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Effect of gamma irradiation on the primary and secondary products of lipid oxidation in raw chicken meat, stored under different temperatures and packaging – a meta-analysis

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ABSTRACT

A meta-analysis on the effect of gamma irradiation on lipid oxidation products in raw chicken meat subjected to different temperatures of storage and packageing was carried out. A total of 11 studies were examined in regard to the peroxide value (POV) and thiobarbituric acid reactive substances (TBARS). The high heterogeneity in the studies was decisive for the selection of the random effects model applied on the raw mean difference (effect size) for the analysis of the data. The results of the meta-analysis showed that gamma irradiation increased the contents of the primary (POV) and secondary products (TBARS) of lipid oxidation in the raw chicken meat ($P<0.001$). Further, meta-regression and the examined covariates indicated significant influence of the dose of radiation on the formation of POV ($P<0.001$), whereas TBARS contents tended to depend on the package of the meat. In most of the studies included in the meta-analysis, the contents of the lipid oxidation products remained in acceptable levels and the treatment with gamma rays did not affect negatively the high nutritive value of the meat.

Keywords: meta-analysis, gamma irradiation, chicken meat, peroxide value, TBARS

INTRODUCTION

Globally, meat consumption has been constantly increasing. For the last 30 years it has doubled and reached 324 000 000 tons for 2020, as the greatest share has been attributed to poultry, mostly chicken meat (over 40%) (OECD, 2022). The main challenge for the meat producers and traders is to preserve its shelf life as long as possible. There is a range of technologies for this purpose. Together with the most widely used freezing and curing, ionising

irradiation has also been applied. The sources of ionising rays, approved *by* the International Atomic Energy Agency (IAEA) are radionuclides Co^{60} and Cs^{137}, x-rays and electron beam. The quantity of irradiated foods is increasing each year as the majority of these foods are treated by gamma rays (IAEA, 2015). Their main advantage is the high penetrating ability which ensures even impact in the whole volume of the product without coming into contact. This allows the process to be carried out in already packed products which decreases the costs and the hazards of physical, chemical and biological contamination. The basis of the food irradiation was laid by the adoption of the World common standard of the Codex Alimentarius for irradiated foods in 1983 (CAC, 1983), which has undergone significant changes in 2003 (CAC, 2003). According to IAEA, the irradiation with doses up to 10 kGy is not dangerous for the human health. Furthermore, FAO/IAEA stated that the total irradiated produce in 2013 was approximately 700 000 tons. The main purpose of the food irradiation is reduction of microorganisms with first attempts dating back in the 1940s (Lawrie, 2006; Zhou et al., 2010). Food and Drug Administration in USA approved the irradiation for control of *Salmonella* and other harmful bacteria in chicken, turkey and other raw and frozen poultry meat in 1990 with highest dose of 3 kGy (Arvanitoyannis and Tserkezou, 2010). The application of gamma irradiation for inhibition of pathogenic and spoiling microflora in chicken meat has been extensively studied. Nevertheless, it is important to monitor also the effects on other quality parameters such as those associated with lipid oxidation. The latter is one of the main reasons for meat quality deterioration (Lorenzo et al., 2012; Domínguez et al., 2019). It shortens significantly the shelf life of meat, when exposed to oxygen and in conditions when the microbial spoilage has been prevented (chilling or freezing). Gamma rays cause radiolysis of the water that is in significant amounts in meat. This generates free radicals further inducing chemical reactions in the nutrients, particularly lipids. The most susceptible place for free radicals' attack in the lipid molecule is the double bond. Hence, the most susceptible lipids during irradiation are those containing polyunsaturated fatty acids (Tao, 2015). The main primary products of lipid oxidation in foods, are hydroperoxides. The measurement of their contents is used as the main indicator for production of primary oxidation products in meat. During the early stages of oxidation, the hydroperoxides increase since the rate of formation is higher than that of decomposition. However, since these compounds are unstable during the later stages of oxidative processes, they decompose rapidly and the lower contents of hydroperoxides might be as well indicator for advanced oxidation (Estévez et al., 2009). For this reason, it is recommended to measure also the secondary oxidative products such as TBARS. Malondialdehyde (MDA, 1,3-Propanedial) is the most important aldehyde formed during secondary oxidation and in highest levels of all TBARS. It is responsible for the rancid odour of foods

including meat (Jones, 2017) and is considered the main marker for lipid oxidation (Pereira and Abreu, 2018). The use of gamma irradiation in meat and meat products mainly aims to reduce the microbial population and prolong the shelf life. The research on the changes of quality characteristics of meat, including lipid oxidation as affected by gamma irradiation remain relatively scarce. Hence the main aim of our study was to assess the effect of the gamma irradiation on the lipid oxidative processes presented by POV and TBARS in raw chicken meat subjected to storage at different temperatures and packaging through meta-analytical approach.

MATERIALS AND METHODS

Selection of studies

Literature sources reporting the effect of the gamma irradiation on the lipid oxidation in raw chicken meat were selected after an exhaustive search in Scopus, Web of Science, Pubmed, Google Scholar databases. Key words used in the search included "gamma irradiation", "chicken meat", "meat", "lipid oxidation", and combinations between them. Studies selected for the meta-analysis were peer reviewed papers, book chapters and reviews in English. Each observation in the meta-analysis corresponded to the mean of each group (control and treated). As additional criteria for inclusion in the meta-analysis, the studies had to provide any measure of variation such as standard deviation (SD), standard error (SE), mean square error (MSE) or root mean square error (RMSE).

Description of the data set

The preliminary goal was to find minimum 6 studies for each of the examined indicators. After the extensive search a total of 17 studies were found to respond to the selection criteria and data about the primary and secondary products of lipid oxidation. Further, after careful examination, two of the studies were excluded due to graphically presented results, other two studies did not report any replicates, one had zero standard deviation, and one reported data of chicken meat treated with grape seed extract prior irradiation. Hence, the final set included 11 studies, 6 presenting data about POV and 10 reporting TBARS. The results were reported as mg of malondialdehyde/kg of meat for TBARS and meq peroxide/kg of meat for POV. Hanis et al. (1989) was the only exception, reporting POV as μequiv O_2/g fat, the data were converted and the study was included in the meta-analysis. Most of the studies did not specify the meat parts used for analysis (Islam et al., 2019; Arshad et al., 2019; Rima et al., 2019; Khalid et al., 2021; Nisar et al., 2019; Hanis et al., 1989). Two of the studies reported use of breast meat (Chouliara et al., 2008; Hassanzadeh et al., 2017), and in other three the analysed meat was minced (Kanatt et al., 1997; Xiao et al. 2011– legs; Bhoir et

al., 2019-breast). In two of the studies the experiments were carried out with chicken, supplied directly by poultry plant/slaughterhouse (Chouliara et al., 2008; Hassanzadeh et al., 2017). The rest of the studies reported commercially obtained chicken meat from local markets or super markets. The studies including more than one dose of radiation or various kinds of packaging were treated as individual studies for the meta-analysis.

Statistical analysis

Statistical analysis was performed through JASP v. 0.16.1 software (JASP team, 2022). Since the primary studies used the same outcome and units of measure, the meta-analysis was performed on the raw difference of means (raw mean difference). The meta-analysis can be carried out through fixed- or random-effects model. According to Borenstein et al. (2010) under the fixed-effect model it is assumed that there is one true effect size that underlies all the studies in the analysis, and that all differences in observed effects are due to sampling error. Studies that are included in meta-analysis often differ considerably in their design and conduct, leading to heterogenous results. The random-effects model accounts for these differences in the underlying study effects and includes between-study variability (true heterogeneity) as well as sampling error. The presence of the true heterogeneity between studies was determined by Q test. Its high values were the reason to select random-effects model for this meta-analysis. The method of restricted maximum likelihood (REML) was applied to estimate heterogeneity variance as recommended by Langan et al. (2019). Quantification of the heterogeneity was done through I^2 as described by Higgins and Thompson (2002). This index describes the proportion of total variation across the studies that is due to heterogeneity. When I^2 was greater than 50%, the sources of variation were explored by meta-regression. The covariates in the model included dose of gamma irradiation (continuous), storage (categorical, refrigerated compared to frozen) and package (categorical, aerobic compared to any other, including vacuum, or modified atmosphere). The covariates were tested for significance at $P<0.05$. 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, MAP1 –modified atmosphere, 30% CO_2/70% N_2, MAP2- 70% CO_2/30% N_2), storage-refrigerated (R, 0-4°C) or frozen (F, -18 to - 20°C). If the package was missing it was accepted as aerobic. Hanis et al. (1989) studied two kinds of storage at different temperatures for 24h prior radiation that are marked as T1(-15°C) and T2(+10°C) in the forest plot.

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.

A positive value of the effect size indicates that chicken treated with gamma irradiation presents higher value of the variables of interest, while a negative value indicates that control samples are superior to the treated ones.

RESULTS AND DISCUSSION

The results of the meta-analysis are presented in Table 1. Gamma irradiation increased significantly POV and TBARS contents in the raw chicken meat ($P<0.001$). This was expected since gamma rays have high energy and enhances free radical formation in meat leading to intensive oxidative processes. I^2 was >99 %, indicating that studies were highly heterogenous.

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

Outcome	ES (95% CI)	SE	df	Z -test (P value)	Q (P value)	I^2 (%)
POV	0.57 (0.26; 0.87)	0.156	24	3.648 (<0.001)	2547.794 (<0.001)	99.932
TBARS	0.22 (0.08; 0.35)	0.069	24	3.193 (<0.001)	1383.440 (<0.001)	99.750

Effect of gamma irradiation on the primary oxidation products (POV)

The random-effects model applied to POV showed a wide range of mean differences (0.03 - 3.01). The effect size at 95% confidence interval was 0.57 (0.26-0.87) These results indicate higher oxidation after gamma irradiation (Fig.1). Studies on meat from other species revealed similar effect of the gamma rays. Quattara et al. (2002) found that gamma irradiation enhanced lipid oxidation in minced beef. Earlier, Lambert et al. (1992), demonstrated fast oxidation of the lipids in beef irradiated with 0.25–1 kGy under oxygen permeable conditions. POV below 5 meq/kg, show that meat is good for eating or the hydroperoxides have converted into ketones. According to Gracey et al. (1999), POV ranging between 5 and 10 meq/kg indicated initial rancidity.

The high heterogeneity of the studies was further explored through meta-regression with dose of radiation, method of storage and packages as covariates (Table 2).

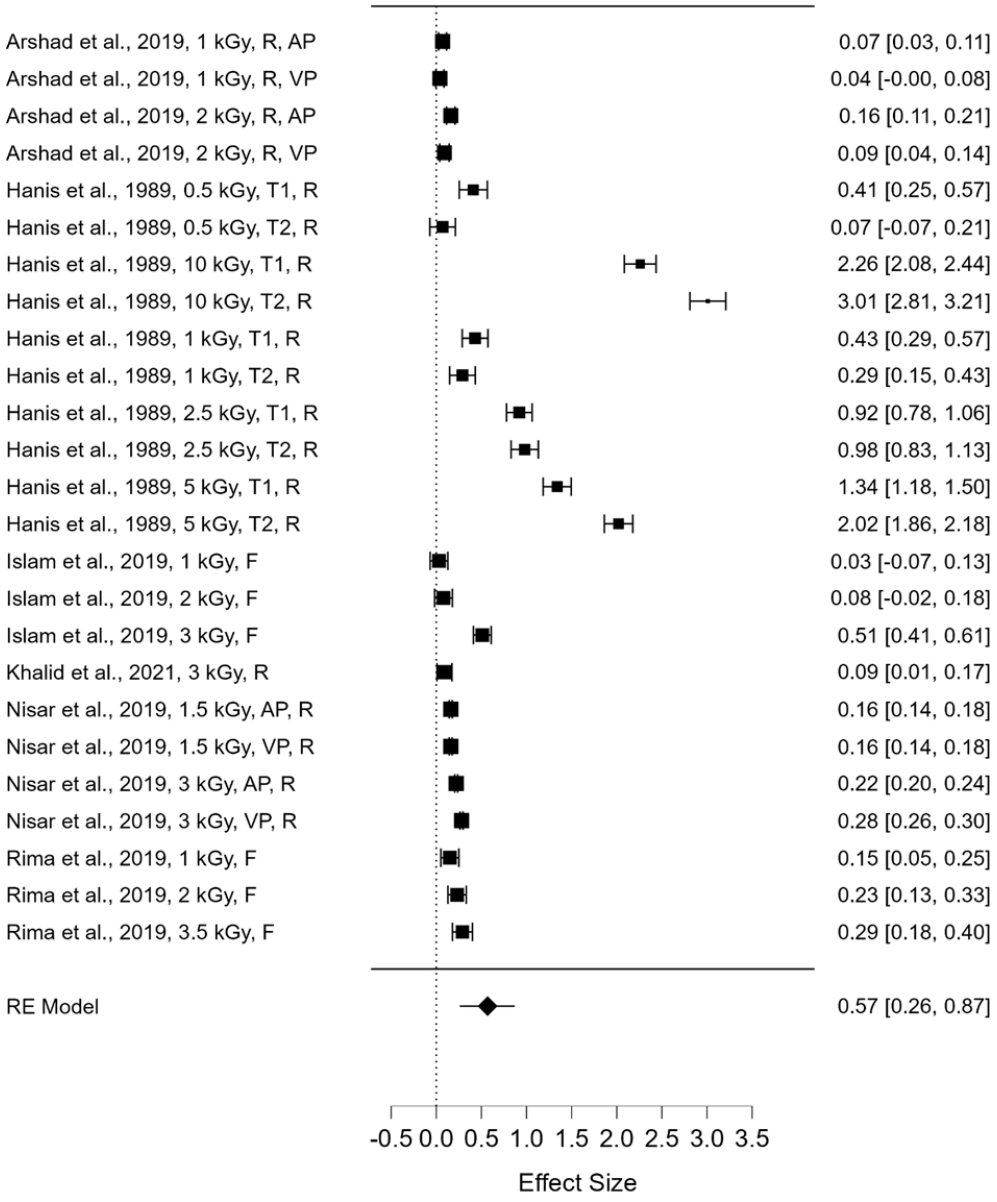

Figure 1. Forest plot of the random-effects models of the effect of the gamma irradiation on Peroxide value in raw chicken meat

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

Covariates	Coefficient (CI 95%)	P value
POV		
Dose	0.266 (0.210; 0.321)	<0.001
Package	0.318 (-0.050; 0.686)	0.090
Storage	0.301 (-0.017; 0.619)	0.064
TBARS		
Dose	0.076 (-0.069;0.222)	0.303
Package	0.272 (-0.023; 0.567)	0.071
Storage	0.042 (-0.272; 0.355)	0.794

The main contributor for the heterogeneity and hence the overall effect was the dose of irradiation (P<0.001). The positive coefficient (0.266) showed that higher dose of gamma rays increased oxidative processes. The higher dose of irradiation means higher energy that causes ruptures of the lipid chains more pronounced in the polyunsaturated fatty acids. The temperature of storage and packages also tended to affect the overall effect. Both covariates had positive coefficients (Table 2), showing increased hydroperoxide formation in the meat under refrigeration and aerobic packaging. The examination of the studentised residuals showed that one of the studies (Hanis et al., 1989, 10 kGy, T2, R) may be a considered a potential outlier in the context of the model and also influential. However, since all the studies showed one-way influence of the gamma irradiation, this outlier will not affect the conclusions that can be derived from this meta-analysis.

Effect of gamma irradiation on the secondary oxidation products (TBARS)

The effect of the gamma irradiation on the formation of the secondary oxidation products is visualised in Figure 2. The applied model revealed that except one, all of the studies showed positive observed differences that varied from –0.03 to 1.39. The effect size was 0.22 (95% CI: 0.08, 0.35), indicating enhanced accumulation of secondary lipid oxidation products in the irradiated meat. This is in line with other studies. Islam et al. (2022) reported higher contents of TBARS (0.42-0.61 mg MDA/kg meat) after irradiating lamb with doses up to 4 kGy at storage temperature -20°C. Kannat et al. (2006) registered increased TBARS in irradiated lamb (2.5 and 5 kGy) by 34% and 89%, respectively. Nam and Ahn (2002) reported 9.83 mg MDA/kg after irradiating (5kGy) turkey meat in aerobic package. At the same conditions, but in vacuum package, the TBARS values were considerably lower – 0,98 mg MDA/kg. Meta-regression showed that the kind of package tended to contribute to the overall effect (P=0.071). The positive regression coefficient (0.272) indicated higher TBARS formation in the meat stored under aerobic

conditions. The dose of radiation and temperature of storage had negligible effects, contrary to POV.

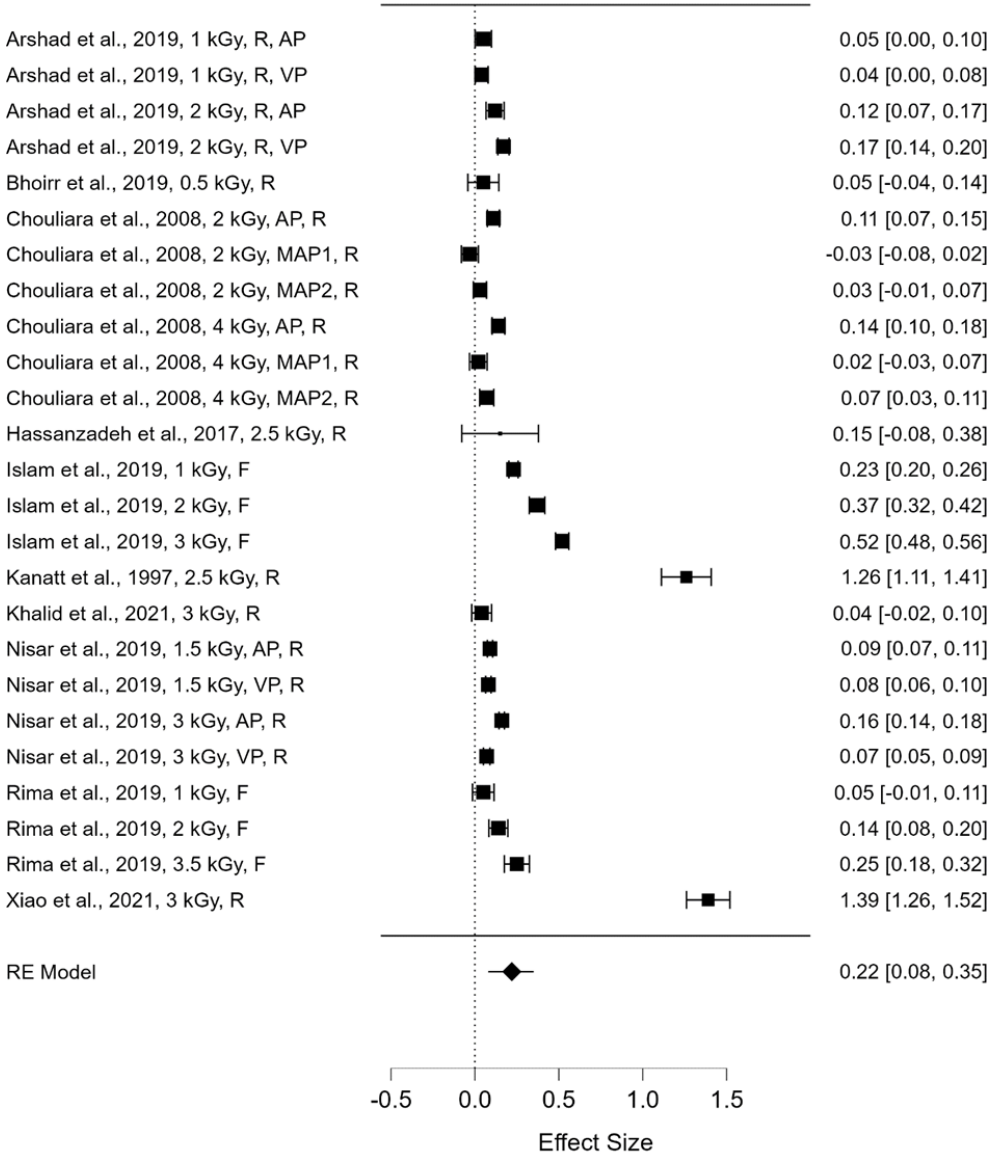

Figure 2. Forest plot of the random-effects models of the effect of the gamma irradiation on TBARS in raw chicken meat.

The studentised residuals and Cook's distance showed that two studies (Kanatt et al., 1997, 2.5 kGy, R; Xiao et al., 2021, 3 kGy, R) could be considered

outliers. As with the POV, these studies could not have significant impact on the final results regarding the direction of the effect of gamma irradiation in the raw chicken meat since 96% of the studies have positive difference.

Various studies showed values of 2–2.5 mg MDA/kg as a limit, at which there is still no rancid odour in the meat and meat products (Zhang et al., 2019). Regardless of the dose of irradiation, the TBARS values reported in the studies included in the meta-analysis were within the range of 0.24-2.61 mg MDA/kg meat. The highest values were reported by Kanatt et al. (1997) in refrigerated chicken meat irradiated with 2.5 kGy. Higher doses of gamma irradiation did not induce dramatic increase in secondary products of oxidation according to the results of Chouliara et al., 2008 (2kGy vs. 4kGy). For aerobically stored and packed in modified atmosphere raw chicken meat the authors reported TBARS values ranging from 0.1 to 0.5 mg/kg. The same was observed by Nisar et al. (2019) who demonstrated lack of difference between samples irradiated with 1.5kGy and 3 kGy stored either aerobically or under vacuum, with TBARS values within the range of 1.68-2.03 mg/kg. This indicates slight effect of the irradiation applied in doses up to 4 kGy on the development of lipid oxidation and its secondary products and hence the flavour of the raw chicken meat.

Conclusion

The meta-analysis was used to assess the effect of gamma irradiation on the lipid oxidation in raw chicken meat as defined by the contents of primary (POV) and secondary (TBARS) products. The considerable heterogeneity of the studies and the overall effect on the POV could be explained mainly by the dose of irradiation, while the packages and temperature of storage had less impact. The higher dose of gamma rays enhanced hydroperoxide formation. On the other hand, the kind of package contributed to the heterogeneity and the overall effect regarding TBARS formation. Aerobic conditions increased TBARS contents. Generally, doses of irradiation up to 4 kGy did not affect adversely the raw chicken meat, kept the oxidation levels low and could be recommended for use in practice.

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