>
<td>4.03 ± 0.28</td>
<td>4.0 ± 0.05</td>
<td>4.68 ± 0.27</td>
<td>3.68 ± 0.06</td>
<td>3.2 ± 0.26</td>
<td></td>
</tr>
<tr>
<td>SPL</td>
<td>4.11 ± 0.12</td>
<td>3.35 ± 0.34</td>
<td>-</td>
<td>5.26 ± 0.08</td>
<td>11.1 ± 0.17</td>
<td></td>
</tr>
<tr>
<td>PLP + PSLn</td>
<td>1.32 ± 0.11</td>
<td>2.19 ± 0.09</td>
<td>5.52 ± 0.07</td>
<td>2.54 ± 0.07</td>
<td>9.8 ± 0.54</td>
<td></td>
</tr>
<tr>
<td>PPO</td>
<td>32.3</td>
<td>2.76 ± 0.22</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>POS</td>
<td>35.6</td>
<td>15.3 ± 0.47</td>
<td>12.8 ± 0.1</td>
<td>5.1 ± 0.30</td>
<td>13.8 ± 0.83</td>
<td>4.6 ± 0.38</td>
</tr>
<tr>
<td>POP + PPoS</td>
<td>37.3 ± 0.26</td>
<td>4.38 ± 0.01</td>
<td>7.89 ± 0.34</td>
<td>4.4 ± 0.74</td>
<td>1.7 ± 0.23</td>
<td></td>
</tr>
<tr>
<td>POA + SSO</td>
<td>37.8 ± 0.40</td>
<td>1.1 ± 0.09</td>
<td>0.85 ± 0.06</td>
<td>-</td>
<td>0.87 ± 0.03</td>
<td>-</td>
</tr>
<tr>
<td>PPS</td>
<td>61.4</td>
<td>0.66 ± 0.11</td>
<td>0.7 ± 0.03</td>
<td>0.68 ± 0.10</td>
<td>0.73 ± 0.06</td>
<td>1.2 ± 0.14</td>
</tr>
<tr>
<td>SPS</td>
<td>67.4</td>
<td>1.29 ± 0.11</td>
<td>1.0 ± 0.14</td>
<td>-</td>
<td>1.04 ± 0.09</td>
<td>-</td>
</tr>
<tr>
<td>DAGs and minor TAG</td>
<td>11.3 ± 0.14</td>
<td>3.17 ± 0.33</td>
<td>3.09 ± 0.90</td>
<td>6.35 ± 1.16</td>
<td>10.7 ± 0.71</td>
<td></td>
</tr>
</tbody>
</table>

*a Abbreviations for fatty acids: P, palmitic acid; Po, palmitoleic acid; S, stearic acid; O, oleic acid; L, linoleic acid; Ln, linolenic acid; A, arachidic acid.
TAGs that are not found.

Melting points of TAGs were acquired from the Triglyceride Property Calculator.

3.3 Microstructure

Overall, it was observed that pâtés which exhibited the highest hardness values (B, C, and E) contained smaller fat globules (Fig. 3.4). This is in agreement with a previous study concerning frankfurter-style products (Zetzl, Marangoni, & Barbut, 2012a). Pâtés A and D showed a larger and more heterogeneous fat globule distribution, as compared to pâtés B, C, and E. Pâté A showed a relatively low hardness value which could be due to a more uneven distribution of fat globules size, and/or resulting from coalescence of smaller fat globules. Even though pâté D had the highest hardness of all samples studied, it also had the lowest fat content (Table 3.1). This discrepancy in behaviour may be attributed to a higher carbohydrate content (double the amount of the other pâtés; Table 3.1). Overall, the presence of a secondary carbohydrate network could add support to weak structures containing larger fat globules such as seen in pâté D. It was also observed that
pâtés with smaller globule sizes (pâtés B, C, and E; Table 3.4) showed higher hardness value at lower temperature (4 °C). High standard deviation within each replicate showed that the fat globule sizes, within each sample, are widely distributed especially in pâtés A and D. This irregularity might also be caused by a history of temperature abuse of the products during transport or storage, which could lead to partial coalescence of some fat globules and creation of bigger globules in these pâtés. This phenomenon is fairly common in oil-in-water emulsion based products such as pâté (Palanuwech, Potineni, Roberts, & Coupland, 2003). In any case, lower standard error of the mean (SEM) indicated that the fat globule size calculations were very reproducible.
Fig. 3.4 Light micrographs of five commercial pâtés (Products A – E). White areas represent fat globules that were removed during sample preparation for microscopy. Scale bar = 100 µm.
Table 3.4 Averaged globules size and numbers of globules base on image analysis data obtained from micrographs such as shown in Figure 3.4.

<table>
<thead>
<tr>
<th>Pâté</th>
<th>Average globule size (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>260.11 ± 32.06¹</td>
</tr>
<tr>
<td>B</td>
<td>77.71 ± 13.58</td>
</tr>
<tr>
<td>C</td>
<td>173.60 ± 26.19</td>
</tr>
<tr>
<td>D</td>
<td>744.58 ± 68.78</td>
</tr>
<tr>
<td>E</td>
<td>86.32 ± 19.37</td>
</tr>
</tbody>
</table>

¹Standard error of the mean.

3.4 Statistical correlations

As shown in Table 3.5, there are strong correlations between hardness and physical as well as chemical characteristics of the pâtés. Hardness was correlated to SFC and high melting TAGs content. Moreover, when tested at 4 °C, the increase in hardness of the pâtés can be correlated to the decrease in fat globule size in addition to a temperature effect. Increase in hardness was also seen in pâtés with smaller and more uniform globules as observed under the light microscope. When tested at 4 °C, the hardness of the pâtés displayed an inverse correlation with the amount of high melting TAGs. At room temperature, there were strong correlations between the increase in hardness and the increase in fat content (FC) and SFC*FC. This was expected, as at this temperature, the SFC in the products was very low, therefore the amount of total fat played a more important role in increasing the hardness of the products than the SFC alone. At room temperature, the increase in hardness is inversely correlated with the increase in saturated fatty acids.
Table 3.5 Correlations among hardness of pâtés and physical as well as chemical parameters.

<table>
<thead>
<tr>
<th></th>
<th>SFC</th>
<th>FC</th>
<th>SFC*FC</th>
<th>Fat Globule size</th>
<th>SATs</th>
<th>HMTAGs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>R</strong></td>
<td>0.91</td>
<td>0.10</td>
<td>0.86</td>
<td>-0.13</td>
<td>0.023</td>
<td>0.87</td>
</tr>
<tr>
<td><strong>P-value</strong></td>
<td>0.0003*</td>
<td>0.780</td>
<td>0.002*</td>
<td>0.718</td>
<td>0.950</td>
<td>0.001*</td>
</tr>
<tr>
<td><strong>R</strong></td>
<td>-0.029</td>
<td>0.36</td>
<td>0.38</td>
<td>-0.92</td>
<td>-0.18</td>
<td>-0.93</td>
</tr>
<tr>
<td><strong>P-value</strong></td>
<td>0.963</td>
<td>0.554</td>
<td>0.523</td>
<td>0.080**</td>
<td>0.777</td>
<td>0.065**</td>
</tr>
<tr>
<td><strong>R</strong></td>
<td>0.25</td>
<td>0.97</td>
<td>0.85</td>
<td>-0.42</td>
<td>-0.97</td>
<td>0.22</td>
</tr>
<tr>
<td><strong>P-value</strong></td>
<td>0.683</td>
<td>0.03*</td>
<td>0.07**</td>
<td>0.583</td>
<td>0.028*</td>
<td>0.717</td>
</tr>
</tbody>
</table>

* Significant correlation at α = 0.05
** Significant correlation at α = 0.1

3.5 Differential Scanning Calorimetry (DSC)

The similarities in the first melting temperatures of extracted fat from pâtés A, B, D, and E (Table 3.6), are consistent with the results of the TAG analysis discussed in the previous section.

Extracted fat from pâtés A, B, D, and E show significantly higher first melting temperatures as well as higher amounts of high melting TAG components. On the other hand, the fat of pâté C mainly consisted of lower melting TAGs, but had a lower melting temperature of around 12.2 °C.

Unlike the melting points of the extracted fat, the first melting point of the pâté samples seem to be lower in most samples, with the exception of pâté A and B. This phenomenon can perhaps be related to the effect of the protein matrix in altering the crystallization behaviour of the fats.

Extracted fat from pâtés B, D, and E showed similar re-melting peaks compared to the first melting peaks. On the other hand, pâtés A and C showed a significantly lower melting peak (-1.57 °C and 1.15 °C), respectively, which correspond to the melting points of lower melting TAGs present in these samples. This may be due to the fact that in the second melting cycle, the fat samples were allowed to crystallize at a much lower temperature (-15 °C), allowing lower melting TAGs to crystallize. The second melting points of the pâtés did not show consistent trends in all pâtés, which perhaps can be correlated to the change in protein matrix after the first heating cycle.

In addition, crystallization peaks of pâtés A, B, and E showed that fats crystallize at lower
temperatures inside of the pâtés. This can perhaps confirm the effect of the protein matrix on the crystallization behaviour of fats.

**Fig. 3.5** Differential scanning calorimetric (DSC) traces for pâté A and its extracted fat melting peaks (A), re-melting peaks (B), and crystallization peaks (C).

**Table 3.6** Major melting and crystallization peaks of pâtés (A – E) and extracted fats from differential scanning calorimetry. Values represent the averages and standard deviations of 3 runs per products.

<table>
<thead>
<tr>
<th></th>
<th>Melting point (°C)</th>
<th>Enthalpy (J/g)</th>
<th>Re-melting Point (°C)</th>
<th>Enthalpy (J/g)</th>
<th>Crystallization point (°C)</th>
<th>Enthalpy (J/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong></td>
<td>Pâté: 38.19 ± 0.24</td>
<td>3.790 ± 0.38</td>
<td>24.27 ± 0.23</td>
<td>4.479 ± 0.19</td>
<td>19.17 ± 0.31</td>
<td>5.25 ± 0.46</td>
</tr>
<tr>
<td></td>
<td>Fat: 27.29 ± 0.50</td>
<td>23.04 ± 0.72</td>
<td>-1.57 ± 0.28</td>
<td>19.17 ± 0.31</td>
<td>5.25 ± 0.46</td>
<td>19.94 ± 1.18</td>
</tr>
<tr>
<td><strong>B</strong></td>
<td>Pâté: 28.13 ± 0.35</td>
<td>5.710 ± 1.40</td>
<td>28.00 ± 0.65</td>
<td>7.100 ± 0.26</td>
<td>1.63 ± 0.29</td>
<td>1.313 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>Fat: 28.04 ± 0.11</td>
<td>19.12 ± 0.99</td>
<td>27.40 ± 0.21</td>
<td>17.25 ± 1.57</td>
<td>6.27 ± 0.28</td>
<td>22.29 ± 0.47</td>
</tr>
<tr>
<td><strong>C</strong></td>
<td>Pâté: -</td>
<td>-</td>
<td>7.57 ± 0.22</td>
<td>1.660 ± 0.16</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Fat: 12.18 ± 0.56</td>
<td>10.74 ± 1.01</td>
<td>1.15 ± 0.05</td>
<td>12.62 ± 0.90</td>
<td>10.7 ± 0.58</td>
<td>3.145 ± 0.22</td>
</tr>
<tr>
<td><strong>D</strong></td>
<td>Pâté: -</td>
<td>-</td>
<td>3.03 ± 0.19</td>
<td>5.473 ± 0.22</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Fat: 28.91 ± 0.25</td>
<td>11.99 ± 0.72</td>
<td>27.29 ± 0.58</td>
<td>9.450 ± 1.23</td>
<td>8.37 ± 0.37</td>
<td>25.29 ± 0.49</td>
</tr>
<tr>
<td><strong>E</strong></td>
<td>Pâté: 17.78 ± 0.30</td>
<td>9.433 ± 0.70</td>
<td>30.64 ± 0.48</td>
<td>9.100 ± 0.83</td>
<td>5.11 ± 0.65</td>
<td>6.911 ± 0.58</td>
</tr>
<tr>
<td></td>
<td>Fat: 30.27 ± 0.32</td>
<td>6.940 ± 0.30</td>
<td>29.24 ± 0.30</td>
<td>6.840 ± 0.27</td>
<td>12.67 ± 0.25</td>
<td>0.3501 ± 0.42</td>
</tr>
</tbody>
</table>

*a* Not detected.
3.6 X-ray Diffraction

Figure 3.6 shows that the x-ray diffraction patterns of the pâtés and that of the corresponding extracted fats have similar polymorphic forms in samples A, B, and C. β crystals are present in all pâtés prepared with lard. β crystals are often present in lard crystallized/cooled down at a slow rate. Lard tends to have a more homogeneous TAG composition, which makes it easier to pack in a more stable β form (Campos et al., 2002). β' crystals are observed in higher quantities compared to β crystals in samples A and B, both in pâté and extracted fat samples. Sample C shows only the β crystal signature in both pâté and extracted fat samples. Sample D shows approximately the same amount of β and β’ crystals in the extracted fat sample, but only β crystals are observed in the pâté sample. However, more β’ crystals are observed in pâté E but absent in the extracted fat sample. This can perhaps be attributed to the effect of the protein matrix on the crystallization behaviour of fat in meat systems. However, further studies are needed to better define the effects of the protein matrix on fat crystallization.
Fig. 3.6 Wide angle X-ray diffraction patterns for commercial brand pâtés (A-E) and their extracted fats.
4. Conclusions

Pâtés tested at room temperature exhibited lower hardness values than those tested at refrigeration temperature. Pâtés with smaller fat globules showed higher hardness values (pâtés B, C, and E). The high hardness value of pâté D was attributed to the presence of a secondary carbohydrate network. Higher overall fat content increased the hardness of pâtés B, C, and E. SFC analysis of all pâtés confirmed higher hardness values as the SFC at 4°C was significantly higher than at 22°C. Higher melting TAGs in pâtés A, B, D, and E were responsible for the higher solid fat contents at both testing temperatures (4°C and 22°C). A higher unsaturated fatty acid content was correlated to a decreased hardness value of pâté C at room temperature (22°C). There was no significant correlation between fat crystal polymorphic form and the hardness values of the pâtés investigated. β crystals were observed in all pâtés made with lard, β’ crystals were found in higher quantities in pâté E but were absent in the extracted fat. Pâté C showed a significantly lower first melting point when compared to the other pâtés as the amount of low melting TAGs were higher in this product. Lower crystallization temperatures were also observed in pâtés A, B, and E. Our study indicates that fat has a major impact on texture of pâté products. A good understanding of fat properties in pâtés would allow for the development of products with better functional and nutritional qualities.
5. References


CHAPTER 4

Fat Replacement in Liver Pâté Using Canola Oil Organogels

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Abstract

Five different canola oil organogel formulations were used to replace pork fat in liver pâté to improve its polyunsaturated fat content and evaluate their effects on texture and sensory properties. Pâtés made with organogels showed similar hardness values as the control pâté. Use of organogels resulted in pâté with better textural properties than another control pâté made with canola oil only. Back extrusion results showed that all pâtés except for pâté made with canola oil only, had significantly higher hardness values when tested at 4°C than at room temperature. Pâtés made using organogels prepared with glycerol monostearate showed lower oil loss compared to the other organogel containing pâtés. Pâtés made with organogels showed higher oil loss overtime compared to control pâté made with pork fat. Image analysis results showed that fat globules size was significantly larger in pâtés made with organogels than those in the pork fat and canola oil control pâtés. Overall, pâtés made using organogels showed comparable textural, physical, and sensory properties as the traditional pâté made with pork fat while reducing the saturated fat content by 62%. Organogel replaced pâtés were perceived to have similar hardness, oiliness, and juiciness in the sensory test compared to control pâté made with pork fat. Control pâté made with pork fat was also perceived as less hard compared to organogel replaced pâtés. In addition, the colour of organogels replaced pâtés were significantly darker compared to pâtés made with pork fat or canola oil only. Control pâté was less red compared to the other pâtés. Sensory data showed that all fat replaced pâtés had very similar flavour profile.
1. Introduction

Trans and saturated fats have been connected to various cardiovascular problems. On the other hands, trans and saturated fats are known to have a positive contribution to the structure and texture of food products. Different governmental institutions, especially in North America and Europe, have suggested reducing the consumption of saturated and trans fatty acids in our diet, and have encouraged people to switch to a diet with higher amount of polyunsaturated fats (WHO, 2013). The challenge with polyunsaturated fats is that they lack the ability to form solid structures at room temperature. Consequently, they can negatively affect textural and sensory properties of food products. The use of ethylcellulose to create organogels could help structure vegetable oils and has been shown to have a great potential in food industry (Rogers et al., 2014; Stortz & Marangoni, 2013; Stortz et al., 2012). There are various known applications for organogels, ranging from stabilizing emulsions, slow release of bioactive components, as well as replacement of saturated and trans fatty acids. By trapping the liquid oil in a gel network, the resulting products can be as firmed at room temperature as saturated fat containing products (Stortz et al., 2012). As a result, these gel structures can be utilized to mimic the properties of solid fat while at the same time maintaining the nutritional properties of the healthier unsaturated fatty acids (Gravelle et al., 2012). Different kinds of vegetable oils (canola, olive, castor, and peanut) have been successfully gelled using ethylcellulose (Gravelle et al., 2014). Recent studies have demonstrated the use of organogels in food products, such as emulsified meat products, heat resistant chocolate, product with controlled release nutraceuticals, pharmaceuticals, and various kind of water in oil emulsions (Hughes, Marangoni, Wright, Rogers, & Rush, 2009).

Over the past 3 decades as the meat sector has been looking at reducing/ replacing saturated fat in various meat products (frankfurters, sausages, etc.) with the aid of different fillers and binders
It was reported that the textural properties of these meat products were somewhat
differenc

from the original products. For example, straight replacement of beef fat with liquid

(canola oil in comminuted products results in a firmer and rubberier product (Barbut et al., 2016),

which is undesirable. On the other hand, a formula that uses canola oil organogel to replace beef

fat in comminuted meat products (e.g., frankfurter) resulted in a more similar organoleptic and
textural properties as the traditional beef fat control (Barbut et al., 2016).

In pâté type products Morales-Irigoyen, Severiano-Perez, Rodriguez-Huezo, & Totosaus (2012) replaced pork fat with vegetable canola oil in order to reduce saturated fat content, they
employed liquid canola oil with the combination of xanthan gum and sodium caseinates to replace
50% of lard from a pâté product. The resulting pâté was softer and showed higher syneresis
compared to the control. It was also reported that the canola, sodium caseinates, and xanthan gum
combination was only able to replace a maximum of 50% lard. Another research attempted to
replace pork fat in pork liver pâtés by a mixture of olive, linseed, and fish oils (Morales-Irigoyen
et al., 2012). They reported negative effects on the rheological properties of the meat systems.

Overall, pâté is classified as an emulsified meat product made primarily from liver, fat,

meat, and spices (Barbut, 2015b). It is commonly consumed around the world due to its rich and
smooth texture. Pâté’s unique texture and taste can be attributed to the type of fat used and to its
relatively high fat content. Various studies have indicated that saturated fats play an important
role in texture, mouthfeel, moistness, and sensory acceptability of such emulsified meat products
(Chin, Keeton, Miller, Longnecker, & Lamkey, 2000; Hughes, Mullen, & Troy, 1998). Unlike
other emulsified meat products, pork meat is added precooked (i.e., denatured proteins) in the pâté
making process. Therefore, liver proteins become the main emulsifying and binding agents.
Temperature control during chopping is also critical in the pâté making process; i.e., the meat and
fat are processed at a relatively high temperature (50-55°C) to ensure proper emulsification of the fat globules. Furthermore, the pâté batter has to be stuffed with sufficient pressure to avoid fat separation to the outer layer of the product. The lack of functional muscle proteins during chopping emphasizes the important contribution of the fat and liver proteins to the formation of an acceptable texture in the final product. These points highlight the importance of understanding the functionality of the fat substitute used to make the fat replace pâté.

Although fat replacement in pâté products has been done in the past times, to the best of our knowledge there are no commercial products utilizing organogelation technology for fat replacement. Therefore, the goal of this study was to look at the use of organogels made from vegetable oil (rich in unsaturated fatty acids) to replace pork fat in liver pâté.

2. Material and Methods

2.1 Organogel Preparation

Organogels were prepared in an oven at a temperature of 140 °C following Gravelle, Barbut, and Marangoni, (2012). Ethylcellulose (EC) with a viscosity of 20 cP (Ethocel™ 20, Dow Chemical, Midland, MI, USA), canola oil (Hela Spice Canada, Uxbridge, ON, CA), glycerol monostearate, (GMS), and crystal promoter (Palsgaard 6111, Palsgaard, Morris Plains, NJ, USA) were used to make the organogels. Briefly, gels were prepared in glass beakers in a bench-top gravity convection oven (Fischer Scientific, Ottawa, ON, Canada) set to 170 °C with constant mixing using an overhead mechanical stirrer (Model L1U10F, Lightnin LabMaster, Wytheville, VA, USA) fitted with a high-shear impeller which was inserted through the roof of the oven, rotating at 175 rpm. The gels reached the target temperature within approximately 50 min, followed by 10 min holding period. Each gel was taken out and cooled down on a tempering table set at 20 °C for approximately 20 min. Each batch was then cut into 2 cm x 2 cm squares and
stored at 5 °C overnight. For this experiment, one control was prepared with the traditional pork back fat and 6 treatments were prepared with canola oil (canola oil control, and T1 – T5 with canola oil organogels). The 7 treatments consisted of two controls consisting of pork back fat (Control “I”), another control consisting of heated canola oil (Control II), and organogels made with 14% EC (T1); 14% EC, 1.5% GMS (T2); 14% EC, 3% GMS (T3); 12% EC, 3% GMS (T4); 12% EC, 3% GMS, 1% Palsgaard 6111 (T5), respectively.

2.2 Pâté Preparation

For each treatment, 700 g of partially frozen pork liver (28%) was cut at high speed setting for 2 min in a bowl chopper (Feuma Gastromaschinen Gmbh, model no. 15L, Gößnitz, Thüringen, Germany). Dry ingredients such as 1.4 % Salt, 0.3 % phosphates mixture (Helabin Ultimal), 0.3% curing salts mixture (Rapid Cure), 0.6% pâté seasoning (Calf Liverwurst Seasoning), 0.5 % roasted onions, 1% powdered whole milk, and 0.8% sugar (Hela Spice Canada) were added into the chopper, and the mixture was cut at low speed for 45 sec. Pork trimmings (80/20 muscle/fat; 20%), pork back fat (20%), pork ham fat (20%), and pork jowls (9%) were cooked at 80°C for 1 hr in a steam jacketed kettle. The bowl chopper was then preheated to ~50°C using hot water (3%). Cooked pork jowls and pork trims were added and chopped at low speed for 40 sec, and cooked ham fat and back fat (or canola oil/organogels for control II and T1-T5) were subsequently added and the mixture was chopped for an additional 50 sec. A spice mix (Hela Spice Canada) was then added to the cutting bowl and chopped at high speed for 1 min. The precut pork liver was then added and cut at high speed for 90 sec. The resulting meat batter was then stuffed using an automatic stuffer (Mainca EB-25, St Louis, MO, USA) into sausage casings (PVDC inside coated fibrous cellulose casing; cal. 60x60; Canada Compound Corp., Woodbridge, Ontario, Canada) and
were cooked in a kettle/hot water bath at 80°C to an internal temperature of 72°C (~2 hrs). Cooked sausages were then cooled in an ice water bath and refrigerated prior to evaluation.

2.3 Back Extrusion

The samples were stuffed into polypropylene test tubes (27 mm diameter) and kept in the refrigerator (4 °C) overnight to allow them to set. The following day, half of the samples were tested directly out of the refrigerator while the other samples were allowed to equilibrate at room temperature (22 °C) for 2 hrs. Back extrusion tests were performed using a texture analyzer (Texture Technologies Corp., Model TA.XT2, Scarsdale, NY, USA). A stainless steel cylindrical probe (height: 9.8 cm, diameter: 1.8 cm) with enlarged spherical tip (height: 1.3 cm, diameter: 2.0 cm) was used to penetrate the product, in the test tubes, for 30 mm at a rate of 1.5 mm/s. Results were recorded as described by Gravelle et al. (2014). Four tubes were analyzed per replicate, and the results were recorded for each of the three individual trials.

2.4 Fatty Acid Composition

The fatty acid composition of extracted pâté fats were determined using gas chromatography (Ghazani et al., 2013). Briefly, samples were subjected to a transmethylation procedure in accordance with Christie, (1982). Fatty acid methyl esters (FAME) were analyzed by using a capillary GC equipped with a BPX70 column, 60 m x 0.22 mm internal diameter and with 0.25 μm film thickness (SGE Inc., Austin, TX, USA). A gas chromatograph (Agilent 6890 Series, Agilent Technologies, Inc., Wilmington, DE, USA) with an auto sampler (7,683 series) was used. The oven temperature was programmed to increase from 110 to 230 °C at a rate of 4 °C/min and held at 230 °C for 10 min. The injector and detector temperatures were 240 and 280 °C, respectively. Hydrogen was used as the carrier gas at an average velocity of 25 cm/s. Peaks were identified via comparison to FAME standards. Samples were measured in triplicates.
2.5 Microstructure

Sample were prepared for light microscopy following the method used by Barbut et al., (2016). Briefly samples (20 × 20 × 5 mm), were cut from the center part of the liver sausages and then fixed and stained using Masson stain. Slides were observed using a light microscope (Model BX60, Olympus Optical Ltd., Tokyo, Japan) and images were captured using a computerized image analysis system (Image-Pro Plus, Version 5.1, Media Cybernetics Inc., Silver Spring, MD).

2.6 Colour Evaluation

Colour of the freshly cut pâté samples was analyzed by a colorimeter (Chroma Meter CR-400, Konica Minolta, NJ, USA). Readings were taken in triplicates and reported as L* (lightness), a* (redness), and b* (yellowness). Four measurements were taken per replicate, and the results were recorded for each of the three individual trials.

2.7 Fat Extraction

Samples were smeared onto the side of fat extraction thimbles, with the total amount not exceeding 4 g, to ensure maximum surface area for the extraction. The thimbles were dried at 60 °C to remove moisture until constant weights were obtained. The thimbles were then placed in the extraction apparatus. Approximately 200 ml petroleum ether was poured into the soxhlet flask, heat turned on, to allow a continual refluxes of petroleum ether, and samples extracted for 3-4 hrs. The petroleum ether was then evaporated in the oven.

2.8 Oil Loss

Oil loss of pâtés were measured at 4°C (storage temperature) by placing triplicate sliced pâté (2 cm thick of about 60 g) sample on 5 filter papers (Whatman 4, 150 mm diameter, Whatman
plc, Sigma Aldrich, Oakville, ON, CA) to absorb the oil. Samples were placed in an enclosed container to minimize moisture loss to the environment. Samples were weighed at 1, 2, 4, 6, and 24 hrs. Measurements were recorded in triplicate.

2.9 Sensory Analysis

Sensory analysis was performed by graduate and undergraduate students from the University of Guelph Food Science Department. The potential panelists were recruited, screened, and trained according to Meilgaard, Carr, & Civille, (2006). The sensory analysis consisted of three sessions including 16 trained panelists. Pâtés were cut into 2 cm cubes, placed inside 75 ml polystyrene cups which were labelled with a random 3-digit code. Panelists were seated in a sensory analysis laboratory in individual booths, with overhead red light to avoid visual bias. Pâté samples were served with a reference sample from the training session (the panelists were trained to use line scale with various food products), a glass of tap water, and unsalted soda crackers. Panelists were instructed to cleanse their palate in between samples. Panelists evaluated the textural properties of the pâtés for hardness, juiciness, oiliness, and cohesiveness. Samples were rated on a 15 cm line scale. Hardness: 0 = very soft, 15 = very hard; Juiciness: 0 = very dry, 15 = very juicy; Oiliness: 0 = not oily, 15 = very oily; Cohesiveness: 0 = not cohesive, 15 = very cohesive. Panelists were also trained to be familiar with potential off-flavors that might be picked up in the samples such as chemical, grassy, rancid, earthy, and cardboardy. Panelists were asked to note these off-flavours if picked up during tasting. Panelists were asked to attend three separate trials (weekly) as replicates.
2.10 Statistical Analysis

The experiment was designed as a complete randomized block, with three separate replications. Statistical analysis of the results was completed using Graphpad Prism 5.0 (GraphPad, San Diego, CA, USA). A one-way ANOVA test was done with a Tukey post test with (P<0.05). Data were graphed using Graphpad Prism 5.0 with error bars indicating standard error of the mean.

3. Results and Discussion

3.1 Texture analysis

Overall, the difference in hardness (obtained using back extrusion method; also an indication of spreadability) between the two temperatures was very noticeable (Fig. 4.1). Pâté tested at 4 °C showed higher hardness values compared to those tested at room temperature (RT), most notably shown in the control pâté made with pork fat. The hardness values at 4 °C were three times higher compared to those tested at RT. This was due to the high amount of saturated fat in the lard compared to lower saturated fat composition of the canola oil based pâté. When tested at room temperature, all pâtés did not show significance differences in hardness compared to control pâté made with pork fat. Back extrusion tests also revealed that all pâtés had similar hardness when tested at 4 °C. In contrast with similar studies in frankfurters, full substitution of pork fat in pâté with only canola oil resulted in softer product( Barbut et al., 2016; Youssef & Barbut, 2009; Zetzl et al., 2012a). Addition of glycerol monostearate (GMS) to the organogel resulted in pâtés with higher overall hardness, as demonstrated in pâtés T1 (0% GMS), T2 (1.5% GMS), and T3 (3% GMS). Addition of a crystal promoter (Palsgaard 6111) did not significantly increase the hardness value. Lastly, T4 pâté had the closest hardness compared to control at both test temperatures. Overall, our instrumental and sensory hardness scores (Fig. 4.2) showed similar trends. These results indicate that hardness values obtained by the texture analyzer instrument matched the
panelists perception. Fatty acid analysis showed that pâté made with pork fat had 35.02 ± 0.074 saturated fat content and 64.98 ± 0.074 unsaturated fat content. In addition, fatty acid analysis of pâté made with organogels resulted in 13.10 ± 0.018 saturated fat content and 86.34 ± 0.016 polyunsaturated fat content. Overall, up to 62% saturated fat reduction was achieved with minimal textural impacts on the products.

3.2 Microstructure

When observed under the microscope, the fat globules in pâtés made with organogels (Fig. 4.3, panels C - G) were notably larger compared to pâtés made with lard and canola oil (panels A and B). Pâté made with canola oil showed smaller fat globules than the other pâtés. This is because during chopping, canola oil presents less resistance to shear, this finding was in line with the study done by Barbut et al., (2016).

On the other hand, the organogel treatments had larger fat globules which could be attributed to the harder nature of the organogels, thus causing them to be more resilient to chopping. This phenomenon can also be observed between organogels with different hardness values. For example, T3-E pâté shows larger fat globules when compared to the other pâtés as hardness value of T3-E pâté is considerably higher than the rest of the pâtés. Also, as will be discussed below the presence of the larger fat globules in organogels based pâtés has probably contributed to the increase oil loss in these treatments as bigger fat globules tend to be less stable.
Fig. 4.1 Hardness of pâtés tested at room temperature (22 °C) and at 4 °C. Bars represent standard error of the mean. n = 12.

a-e Bars with different superscripts are significantly different (P < 0.05).

Pork fat control (Ctr); Canola control (Can); 14% EC (T1); 14% EC, 1.5% GMS (T2); 14% EC, 3% GMS (T3); 12% EC, 3% GMS (T4); 12% EC, 3% GMS, 1% Palsgaard 6111 (T5).

Fig. 4.2 Left figure shows hardness values of pâtés obtained by the sensory analysis panel (0 = very soft; 15 = very hard). Right figure shows hardness values of pâtés determined using texture analyzer. Both tests were performed at room temperature. Bars indicate means and standard error of the mean. n = 48 for the left figure and n = 16 for the right figure.

a Bars with similar superscripts are not statistically different (P > 0.05).

Pork fat control (Ctr); Canola control (Can); 14% EC (T1); 14% EC, 1.5% GMS (T2); 14% EC, 3% GMS (T3); 12% EC, 3% GMS (T4); 12% EC, 3% GMS, 1% Palsgaard 6111 (T5).
Fig. 4.3 Light micrographs of pâté samples (A = Control, B = Canola, C = T1, D = T2, E = T3, F = T4, G = T5). White areas represent fat globules that were removed during sample preparation for microscopy. Bar = 200 µm.

Pork fat control (Ctr); Canola control (Can); 14% EC (T1); 14% EC, 1.5% GMS (T2); 14% EC, 3% GMS (T3); 12% EC, 3% GMS (T4); 12% EC, 3% GMS, 1% Palsgaard 6111 (T5).
3.3 Sensory Analysis

Organogels based pâtés were perceived to have similar hardness, oiliness, and juiciness as the lard and the canola oil pâtés (Table 4.1). However, T3 pâté was perceived to be less cohesive compared to control pâté made with lard, while canola oil only pâté and the other organogels pâté had the same cohesiveness as the control pâté made with lard. T1 and T4 had the closest hardness values to the lard control pâté when compared to the other pâtés. T1 and T4 also showed very similar oiliness scores compared to control pâté. In terms of juiciness, T1 and T4 pâtés showed the closest scores to the control pâté made with lard compared to the rest of the treatments. Finally, T1, T4, and T5 had the most similar cohesiveness scores to the control pâté made with lard. Overall, T1 and T4 had very similar sensory properties compared to control pâté made with pork fat.

As shown in Table 4.2, canola oil pâté, T2, T4, and T5 pâtés had equal to or lower perceived off-flavours score to pâté made with pork fat. This sensory analysis results showed that at least 4 pâtés made using organogels were perceived to have very similar sensory characteristics to control pâté made with pork fat. T1 and T3 showed higher overall perceived off-flavour scores. T1 was perceived to have more chemical, rancid, and earthy flavours compared to the control. T3 was perceived to be more cardboardy and chemically than the control. Higher chemical and rancid flavours perceived in T1 and T3 can be attributed to higher EC content in the organogels used to make these pâtés (14%). However, by lowering the amount of EC in the formula, the amount of perceived off flavours can be minimized as demonstrated in T4 and T5.

Statistically, the outcome of this sensory analysis confirmed that the panel could not detect any differences in hardness, oiliness, juiciness, and cohesiveness in pâtés made using organogels.
compared to the control pâté made using pork fat.

**Table 4.1** Sensory analysis results of the seven test pâtés evaluated by the 16 train panellists averaged across the three trials.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Canola</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hardness</td>
<td>5.67 ± 1.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.13 ± 1.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.32 ± 0.62&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.22 ± 0.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.68 ± 0.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.48 ± 0.48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.21 ± 0.53&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Oiliness</td>
<td>8.47 ± 0.30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.47 ± 0.91&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.06 ± 0.66&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.79 ± 0.93&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.48 ± 1.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.84 ± 0.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.94 ± 0.30&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Juiciness</td>
<td>7.45 ± 0.38&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.26 ± 1.19&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.78 ± 0.79&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.48 ± 0.71&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.18 ± 1.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.04 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.36 ± 0.10&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cohesiveness</td>
<td>6.09 ± 0.26&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.71 ± 0.51&lt;sup&gt;de&lt;/sup&gt;</td>
<td>5.12 ± 0.39&lt;sup&gt;de&lt;/sup&gt;</td>
<td>4.98 ± 0.34&lt;sup&gt;de&lt;/sup&gt;</td>
<td>4.35 ± 0.06&lt;sup&gt;e&lt;/sup&gt;</td>
<td>5.09 ± 0.23&lt;sup&gt;de&lt;/sup&gt;</td>
<td>5.34 ± 0.41&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a-e</sup> superscripts are significantly different (P < 0.05).

Pork fat control (Ctr); Canola control (Can); 14% EC (T1); 14% EC, 1.5% GMS (T2); 14% EC, 3% GMS (T3); 12% EC, 3% GMS (T4); 12% EC, 3% GMS, 1% Palsgaard 6111 (T5).

**Table 4.2** Perceived off-flavours during sensory analysis of the seven test pâtés evaluated by the 16 train panellists.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Control</th>
<th>Canola</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grassiness</td>
<td>1.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.67&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.67&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.67&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Rancidity</td>
<td>1.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.67&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.67&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.00&lt;sup&gt;f&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Earthiness</td>
<td>3.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.67&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.00&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14.0&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Cardboardiness</td>
<td>1.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.00&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.00&lt;sup&gt;f&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.67&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.67&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.67&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12.33&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>2.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.67&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.00&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.67&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.67&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>T5</td>
<td>1.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.67&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.67&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>8.33&lt;sup&gt;f&lt;/sup&gt;</td>
<td></td>
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</tbody>
</table>

<sup>a-g</sup> values followed by superscripts are significantly different (P < 0.05).

Pork fat control (Ctr); Canola control (Can); 14% EC (T1); 14% EC, 1.5% GMS (T2); 14% EC, 3% GMS (T3); 12% EC, 3% GMS (T4); 12% EC, 3% GMS, 1% Palsgaard 6111 (T5).

3.4 Oil loss

The oil loss results showed that control lard pâté had the lowest oil loss over the period of 24 hr (Fig. 4.4). Pâté made by EC only (T1) showed the highest overall oil loss. Pâté made with canola oil only showed high oil loss in the first 4 hr but had low overall oil loss after 24 hr. As indicated before, high oil loss values of organogels based pâtés can be attributed to formation of larger fat globules that could be more vulnerable to oil separation. Addition of GMS to the organogels also improve oil retention performance as demonstrated in T1, versus T2, and T3. However, addition of crystal promoter did not show any improvement in oil retention. Overall, canola oil, T3, and T4 pâtés had the closest oil loss performance compared to pâté made with pork.
fat. High oil retention in pâté made using lard was mainly attributed to the high amount of saturated fat contents (three times as much as the canola oil based pâtés) which were solid at the test temperature (4 °C). On the other hand, pâtés made with both liquid canola oil (Control II) and canola oil organogels (T1-T5) contained low amount of saturated fats and high polyunsaturated fats which are mainly liquid at this temperature.

**Fig. 4.4** Oil loss of pâtés tested at 4 °C over 24 hrs period. Bars represent standard error of the means. n = 3.
Pork fat control (Ctr); Canola control (Can); 14% EC (T1); 14% EC, 1.5% GMS (T2); 14% EC, 3% GMS (T3); 12% EC, 3% GMS (T4); 12% EC, 3% GMS, 1% Palsgaard 6111 (T5).
Canola oil pâté showed a higher L* value compared to organogels based pâtés. This result is in agreement with Youssef & Barbut, (2009) who noted that the higher lightness value in the canola based product is due to the differing distribution and light reflectance of smaller fat globules compared to the larger fat particles in control product. The higher amount of organogel particles (translucent gels) affected the colour of the products, which caused the organogels based pâtés to appear darker and had higher a* and b* values as well as lower L values (Table 4.3). It was also observed that the three pâtés made with higher concentration of EC (T1, T2, and T3; contained 14% EC) have slightly higher L* values compared to those made with less EC (T4 and T5; 12% EC). This could be attributed to higher EC content which resulted in gels with more opaque colour.

Table 4.3 Colour analysis of pâtés reported as L* = lightness, a* = redness, b* = yellowness. n = 15.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>61.29±0.38</td>
<td>13.36±0.15</td>
<td>12.02±0.16</td>
</tr>
<tr>
<td>Canola</td>
<td>58.15±0.53</td>
<td>15.22±0.27</td>
<td>13.75±0.18</td>
</tr>
<tr>
<td>T1</td>
<td>55.46±0.56</td>
<td>15.64±0.18</td>
<td>12.94±0.08</td>
</tr>
<tr>
<td>T2</td>
<td>55.64±0.31</td>
<td>15.61±0.22</td>
<td>13.02±0.11</td>
</tr>
<tr>
<td>T3</td>
<td>55.21±0.36</td>
<td>15.60±0.30</td>
<td>13.23±0.17</td>
</tr>
<tr>
<td>T4</td>
<td>54.96±0.31</td>
<td>15.59±0.24</td>
<td>13.17±0.15</td>
</tr>
<tr>
<td>T5</td>
<td>54.99±0.40</td>
<td>15.58±0.24</td>
<td>13.26±0.12</td>
</tr>
</tbody>
</table>

a-c values followed by superscripts are significantly different (P < 0.05).

Pork fat control (Ctr); Canola control (Can); 14% EC (T1); 14% EC, 1.5% GMS (T2); 14% EC, 3% GMS (T3); 12% EC, 3% GMS (T4); 12% EC, 3% GMS, 1% Palsgaard 6111 (T5).
4. Conclusion

Overall results of the sensory panels showed that 4 out of 5 pâtés made using organogels had the same sensory characteristics as the control pâté made with pork fat. Pâtés made with organogel also had a relatively low off-flavours perception. Pâtés made with organogels had similar hardness compared to pâté made with pork fat when tested at RT and 4 °C. In terms of microstructure, pâté made with EC only organogel (T1) had the highest oil loss over a 24 hrs period. Pâté made using organogels showed bigger fat globules size compared to the canola oil only treatment. The colour of pâtés made using organogels appeared to be darker, redder and yellower. Overall, the pâté made with 12% EC and 3% GMS (T4) organogel showed the best performance in matching the control pâté made with pork fat while at the same time successfully reducing the saturated fat content by 62%. Successful addition of organogels to liver pâté showed the potential of organogel applications in a highly emulsified meat system.
5. References


CHAPTER 5

Partial Fat Replacement in Liver Pâté Using Canola Oil Organogel

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Abstract
Partial replacement of pork fats with canola oil organogels was done to improve oil retention, sensory and textural properties of liver pâté. Sensory analysis results indicated that full replacement as well as partial replacement of pork fat up to 80% could not be differentiated from pâté made 100% from lard by the panelists in terms of hardness, oiliness, cohesiveness, and perceived off-flavours. In addition, oil loss results showed high oil retention for pâtés made with up to 60% organogels and 40% lard. Light microscopy showed a gradual increase in fat globules size as more pork fats were replaced by organogels. Based on texture analysis results using the back extrusion method, hardness of the fat replaced pâtés were statistically similar up to 100% replacement. However, colour analysis suggested that addition of canola oil organogels lower the L* value and increase a* and b* values when compared to pâté made with 100% pork fat. Overall, oil retention performance could be retained at 60% pork fat replacement while maintaining the textural properties and having minimal effect on the sensory properties and colour of the products.

1. Introduction
The demand for low saturated fat meat products is continually increasing as the consumer becomes more health conscious (Marchetti et al., 2014). However, various studies have suggested that saturated fats play a big role in texture, mouthfeel, moistness, and sensory acceptability of emulsified meat products (Chin et al., 2000; E. Hughes et al., 1998). The World Health Organization has suggested reducing the consumption of dietary saturated and trans fatty acids,
and have encouraged people to switch to a diet with higher amounts of polyunsaturated fats (WHO, 2013). However, polyunsaturated fats lack the ability to form the solid fat structure that is essential for the sensory and textural properties of high fat meat products such as ice cream, cheese, salad dressings, and processed meat products.

Replacement of saturated fats has been done in different comminuted meat products (i.e., frankfurters, sausages) with liquid unsaturated vegetable oils such as canola oil and olive oil with the aid of hydrocolloids, in an attempt to improve the fatty acid compositions of the product. It was reported that the textural properties of these meat products were significantly different than the original products (Barbut et al., 2016; Delgado-Pando et al., 2012). It was also reported that the straight replacement of beef fat with liquid canola oil in comminuted products results in a firmer and more rubbery product, which is undesirable (Barbut et al., 2016). In pâté type products, in order to reduce saturated fat content, Morales-Irigoyen, Severiano-Perez, Rodriguez-Huezo, & Totosaus (2012) employed liquid canola oil with the combination of xanthan gum and sodium caseinate to replace 50% of the lard in a pâté. The resulting pâté product was softer and showed higher syneresis compared to the control. It was also reported that the canola, sodium caseinate, and xanthan gum combination was only able to replace a maximum of 50% lard (Morales-Irigoyen et al., 2012). Other researchers attempted to replace pork fat in pork liver pâtés with a mixture of olive, linseed, and fish oils (Morales-Irigoyen et al., 2012). They reported negative effects on the rheological properties of the meat systems.

Nanostructuring of unsaturated vegetable oils into solid functional fats has been showing great promise. This could be the solution to replacing fat in products that require the unique characteristics, structure, and mouthfeel provided by saturated fat (Zetzl & Marangoni, 2012). Different known methods include structuring of emulsions, interesterification, and organogelation.
Organogelation is considered to be the most novel technique and also been proven previously to show positive results. The use of ethylcellulose to create organogels by structuring vegetable oils has been shown to have great potential for the food industry (Rogers et al., 2014; Stortz & Marangoni, 2013; Stortz et al., 2012). In a previous study by Barbut et al. (2016), a use of canola oil organogels to replace beef fat in comminuted meat products resulted in similar organoleptic and textural properties to the control. By trapping the liquid oil in a gel network, the resulting products can be as firm as saturated fat at room temperature (Stortz et al., 2012). As a result, these gel structures can be utilized to imitate the properties of solid fat while at the same time maintaining the nutritional properties of the healthier unsaturated fatty acids (Gravelle et al., 2012).

Overall, pâté is classified as an emulsified meat product made primarily from liver, fat, meat, and spices (Barbut, 2015b). It is commonly consumed around the world due to its rich and smooth texture. Pâté’s unique texture and taste can be attributed to the type of fat used and to its relatively high fat content. Various studies have indicated that saturated fats play an important role in texture, mouthfeel, moistness, and sensory acceptability of emulsified meat products (Chin, Keeton, Miller, Longnecker, & Lamkey, 2000; Hughes, Mullen, & Troy, 1998). Unlike other emulsified meat products, pork meat is added precooked (i.e., denatured proteins) in pâté production. Therefore, liver proteins become the main emulsifying and binding agents. Temperature control during chopping is also critical in the pâté making process; i.e., the meat and fat are processed at a relatively high temperature (50-55°C) to ensure proper emulsification of the fat globules. Furthermore, the pâté batter has to be stuffed with sufficient pressure to avoid fat separation to the outer layer of the product. The lack of functional muscle proteins, during chopping, emphasizes the important contribution of the fat and liver proteins to the formation of
acceptable texture in the final product. These issues highlight the importance of understanding the
functionality of the fat substitute used to make the fat replaced pâté.

In the previous study, the authors reported on the full replacement of pork fat with different
organogels preparation in pâté products. It was reported that the use of ethylcellulose based
organogels to reduce saturated fat content in pâté yielded positive sensory analysis results.
However, full replacement of pork fat with organogels resulted in pâtés that were more vulnerable
to oil separation. In this current study, the authors used one of the successful organogel formula to
employ partial replacement of pork fat to solve the low oil retention problem as well as to improve
sensory and textural properties of organogel based pâté even further.

2. Material and Methods

2.1 Organogel preparation

Organogels were prepared in an oven at a temperature of 140 °C following Gravelle, Barbut, and Marangoni, (2012). Ethylcellulose (EC) with a viscosity of 20 cP (Ethocel™ 20, Dow Chemical, Midland, MI, USA), canola oil (Hela Spice Canada, Uxbridge, ON, CA), glycerol monostearate, (GMS), and crystal promoter (Palsgaard 6111, Palsgaard, Morris Plains, NJ, USA) were used to make the organogels. Briefly, gels were prepared in glass beakers in a bench-top gravity convection oven (Fischer Scientific, Ottawa, ON, Canada) set to 170 °C with constant mixing using an overhead mechanical stirrer (Model L1U10F, Lightnin LabMaster, Wytheville, VA, USA) fitted with a high-shear impeller which was inserted through the roof of the oven, rotating at 175 rpm. The gels reached the target temperature within approximately 50 min, followed by 10 min holding period. Each gel was taken out and cooled down on a tempering table set at 20 °C for approximately 20 min. Each batch was then cut into 2 cm x 2 cm squares and stored at 5 °C overnight. For this experiment, an organogel formulation was prepared with 12%
EC and 3% GMS. Six formulations for partial fat replacement was prepared with increasing organogel concentration (T1: 100% lard, 0% organogel; T2: 80% lard, 20% organogel; T3: 60% lard, 40% organogel; T4: 40% lard, 60% organogel; T5: 20% lard, 80% organogel; T6: 0% lard, 100% organogel).

2.2 Pâté preparation

For each treatment, 700 g of partially frozen pork liver (28%) was cut at high speed setting for 2 min in a bowl chopper (Feuma Gastromaschinen Gmbh, model no. 15L, Gößnitz, Thüringen, Germany). Dry ingredients such as 1.4 % Salt, 0.3 % phosphates mixture (Helabin Ultimal), 0.3% curing salts mixture (Rapid Cure), 0.6% pâté seasoning (Calf Liverwurst Seasoning), 0.5 % roasted onions, 1% powdered whole milk, and 0.8% sugar (Hela Spice Canada) were added into the chopper, and the mixture was cut at low speed for 45 sec. Pork trimmings (80/20 muscle/fat; 20%), pork back fat (20%), pork ham fat (20%), and pork jowls (9%) were cooked at 80°C for 1 hr in a steam jacketed kettle. The bowl chopper was then preheated to ~50°C using hot water (3%). Cooked pork jowls and pork trimns were added and chopped at low speed for 40 sec, and cooked ham fat and back fat (or canola oil/ organogels for control II and T1-T5) were subsequently added and the mixture was chopped for an additional 50 sec. A spice mix (Hela Spice Canada) was then added to the cutting bowl and chopped at high speed for 1 min. The precut pork liver was then added and cut at high speed for 90 sec. The resulting meat batter was then stuffed using an automatic stuffer (Mainca EB-25, St Louis, MO, USA) into sausage casings (PVDC inside coated fibrous cellulose casing; cal. 60x60; Canada Compound Corp., Woodbridge, Ontario, Canada) and were cooked in a kettle/ hot water bath at 80°C to an internal temperature of 72°C (~2 hrs). Cooked sausages were then cooled in an ice water bath and refrigerated prior to evaluation.
2.3 Back Extrusion

The samples were stuffed into polypropylene test tubes (27 mm diameter) and kept in the refrigerator (4 °C) overnight to allow them to set. The following day, half of the samples were tested directly out of the refrigerator while the other samples were allowed to equilibrate at room temperature (22 °C) for 2 hrs. Back extrusion tests were performed using a texture analyzer (Texture Technologies Corp., Model TA.XT2, Scarsdale, NY, USA). A stainless steel cylindrical probe (height: 9.8 cm, diameter: 1.8 cm) with enlarged spherical tip (height: 1.3 cm, diameter: 2.0 cm) was used to penetrate the product, in the test tubes, for 30 mm at a rate of 1.5 mm/s. Results were recorded as described by Gravelle et al. (2014). Four tubes were analyzed per replicate, and the results were recorded for each of the three individual trials.

2.4 Fatty Acid Composition

The fatty acid composition of extracted pâté fats were determined using gas chromatography (Ghazani et al., 2013). Briefly, samples were subjected to a transmethylation procedure in accordance with Christie, (1982). Fatty acid methyl esters (FAME) were analyzed by using a capillary GC equipped with a BPX70 column, 60 m × 0.22 mm internal diameter and with 0.25 µm film thickness (SGE Inc., Austin, TX, USA). A gas chromatograph (Agilent 6890 Series, Agilent Technologies, Inc., Wilmington, DE, USA) with an auto sampler (7,683 series) was used. The oven temperature was programmed to increase from 110 to 230 °C at a rate of 4 °C/min and held at 230 °C for 10 min. The injector and detector temperatures were 240 and 280 °C, respectively. Hydrogen was used as the carrier gas at an average velocity of 25 cm/s. Peaks were identified via comparison to FAME standards. Samples were measured in triplicates.

2.5 Microstructure

Sample were prepared for light microscopy following the method used by Barbut et al., (2016). Briefly samples (20 × 20 × 5 mm), were cut from the center part of the liver sausages and
then fixed and stained using Masson stain. Slides were observed using a light microscope (Model BX60, Olympus Optical Ltd., Tokyo, Japan) and images were captured using a computerized image analysis system (Image-Pro Plus, Version 5.1, Media Cybernetics Inc., Silver Spring, MD).

2.6 Colour Evaluation

Colour of the freshly cut pâté samples was analyzed by a colorimeter (Chroma Meter CR-400, Konica Minolta, NJ, USA). Readings were taken in triplicates and reported as L* (lightness), a* (redness), and b* (yellowness). Four measurements were taken per replicate, and the results were recorded for each of the three individual trials.

2.7 Fat Extraction

Samples were smeared onto the side of fat extraction thimbles, with the total amount not exceeding 4 g, to ensure maximum surface area for the extraction. The thimbles were dried at 60 °C to remove moisture until constant weights were obtained. The thimbles were then placed in the extraction apparatus. Approximately 200 ml petroleum ether was poured into the soxhlet flask, heat turned on, to allow a continual refluxes of petroleum ether, and samples extracted for 3-4 hrs. The petroleum ether was then evaporated in the oven.

2.8 Oil loss

Oil loss of pâtés were measured at 4°C (storage temperature) by placing triplicate sliced pâté (2 cm thick of about 60 g) sample on 5 filter papers (Whatman 4, 150 mm diameter, Whatman plc, Sigma Aldrich, Oakville, ON, CA) to absorb the oil. Samples were placed in an enclosed container to minimize moisture loss to the environment. Samples were weighed at 1, 2, 4, 6, and 24 hrs. Measurements were recorded in triplicate.
2.9 Sensory Analysis

Sensory analysis was performed by graduate and undergraduate students from the University of Guelph Food Science Department. The potential panelists were recruited, screened, and trained according to Meilgaard, Carr, & Civille, (2006). The sensory analysis consisted of three sessions including 16 trained panelists. Pâtés were cut into 2 cm cubes, placed inside 75 ml polystyrene cups which were labelled with a random 3-digit code. Panelists were seated in a sensory analysis laboratory in individual booths, with overhead red light to avoid visual bias. Pâté samples were served with a reference sample from the training session (the panelists were trained to use line scale with various food products), a glass of tap water, and unsalted soda crackers. Panelists were instructed to cleanse their palate in between samples. Panelists evaluated the textural properties of the pâtés for hardness, juiciness, oiliness, and cohesiveness. Samples were rated on a 15 cm line scale. Hardness: 0 = very soft, 15 = very hard; Juiciness: 0 = very dry, 15 = very juicy; Oiliness: 0 = not oily, 15 = very oily; Cohesiveness: 0 = not cohesive, 15 = very cohesive. Panelists were also trained to be familiar with potential off-flavors that might be picked up in the samples such as chemical, grassy, rancid, earthy, and cardboardy. Panelists were asked to note these off-flavours if picked up during tasting. Panelists were asked to attend three separate trials (weekly) as replicates.

2.10 Statistical Analysis

The experiment was designed as a complete randomized block, with three separate replications. Statistical analysis of the results was completed using Graphpad Prism 5.0 (GraphPad, San Diego, CA, USA). A one-way ANOVA test was done with a Tukey post test with (P<0.05). Data were graphed using Graphpad Prism 5.0 with error bars indicating standard error of the mean.
3. Results and Discussion

3.1 Texture and microstructure

The hardness values of pâtés tested at both temperatures were not significantly different (Fig. 5.1). However, at room temperature (RT), gradual increase in hardness was observed as more pork fat was replaced by organogel. This was expected since at this temperature, most of the saturated fats (higher in pâté made with more pork fat) were in liquid form which amplified the effect of organogel in increasing hardness to be more apparent (organogel remained in a solid gel form at both temperatures). The formulation used to make the organogel in this experiment was previously determined (12% EC and 3% GMS) to be acceptable to a sensory panel and to have similar effect on textural properties with pâté made with 100% lard. Based on the back extrusion results, up to 100% pork fat replacement was possible without changing the textural properties of the products, it will be discussed in more detail in the sensory analysis section. However, this experiment was conducted to also find a way to improve oil loss performance of the pâtés during storage which will be discussed in more detail in the next section. In addition, our instrumental and sensory hardness score showed similar trends. These results confirmed that the instrumental values obtained from the texture analyzer matched the panelists perception (Fig. 5.2).

When observed under the light microscope, the fat globules of pâtés made with 100% pork fat (T1) appeared to be smaller compared to those made with organogel (T2-T6; Fig. 5.3). The bigger globules shown in pâtés made with organogel could be attributed to the harder organogel texture, thus making them more resilient to shear during chopping. It was also observed that there was a gradual increase in the number of bigger fat globules as more pork fat was replaced with
organogel. The presence of more large fat globules could also be related to the increase in oil loss as bigger fat globules tend to be less stable and more vulnerable oil to oil separation.

Fig. 5.1 Hardness of pâtés tested at room temperature (22 °C) and 4 °C. Bars represent standard error of the means. n = 12.

a-b Bars with superscripts are significantly different (P < 0.05).
(T1: 100% lard, 0% organogel; T2: 80% lard, 20% organogel; T3: 60% lard, 40% organogel; T4: 40% lard, 60% organogel; T5: 20% lard, 80% organogel; T6: 0% lard, 100% organogel).

Fig. 5.2 Left figure shows hardness values of pâtés obtained from sensory analysis (0 = very soft; 15= very hard). Right figure shows hardness values of pâtés determined using texture analyzer. Both tests were performed at room temperature. n = 48 for the left figure and n = 16 for the right figure.
(T1: 100% lard, 0% organogel; T2: 80% lard, 20% organogel; T3: 60% lard, 40% organogel; T4: 40% lard, 60% organogel; T5: 20% lard, 80% organogel; T6: 0% lard, 100% organogel).
Fig. 5.3 Light micrographs of pâté samples (A = T1, B = T2, C = T3, D = T4, E = T5, F = T6). White areas represent fat globules that were removed during sample preparation for microscopy. Bar = 200 µm.

(T1: 100% lard, 0% organogel; T2: 80% lard, 20% organogel; T3: 60% lard, 40% organogel; T4: 40% lard, 60% organogel; T5: 20% lard, 80% organogel; T6: 0% lard, 100% organogel).
3.2 Sensory Analysis

Pâtés made with organogels (T2-T6) were perceived to have similar oiliness and cohesiveness compared to pâté made with 100% pork fat (Table 5.1). All pâtés that contained organogels were also perceived to be as hard as pâté made with 100% pork fat (T1), except for T2 which was perceived to be harder than T1. T1 pâté was perceived to be juicier compared to the other pâtés (T2-T6). This could be attributed to a higher amount of liquid unsaturated fat in pâté made with pork fat (35.02% pâté made with pork fat and 12.10% in organogels based pâté). As opposed to the pâtés made with organogel in which the vegetable oil would be bound to the EC network and less “mobile”, and hence perceived as drier.

As shown in Table 5.2, panelists were not able to perceive any difference in off-flavours between pâté samples in general. However, it was observed that pâtés containing organogels had slightly elevated chemical, grassy, and rancid smells compared to pâté made with 100% pork fats. Total perceived off flavours were also reported to be slightly higher in pâtés containing organogels. However, these differences were not statistically significant. Overall, the final results of this sensory analysis confirmed that full replacement as well as partial replacement of pork fat up to 80% could not be differentiated by the panelists in terms of hardness, juiciness, cohesiveness, and perceived off-flavours.

Table 5.1 Averages sensory analysis results of six test pâtés evaluated by 16 trained panels in three separate replicates.

<table>
<thead>
<tr>
<th></th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
<th>T6</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hardness</strong></td>
<td>5.852 ± 0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.542 ± 0.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.604 ± 0.25&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.573 ± 0.69&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.348 ± 0.10&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.146 ± 0.57&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Oiliness</strong></td>
<td>9.050 ± 0.17&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.350 ± 0.25&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.650 ± 0.15&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.448 ± 0.45&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.042 ± 0.53&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.494 ± 0.10&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Juiciness</strong></td>
<td>7.903 ± 0.17&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.321 ± 0.16&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.735 ± 0.09&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.163 ± 0.15&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.925 ± 0.27&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.513 ± 0.34&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Cohesiveness</strong></td>
<td>5.902 ± 0.17&lt;sup&gt;e&lt;/sup&gt;</td>
<td>5.058 ± 0.34&lt;sup&gt;e&lt;/sup&gt;</td>
<td>5.290 ± 0.25&lt;sup&gt;e&lt;/sup&gt;</td>
<td>5.163 ± 0.13&lt;sup&gt;e&lt;/sup&gt;</td>
<td>5.248 ± 0.18&lt;sup&gt;e&lt;/sup&gt;</td>
<td>5.529 ± 0.11&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a-e</sup> values followed by superscripts are significantly different (P < 0.05).

(T1: 100% lard, 0% organogel; T2: 80% lard, 20% organogel; T3: 60% lard, 40% organogel; T4: 40% lard, 60% organogel; T5: 20% lard, 80% organogel; T6: 0% lard, 100% organogel).
Table 5.2 Perceived off-flavours during sensory analysis of six test pâtés evaluated by 16 trained panels.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Grassy</th>
<th>Rancid</th>
<th>Earthy</th>
<th>Cardboardy</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T1</strong></td>
<td>0.33(^a)</td>
<td>0.00(^b)</td>
<td>1.00(^c)</td>
<td>2.33(^d)</td>
<td>1.33(^e)</td>
</tr>
<tr>
<td><strong>T2</strong></td>
<td>1.67(^a)</td>
<td>0.33(^b)</td>
<td>2.33(^c)</td>
<td>1.67(^d)</td>
<td>1.00(^e)</td>
</tr>
<tr>
<td><strong>T3</strong></td>
<td>1.67(^a)</td>
<td>1.00(^b)</td>
<td>3.00(^c)</td>
<td>1.67(^d)</td>
<td>1.33(^e)</td>
</tr>
<tr>
<td><strong>T4</strong></td>
<td>1.33(^a)</td>
<td>0.33(^b)</td>
<td>1.30(^c)</td>
<td>2.00(^d)</td>
<td>1.33(^e)</td>
</tr>
<tr>
<td><strong>T5</strong></td>
<td>2.00(^a)</td>
<td>1.00(^b)</td>
<td>2.33(^c)</td>
<td>2.33(^d)</td>
<td>1.00(^e)</td>
</tr>
<tr>
<td><strong>T6</strong></td>
<td>1.33(^a)</td>
<td>1.67(^b)</td>
<td>1.33(^c)</td>
<td>1.33(^d)</td>
<td>1.67(^e)</td>
</tr>
</tbody>
</table>

\(^a-f\) values followed by superscripts are significantly different (\(P < 0.05\))

(T1: 100% lard, 0% organogel; T2: 80% lard, 20% organogel; T3: 60% lard, 40% organogel; T4: 40% lard, 60% organogel; T5: 20% lard, 80% organogel; T6: 0% lard, 100% organogel).

3.3 Oil loss

As shown in Fig. 5.4, pâté made with more pork fat (T1, T2, T3, and T4) had the highest oil retention over the period of 24 hrs. This was expected as at storage temperature (4 °C), saturated fat in pâtés containing pork fat was mostly in solid form. On the other hand, pâtés made with more organogel showed higher oil loss over time. This could be related to the larger size of fat globules in organogel based pâtés that inevitably would lead to a more unstable emulsion that was more vulnerable to oil separation. It is also suspected that high enough pork fat in the pâté would work efficiently to help prevent oil separation from the larger, more unstable organogel fat globules. It was observed that with as high as 60% pork fat replacement, oil loss was considerably low and comparable to 100% pork fat.
Fig. 5.4 Oil loss of pâtés tested at 4 °C over 24 hrs period. Bars represent standard error of the means. n = 3.
(T1: 100% lard, 0% organogel; T2: 80% lard, 20% organogel; T3: 60% lard, 40% organogel; T4: 40% lard, 60% organogel; T5: 20% lard, 80% organogel; T6: 0% lard, 100% organogel).

3.4 Colour

As shown in Table 5.3, pâté made with 100% pork fat had the highest L* value (lightness). It was observed that the L* values decreased gradually as more pork fat was replaced with organogel. This was expected as gels made with ethylcellulose (EC) were more translucent compared to solid pork fat. In contrast to opaque pork fat, translucent materials such as organogels would not reflect as much light which translated into lower L* reading in pâté made with more organogel. This is in line with a work in progress by the author, as pâté made with organogels showed darker colour compared to pâté made with pork fat (Tien, Marangoni, & Barbut, 2017).

Moreover, as more organogels were added into the pâtés, the redness (a*) and yellowness (b*) values gradually increased. The increase in a* and b* values could also be connected to a more
translucent nature of the organogels, causing the meat colour to be more apparent. Note that L* and a* values started to increase significantly with as low as 20% pork fat replacement.

### Table 5.3 Colour analysis of pâtés reported as L* = lightness, a* = redness, b* = yellowness. n = 15.

<table>
<thead>
<tr>
<th></th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>60.86 ± 0.12a</td>
<td>13.32 ± 0.20fc</td>
<td>11.90 ± 0.03f</td>
</tr>
<tr>
<td>T2</td>
<td>56.36 ± 0.26b</td>
<td>14.98 ± 0.03d</td>
<td>12.06 ± 0.36c</td>
</tr>
<tr>
<td>T3</td>
<td>55.16 ± 0.53b</td>
<td>15.38 ± 0.07cd</td>
<td>12.36 ± 0.12c</td>
</tr>
<tr>
<td>T4</td>
<td>55.33 ± 0.42b</td>
<td>15.39 ± 0.00cd</td>
<td>12.76 ± 0.16c</td>
</tr>
<tr>
<td>T5</td>
<td>54.94 ± 0.64b</td>
<td>15.57 ± 0.01 systematically</td>
<td>12.84 ± 0.30c</td>
</tr>
<tr>
<td>T6</td>
<td>54.45 ± 0.45b</td>
<td>15.98 ± 0.11c</td>
<td>13.09 ± 0.04f</td>
</tr>
</tbody>
</table>

* a-g values followed by superscripts are significantly different (P < 0.05).

(T1: 100% lard, 0% organogel; T2: 80% lard, 20% organogel; T3: 60% lard, 40% organogel; T4: 40% lard, 60% organogel; T5: 20% lard, 80% organogel; T6: 0% lard, 100% organogel).

### 4. Conclusion

Overall, the final results of this sensory analysis confirmed that full replacement as well as partial replacement of pork fat up to 80% could not be differentiated from pâté made with 100% lard by the panelists in terms of hardness, oiliness, cohesiveness, and perceived off-flavours. When observed under the microscope, there was a gradual increase in the number of bigger fat globules as more pork fat was replaced with organogel. Based on the back extrusion results, up to 100% pork fat replacement was possible without changing the textural properties of the products. It was observed that with as high as 60% pork fat replacement, oil retention was comparable to 100% pork fat. Colour analysis results showed a gradual decrease in L* value and a gradual increase in both a* and b* values as more organogels were added to the pâté. To summarize, oil retention performance could be retained at 60% pork fat replacement while at the same time maintaining textural properties and having minimal effects on sensory properties and colour of the products.
5. References


CHAPTER 6 Conclusions and Future Works

In this work, it was discovered that pâtés tested at room temperature showed lower hardness values than those tested at refrigeration temperature due to difference in saturated fat compositions. The high hardness value of pâté D was connected to the presence of a secondary carbohydrate network due to significantly higher carbohydrate content in this particular pâté. Higher overall fat content increased the hardness of pâtés B, C, and E. SFC analysis of all pâtés confirmed higher hardness values as the SFC at 4°C was significantly higher than at 22°C. Higher melting TAGs in pâtés A, B, D, and E were responsible for the higher solid fat contents at both testing temperatures (4°C and 22°C). A higher unsaturated fatty acid content was correlated to a decreased hardness value of pâté C at room temperature (22°C). Pâtés with smaller fat globules showed higher hardness values (pâtés B, C, and E). No significant correlation was found between fat crystal polymorphic form and the hardness values of the pâtés investigated. β crystals were observed in all pâtés made with lard. β’ crystals were found in higher quantities in pâté E but were absent in the extracted fat. Pâté C showed a significantly lower first melting point when compared to the other pâtés as the amount of low melting TAGs were higher in this product. Lower crystallization temperatures were also observed in pâtés A, B, and E. Our study indicates that fat has a major impact on texture of pâté products. Understanding the factors that influence pâté textural properties is important in determining the formula to replace saturated fat in pâté.

Full replacement of saturated fat in liver pâté showed that 4 out of 5 pâtés made using organogels had the same sensory characteristics as the control pâté made with pork fat. Pâtés made with organogels also had a relatively low off-flavours perception. Pâtés made with organogel had similar hardness compared to those made with pork fat when tested at RT and 4 °C. Pâté made with EC only organogel (T1) had the highest oil loss over a period of 24 hrs.
organogels had bigger fat globule size compared to the canola oil only treatment. Pâtés made using organogels appeared to be darker, redder and yellower due to the translucent nature of the organogels. Overall, the pâté made with T4 (12% EC and 3% GMS) organogel showed the best performance in matching the control pâté made with pork fat while at the same time successfully reducing the saturated fat content by 62%.

Overall, the final results of partial replacement of saturated fat in liver pâté confirmed that fat replacement up to 100% could not be differentiated from pâté made with 100% lard by the panelists in terms of hardness, oiliness, cohesiveness, and perceived off-flavours. Based on the back extrusion results, full replacement as well as partial replacement of pork fat up to 80% were also possible without changing the textural properties of the products. It was observed that with as high as 60% pork fat replacement, oil retention performance was high and comparable to 100% pork fat. When observed under the microscope, there was a gradual increase in the number of bigger fat globules as more pork fat was replaced with organogel. Colour analysis results showed a gradual decrease in L* value and a gradual increase in both a* and b* values as more organogels were added to the pâté. To summarize, oil retention performance could be retained at 60% pork fat replacement while maintaining textural properties and having minimal effect on sensory properties and colour of the products.

In order to improve the quality of fat replaced pâté even further, there are other methods that could be employed. It would be interesting to see the effect of chopping time on the oil retention performance of the pâté, since further chopping might decrease the large fat globules found in fat replaced pâtés and hence decrease oil separation during storage. Moreover, there are other organogelators that could be used to improve the oil retention capacity of the fat replaced pâté. Although, some of these gelators are not food grade at the moment, gelators such as stearic
acid and stearyl alcohol (SOSA) and mono-and diglycerides can be used to replace saturated fat while at the same time maintaining the plasticity and oil retention capabilities of saturated fat.

In conclusion, the fatty acid composition of the fat can be very important in dictating the final texture of the finished pâté products. Temperature also plays an important role in texture in high fat content products such as pâté. However, polymorphism of the fat within the pâté did not have significant influence on the hardness of the pâté. It was also concluded that saturated fat replacement using canola oil organogels was possible and showed positive results in most tested parameters except for oil retention. Partial replacement of up to 60% saturated fat in pâté solved the oil retention problem.
CHAPTER 7 Appendix I

7.1 Pate Sensory Analysis Session # 1

STUDY CODE: ____________________________ DATE: ____________

On the scale below, please evaluate samples. Place a mark on the line with corresponding sample number writer over it. The cup marked R is the reference sample that was used during training.

Sample numbers: R, 121, 697, 518, 968, 735, 867, 208

1. Hardness

Very soft R = 5.7 Very hard

[1_________________________1______________________________15]

2. Oiliness

Not oily R = 9.4 Very oily

[1_________________________1______________________________15]

3. Juiciness

Not juicy R = 8.0 Very juicy

[1_________________________1______________________________15]

4. Cohesiveness

Not cohesive R = 5.3 Very cohesive

[1_________________________1______________________________15]

5. Off-flavour

Please write down off flavour(s) note that is perceived in each sample (the potential off flavours are chemical, grassy, rancid, earthy, and cardboardy). You can write down more than one off-flavour per sample. It is acceptable to leave it blank if there is no off-flavour perceived.

Sample #121

Sample #697

Sample #518

Sample #968

Sample #735

Sample #867

Sample #208
7.2 Pate Sensory Analysis Part 2 Session # 2

STUDY CODE: ____________________________ DATE: ____________

On the scale below, please evaluate samples. Place a mark on the line with corresponding sample number writer over it. The cup marked R is the reference sample that was used during training.

Sample numbers: R, 121, 697, 518, 968, 735, 867

6. Hardness

Very soft  R = 5.7  Very hard

[1____________________ _______________ 15]

7. Oiliness

Not oily  R = 9.4  Very oily

[1____________________ _______________ 15]

8. Juiciness

Not juicy  R = 8.0  Very juicy

[1____________________ _______________ 15]

9. Cohesiveness

Not cohesive  R = 5.3  Very cohesive

[1____________________ _______________ 15]

10. Off-flavour

Please write down off flavour(s) note that is perceived in each sample (the potential off flavours are chemical, grassy, rancid, earthy, and cardboardy). You can write down more than one off-flavour per sample. It is acceptable to leave it blank if there is no off-flavour perceived.

Sample #121
Sample #697
Sample #518
Sample #968
Sample #735
Sample #867

Thank you for your participation!
7.3 Pate Sensory Panel Training Form

STUDY CODE: ____________________________ DATE: ____________

Please mark x for pâté on the line scale for question 1 to 5. Pâté sample should fall somewhere in between the two anchor points. Please wait for the researcher’s instructions to start the training.

11. Hardness: Whipped cream, frankfurter, pâté

<table>
<thead>
<tr>
<th>Whipped cream</th>
<th>Frankfurter</th>
</tr>
</thead>
<tbody>
<tr>
<td>[x______________________________x]</td>
<td></td>
</tr>
</tbody>
</table>

12. Oiliness: Bread cube, bread cube with olive oil, pâté

<table>
<thead>
<tr>
<th>Bread cube</th>
<th>Bread cube w/ oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>[x______________________________x]</td>
<td></td>
</tr>
</tbody>
</table>

13. Juiciness: Cracker, ham, pâté

<table>
<thead>
<tr>
<th>Cracker</th>
<th>Ham</th>
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<tbody>
<tr>
<td>[x______________________________x]</td>
<td></td>
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</tbody>
</table>

14. Cohesiveness: Muffin, dried fruit, pâté

<table>
<thead>
<tr>
<th>Muffin</th>
<th>dried fruit</th>
</tr>
</thead>
<tbody>
<tr>
<td>[x______________________________x]</td>
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Odour test

15. Please smell all five cups in the order given with a 10 seconds interval in between samples and describe what smells are perceived. The smells are chemical, grassy, rancid, earthy, and old cardboard.

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<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
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<table>
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<tr>
<th>D</th>
<th>E</th>
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<tbody>
<tr>
<td>[________]</td>
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</tr>
</tbody>
</table>
7.4 Screening questionnaire I

STUDY CODE: __________________________ DATE: __________

Please answer the questions by marking a checkmark for each question in the spaces provided (one checkmark for one question).

1. How often do you consume meat products?
   - Very rare
   - Very often

2. How often do you consume pork?
   - Very rare
   - Very often

3. How would you rate your sensory evaluation skills?
   - 1
   - 2
   - 3
   - 4
   - 5

4. How sensitive are you to smell?
   - Very insensitive
   - Very sensitive

5. Are there any weekdays (M-F) that you will not be available on a regular basis?

6. Is your sensitivity to textural characteristics in foods
   a. Better than average
   b. Average
   c. Worse than average

7. Do you have any known food allergies?
   - Yes
   - No

Please describe allergy:

Note: If participants are not eligible to participate in the study, this screening questionnaire will be destroyed.
7.5 Screening questionnaire II

STUDY CODE: ____________________________ DATE: __________

1. Sucrose solutions
Please taste the samples and write down the code of the sample that is different from the others.

Why________________________________________________________

2. NaCl solutions
Please taste the samples and write down the code of the sample that is different from the others.

Why________________________________________________________

3. Citric acid solutions
Please taste the samples and write down the code of the sample that is different from the others.

Why________________________________________________________

4. Pâté
Please taste the samples and write down the code of the sample that has a coarser texture.

5. Cream cheese
Please taste the samples and write down the code for the sample that is more fatty.

6. Soybean oil
Please smell the samples and write down the code for the sample that is oxidized.
7.6 Letter of Information

Addition of Canola Oil Gels to the Flavour and Textural Properties of Pâté Study

The research proposal deals with the reduction of saturated fat in meat products and examining the sensory and stability qualities of pork liver pâté produced with canola oil based gels as a full or partial replacement for animal fat. The meat provided for this study is obtained from the University of Guelph Meat Laboratory Guelph. Also known as the Canadian Food Inspection Agency (CFIA) Establishment #183, a federally inspected, Hazard Analysis and Critical Control Point (HACCP) approved slaughter and meat processing facility on campus. The products will also be prepared at the Federally inspected plant. A taste panel will evaluate the meat products for texture, flavour, mouthfeel, off-flavours, and overall acceptability. The goals of this research project are to reduce saturated fat in meat products and develop products with highly acceptable sensory qualities.

The liver pâté consists of various commonly used ingredients for sausage making.

Ingredients list:

Pork liver, pork fat, pork trim, water, canola oil, salt, sodium nitrites, sodium phosphates, liverwurst seasoning, roasted onions, powdered whole milk, sugar, ethylcellulose, surfactants (e.g. glycerol mono-stearate, sorbitan mono-stearate.)

If you have certain allergies or sensitivity to any of the ingredient(s) please inform one of the researchers.
7.7 Consent Form

Addition of Canola Oil Gels to the Flavour and Textural Properties of Pâté Study

The purpose of this letter is to provide you with the information you require to make an informed decision on participating in this research. The goal of the research project is to understand the effect of adding canola oil gels to texture, flavour, and overall acceptability of pâté products.

I am a Masters student in the Department of Food Science at the University of Guelph and the information I am collecting will be used in my thesis. This project is funded by Ontario Ministry of Agriculture, Food, and Rural Affairs (OMAFRA).

While there are no direct benefits for participants, your contribution will have a direct effect on the development of the new pâté products. We are looking for people who consume processed meat products regularly and have an understanding about sensory qualities of food. People with allergies to ingredients listed in the information letter should not participate in the studies.

Although the products are cooked to the minimum temperature of 72°C and handled properly, you should be aware that there are certain risks in taking part of this study (e.g. Allergy reactions and stomach upset). As with all ingestion of food, there is always a risk of choking.

We expect to have a total of 14 sessions including training and recruitment over the course of 6 months. Each session will take approximately about 30 minutes of your time. You will be invited to join a dozen other people at the sensory lab in the Food Science building. The researchers will introduce the topic and explain the procedures of the testing to the participants.

Participants will be asked to consume approximately half a dozen pâté products per session (approximately 10 g each). Water and crackers will be provided to cleanse palate.

Participants will be compensated at $7 per session. The Participants will also be given a candy and a pop after each session is finished. Participants will be asked to sign a receipt of payment. Cash payments will be provided at the end of the study.

Participation in this study is voluntary. You may decline to participate, decline to answer any questions or withdraw from the study at any time with no consequences.

If you have already completed and submitted the survey, and you change your mind about taking part, you can contact the researcher and ask to have your data removed. Your data will be anonymized (removal of your name) after 1 year. Any time prior to this time, you can request to have the data destroyed. After anonymization we will not be able to tell which data is yours and you will no longer be able to withdraw.

The faculty advisor, and a graduate student will be the only individuals who can access the information you provide. The information you provide will be stored on
password protected laboratory computers, which can be accessed only by qualified laboratory personnel under the supervision of the faculty advisor.

If the results of the study are published, your name will not be used and no information that discloses your identity will be released or published. If the research is successful, there might be a potential commercialization of this type of product. Outcomes of the research will be published in the scientific literatures and food industries will have access and will be able to commercialize this product.

If you chose to, you will be notified when the results of the study are published. You can also get a copy of the results by contacting the researchers.

You do not waive any legal rights by agreeing to take part in this study.

This project has been reviewed by the Research Ethics Board for compliance with federal guidelines for research involving human participants.

If you have questions regarding your rights and welfare as a research participant in this study (REB#16JN024), please contact: Director, Research Ethics; University of Guelph; reb@uoguelph.ca; (519) 824-4120 (ext. 56606)

If you have any question about the study, please contact:

**Brian Tiensa, Master’s student**, Department of Food Science, University of Guelph, btiensa@uoguelph.ca; (647)222-8468

**Shai Barbut, Professor**, Department of Food Science, University of Guelph, sbarbut@uoguelph.ca; X53669

**Alejandro Marangoni, Professor**, Department of Food Science, University of Guelph, amrango@uoguelph.ca; X54340

<table>
<thead>
<tr>
<th>Participant Name</th>
<th>Witness</th>
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<tr>
<td>_________________</td>
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<tr>
<th>Participant Signature</th>
<th>Date</th>
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CHAPTER 8 Appendix II

Research Note

Texture Improvement in Organogel Based Liver Pâté Using Alternate Gelators and Chopping Time Extension

Brian E. Tiensa, Alejandro G. Marangoni, & Shai Barbut
Department of Food Science, University of Guelph, Guelph, ON, Canada, N1G 2W1
E-mail: amarango@uoguelph.ca, sbarbut@uoguelph.ca

Abstract

Four different organogels formulations were added and compared to pâté made with 100% pork fat. Five different chopping times were employed to see the effect of chopping time on pâté texture. All four treatment pâtés showed similar hardness values as control pâté made with pork fat. Prolong chopping of pâté batter did not show any effect on the hardness of the final products.

Fat globules of pâté made with 100% pork fat had similar globules size compared to organogel based pâtés containing SOSA as well as mono and di-glycerides. On the other hand, extension of chopping time did not reduce the size of the fat globules. Pâtés made with SOSA and mono and di-glycerides showed the best oil retention performance amongst organogel based pâtés and were comparable to pâté made with 100% pork fat. In contrast, prolonged chopping of the pâté batter did not improve oil loss performance. Pâté made with 100% pork fat had similar L* value (lightness) to pâtés made with organogels high in SOSA and mono and di-glycerides. To summarize, addition of SOSA as well as mono and di-glycerides to organogels resulted in pâtés with smaller fat globules, better oil retention, and better colour.

1. Introduction

Trans and saturated fats have been connected to various cardiovascular problems. On the other hands, trans and saturated fats are known to have a positive contribution to the structure and texture of food products. Different governmental institutions, especially in North America and
Europe, have suggested reducing the consumption of saturated and trans fatty acids in our diet, and have encouraged people to switch to a diet with higher amount of polyunsaturated fats (WHO, 2013). The challenge with polyunsaturated fats is that they lack the ability to form solid structures at room temperature. Consequently, they can negatively affect textural and sensory properties of food products. The use of ethylcellulose to create organogels could help structure vegetable oils and has been shown to have a great potential in food industry (Rogers et al., 2014; Stortz & Marangoni, 2013; Stortz et al., 2012). There are various known applications for organogels, ranging from stabilizing emulsions, slow release of bioactive components, as well as replacement of saturated and trans fatty acids. By trapping the liquid oil in a gel network, the resulting products can be as firmed at room temperature as saturated fat containing products (Stortz et al., 2012). As a result, these gel structures can be utilized to mimic the properties of solid fat while at the same time maintaining the nutritional properties of the healthier unsaturated fatty acids (Gravelle et al., 2012). Recent studies have demonstrated the use of organogels in food products, such as emulsified meat products, heat resistant chocolate, product with controlled release nutraceuticals, pharmaceuticals, and various kind of water in oil emulsions (Hughes, Marangoni, Wright, Rogers, & Rush, 2009).

In the previous study, the authors reported on the full replacement of pork fat with different organogels preparation in pâté products. It was reported that the use of ethylcellulose based organogels to reduce saturated fat content in pâté yielded positive sensory analysis results. However, full replacement of pork fat with EC based organogels resulted in pâtés that were more vulnerable to oil separation. In this current study, the authors explored alternative organogelators (i.e. mono and di-glycerides, stearic acid-stearyl alcohol combinations or SOSA) and alternative processing method (i.e. chopping time extension) to solve the low oil retention problem as well as
to improve sensory and textural properties of organogel based pâté even further.

2. Material and Methods

2.1 Organogel preparation

Organogels were prepared in an oven at a temperature of 140 °C following Gravelle, Barbut, and Marangoni, (2012). Ethylcellulose (EC) with a viscosity of 20 cP (Ethocel™ 20, Dow Chemical, Midland, MI, USA), canola oil (Hela Spice Canada, Uxbridge, ON, CA), glycerol monostearate (GMS), mono-diglycerides (Danisco, Dupont, Mississauga, ON, Canada), stearyl alcohol (st-OH, or SO; 1-octadecanol; 95% purity, Acros Organics, Fisher Scientific, Ottawa, ON, Canada), and stearic acid (st-acid or SA; 1-octadecanoic acid; 97% purity, Acros Organics, Fisher Scientific, Ottawa, ON, Canada) were used to make the organogels. Briefly, gels were prepared in glass beakers in a bench-top gravity convection oven (Fischer Scientific, Ottawa, ON, Canada) set to 170 °C with constant mixing using an overhead mechanical stirrer (Model L1U10F, Lightnin LabMaster, Wytheville, VA, USA) fitted with a high-shear impeller which was inserted through the roof of the oven, rotating at 175 rpm. The gels reached the target temperature within approximately 50 min, followed by 10 min holding period. Each gel was taken out and cooled down on a tempering table set at 20 °C for approximately 20 min. Each batch was then cut into 2 cm x 2 cm squares and stored at 5 °C overnight. For this experiment, one control (T1) was prepared with the traditional pork back fat and 4 treatments were prepared with canola oil (T2 – T5 with canola oil organogels). The 5 treatments consisted of one control consisting of pork back fat (T1) and organogels made with 12% EC, 3% GMS (T2); 20% mono and di-glycerides (T3); 6% EC, 8% Sosa (T4); 9% EC, 3% Sosa (T5), respectively. For chopping time experiment, 12% EC, 3% GMS (successful formulation used in previous study) formulation in used.
2.2 Pâté preparation

For each treatment, 700 g of partially frozen pork liver (28%) was cut at high speed setting for 2 min in a bowl chopper (Feuma Gastromaschinen GmbH, model no. 15L, Gößnitz, Thüringen, Germany). Dry ingredients such as 1.4 % Salt, 0.3 % phosphates mixture (Helabin Ultimal), 0.3% curing salts mixture (Rapid Cure), 0.6% pâté seasoning (Calf Liverwurst Seasoning), 0.5 % roasted onions, 1% powdered whole milk, and 0.8% sugar (Hela Spice Canada) were added into the chopper, and the mixture was cut at low speed for 45 sec. Pork trimmings (80/20 muscle/fat; 20%), pork back fat (20%), pork ham fat (20%), and pork jowls (9%) were cooked at 80°C for 1 hr in a steam jacketed kettle. The bowl chopper was then preheated to ~50°C using hot water (3%). Cooked pork jowls and pork trims were added and chopped at low speed for 40 sec, and cooked ham fat and back fat (or canola oil/ organogels for control II and T1-T5) were subsequently added and the mixture was chopped for an additional 50 sec. A spice mix (Hela Spice Canada) was then added to the cutting bowl and chopped at high speed for 1 min. The precut pork liver was then added and cut at high speed for 90 sec. For chopping time experiment, pâté batter was chopped at an extended period of time before stuffing (0 min: T1, 2.5 min: T2, 5 min: T3, 10 min: T4, and 15 min: T5). The resulting meat batter was then stuffed using an automatic stuffer (Mainca EB-25, St Louis, MO, USA) into sausage casings (PVDC inside coated fibrous cellulose casing; cal. 60x60; Canada Compound Corp., Woodbridge, Ontario, Canada) and were cooked in a kettle/ hot water bath at 80°C to an internal temperature of 72°C (~2 hrs). Cooked sausages were then cooled in an ice water bath and refrigerated prior to evaluation.
2.3 Back Extrusion

The samples were stuffed into polypropylene test tubes (27 mm diameter) and kept in the refrigerator (4 °C) overnight to allow them to set. The following day, half of the samples were tested directly out of the refrigerator while the other samples were allowed to equilibrate at room temperature (22 °C) for 2 hrs. Back extrusion tests were performed using a texture analyzer (Texture Technologies Corp., Model TA.XT2, Scarsdale, NY, USA). A stainless steel cylindrical probe (height: 9.8 cm, diameter: 1.8 cm) with enlarged spherical tip (height: 1.3 cm, diameter: 2.0 cm) was used to penetrate the product, in the test tubes, for 30 mm at a rate of 1.5 mm/s. Results were recorded as described by Gravelle et al. (2014). Four tubes were analyzed per replicate, and the results were recorded for each of the three individual trials.

2.4 Fatty Acid Composition

The fatty acid composition of extracted pâté fats were determined using gas chromatography (Ghazani et al., 2013). Briefly, samples were subjected to a transmethylation procedure in accordance with Christie, (1982). Fatty acid methyl esters (FAME) were analyzed by using a capillary GC equipped with a BPX70 column, 60 m × 0.22 mm internal diameter and with 0.25 µm film thickness (SGE Inc., Austin, TX, USA). A gas chromatograph (Agilent 6890 Series, Agilent Technologies, Inc., Wilmington, DE, USA) with an auto sampler (7,683 series) was used. The oven temperature was programmed to increase from 110 to 230 °C at a rate of 4 °C/min and held at 230 °C for 10 min. The injector and detector temperatures were 240 and 280 °C, respectively. Hydrogen was used as the carrier gas at an average velocity of 25 cm/s. Peaks were identified via comparison to FAME standards. Samples were measured in triplicates.
Sample were prepared for light microscopy following the method used by Barbut et al., (2016). Briefly samples (20 × 20 × 5 mm), were cut from the center part of the liver sausages and then fixed and stained using Masson stain. Slides were observed using a light microscope (Model BX60, Olympus Optical Ltd., Tokyo, Japan) and images were captured using a computerized image analysis system (Image-Pro Plus, Version 5.1, Media Cybernetics Inc., Silver Spring, MD).

Colour of the freshly cut pâté samples was analyzed by a colorimeter (Chroma Meter CR-400, Konica Minolta, NJ, USA). Readings were taken in triplicates and reported as L* (lightness), a* (redness), and b* (yellowness). Four measurements were taken per replicate, and the results were recorded for each of the three individual trials.

Samples were smeared onto the side of fat extraction thimbles, with the total amount not exceeding 4 g, to ensure maximum surface area for the extraction. The thimbles were dried at 60 °C to remove moisture until constant weights were obtained. The thimbles were then placed in the extraction apparatus. Approximately 200 ml petroleum ether was poured into the soxhlet flask, heat turned on, to allow a continual refluxes of petroleum ether, and samples extracted for 3-4 hrs. The petroleum ether was then evaporated in the oven.

Oil loss of pâtés were measured at 4°C (storage temperature) by placing triplicate sliced pâté (2 cm thick of about 60 g) sample on 5 filter papers (Whatman 4, 150 mm diameter, Whatman plc, Sigma Aldrich, Oakville, ON, CA) to absorb the oil. Samples were placed in an enclosed
container to minimize moisture loss to the environment. Samples were weighed at 1, 2, 4, 6, and 24 hrs. Measurements were recorded in triplicate.

2.9 Statistical Analysis

The experiment was designed as a complete randomized block, with three separate replications. Statistical analysis of the results was completed using Graphpad Prism 5.0 (GraphPad, San Diego, CA, USA). A one-way ANOVA test was done with a Tukey post test with (P<0.05). Data were graphed using Graphpad Prism 5.0 with error bars indicating standard error of the mean.

3. Results and Discussion

3.1 Texture analysis

Fig. 8.1 shows difference in hardness between various pâtés made with alternate gelators. T1 – T4 showed drastic changes in hardness at the two test temperatures. However, T3 showed minimal change in hardness at the two test temperatures. T1 – T4 hardness values are in agreement with previous finding, the drastic change in hardness was mostly caused by the change of solid fat content at the 2 different temperature points (Tiensa et al., 2017). All treatment pâtés showed similar hardness values as control pâté made with pork fat (T1). However, in this current study, the authors attempted to improve oil retention performance of the treatment pâtés which will be discussed in more detail in the next section.

Fig 8.2 shows difference in hardness of pâtés chopped at different times (0 min: T1, 2.5 min: T2, 5 min: T3, 10 min: T4, and 15 min: T5). Prolong chopping of pâté batter did not show any effect on the hardness of the products both at room temperature (RT) and at 4°C.
Fig. 8.1 Hardness of pâtes made with alternate gelators tested at room temperature (22 °C) and at 4 °C. Bars represent standard error of the mean. n = 9.
Pork fat control (T1); 12% EC, 3% GMS (T2); 20% mono and di-glycerides (T3); 6% EC, 8% SOSA (T4); 9% EC, 3% SOSA (T5).

Fig. 8.2 Hardness of pâtes chopped for 0 min: T1, 2.5 min: T2, 5 min: T3, 10 min: T4, and 15 min: T5 tested at room temperature (22 °C) and at 4 °C. Bars represent standard error of the mean. n = 9.
3.2 Microstructure

When observed under the light microscope, the fat globules of pâté made with 100% pork fat (T1) showed similar globules size compared to pâtés containing SOSA as well as mono and di-glycerides organogels (T3 and T4; Fig. 8.3). However, pâtés made with high EC organogels (T2 and T5) showed higher fat globules size. This is in line with the previous study done by Tiensa et al. (2017). The bigger globules shown in pâtés made with EC based organogel could be attributed to the harder organogel texture, thus making them more resilient to shear during chopping. The presence of more large fat globules could also be related to the increase in oil loss as bigger fat globules tend to be less stable and more vulnerable oil to oil separation. It is suspected that the more pseudoplastic property of organogels made with SOSA and mono and di-glycerides behaved more similarly to pork fat during chopping, resulting in organogels that is less resilient to shear force and led to the formation of smaller and more stable fat globules in the final products. Fig 8.4 shows images of fat globules in pâtés chopped at different time points observed under light
microscope. It was observed that extension of the chopping time did not reduce the size of the fat globules. This was reflected in high oil loss performance in all pâtés.

Fig. 8.3 Light micrographs of pâté samples (A = T1, B = T2, C = T3, D = T4, E = T5, F = T6). White areas represent fat globules that were removed during sample preparation for microscopy. Bar = 100 µm.
Pork fat control (T1); 12% EC, 3% GMS (T2); 20% mono and di-glycerides (T3); 6% EC, 8% SOSA (T4); 9% EC, 3% SOSA (T5).
Fig. 8.4 Light micrographs of pâté samples (A = T1, B = T2, C = T3, D = T4, E = T5, F = T6). White areas represent fat globules that were removed during sample preparation for microscopy. Bar = 100 µm.

Pâtés chopped for 0 min: T1, 2.5 min: T2, 5 min: T3, 10 min: T4, and 15 min: T5.
3.3 Oil loss

Oil loss performance in pâtés made with alternate gelators is in line with the results observed in the microstructure section. As observed in Fig 8.5, T3 and T4 showed the best oil retention performance amongst organogel based pâtés and are comparable to pâté made with 100% pork fat. However, pâtés made with high ethylcellulose content (T2 and T5) have a considerably higher oil loss compared to the other three pâtés. This was expected as T2 and T5 pâtés had larger fat globules size making them more vulnerable to oil separation. Fig 8.6 showed oil loss performance of pâtés chopped at different time points. In contrast with a study done in meat batter by Barbut (1988), prolong chopping of the pâté batter did not improve oil loss performance. In summary, addition of SOSA as well as mono and di-glycerides showed promising results in improving oil retention performance of organogels based pâtés.
**Fig. 8.5** Oil loss of pâtés tested at 4 °C over 24 hrs period. Bars represent standard error of the means. n = 3.

Pork fat control (T1); 12% EC, 3% GMS (T2); 20% mono and di-glycerides (T3); 6% EC, 8% SOSA (T4); 9% EC, 3% SOSA (T5).
Fig. 8.6 Oil loss of pâtés chopped for 0 min: T1, 2.5 min: T2, 5 min: T3, 10 min: T4, and 15 min: T5 tested at 4 °C over 24 hrs period. Bars represent standard error of the means. n = 3.

3.4 Colour

As shown in Table 8.1, pâté made with 100% pork fat had similar L* value (lightness) to pâtés made with organogels high in SOSA and mono and di-glycerides. This was expected as SOSA and mono and di-glycerides organogels had similar opaque colour as pork fat when cooled/crystalized. However, pâtés made with high EC content organogels showed lower L values (T2 and T5). This was expected as gels made with ethylcellulose (EC) were more translucent compared to solid pork fat. This is in line with a work in progress by the author, as pâté made with organogels showed darker colour compared to pâté made with pork fat (Tiensa et al., 2017). Moreover, T2 and T5 pâtés had higher redness value (a*). The increase in a* value could also be connected to a more translucent nature of the organogels, causing the meat colour to be more...
apparent. Lastly, all pâtés had similar b* value (yellowness).

Table 8.1 Colour analysis of pâtés made with alternate gelators reported as L* = lightness, a* = redness, b* = yellowness. n = 15.

<table>
<thead>
<tr>
<th></th>
<th>L</th>
<th>a*</th>
<th>b*</th>
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<tbody>
<tr>
<td>T1</td>
<td>61.31 ± 0.48</td>
<td>12.95 ± 0.13</td>
<td>12.90 ± 0.23</td>
</tr>
<tr>
<td>T2</td>
<td>55.84 ± 0.38</td>
<td>14.62 ± 0.10</td>
<td>13.63 ± 0.51</td>
</tr>
<tr>
<td>T3</td>
<td>60.34 ± 0.49</td>
<td>13.03 ± 0.18</td>
<td>13.87 ± 0.25</td>
</tr>
<tr>
<td>T4</td>
<td>61.22 ± 0.47</td>
<td>12.69 ± 0.06</td>
<td>13.50 ± 1.31</td>
</tr>
<tr>
<td>T5</td>
<td>56.68 ± 1.21</td>
<td>14.47 ± 0.14</td>
<td>13.83 ± 0.07</td>
</tr>
</tbody>
</table>

Pork fat control (T1); 12% EC, 3% GMS (T2); 20% mono and di-glycerides (T3); 6% EC, 8% SOSA (T4); 9% EC, 3% SOSA (T5).

4. Conclusion

All treatment pâtés showed similar hardness values as control pâté made with pork fat (T1).

Prolong chopping of pâté batter did not show any effect on the hardness of the products both at room temperature (RT) and at 4°C. When observed under the light microscope, the fat globules of pâté made with 100% pork fat (T1) had similar globules size compared to pâtés containing SOSA as well as mono and di-glycerides organogels (T3 and T4). However, it was observed that extension of the chopping time did not reduce the size of the fat globules. T3 and T4 showed the best oil retention performance amongst organogel based pâtés and are comparable to pâté made with 100% pork fat. Prolong chopping of the pâté batter did not improve oil loss performance. Finally, pâté made with 100% pork fat had similar L* value (lightness) to pâtés made with organogels high in SOSA and mono and di-glycerides. In conclusion, addition of SOSA as well as mono and di-glycerides to organogels resulted in pâtés with smaller fat globules, better oil retention, and better colour. However, chopping time extension did not affect hardness, oil loss performance, and fat globules size.
5. References


