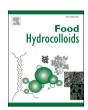
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# Effect of different vegetable oils on extruded plant-based meat analogs: Evaluation of oxidative degradation, textural, rheological, tribological and sensory properties

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#### ABSTRACT

Vegetable oils are often added during high moisture extrusion of plant-based meat analogs to improve their textural and sensory quality. However, the impact of extrusion processing on lipid degradation often receives minimal attention, despite its potential to reduce the nutritional and sensory quality of the product. Therefore, in this study the influence of four different oil types (MCT, coconut, olive and grapeseed oil) at different oil concentrations (0, 3, 6%) on the extrusion process and relevant quality characteristics of wheat gluten-based meat analogs was evaluated. The vegetable oils were added as emulsions, and the die pressure measured during processing, as well as the rheological, textural, tribological and sensory properties of the extrudates were characterized as a function of oil concentration and oil type. Extrudates without oil showed significantly higher storage moduli, which correlated with higher firmness and chewiness as measured by texture profile analysis. With increasing oil concentration, storage modulus, firmness and chewiness decreased significantly for all oil types, which was reflected in a higher perception of tenderness during sensory evaluation. Determination of polar compounds of the oils before and after extrusion processing showed that oils with a high degree of unsaturation, i.e., olive oil and grapeseed oil, are more susceptible to lipid oxidation caused by high moisture extrusion. However, sensory evaluation did not reveal any rancid off-flavors associated with lipid oxidation in any of the extrudates, irrespective of oil type. Future studies could involve the evolution of oxidative degradation during shelf-life, and its implications on sensory properties.

#### 1. Introduction

Plant-based meat analogs are becoming increasingly important as more sustainable alternatives to meat products. A widely used process for the production of plant-based meat analogs is high moisture extrusion. During extrusion, protein-rich powdered raw materials are mixed with water, heated, sheared by the rotation of the screws and forced through a cooling die where the product acquires its anisotropic, fibrous structure and final shape. The thermomechanical energy input in the extruder induces significant physical and chemical changes in the ingredients, e.g. protein denaturation and cross-linking reactions (Cheftel et al., 1992; De Angelis et al., 2024; Guyony et al., 2023; Zhang et al., 2023). For meat analog applications, plant proteins from various sources, such as cereals (wheat or maize), oilseeds (sunflower or rapeseed) or

legumes (soy or pea) are commonly used (Kurek et al., 2022). Wheat gluten, which is widely used in the production of meat analogs, is very sensitive to thermal treatment due to its native conformation, which is mainly stabilized by non-covalent bonds. During extrusion, wheat gluten unfolds, exposing previously hidden functional groups and promoting the formation of intermolecular cross-links. This aggregation, dominated by covalent, irreversible disulfide bonds, results in a three-dimensional protein network (Pietsch et al., 2017; Schofield et al., 1983; Weegels et al., 1994).

A great number of research has been conducted on how to improve the sensory quality of meat analogs in order to increase consumer acceptance (De Angelis et al., 2024). The main goal is to create sensory appealing products that resemble meat in terms of taste, flavor, texture and mouthfeel (Chen, Feng, et al., 2022; Egbert and Borders, 2006). In

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order to generate meat analogs with a tender and juicy mouthfeel, various functional ingredients such us enzymes, binding agents, hydrocolloids or lipids and fats can be added to the texturized proteins for the production of meat analogs (Bohrer, 2019; Kyriakopoulou et al., 2021; Singh et al., 2021). This study focuses on the addition of vegetable oils or fats as potential ingredients to enhance the tenderness and juiciness of meat analogs. Oils or fats can be added either before or during the extrusion process or during post-processing steps. To add oil or fat prior to post-processing, it can be premixed with the powdered raw materials prior to extrusion (Gwiazda et al., 1987) or added as a second liquid feed either directly (Kendler et al., 2021; Opaluwa et al., 2023; Saavedra Isusi et al., 2023; Stehle et al., 2024) or as an emulsion (Stehle et al., 2024; Wang et al., 2022a) during extrusion. The advantage of adding oil or fat during the extrusion process, rather than post-processing, is that the lipid phase can be better incorporated and more homogeneously distributed in the protein matrix, more closely resembling the intramuscular fat of real meat.

For meat, it is generally accepted that intramuscular fat has a positive effect on meat quality in terms of flavor, juiciness and tenderness, leading to an overall improvement in consumer sensory perception (Hocquette et al., 2010). Whether this also applies to meat analogs in which oils or fats are incorporated by high moisture extrusion has hardly been investigated in the literature to date. It should be noted that the addition of oils and fats during extrusion can also introduce undesirable lipid-derived off-flavors, such as rancidity, caused by volatile compounds originating from lipid oxidation (Wang et al., 2022b). In addition, lipid degradation caused by thermal treatments, such as during extrusion processing, can lead to the formation of polar compounds, including triacylglycerol polymeric compounds and oxidized triacylglycerol (Caponio et al., 2007; Chen et al., 2021; Rodríguez et al., 2021). The polar compounds, as non-volatile molecules, are not only indicators of the overall oxidative degradation of oils, but are also of interest for their potential adverse effects on human health. Although the evidence is limited or debated (Billek, 2000; Chen et al., 2021; Ju et al., 2019), these health concerns highlight the need for further research in this area.

As different vegetable oils or fats differ not only in taste, but also in physical properties (e.g. melting behavior) and chemical composition (e.g. degree of unsaturation or free fatty acid concentration), the type of oil can also have a major impact on the entire production process. Two recent studies of Chen et al. (2022a, 2023) investigated the influence of the addition of free fatty acids with different degrees of unsaturation (stearic, oleic, and linoleic acids) on the high moisture extrusion of pea protein. It was reported that the degree of unsaturation of the fatty acids had a significant effect on the viscosity, process conditions, protein denaturation and fibrous structure of the extrudates. While this approach is valuable for research purposes, the use of food-grade vegetable oils containing mixtures of fatty acids is more practical for industrial applications.

The extent of lipid oxidation during the production of high moisture meat analogs and its effect on textural, rheological and sensory quality has received limited research attention. Only one recent study by Chen, Liang et al. (2022) focused on the lipid oxidation of meat analogs based on wheat gluten and peanut oil. They reported that the degree of oxidation of peanut oil increased by extrusion processing as a function of extrusion temperature. However, this study does not provide any information on the influence of lipid oxidation on the sensory attributes of meat analogs, nor does it consider the influence of different oil types with different degrees of unsaturation.

In conclusion, the addition of lipids to meat analogs can have both positive and negative effects on product quality. While lipids can enhance tenderness and juiciness, lipid oxidation can lead to undesirable quality losses. This study hypothesizes that both oil concentration and oil type have a significant effect on the quality of meat analogs. To test this, the effect of four different vegetable oils on the quality of wheat gluten-based meat analogs produced by high moisture extrusion was

studied. Specifically, the oils were selected for their fatty acid profiles, representing four scenarios relevant to the industry: a medium-chain saturated oil (MCT oil), a saturated oil commonly used for meat analogs (coconut oil), a monounsaturated fatty acid (MUFA)-rich oil (olive oil), and a polyunsaturated fatty acid (PUFA)-rich oil (grapeseed oil). Literature suggests that the degree of unsaturation of the fatty acids may influence product properties, and this selection allows for an in-depth examination of extrusion-related phenomena. The meat analogs were characterized in terms of total polar compounds in the lipid fraction to assess the oxidative phenomena occurring during extrusion. In addition, textural, rheological and tribological properties were evaluated, as these are critical to the quality of the meat analogs in terms of tenderness and juiciness. Finally, a sensory evaluation was carried out to correlate these parameters with the general sensory perception of the meat analogs.

This study aims to provide a more comprehensive understanding of the effects of oil addition on the sensory properties of meat analogs. Overall, it suggests additional areas for future research and highlights the challenges of monitoring the formation of undesirable compounds in meat analogs and their impact on textural and sensory properties.

#### 2. Materials and methods

#### 2.1. Materials

Kröner Stärke (Ibbenbüren, Germany) kindly provided vital wheat gluten. Wheat gluten was chosen because of its high availability and widespread use in the production of meat analogs, making it an industrially relevant raw material for high moisture extrusion. According to the manufacturer, the vital wheat gluten contains max. 8 g/100 g moisture and 83 g/100 g protein on a dry matter basis. Additionally, lipid fraction was extracted using a Randall apparatus (SER 148 extraction system, Velp Scientifica srl, Usmate, Italy) according to method AOAC 945.38F (AOAC, 2006), with diethyl ether as extracting solvent. Wheat gluten had a lipid concentration of 3 g/100 g on dry matter basis. The lipid composition of medium-chain triacylglycerol oil (MCT oil, WITARIX®60/40, IOI Oleo GmbH, Hamburg, Germany), virgin coconut oil (Nature et Plantes, Magescq, France), olive oil (Olearia De Santis SpA, Bitonto, Italy) and grapeseed oil (Benvolio 1938; Treviso, Italy) is reported in Supplementary Table S1. The sum of monounsaturated fatty acids was 0.0%, 5.53%, 72.03% and 16.63%, while the sum of polyunsaturated fatty acids was 0.0%, 0.97%, 10.72% and 72.30% for MCT, coconut, olive and grapeseed oil, respectively. The lipid composition of the oil extracted from wheat gluten and the vegetable oils was determined by gas-chromatographic analysis of fatty acid methyl esters using the method and equipment previously described by De Angelis et al. (2021) with some modifications. The temperature of the injector was 210 °C and the column was subjected to a temperature of 80  $^{\circ}$ C for 5 min, followed by an increase from 80  $^{\circ}$ C to 200  $^{\circ}$ C at a rate of 2 °C per minute. The column was held at 200 °C for 5 min and finally the temperature was raised to 240 °C at a rate of 10 °C per minute. Helium was used as carrier gas at a constat flow rate of 1 ml per minute. The Flame Ionization Detector (FID) was set at 220 °C with an air flow of 400 ml per minute and a hydrogen flow of 40 ml per minute.

## 2.2. Preparation of extruded samples

## 2.2.1. Preparation of emulsions

Oil-in-water emulsions with two different oil concentrations (6.2 wt % and 11.9 wt%) were prepared from all oil types. These two oil concentrations were chosen to achieve the oil concentrations of 3.0 wt% and 6.0 wt% in the extrudate, both of which are within the range of intramuscular fat contents of real meat products (Hocquette et al., 2010) and were found to be suitable in preliminary tests. To ensure that the coconut oil was liquid, it was heated to 30 °C prior to emulsification. First, 3.3 wt% of Tween20® was dissolved in water. The oil was then dispersed in water using an Ultra-Turrax T25 (IKA Werke GmbH & Co.

KG, Staufen, Germany) at a rotor speed of 5000 rpm for 5 min. Subsequently, the pre-emulsion was homogenized for 10 min using a colloid mill (IKA magic LAB®, IKA®-Werke GmbH & Co. KG, Germany), which was operated at a gap width of 0.16 mm and a rotor speed of 20.000 rpm. The oil droplet size distributions of the final emulsions were determined by laser diffraction spectroscopy (HORIBA LA950, Retzsch Technology GmbH, Haan, Germany) and are shown in Supplementary Figure S2 (a).

## 2.2.2. High moisture extrusion

High moisture extrusion was performed using a laboratory scale corotating twin-screw extruder (Process11, ThermoFisher Scientific Inc., Waltham, MA, USA) with a length to diameter (L/D) ratio of 40 and a screw diameter of 11 mm. The extruder barrel consisted of eight barrel segments that could be heated or cooled, and a die adapter that could only be heated. A slit die measuring  $125 \times 19 \times 4$  mm (L  $\times$  W  $\times$  H) was attached to the die adapter and cooled to 10  $^{\circ}\text{C}$  using a refrigeration unit (Presto Plus LH 47, Julabo GmbH, Seelbach, Germany). Wheat gluten was fed into the first barrel segment by a gravimetrically controlled feeder (Brabender Technology GmbH, Duisburg, Germany). Oil-in-water emulsions were dosed into the third barrel segment using a peristaltic pump ("Masterflex L/S", Cole Parmer, Vernon Hills, IL, USA). For all samples, the water and gluten concentrations were reduced to the same extent as the oil concentration was increased from 0 wt% to 6 wt%, resulting in a constant water-to-protein ratio. All the samples are listed in Table 1.

During the extrusion process, the material temperature and the die pressure at the end of the extruder barrel were recorded. For the extrusion trials, the temperature profile was set to  $25/25/40/80/120\,^{\circ}$ C for barrel segments 2/3/4/5/6. The temperature of the last two segments and the die adapter was adjusted so that the material temperature was  $135\,^{\circ}$ C for all samples. This setup was chosen to ensure that all samples experienced the same maximum temperature, which is a critical parameter for lipid oxidation. Trials were carried out with otherwise constant process parameters with the screw speed set at 700 rpm and the total mass flow rate set at  $1\,$ kg/h. Samples were taken after the process conditions had been stable for at least  $3\,$ min.

## 2.3. Analysis of rheological and textural properties

The rheological properties of the samples were characterized using an oscillating closed cavity rheometer (CCR) (RPA elite, TA Instruments, New Castle, DE, USA). A 5.5 g sample was placed between the two cones and preheated at 30 °C for 2 min without shear. Strain sweeps were then performed at a frequency of 1.0 Hz over a strain range of 0.1–1000% at 30 °C. All strain sweeps were performed in triplicate. The critical stress, i.e., the value of the shear stress (kPa) at the end of the linear viscoelastic region, and the corresponding critical strain (%) were recorded and used to plot the texture map of the meat analogs (De Angelis et al., 2023; Schreuders et al., 2022). The textural properties were evaluated using a ZI.0 TN texture analyzer (ZwickRoell GmbH & Co. KG, Ulm, Germany), equipped with a 1 kN load-cell and a 36 mm diameter compression probe under the conditions described in De Angelis et al. (2023).

**Table 1** Overview of samples.

Sample name	Added oil/wt%	Wheat gluten/wt%	Added water/wt%
0% oil (control)	0	55	45
3% MCT oil	3	53	44
6% MCT oil	6	51	43
3% coconut oil	3	53	44
6% coconut oil	6	51	43
3% olive oil	3	53	44
6% olive oil	6	51	43
3% grapeseed oil	3	53	44
6% grapeseed oil	6	51	43

Specifically, the specimens were cut into pieces of  $19 \times 19 \times 4$  mm (L  $\times$  w  $\times$  h) and subjected to double compression up to 75% of deformation at a speed of 1 mm/s with a 5 s pause between the two compressions. The following parameters were evaluated: firmness (N), indicating the maximum force recorded during the first compression; springiness, measured as the height recorded during the second compression divided by the height of the specimen; cohesiveness, measured as the area of work during the second compression divided by the area of work during the first compression; chewiness (N), calculated as firmness  $\times$  cohesiveness  $\times$  springiness. The analysis was repeated four times.

## 2.4. Tribological measurements

A stress-controlled HAAKE Mars rheometer 40/60 (Thermo Fisher Scientific, Karlsruhe, Germany) equipped with a single ball TR13 45° tribology measurement geometry was used to measure the friction coefficient of the samples. The samples were cut into pieces of  $19\times19\times4$  mm (L  $\times$  W  $\times$  H) and placed directly under the measurement geometry on a serrated plate to prevent the samples from slipping during the measurements. All measurements were performed over a relative sliding velocity range of 0.01--100 mm/s. The normal force  $F_N$  was set at 1 N and the temperature at 23 °C. Tribological measurements were repeated three times.

## 2.5. Analysis of the total polar compounds of the lipid fraction

Extrudates and emulsions were lyophilized (Lyovapor L-200; BÜCHI Labortechnik AG, Flawil, Switzerland) and then, the lipid fraction was extracted using a Randall apparatus (SER 148 extraction system, Velp Scientifica srl, Usmate, Italy) according to the method AOAC 945.38F (AOAC, 2006), with diethyl ether as extracting solvent. The total polar compounds consisting of triacylglycerol oligopolymers (TAGP), oxidized triacylglycerols (ox-TAG) and diacylglycerols (DAG) were determined by high-performance size-exclusion chromatography according to the method described by Rodríguez et al. (2021). The determination was also carried out on the wheat gluten and the emulsions used for extrusion (data shown in Supplementary Table S6). The analysis was repeated two times. The results were expressed as g/100 g extracted oil.

## 2.6. Sensory evaluation

The quantitative descriptive analysis of the meat analogs was carried out by a trained sensory panel of 8 people (6 men and 2 women), according to the ethical guidelines of the laboratory of Food Science and Technology of the Department of Soil, Plant and Food Science (Di.S.S.P. A., University of Bari, Bari, Italy). The panelists already had experience in sensory analysis of extruded products and did not suffer from any food intolerances or allergies. They were informed about the aims of the study and signed an individual written informed consent. A training session was conducted in order to familiarize them with the descriptors best suited to the products. Verbal descriptors were used to explain how to interpret the scales, and reference samples such as lean boiled pork meat, fried bacon or pulled pork were used to better understand the anchors. Specifically, the descriptors, scale anchors and reference samples used for each parameter are reported in Table 2, and they were selected according to previous works (De Angelis et al., 2020; Zhang et al., 2024) with the aim of understanding the effect of oils on the presence of off-odor and evaluating the texture of the products. The sensory assessment was carried out serving the panelists 5 g of samples codified by a three-digit alphanumeric code in randomized order, and the intensity of each descriptor was evaluated using a 10-point structured scale ranging from 0 to 9.

**Table 2**Sensory attributes, definition and scale anchors for the evaluation of meat analogs.

Descriptor	Definition	Scale anchors (0–9)
Visual/tactil	e aspect	
Fattiness	Perception of oil/fat on the surface of the product	0: no perception (lean and boiled pork meat) - 3: weakly perceived - 6: clearly perceived - 9: intensively perceived (fried bacon with visible oil on the surface)
Tenderness	Sensation related to how easily the product splits in the hands (when pulled)	0: extremely cohesive and compact (lean and boiled pork meat) - 3: difficult to split - 6: easy to split - 9: very easy to split (pulled pork)
Odor		
Rancidity	Perceived intensity of rancidity	0: no perception - 3: weakly perceived - 6: clearly perceived - 9: intensively perceived (rancid olive oil)
Texture/flav	or upon tasting	
Hardness	Force applied during chewing to break the product	0: soft (almost no effort required, similar to tofu) - 3: easy to compress - 6: moderately hard to compress - 9: very hard to compress (lean and boiled pork meat)
Juiciness	Sensation of moisture/liquids released during chewing	0: no fluids released (lean and boiled pork meat) - 3: small volume of fluids released - 6: discrete volume of fluids released - 9: large volume of fluids released during chewing (pulled pork)
Fattiness	Sensation of fattiness in the mouth	0: no perception (lean and boiled pork meat) - 3: weakly perceived - 6: clearly perceived - 9: intensively perceived (fried bacon with visible oil on the surface)
Fibrousness	Sensation related to the presence of elongated or fibrous structures during chewing	0: no fibers perceived (finely ground meat) 3: low perception of fibers - 6: clear perception of fibers - 9: strong perception of fibers (pulled pork)
Astringency	Puckering sensation in mouth/tongue	0: no perception - 3: weakly perceived - 6: clearly perceived - 9: intensively perceived (grape seeds)

## 2.7. Statistical analysis

OriginPro 2020 software (version 9.7.) from OriginLab Corporation (Northampton, USA) was used for statistical analysis. Differences between the oil-containing samples and the control produced without oil addition in terms of extruder response, rheological and textural properties, polar compounds and sensory profile were evaluated using Dunnett's multiple comparisons at p < 0.05. For the oil-containing samples, data were subjected to two-way ANOVA with Tukey's HSD post hoc test for multiple comparisons of means at p < 0.05 with oil type and oil concentration and their interaction as factors to identify significant differences between samples.

## 3. Results and discussion

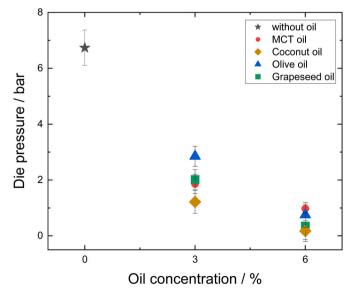
## 3.1. Effect of oil concentration and oil type on the die pressure

In the extrusion trials, different oils were added in the form of emulsions. All emulsions had narrow droplet size distributions with median droplet sizes between 1 and 3  $\mu m$  (data reported in

Supplementary Figure S2 a). During extrusion, the material temperature at the end of the barrel was adjusted to 135 °C for all oil types and concentrations, ensuring that all oils were exposed to the same maximum material temperature (data reported in Supplementary Figure S2 b). Fig. 1 shows the die pressure as a function of oil concentration and oil type. The results of the statistical analysis of the effect of oil type, oil concentration and their interaction on the die pressure are presented in Supplementary Table S3. As the oil concentration increases from 0% to 6%, the pressure drops significantly for all oil types. This observation corroborates previous findings (Kendler et al., 2021; Wang et al., 2022a). Die pressure in the extrusion process is a function of material viscosity, so a decrease in die pressure implies a decrease in viscosity. The material viscosity is dependent on die geometry, flow rate, material temperature and composition (Akdogan, 1999). As the geometry, material temperature and flow rate were kept constant, the change in die pressure can be related to the composition of the material, i.e., the different oil concentrations and oil types.

As the oil is added at the beginning of the extrusion process, it affects the cross-linking of the wheat gluten. Kendler et al. (2021) described that oil not only reduces the viscosity of the material by a diluting and plasticizing effect, but also reduces the cross-linking density of the wheat gluten due to a lower energy input during the extrusion process as a result of lubrication. The differences between the different oil types were significant and could be attributed to the fact that they differ in their chemical composition, e.g. degree of saturation and polarity, which could affect the lubrication in the extruder barrel, as well as how strongly they interact with the wheat gluten network, resulting in different rheological properties. In section 3.2, the effect of different oil types on rheological properties will be evaluated in more detail.

The effect of oils at different degrees of saturation on extruder response has not been previously investigated. Chen et al. (2022a) studied the effect of free fatty acids, i.e., stearic, oleic and linoleic acids, on various system parameters, i.e., die pressure, die temperature, torque and specific mechanical energy input (SME) during the extrusion of pea protein isolate. The authors reported that die pressure was lowest with linoleic acid and highest with stearic acid, which has the highest degree of saturation between them. In contrast, the opposite behavior was found in this study, with coconut oil, as an oil with a high proportion of saturated fatty acids, causing the lowest die pressure. However, comparing the results is rather difficult because of the different experimental set-ups used. Whereas Chen et al. (2022a) operated at constant



**Fig. 1.** Effect of oil concentration and oil type on die pressure during high moisture extrusion of wheat gluten. Total mass flow was 1 kg/h and water concentration was 51–53% for all samples.

barrel temperatures, resulting in different die temperatures in the process, in this study the barrel temperatures were adjusted to give constant die temperatures for all oil types. In addition, Chen et al. (2022a) used free fatty acids which differ significantly in structure, polarity and molecular weight from the triacylglycerols used in this study. Fatty acids are small molecules with low molecular weights. They can therefore act as plasticizers in highly concentrated wheat gluten, lowering its glass transition temperature and increasing its cross-linking density (Pommet et al., 2003). In contrast, triacylglycerols have no plasticizing effect on highly concentrated wheat gluten due to their much higher molecular weights (Kalichevsky et al., 1992), making it difficult to compare results obtained with free fatty acids and different vegetable oils.

To date, knowledge of the effect of vegetable oils with different levels of unsaturation on extruder response is still lacking in the literature. However, as the results of this study show, the type of oil has a significant effect on the overall extrusion process, highlighting the need for further research in this area.

## 3.2. Rheological and textural properties of the extrudates

Strain sweeps were performed to evaluate the effect of different oil types and oil concentrations on the deformability and strength of the samples. In Fig. 2 (a) the storage modulus G' and the loss modulus G" of the samples containing 0%, 3% and 6% coconut oil are plotted as a function of strain. The strain sweeps of the samples containing MCT, olive and grapeseed oil show similar behavior and are shown in Supplementary Fig. S4. For all samples, the storage modulus G' is higher than the loss modulus G" in the linear viscoelastic (LVE) region. This is characteristic of meat analogs, which consist of highly concentrated protein networks and exhibit solid-like elastic behavior (De Angelis et al., 2023; Opaluwa et al., 2024; Wittek et al., 2021). Furthermore, the control sample without oil showed the highest values of G' and G". As the oil content increases, the absolute values of the moduli, especially G', decrease significantly, indicating a reduction in viscosity. In the LVE region, G' is independent of strain as the applied strain causes a reversible deformation of the protein network. Once a critical strain is exceeded, the deformation is no longer reversible and G' and G" decrease significantly due to strain thinning behavior (Hyun et al., 2011). Stress and strain at the end of the LVE region, defined as critical stress and critical strain, were determined for all samples and plotted as a texture map in Fig. 2 (b). The results of the statistical analysis of the effect of oil type, oil concentration and their interaction on the critical stress and strain of meat analogs are presented in Supplementary Table S5.

Looking at the effect of oil concentration, the control sample without oil exhibits significantly higher critical stress values compared to the oil-containing samples. As the oil concentration increases from 3% to 6%, the critical stress values decrease significantly for all samples regardless

of oil type, resulting in more mushy extrudates. These results are in line with the decrease in die pressure described in the previous section. A reduction in storage modulus has also been previously reported in the literature by Wang et al. (2022a) when maize oil was added to soy protein/wheat gluten extrudates and by Saavedra Isusi et al. (2023) when rapeseed oil was added to pea protein extrudates. The reduction in critical stress can be attributed to a dilution of the high viscosity gluten matrix due to a reduction in protein concentration per unit volume. In addition, the lower critical stress of the oil-containing samples may be explained by a lower cross-linking density of the gluten network. As the oil acts as a lubricant during extrusion, the reduced friction reduces polymerization and cross-linking of the wheat gluten, resulting in more mushy extrudates (Kendler et al., 2021).

Regarding the effect of oil type, no significant differences were found between the samples, except for the sample containing coconut oil, which has significantly lower critical stress values. These results are consistent with the extruder response described above, where coconut oil causes the highest pressure drop during extrusion processing. By combining CLSM imaging and rheological measurements, Opaluwa et al. (2024) found that oil droplet size, rather than the oil type (MCT, rapeseed or purified rapeseed oil), significantly influenced the rheological behavior of highly concentrated wheat gluten doughs under extrusion-relevant conditions. This may suggest that the similar critical stresses found for samples containing MCT, olive and grapeseed oil (Fig. 2, b) derive from similar oil droplet sizes. However, to confirm this hypothesis further analysis such as CLSM imaging would be required to determine the oil droplet size distributions of the extrudates. The fact that coconut oil is the only oil that solidifies at room temperature, which may affect the size and shape of the oil droplets formed in the extrudates, may explain why coconut oil gives significantly different stress values to the other oils. However, further studies on the size distribution and shape of the oil droplets would be required to confirm this hypothesis.

In addition to the critical stress, the critical strain provides further insight into the properties of the wheat gluten network of the extrudates. The critical strain is used as a measure of the deformability and structural strength of a network. The deformability of a protein network is determined by the cross-linking density and pore size, as well as the homogeneity of the network. Homogeneous and elastic networks can resist large deformations before irreversible changes in the material occur, which is reflected in high critical strains (Hyun et al., 2011; Ross-Murphy, 1995). When oil is added to a protein network, these oil droplets can act as defects and inhomogeneities in the protein matrix and thus be the starting point for irreversible deformation, leading to lower critical strains as the oil concentration increases (Opaluwa et al., 2024; Sala et al., 2009). However, this trend is only partially reflected in this study depending on the oil type. No significant difference in critical strain was found between the control sample without oil and the samples with oil. When increasing from 3% to 6% oil, a slight decrease in critical

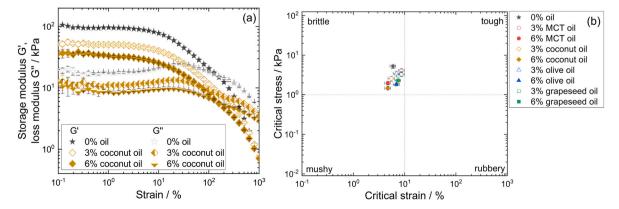


Fig. 2. (a) Storage modulus (G') and loss modulus (G'') as a function of strain for samples containing 0%, 3% and 6% coconut oil. (b) Texture map showing the critical stress and strain at the end of the LVE region for samples containing 0%, 3% and 6% MCT, coconut, olive and grapeseed oil.

strain was observed for all oil types. However, the differences in critical strain were only significant for the samples containing MCT oil, whereas they were not significant for the samples containing coconut, olive and grapeseed oil. Although oil droplets can cause network inhomogeneities and thus reduce the critical strain of the extrudates, the oil also leads to a lower cross-linking density of the gluten network due to the lubrication during processing, thus providing higher elasticity and deformability compared to the sample without oil. These opposing effects may explain why the critical strain in this study was not found to be a linear function of oil concentration.

The effect of oil type, oil concentration and their interaction on the textural properties of meat analogs is reported in Table 3. The meat analogs without oil showed the highest firmness and chewiness, indicating that these products require a higher mechanical energy input during mastication compared to those containing oil. As the oil concentration increased, the firmness and chewiness of the extrudates decreased significantly. Consequently, the products containing 6% oil were characterized by the lowest firmness and chewiness. This finding is consistent with the literature (Saavedra Isusi et al., 2023; Wang et al., 2022a) and also with the previous results of the rheological evaluation, which indicated a reduction of the critical stress in oil-containing samples.

Oil type also had a significant effect on textural properties, with both MCT oil and grapeseed oil imparting a softer texture compared to

Table 3
Results of texture profile analysis of meat analogs containing different oil types (MCT, coconut, olive and grapeseed) and oil concentrations (3% and 6%). Each section reports the mean value, standard deviation and results of two-way ANOVA considering oil type, oil concentration and their interactions.

	Firmness/N	Springiness	Chewiness/ N	Cohesiveness
0% oil	444 ±	$0.83\pm0.01^{\text{a}}$	$208\pm13.6^{\text{a}}$	$0.56\pm0.03^{\text{a}}$
	23.1ª	0.00	70.4	0.55   0.01
3% MCT oil	160 ±	0.80 ±	70.4 ±	$0.55 \pm 0.01d^{\circ}$
604 3 5 0m . H	8.39bc	0.04b <sup>a</sup>	4.69c	
6% MCT oil	73.8 ±	$0.83 \pm 0.02$	29.2 ±	$0.48\pm0.02e$
	1.63e	ab <sup>a</sup>	2.00e	
3% coconut oil	169 ±	$0.83 \pm 0.02$	85.7 ±	$0.61 \pm$
	9.11b	ab <sup>a</sup>	6.34b	0.01bc <sup>a</sup>
6% coconut oil	$103~\pm$	$0.81~\pm$	54.2 $\pm$	$0.65 \pm 0.02b$
	4.73d	0.03b <sup>a</sup>	2.42d	
3% olive oil	$237~\pm$	$0.89~\pm$	$126\pm11.4\mathrm{a}$	$0.60 \pm$
	11.2a	0.04a <sup>a</sup>		0.02cd <sup>a</sup>
6% olive oil	86.6 $\pm$	$0.85\pm0.03$	54.7 $\pm$	$0.74 \pm 0.03a$
	5.24de	ab <sup>a</sup>	3.03d	
3% grapeseed oil	$151~\pm$	$0.84\pm0.01$	$68.9 \pm$	$0.54 \pm 0.04d$
	9.07c	ab <sup>a</sup>	5.88c	
6% grapeseed oil	101 $\pm$	$0.22\pm0.05c$	17.2 $\pm$	$0.77\pm0.02a$
	7.05d		2.91e	
Oil type ×	p < 0.001	p < 0.001	p < 0.001	p < 0.001
concentration				
MCT oil	$117~\pm$	$0.81\pm0.03b$	49.8 $\pm$	$0.52 \pm 0.04c$
	46.2c		22.2c	
Coconut oil	$136 \pm$	$0.82 \pm 0.02b$	69.9 $\pm$	$0.63 \pm 0.03b$
	36.3b		17.4b	
Olive oil	$162 \pm$	$0.87 \pm 0.04a$	90.2 $\pm$	$0.67 \pm 0.08a$
	81.0a		38.7a	
Grapeseed oil	126 ±	$0.53 \pm 0.33c$	43.0 ±	$0.66 \pm 0.13$
	27.9bc		27.9c	ab
Oil type	p < 0.001	p < 0.001	p < 0.001	p < 0.001
3%	179 ±	$0.84 \pm 0.04a$	87.6 ±	$0.58 \pm 0.04b$
	36.2a		24.6a	
6%	90.9 $\pm$	$0.68 \pm 0.27 b$	38.8 $\pm$	$0.66\pm0.12a$
	12.9b		16.9b	
Oil concentration	p < 0.001	p < 0.001	p < 0.001	p < 0.001

Different letters for the same parameter indicate significant differences between oil-containing samples according to two-way ANOVA with interaction at p < 0.05 followed by Tukey's test.

coconut oil and olive oil. This result was partly unexpected as previous reports suggested that the addition of fatty acids with a high degree of unsaturation, such as linoleic acid (C18:2), resulted in a greater reduction in firmness and chewiness compared to stearic and oleic acid (Chen et al., 2022a), suggesting that a higher concentration of polyunsaturated fatty acids results in softer products. This emphasizes the importance of working with oils that contain mixtures of triacylglycerols with different fatty acids, providing valuable insights for real case applications. In addition, it should be noted that the stearic acid (C18:0) used by Chen et al. (2022a) is typically found at very low levels in food-grade oils, with saturated fatty acids usually represented by other acids such as capric (C10:0), lauric (C12:0), myristic (C14:0) and palmitic acid (C16:0) (Giakoumis, 2018).

Interestingly, the addition of oil resulted in products with similar springiness and cohesiveness to the control sample without oil, except for the meat analog containing grapeseed oil at 6% concentration. This suggests that although the extrudates had a softer texture compared to the control sample without oil, they retained high springiness and elasticity. The previous results on critical strain also showed only slight changes in elasticity and deformability of meat analogs with different oil concentrations and oil types, which supports these results. Firmness of meat analogs, together with juiciness, is one of the most important quality parameters of the products (Xu and Falsafi, 2024). In fact, lower firmness correlates with greater tenderness in meat analogs, potentially resulting in increased juiciness (Xu and Falsafi, 2024). Therefore, the results obtained with the addition of oil could improve the textural properties of the products by reducing the firmness. At the same time, a detailed discussion of the sensory perceptions is given in section 3.5. Overall, the combination of both rheological and textural measurements provided a detailed characterization of the structural and microstructural behavior of the products as influenced by different oil types at different concentrations, underlining the importance of performing more than one test to evaluate the properties of the product, as already reported by De Angelis et al. (2023).

#### 3.3. Tribological properties of the extrudates

Many studies have characterized the tribological properties of different food systems, such as milk, mayonnaise or bread, and found a good correlation with their sensory properties, e.g. the perception of creaminess and juiciness (Chojnicka-Paszun et al., 2012; Kiumarsi et al., 2019; de Wijk & Prinz, 2005). However, only one recent study has focused on the tribological properties of commercial sliced sausages and sausage alternatives (Ghebremedhin et al., 2022). This study did not include an additional sensory evaluation. There is still a great lack of knowledge about the extent to which the addition of oil can improve the sensory properties of meat analogs, such as the juiciness, and whether the instrumental tribological measurements can be correlated with sensory perception. Therefore, in this study, both tribological and sensory properties were evaluated to assess the effect of different oil concentrations and oil types on wheat gluten meat analogs.

In this study, the friction behavior was determined directly on the intact meat analog surface. Thus, the results correspond to the first touch mouthfeel and should additionally provide information on the degree of incorporation of the oils into the protein matrix. Fig. 3 shows friction curves plotting the friction coefficient against the sliding viscosity for samples containing 0%, 3% and 6% (a) MCT oil, (b) coconut oil, (c) olive oil and (d) grapeseed oil. At low sliding velocities the samples are still intact, and the friction coefficient reflects the initial lubricating properties of the samples. At higher speeds of the measuring geometry, particles are removed from the surface, so that not only the frictional properties of the surface are measured, but also those of the deeper layers and the friction caused by the removed particle-lubricant mixture. In the friction curves of all samples in Fig. 3(a–d), this effect is reflected in small plateaus or kinks at sliding velocities in the range between 4 and 10 mm/s. Ghebremedhin et al. (2022), who studied the tribological

<sup>&</sup>lt;sup>a</sup> The value is similar to the control (0% oil) according to the Dunnett's test (p > 0.05).

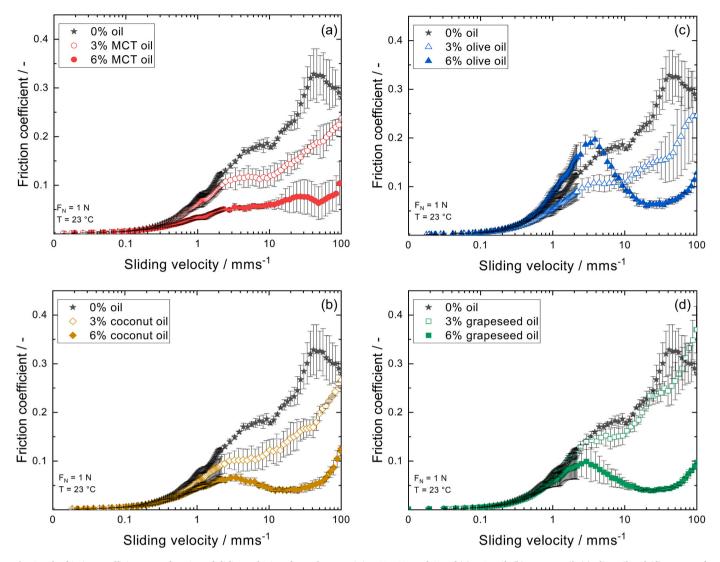


Fig. 3. The friction coefficients as a function of sliding velocity of samples containing 0%, 3% and 6% of (a) MCT oil, (b) coconut oil, (c) olive oil and (d) grapeseed oil. Normal force ( $F_N$ ) was set at 1 N and temperature at 23 °C.

properties of sausage and sausage alternatives, also reported abrasion of particles from the surface during the measurement.

In Fig. 3 (b), it can be seen that at sliding velocities of less than 1 mms<sup>-1</sup>, the 0%, 3% and 6% coconut oil samples show similar behavior, i.e., an increase in the friction coefficient. At a sliding velocity of 2 mm/ s, the 6% coconut oil sample reaches a small plateau, followed by the 3% coconut at about 4 mm/s and finally the 0% sample at about 10 mm/s. These plateaus mark the sliding velocity at which particle abrasion begins. The fact that abrasion starts first with the 6% coconut oil sample, then with the 3% coconut oil sample and finally with the 0% oil sample agrees well with the rheological results in section 3.2 which showed that the 6% coconut oil sample is more mushy and can withstand lower critical stresses and strains before irreversible deformation occurs. After reaching the plateaus, the three samples show remarkably different behavior. The 0% oil sample shows a further increase in the friction coefficient due to the friction caused by the abraded particles. This may reflect a dry mouthfeel during mastication rather than a juicy perception of the meat analog. A different behavior can be observed for the samples containing oil. After the plateau, the friction coefficients for both samples are significantly lower than for the 0% oil sample. The sample with 6% coconut oil even shows a decrease in the friction coefficient after the surface particles have been abraded. This can be explained by the fact that the oil encapsulated in deeper layers is now exposed. As oil is known

to be a very effective lubricant, it is reasonable to assume that the sample with the higher oil concentration would have the highest lubrication. Whether this higher lubrication is also reflected in the sensory perception as juiciness will be assessed in section 3.5.

The samples with different oil types (Fig. 3) show a similar behavior to the samples with coconut oil, i.e., an increase in lubrication with increasing oil concentration at higher sliding velocities, with some differences to be highlighted. For example, the sample with 6% olive oil shows a particularly high friction coefficient at intermediate sliding velocities (1–10 mms<sup>-1</sup>), which is even higher than that of the control sample without oil. This indicates that particle abrasion is increased in the 6% olive oil sample compared to the other oil types. At sliding velocities above 10 mms<sup>-1</sup>, the friction coefficient decreases significantly, indicating that oil from deeper layers is now exposed, resulting in strong lubrication of the system. The samples containing grapeseed oil also show slight differences, i.e., the sample with 3% grapeseed oil (see Fig. 3 d) has a very similar friction coefficient to the sample with 0% oil. Contrary to the other samples with 3% oil, there is no decrease in the friction coefficient with increasing oil concentration. This result suggests that the grapeseed oil is better encapsulated and bound to the protein matrix, resulting in less free oil after particle abrasion. This would be consistent with the results of Liu et al. (2015), who reported that emulsion-filled gels with bound oil droplets had higher friction

coefficients than those with unbound oil droplets. It is possible that the grapeseed oil has a higher compatibility with the protein matrix, resulting in better encapsulation, leading to less surface oil on the extrudate.

To gain further insight, measurements of oil encapsulation efficiency, determined by solvent extraction of unencapsulated surface oil, may be useful. Saavedra Isusi et al. (2023) found that the encapsulation efficiency of rapeseed oil in extrudates differed significantly for different types of protein, i.e., soy and pea protein. They attributed this to the different compatibility and affinity of the proteins to the rapeseed oil due to the different chemical composition of the proteins. It is therefore reasonable to expect that wheat gluten will also have different affinities for oils of different chemical composition and degree of unsaturation.

#### 3.4. Polar compounds of the lipid fraction

Polar compounds are molecules with a higher polarity than unaltered triacylglycerols (TAG) and are formed as a result of lipid degradation reactions. Therefore, their content can give an overall idea of the oxidative phenomena that have occurred in the products. Specifically, diacylglycerols (DAG) are formed by the hydrolytic degradation of triacylglycerols (Chen et al., 2021), while oxidized triacylglycerols (ox-TAG) are formed by the interaction between radicals and hydrogen during the primary oxidation reactions. Finally, triacylglycerol oligopolymers (TAGP) are products of advanced secondary reactions that occur due to oxidative and thermal alterations (Caponio et al., 2007; Chen et al., 2021; Rodríguez et al., 2021). The results for polar compounds determined on the lipid fraction extracted from the meat analogs are presented in Table 4. The effect of oil type and oil concentration was always significant for all classes of polar compounds.

Regarding the influence of oil type, the highest presence of polar compounds was found in grapeseed oil, followed by olive, coconut and MCT oil extracted from the meat analogs. However, these differences were already evident in the oils used for the emulsion preparation and consequently in the raw mixes (Supplementary Table S1). Considering the fatty acid composition of the oils, a high concentration of polar compounds was expected in the mixes containing grapeseed oil due to the higher presence of polyunsaturated fatty acid, i.e., linoleic acid, compared to the other oils (Supplementary Table S1). Indeed, polyunsaturated fatty acids are prone to oxidation reactions (Ben Hammouda et al., 2018; Chen et al., 2021). In contrast, MCT oil composed of only saturated fatty acids is known to have very high oxidative stability (Nimbkar et al., 2022), suggesting its potential to minimize oxidative processes during product storage. This could be the subject of further investigation in the field. In addition to the fatty acid composition, it should be considered that refined oils, such as the olive oil or grapeseed oil used in this study, are usually characterized by higher concentrations of polar compounds compared to virgin oils, as the refining process leads to oxidation and polymerization phenomena (Chen et al., 2021).

It is interesting to note that there is no standardized or established regulation regarding the content of polar compounds in oils. Usually, only the total content of polar compounds is used as a threshold to delimit the non-conformity of edible vegetable oils for frying. According to Chen et al. (2021), this threshold is between 24 and 27 g/100 g, while some countries also set limits for TAGP in the range of 10-16% (Chen et al., 2021). These values are considerably higher than those found in extruded meat analogs. Interestingly, a recent study by Yuan et al. (2021) suggests that the total polar compound contents, as proposed in the current regulations, may be an inaccurate indicator for assessing oil degradation, as TAGP, DAG and ox-TAG have different health effects, with ox-TAG having the strongest cytotoxic effect. This emphasizes the need for additional research on the occurrence and formation of polar compounds or other harmful compounds in meat analogs, as insufficient evidence can be found in the literature (Billek, 2000; Chen et al., 2021; Ju et al., 2019). Furthermore, from a technological point of view, their pro-oxidant role may raise concerns regarding the shelf-life of the

Table 4

Polar compounds determined on the lipid fraction extracted from the meat analogs containing different oil types (MCT, coconut, olive, and grapeseed) and oil concentrations (3% and 6%). Each section reports the mean value, standard deviation, and results of two-way ANOVA considering oil type, oil concentration and their interactions. Data are expressed as g/100 g of extracted oil. TAGP, triacylglycerol oligopolymers; ox-TAG, oxidized triacylglycerols; DAG, diacylglycerols.

under 1819 cerois.				
	TAGP	ox-TAG	DAG	$\begin{array}{l} {\rm TAGP} + {\rm ox} \\ {\rm TAG} \end{array}$
0% oil	0.07 ±	3.53 ±	4.87 ±	$3.60 \pm 0.08^{a}$
	0.01	$0.08^{a}$	$0.03^{a}$	
3% MCT oil	0.13 $\pm$	$1.93 \pm$	$2.55 \pm$	2.07 $\pm$
	0.00f	0.02d	0.01cd	0.02ef
6% MCT oil	$0.09 \pm$	$1.45 \pm$	$1.95 \pm$	$1.54 \pm 0.02 g$
	0.00g	0.02e	0.01d	
3% coconut oil	$0.17~\pm$	2.26 $\pm$	2.86 $\pm$	$2.43\pm0.04\textrm{d}$
	0.00e	0.03c	0.06c	
6% coconut oil	0.10 $\pm$	1.77 $\pm$	$1.89~\pm$	$1.87 \pm 0.03 f$
	0.00g	0.03d	0.04d	
3% olive oil	0.42 $\pm$	2.43 $\pm$	4.82 $\pm$	$2.85\pm0.06c$
	0.00c	0.05c	0.01b <sup>a</sup>	
6% olive oil	0.34 $\pm$	$1.89~\pm$	4.19 $\pm$	2.24 $\pm$
	0.01d	0.16d	0.03b <sup>a</sup>	0.17de
3% grapeseed oil	0.77 $\pm$	3.65 $\pm$	8.64 $\pm$	$4.42\pm0.00a$
	0.00b	0.00a <sup>a</sup>	0.01a	
6% grapeseed oil	$0.80~\pm$	$2.89~\pm$	8.43 $\pm$	3.68 $\pm$
	0.00a	0.15b	0.85a	$0.15b^a$
Oil type $\times$	p < 0.001	p = 0.029	p = 0.228	p = 0.197
concentration .				
MCT oil	0.11 $\pm$	$1.69~\pm$	$2.25~\pm$	$1.8 \pm 0.29 d$
	0.03d	0.26d	0.33c	
Coconut oil	0.13 $\pm$	2.02 $\pm$	$2.37~\pm$	$2.15\pm0.31c$
	0.04c	0.27c	0.53c	
Olive oil	0.38 $\pm$	2.16 $\pm$	4.50 $\pm$	$2.54 \pm 0.36b$
	0.05b	0.31b	0.34b	
Grapeseed oil	0.79 $\pm$	3.27 $\pm$	8.53 $\pm$	$4.05\pm0.41a$
	0.01a	0.43a	0.55a	
Oil type	p < 0.001	p < 0.001	p < 0.001	p < 0.001
3%	0.37 $\pm$	2.57 $\pm$	4.71 $\pm$	$2.94 \pm 0.94a$
	0.27a	0.69a	2.53a	
6%	0.33 $\pm$	2.00 $\pm$	4.11 $\pm$	$2.33 \pm 0.86b$
	0.30b	0.57b	2.80b	
Oil concentration	p < 0.001	p < 0.001	p < 0.001	p < 0.001

Different letters for the same parameter indicate significant differences between oil-containing samples according to two-way ANOVA with interaction at p < 0.05 followed by Tukey's test.

## products (Caponio et al., 2007).

Considering the effect of oil concentration, a significantly lower concentration of polar compounds was observed in the extrudates with 6% oil compared to those with 3% oil. This result could be attributed to a combination of factors, on the one hand to the lower concentration of polar compounds in the mixes with 6% oil added and on the other hand to the overall lower stress experienced by the material during extrusion, due to lower viscosities and increased lubrication.

It is noteworthy that the lipid fraction of the wheat gluten used in this study was characterized by a high presence of ox-TAG. The formation of such compounds could be hypothesized to occur during the manufacturing process, especially due to the high temperatures used for protein drying (Kaushik et al., 2015), or possibly due to prolonged storage (Chen et al., 2021). This aspect underlines the importance of better investigating the quality of the protein ingredients used for the development of plant-based foods. Indeed, other authors had also reported the lack of sufficient knowledge and studies in this rapidly developing sector (Wickramasinghe et al., 2021).

During thermal treatment, ox-TAG can be newly formed and existing ox-TAG can polymerize to form TAGP. Therefore, the sum of TAGP and ox-TAG must be considered to assess the oxidation that occurs in the

 $<sup>^{\</sup>rm a}$  The value is similar to the control (0% oil) according to the Dunnett's test (p > 0.05).

lipid fraction due to extrusion processing (Caponio et al., 2007). The percentage increase observed in the extruded products compared to the raw mixes is shown in Fig. 4. In all extrudates there was a percentage increase in the sum of TAGP and ox-TAG. This increase can be attributed to the thermomechanical treatments during extrusion, as it is known that oxidation reactions are favored by the combination of high temperatures, mechanical stresses from the screw and the presence of moisture (Amft et al., 2019; Lampi et al., 2015).

When comparing different oil types, the percentage increase rises with the degree of unsaturation of the oils, as oxidation reactions are a function of the degree of unsaturation of the fatty acids, and the rate of oxidation increases with the number of double bonds in the fatty acids (Choe and Min, 2006). Oxidation phenomena during extrusion have been reported for peanut oil extruded with wheat gluten at different temperatures (Chen et al., 2022). However, to the best of the author's knowledge, the formation of polar compounds during high moisture extrusion with different oil types has not been investigated. The results presented in Fig. 4 suggest that using oils with a higher degree of saturation can help to limit the degradation phenomena of the lipid fraction during high moisture extrusion. Nevertheless, it needs to be considered that lipids with a high content of saturated fatty acids, which are predominant in animal-derived fats, are known to have negative effects on human health (Ruiz-Núñez et al., 2016). For this reason, coconut oil, which is widely used in the formulation of meat analogs and is high in saturated fatty acids, is a cause for concern (Boemeke et al., 2015; Yang et al., 2023). This motivates the need to investigate and develop healthier alternatives. In this respect, the incorporation of MCT oil could be particularly advantageous, not only due to its high resistance to lipid oxidation, but also due to its recognized benefits for human health, including positive effects on body weight, cholesterol-lowering effects, benefits for neurological and gastrointestinal disorders (Nimbkar et al., 2022). Furthermore, the use of oils extracted without refining processes, such as extra virgin olive oil, which are also rich in antioxidant compounds, may offer advantages in mitigating oxidation phenomena and developing healthier solutions. This could be explored in further research.

In addition, there is a lack of research on potential interactions between polar compounds derived from lipid oxidation and proteins. In this respect, the reactive oxygen species formed during oxidative phenomena could interact with proteins and subsequently oxidize them. This could lead to protein aggregation and disulfide bonding, altering secondary structures and exposing internal hydrophobic groups (Geng

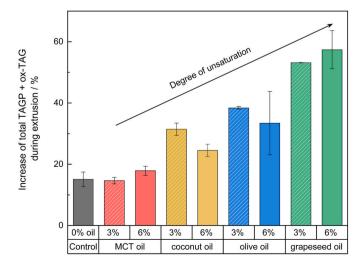


Fig. 4. Percentage increase in the sum of triacylglycerol oligopolymers and oxidized triacylglycerols (TAGP + ox-TAG) extracted from the extrudates (Table 4) compared to the raw mixes (data reported in Supplementary Table S6).

et al., 2023; Guo et al., 2022), with potential effects on overall textural properties. This represents an interesting and valuable subject for future studies.

The variation in the concentration of polar compounds in the extrudates provides valuable insights into the lipid stability of the products over time. Indeed, previous studies have indicated that lipid stability is influenced by the initial quality of the lipid and the intensity of the treatment during extrusion (Ying et al., 2015). Consequently, if the oil used in the product is already oxidized, it will be more susceptible to oxidative phenomena. In fact, TAGP and ox-TAG demonstrated a pro-oxidant activity (Gomes et al., 2008), suggesting that monitoring the quality of the lipid fraction of the raw material is crucial to guarantee the quality of the products.

#### 3.5. Sensory evaluation

The results of the quantitative descriptive analysis of the most relevant sensory attributes of the meat analogs are presented in Fig. 5. A complete overview of all attributes, including the results of the statistical analysis of oil type, oil concentration and their interaction, is presented in Supplementary Table S7. During the visual evaluation, the products containing oil were always perceived as fattier than the control sample, with scores close to 5-7 indicating a clear perception of this attribute. Moreover, the products containing oil were perceived as more tender compared to the control sample without oil, indicating a tendency to split more easily when pulled by hand. This observation could be related to the results of the rheological evaluation, which showed a more pronounced mushy behavior in the samples with oil, suggesting that the sensory perception of tenderness could be related to a lower critical stress of the products. According to these results, the extrudates containing oil were rated as less hard compared to the control, except for the samples containing 3% coconut oil and 3% olive oil, which were similar to the control. The type of oil and its concentration had a significant effect on the tenderness of the products, which was higher in extrudates containing 6% oil than in extrudates containing 3% oil. Moreover, products containing MCT oil and grapeseed oil showed the highest tenderness. This result was in line with the results of the texture analysis that showed the lowest firmness and chewiness for these samples.

In terms of juiciness and fat perception during chewing, all oilcontaining samples showed slightly higher values compared to the control sample. However, the effect of oil type, oil concentration or their interaction was not significant, and the overall scores were low, with values below 3, indicating a low amount of liquid released during chewing. This indicates that the results of the tribological evaluation, which showed a remarkable increase in lubrication with increasing oil concentration for all samples except grapeseed oil, correlate only to a limited extent with the actual sensory perception of juiciness and fattiness. The results suggest the need for further research into strategies to enhance the juiciness perception of these types of products. For example, further increasing the oil concentration to higher levels may enhance the perception of juiciness (Zhang et al., 2024). It should be noted that the extrudates produced in this research are only intermediate products, which will be further processed by the addition of several other ingredients for the development of plant-based meat analogs. The cooking process, and therefore the serving temperature, may also affect sensory perception, particularly the juiciness (Zhang et al., 2024).

The low juiciness perception may also be related to the high astringency of the products, which was always rated close to or above 7, indicating a clear and intense perception. Indeed, astringency is described as a puckering and dryness sensation (De Angelis et al., 2020; Wang et al., 2022b) and is commonly perceived in plant proteins such as pea and soy (Wang et al., 2022b). As the astringency was also perceived in the control, it can be hypothesized that it is derived from the wheat gluten. The literature suggests that polyphenols, tannins, proteins, organic acids and salts are the compounds responsible for astringency

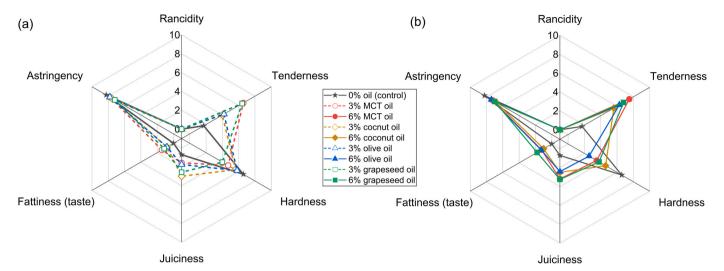


Fig. 5. Radar chart describing the results of the quantitative descriptive analysis of the meat analogs containing different oil types at concentrations of (a) 3% and (b) 6%.

(Pires et al., 2020). Additionally, previous studies suggest that compounds derived from lipid oxidation may contribute to the bitter taste of products (Gläser et al., 2021; Molteberg et al., 1996), although this was not evaluated in the present study. In particular, Molteberg et al. (1996) reported a positive correlation between volatile compounds from lipid oxidation and astringency, suggesting a potential effect. Overall, the lack of definitive information on the role of oxidation compounds in astringency highlights the need for further research.

The presence of fibrous structures was rated low in all samples. Remarkably, rancidity was not perceived in any of the samples, which is an important indication that the oil addition did not cause any off-flavor or off-odor related to lipid oxidation. In fact, aldehydes formed during advanced oxidation processes are known to contribute to off-odor development (Neugebauer et al., 2020). Such molecules may not be generated during extrusion but could potentially be formed even at room temperature or during storage. This issue is particularly complex due to several factors, including the amount of oil used, the composition of the original oils—which determines their susceptibility to oxidation—and their oxidative status, including the presence of pro-oxidant compounds. These aspects underscore the need for further research on meat analogs.

## 4. Conclusion

Vegetable oils are commonly added during the high moisture extrusion of meat analogs with the intention of improving their quality, particularly by increasing tenderness and juiciness. However, the potential effects of extrusion processing on lipid degradation, which can adversely affect both the nutritional value and sensory properties of meat analogs, often receive minimal attention. This study evaluates the effect of vegetable oils with different degrees of unsaturation and different oil concentrations on extruder response and the resulting physicochemical and sensory properties of meat analogs.

Increasing the oil concentration resulted in a decrease in die pressure during high moisture extrusion for all oil types, which was attributed to the viscosity reducing effect of the oils. This was in line with the rheological, textural and sensory evaluation of the extrudates, which showed that storage modulus, firmness and chewiness decreased, while tenderness increased significantly with increasing oil concentration. The sensory perception of juiciness and fattiness increased only slightly and not significantly with increasing oil concentration, which was rather unexpected given the results of the tribological measurements, which showed a distinct reduction in friction with increasing oil concentration. However, high astringency was found in all samples, which may have

impaired the sensory perception of juiciness. Considering the high astringency perceived in the control sample without oil, it was hypothesized that the astringency was mainly caused by wheat gluten, underscoring the importance of raw material quality. While oil type had no significant effect on the rheological properties of the extrudates, it had a major influence on lipid degradation during extrusion. Vegetable oils with a higher degree of unsaturation, such as grapeseed oil, were more susceptible to lipid degradation and the formation of polar compounds. No off-flavors, such as rancidity, were detected in any of the samples, indicating that lipid oxidation did not negatively affect the sensory perception of the products.

However, it is important to consider that high levels of polar compounds resulting from oxidative degradation may have potential negative health implications. Moreover, their pro-oxidant role may negatively affect the shelf-life. These aspects, together with the effect of lipid oxidation on the structure formation of the products, emerged as valuable points for future research in the field.

When discussing the findings of this study, we conclude that the use of vegetable oil can contribute to the development of meat analogs with higher sensory quality, particularly by increasing their tenderness. This outcome can be exploited to develop products with improved textural characteristics to meet consumer expectations. However, we strongly encourage food developers to consider factors such as the oxidative stability of the products and the potential implications of raw material quality on their nutritional profile. In fact, while oil concentration has a stronger effect on textural and sensory properties, the oil type, particularly its degree of unsaturation, plays a significant role in the oxidative stability of lipids. Understanding the parameters that influence the sensory perception of juiciness has emerged as a key area for improving the overall quality of meat analogs.

## CRediT authorship contribution statement

Christina Opaluwa: Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. Davide De Angelis: Writing – original draft, Methodology, Investigation, Data curation. Carmine Summo: Writing – review & editing, Resources, Project administration. Heike P. Karbstein: Writing – review & editing, Resources, Project administration.

#### **Ethical Statement**

Hereby, I, Christina Opaluwa, consciously assure that for the manuscript ': Effect of different vegetable oils on extruded plant-based

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meat analogs: Evaluation of oxidative degradation, textural, rheological, tribological and sensory properties' the following is fulfilled:

- This material is the authors' own original work, which has not been previously published elsewhere.
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## **Declaration of competing interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix ASupplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodhyd.2024.111038.

## Data availability

Data will be made available on request.

## References

- Akdogan, H. (1999). High moisture food extrusion. *International Journal of Food Science and Technology*, 34(3), 195–207. https://doi.org/10.1046/j.1365-2621.1999.00256.
- Amft, J., Bauer, J. L., Rostek, J., Spielvogel, S., & Schwarz, K. (2019). Effect of water addition on the microstructure, lipid incorporation, and lipid oxidation of corn extrudates. European Journal of Lipid Science and Technology, 121(9), Article 1800433. https://doi.org/10.1002/ejlt.201800433
- AOAC. (2006). Official methods of analysis of AOAC international (18th ed.). Gaithersburg, MD, USA: AOAC International.

Ben Hammouda, I., Triki, M., Matthäus, B., & Bouaziz, M. (2018). A comparative study on formation of polar components, fatty acids and sterols during frying of refined olive pomace oil pure and its blend coconut oil. *Journal of Agricultural and Food Chemistry*, 66(13), 3514–3523. https://doi.org/10.1021/acs.jafc.7b05163

- Billek, G. (2000). Health aspects of thermoxidized oils and fats. European Journal of Lipid Science and Technology, 102(8-9), 587–593. https://doi.org/10.1002/1438-9312 (200009)102:8/9<587::AID-EJLT587>3.0.CO;2-#
- Boemeke, L., Marcadenti, A., Busnello, F. M., & Gottschall, C. B. A. (2015). Effects of coconut oil on human health. *Open Journal of Endocrine and Metabolic Diseases*, 5(7), 84–87. https://doi.org/10.4236/ojemd.2015.57011
- Bohrer, B. M. (2019). An investigation of the formulation and nutritional composition of modern meat analogue products. Food Science and Human Wellness, 8(4), 320–329. https://doi.org/10.1016/j.fshw.2019.11.006
- Caponio, F., Gomes, T., Pasqualone, A., & Summo, C. (2007). Use of the high performance size exclusion chromatography analysis for the measurement of the degree of hydrolytic and oxidative degradation of the lipid fraction of biscuits. *Food Chemistry*, 102(1), 232–236. https://doi.org/10.1016/j.foodchem.2006.04.047
- Cheftel, J. C., Kitagawa, M., & Quéguiner, C. J. F. R. I. (1992). New protein texturization processes by extrusion cooking at high moisture levels. Food Reviews International, 8 (2), 235–275. https://doi.org/10.1080/87559129209540940
- Chen, Y. P., Feng, X., Blank, I., & Liu, Y. (2022). Strategies to improve meat-like properties of meat analogs meeting consumers' expectations. *Biomaterials*, 287, Article 121648. https://doi.org/10.1016/j.biomaterials.2022.121648
- Chen, Y., Liang, Y., Tian, X., Wang, X., Liu, H., Jia, F., Zhang, X., & Wang, J. (2022). Effect of oil oxidation on aggregation of wheat gluten-peanut oil complexes during extrusion. *International Journal of Food Science and Technology*, 57(4), 2467–2478. https://doi.org/10.1111/jjfs.15611
- Chen, J., Zhang, L., Li, Y., Zhang, N., Gao, Y., & Yu, X. (2021). The formation, determination and health implications of polar compounds in edible oils: Current status, challenges and perspectives. Food Chemistry, 364, Article 130451. https://doi. org/10.1016/j.foodchem.2021.130451
- Chen, Q., Zhang, J., Zhang, Y., Liu, H., Li, T., Wang, Q., & Kaplan, D. L. (2023). Microscopic insight into the interactions between pea protein and fatty acids during high-moisture extrusion processing. Food Chemistry, 404, Article 134176. https:// doi.org/10.1016/j.foodchem.2022.134176
- Chen, Q., Zhang, J., Zhang, Y., & Wang, Q. (2022a). Effect of fatty acid saturation degree on the rheological properties of pea protein and its high-moisture extruded product quality. Food Chemistry, 390, Article 133139. https://doi.org/10.1016/j. foodchem.2022.133139
- Choe, E., & Min, D. B. (2006). Mechanisms and factors for edible oil oxidation. Comprehensive Reviews in Food Science and Food Safety, 5(4), 169–186. https://doi.org/10.1111/j.1541-4337.2006.00009.x
- Chojnicka-Paszun, A., De Jongh, H. H. J., & De Kruif, C. G. (2012). Sensory perception and lubrication properties of milk: Influence of fat content. *International Dairy Journal*, 26(1), 15–22. https://doi.org/10.1016/j.idairyj.2012.04.003
- De Angelis, D., Kaleda, A., Pasqualone, A., Vaikma, H., Tamm, M., Tammik, M. L., Squeo, G., & Summo, C. (2020). Physicochemical and sensorial evaluation of meat analogues produced from dry-fractionated pea and oat proteins. *Foods*, 9(12), 1754. https://doi.org/10.3390/foods9121754
- De Angelis, D., Opaluwa, C., Pasqualone, A., Karbstein, H. P., & Summo, C. (2023). Rheological properties of dry-fractionated mung bean protein and structural, textural, and rheological evaluation of meat analogues produced by high-moisture extrusion cooking. Current Research in Food Science, 7, Article 100552. https://doi. org/10.1016/j.crfs.2023.100552
- De Angelis, D., Pasqualone, A., Costantini, M., Ricciardi, L., Lotti, C., Pavan, S., & Summo, C. (2021). Data on the proximate composition, bioactive compounds, physicochemical and functional properties of a collection of faba beans (Vicia faba L.) and lentils (Lens culinaris Medik.). Data in Brief, 34, Article 106660. https://doi.org/10.1016/j.dib.2020.106660
- De Angelis, D., van der Goot, A. J., Pasqualone, A., & Summo, C. (2024). Advancements in texturization processes for the development of plant-based meat analogs: A review. Current Opinion in Food Science, 58, Article 101192. https://doi.org/ 10.1016/j.cofs.2024.101192
- de Wijk, R. A., & Prinz, J. F. (2005). The role of friction in perceived oral texture. Food Quality and Preference, 16(2), 121–129. https://doi.org/10.1016/j. foodqual.2004.03.002
- Egbert, R., & Borders, C. (2006). Achieving success with meat analogs. *Food Technology*, 60(1), 28–34.
- Geng, L., Liu, K., & Zhang, H. (2023). Lipid oxidation in foods and its implications on proteins. *Frontiers in Nutrition*, 10, Article 1192199. https://doi.org/10.3389/ few.2023.110316.
- Ghebremedhin, M., Baechle, M., & Vilgis, T. A. (2022). Meat-, vegetarian-, and vegan sausages: Comparison of mechanics, friction, and structure. *Physics of Fluids*, 34(4). https://doi.org/10.1063/5.0083730
- Giakoumis, E. G. (2018). Analysis of 22 vegetable oils' physico-chemical properties and fatty acid composition on a statistical basis, and correlation with the degree of unsaturation. *Renewable Energy*, 126, 403–419. https://doi.org/10.1016/j. renepa. 2018.03.057.
- Gläser, P., Mittermeier-Kleßinger, V. K., Spaccasassi, A., Hofmann, T., & Dawid, C. (2021). Quantification and bitter taste contribution of lipids and their oxidation products in pea-protein isolates (Pisum sativum L.). *Journal of Agricultural and Food Chemistry*, 69(31), 8768–8776. https://doi.org/10.1021/acs.jafc.1c02889
- Gomes, T., Delcuratolo, D., & Paradiso, V. M. (2008). Pro-oxidant action of polar triglyceride oligopolymers in edible vegetable oils. European Food Research and Technology, 226, 1409–1414. https://doi.org/10.1016/j.lwt.2010.12.004

- Guo, Y., Wang, Z., Hu, Z., Yang, Z., Liu, J., Tan, B., Guo, Z., Li, B., & Li, H. (2022). The temporal evolution mechanism of structure and function of oxidized soy protein aggregates. Food Chemistry X, 15, Article 100382. https://doi.org/10.1016/j. fochx 2022 100382
- Guyony, V., Fayolle, F., & Jury, V. (2023). High moisture extrusion of vegetable proteins for making fibrous meat analogs: A review. Food Reviews International, 39(7), 4262–4287. https://doi.org/10.1080/87559129.2021.2023816
- Gwiazda, S., Noguchi, A., & Saio, K. J. F. S. (1987). Microstructural studies of texturized vegetable protein products: Effects of oil addition and transformation of raw materials in various sections of a twin screw extruder. Food Structure, 6(1), 8.
- Hocquette, J. F., Gondret, F., Baéza, E., Médale, F., Jurie, C., & Pethick, D. W. (2010). Intramuscular fat content in meat-producing animals: Development, genetic and nutritional control, and identification of putative markers. *Animal*, 4(2), 303–319. https://doi.org/10.1017/S1751731109991091
- Hyun, K., Wilhelm, M., Klein, C. O., Cho, K. S., Nam, J. G., Ahn, K. H., Lee, S. J., Ewoldt, R. H., & McKinley, G. H. (2011). A review of nonlinear oscillatory shear tests: Analysis and application of large amplitude oscillatory shear (Laos). *Progress in Polymer Science*, 36(12), 1697–1753. https://doi.org/10.1016/j. progpolymsci.2011.02.002
- Ju, J., Zheng, Z., Xu, Y. J., Cao, P., Li, J., Li, Q., & Liu, Y. (2019). Influence of total polar compounds on lipid metabolism, oxidative stress and cytotoxicity in HepG2 cells. *Lipids in Health and Disease*, 18, 1–13. https://doi.org/10.1186/s12944-019-0980-0
- Kalichevsky, M. T., Jaroszkiewicz, E. M., & Blanshard, J. M. V. (1992). Glass transition of gluten. 2: The effect of lipids and emulsifiers. *International Journal of Biological Macromolecules*, 14(5), 267–273. https://doi.org/10.1016/s0141-8130(05)80039-x
- Kaushik, R., Kumar, N., Sihag, M. K., & Ray, A. (2015). Isolation, characterization of wheat gluten and its regeneration properties. *Journal of Food Science & Technology*, 52, 5930–5937. https://doi.org/10.1007/2Fs13197-014-1690-2
- Kendler, C., Duchardt, A., Karbstein, H. P., & Emin, M. A. (2021). Effect of oil content and oil addition point on the extrusion processing of wheat gluten-based meat analogues. *Foods*, 10(4), 697. https://doi.org/10.3390/foods10040697
- Kiumarsi, M., Shahbazi, M., Yeganehzad, S., Majchrzak, D., Lieleg, O., & Winkeljann, B. (2019). Relation between structural, mechanical and sensory properties of glutenfree bread as affected by modified dietary fibers. Food Chemistry, 277, 664–673. https://doi.org/10.1016/j.foodchem.2018.11.015
- Kurek, M. A., Onopiuk, A., Pogorzelska-Nowicka, E., Szpicer, A., Zalewska, M., & Półtorak, A. (2022). Novel protein sources for applications in meat-alternative products—insight and challenges. *Foods*, 11(7), 957. https://doi.org/10.3390/foods11070957
- Kyriakopoulou, K., Keppler, J. K., & van Der Goot, A. J. (2021). Functionality of ingredients and additives in plant-based meat analogues. *Foods*, 10(3), 600. https:// doi.org/10.3390/2Ffoods10030600
- Lampi, A. M., Damerau, A., Li, J., Moisio, T., Partanen, R., Forssell, P., & Piironen, V. (2015). Changes in lipids and volatile compounds of oat flours and extrudates during processing and storage. *Journal of Cereal Science*, 62, 102–109. https://doi.org/10.1016/j.jcs.2014.12.011
- Liu, K., Stieger, M., van der Linden, E., & van de Velde, F. (2015). Fat droplet characteristics affect rheological, tribological and sensory properties of food gels.
- Food Hydrocolloids, 44, 244-259. https://doi.org/10.1016/j.foodhyd.2014.09.034
  Molteberg, E. L., Magnus, E. M., Bjørge, J. M., & Nilsson, A. (1996). Sensory and chemical studies of lipid oxidation in raw and heat-treated oat flours. Cereal Chemistry, 73(5), 579-587.
- Neugebauer, A., Granvogl, M., & Schieberle, P. (2020). Characterization of the key odorants in high-quality extra virgin olive oils and certified off-flavor oils to elucidate aroma compounds causing a rancid off-flavor. *Journal of Agricultural and Food Chemistry*, 68(21), 5927–5937. https://doi.org/10.1021/acs.jafc.0c01674
- Nimbkar, S., Leena, M. M., Moses, J. A., & Anandharamakrishnan, C. (2022). Medium chain triglycerides (MCT): State-of-the-art on chemistry, synthesis, health benefits and applications in food industry. Comprehensive Reviews in Food Science and Food Safety, 21(2), 843–867. https://doi.org/10.1111/1541-4337.12926
- Opaluwa, C., Deskovski, S., Karbstein, H. P., & Emin, M. A. (2024). Effect of oil on the rheological properties and reaction behavior of highly concentrated wheat gluten under conditions relevant to high moisture extrusion. *Future Foods*, 9, Article 100307. https://doi.org/10.1016/j.fufo.2024.100307
- Opaluwa, C., Lott, T., Karbstein, H. P., & Emin, M. A. (2023). Encapsulation of oil in the high moisture extrusion of wheat gluten: Interrelation between process parameters, matrix viscosity and oil droplet size. *Future Foods*, 7, Article 100222. https://doi.org/ 10.1016/j.fufo.2023.100222
- Pietsch, V. L., Emin, M. A., & Schuchmann, H. P. (2017). Process conditions influencing wheat gluten polymerization during high moisture extrusion of meat analog products. *Journal of Food Engineering*, 198, 28–35. https://doi.org/10.1016/j. ifoodeng.2016.10.027
- Pires, M. A., Pastrana, L. M., Fuciños, P., Abreu, C. S., & Oliveira, S. M. (2020). Sensorial perception of astringency: Oral mechanisms and current analysis methods. *Foods*, 9 (8), 1124. https://doi.org/10.3390/foods9081124

- Pommet, M., Redl, A., Morel, M. H., & Guilbert, S. (2003). Study of wheat gluten plasticization with fatty acids. *Polymer*, 44(1), 115–122. https://doi.org/10.1016/ s0032-3861(02)00746-2
- Rodríguez, G., Squeo, G., Estivi, L., Berru, S. Q., Buleje, D., Caponio, F., Brandolini, A., & Hidalgo, A. (2021). Changes in stability, tocopherols, fatty acids and antioxidant capacity of sacha inchi (Plukenetia volubilis) oil during French fries deep-frying. Food Chemistry, 340, Article 127942. https://doi.org/10.1016/j.foodchem.2020.127942
- Ross-Murphy, S. B. (1995). Structure–property relationships in food biopolymer gels and solutions. *Journal of Rheology*, *39*(6), 1451–1463. https://doi.org/10.1122/
- Ruiz-Núñez, B., Dijck-Brouwer, D. J., & Muskiet, F. A. (2016). The relation of saturated fatty acids with low-grade inflammation and cardiovascular disease. *The Journal of Nutritional Biochemistry*, 36, 1–20. https://doi.org/10.1016/j.jnutbio.2015.12.007
- Saavedra Isusi, G. I., Pietsch, V., Beutler, P., Hoehne, S., & Leister, N. (2023). Influence of rapeseed oil on extruded plant-based meat analogues: Assessing mechanical and rheological properties. *Processes*, 11(7), 1871. https://doi.org/10.3390/pr11071871
- Sala, G., Van Vliet, T., Stuart, M. A. C., Van Aken, G. A., & Van de Velde, F. (2009). Deformation and fracture of emulsion-filled gels: Effect of oil content and deformation speed. *Food Hydrocolloids*, 23(5), 1381–1393. https://doi.org/10.1016/ j.foodhyd.2008.11.016
- Schofield, J. D., Bottomley, R. C., Timms, M. F., & Booth, M. R. (1983). The effect of heat on wheat gluten and the involvement of sulphydryl-disulphide interchange reactions. *Journal of Cereal Science*, 1(4), 241–253. https://doi.org/10.1016/S0733-52106-29829829900125
- Schreuders, F. K., Sagis, L. M., Bodnár, I., Boom, R. M., & van der Goot, A. J. (2022). Non-linear rheology reveals the importance of elasticity in meat and meat analogues. Scientific Reports, 12(1), 1334. https://doi.org/10.1038/s41598-021-04478-z
- Singh, M., Trivedi, N., Enamala, M. K., Kuppam, C., Parikh, P., Nikolova, M. P., & Chavali, M. (2021). Plant-based meat analogue (PBMA) as a sustainable food: A concise review. European Food Research and Technology, 247, 2499–2526. https://doi.org/10.1007/s00217-021-03810-1
- Stehle, F., Woern, C., Tan, N. K., Weiss, J., & Grossmann, L. (2024). Effect of oil-water colloidal states in liquid feeds on extrudability and textural attributes of highmoisture meat alternatives. Sustainable Food Technology, 2(5), 1569–1582. https:// doi.org/10.1039/D4FB00096J
- Wang, Y., Tuccillo, F., Lampi, A. M., Knaapila, A., Pulkkinen, M., Kariluoto, S., Coda, R., Edelmann, M., Jouppila, K., Sandell, M., Piironen, V., & Katina, K. (2022b). Flavor challenges in extruded plant-based meat alternatives: A review. Comprehensive Reviews in Food Science and Food Safety, 21(3), 2898–2929. https://doi.org/10.1111/1541.4337.1964
- Wang, H., Zhang, L., Czaja, T. P., Bakalis, S., Zhang, W., & Lametsch, R. (2022a). Structural characteristics of high-moisture extrudates with oil-in-water emulsions. Food Research International, 158, Article 111554. https://doi.org/10.1016/j. foodres.2022.111554
- Weegels, P. L., De Groot, A. M. G., Verhoek, J. A., & Hamer, R. J. (1994). Effects on gluten of heating at different moisture contents. II. Changes in physico-chemical properties and secondary structure. *Journal of Cereal Science*, 19(1), 39–47. https://doi.org/10.1006/icrs.1994.1006
- Wickramasinghe, K., Breda, J., Berdzuli, N., Rippin, H., Farrand, C., & Halloran, A. (2021). The shift to plant-based diets: Are we missing the point? Global Food Security, 29, Article 100530. https://doi.org/10.1016/J.GFS.2021.100530
- Wittek, P., Walther, G., Karbstein, H. P., & Emin, M. A. (2021). Comparison of the rheological properties of plant proteins from various sources for extrusion applications. *Foods*, 10(8), 1700. https://doi.org/10.3390/foods10081700
- Xu, S., & Falsafi, S. R. (2024). Juiciness of meat, meat products, and meat analogues: Definition, evaluation methods, and influencing factors. Food Reviews International, 40(8), 2344–2377. https://doi.org/10.1080/87559129.2023.2273923
- Yang, Y., Zheng, Y., Ma, W., Zhang, Y., Sun, C., & Fang, Y. (2023). Meat and plant-based meat analogs: Nutritional profile and in vitro digestion comparison. *Food Hydrocolloids*, 143, Article 108886. https://doi.org/10.1016/j. foodbyd.2023.108886
- Ying, D., Edin, L., Cheng, L., Sanguansri, L., & Augustin, M. A. (2015). Enhanced oxidative stability of extruded product containing polyunsaturated oils. LWT-Food Science and Technology, 62(2), 1105–1111. https://doi.org/10.1016/j. lwt.2015.02.005
- Yuan, L., Xu, Z., Tan, C. P., Liu, Y., & Xu, Y. J. (2021). Biohazard and dynamic features of different polar compounds in vegetable oil during thermal oxidation. Lwt, 146, Article 111450. https://doi.org/10.1016/j.lwt.2021.111450
- Zhang, Y., Brouwer, R., Sala, G., Scholten, E., & Stieger, M. (2024). Exploring relationships between juiciness perception, food and bolus properties of plant-based meat analogue and beef patties. *Food Hydrocolloids*, 147, Article 109443. https://doi. org/10.1016/j.foodhyd.2023.109443
- Zhang, Z., Zhang, L., He, S., Li, X., Jin, R., Liu, Q., Chen, S., & Sun, H. (2023). High-moisture extrusion technology application in the processing of textured plant protein meat analogues: A review. Food Reviews International, 39(8), 4873–4908. https://doi.org/10.1080/87559129.2021.2024223