Novel biphasic gels can mimic and replace animal fat in fully-cooked coarse-ground sausage

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ABSTRACT

Four biphasic gels (BPG) were developed and tested as pork fat replacers in coarse-ground fully-cooked sausages. An oleogel (OG) phase (92.5% high-oleic soybean oil, 7.5% rice bran wax) and one of two hydrogel (HG) phases (water and 7% or 8% gelatin) were combined in 7:3 or 6:4 OG:HG ratios, for a total of four test formulations. Control sausages were formulated to 27.5% fat and stored at 0 – 2 °C for 98 d. BPGs allowed for fat reductions of up to 26%. Visually, all BPGs resembled pork fat. There were no differences in external L* and a* but, internally, controls were darker and redder. Except for one control, there were no differences in Texture Profile Analysis (TPA) hardness, cohesiveness, springiness, and chewiness. Warner-Bratzler Shear (WBS) force was highest in 6:4 samples, which were also highest in Sensory First Bite Firmness and lowest in Smoked Sausage Aroma and Smoked Sausage Flavor. TBARS values remained steady, with no rancid flavors detected by the sensory panel.

1. Introduction

In order to maintain a healthy dietary pattern, the World Health Organization (WHO) recommends limiting intake of total fat to <30%, and of saturated fat to <10%, of total energy intake (WHO, 2020). The Dietary Guidelines for Americans (USDHHS/USDA, 2020) offer similar recommendations on saturated fats and suggest several strategies to achieve this target, such as (1) “reading food labels to choose packaged foods lower in saturated fats” and (2) “cook[ing] and purchas[ing] products made with oils higher in polyunsaturated and mono-unsaturated fat (e.g., canola, corn, olive, peanut, safflower, soybean, and sunflower) rather than butter, shortening, or coconut or palm oils.”

From a functional perspective, however, animal fat is essential in many sausage products due to their important contributions to texture, mouthfeel, flavor and juiciness (Barbut, 2011), which result primarily from their relatively high proportion of saturated fatty acids and the presence of important meat flavor compounds (Breuer, 2010). Replacing them is, therefore, challenging for the processed meat industry. Most non-animal food lipids are mixtures of triacylglycerols containing fatty acids of varying chain length and degree of saturation. The specific mixture of fatty acids, and their position on the glycerol backbones, determine the physicochemical and rheological properties of the lipids—including their melting and crystallization temperatures—and, hence, their functional properties in food systems and during processing (O’Brien, 2009). The fact that they are composed mostly of triacylglycerols also gives them a more homogenous character and makes their behavior easier to predict and manipulate. Animal fat, on the other hand, is a much more complex material. It is primarily derived from adipose tissue, which constitutes a network of monoglobular fat cells (adipocytes) surrounded and connected by a mesh of connective tissue make up mostly of collagen. Although the lipid portion of adipose tissue is also composed mostly of triacylglycerols, the overall complexity of its structure allows it to exhibit elastic deformation behavior (Comley & Fleck, 2010) at shear stress values typical of many food processing conditions, such as grinding and mixing. For this reason, the many strategies attempted over the years to reduce saturated fats in high-fat processed meat products have encountered significant challenges. Research on replacement of animal fat with water and combinations of water and water-binders has historically met with varying degrees of success, in many cases resulting in changes in hardness, mouthfeel, creaminess and juiciness, shorter shelf-life or higher cooking losses (Claus & Hunt, 1991; Keeton, 1994; Troutt et al., 1992a; Troutt et al., 1992b; Troutt et al., 1992c).

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and easily-deformed to be of use in other important meat product applications, they are limited by the fact that they are too plastic to be used in ground products such as bratwurst, chorizo, pepperoni, mortadella, salami, and ground mustard, garlic powder, monosodium glutamate, and liver.

As a potential solution to this challenge, we investigated the novel use of biphasic gel (BPG) formulations made with a high-oleic soybean oil (HOSO)/rice bran wax (RBW) oleogel (OG) phase and a water/gelatin hydrogel (HG) phase, when used as complete pork fat replacers in coarse-ground sausages. It was hypothesized that these biphasic gels would provide processing characteristics similar to animal fat, and physical and sensory attributes similar to those of a similar product made with pork fat. HOSO was chosen as the lipid source, as high-oleic vegetable oils have been shown to reduce cardiovascular disease risk factors, by decreasing levels of total cholesterol, LDL cholesterol, and apolipoprotein B (apoB), while not changing levels of HDL cholesterol, triglycerides and apoA (Bowen et al., 2019; Huth, Fulgoni III, & Larson, 2015). This study constitutes the first time BPGs have been researched as total fat replacements in a coarse-ground meat product of any kind.

### Materials and methods

#### 2.1. Sample preparation

##### 2.1.1. Raw materials and formulations

Trimmed pork knuckles (PKN) (v. intermedium, v. lateralis, v. medialis), pork picnic trimmings (PPT), and pork regular trimmings (PRT) were obtained from a commercial pork processing plant. Pork back fat (PBF) was obtained from the Iowa State University Meat Laboratory (Ames, IA, USA); high-oleic soybean oil (HOSO; Plenish® brand) from Corteva Agriscience (Johnston, IA, USA); rice bran wax (RBW) from Koster Keunen, Inc. (Watertown, CT, USA); 250 bloom pork skin gelatin (PSG) from Nitta Gelatin NA, Inc. (Morrisville, NC, USA); sodium phosphates (Bifisol 414 blend) from BK Giulini GmbH (Ladenburg, Germany); dried vinegar (Verdad® Powder N6, Corbion, Lenexa, KS, USA); and salt, seasoning blend (corn syrup solids, dextrose, ground black pepper, monosodium glutamate, ground mustard, garlic powder, ground ginger), curing salt (Koch, 2010; Youssef et al., 2016; Rehman et al., 2014; Satapathy et al., 2015), but there are limited reports on their use as fat replacers in food or meat products (Ghiasi & Golmanki, 2022).

The purpose of this study was to manufacture and evaluate the performance of four BPG formulations made with a high-oleic soybean oil (HOSO)/rice bran wax (RBW) oleogel (OG) phase and a water/gelatin hydrogel (HG) phase, when used as complete pork fat replacers in coarse-ground sausages. It was hypothesized that these biphasic gels would provide processing characteristics similar to animal fat, and physical and sensory attributes similar to those of a similar product made with pork fat. HOSO was chosen as the lipid source, as high-oleic vegetable oils have been shown to reduce cardiovascular disease risk factors, by decreasing levels of total cholesterol, LDL cholesterol, and apolipoprotein B (apoB), while not changing levels of HDL cholesterol, triglycerides and apoA (Bowen et al., 2019; Huth, Fulgoni III, & Larson, 2015). This study constitutes the first time BPGs have been researched as total fat replacements in a coarse-ground meat product of any kind.

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2.1.3. Sausage formulation and processing

Smoked sausage formulations are shown on Table 3. Control 1 (C1) was formulated with PPT and PRT, which are raw materials similar to those used commercially in this type of product in the United States. However, since the fat content of both of these raw materials is considerable (Table 1), replacement of 100% of the fat is not possible using them. Therefore, Control 2 (C2) was formulated with a very lean material (PKN; 2.2 ± 0.4% lipid) and a high-fat material (PBF; 84.3 ± 1.2% lipid), such that most of the fat was provided by a single raw material (PBF), thus allowing for replacement of most of the product’s fat content (in the form of PBF) with BPGs. Both controls were formulated to a finished target fat content of 28.5%, assuming a processing yield of 93% (Table 3). After preliminary evaluation of various BPG formulations, four were selected (Table 2), and used as total replacements of the PBF in C2 (Table 3). Given that the composition of the BPGs differed from each other (Table 2) and from that of PBF (Table 1), their use to replace pork fat in equal proportions would be expected to result in formulated sausages of varying composition (Table 3).

Meat raw materials PKN, PPT, and PRT were sourced 10 d prior to sausage manufacturing, packaged under vacuum, and stored at −40 °C. They were allowed to thaw for 3 d (2 d at 10 °C followed by 1 d at 4–5 °C) prior to use. PBF was obtained 14 d prior to use, stored at −2 °C, and allowed to thaw for 2 d at 4–5 °C prior to use. All sausage treatments were manufactured in the Iowa State University Meats Laboratory (Ames, IA, USA) in batches of 13.1 kg. All meat materials and BPGs were ground through a 9.5-mm grinding plate (grinder model 7542, Biro Manufacturing Co., Marblehead, OH, USA), placed in a single-shaft paddle mixer (type AV-80, Fatos A.S., Barcelona, Spain), and mixed with all other ingredients for 2 min at 18 rpm. The mixtures were then ground through a 3.2-mm grinder plate, stuffed into 28 mm (dia.) clear edible bovine collagen casings (FINE-TF, Devro s.r.o., Jilemnice, Czech Republic), and portioned into links 15-cm in length and 87.5 cm³ in volume using a vacuum stuffer/linker (Handtmann F608 plus, Albert Handtmann Maschinenfabrik GmbH & Co. KG, Riss, Germany). Sausage links were hung on metal dowels and weighed; dowels with sausage links were then positioned on an oven truck in a random arrangement and thermally processed in a single-truck oven (Alkar, DEC International, Inc., Lodi, WI, USA) according to the schedule shown in Table 4. Smoke was generated by pyrolyzing natural hickory wood chips (Chips n’ Chunks Hickory All Natural Wood Chips, Smokehouse Products, LLC, Hood River, OR, USA) in an Alkar Smokemaster natural smoke generator (DEC International Inc., Lodi, WI, USA). Sausage treatments were manufactured in random order, and thermally processed at the same time, with thermal process starting approximately 2.5 h after the first treatment was stuffed. After cooking, sausages were chilled for approximately 18 h in a cooler at 0–2 °C, weighed to determine cooked and chilled yields, packaged (4 links per package) in plastic packages (oxygen transmission rate of 3–6 cm³/m²/24 h at 23 °C, 0% RH and water vapor transmission rate of 7.8–9.3 g/m²/24 h at 38 °C, 100% RH; Cryovac, Sealed Air Corporation, Duncan, SC, USA) and vacuum sealed (Ultravac UV 2100 packaging machine, UltraSource LLC, Kansas City, MO, USA). Packages were then shrink-wrapped by dipping them into

### Table 3

<table>
<thead>
<tr>
<th>Ingredients, g/100 g</th>
<th>Treatments</th>
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<tbody>
<tr>
<td>Pork picnic trimmings (PPT)</td>
<td>SC1</td>
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<tr>
<td>Pork regular trimmings (PRT)</td>
<td>56.86</td>
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<tr>
<td>Pork knuckles (PKN)</td>
<td>36.06</td>
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<tr>
<td>Pork fat (PBF)</td>
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<tr>
<td>BPG 7/3/7</td>
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</tr>
<tr>
<td>BPG 7/3/8</td>
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</tr>
<tr>
<td>BPG 6/4/7</td>
<td>–</td>
</tr>
<tr>
<td>BPG 6/4/8</td>
<td>–</td>
</tr>
<tr>
<td>Seasoning blend</td>
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<tr>
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<tr>
<td>Curing salt</td>
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<tr>
<td>Sodium erythorbate</td>
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<td>Dried vinegar</td>
<td>0.40</td>
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### Table 4

<table>
<thead>
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<th>Compositional targets, g/100 g</th>
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<tr>
<td>Lipid</td>
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<tr>
<td>Moisture</td>
<td>48.4</td>
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<td>Protein</td>
<td>14.7</td>
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### Table 4

<table>
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<tr>
<th>Step</th>
<th>Step time (min)</th>
<th>Dry bulb (°C)</th>
<th>Wet bulb (°C)</th>
<th>Relative humidity (%)</th>
<th>Product Internal (°C)</th>
<th>Main blower</th>
<th>Exhaust damper</th>
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<tbody>
<tr>
<td>Steam Cook</td>
<td>45</td>
<td>52</td>
<td>52</td>
<td>100</td>
<td>–</td>
<td>8</td>
<td>Closed</td>
</tr>
<tr>
<td>Cook</td>
<td>5</td>
<td>62</td>
<td>42</td>
<td>31</td>
<td>–</td>
<td>10</td>
<td>Auto</td>
</tr>
<tr>
<td>Steam Cook</td>
<td>5</td>
<td>74</td>
<td>74</td>
<td>100</td>
<td>–</td>
<td>8</td>
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<tr>
<td>Cook</td>
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<td>62</td>
<td>42</td>
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<tr>
<td>Smoke Cook</td>
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<tr>
<td>Smoke Cook</td>
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<td>51</td>
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<tr>
<td>Cook</td>
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<td>10</td>
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<td>Auto</td>
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<tr>
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<td>0</td>
<td>0</td>
<td>–</td>
<td>0</td>
<td>Auto</td>
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</table>
water at 78 °C and stored inside cardboard boxes at 0–2 °C for up to 98 d. Sampling days were day 0, 14, 28, 42, 56, 70, 84, and 98, and depended on the analyses performed, as described below. Day of packaging was designated as day 0.

2.2. Sausage analysis

2.2.1. Proximate analysis and pH

Raw and cooked sausage samples were finely-ground using a KFP715WH2 food processor (KitchenAid, St. Joseph, Michigan, USA). Moisture content was determined by the CEM SMART 6 system (Official Method 2008.06, AOAC International), fat content by the CEM ORACLE system (Official Method 2008.06, AOAC International), and protein content by the CEM Sprint Rapid Protein Analyzer (Official Method 2011.04, AOAC International) (CEM Corporation, Matthews, NC, USA). All proximate analyses were done in triplicate.

For pH measurement, 10 g of ground sample were mixed with 90 mL distilled water and stirred for 60 s. The mixture was then filtered through 11-μm filter paper (Whatman Grade 1, GE Healthcare Life Sciences, Pittsburgh, PA, USA) and the pH of the filtrate was then measured by a SevenMulti pH meter equipped with an InLab Solids Pro-ISM electrode (Mettler Toledo, Columbus, OH, USA). pH samples were done in duplicate.

2.2.2. Fatty acid profile

Fatty acids were extracted from sausage samples and formulation raw materials (meat, HOSO) and methylated according to O’Fallon, Busboom, Nelson, and Gaskins (2007). Fatty acid profile was determined by gas chromatography mass spectrometry (model 6890 gas chromatograph coupled to a model 5973 mass selective detector, Agilent Technologies, Inc., Santa Clara, CA, USA). The gas chromatograph (GC) was equipped with a 100 m × 0.25 μm × 0.2 μm high-polarity (88% cyanopropyl) aryl-polysiloxane column (J&W HP-88, Agilent Technologies, Inc., Santa Clara, CA, USA). The GC oven temperature program consisted of an initial temperature of 95 °C for 1 min; heating to 200 °C at 3 °C min⁻¹; holding for 5 min; heating to 230 °C at 10 °C min⁻¹; and holding for 1 min. One μL of sample was injected at a split ratio of 15:1 (product samples and pork raw materials) or 500:1 (HOSO). The retention index was calibrated with a hydrocarbon ladder. Fatty acid methyl ester (FAME) peaks were identified using a Supelco 37 Component FAME Mix (Millipore Sigma, Damstadt, Germany) and fatty acid identification was done using version 2.3 of the NIST/EPA/NIH Tandem Mass Spectral Library (NIST 17) of the National Institute of Standards and Technology (United States) (NIST, 2017). Iodine values (IV) were calculated as described in AOCS Recommended Practice Cd 1e-85 (American Oil Chemists’ Society, 2017). Sausage samples from three replications were analyzed in duplicate. Since all raw materials were from a single production lot, there was no replication.

2.2.3. Lipid oxidation

Lipid oxidation was measured on storage days 0, 14, 28, 42, 56, 70, 84, and 98, according to the 2-thiobarbituric acid (TBARS) method of Tarladgis, Watts, Younathan, and Dugan Jr. (1960), as modified by Zipser and Watts (1962). Absorbance was measured at 532 nm by a Genesys UV–Visible Spectrophotometer (Genesys 150, model 240,300,000, Thermo Fisher Scientific, Madison, WI, USA). Analyses were performed in duplicate and averaged, and results were reported as μg of malondialdehyde per g of sample.

2.2.4. Instrumental color

Color was measured on storage days 0, 14, 28, 42, 56, 70, 84, and 98, by a LabScan XE colorimeter (model LS 1500, Hunter Associates Laboratory, Inc., Reston, VA, USA), using illuminant D65 (daylight at 6500 K), 10° observer angle and a 12.7-mm aperture, with color represented by the coordinates of the CIE Lab (L*a*b*) color space. External color measurements were taken from two locations of each of three sausage links obtained from the same package. For internal color, the same sausages were cut perpendicular to their length and one measurement was taken from each of the two exposed faces of each sausage link. Results of the six scans per treatment were averaged for both external and internal color.

2.2.5. Texture analysis

Texture analyses were done on storage days 0, 14, 28, 42, 56, 70, 84, and 98, using a TA-XT2 Texture Analyzer equipped with a 30-kg load cell (Stable Micro Systems, Surrey, UK). Prior to testing, the remaining 3 sausage links from the package used for color analysis were removed from refrigeration and allowed to equilibrate to room temperature for 2 h.

For Texture Profile Analysis (TPA) testing, links were cut perpendicular to their length into 25.4-mm-thick sections. One randomly-selected section from each link was then placed vertically and compressed twice, with a 50.8 mm (diameter) × 20 mm (height) cylindrical aluminum probe (TA-25, Texture Technologies Inc., Scarsdale, NY, USA), to 30% of its original height at a test speed of 5.0 mm s⁻¹ and trigger force of 0.049 N (compression beyond 30% caused some sausage sections to fracture). The TPA parameters hardness, cohesiveness, springiness, resilience, and chewiness were calculated, as described by Bourne (1978).

For Warner-Bratzler shear force analysis, a Warner-Bratzler blade with a guillotine block (TA-7, Texture Technologies Inc., Scarsdale, NY, USA) was used to shear through a sausage section placed perpendicular to the blade, at a test speed of 4.0 mm s⁻¹. For each treatment, 3 sections obtained from the same package were measured and the results averaged.

2.2.6. Sensory analysis

Descriptive sensory evaluation was performed on storage days 14, 42, 70 and 98, by a nine-member trained panel comprised of students and staff of Iowa State University. Three training sessions were held, utilizing sausage samples manufactured for this study as references, during which descriptive terms were developed and panel responses calibrated. At the 3 training sessions and 12 test sessions, panel participants were asked to chew and expectorate the samples. Prior to serving, sausage links from one randomly-selected package (6 links) from each treatment were heated to 60 °C in a 1.5-kW microwave oven (model RSW458P, Amana Refrigeration, Inc., Amana, IA, USA) and cut perpendicular to their length into sections 25.4 mm in length. Sausage end pieces were discarded and the remaining pieces were placed in a bowl and mixed. Two randomly-selected sausage sections were then placed in an expanded polystyrene foam cup with a plastic lid, and served to each panelist, one treatment at a time. Treatments were randomized for each test session and assigned a three-digit random code. Sensory attributes were evaluated on a 15-cm-long unstructured line scale anchored with descriptive terms on the left (low degree of the attribute) and right (high degree of the attribute). The sensory attributes evaluated were (descriptive terms in parentheses): smoked sausage aroma (none to intense), other aroma (none to intense), first bite firmness (soft to firm), creaminess (none to intense), moisture release (none to intense), smoked sausage flavor (none to intense), off-flavor (none to intense), external color (light to dark), external appearance (discrete particles not visible to discrete particles very visible), and internal appearance (discrete particles not visible to discrete particles very visible). Testing booths equipped with sensory evaluation computer software and color-masking lights were not used, in compliance with laboratory and workplace safety restrictions in place due to the COVID-19 pandemic. Therefore, evaluation took place in a large room under white fluorescent lights, and data was collected and compiled manually.
The sensory analysis protocol was reviewed and approved by the Iowa State University Institutional Review Board (25 September 2020; IRB ID 20-353) and signed informed consent was obtained from all panelists prior to initiation of the study. The consent document specifically made panel participants aware of the inclusion of RBW in some of the samples (since its use is presently not permitted in meat products in the USA), but the identity of samples that contained it, as well as usage levels, were not disclosed.

2.3. Experimental design and statistical analysis

The experiment was designed as a randomized complete block (RCBD) and was replicated three times, with each replication corresponding to one of three consecutive sausage manufacturing days. All meat raw materials and ingredients were obtained from a single production lot in order to reduce within block variation. For each replication, experimental treatments were randomly assigned to production batches, and placement of stuffed sausage links in smokehouse trucks was done following a random arrangement. Data were analyzed as a mixed model using JMP Pro statistical software, version 15.1.0 (SAS Institute, Cary, NC, USA), with treatment and storage time as fixed factors and replication as random factor; for sensory analysis data, panelists were treated as a random factor. Where storage time was a factor (color, texture, lipid oxidation, sensory) the RCBD was arranged as a split-plot in time, with treatment as the whole-plot factor and storage time as the subplot factor. The Tukey-Kramer pairwise comparison method was used to determine differences between means. Significance was determined at $P < .05$. $P$ values for effects of fixed main factors (treatment and storage time) and their interaction are shown in Table 5.

### 3. Results & discussion

#### 3.1. Biphasic gels

The biphasic gel (BPG) formulations tested were fully characterized and studied in a companion study (Cho, Tarté, & Acevedo, 2022). The specific OG/HG ratios used in this study (Table 2) were selected based on the gels’ mechanical and visual properties for this specific application. All four gels tested were firm and elastic, a necessary condition for grinding, and opaque, a necessary condition for the visual simulation of animal fat. They were all milky white in color (Fig. 1), with the 7:3 BPGs being slightly yellower and greasier to the touch than the 6:4 BPGs, due to their higher oil content, and withstood the mixing and grinding treatments as well as pork fat (Fig. 2). All gels performed well upon grinding and mixing, without any visible phase separation or degradation. Microscopic examination of the gels revealed them to be biphasic systems with the OG phase dispersed in the HG phase.

#### 3.2. Proximate analysis, pH, and yields

Due to the differences in composition among BPGs (Table 2) and between BPGs and pork fat, differences in composition among treatments were expected (Table 3). Total replacement of pork fat with BPGs was expected to decrease product total lipid content, and increase total moisture content (Table 3), given the fact that BPGs contain both lipid and water. The largest difference was expected when 60:40 BPGs were used (Table 3), given they were lower in OG and higher in HG than 70:30 BPGs (Table 2). The differences in protein content among BPGs would also be expected to result in small differences in the protein content of the final sausage product samples.

Proximate analysis results are shown in Table 6. The main effect of treatment was significant ($P < .05$) for all proximate parameters, both for cooked and raw sausages (Table 5), which was expected, as previously explained. Though not significant in every instance, raw and cooked moisture content of the sausages followed the progression S6:4/7 > S6:4/8 > S7:3/7 > S7:3/8 > C2 > C1, which, with the exception of the moisture content of C1 being lower than that of C2, agrees with the expected results (Table 3). Protein content of cooked sausages was S6:4/8 > S6:4/7 > S7:3/7 > C2 > S7:3/7 > C1, as expected, once again with the exception of C1, which was expected to have the highest protein content (Table 3). Lipid content results were C1 > C2 > S7:3/7, S7:3/8 > S6:4/7, S6:4/8, once again, with the exception of C1, in agreement with the expected results. As mentioned, C1—though formulated to have the same lipid (28.5%) and moisture (52.0%) contents as C2, and higher protein content—was higher in lipid (31.3% vs. 26.8%), lower in

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Treatment (lipid source)</th>
<th>Storage time</th>
<th>Treatment x storage time</th>
</tr>
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<tbody>
<tr>
<td>Lipid, raw</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Moisture, raw</td>
<td>$&lt;.001$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein, raw</td>
<td>$&lt;.001$</td>
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</tr>
<tr>
<td>Lipid, cooked</td>
<td>$&lt;.001$</td>
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</tr>
<tr>
<td>Moisture, cooked</td>
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</tr>
<tr>
<td>Protein, cooked</td>
<td>$&lt;.001$</td>
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<td></td>
</tr>
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<td>pH</td>
<td>$&lt;.001$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yield</td>
<td>$&lt;.001$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5

$P$ values of fixed main factor and interaction effects.$^a$

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$^a$ Statistically significant values ($P < .05$) shown in bold.


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**Fig. 1.** Representative biphasic gels (BPG) prior to grinding. Left: BPG made with 60% OG phase/40% HG phase; right: BPG made with 70% OG phase/30% HG phase. HG phases of both BPGs shown contained 7% pork skin gelatin.

$7 > S6:4/8 > S7:3/7 > S7:3/8 > C2 > C1$, which, with the exception of the moisture content of C1 being lower than that of C2, agrees with the expected results (Table 3). Protein content of cooked sausages was S6:4/8 > S6:4/7 > S7:3/7, C2 > S7:3/7 > C1, as expected, once again with the exception of C1, which was expected to have the highest protein content (Table 3). Lipid content results were C1 > C2 > S7:3/7, S7:3/8 > S6:4/7, S6:4/8, once again, with the exception of C1, in agreement with the expected results. As mentioned, C1—though formulated to have the same lipid (28.5%) and moisture (52.0%) contents as C2, and higher protein content—was higher in lipid (31.3% vs. 26.8%), lower in
however, 100% replacement was not possible due to small amounts of lipid still present in this material (2.5%, Table 1). Therefore, the amount of animal fat replaced is estimated to be 98.4% (63.33% PKN x 2.5% lipid).

The lack of difference in yield despite the higher moisture content of the BPG-containing treatments can be explained by the physical entrapment of the BPGs in the meat matrix, which would make their moisture less available for evaporation during cooking.

### 3.3. Fatty acid profile

Fatty acid profiles of raw materials and sausage samples are shown in Table 7. The fatty acid profiles of the sausage samples were similar to those of the lipid sources used in their formulation. The lipid fractions of BPG-containing samples were lower in palmitic (C16:0), stearic (C18:0), and linoleic (C18:2n-6c) acids and higher in oleic (C18:1n-9c) and α-linolenic (C18:3n-3) acids. The contents of myristic (C14:0) and palmitoleic (16:1n-7) acids were also lower in BPG treatments, although they were very low in all samples. The concentrations of most of the other fatty acids detected were very small in all treatments and, hence, any differences in these among treatments can be considered trivial, even when statistically significant. Iodine values (IV) were higher in BPG treatments, as expected, due to the higher IV of HOSO when compared to pork fat. The n-6:n-3 fatty acid ratio was lower in BPG treatments than in the controls, which can be considered favorable given current dietary recommendations (USDHHS/USDA, 2020).

### 3.4. Lipid oxidation

Lipid oxidation results are shown in Fig. 3. The main effect of treatment was significant (P < .05), but that of storage time, and the interaction effect of treatment x storage time, were not (P ≥ .05) (Table 5). TBARS values of BPG treatments were not different among each other over the storage period, but were significantly higher (P < .05) than for C1 and C2, with C2 being higher than C1. The higher TBARS values of BPG treatments could be explained by the heat applied during production of the BPGs, as suggested by Park, Bemer, and Maleky (2018) for oleogel systems. Over the entirety of the 98-d storage period, however, lipid oxidation remained unchanged and below sensory detection levels in all treatments, suggesting that rancid flavor notes due to lipid oxidation were not a concern in these products. These results are
not surprising, given that these same gels were found to be oxidatively stable throughout a 6-mo. storage period at 22 °C (Cho et al., 2022), and that in our previous studies utilizing oleogels containing conventional or high-oleic soybean oil in frankfurters (Wolfer et al., 2018) and bologna (Tartt et al., 2020), TBARS values of oleogel-containing treatments, while higher than in controls, remained under the sensory detection threshold. However, it must be noted that the TBARS values obtained for BPG treatments in this study were still considerably higher (0.85 mg g⁻¹ being the highest) than the highest observed in those two studies (0.20 and 0.28 mg g⁻¹, respectively), suggesting that the BPG manufacturing procedure (which includes high-shear homogenization in addition to heat) may have a greater destabilizing impact on the oxidative stability of the oil. Recently, Ghiasi & Golmakani (2022) reported increasing TBARS values as greater amounts of a 75:25 BPG were incorporated into beef burgers, which they attributed to the very high temperature (145 °C) used during preparation of the ethylcellulose oleogel phase. However, in contrast to our results, they observed that TBARS values continued to increase during a 3-month frozen storage period. We
attribute this difference in lipid stability over time to any one or more of a combination of factors: (i) our use of high-oleic soybean oil (HOSO), which is less prone to oxidation than the sunflower oil Ghiasi & Golmakan (2022) used, (ii) the antioxidant properties of sodium nitrite and sodium erythorbate, which their beef burgers did not contain, and (iii) the lower temperature (90° C) required to melt RBW during preparation of our BPGs.

Future work, however, should further explore the extent of any potential oxidative rancidity problems and the need for adding antioxidants, should it be necessary. The need for antioxidants will likely be product-dependent and could be required by commercial handling, storage and distribution conditions.

3.5. Appearance and color

Numerical results are shown in Table 8 and visual appearance in Fig. 4. The main effects of treatment and storage time on both external an internal appearance were significant, but not their interaction (Table 5). Both externally and internally, S6:4 treatments had significantly more visible discrete particles than both S7:3 samples and both controls, a difference that is visually appreciated in Fig. 4. The more elastic texture of 6:4 BPGs, documented in a companion study (Cho et al., 2022), made them much more resistant to grinding and shear throughout the process, whereas the higher plasticity of the 7:3 BPGs made them less resistant to shear and, therefore, enabled them to more closely simulate pork fat in the final sausage product. A higher OG:HG ratio, then, increases plastic behavior and reduces elastic behavior of the BPG, and is, therefore, critical to the properties of these BPGs and their overall performance as semi-solid fat replacers (Cho et al., 2022). This effect has also been reported in similar BPGs of OG:HG ratios lower (5:5, 4:6, 3:7, 2:8) than the ones used in this study (Saffold & Acevedo, 2021). In a recent study, emulsified fat crystal networks composed of canola oil, fully hydrogenated canola oil, soy protein isolate, and transglutaminase (added for protein crosslinking)—also formulated as fat mimetics—exhibited increased plastic behavior with increasing solid fat content, in this case in the form of sol fat (Dreher, Blach, Terjung, Gibis, & Weiss, 2020).

Color results are shown in Table 8. Main effect of treatment on instrumental external color was significant (P < .05) only for b* values, which were higher in S7:3/8 than SC2, S6:4/7 and S6:4/8. The lack of difference in L* and a* values among treatments could be attributed to the smoke deposited on the surface of the samples during thermal processing, which may have covered up any differences that might have been observable otherwise. In contrast, the main effect of treatment on external sensory color was significant, with the two S6:4 treatments being darker than S7:3 samples and C1, with the color intensity of SC2 falling in between these. We theorize that the sensory perception of color may be affected by the stark contrast between the very discrete 6:4 BPG particles and the lean portion of the product, leading sensory panelists to perceive the lean meat, and thus the overall product, as darker, in contrast to instrumental color measurement, which objectively detects light reflected from both lean meat and lipid particles. For internal L*, a* and b*, main effects of treatment and storage time were significant, but their interaction was not. Internal L* values were higher in both S6:4 treatments and lowest in both controls, with S7:3 treatments falling in between. The inverse relationship was observed for a* (P < .05), indicating that pork fat replacement with BPGs resulted in increased lightness and reduced redness, and that this effect became

| Table 8 |

Least squares means 1 for main effect of treatment and storage time on instrumental and sensory color and appearance attributes of coarse-ground sausage stored at 0–2°C.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ext. L*</th>
<th>Ext. a*</th>
<th>Ext. b*</th>
<th>Int. L*</th>
<th>Int. a*</th>
<th>Int. b*</th>
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</thead>
<tbody>
<tr>
<td>SCI</td>
<td>47.23a</td>
<td>18.37a</td>
<td></td>
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<tr>
<td>SC2</td>
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<td>S7:3/7</td>
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<td></td>
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</tr>
<tr>
<td>S1.3</td>
<td>1.432</td>
<td>0.741</td>
<td>0.849</td>
<td>0.568</td>
<td>0.215</td>
<td>0.159</td>
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<tr>
<td>S.E.M.</td>
<td>1.432</td>
<td>0.741</td>
<td>0.849</td>
<td>0.568</td>
<td>0.215</td>
<td>0.159</td>
</tr>
</tbody>
</table>

Storage time (d) 2

<table>
<thead>
<tr>
<th>Ext. L*</th>
<th>Ext. a*</th>
<th>Ext. b*</th>
<th>Int. L*</th>
<th>Int. a*</th>
<th>Int. b*</th>
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<td>44.64b</td>
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<tr>
<td>S.E.M.</td>
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<td>0.676</td>
<td>0.835</td>
<td>0.427</td>
<td>0.152</td>
</tr>
</tbody>
</table>

Int.: internal; Ext.: external.

S.E.M.: standard error of mean.

1 Mean of three replications.
2 Means averaged across all storage time sampling points.
3 Means averaged across all treatments.
4 Evaluated on 15-cm-long unstructured line scale; only on days 14, 42, 70, and 98.
5 Unstructured scale: 0 = light, 15 = dark.
6 Unstructured scale: 0 = discrete particles not visible, 15 = discrete particles very visible.
HOSO and/or CSO in finely comminuted meat products (Tart et al., 2022) to the melting of gelatin at around 35°C and of RBW at around 66°C during cooking. This effect was visible only upon cutting of cold sausages (as in Fig. 4). As during the first cook, gelatin and RBW would be expected to re-melt upon heating by consumers, thus providing the creamy mouthfeel reported by the sensory panel (see 3.7). The BPG particles inside the sausage links appeared intact after cooling and re-gelatinization, which we hypothesize was due to the physical entrapment and immobilization of the BPG particles inside the product matrix.

Close examination of the internal physical appearance of the cooked sausage products (Fig. 4) revealed the occurrence of slight phase separation in some of the BPG particles, which is attributed (based on DSC studies of these gels by Cho et al., 2022) to the melting of gelatin at around 35°C and of RBW at around 66°C during cooking. This effect was visible only upon cutting of cold sausages (as in Fig. 4). As during the first cook, gelatin and RBW would be expected to re-melt upon heating by consumers, thus providing the creamy mouthfeel reported by the sensory panel (see 3.7). The BPG particles inside the sausage links appeared intact after cooling and re-gelatinization, which we hypothesize was due to the physical entrapment and immobilization of the BPG particles inside the product matrix.

3.6. Texture

All instrumental and sensory texture results are shown in Fig. 5. Main effect of treatment was significant (P < .05) for TPA hardness, TPA resilience, TPA chewiness, WBS peak force (WBSF), and sensory first bite firmness, and main effect of storage time was significant for TPA hardness, TPA chewiness, and WBSF (Table 5). No significant main effects were observed for TPA cohesiveness and TPA springiness. The treatment x storage time interaction effect was not significant for any of the instrumental or sensory texture parameters evaluated (Table 5).

TPA hardness values were higher for both controls than for all BPG-containing treatments, though significantly only between SC1 and both S7:3 treatments, suggesting that, while BPGs result in softer sausages, OG:HG ratio has an impact. In a separate companion study (Cho et al., 2022), as the OG:HG ratio in the BPG increased, storage modulus (G”) increased and yield stress (τ0) decreased, indicating irreversible deformation of the gels at lower stress values as OG content increased. Therefore, a BPG higher in HG phase would exhibit more elastic behavior, which would make it more resilient to permanent deformation, and can be expected to result in increased hardness of the BPG-containing sausages. During the storage period, an overall increase was observed between d 0 and d 56, after which TPA hardness values stabilized. TPA resilience was higher in the controls (significantly higher in SC1 than SC2) than in S7:3 samples, with S6:4 samples falling in between, and did not change over the storage time period. TPA chewiness results were similar to those of TPA hardness, being significantly higher for SC1 than for S7:3/7, and increased overall from d 0, peaked at d 56, and decreased slightly and remained stable thereafter. WBSF values (Fig. 5E) were higher for S6:4/7 than S7:3/7 and SC1, and not different between SC2 and the all BPG-containing treatments. Over the storage time period it increased until d 56, after which it stabilized. Sensory first bite firmness was higher in S6:4 than in S7:3 treatments and SC1 and did not change significantly over time. However, there was no significant difference between SC2 and the 6:4 BPG treatments. In general, these results are consistent with the measured mechanical properties of these gels (Cho et al., 2022), as discussed above.

Overall, replacement of pork fat with the BPG compositions tested resulted in products of comparable texture, which is not surprising given their documented solid-like rheological and mechanical properties (Cho et al., 2022). SC1, in which the fat was attached to the pork trimmings used (PPT and PRT; Table 3), tended to be slightly harder and more resilient, but lower in WBSF peak force and sensory first bite firmness, than SC2 and BPG treatments, in which the lipid portion, regardless of source, was incorporated as a separate, stand-alone material.

3.7. Sensory flavor, aroma and creaminess

Results are shown in Table 9. Main effect of treatment was significant (P < .05) for all attributes, and main effect of storage time was significant only for aroma and flavor attributes. The treatment x storage time interaction was significant for sensory other flavor only. For intensity of all attributes except moisture release, the six treatments were clearly segregated into three groups: the two controls, S7:3s, and S6:4s. For intensity of smoked sausage aroma and smoked sausage flavor, SC1/SC2 > S7:3s > S6:4s. The inverse relationship was observed for other aroma, other flavor, and creaminess. These results are consistent with a lack of meat flavor precursor molecules in the BPGs and potential contribution of other flavor notes by the components of the BPGs (primarily HOSO and RBW), and the effect was more pronounced in the samples containing BPGs higher in hydrogel phase. Therefore, inclusion of flavoring ingredients may be necessary in some applications, depending on the type of meat product and the specific make-up of the BPG utilized.
4. Conclusions

The biphasic gels utilized in this study maintained their textural and visual properties after being subjected to the processing conditions of a fully-cooked, coarse-ground sausage, which involve grinding, mixing, re-grinding, stuffing, cooking, and re-heating. The properties of the BPGs were affected by the OG:HG ratio, but not by the amount of gelatin in the HG phase. It has been demonstrated that these gels become more plastic and less elastic as the OG:HG ratio increases (Cho et al., 2022; Saffold & Acevedo, 2021). In the present study, 7:3 BPGs more closely simulated the properties of pork fat, having enough elasticity to maintain an animal fat-like appearance, but enough plasticity to become partially smeared throughout the process, in much the same way pork fat does. On the other hand, the greater elasticity of 6:4 BPGs allowed them to be so resilient to sausage processing conditions that the fat particle definition of the resulting products was extreme and deviated significantly from that of the pork fat controls.

Our results demonstrate that the biphasic gels used in this study can effectively replace animal fat in coarse-ground meat products and, likely, other food products (e.g., plant-based meat analogs) in which discrete semi-solid fat particles are desired. They also suggest that the mechanical properties of the biphasic gels can, and should, be tailored and optimized to the desired final product characteristics (visual, sensory, compositional), in accordance with the specific processing conditions. While the preparation process of these biphasic gels is simple and straightforward, they may require incorporation of structuring agents other than the ones herein described, depending on the mechanical properties desired and the formulation/labeling requirements of each application. When formulating the final meat product, the moisture content of the BPGs should be considered in order to determine the
correct usage levels of antimicrobial agents.

As follow-up to this work, ongoing research should focus on how the mechanical properties of the BPG systems are affected by other oleogel and hydrogel structuring agents, lipid sources of varying composition, varying pH, and extended aging time (> 3 months), as well as how these factors impact the quality characteristics of the final product. This understanding will pave the way for application of the technology to other product types, such as fermented and aged meat products, plant-based meat analogs, and others.

Declaration of Competing Interest
None.

Data availability
Data will be made available on request.

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References


S.E.M.: standard error of mean.

**Within main effect, means in the same column with different superscripts are significantly different (P < .05).

a Means of three replications.

Evaluated on 15-cm-long unstructured line scale; only on days 14, 42, 70, and 98.

2 Scale: 0 = none, 15 = intense.

3 Sensory attributes related to appearance/color and texture are shown in Table 8 and Fig. 3, respectively.

4 Means averaged across all storage time sampling points.

5 Means averaged across all treatments.


