**Protection of Camel Meat Against Heat Stress by Vitamins C and E**

**ABSTRACT**

**Background**: Heating or cooking of meat could alter its chemical composition and sensory profile. This work evaluated the oxidizing effect of heating on the camel meat and the protective effect of vitamins C (Vit C) and E (Vit E). **Methods**: Meat samples were pretreated by Vit C or Vit E or simultaneously by both vitamins C and E, then were heated until reaching increasing internal temperatures. Thiobarbituric acid-reactive substance (TBARS) and carbonyls contents, enzymatic activities of catalase (CAT), glutathion peroxidase (GSHPx) and superoxide dismutase (SOD), and sensory score (SS) were analyzed in meat samples. **Results**: Heating induced a significant increase in TBARS and carbonyls, and a significant decrease in CAT and SOD activities, and SS. TBARS and carbonyls in samples pretreated with Vit C or Vit E before heating were significantly (p<0.05) lower than untreated samples, and decreased more and more in presence of both vitamins at the same time. In samples pretreated with Vit C or Vit E, CAT and SOD activities, and SS were significantly (p<0.05) higher than those observed in untreated samples, and increased more and more with both vitamins treatment. **Conclusion**: The results of this study highlight the antioxidant potential of vitamins C and E and their synergistic effect in preserving the organoleptic and sensory characteristics of camel meat exposed to heat stress. These vitamins could be recommended as dietary supplements for a few months before slaughter to reduce the impact of environmental heat in camels.

**Key words**: Camel, heat stress, meat, oxidative stress, vitamins.

**1. INTRODUCTION**

Meats are an excellent source of nutritional and bioactive compounds for the consumer [1]. Camel meat has excellent nutritional and dietary properties and is important in human diets due to their low cholesterol and high unsaturated fatty acid and amino acid contents [2,3]. Camel meat compounds are largely influenced by preslaughter heat stress, cooking and ageing [4-9] during which a number of physiological, metabolic, and biochemical changes occur in meat, mainly involving lipid and protein oxidation which affect water-holding capacity, pH value and losses during storage and cooking. In these situations, an oxidative stress (OS) appears and deteriorate the meat organoleptic composition, then increases thiobarbituric acid-reactive substance (TBARS) and carbonyls contents, and decreases endogenous enzymatic substances like catalase (CAT), glutathione peroxidase (GSHPx) and superoxide dismutase (SOD) in the camel meat [4,5,9,10].

The addition of vitamin C (Vit C) and/or vitamin E (Vit E) directly to meat, or to the animal's diet, remains an effective means of minimizing the development of OS by heating or cooking, extending shelf life and storage time, improving oxidative stability and sensory characteristics, and preserving organoleptic and nutritional quality of livestock meat [11-13]. In the dromedary camel, administration of vitamin C and E dietary supplements increased vitamin E content and improved oxidative stability of lipids in muscle, and this content was negatively correlated with MDA content [14]. To our knowledge, no study has investigated the effect of Vit C and/or Vi E in mitigating heat stress and improving the oxidative stability of camel meat. Thus, the objective of the present investigation was to evaluate the oxidizing effect of heating on the meat, and the protective effect of vitamins C and E against heat stress, by analysis of MDA and carbonyl contents, enzymatic activities of CAT, GSHPx and SOD, and sensory profile in the dromedary camel.

**2. MATERIALS AND METHODS**

**2.1 Study Site and Animals**

The study was carried out on 5 healthy male camels (*Camelus dromedarius*) from the municipal slaughterhouse of Casablanca, Morocco (2 to 3 years, 170 to 260 kg). Casablanca is located in the west of Morocco (North Africa, latitude 33° 34' 42'' N, longitude 7° 36' 24'' W). The animals lived in similar conditions, were exposed to the same conditions before slaughter and were fed respectively barley concentrate and dry hay straw. They were deprived of water and food, transported and carefully unloaded upon arrival. They were calmly handled and then slaughtered using the halal method without any stunning. The slaughter and then all handling of the carcasses were carried out according to common traditional manual practice. No ethical approval was obtained because this study only involved non-invasive procedures in the form of meat samples collection.

**2.2 Muscle Sampling and Treatment**

Four hours after slaughter, skinning, evisceration and then cutting of the camel carcasses, a portion of the long dorsal muscle (longissimus or *Longissimus dorsi*) on the right side of the carcass of each animal was collected in a sterile plastic bag, using a sharp knife. The muscles were transported for 10 to 15 minutes in a cooler at 4°C, from the slaughterhouse to the Ecology and Environment laboratory in Ben M'Sik Faculty of Sciences, Casablanca (Morocco), and were stored at 4°C until the 24-hour *post-mortem* time. After this time, all external fat and connective tissue were removed. A meat portion of approximately 500 g from each animal was ground twice at 4 °C through a plate with 6 mm holes of a laboratory meat grinder, and then 200 g were divided into 8 groups (Gr) (numbered Gr1 to Gr8) for the biochemical analysis, and 300 g were divided between groups 4 to 8 for the sensory profile determination.

D-alpha-tocopherol was prepared at a concentration of 0.6 mg/mL in white mineral oil (WMO) and was added to a concentration of 6 mg/kg of minced meat. L-ascorbic acid sodium solution was prepared in 0.9% NaCl solution at a concentration of 50 mg/mL, and was used at a dose of 500 mg/kg of minced meat. Immediately after the addition of these solutions, each sample was thoroughly mixed by hand. The eight minced meat groups of each animal were allotted to the following treatments:

Gr1 (control 1) was pretreated with 0.5 mL NaCl 0.9%.100 g-1 and was unheated.

Gr2, Gr3 and Gr4 were pretreated with 0.5 mL NaCl 0.9%.100 g-1 and were heated to reach different internal T° (respectively 60 °C, 70 °C and 80 °C).

Gr5 (control 2) was pretreated with 0.5 mL of WMO and heated to reach 80°C.

Gr6 and Gr7 were pretreated with 0.5 mL of vitamin C solution (50 mg.100 g-1) and 0.5 mL of vitamin E solution (0.6 mg.100 g-1), respectively, then were heated to reach 80 °C.

Gr8 was pretreated with both vitamins C (50 mg.100 g-1) and E (0.6 mg.100 g-1), then heated to reach 80 °C.

Thirty minutes after treatment, meat groups were shaped into patties then heated. After cooling immediately on ice, all groups were vacuum-packed separately then stored in plastic bags in the freezer at -80°C until analysis of TBARS, carbonyls, CAT, GSHPx and SOD, except the samples recovered from Groups 4, 5, 6, 7 and 8 intended for the sensory profile determination.

**2.3 Heating Procedure of Meat Samples**

In the absence of ingredients, the meat samples were packed separately in a polyethylene bag and completely immersed in a water bath preheated to 100°C for 10 min until reaching different temperatures inside these samples. These temperatures were monitored using a Digital Kitchen Cooking Thermometer (model Alla France, Reference 91000AF003) with the food-grade stainless steel percing probe and a measurable temperature range from -50 to +300 °C (ac- curacy: ± 1 °C). After heating, the bags were removed from the water bath and immediately immersed in an ice bath for 15 min to cool the samples.

**2.4 Estimation of Carbonyl and Protein Content**

Protein oxidation was assessed by the absorbance of protein carbonyl groups at 370 nm (spectrophotometer JENWAY6320D visible range, JENWAY, GB), using the molar extinction coefficient of the hydrazone (22000 L.M-1cm-1 formed after derivatization of the carbonyl groups with 2,4-dinitrophenyl hydrazine (DNPH) [15]. The carbonyl content was expressed as nmole of incorporated DNPH/mg protein.

Protein concentration was calculated by measuring the absorbance at 280 nm (UV-visible spectrophotometer, model UVILINE9400, Ref: SOC-UVILINE9400, France) and comparing it with that obtained in the case of bovine serum proteins used as standards.

**2.5 Determination of Thiobarbituric Acid Reactive Substances Values**

Lipid oxidation of meat samples was estimated by the TBARS assay using the method described by Botsoglou et al. [16] with slight modifications. Briefly, 3 g of meat samples were homogenized at 37,000 x g for 1 min, with 20 mL of ultrapure water. After adding 5 mL of 25% trichloroacetic acid, the homogenate was centrifuged (SIGMA centrifuge, model 2-16 K, Germany) at a temperature of 4 °C for 15 min at 10,000 x g, and then the supernatant was filtered. In a test tube, 3.5 mL of the extract solution was added to 1.5 mL of 0.6% aqueous 2-thiobarbituric acid, and the solution was kept in a water bath at a temperature of 70 °C for 30 min, then cooled in tap water for 10 min. The absorbance was measured at 532 nm (spectrophotometer JENWAY6320D visible range, JENWAY, GB) to calculate the TBARS value, and the results were expressed as nmoles of malondialdehyde/mg of proteins.

**2.6 Analysis of Catalase Activity**

An enzymatic fraction was prepared from 2 g of sample homogenized in 6 ml of ice-cold phosphate buffer (100 mM, pH 7.4) using a homogenizer IKA model T18 Digital Ultra-Turrax (IKA-Werke GmbH & Co. KG, Germany) at 13,000 rpm for 1 minute. The homogenate obtained was centrifuged at 2,000 rpm for 30 minutes (SIGMA centrifuge, model 2-16 K, Germany) at a temperature of 4°C. CAT activity was analyzed in the final supernatant. This activity was continuously monitored by the decomposition of hydrogen peroxide (H2O2) into H2O and O2, using the method of Sinha [17]. Fifty microliters of meat extract was placed in a UV cuvette with 2.9 ml of H2O2 solution (11 mM H2O2 in 50 mM phosphate buffer) and the absorbance kinetics at 240 nm were monitored at 1-s intervals for 3 minutes (UV-visible spectrophotometer, model UVILINE9400, Ref: SOC-UVILINE9400, France). The same phosphate buffer was used to prepare a blank. CAT activity was calculated using the molar extinction coefficient of H2O2 (39.5 L. M-1cm-1) in U/g, with U corresponding to the amount of enzyme required to decompose 1 μmol of H2O2 per minute, and was expressed in μmoles of H2O2/min/mg of protein.

**2.7 Analysis of Superoxide Dismutase Activity**

Total SOD activity was quantified according to the method of Paoletti et al. [18]. The procedure is based on the oxidation of NADH in the presence of superoxide anions generated by EDTA, MnCl2 and β-mercaptoethanol. As SOD transforms superoxide anions into hydrogen peroxide and the oxidation of NADH is itself linked to the availability of these anions in the medium, then, as soon as SOD is added to the reaction mixture it inhibits the oxidation of the nucleotide. Therefore, at high concentrations of the enzyme, the absorbance at 340 nm (spectrophotom- eter JENWAY6320D visible range, JENWAY, GB) remains stable, however, in the control (not added to SOD) it decreases. Twenty microliters of meat extract was placed in a UV tank with 167 μl of TDB (100 mM triethanolamine buffer, 100 mM diethanolamine, pH 7.4), 5 μl of 100 mM EDTA/50 mM MnCl2 pH 7 and 8 μl of 7.5 mM NADH. The decrease in absorbance at 340 nm was monitored in kinetic mode during each 42-second cycle for 21 minutes. One unit (1 U) of SOD activity was defined as the amount of enzyme required to inhibit the NADPH oxidation rate of the control by 50%. Enzyme activity was calculated in μmol/min/mg protein.

**2.8 Analysis of Glutathione Peroxidase Activity**

GSHPx activity was determined according to the method described by Chen et al. [19]. Meat extract was obtained from 5 g samples homogenized in ice-cold phosphate buffer (50 mM, pH 7) using a homogenizer IKA model T18 Digital Ultra-Turrax (IKA-Werke GmbH & Co. KG, Germany) at 13,000 rpm for 1 minute. Sample tubes were always kept in an ice-water bath during homogenization. After centrifugation at 2,800 g for 20 minutes at a temperature of 4 °C, supernatants were collected in microtubes and centrifuged again (10,000 g, 10 minutes, a temperature of 4°C) and stored at -80 °C until analysis. Twenty microliters of meat extract was placed in a UV cuvette with 80 μl of 50 mM phosphate buffer, 500 μl of the assay medium (100 mM potassium phosphate buffer, pH 7, 1 mM EDTA, 2 mM NaN3), 100 μl of glutathione reductase (2.4 U/ml), 100 μl of 10 mM L-glutathione, and 100 μl of NADPH (1.5 mM NADPH in 0.1% NaHCO3). After 1 min, 100 μl of 1.5 mM H2O2 was added and the kinetics of absorbance at 340 nm (spectrophotometer JENWAY6320D visible range, JENWAY, GB) were monitored at 20-s intervals for 2 min and compared to a blank consisting of the phosphate buffer. GSHPx activity was calculated using the molar extinction coefficient of NADPH (6,220 L.M-1cm-1) and expressed as units (U)/g protein, with U corresponding to the amount of enzyme required to oxidize 1 μmol NADPH/min.

**2.9 Sensory Profile**

Finally, five sensory evaluators specialized in food science participated in the evaluation of the meat sensory profile. This evaluation was carried out using a descriptive hedonic scale from 1 to 9 (9 being the highest sensory quality, while 1 corresponds to the lowest sensory quality) [20]. Sensory scores were assigned to the six sensory parameters (odor, color, elasticity, viscosity, and texture) of the meat samples of the Groups 4, 5, 6, 7 and 8 which were heated until reaching an internal T° of 80 °C.

**2.10 Data Analysis**

For each of the analyzed parameters of each animal, measurements were performed in duplicate on five animals and SD values were therefore calculated on ten values. The results are presented as mean ± standard deviation (SD). The effect of two factors: antioxidant treatment (Vit C, Vit E, Vit C + E and control) and heating T° (60°C, 70°C, 80°C and control) were evaluated by ANOVA (analysis of variance), followed by Tukey's post-hoc test on GraphPad Prism 8. A p-value of less than 0.05 was considered statistically significant. Data were analyzed with Statgraphics Centurion XV (StatPoint Tech, Inc., Warrenton, VA, USA).

**3. RESULTS**

**3.1 Heating of Unpre-treated Meat Samples with Vitamins**

**3.1.1 Thiobarbituric Acid Reactive Substances and Carbonyls**

In camel, the contents of MDA and carbonyls as indicators of peroxidation of lipids and proteins respectively, increased in the heated meat samples by comparison to unheated ones, and becomed more and more higher when the heating T° increased (**Figure 1**). MDA and carbonyl levels were significantly (p<0.05) higher in samples that reached an internal temperature of 60°C (2.23±0.15 and 2.16±0.14, respectively) and much higher (p<0.005) at 80°C (7.81±0.55 and 7.12±0.51, respectively) than those measured in unheated samples (1.75±0.16 and 1.64±0.15, respectively) (**Figure 1**).

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**Fig. 1.** TBARS (nmoles of MDA/mg of proteins) and carbonyls (nmoles of incorporated DNPH/mg protein) in pretreated camel meat with 0.5 mL NaCl 0.9%.100 g-1, then heated until reaching different internal temperatures. The data are expressed as mean and SD (n = 5 camels). (DNPH: 2,4-dinitrophénylhydrazine, MDA: malondialdehyde, TBARS: thiobarbituric acid reactive substances).

**3.1.2 Enzymatic Antioxidants Activities**

In the same heating conditions, meat enzyme activities of CAT and SOD, decreased by comparison to unheated samples, and becomed more and more lower when the heating T° increased. However, GSHPx activity showed no significant variation (**Figure 2**). CAT and SOD activities were significantly (p<0.05) lower when the internal temperature was 60°C (8.81±0.31 and 8.22±0.31, respectively) and much lower (P<0.005) at 80°C (4.31±0.32 and 5.23±0.36, respectively) than those observed in unheated samples (10.34±0.39 and 10.42±0.33, respectively) (**Figure 2**).

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**Fig. 2.** Catalase (CAT) (μmoles of H2O2/min/mg of protein), Glutathion peroxidase (GSHPx) (U)/g protein) and superoxide dismutase (SOD) (μmol/min/mg protein) activities in pretreated camel meat with 0.5 mL NaCl 0.9%.100 g-1, then heated until reaching different internal temperatures. The data are expressed as mean and SD (n = 5 camels).

**3.2 Heating of Pre-treated Meat Samples with Vitamins**

**3.2.1 Malondialdehyde and Carbonyls**

MDA and carbonyl contents in pre-treated meat samples with Vit C or Vit E, then heated at 80 °C, were lower than those analyzed in unpre-treated and heated samples, and were more lower in pre-treated simultaneously with both vitamins C and E then heated samples (**Figure 3**).

For the same heating internal temperature (80°C) and by comparison to controls (unpre-treated meat samples), MDA and carbonyl levels were significantly (p<0.05) lower in Vit C pre-treated samples (3.52±0.42 vs 7.31±0.55 and 3.47±0.41 vs 6.52±0.51, respectively) and Vit E pre-treated ones (4.91±0.41 vs 7.31±0.55 and 4.75±0.43 vs 6.52±0.51, respectively) and were much lower (p<0.005) in the presence of both Vit C and Vit E (2.07±0.35 vs 7.31±0.55 and 2.11±0.32 vs 6.52±0.51, respectively) (**Figure 3**).

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**Fig. 3.** TBARS (nmoles of MDA/mg of proteins) and carbonyls (nmoles of incorporated DNPH/mg protein) in camel meat after treatment with vitamin C (50 mg.100 g-1) and/or vitamin E (0.6 mg.100 g-1) then heating to reach 80°C. Data are means and SD (n= 5 camels). Vitamins C and E were prepared respectively in salt solution (NaCl 0.9%) and white mineral oil (WMO). The control was mixed with 0.5 mL WMO.100 g-1. (DNPH: 2,4-dinitrophénylhydrazine, MDA: malondialdehyde, TBARS: thiobarbituric acid reactive substances).

At the same internal temperature (80°C) and by comparison to controls (unpre-treated meat samples), CAT and SOD activities were significantly (p<0.05) higher in Vit C pre-treated samples (7.68±0.61 vs 5.12±0.55 and 8.73±0.67 vs 6.11±0.61, respectively) and Vit E pre-treated ones (6.75±0.56 vs 5.12±0.55 and 7.78±0.66 vs 6.11±0.61, respectively) and were much higher (p<0.005) in the presence of both Vit C and Vit E (9.25±0.67 vs 5.12±0.55 and 9.37±0.71 vs 6.11±0.61, respectively) (**Figure 4**).

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**Fig. 4.** Catalase (CAT) (μmoles of H2O2/min/mg of protein), Glutathion peroxidase (GSHPx) (U)/g protein) and superoxide dismutase (SOD) (μmol/min/mg protein) activities in camel meat after treatment with vitamin C (50 mg.100 g-1) and/or vitamin E (0.6 mg.100 g-1) then heating to reach 80°C. Data are means and SD (n= 5 camels). Vitamin C and vitamin E were prepared respectively in salt solution (NaCl 0.9%) and white mineral oil (WMO). The control was mixed with 0.5 mL WMO.100 g-1.

**3.2.2 Enzymatic Antioxidants Activities**

CAT and SOD activities in camel meat samples pre-treated with Vit C or Vit E, then heated at 80°C were higher than those analyzed in unpre-treated and heated samples, and were more higher in pre- treated simultaneously with both vitamins C and E and heated samples (**Figure 4**).

**3.2.3 Sensory Profile**

Overall acceptability was significantly (p<0.05) higher in meat samples pretreated with Vit C and/or Vit E and then cooked until reaching an internal temperature of 80°C, compared to the control (**Table 1**).

**TABLE 1.** Sensory score of camel meat after pretreatment with vitamin C and/or E then heating to reach 80°C. *Data are means and SD (n= 5 camels). Vitamins C and E were prepared respectively in salt solution (NaCl 0.9%) and white mineral oil (WMO). The control was mixed with 0.5 mL WMO.100 g-1*.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| 0.5 mL NaCl 0.9%.100 g-1 | 0.5 mLWMO.100 g-1 | 50 mgVit C.100 g-1 | 0.6 mgVit E.100 g-1 | 50 mg Vit C.100 g-1 + 0.6 mg Vit E.100 g-1 |
| 6.8 ± 0.26 | 7.1 ± 0.27 | 7.3 ± 0.37 | 7.7 ± 0.33 | 8.2 ± 0.41 |

**4. DISCUSSION**

In the present study, heating of camel meat induced a significant increase in the amounts of MDA and carbonyls, associated with a significant decrease in the activities of CAT and SOD. These observations could reveal an attack on the meat by the induced thermal stress [21,22]. The significant increase of MDA and carbonyls contents in heated camel meat, may be induced by reactive oxygen species and other oxidizing agents affecting the structure, function and biological activity of lipids and proteins [5,6]. These negative effects may be attributed to the presence of high iron-rich myoglobin, hemoglobin, haem prooxidants, polyunsaturated fatty acids and transition metal ions contents in the camel meat [3,10,23,24] which could promote oxidation of the meat [25].

In the camel meat samples heaving been treated with vitamins C and/or E, then heated, the MDA and carbonyl contents were lower, and the enzymatic antioxidant activities, and sensory characteristics were higher by comparison to untreated samples, suggesting that these vitamins might promote a significant protection of camel meat against OS. The activities of these enzymes inhibit oxidative damage to cell membranes caused by lipid peroxides [26]**.** Indeed, direct addition of Vit C to beef meat was responsible for greater pigment stability and lower lipid oxidation values ​​during refrigerated storage [27,28]. Furthermore, according to Realini et al. [29], the addition of Vit C to ground beef patties exposed to heat stress reduced their lipid peroxidation. On the other hand, a supply of supranutritional levels of vitamin E extended the shelf life and storage and improved the sensory characteristics of raw or cooked meat in pigs [30] and beef [31]. In rabbit meat [32]) and camel meat [14], the content of Vit E was negatively correlated with that of MDA. Indeed, Vit E is a fat-soluble vitamin capable of stopping the propagation of free radicals in cell membranes [12], reducing the oxidation of meat lipids and increasing its color stability [33]. In addition, in camels, vitamin E and vitamin C were able to protect red blood cells against hydrogen peroxide-induced hemolysis and reduce the formation of MDA [34]. In the present study, the simultaneous presence of both vitamins C and E showed a synergistic action to reduce the oxidation of camel meat induced by heating. A study using dietary supplementation of both Vit C and Vit E in pigs showed that MDA concentrations in meat were reduced compared to the control [11]. Furthermore, Vit C has been shown to enhance the antioxidant activity of Vit E, particularly the radical tocopheroxyls to their active form of Vit E [35] or by sparing available Vit E [36]. Furthermore, Yin et al. [37] reported that a mixture of both vitamins C and E delayed myoglobin oxidation, while Vit C or Vit E alone could not delay metmyoglobin formation. Furthermore, the simultaneous administration of both vitamins in the diet increased the oxidative stability of rabbit meat during cold storage [38]. Regarding the synergistic interaction of the 2 vitamins, according to Wijesundar and Berger [39], Vit C could be responsible for the regeneration of the active form of Vit E, thus increasing protection against lipid peroxidation. This regeneration seems to occur *in vitro* through the reaction of the tocopheryl radical with ascorbate [40]. Furthermore, the two vitamins could interact with each other and exert a synergistic effect to increase the Vit E content and reduce that of MDA in the muscle of rabbits [31]) and camels [14]. In addition, dietary supplementation of Vit E associated with intravenous infusion of Vit C immediately before slaughter could increase their concentrations and improve lipid stability in beef skeletal muscle [41].

**5. CONCLUSION**

The protective effect of Vit C and Vit E against OS during heating of camel meat was investigated for the first time. The study showed on the one hand, the presence of OS in camel meat induced by heating, in a reciprocal relationship between MDA and carbonyls, and CAT, GSHPx and SOD, and on the other hand, the protective role of vitamins C and E against this OS, by decreasing lipid and protein oxidation and increasing antioxidant enzymes activities, and sensory score. The improvement in the stability of lipids and proteins of camel meat exposed to heat was much greater in the simultaneous presence of the two vitamins, thus proving their synergistic action. The results of this study allow us to consider the use of vitamin C and/or vitamin E as food supplement(s) for 1 to 3 months before slaughter in order to minimize the impact of HS on the organoleptic and sensory characteristics of meat in dromedaries.

**Abbreviations:** CAT, catalase; GSHPx, glutathione peroxidase; MDA, malondialdehyde; OS, oxidant stress; pHu, ultimate pH; SS, sensory score; SOD, superoxide dismutase; TBARS, thiobarbituric acid-reactive substance; Vit C, vitamine C; Vit E, vitamin E; WMO, white mineral oil.

**Data Availability Statement**

All relevant data can be found within the article.

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