

Article

# Lipid Oxidation, pH and Colour in Beef as Affected by Gamma Irradiation—A Meta-Analysis

Krasimir Dimov <sup>1,\*</sup> and Teodora Popova <sup>2</sup><sup>1</sup> Agricultural Academy, Institute of Cryobiology and Food Technologies, 1407 Sofia, Bulgaria<sup>2</sup> Agricultural Academy, Institute of Animal Science, 2232 Kostinbrod, Bulgaria\* Correspondence: [krasimir.dimov@ikht.bg](mailto:krasimir.dimov@ikht.bg)**How To Cite:** Dimov, K.; Popova, T. Lipid Oxidation, pH and Colour in Beef as Affected by Gamma Irradiation—A Meta-Analysis. *Food Science and Processing* **2026**, *2*(1), 2. <https://doi.org/10.53941/fsp.2026.100002>

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**Abstract:** A meta-analysis of the effect of gamma irradiation on the accumulation of primary and secondary products of oxidation, as well as pH and instrumental colour characteristics (lightness—L\*; redness—a\* and yellowness—b\*) in beef stored at different temperatures and packaging was performed. A total of 15 studies were included, each concerning at least one of the examined indicators. The number of independent experiments according to the covariates (dose of irradiation, temperature of storage, and packaging) varied between 16 and 25 for the individual traits. The heterogeneity between studies determined the selection of the random effects model to be applied on the raw mean difference (effect size) in the analysis of the data. The results of the meta-analysis showed that the gamma irradiation of beef is associated with a significant increase in the peroxide value (POV) ( $p = 0.046$ ) and the content of thiobarbituric acid reactive substances (TBARS) ( $p < 0.001$ ) as well as a decrease in pH ( $p < 0.001$ ). The colour parameters L\*, a\*, and b\* varied insignificantly. While no effect of the dose of applied radiation and the type of packaging was found on the studied parameters, the storage conditions affected the TBARS content, which, however, remained at acceptable levels without negative influence on the high nutritional value of the meat.

**Keywords:** meta-analysis; gamma irradiation; beef; meat quality

## 1. Introduction

Global beef consumption has been constantly increasing. For the period 2013–2023, it rose from 63.02 to 69.46 billion tons [1], ranking it third after chicken and pork. The main challenge for the beef producers is the long-term storage of the meat. Several technologies for extending its shelf life exist, including the traditional freezing and canning, as well as the novel ultrasound, high hydrostatic pressure, infrared light processing, and gamma irradiation. The latter has two main advantages over the others—ensuring equal treatment in the whole product volume and allowing the treatment to be performed on already packed product, thus decreasing the cost and hazards of physical, chemical, and biological contamination. Food irradiation dates back over 100 years and has been considered a safe technology since the second half of XX century [2]. The main sources of ionizing radiation used in food are photons induced by the radionuclides  $\text{Co}^{60}$  and  $\text{Cs}^{137}$ , X-rays with a maximum power of up to 5 MeV, and accelerated electrons with a maximum kinetic energy of up to 10 MeV [3]. After many years of research and monitoring carried out jointly with FAO and IAEA, in 1981 World Health Organization (WHO) issued a final report, stating that irradiation of food with doses up to 10 kGy absolutely guarantees its safety [4]. Gamma radiation is most commonly used to inhibit pathogenic microflora in meat. However, it is important to monitor the impact on other quality parameters, such as those related to lipid oxidation. The latter is one of the main reasons for the deterioration of its quality [5]. Meat is a good source of both unsaturated and saturated fatty acids, which are contained in neutral lipids and phospholipids. Phospholipids constitute a relatively small part of



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the total lipids in meat (0.5–1%). However, they have a high content of polyunsaturated fatty acids [6]. The most vulnerable site for lipid oxidation is the double bond of polyunsaturated fatty acids. The primary products of lipid oxidation in foods are hydroperoxides. Measurement of their content is used as a primary indicator of the production of primary oxidation products in meat. During the early stages of oxidation, hydroperoxides increase because the rate of formation is higher than that of decomposition. However, since these compounds are unstable during the later stages of the oxidation, it is recommended to also measure secondary oxidation products, such as TBARS. Malondialdehyde (MDA, 1,3-propanedial) is the most important aldehyde formed during secondary oxidation and is present in the highest levels of all TBARS. It is responsible for the rancid flavour of foods. According to Arshad et al. [7], gamma irradiation of 7 kGy of duck meat reduced MUFA content. In another study, gamma irradiation in the range (1.13–3.17 kGy) was found to reduce polyunsaturated fatty acids in both neutral lipids and phospholipids of beef meat, regardless of the dose [8]. Gamma irradiation also affects quality characteristics—such as color, which is very important, as it is the first perception consumers have when purchasing a product. There are many publications in the literature that investigate the effect of gamma radiation on meat quality. However, most of them have contradictory results and also focus more on qualitative rather than quantitative assessment of the effect. The use of meta-analysis allows a quantitative assessment of the effect obtained from the results of individual studies. The advantage of meta-analysis over a single study with a small sample size is its higher power (i.e., combined sample size from individual studies) [9], which allows a more convincing characterization of the relationships between the variables in a certain field and the variables determining these relationships [10]. There is no consensus on the minimum number of studies to estimate the effect size in a meta-analysis. The minimum number may be two, but it has been found that this threshold may not fully reflect the complexity of the relationships [11]. Therefore, other researchers suggest at least five studies to estimate the effect in a meta-analysis [12]. The aim of this meta-analysis was to quantify the effect of gamma irradiation on lipid oxidation and quality characteristics (pH and colour) of beef.

## 2. Materials and Methods

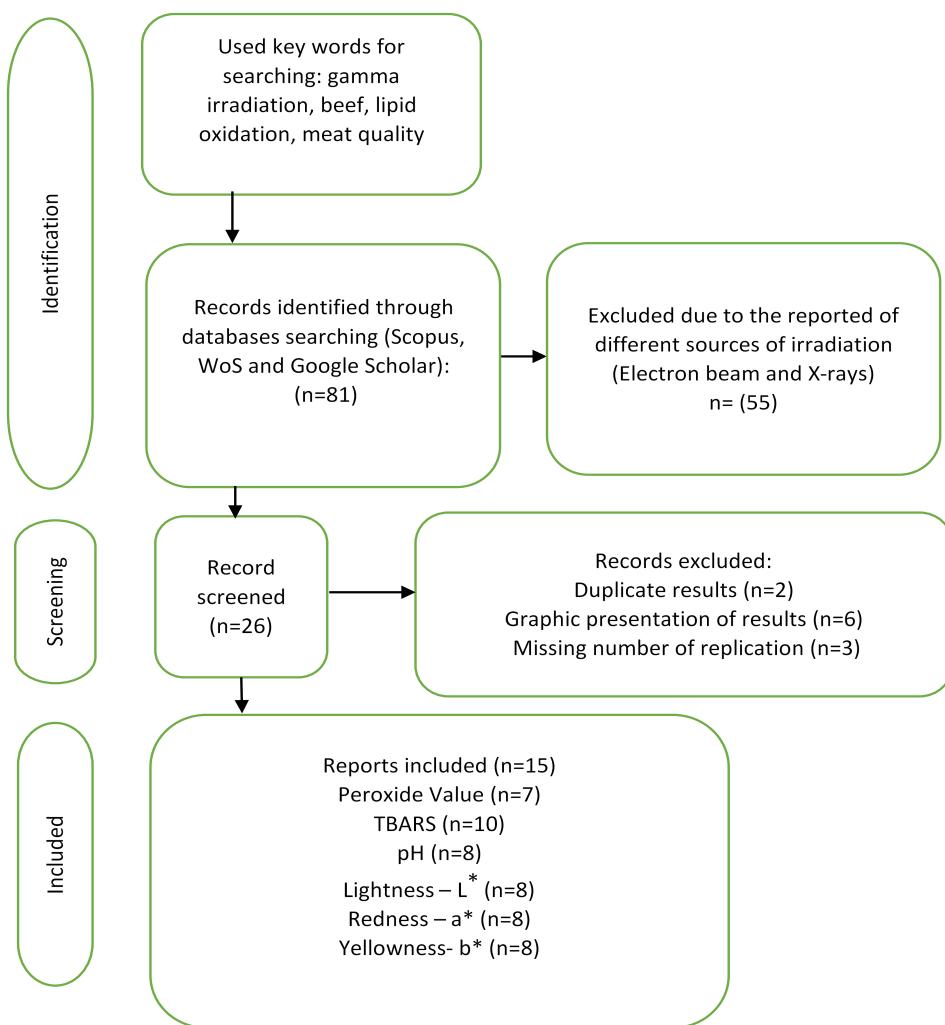
### 2.1. Selection of Studies

This work is based on an analysis of previously published studies. Literature sources reporting the effect of gamma irradiation on the studied parameters were selected after an exhaustive search in the Web of Science, Scopus, and Google Scholar databases in the period 1995–2024. Results obtained after irradiation with X-rays and linear electron accelerators were not used in the meta-analysis. The key words and expressions used were “beef”, “gamma irradiation”, “gamma rays”, “lipid oxidation”, “pH”, “color characteristics”, as well as combinations between them. The studies selected for the meta-analysis were peer-reviewed articles and book chapters in English.

Each observation in the meta-analysis included an assessment of the differences in mean values between the control and experimental groups, with studies necessarily including one of the measures of variation—standard deviation (SD), standard error (SE), mean square error (MSE), or root mean square error (RMSE).

### 2.2. Description of the Data Set

The preliminary goal was to find a minimum of six studies for each of the investigated parameters. After a detailed search in the used databases, schematized in Figure 1, 15 studies were found that met the data selection criteria. The units of measurement used for all reported results of primary and secondary lipid oxidation products were meq peroxide/kg of meat of PV and mg of malondialdehyde/kg of meat of TBARS. The values of the color parameters were presented as CIELAB coordinates. Raw beef was used in six of the studies [13–18] without specifying which muscle group was studied, *m. longissimus dorsi* was used by Sadakuzzaman et al. [19], Sales et al. [20] used beef strip loins (*Longissimus lumborum muscle*, LL), right and left, and Cap et al. [21] used beef trimmings. In two of the studies, minced meat was specified [22,23]. In the other two studies, the product used was beef pâté [24,25]. The last two studies reported products derived predominantly from beef sausage [26] and beef biltong [27]. Studies involving more than one radiation dose or different types of packaging were treated as separate studies for the meta-analysis. In studies reporting results at different storage intervals, the latter were averaged for each type of storage (refrigerated or frozen) according to the applied dose. In the studies [18,21,26], storage conditions and type of packaging were not reported. We assume that in these studies, the packaging is aerobic and the meat is refrigerated.



**Figure 1.** Flowchart of selection of the studies included in the meta-analysis on the effects of gamma irradiation in beef on lipid oxidation, pH, and color parameters.

### 2.3. Statistical Analysis

Statistical analysis was performed using JASP v.0.19.3.0 (the JASP team, 2018) and jamovi v.26.44 software. Since the primary studies used the same outcomes and units of measurement, the meta-analysis was performed on the raw mean difference (difference in raw mean values).

Most meta-analyses are based on one of two statistical models: a fixed-effects model or a random-effects model. The fixed-effects model assumes that the true effect size is the basis of all studies in the meta-analysis, hence the term “fixed effect”. Any differences in observed effects are due to sampling error. The random-effects model assumes that the true effect may vary from study to study due to differences (heterogeneity) between studies [28]. The presence of true heterogeneity between studies was identified using the Q test.

Random-effects model was opted for this meta-analysis due to heterogeneous data. The method of restricted maximum likelihood (REML) was applied to estimate heterogeneity variance as recommended by Langan et al. [29]. Quantification of the heterogeneity was done using the  $I^2$  index [30], describing the proportion of total variation across the studies that is due to heterogeneity. Whenever the values of  $I^2$  were greater than 50%, indicating substantial heterogeneity, meta-regression was applied to explore the sources of heterogeneity. Covariates included in the model were dose of gamma irradiation (continuous), storage (categorical, refrigerated compared to frozen), and package (categorical, vacuum compared to any other). The covariates were tested for significance at  $p < 0.10$ . The individual estimated effect sizes within studies were illustrated by forest plots, which were constructed for each outcome variable. In the forest plots after the reference, indicators have been used as follows: dose of irradiation, kGy, package (AP –aerobic, VP –vacuum), storage- refrigerated (R, 0–4 °C) or frozen (F, –18 to –20 °C). If the package was missing it was accepted as aerobic. If the storage was not indicated, we accepted it as refrigerated.

Additionally, studentized residuals and Cook's distances were used to examine whether studies may be outliers and/or influential in the context of the model.

### 3. Results and Discussion

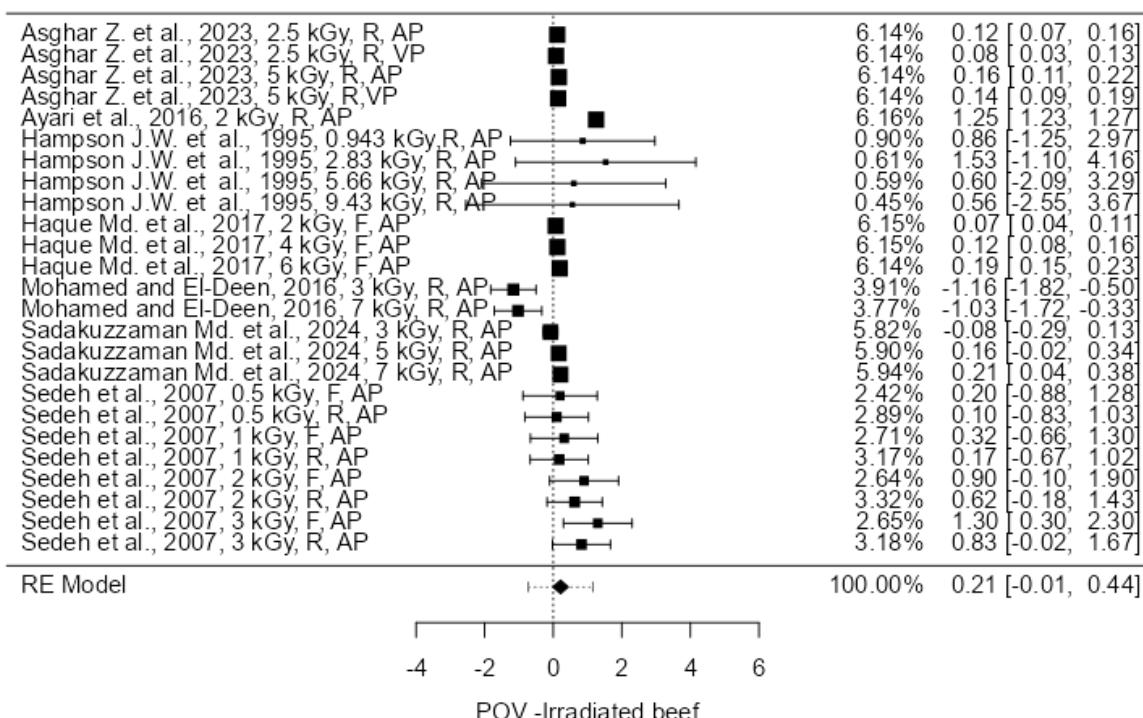
#### 3.1. Lipid Oxidation

The results of the meta-analysis concerning primary and secondary products of lipid oxidation are presented in Table 1. Gamma irradiation significantly increased the content of hydroperoxides ( $p = 0.046$ ) and TBARS ( $p < 0.001$ ). This could be attributed to the high energy of the gamma rays that contribute to free radical formation in meat that induces oxidation. The results revealed very high heterogeneity  $I^2$  in POV and TBARS (>90%).

**Table 1.** Effect size estimates of gamma irradiation on the primary and secondary products of lipid oxidation in beef meat.

Outcome	ES (95% CI)	SE	df	Z-Test ( $p$ -value)	Q ( $p$ Value)	$I^2$ (%)
POV	0.21 (0.00; 0.42)	0.11	24	2.00 (0.046)	9521.78 (<0.001)	99.38
TBARS	0.13 (0.09; 0.18)	0.02	23	5.58 (<0.001)	200.61(<0.001)	90.31

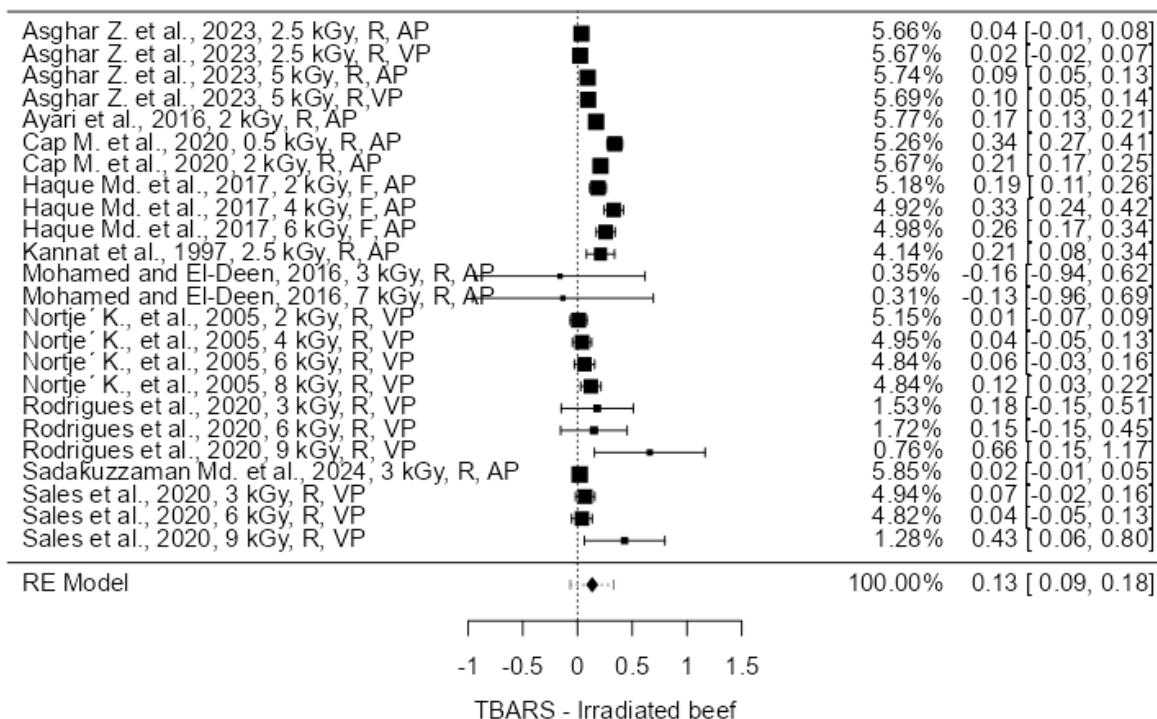
A total of 25 experiments were included in the analysis of POV. The random effects model showed significant mean differences among the individual trials (-1.16; 1.53). The effect size at CI 95% was 0.213 (0.00; 0.42). These results are evidence for a higher degree of peroxide formation following gamma irradiation. (Figure 2).



**Figure 2.** Forest plot of the random-effects models of the effect of the gamma irradiation on Peroxide value (POV) in beef meat [14–19,23,25].

Examination of the studentized residuals revealed that one study [23] had a value greater than  $\pm 3.0902$  and could be a potential outlier in the context of this model. According to Cook's distances, two studies ([15,23], 3 kGy, R, AP) could be considered overly influential.

The experiments on the effect of gamma irradiation on the TBARS in beef involved in the meta-analysis were 24. The random-effects model revealed mean differences among the experiments (-0.16; 0.66). The effect size at CI 95% was 0.13 (0.09; 0.18), showing higher TBARS formation after the irradiation (Figure 3). The studentized residuals showed no indication of deviation in the context of the model, and none of the trials could be considered overly influential according to Cook's distances.



**Figure 3.** Forest plot of the random-effects models of the effect of the gamma irradiation on TBARS in beef meat [13,15–17,19–21,23,25,27].

The high heterogeneity of the studies was additionally analysed through meta-regression, with dose of irradiation, storage method, and package as covariates (Table 2).

**Table 2.** Covariates affecting the effect of the gamma irradiation on the product of lipid oxidation in beef meat.

Covariates	Coefficient (CI 95%)	p Value
POV		
Dose	-0.08 (-0.20; 0.05)	0.208
Package (aerobic vs. vacuum)	0.11 (-0.54; 0.77)	0.719
Storage (refrigerated vs. frozen)	0.23 (-0.27; 0.73)	0.352
TBARS		
Dose	0.01 (-0.02; 0.03)	0.581
Package (aerobic vs. vacuum)	0.08 (-0.03; 0.19)	0.158
Storage (refrigerated vs. frozen)	-0.10 (-0.24; 0.04)	0.143

The studied covariates did not show a significant effect on POV. While the effect of the package (aerobic vs. vacuum) and storage (refrigerated vs. frozen) is expected, the result of the influence of the dose on the hydroperoxide formation is surprising, showing lower POV at higher doses of irradiation. In a study of lean ground beef irradiated with 1, 2.5, and 5.0 kGy, Lefebvre et al. [31] found a 9–12-fold increase in peroxide value compared to unirradiated meat. However, this study did not show any difference in the initial peroxide values induced by the irradiation doses. When stored at 4 °C, the authors found a further increase in POV compared to unirradiated meat, but these values were also independent of the dose of irradiation.

The increase of POV leads to deterioration in meat quality [32,33]. This is particularly visible in meat rich in fat and unsaturated fatty acids, since the oxidation generates numerous free radicals [34]. When they react with oxygen, hydroperoxides are formed. When studying the effect of gamma irradiation on other meats, Hashem et al. [32] found a significant increase in POV after irradiation with 1.5, 2, and 4 kGy of mutton stored for 60 days at -20 °C. According to Gracey et al. [35], POV up to 5 meq peroxide/kg in meat shows that it is still acceptable for consumption, when the POV is within the range of 5–10 meq peroxide/kg, initial rancidity is noticed. In this meta-analysis, the reported values for the hydroperoxide content were within 0.26–5.14 meq peroxide/kg.

Similar to POV, the studied covariates did not show a significant effect on the TBARS (Table 2). In regard to the formation of secondary products of lipid oxidation, Arshad et al. [36] found that gamma irradiation led to an increase in TBARS in raw and cooked beef when aerobically packaged. In the course of storage, the levels of TBARS increased together with the oxidation rate in the irradiated samples [37]. In a study of Khalid et al. [38] it

was demonstrated that the higher doses of gamma rays increased TBARS in ostrich and chicken meat. When irradiating vacuum-packed chicken thighs with 3 kGy, Du et al. [39] recorded much lower TBARS when compared to aerobically packed (0.90 mg/kg vs. 8.17 mg/kg, respectively). Other researchers showed increased TBARS after irradiation in broiler meat [40], duck [7], rabbit [41], and lamb [42]. All but one trial included in the meta-analysis showed levels of TBARS after irradiation  $<1.0$  mg/kg, which is considered a threshold for rancidity. Haque et al. [17] reported TBARS levels above 1.0 mg/kg; however, they exceeded this threshold before irradiation. Hence, we can accept that the irradiation doses of up to 9 kGy, especially combined with a vacuum package, do not deteriorate the quality of the beef.

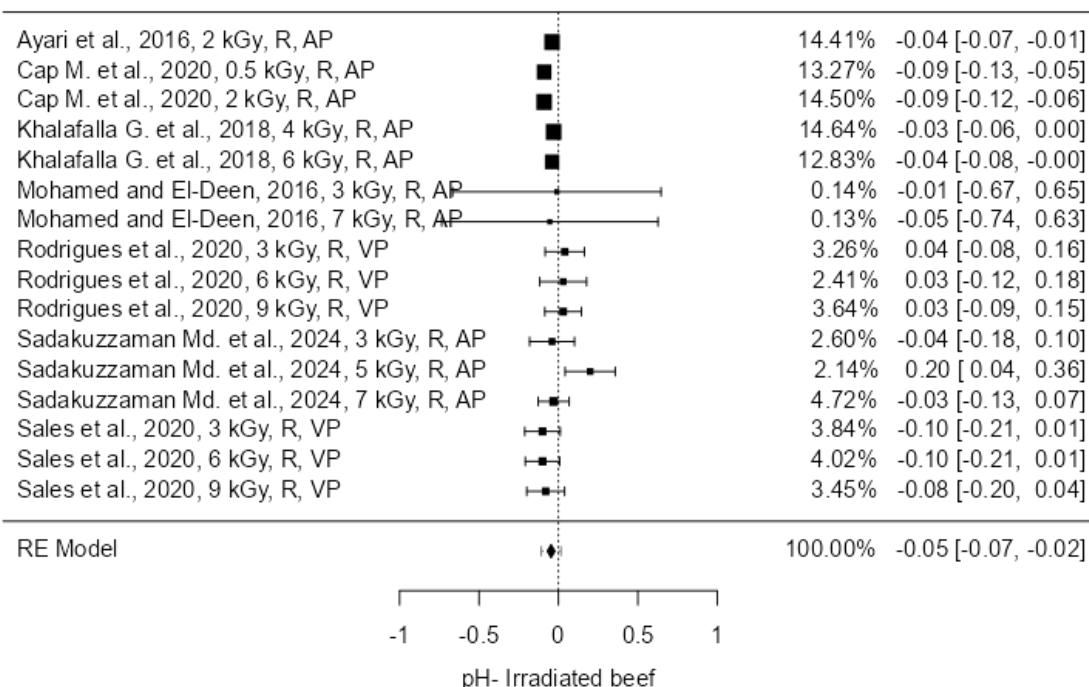
### 3.2. pH and Instrumental Colour

The results of the meta-analysis on pH and color are presented in Table 3. The results show very high heterogeneity  $I^2$  in  $a^*$  ( $>90\%$ ), high in  $L^*$  and  $b^*$  ( $70\text{--}75\%$ ), and medium in pH (46.78%).

**Table 3.** Effect size estimates of gamma irradiation on the pH and color parameters in beef meat.

Outcome	ES (95% CI)	SE	df	Z-Test (p-Value)	Q (p Value)	$I^2$ (%)
pH	-0.05 (-0.07; 0.02)	0.01	15	-3.69 (<0.001)	29.39 (0.014)	46.78
$L^*$	0.606 (-0.251; 1.462)	0.44	22	1.39 (0.166)	74.64 (<0.001)	74.65
$a^*$	-0.759 (-1.660; 0.141)	0.46	22	-1.65 (0.098)	345.26 (<0.001)	94.13
$b^*$	-0.312 (-0.704; 0.080)	0.20	22	-1.56 (0.119)	76.47 (<0.001)	70.95

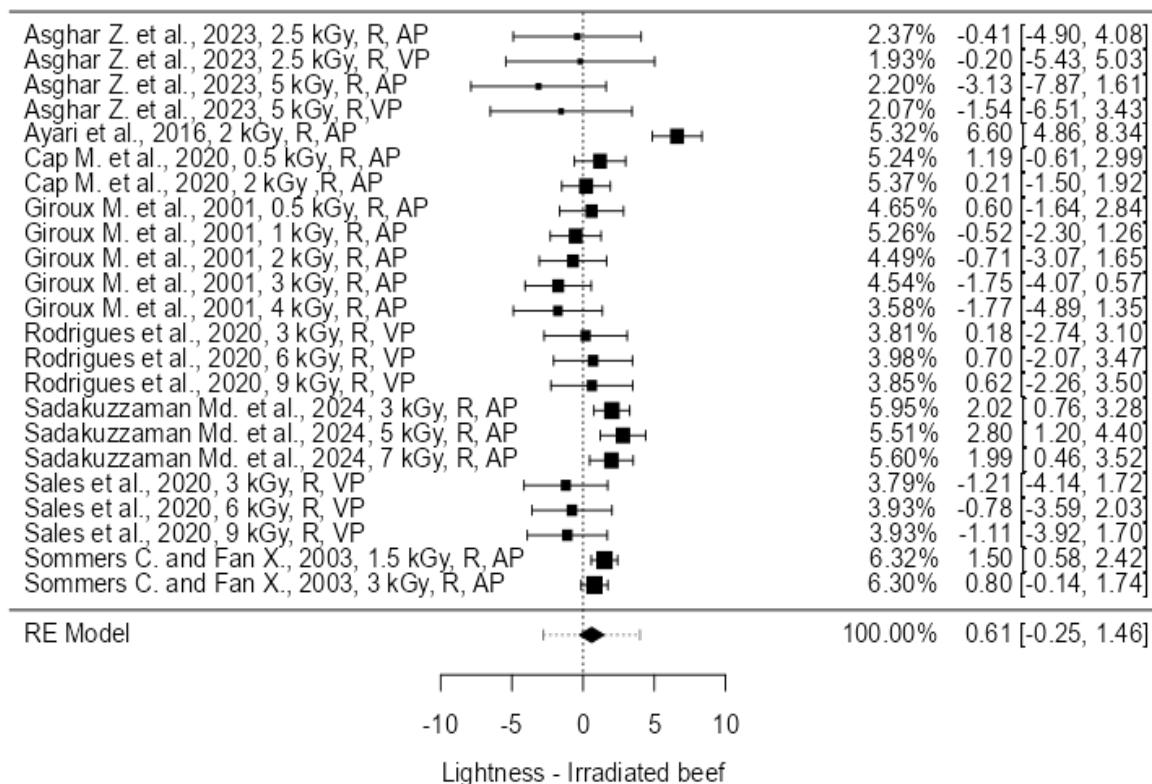
A total of 16 studies were included in the analysis of pH. The random-effects model applied to pH showed differences between studies (-0.09; 0.20). The effect size at 95% confidence interval was -0.05 (-0.07; 0.02) towards decreasing pH values (Figure 4). The examination of the studentized residuals revealed that one study ([19], 5 kGy, R, AP) could be a potential outlier in the context of this model, while according to Cook's distances, none of the studies could be considered overly influential.



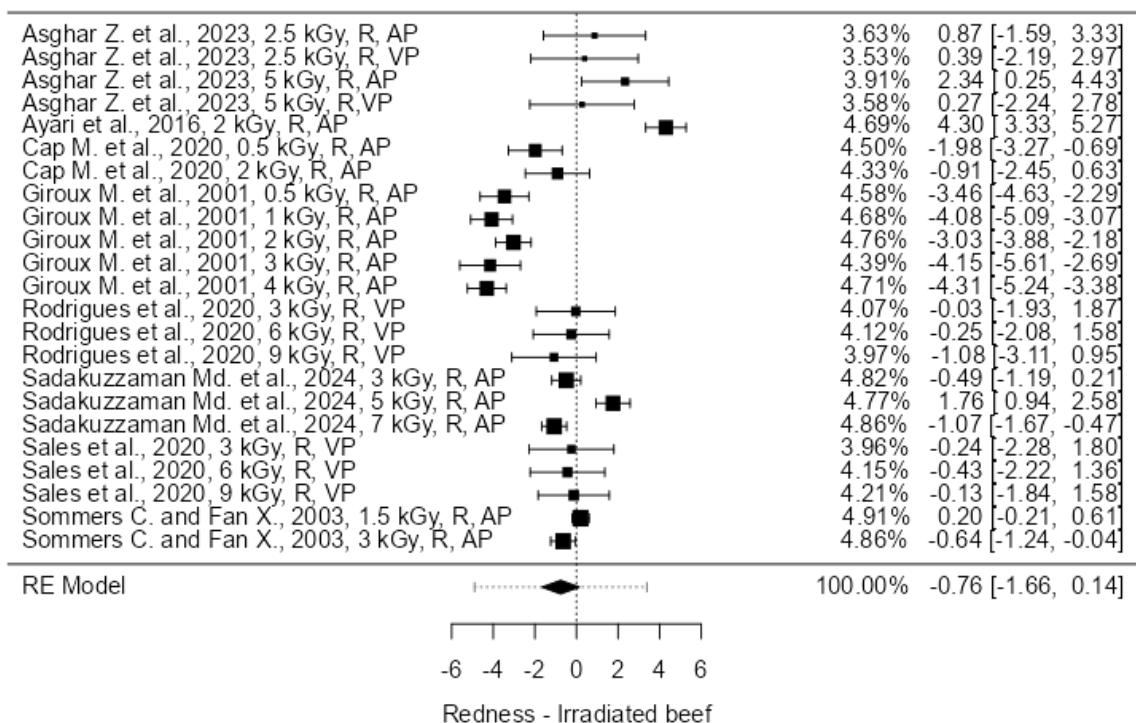
**Figure 4.** Forest plot of the random-effects models of the effect of gamma irradiation on pH in beef meat [15,16,19–23].

A total of 23 studies were included in the analysis of the colour characteristics. The random effects model applied to the colour characteristics showed significant variation between studies (-3.13; 6.60) for  $L^*$ , (-4.31; 4.30) for  $a^*$ , and (-2.76; 0.95) for  $b^*$ . The effect size at 95% confidence interval was 0.606 (-0.25; 1.46) for  $L^*$ , -0.76 (-1.66; 0.14) for  $a^*$  and -0.31 (-0.70; 0.08) for  $b^*$ , respectively, and in the direction of increasing lightness and decreasing the values of the red and yellow components of the irradiated meat (Table 4). The results of the meta-analysis of gamma irradiation on these indicators are demonstrated in Figures 5–7. According to the results obtained, gamma irradiation did not significantly affect  $L^*$  and  $b^*$  of the meat, with only  $a^*$  showing a trend ( $P = 0.098$ ).

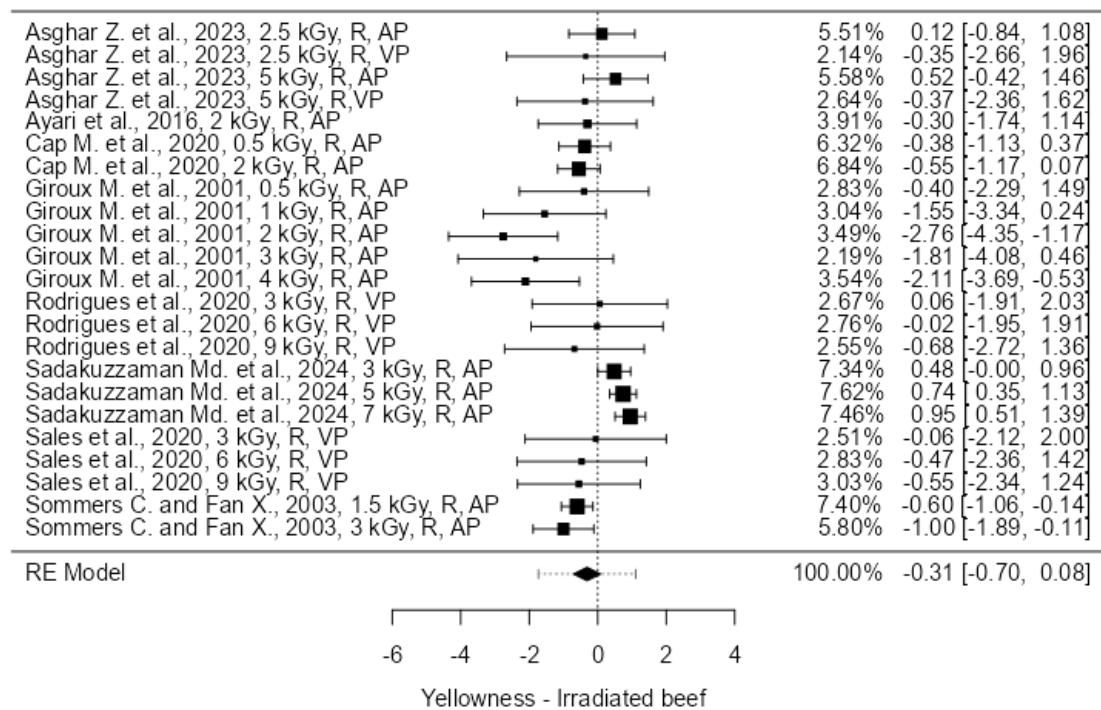
Examination of the studentized residuals revealed that one study ([23]) could be a potential outlier in the context of this model with respect to  $L^*$ . According to the Cook's distances, none of the studies could be considered to be overly influential.



**Figure 5.** Forest plot of the random-effects model of the effect of the gamma irradiation on lightness ( $L^*$ ) in beef meat [16,19–21,23–26].



**Figure 6.** Forest plot of the random-effects model of the effect of the gamma irradiation on redness ( $a^*$ ) in beef meat [16,19–21,23–26].



**Figure 7.** Forest plot of the random-effects models of the effect of the gamma irradiation on yellowness ( $b^*$ ) in beef meat [16,19–21,23–26].

The high heterogeneity of the studies was further investigated by meta-regression, with the covariates studied being the dose of irradiation and packaging (Table 4).

**Table 4.** Covariates affecting the effect of the gamma irradiation on the physical characteristics of beef meat.

Covariates	Coefficient (CI 95%)	p Value
pH		
Dose	0.01 (-0.00; 0.02)	0.133
Package (aerobic vs. vacuum)	0.01 (-0.07; 0.09)	0.734
Lightness (L*)		
Dose	0.05 (-0.39; 0.48)	0.830
Package (aerobic vs. vacuum)	1.46 (-0.87; 3.79)	0.206
Redness (a*)		
Dose	0.14 (-0.34; 0.62)	0.555
Package (aerobic vs. vacuum)	-0.44 (-2.87; 1.99)	0.709
Yellowness (b*)		
Dose	0.19 (0.01; 0.37)	0.040
Package (aerobic vs. vacuum)	0.56 (-0.49; 1.61)	0.282

No significant effect of dose and packaging type on pH values was found. In the available literature there are studies reporting a lack of changes in pH after irradiation. In studies of Lasmawati et al. [43] and Sadakuzzaman et al. [44] it was demonstrated that the exposure of fresh beef and meatballs to medium (3, 5, and 7 kGy) and high (20, 25, and 35 kGy) doses of gamma radiation did not lead to a significant impact on pH values. Results reported by Abdel-Khalek et al. [45] showed that the pH of chicken breasts remained unchanged after exposure to gamma radiation at 2 and 4 kGy. Studies on other types of meat also showed that gamma irradiation at different doses did not affect pH, for example, in mutton [46], camel meat [47], and pork meat [48]. On the other hand, a study conducted by Hashem et al. [32] showed a significant decrease in pH after irradiation with doses of 1.5, 2, and 4 kGy stored for up to 60 days at a temperature of  $-20^{\circ}\text{C}$  for the entire period studied. This may be due to ionic imbalance (release of hydrogen ions) caused by freezing-induced denaturation of buffering proteins [49]. The pH of gamma-irradiated meat may decrease during storage due to several factors, such as protein degradation or proteolysis [43,50], microbial activity, an increase in free fatty acids content, and reactions leading to rancidity.

The decreasing values of pH might be associated with significant changes in meat characteristics, and in particular, the water-holding capacity. The latter is one of the most important quality attributes for its crucial effects

on the product yield and hence the economic implications [51]. Water-holding capacity of meat can also influence its processing characteristics, and products derived from meat with reduced ability to retain water often are of inferior quality. From the studies included in the meta-analysis, only a few reported results on the water-holding capacity of irradiated meat or associated losses of water. Sales et al. [20] found a decrease in pH in irradiated beef and also a decrease in the values of the water holding capacity, but higher purge losses. On the other hand, Rodrigues et al. [16] did not observe the effect of irradiation on pH in beef, they reported decreased water-holding capacity and higher purge losses in the meat that was irradiated.

An investigation of the influence of the covariates dose and packaging on the color characteristics showed a significant influence of dose only on the yellow component of the meat colour towards increasing the values of the indicator ( $P = 0.040$ ). Several factors influence the color of irradiated meat, including the concentration of heme pigment (especially myoglobin), the oxidation status, the formation of ligands, and the physical characteristics (irradiation dose, pH, temperature, and storage time). One of the reasons for the increase in the values of the red component of the meat after irradiation is the formation of carbon monoxide (CO) [52]. According to Lycometros and Brown [53], who studied the behavior of the red component in model systems, the change from oxymyoglobin to metmyoglobin on the surface can explain the lower values of  $a^*$  on the external surface of irradiated beef. According to Rodrigues et al. [16], the effect of irradiation on the color of chilled beef depends on several factors, such as muscle type, irradiation type/source, and irradiation time, but appears to be evident only when doses up to 6 kGy are applied. These authors reported that chilled beef irradiated with 9 kGy showed significant surface discoloration, with higher proportions of metmyoglobin. Irradiation of chicken breasts with a dose of 4 kGy of gamma rays resulted in a slight increase in  $a^*$  values, but  $L^*$  and  $b^*$  values were unchanged [54]. Some studies have shown that the packaging atmosphere (aerobic or anaerobic) has a greater effect on meat color than irradiation itself [55,56].

#### 4. Conclusions

Meta-analysis was used to assess the effect of gamma irradiation on lipid oxidation in beef, as determined by the content of primary (POV) and secondary (TBARS) products, as well as on the pH and colour. There is high variability between studies, which is partly explained by the inclusion of covariates, such as irradiation dose, storage method, and packaging. Significant effect of irradiation was found towards increasing POV and TBARS values and decreasing pH, while color characteristics remained without significant changes. However, lipid oxidation of gamma-irradiated beef remained within normal limits and preserved its nutritional value. This gives reason to conclude that gamma irradiation with doses up to 9 kGy can be recommended for use in practice.

#### Author Contributions

K.D.: conceptualization, data curation, writing—original draft preparation; T.P.: methodology, writing—reviewing and editing. All authors have read and agreed to the published version of the manuscript.

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#### Data Availability Statement

The data are available from the corresponding author upon request.

#### Conflicts of Interest

The authors declare no conflict of interest.

#### Use of AI and AI-Assisted Technologies

No AI tools were utilized for this paper.

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