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Structure, Microbiology and Sensorial Evaluation of Bologna-Style Sausages in a Kilohertz Ohmic Heating Process

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Abstract: Ohmic heating (OH) is a sustainable heating technology with a high potential in terms of energy and time efficiency. However, its industrial application for solid or semi-solid foods is not widespread yet. This study evaluates the pilot-scale production of Bologna-style sausages (2.3 kg weight) via conventional heating (COV) and OH at an electrical frequency of 10 kHz. Sausages with a diameter of 110 mm heated via OH were produced in approximately 5% of the time (i.e., 10 min) needed to produce sausages heated via COV. OH-treated samples showed a higher moisture content and an increased water holding capacity. A texture profile analysis revealed OH sausages as possessing a lower hardness, springiness, and chewiness. The microbiological load of the samples was identical, regardless of the heating technology. Color measurements (L*a*b* values) showed OH-treated samples to be less red. However, this difference could not be confirmed during sensorial evaluation. Temporal sensation of dominance and descriptive sensory analyses were conducted and revealed a decreased solid consistency but an increased meat taste when sausages were heated via OH. The gel network structures obtained via scanning microscopic analysis showed an increased size of fat globules within OH-treated samples. The results indicated that OH can be used as an alternative heating method to produce Bologna-style sausages.

Keywords: meat emulsion batter; resistance heating; texture; sensory; Joule heating; microbiology; meat color; scanning electron microscopy; confocal laser scanning microscopy; meat quality

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1. Introduction

Thermal treatment constitutes a pivotal process in the production of meat products, with many such products undergoing heating steps as part of their manufacturing process, as exemplified by cooked sausages. This treatment is indispensable for imparting characteristic textures and ensuring the microbiological safety of meat products [1,2]. Notable examples of cooked sausages include frankfurters, wieners, hot dogs, mortadella, and Bologna-style sausages, which collectively represent approximately 45% of all sausage products sold in the USA [3,4].

Ohmic heating (OH) has emerged as a promising heating technology, offering potential as an alternative method with advantages over conventional heating (COV) technologies in terms of time and energy efficiency [5]. During OH, a food product is placed between two electrodes and used as a resistor in an electrical circuit; thus, an electrical field is applied to the food. As the electrical current runs through the product, electrical energy dissipates into thermal energy within the whole body of the food [6]. Thus, OH is described as a volumetric heating process. Moreover, OH, being independent of fossil fuels, presents sustainability potential as a heating method [7].

Various applications of OH in food heating, including cooking, blanching, defrosting, and baking, have been documented in the recent scientific literature and reviewed comprehensively by Jaeger et al. [8]. Nonetheless, industrial applications and OH plants are predominantly tailored for pumpable or fluid foods, owing in part to a dearth of data on OH processes for solid or semi-solid foods at pilot or industrial scales.

The application of OH for heating meat emulsion batters (e.g., Bologna-style sausages) has been investigated by Shirsat et al. and Piette et al., who analyzed parameters such as color, texture, water holding capacity, sensory attributes, and microbiological inactivation of OH-treated versus COV-treated sausages [9–12]. These studies suggest OH as a viable heating alternative for meat emulsion batters, resulting in subtle differences (e.g., reduced product hardness) while maintaining overall comparable product attributes and sensory acceptance [9,11,12]. Notably, these investigations were conducted using laboratory-scale OH facilities, with an electrical frequency of 50 Hz, akin to domestic electricity. However, utilizing low electrical frequencies like 50 Hz during OH has been associated with a significantly higher release of metals from electrodes into the food product [13,14], thereby increasing electrode corrosion, shortening the electrode lifespan, and increasing the risk of electrode fouling or product contamination [13]. This phenomenon has been demonstrated by Pataro et al. and Pereira et al., who employed stainless-steel electrodes [13,14], a commonly recommended and applied electrode material for OH processes [15,16]. Pataro et al. identified electrical frequency as the most influential factor governing metal release from electrodes into food products, superseding factors such as the pH, electrical conductivity of the food, or applied electrical field strength. Conversely, employing higher frequencies in the kilohertz range during OH markedly diminishes electrode corrosion and metal leakage into food [13,14]. Thus, using higher frequencies in the kilohertz rage leads to a more product-safe process with a decreased potential of contamination.

In addition to heightened electrode corrosion, low-frequency OH processes (e.g., between 1 and 100 Hz) are reported to impact food constituents, particularly proteins. Samaranayake and Sastry demonstrated that dissolved protein molecules exhibit increased rotational and translational motion within the electric field during OH at low frequencies of 1–60 Hz compared to high-frequency OH in the kilohertz range [17]. Thus, the molecular motion of proteins at, e.g., 10 kHz, differs from the motion of protein molecules at 50 Hz. Furthermore, Vicente et al. observed a notably reduced thermal stability of beta-lactoglobulin when subjected to OH at low frequencies of 50–100 Hz, leading to structural alterations at lower temperatures [18]. Conversely, heating at high frequencies (e.g., between 1 and 100 kHz) yielded thermal transition temperatures comparable to COV-treated samples.

To the best of our knowledge, the influence of OH on meat emulsion batters in the kilohertz range has not been described yet. Therefore, this study aims to assess the impact of OH at a high frequency on the structure, microbiology, and sensory attributes of Bologna-style sausages. Therefore, a generator of the DIL German Institute of Food Technologies e.V. was used to provide an electrical frequency of 10 kHz. We hypothesize that OH treatment of Bologna-style sausages at 10 kHz will yield comparable product attributes, microbiological safety, and sensory acceptance compared to COV treatment, albeit in significantly reduced processing times. Furthermore, a pilot-scale OH plant is utilized to gather data on OH processes, thereby enhancing the database necessary for the integration of energy- and time-efficient, sustainable technologies such as OH into industrial food production.

2. Materials and Methods

2.1. Preparation of Meat Emulsion Batter

The meat emulsion batter was prepared using a vacuum chopper (KILIA Vakuum Schnellkutter 30 Ltr. 5000 Express, Heltek Maschinenbau GmbH & Co KG, Bad Fallingbostel, Germany) by combining the following ingredients: 50 wt% lean pork meat SIII, 15 wt% pork cheeks SVI, 15 wt% pork back fat SVII (sourced from a local producer), 17.5 wt% crushed ice, 1 wt% NaCl (Suprasel Classic, Suprasel, Amsterdam, The Nether-

lands), and 1 wt% sodium nitrite salt (Nitritpökelsalz 0.44–0.55%, Salinen Austria AG, Ebensee am Traunsee, Austria). Additional additives procured from NovaTaste Austria GmbH (Salzburg, Austria) were incorporated in the following quantities: 0.002 wt% sodium diphosphate (Brätfix), 0.0005 wt% sodium ascorbate (Natriumascorbat), and 0.005 wt% seasoning (Aufschnitt/Lyoner Gewürzaromazubereitung). The ingredients were pre-mixed and subsequently vacuum-chopped for 3 min. The resulting meat emulsion batter was then filled into polyamide casings and the OH cell, as detailed in the subsequent sections.

2.2. Heat Treatments

Heat treatments were conducted via OH and COV to achieve a final core temperature of 76 °C, as elaborated below. Following heating, samples were extracted from the ohmic heating cell and casings (for COV heating), respectively, and cut into 10 cm chunks. These chunks were subsequently cooled in 20 °C rinsed water (without packing) for 10 min. Rinsing without packages was performed as OH-treated samples were heated in an OH cell (as described in Section 2.2.2) and COV-treated samples were heated in the casing. Thus, comparable cooling was ensured. After water cooling, the chunks were vacuum-sealed and stored at 4 °C for 48 h for further analysis.

2.2.1. Conventional Heat Treatment

Conventional heating was executed using an ASR 1297 EL/WA AlroundSystem RONDETTE smoking and steam oven from Maurer-Atmos GmbH (Reichenau, Germany). Approximately 2.3 kg of meat batter was filled into 110 mm diameter polyamide casings (Budenheimer Kunststofftechnik GmbH, Schweighofen, Germany) and placed in the cooking chamber, as illustrated in Figure 1. Steam with approximately 98% saturation was utilized for heating within the chamber. The temperature profile of the steam oven consisted of 50 °C for 1 h, followed by heating at 80 °C until the final core temperature of 76 °C was attained, with an additional holding time of 2 min. Temperature monitoring was conducted using the oven's pre-installed thermocouple.

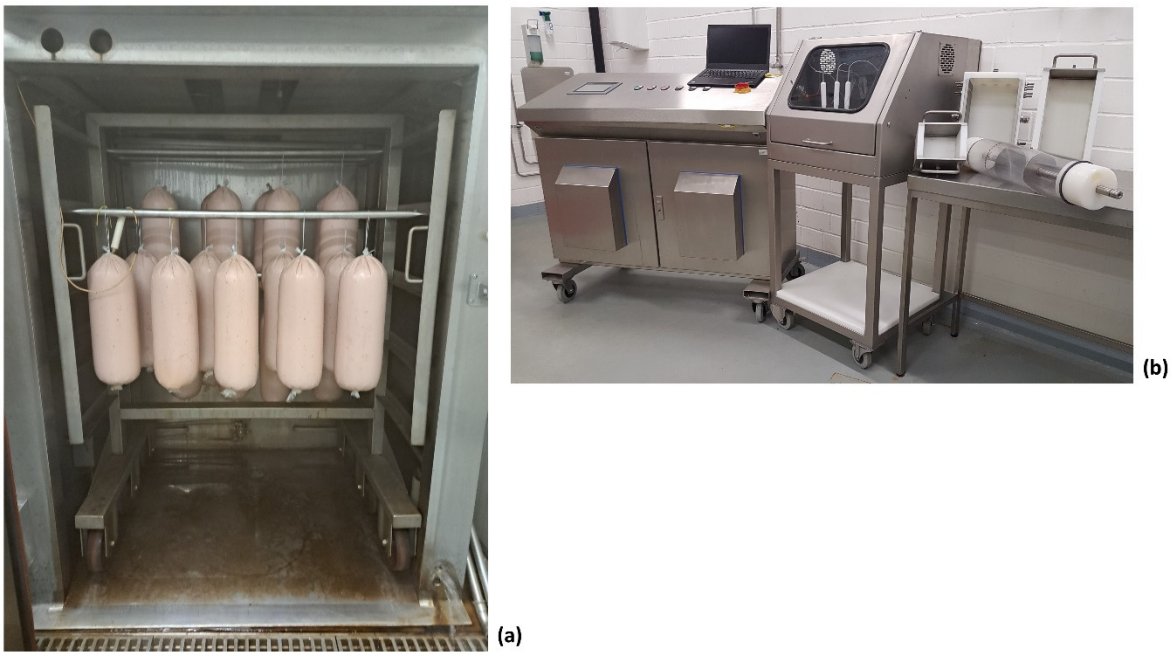

Figure 1. (**a**) Conventional cooking process of Bologna-style sausages in a steam oven, (**b**) ohmic heating (OH) plant, consisting of a generator, a datalogger, an OH chamber with three installed PT-100 thermocouples, and various OH cells (from left to right). The OH cell used was the cylindrical-shaped cell (front left).

2.2.2. Ohmic Heating Treatment

A pilot-scale OH plant provided by the German Institute of Food Technology—DIL e.V. was employed for OH treatments. The OH plant comprises a function generator and an OH chamber equipped with an OH cell. The function generator applies an alternating current through rectangular bipolar direct-current pulses, regulated by the pulse height and controlled via a proportional integral derivative controller. The alternating-current flow occurs at a frequency of 10 kHz. The cylindrical OH cell, as depicted in Figure 1, was constructed from 5 mm thick polymethylacrylate, with an internal diameter of 11 mm for the cell and electrodes. Electrodes, fashioned from 2 mm thick stainless steel, were securely inserted into the sides of the cell. Temperature measurements are facilitated by PT-100 thermocouples (type HET/E, FuehlerSysteme eNet International GmbH, Nürnberg, Germany) positioned at three distinct measuring points, namely core, halfway, and outer temperatures, as shown in Figure 2d.

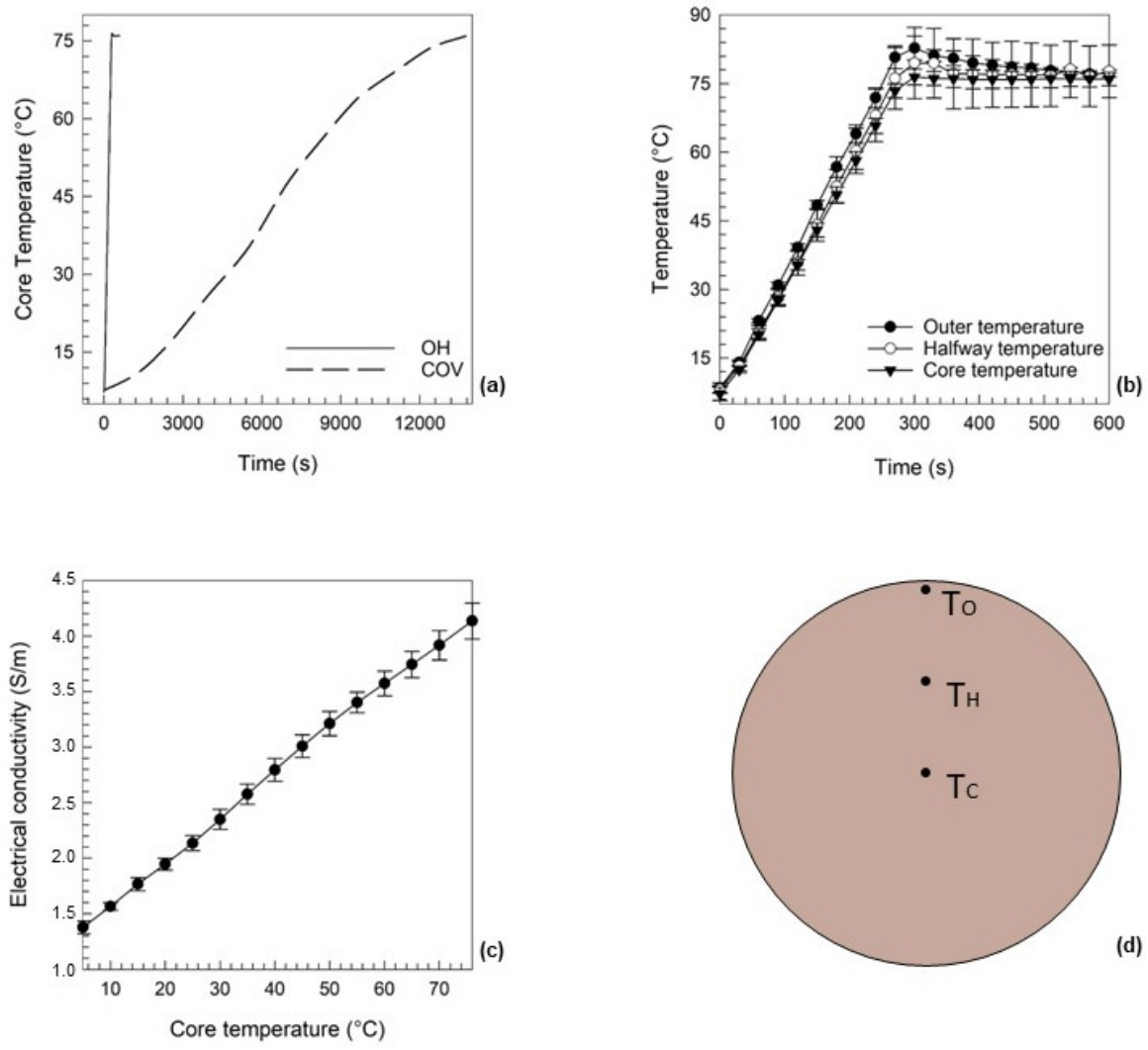

Figure 2. (**a**) Time–temperature profile of the core temperature ohmic and conventionally heated (OH and COV, respectively) Bologna-style sausages, (**b**) temperature distribution of OH-treated Bologna-style sausages. Outer temperature (T_O), halfway temperature (T_H) and core temperature (T_C) are located as shown in (**d**). (**c**) Electrical conductivity of Bologna-style sausages during the OH process.

For heating, 2.3 kg of meat batter was placed in the cell, with electrodes maintained at a fixed position during heating. Heating was initiated at a constant power of 2.3 kW until

reaching a final core temperature of 76 °C. A holding time of 5 min at the final temperature was achieved by reducing the applied power to 0.1 kW.

According to Zareifard et al. [6], the electrical conductivity of the Bologna-style sausages was calculated using Equation (1):

$$\sigma = \frac{I * L}{V * A} \quad (1)$$

where σ is the electrical conductivity (S/m), I is the electrical current (A), L is the distance between the electrodes (m), V is the voltage (V) and A is cross-section surface area of the electrodes (m^2).

2.3. Sample Analysis

2.3.1. L*a*b* Value Determination

Color measurements were performed using a CM-600 d from Konica Minolta Sensing, Inc. (Marunouchi, Japan), with an ⌀ 11 mm illumination area and a calibration cap CM-A177. Before measurements, samples of both treatments were taken from 4 °C storage and immediately cut, and freshly cut surfaces were used to determine luminosity coordinates (L*), red-green coordinates (a*), and yellow-blue coordinates (b*). The illuminant used was a D65 and the observer angle was 10°. Reflection spectra were acquired between 400 and 700 nm with reflectance values every 10 nm. The software used was Spectra Magic NX (Konica Minolta Sensing, Inc., Marunouchi, Japan, https://www.konicaminolta.eu/eu-en/hardware/measuring-instruments/colour-measurement/software-colour-appearance/spectramagic-nx).

2.3.2. Moisture Content

For the determination of moisture content, 3 g of sausage sample was initially weighed, ground, and homogenously mixed with a specified amount of arenaceous quartz. Subsequently, drying was conducted in glass beakers within a UL 50 drying oven (Memmert GmbH & Co. KG, Schwabach, Germany) at 105 °C for 3 h. Following the initial drying period, the combined sample and arenaceous quartz mixture underwent reweighing and were subjected to an additional hour of drying at the same temperature. This iterative process continued until no discernible further weight loss was observed. Cooling of samples post-heating and weighing procedures was consistently performed within a desiccator environment. Upon reaching a state of equilibrium, the moisture content was determined utilizing Equation (2):

$$c_{H2O} = 100\% - \left(m_d \frac{m_d}{m_i} 100 \right) \quad (2)$$

where C_{H2O} is the moisture content (wt%) of the sample, and m_i and m_d are the weighed mass (g) of the sample/arenaceous quartz mixture of the initial sample and after drying, respectively.

2.3.3. Water Holding Capacity

The water holding capacity (WHC) of the samples was assessed by employing a centrifugation technique, as outlined by Qi et al., with minor adjustments [19]. Ten grams of the sample was placed in a centrifugation tube and sealed before heating for 10 min at 90 °C in a water bath. Subsequently, samples were cooled down to room temperature before centrifugation was performed. Centrifugation was conducted at 9000× g at 4 °C for a duration of 10 min utilizing a Sorvall RC-6 centrifuge (Thermo Fisher Scientific Inc., Waltham, MA, USA). Following centrifugation, the supernatant was decanted from the solid gel components, and the remaining solid gel was reweighed. The WHC was calculated as the percentage ratio of the weight of the supernatant to the initial weight of the gel sample, as defined by Equation (3):

$$WHC = \left(1 - \frac{m_i - m_s}{m_{H2O}} \right) * 100 \quad (3)$$

where *WHC* is the water holding capacity (wt%), m_s is the mass of remaining solids after centrifugation (g), m_i is the initial mass of the gel sample (g) and m_{H2O} is the total water content of the sample (g).

2.3.4. Texture Profile Analysis

A TPA was conducted utilizing a TA.TX2 texture analyzer and Texture Expert Exceed software from Stable Micro Systems (Godalming, UK, https://www.stablemicrosystems.com/SoftwareUpdateTextureExpertExceed.html). From all three replicates of each heating method, six quadratic cubes measuring 1 cm in length were prepared and allowed to equilibrate to room temperature. Subsequently, the cubes were positioned into the texture analyzer and subjected to compression to 40% of their initial height using a cylindrical, flat-ended probe with a diameter of 40 mm, constructed from acrylic material. The test parameters employed were as follows: pre-test speed = 3.0 mm/s, test speed = 1.0 mm/s, post-test speed = 3.0 mm/s, and trigger value = 0.05 N, with a resting time of 10 s between the two cycles.

2.3.5. Microbiological Evaluation

The microbiological evaluation was performed by measuring the count for various microorganisms as follows (specific method of determination is given in brackets for each microorganism): aerobic total bacteria count (DIN EN ISO 4833-2 2022-05 [20]), Enterobacteriaceae (DIN EN ISO 21528-2 2019-05 [21]), *Escherichia coli* (DIN ISO 16649-2 2020-12 [22]), coagulase-positive *Staphylococci* (DIN EN ISO 6888-1 2022-06 [23]), and sulfite-reducing mesophilic *Chlostridia* (BVL L 06.00-39:1994-5 [24])). Furthermore, presence/absence tests were performed for *Salmonella* spp. (DIN EN ISO 6579 2020-08 [25]), *Listeria* spp., and *Listeria monocytogenes* (DIN EN ISO 11290-1 2017-09 [26]).

2.3.6. Sensorial Evaluation

A sensorial evaluation was performed via quantitative descriptive analysis (QDA) and temporal dominance of sensations (TDS). For both analyses, Smart Sensory Solution Software (Smart Sensory Solution S.l.r., Sassari, Italy, https://www.smartsensorysolutions.com/) was used. Twenty trained panelists (trained via DIN EN ISO 8586:2014-05 [27] and introduced to Smart Sensory Solution Software) assessed the sensorial evaluation via QDA and TDS. For QDA, panelists were given cubes of 1 cm long OH- and COV-treated Bologna-style sausage. Attributes to be evaluated were color, odor, taste, hardness, juiciness, saltiness, and overall score. The scale used had seven increments, going from 1 = do not like at all/not intense at all to 7 = like very much/very intense. TDS was performed for 30 s. Panelists were also given sample cubes of 1 cm in length for evaluation. The sensations tested were hardness, greasy, meaty, juicy, salty, and sour. Prior to initiating TDS, all panelists became familiar with the following attributes: hardness, greasy, meaty, juicy, salty, and sour, extracted from previous research [28,29]. Upon becoming accustomed to the selected sensations, the temporal dominance of sensations was determined using the designated software. The panel was asked to select a dominant attribute, pointing out that a sensation could be selected several times. The serving was performed randomly.

2.3.7. Confocal Laser Scanning Microscopy

CLSM was performed as described by Baune et al. [30]. Before microscopic analysis, samples underwent staining procedures as follows: Samples were manually cut to slices of approx. 0.5 mm thickness and 1 cm^2 area and placed on a slide. Subsequently, the slices were stained by adding three drops of the respective stain. The protein fraction was stained utilizing fluorescein isothiocyanate isomer I (FITC; sourced from AppliChem GmbH, Darmstadt, Germany), while the fat fraction was stained using Nile red (procured from Fluka® Analytical, St. Gallen, Switzerland). Slides were left overnight at room temperature in darkness before imaging. Imaging was conducted utilizing a Nikon ECLIPSE E 600 model (Nikon Corporation, Tokyo, Japan), equipped with an oil-corrected 60× objective.

Fluorescence excitation was achieved via an argon (Ar) laser emitting light at 488 nm and a helium–neon (He-Ne) laser emitting light at 543 nm.

2.3.8. Scanning Electron Microscopy

The cryo-preparation system utilized for the preparation of gel samples was the K1250 model (Quorum Technologies Ltd., Laughton, UK). Initially, the samples were immobilized in liquid nitrogen. Following this, the frozen specimens were sectioned into 2 mm fragments under vacuum conditions to facilitate the sublimation of frozen water. Subsequently, the protein network structures were coated with a layer of gold and mounted onto the stage of a Jeol JSM-6460LV microscope (Jeol Ltd., Akishima Tokyo, Japan). Scanning electron microscopy (SEM) images were captured at various magnifications and subjected to analysis.

2.3.9. Statistical Analysis

All samples were prepared in triplicate execution for each heating method. When possible, two or more technical duplicates were taken for data collection. To conduct statistical analysis, a Sigma Plot 13 (Systat Software Inc., San Jose, CA, USA) was used by performing Shapiro–Wilk normality tests and an ANOVA, followed by Dunn's or Holm–Sidak post hoc tests. Standard deviations are expressed as error bars in plots or given as means in tables. Significant statistical differences with a significance level of $p < 0.05$ are indicated via superscripts.

3. Results and Discussion

3.1. Heat Treatments

For both heating methods, Bologna-style sausages were produced with a final core temperature of 76 °C. Time–temperature profiles are displayed in Figure 2a. Using OH at a frequency of 10 kHz and 2.3 kW to heat a mass of 2.3 kg of meat emulsion batter significantly reduced the treatment time from approx. 230 min to 10 min. Noteworthy, both heating methods, i.e., OH and COV, could be further reduced with regard to treatment time. In this case, the preinstalled heating program of the steam oven was used, leading to a 230 min heating duration. This program was also prolonged as a reddening step at 50 °C for 60 min is implemented in the program. Furthermore, the OH treatment was chosen in such a way that a treatment time of approx. 5% of the time needed for COV treatment was reached.

The electrical conductivity showed a linear increase over temperature (as shown in Figure 2c), ranging from 1.38 ± 0.06 S/m at 5 °C to 4.14 ± 0.16 S/m. This linear increase is a common observation appearing during OH processes for emulsions, meat emulsion batters, and other food products, e.g., potatoes or hydrocolloid solutions such as gelatin or starch [10,31–33]. Taking a closer look at the temperature distribution during OH treatment (given in Figure 2b), it appears that higher values were constantly measured for the outer temperature than for the halfway or the core temperature. Thus, a small heat gradient was measurable from higher temperatures on the outside to lower temperatures in the core of the sausage when heated via OH. This is contrary to what was initially expected, as during OH, the coldest spot is rather on the outside where the food product is in contact with a colder surface or environment [12,34]. A higher temperature in the outer layer of the sausage can be explained by the initial temperature difference between the meat batter and the OH cell. The meat emulsion batter was filled into the OH cell at approx. 5–6 °C. The OH cell was stored at room temperature without any precooling or preheating. Thus, the temperature of the OH cell was approx. 21 °C. After filling the meat emulsion batter into the OH cell, the cell was installed into the OH chamber, and electrodes were fixed before starting the OH process. Thus, approx. 3–5 min passed between taking the meat emulsion batter from the cooling chamber and starting the flow of electrical current. In this time, the meat emulsion batter in the outer layer of the sample was in contact with a warmer surface, i.e., the OH cell. This time was enough to let the temperature of the meat emulsion

batter rise in this area, leading to a temperature difference of approx. 1.6 ± 0.49 °C between the outer layer and the core of the sausage. We assume that from the start of the OH process until the meat emulsion batter had reached an outer temperature of approx. 21 °C, i.e., room temperature, the temperature gradient between the OH cell/environment and the colder meat emulsion batter led to a faster warming of the outer layer of the sausage. Given that the electrical conductivity of the meat emulsion batter rises with temperature (as shown in Figure 2c), a higher initial temperature led to a higher electrical conductivity in the outer layer of the sausage. Thus, a higher flow of electrical current occurred in this outer layer from the beginning of the OH process, which led to a faster temperature rise. As shown in Figure 2b, this phenomenon potentiated during the heating-up time and reached its maximum towards the end of the heating-up phase. Here, the temperature difference between the outer layer and the core was approx. 7.4 ± 1.81 °C. In turn, during the holding time, the power input was reduced from 2.3 to 0.1 kW, which led to no further temperature rise. Thus, the temperature gradient between the outer layer and the core of the sausage converged until an equilibrium was reached during the holding time.

*3.2. L*a*b* Value Determination*

As shown in Figure 3, the color and structure of samples heated by either heating method, i.e., OH or COV, did not differ when evaluated by eye. This was also confirmed by visual evaluations during the descriptive sensorial testing by the panelists, as no significant differences in color were scored. Using a colorimeter to measure the L*a*b* values of the samples, however, revealed a significant difference between the two heating methods. OH samples had decreased a and b values and thus less red and yellow color, but more grayish attributes compared to COV-heated samples. Additionally, OH-treated samples had a brighter appearance compared to COV-treated samples, evidenced by higher L values. Shirsat et al. also describe OH-treated meat batter emulsions as having less color development compared to COV-treated samples when heated to equal final temperatures and at comparable treatment times to this study (i.e., when an electric field of 3 V/cm was applied) [11]. Notably, the authors did not measure significant differences. However, when heated at faster heating rates (i.e., when electric fields of 5 V/cm and 7 V/cm were applied), Shirsat et al. describe an increased a value [11], which is contrary to the findings of this study.

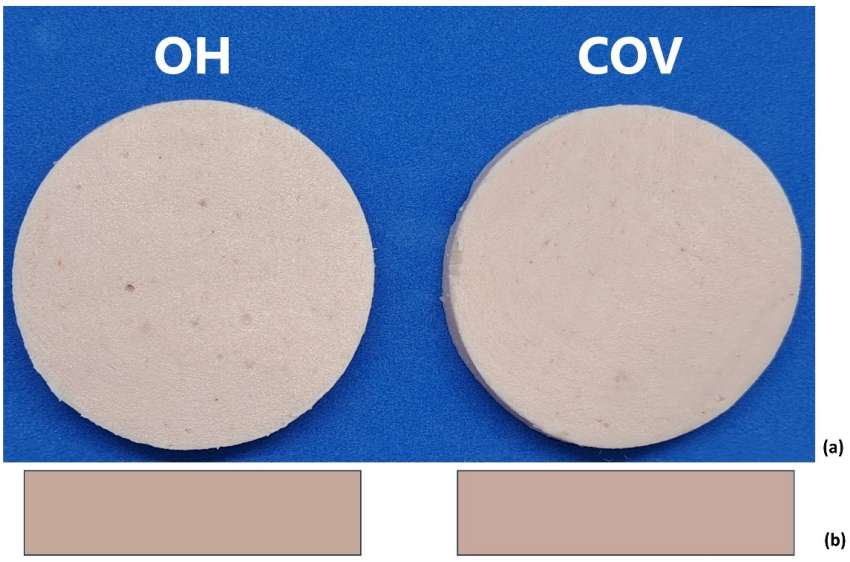

Figure 3. (**a**) Photograph of freshly cut Bologna-style sausages heated via Ohmic and conventional heating (OH and COV, respectively), which were used for L*a*b* value determination. (**b**) Graphic representation of colors of OH- and COV-treated samples by L*a*b* values from Table 1.

Table 1. Properties of Bologna-style sausage after ohmic and conventional heating (OH and COV, respectively). Different superscript letters within each column indicate statistically significant differences between samples.

	Moisture (%)	Water Holding Capacity (%)	Color (L*a*b* Value Determination)		
OH	67.28 ± 0.66 ^{b}	73.38 ± 1.18 ^{b}	L: 71.29 ± 0.4 ^{b}	a: 7.08 ± 0.14 ^{a}	b: 8.69 ± 0.19 ^{a}
COV	66.55 ± 0.91 ^{a}	68.03 ± 1.70 ^{a}	L: 70.71 ± 0.64 ^{a}	a: 7.42 ± 0.04 ^{b}	b: 8.95 ± 0.20 ^{b}

The color differences measured in this study via a colorimeter are attributed to a shorter heating process during OH treatment, which was less than 5% of the time needed to heat the COV-treated samples. During the cooking of meat emulsion batters, reddening of the batter occurs. Commonly, heating of meat emulsion batters leads to a loss of red color when no sodium nitrite is added. $NaNO_2$ helps stabilize myoglobin, which has the biggest impact on the red color of the sausage [35]. Reddening is enhanced by time and temperature. Temperatures between 40 and 50 °C or an increased duration at these temperatures will increase the stability of myoglobin, leading to preservation of the red color [36]. During OH, the residence time at these temperatures is significantly shortened, and this will lead to a less red and saturated color with more grayish attributes.

3.3. Moisture Content and Water Holding Capacity

Sausages produced via ohmic heating (OH) exhibited both a significantly higher moisture content and water holding capacity (WHC) compared to samples treated with conventional heating (COV). The disparity in moisture content is less pronounced than that in the WHC between OH- and COV-treated samples, as illustrated in Table 1. Shirsat et al. also investigated the WHC, as well as the quantity of expressible fluids and solids, of OH- and COV-treated meat emulsion sausages [9]. The authors noted that COV-treated samples contained more loosely bound water compared to OH-treated ones, resulting in a higher level of water that can be drained from COV-treated sausages.

In this study, corresponding samples after ohmic heating (OH) and conventional heating (COV) were produced from the same meat emulsion batters, resulting in comparable moisture contents for both heating methods. However, OH-treated samples consistently exhibited a slightly higher moisture content, attributed to a lower degree of water bound to the proteins. Several authors have noted that OH can lead to less denaturation of proteins, resulting in a state less capable of binding water molecules [37–41]. This less denatured state is less capable of binding water molecules [9]. Consequently, more water can be classified as retained water, which is easier to mobilize from the gel network [42] and which is more likely to drain due to syneresis from COV samples during storage, which decreases the total moisture content compared to OH samples. Another influencing factor might be the cooling process applied in this study. To ensure comparable cooling behavior, samples from both heating methods were removed from the cooling cell or casing, respectively, and cooled under rinsed water. Consequently, OH samples might have a slightly higher water uptake as the proteins are less denatured and thus more capable of binding additional water.

Similar to the moisture content, the water holding capacity (WHC) of a protein network depends on the amount of absorbed and retained water, i.e., water molecules bound to proteins and water trapped in the protein matrix, respectively [43]. The higher water holding capacity of OH-treated samples is also related to a lower degree of protein denaturation, resulting in more water molecules being absorbed by the protein molecules and remaining immobile during centrifugation, leading to the higher WHC of OH-treated samples. Conversely, more loosely bound water is expected in COV-treated samples, as described by Shirsat et al. [9]. This water is easier to mobilize during centrifugation, thus decreasing the WHC of COV-treated samples.

3.4. Texture Profile Analysis

A TPA of Bologna-style sausages heated via OH and COV revealed that OH-treated samples generally had a less hard texture, with less capability to spring back to their original shape. This is reflected in significantly lower hardness and springiness values, respectively, as shown in Table 2. Shirsat et al. also describe lower springiness values for OH-treated meat emulsion batters, whereas a comparable hardness to COV-treated samples was determined [11]. In contrast, Piette et al. measured a lower hardness of Bologna-style sausages heated via OH [12], which is in accordance with results from this study. Further attributes that were significantly lower for OH-treated samples in this study are gumminess and chewiness, which is in line with the generally lower hardness of the product. Thus, less force needs to be applied when simulating the chewing process of OH-treated samples compared to COV-treated samples.

Table 2. Texture profile analysis results for Bologna-style sausages heated via ohmic heating (OH) and conventional heating (COV). Different superscript letters within each column indicate statistically significant differences between samples.

	Hardness (N)	Adhesiveness (Ns)	Cohesiveness (/)	Springiness (%)	Gumminess (N)	Chewiness (N)	Resilience (/)
OH	9.64 ± 1.83 ^{a}	0.26 ± 0.12 ^{a}	0.56 ± 0.00 ^{a}	91.62 ± 3.86 ^{a}	5.40 ± 1.02 ^{a}	15.65 ± 2.54 ^{a}	0.42 ± 0.02 ^{b}
COV	11.53 ± 1.24 ^{b}	0.27 ± 0.04 ^{a}	0.56 ± 0.01 ^{a}	93.08 ± 2.90 ^{b}	6.46 ± 0.70 ^{b}	19.33 ± 2.82 ^{b}	0.42 ± 0.01 ^{a}

The lower hardness of OH-treated samples is mainly ascribed to two reasons: (i) a decreased heating time when heated via OH and (ii) lower intermolecular interactions within the OH-treated sausage gel network. With regard to (i), aggregation and the formation of gel network structures in meat emulsion sausages start at temperatures surpassing 40 °C [44]. As shown in Figure 2a, the duration that samples were above 40 °C during OH is significantly lower compared to COV samples. In turn, OH-treated samples had a longer holding time after reaching the final temperature of 76 °C (i.e., 5 min for OH and 2 min for COV). However, the prolonged holding time at the final temperature did not lead to a similar hardness of the OH samples compared to the COV-treated samples. A shorter total treatment time, as applied in this study, leads to a lower total thermal energy input. Thus, less energy is available for protein denaturation besides the overall heating of the sample [45]. Less denaturation of a protein molecule is described as a less reactive state for protein–protein interactions and hence for aggregation and the subsequent formation of gel network structures [46]. Thus, a lower total thermal energy input due to shorter durations at denaturation temperatures reduces the likelihood of intermolecular interactions, e.g., hydrophobic interactions, which help stabilize a dense protein gel network.

With regard to (ii), besides a lower total energy input, OH has also been demonstrated to decrease protein denaturation and aggregation or the formation of gel network structures due to non-thermal effects [37–41]. These effects are described, e.g., as a diverging motion of protein molecules due to interactions with the electric field [47]. At electrical frequencies in the kilohertz range, an oscillating motion of molecules is likely, as described by Samaranayake and Sastry [17]. This oscillating motion can lead to a constant reorientation of hydrophobic clusters, as described by Rodrigues et al. [37]. Furthermore, the oscillatory motion at kilohertz frequencies can reduce the rotational motion of the proteins, leading to a local restriction in dependence on the frequency applied [17,48]. Thus, the possibility of physical interactions between proteins (e.g., via hydrophobic interactions) can be decreased due to non-thermal effects, which also negatively affect the sample's hardness when heated via OH.

Besides hydrophobic interactions, disulfide bonds also play a crucial role in forming the gel network structures of meat emulsion batters, as they enhance the gel's hardness [44]. According to the literature, OH can reduce the number of disulfide bonds in these gel structures [39]. Therefore, we assume that the number of disulfide bonds in the OH-treated

Bologna-style sausages will be decreased for the same reasons as described above. Thus, fewer disulfide bonds would contribute to a lower hardness of OH-treated samples.

3.5. Microbiological Evaluation

For both heating methods, no differences concerning the microbiological count were measurable, as shown in Table 3.

Table 3. Determination of microbiological contamination of ohmic and conventionally heated (OH and COV, respectively) Bologna-style sausages after 7 days of storage.

Parameter	Unit	OH	COV
Aerobic total bacteria count	(cfu/g)	1.7×10^1	3.0×10^1
Enterobacteriaceae	(cfu/g)	$<1.0 \times 10^1$	$<1.0 \times 10^1$
Escherichia coli	(cfu/g)	$<1.0 \times 10^1$	$<1.0 \times 10^1$
Coagulase-positive *Staphylococci*	(cfu/g)	$<1.0 \times 10^1$	$<1.0 \times 10^1$
Sulfite-reducing mesophilic *Chlostridia*	(cfu/g)	$<1.0 \times 10^1$	$<1.0 \times 10^1$
Salmonella spp.	in 25 g sample	0	0
Listeria spp.	in 25 g sample	0	0
Listeria monocytogenes	in 25 g sample	0	0
Listeria spp.	(cfu/g)	$<1.0 \times 10^1$	$<1.0 \times 10^1$
Listeria monocytogenes	(cfu/g)	$<1.0 \times 10^1$	$<1.0 \times 10^1$

Thus, both heating methods were sufficient to produce a consumer-safe meat product, which could be used for sensorial analysis. Several authors have already described microbiological inactivation after OH processes, which was also reviewed by Müller et al. and Makroo et al. [49,50]. Commonly, OH leads to comparable or enhanced inactivation compared to COV treatment, which is in line with our results. Furthermore, several authors have described the influence of the electrical frequency during OH processes on microbiological inactivation. Thereby, the results partially differ, as some authors report lower frequencies (e.g., 50–500 Hz), while other authors describe frequencies in the kilohertz range that lead to better or faster inactivation [51–55]. Thus, the influence of the electrical frequency on microbiological inactivation is not definite but described to be dependent, e.g., on the type of organism or environment, and needs further research [50]. In this study, the applied frequency of 10 kHz did not negatively impact microbiological inactivation. This is also reasonable as the main factor for microbiological inactivation in an OH heating process is the temperature itself, which was comparable to the temperature profile used in this study. Additionally, the unexpectedly hotter outer layer of the sausage during OH treatment (as described in Section 3.1) decreased the occurrence of underprocessed cold spots, which might occur on the OH cell/product interface during OH processes. This phenomenon has most likely reduced the possibility of microbiological contamination, which could have been induced by the method of heating.

3.6. Sensorial Evaluation

QDA and TDS were chosen as sensory evaluation tests, as these two methods provide complementary information about the sensory perception of the consumer during and after sensory stimulation [56]. During QDA, the sensory profile is both qualified and quantified after the chewing and swallowing process [57], whereas during TDS, the evolution of the dominant attribute during the chewing process is documented [58].

The attributes of color, odor, taste, hardness, juiciness, saltiness, and the overall score were recorded during QDA. These attributes were generally assessed as having low values, indicating a lower intensity of the respective attribute when heated via OH, except for juiciness. However, the difference in juiciness was not significant; the higher value is attributed to the higher moisture content of the OH-treated samples. The attributes of taste, color, and saltiness also did not show significant differences; thus, a comparable perception

of these attributes is concluded. Significant differences, however, were observed for the attributes of odor, hardness, and the overall score.

The hardness of OH-treated samples was also rated lower compared to COV-treated samples. This observation is in line with Shirsat et al. and Piette et al., who also describe lower hardness values after sensory evaluation of OH-treated Bologna-style sausages [11,12]. Furthermore, the sensory evaluation of this study aligns well with results obtained during TPA, as a lower hardness or a lower gumminess were measured for OH-treated samples. Lower hardness values in sensory evaluation are therefore also attributed to the reasons given in Section 3.4 The lower overall score for OH-treated samples is expected to occur as a combination of the mainly lower attribute intensities, which led to a general lower acceptance of OH-treated sausages.

TDS evaluation confirmed TPA and QDA results for the lower hardness of OH-treated samples during the chewing process. This attribute was significant during the chewing process of COV-treated samples but was not dominant during the chewing of OH-treated samples. Furthermore, the attributes greasy, meaty juicy, salty, and sour were assessed during TDS. Thereby, the attributes sour and meaty were rated similarly between samples of both heating methods. The temporal dominance of saltiness occurred also at a comparable timescale and intensity between OH- and COV-treated sausages. Differences, however, were obtained for the attributes of juicy and greasy. Here, the dominance of sensation occurred at an earlier stage of chewing for OH-treated samples compared to COV-treated samples. Furthermore, for OH-treated samples, the attribute juicy had two noteworthy appearances, i.e., two peaks as shown in Figure 4. Notably, both attributes are rated at a level of chance. A faster dominance and a double appearance of the attribute juicy could explain the results obtained during QDA, where juiciness was the only attribute that had a higher value for OH-treated samples compared to COV. The attribute greasy did appear earlier and decreased towards the end of the chewing process for OH-treated samples. In turn, the perception of greasiness increased and reached its maximum at the end of the chewing process for COV-treated samples. As the greasiness is strongly related to the appearance of fat droplets [59], this indicates a difference in fat distribution within the gel network between samples produced by the two heating methods, which is addressed in detail in the following section. Additionally, a lower hardness of OH-treated samples could have enhanced the faster release of fat droplets when similar chewing forces were applied, leading to a faster greasier sensation.

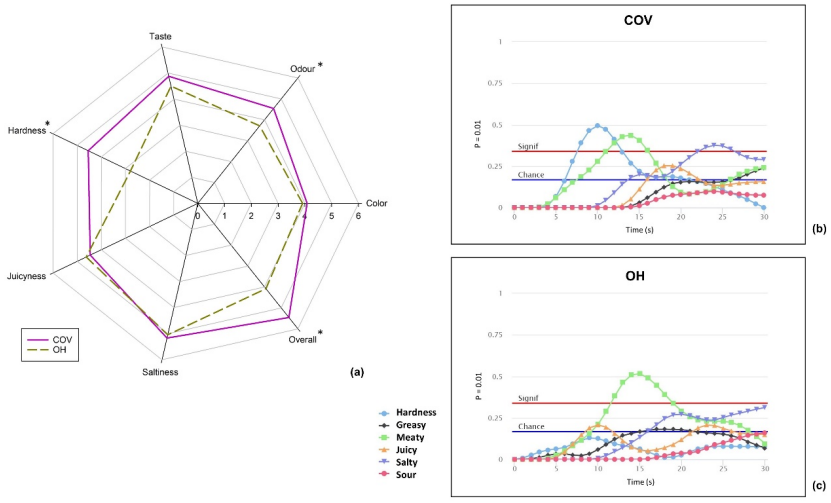

Figure 4. (**a**) Results from a quantitative descriptive analysis of Bologna-style sausages heated via ohmic and conventional heating (OH and COV, respectively), (**b**) and (**c**) results from temporal dominance of sensation evaluations of COV-treated and OH-treated samples, respectively. * indicates statistically significant differences ($p < 0.05$) between OH- and COV-treated samples for the respective attribute.

3.7. Microscopic Evaluation

A microscopic evaluation was conducted using CLSM and SEM. For CLSM, the protein and fat fractions were stained green and red, respectively. As shown in Figure 5, the obtained structures were mainly comparable. However, both CLSM and SEM micrographs revealed that OH-treated samples tended to contain larger fat droplets within the protein gel matrix compared to COV-treated samples. This includes, for example, more fat droplets surpassing a diameter of 50 µm. This finding is assumed to cause the faster greasy perception of OH-treated samples during TDS. As shown by Lett et al. [59], the oiliness of a flavored emulsion increased during sensory evaluation when oil droplet sizes increased from 20 to 50 µm.

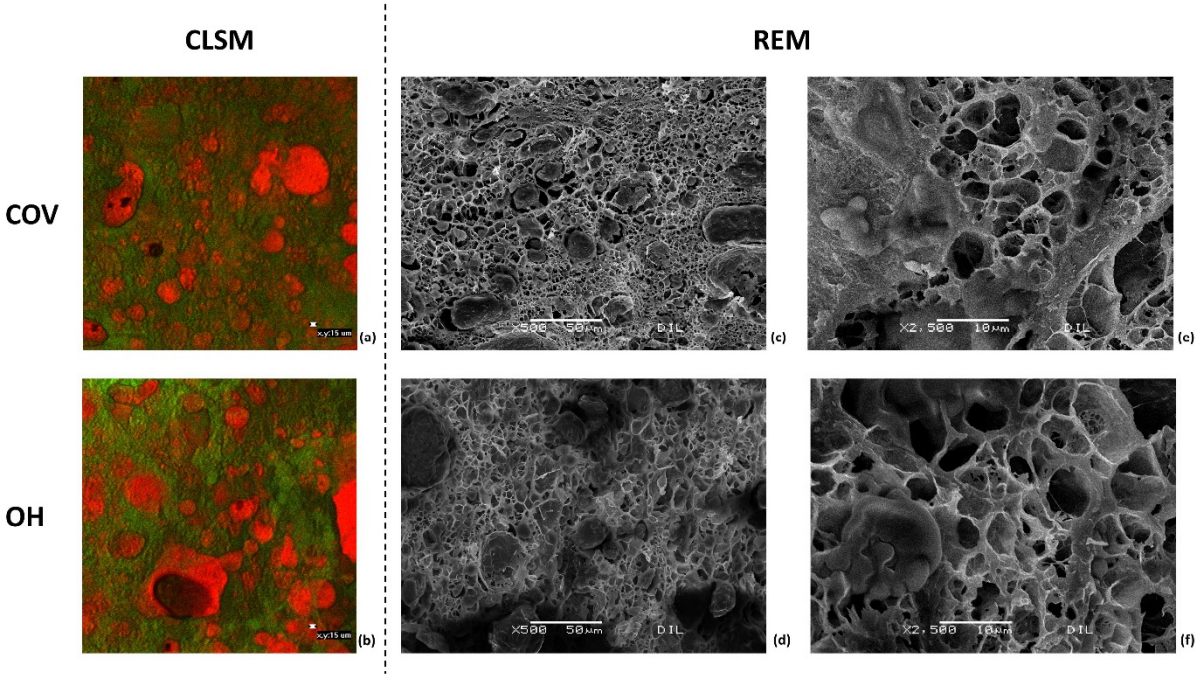

Figure 5. Representative micrographs of Bologna-style sausages heated via ohmic and conventional heating (OH and COV, respectively). (**a**,**b**) Micrographs taken via CLSM (proteins are stained green and fat is stained red); scale bar represents 15 µm. (**c**–**f**): Micrographs taken via SEM at different magnifications as indicated; scale bars are given in the micrographs.

A more porous network, as well as a decreased integrity of the stabilizing protein matrix which surrounds each fat globule, is expected to cause the observed increase in fat droplet size of OH-treated samples. Fat droplets in meat emulsions are surrounded by protein layers that prevent the coalescence of fat droplets during the cooking of meat emulsion batters [9]. Given that OH leads to less denaturation, as described in Section 3.3 and 3.4, this will have a destabilizing effect on the protein layers and, hence, also on the fat droplet integrity. Thus, the likelihood of fat droplets to coagulate and form bigger-sized fat droplets is enhanced when heated via OH. In addition, the thermally induced protein gel networks under OH are more open and porous compared to COV-treated gel network structures [39,40]. A more open and porous gel network under OH will enhance the possibility of incorporating larger fat droplets compared to COV.

In contrast to the results from this study, Shirsat et al. describe smaller fat droplets in OH-treated meat emulsions batters compared to COV-treated samples, as determined via light microscopy [9]. The authors ascribe smaller fat droplets in OH-treated samples to occurring as a result of "...slightly greater amounts of interfacial protein material" [9]. This difference could be attributed to the applied electrical frequency, as Shirsat et al. used a low frequency of <100 Hz. As described by Vicente et al., low frequencies can lead to protein alterations due to a lower thermal stability [18], which could result in a slightly thicker

layer of denatured proteins. In contrast, an electrical frequency of 10 kHz is likely to lead to less protein denaturation and a destabilizing effect on the protein layer, as described above.

4. Conclusions

This study demonstrated the feasibility of pilot-scale production of Bologna-style sausages via ohmic heating. The results have shown that a microbiologically safe product with comparable product attributes can be produced in a timeframe that is less than 5% of the time needed to produce comparable-sized sausages via conventional heating (COV) in a pilot-scale steam oven with a conventional heating program. Thus, the possibility of heating Bologna-style sausages at an electrical frequency of 10 kHz is high.

The distinctive gel properties, like a lower hardness and chewiness, of OH-treated samples can be utilized to produce products with softer and juicier textures when desired. Furthermore, a higher water holding capacity could reduce the water release in packages and enhance the water regulation of Bologna-style sausages during storage. When differences in product attributes between OH- and COV-treated sausages are undesired (e.g., a less intense red color or a lower hardness of the OH-treated sausage), an adaptation of the OH process can be considered to reduce these differences. This could include, for example, the addition of an integrated holding time at 40–50 °C to increase reddening or an increased holding time to further stabilize protein–protein interactions.

Furthermore, we conclude that the technical setup, i.e., the OH cell, as well as the environmental conditions, i.e., the surrounding temperature, can be used to manage the occurrence of cold spots. This study demonstrated that a temperature gradient from the cold meat emulsion batter to the warmer cell surface/environment can improve current flow and thus heat generation within the outer layer of the sausage. This finding is of interest when producing consumer-safe products industrially via OH. Further research is needed to identify the limits and boundaries of using an initial heat gradient to decrease cold spot occurrences at the product/cell interface during an OH process. Finite element modeling is proposed at this point as a fast approach to gain deeper insights into this phenomenon.

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