TITLE PAGE
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ARTICLE INFORMATION	Fill in information in each box below
Article Type	Research article
Article Title	Antioxidant activity of radish seed oil and the quality and storage characteristics of pork patties with added radish seed oil
Running Title (within 10 words)	Antioxidant activity of radish seed oil in Pork Patties
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Special remarks – if authors have additional information to inform the editorial office	
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Conflicts of interest List any present or potential conflict s of interest for all authors. (This field may be published.)	The authors declare no potential conflict of interest.
Acknowledgements State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available. (This field may be published.)	This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture and Forestry(IPET) through the High Value-added Food Technology Development Project, funded by Ministry of Agriculture, Food and Rural Affairs(MAFRA)(321028-5). This work was supported by "Regional Innovation Strategy (RIS)" through the National Research Foundation of Korea(NRF) funded by the Ministry of Education(MOE)(2021RIS-001).
Author contributions (This field may be published.)	Conceptualization: Jang SY Data curation: Lim YH Formal analysis: Choi NY Methodology: Park SH Software: Park YH Validation: Oh SH Investigation: Park GT Writing - original draft: Jang SY, Kim CR Writing - review & editing: Jang SY, Kim CR, Park SH, Park YH, Park GT, Oh SH, Choi NY, Lim YH
Ethics approval (IRB/IACUC) (This field may be published.)	This article does not require IRB/IACUC approval because there are no human and animal participants.

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Abstract (within 250 words)

This study investigated the antioxidant activity of radish seed oil (RSO) and its effects on the quality and storage characteristics of pork patties. To assess the antioxidant capacity of RSO, this study analyzed fatty acid composition, peroxide value (PV), and DPPH radical scavenging activity. Pork patties were manufactured with the addition of RSO—0.4%, 0.8%, 1.6%, and 2.4%—and measured in terms of proximate composition, pH, water holding capacity (WHC), cooking loss (CL), color, texture profile analysis (TPA), and a sensory evaluation. Total microbial count (TMC), volatile basic nitrogen (VBN), thiobarbituric acid reactive substances (TBARS), and PV were measured at 1, 3, and 7 days of refrigerated storage. The DPPH radical scavenging activity of RSO was found to be 75.46%. In the cases of WHC and CL, there was no significant differences observed between RSO0.4%, RSO0.8%, and positive control (PC) (p>0.05). Meanwhile, RSO2.4% showed significantly lower hardness, springiness, gumminess, and chewiness than PC (p<0.05), and these values tended to decrease with the addition of increasing RSO. In terms of storage characteristics, with an increase in the amount of RSO added, TMC, VBN, TBARS, and PV all decreased; among the treatment groups, RSO2.4% showed the lowest values. In conclusion, RSO exhibits antioxidant activity, but when added in large amounts, it negatively affects the quality characteristics of patties while positively impacting their storage properties, thus necessitating a balanced

consideration of both outcomes. Therefore, adding 1.6% RSO is considered to be the most appropriate level for formulations to be used in practice.

Keywords: (3-5 keywords)

meat product, radish seed oil, antioxidant, quality characteristics, storage characteristics

Introduction

The worldwide consumption of meat products has been gradually increasing with population growth and rising average personal incomes (Godfray et al., 2018). Accordingly, the meat processing industry has also rapidly increased in recent years, and it is now expected to meet the needs of consumers interested in health (Jeong, 2016). Health functional food may vulnerable to a risk of microbial growth and fatty acid spoilage because it does not initially contain compounds such as preservatives or antioxidants. Microbial growth and fatty acid oxidation are thus considered to be major problems that impair the storage properties of these ground meat products (Shan et al., 2009). Therefore, when manufacturing processed meat products, chemical food additives are added to improve storage properties. However, nitrite—which is an additive that is mainly added to improve the functionality of processed meat products—has been suggested to form N-nitroso compounds in the body, and substances including nitrosamine can cause carcinogenicity (WHO, 2022). Although the amount of such food additives is regulated by the Food Sanitation Act (KFDA,

2023), consumers prefer processed meat products using natural additives because of negative perceptions of chemical additives and concerns about the adverse effects of ingesting large amounts of such additives. This had led to active research examining the founding of new types of value using food byproducts.

Due to their antioxidant and antibacterial properties, essential oils and various plant extracts can serve as alternatives to chemical additives (Banon et al., 2007). Vegetable oil extracted from seeds has the advantage of containing a high proportion of mono- or polyunsaturated fatty acids and no cholesterol, and when added to meat, it can increase the proportion of monounsaturated fatty acids such as oleic acid (Bloukas et al., 1993). Among these vegetable oils, radish seed oil and canola oil have been traditionally used; cruciferous oils have yet to be popularized despite having similar properties (Ratanapariyanuch et al., 2013). Cruciferous species are reported to confer several health benefits, including antioxidant and antibacterial activity (Avato & Argentieri, 2015).

Radish (*Raphanus sativus* L.) is a vegetable belonging to the cruciferous species that is consumed worldwide, and it contains bioactive compounds that are beneficial to human health. Radish seeds have also been reported to reduce the level of tumors and to be effective in preventing diabetes (Banihani, 2017). Radish seed oil is composed of unsaturated fatty acids, which can reduce the cholesterol content of meat products. Moreover, radish seed oil includes compounds such as Tocopherol, Glucosinolate, and Sulforaphene, which have antioxidant and antibacterial activities which are beneficial to the human body (Zhao et al., 2017; Carlson et al., 1985).

Therefore, the current study investigated the antioxidant activity of radish seed oil by analyzing the quality and storage characteristics of pork patties prepared with various amounts of radish seed oil.

Materials and Methods

Radish seed oil extraction method

The radish seeds used in this study were provided by PPS Co. Ltd. For low-temperature cold pressing extraction, 200 g of seeds was extracted using a cold press machine (NF-80, Karaerler, Ankara, Turkey) set at 18 Hz and 49℃. During the oil extraction process, a thermometer was used to ensure that the temperature of the machine and oil did not exceed 49℃. The extracted oil was collected using a vacuum filtration pump and filter paper to remove impurities, resulting in the collection of pure oil. The oil extraction yield was measured to be 20.84%, while the oil moisture content was measured to be 1.12%.

Fatty acid composition analysis

The fatty acid composition was analyzed using the AOAC validated method, which involved methylation followed by gas chromatography. For fat hydrolysis, a sample of 25 μL was treated with 1.5 mL of 0.5 N NaOH·MeOH. Next, a mixture of 2 mL of 14% boron trifluoride methanol solution, 2 mL of 2,2,4-trimethyl pentane, and 5 mL of saturated NaCl was added, and this mixture was then kept at room temperature for 15 min for fatty acid derivatization. Once the layers had separated, the upper layer

was filtered through a 0.45 μM membrane filter (Millipore, Billerica, MA, USA) and used as the sample for fatty acid composition analysis. At this point, the sample was injected into a gas chromatography instrument (HP 6890N/5973N MSD, Agilent Technologies, Wilmington, DE, USA) equipped with a flame ionization detector (FID) for analysis of the fatty acid composition. The gas chromatography column used was HP-INNOWAX (60 m × 0.25 mm × 0.25 nm, Agilent, Santa Clara, CA, USA). The column was held at 120℃ for 5 min, after which the temperature was increased at a rate of 5℃ per min until reaching 240℃, where it was maintained for 20 min. The injection temperature was set at 250℃, while the detector temperature was set at 300℃. Nitrogen (N2) was used as the carrier gas. To determine the fatty acid composition, the peaks corresponding to fatty acids in the sample were identified through comparison with a standard (CRM18918, Sigma-Aldrich, St. Louis, MO, USA). The relative composition of each fatty acid was calculated as the ratio of the area sum of each fatty acid to the total area sum of all fatty acids.

DPPH radical scavenging activity

The radical scavenging activity was measured using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. For the DPPH test, a small amount of DPPH solution was dissolved in ethyl acetate and adjusted to an absorbance of 0.700 ± 0.020 at 520 nm. The DPPH solution was then diluted with ethyl acetate. Next, 40 mg of the oil sample was weighed in a test tube, to which 160 μL of ethyl acetate and 5.8 mL of DPPH• free radical solution were added. The sample was then vortexed for 20 s.

After incubation in a dark room for 30 min, the absorbance of ethyl acetate was measured at 520 nm.

Peroxide value (PV)

To begin, 1.0g of the sample was placed in a triangular flask. Then, 10mL of chloroform was added to dissolve the sample completely, after which 15mL of acetic acid was added for mixing. A saturated KI solution was prepared by dissolving potassium iodide (99%) and distilled water in a 7:3 ratio. Next, 1mL of the saturated KI solution was added to the flask, which was then sealed and vigorously shaken for about 1 min, followed by incubation at room temperature for 10 min in a dark room. After that, 30mL of distilled water was added, and the mixture was homogenized again. At this point, 1mL of 1% starch indicator solution was added, and the mixture was titrated with 0.01N $Na_2S_2O_3$ standard solution until it turned colorless. A blank test was also conducted in parallel with the actual experiment.

Preparation of pork patties

Pork patties with RSO (RSO) added were prepared as described in Table.1. There are six treatments in total: PC (Tocopherol 0.1% + Sodium Nitrite 0.01%), NC, RSO4 (RSO 0.4%), RSO8 (RSO 0.8%), RSO16 (RSO 1.6%), and RSO24 (RSO 2.4%). The ground pork and materials were mixed for 5 min, then divided into 100g each and molded into a 10 cm diameter and a 1 mm thickness using a patty molding machine. The prepared patty was aged for one day through refrigeration for 24 h before being used as a sample for the experiment. After aging for one day after manufacturing, proximate composition, pH, water holding capacity, cooking loss, color, texture

profile analysis, and sensory evaluation were conducted. Total Microbial Count (TMC), Volatile Basic Nitrogen (VBN), Thiobarbituric Acid Reactive Substances (TBARS) and PV were measured while they were frozen for the storage period (1, 3, and 7 days). All experiments were performed in triplicate.

Proximate composition

Proximate component analysis was performed according to the Association of Official Analytical Chemists (AOAC, 2012) method. Moisture was analyzed using the atmospheric pressure drying method at 105℃, protein was analyzed using the Kjeldahl method, crude fat was analyzed using the Folch extraction method, and ash was analyzed using the direct ashing method.

pH

After taking 5 g of the sample, the mixture with 50 mL of distilled water was homogenized with a Stomacher for 30 s. Then, after immersing the electrode in the sample solution so that it was submerged, the value was derived by reading the value displayed on the pH meter.

Water holding capacity (WHC)

0.5g of ground meat was placed in the upper filter tube of a centrifuge tube. The weight was measured, and then the filter tube was heated in an 80℃ water bath for 20 min. After that, the filter tube was placed in the lower part of the centrifuge tube and centrifuged at 2000 rpm for 10 min. The filter tube was then removed, and the weight was measured again.

Water holding capacity (%) = (Total moisture – Free moisture) / Total moisture x 100

Free moisture = {(sample weight before centrifugation) – sameple weight aftger centirifugation) x 100} / (sample weight x fat coefficient)

Cooking loss (CL)

The cooking loss of the meat was determined by cutting the sample into cubic shapes that were approximately 50g. The sample was then heated in a 70℃ water bath for 30 min. The percentage of cooking loss was calculated by subtracting the weight after cooking from the weight before cooking, dividing it by the weight before cooking, and multiplying by 100.

Cooking Loss (%) = (Weight before cooking - Weight after cooking) / Weight before cooking x 100

Color

The color measurements were carried out using a color meter or spectrophotometer (CM-26d, Minolta, Japan) according to the specifications set by the International Commission on Illumination (CIE). The measurements included the values for lightness (CIE L^*), redness (CIE a^*), and yellowness (CIE b^*). The average values were recorded to represent the color characteristics of the meat. Color was measured after cooking the pork patties.

Texture profile analysis (TPA)

The texture profile analysis was conducted using a rheometer (Model Compac-100, SUN SCIENTIFIC Co., LTD. USA). The measurements included hardness,

springiness, cohesiveness, chewiness, and gumminess. The load cell was set at 10kg, while the cross-head speed was set at 200mm/min. TPA was measured after cooking the pork patties.

Total Microbial Count (TMC)

A sample of 10g is taken and mixed with 90mL of 0.1% peptone solution in a stomacher. The mixture is homogenized at 200rpm for 30 s. After that, the sample is serially diluted and inoculated onto Plate Count Agar (PCA) plates, which are then incubated at 36°C for 48 h. After 48 h, the microbial count is determined using a colony counter. The total microbial count is expressed in Log CFU/g.

Volatile Basic Nitrogen (VBN)

A sample of 10g is mixed with 90mL of distilled water. The mixture is then homogenized at 10,000 rpm using a homogenizer, before being filtered using a Whatman No. 2 filter. At this point, 3 mL of the filtrate is transferred to the outer chamber of a Conway unit, and 1mL of 0.01N boric acid and two to three drops of an indicator are added to the inner chamber. The unit is sealed, and 1mL of 50% K_2CO_3 is quickly injected into the outer chamber. After sealing the unit with a clip, it is incubated at 36°C for 2 h. The boric acid solution in the inner chamber is then titrated with 0.02N H_2SO_4. The VBN value is expressed as mg percent 100g of sample.

Thiobarbituric Acid Reactive Substances (TBARS)

A sample of 10g is mixed with 15mL of cold 10% perchloric acid and 25mL of 3rd-distilled water in a homogenizer. Next, the mixture is homogenized at 10,000 rpm for 10 s. The homogenate is then filtered using Whatman No. 2 filter paper. At this point,

5mL of 0.02M thiobarbituric acid (TBARS) solution is added to 5mL of the filtrate, and they are thoroughly mixed. After the mixture is allowed it to stand for 16 h in a cold room, the absorbance at 529nm is measured using a spectrophotometer. A blank using 3rd-distilled water is also measured. The TBARS value is expressed in terms of TBARS values, specifically as mg malonaldehyde per kg of sample. The standard curve used for calculation is y = 0.1975x-0.0011 (r = 0.999), where y represents the absorbance and x represents the TBARS concentration.

Sensory evaluation

The researchers conducted a sensory evaluation to assess the overall preference of the product. The following attributes were measured using a nine-point scale: color, flavor, smell, juiciness, texture, and overall preference. For color, a score of 1 indicated a lighter shade, while a score of 9 indicated a darker shade. For flavor, a score of 1 represented poor taste, while a score of 9 represented excellent taste. For smell, a score of 1 indicated an unpleasant odor, while a score of 9 indicated a pleasant odor. For juiciness, a score of 1 represented dryness, while a score of 9 represented high juiciness. For texture, a score of 1 indicated softness, while a score of 9 indicated firmness. Lastly for overall preference, a score of 1 represented a negative acceptability, while a score of 9 represented a positive acceptability. This study was approved by the institutional review board (IRB) at Chungbuk National University (IRB approval number: CBNU-202302-HR-0016).

Statistical analysis

All experiments were measured in triplicate. Statistical analysis was performed using the GLM (General Linear Model) method run in the SAS 9.4 program (SAS Institute, Cary, NC, USA). Mean comparisons within traits grousps were conducted using one-way ANOVA followed Duncan's Multiple Range Test ($p<0.05$).

Results & Discussion

Fatty acid composition

Table 2 presents the fatty acid composition of RSO. The identified fatty acids in RSO are classified into groups such as saturated, monounsaturated, and polyunsaturated fatty acids. Of the total fatty acid content, saturated fatty acids account for 9.53%, monounsaturated fatty acids account for 67.10%, and polyunsaturated fatty acids account for 23.38%. It is evident that RSO has a higher content of unsaturated fatty acids than saturated fatty acids. The major fatty acids present include oleic acid, linoleic acid, linolenic acid, and erucic acid. They respectively constitute 46.13%, 13.16%, 10.22%, and 20.75% of the total fatty acids.

This fatty acid composition is similar to that of RSO as reported by Kazlauskienė (2021) and Uluata (2012). Linoleic acid and linolenic acid have been reported to reduce the incidence of cancer and cardiovascular diseases (Parker et al., 2003). However, RSO contains erucic acid, which is a naturally occurring toxic substance that includes the accumulation of triacylglycerol in the heart as a result of insufficient oxidation (Waheed, A et al., 2019). Large amounts of erucic acid are also present in radish seed oil, but research suggests that by carefully considering the variety of

radish seed oil and the beneficial components present, the intake of erucic acid can be reduced when used for consumption (Wendlinger et al., 2014). It is therefore recommended that the variety of radish seeds should be carefully considered to minimize the intake of toxic substances.

Antioxidant test and oxidative stability test of radish seed oil

The PV and DPPH measurements of RSO are as listed in Table 3. Measuring hydroperoxides, which are the primary oxidation products of oil, is an important aspect of evaluating the oxidative status and quality of the oil. PV is commonly measured to assess the degree of lipid oxidation, with higher values indicating higher levels of oxidative rancidity in vegetable oils (Kyari, 2008). The PV of RSO was measured at 2.75±0.15 meq/kg, which is within the Codex standard value of 10 meq/kg.

The DPPH assay is used to measure the free radical scavenging ability of antioxidants, and it is useful for assessing the antioxidant activity of food (Marinova & Batchvarov, 2011). DPPH is a stable free radical, and its proton is reduced by the electron-donating ability of substances with antioxidant activity, thus allowing for estimation of the sample's antioxidant capacity (Katsube et al., 2004). The DPPH value of RSO was measured at 75.46±3.7%. Compared to grape seed oil and sesame oil, both of which are known for their high antioxidant activity with radical scavenging abilities of 43.50% and 63.88%, respectively (Kim et al., 2019), the DPPH value of RSO is relatively high. The relatively high antioxidant activity of RSO

is believed to be due to the high contents of vitamin E and unsaturated fatty acids present in cold-pressed extracted seed oil.

Proximate compositions

Table 4 presents the results of measuring the proximate composition of pork patties prepared with varying amounts of RSO. There was no significant difference in moisture content between PC and RSO4 (p>0.05), while moisture content showed a decreasing trend with increasing levels of tocopherol and RSO added. Regarding protein content, PC and RSO24 showed similar values (p>0.05). Overall, the addition of RSO resulted in an increase in fat content, which led to relative decreases in both moisture and protein content. Ash content did not show significant differences among all treatment groups (p>0.05).

pH, WHC and CL

Table 5 presents the results of measuring the pH, water-holding capacity (WHC), and cooking loss (CL) of pork patties manufactured with varying levels of radish seed oil addition. There were no significant differences in pH between PC, NC, and RSO4 (p>0.05). The pH of the RSO used in this experiment was 4.58, thus indicating slight acidity. Therefore, as the level of added RSO increased, there was a tendency for pH to decrease.

Regarding water-holding capacity (WHC), there were no significant differences between PC, NC, RSO4 and RSO8 (p>0.05); however, there was a decreasing trend with increasing levels of added RSO (p>0.05). WHC shows a minimum value around the isoelectric point of myosin and actomyosin, which is around pH 5.2-5.4. When pH

296 either increases above or decreases below this point, the meat's water-holding
297 capacity increases (Zayas & Zayas, 1997). It can therefore be inferred that the
298 decrease in pH influenced the decrease in WHC.
299 There were no significant differences in cooking loss between PC, NC, RSO4 and
300 RSO8 (p>0.05), but cooking loss was significantly lower in RSO16 and RSO24
301 (p<0.05). These results are consistent with the findings of Dzudie et al. (2004), who
302 reported that vegetable fat has lower water-holding capacity and higher cooking loss
303 than animal fat. Similarly, Paneras et al. (1994) and Lopez et al., (2011) found that
304 cooking loss increases when vegetable fat is added during the production of meat
305 products. The addition of plant fibers such as rice bran fiber, locust bean gum, and
306 xanthan gum has been reported to help preserve cooking loss when replacing pork
307 fat with vegetable fat (Luruena-Martinex et al., 2004). Therefore, when
308 manufacturing pork patties, the addition of plant fiber can enhance cohesion with
309 vegetable oil, maintain WHC, and improve emulsion stability to compensate for
310 cooking losses.
311 **Color**
312 Table 6 lists the results of measuring the color of uncooked pork patties
313 manufactured with varying levels of radish seed oil addition. In terms of lightness
314 (L^*), the treatment groups with added RSO showed significantly higher values
315 compared to PC (p>0.05). For redness (a^*), there were no significant differences
316 between NC, RSO4, RSO8, and RSO16, but PC showed significantly lower values
317 (p<0.05). The lower values of lightness (L^*) and redness (a^*) in PC can be attributed

318 to the addition of dark-colored tocopherol. For yellowness (b^*), RSO4 showed
319 significantly lower values ($p<0.05$) than other samples; on the other hand, there were
320 no significant differences in yellowness between PC and the other treatment groups
321 ($p>0.05$). Yellowness (b^*) tended to increase slightly with increasing levels of added
322 RSO, as RSO contains carotenoids, which are natural pigments that contribute to the
323 yellow color (Zhao et al., 2017). Therefore, RSO can be used as an additive to
324 increase the yellowness (b^*) of meat products.

325 **TPA**

326 Table 7 presents the results of measuring the TPA of pork patties manufactured
327 with varying levels of RSO added. In terms of hardness, there was no significant
328 difference between PC and RSO16 ($p>0.05$), but RSO24 showed a lower value than
329 PC ($p<0.05$). Regarding cohesiveness, no significant difference was observed
330 between PC and the treatment group ($p>0.05$). For springiness, there was no
331 significant difference between PC and RSO8 or RSO16 ($p>0.05$), but RSO24
332 showed a lower value than PC ($p<0.05$). In terms of gumminess, there was no
333 significant difference between PC and RSO16 ($p>0.05$), but RSO24 showed a lower
334 value ($p<0.05$). There was no significant difference in chewiness between PC and
335 RSO16 ($p>0.05$). These findings are consistent with the results reported by Park et
336 al. (2005) and Matulis et al. (1995), who observed a significant decrease in the
337 chewiness of beef patties with the addition of vegetable oil. These results are also
338 consistent with the results reported by Monteiro et al. (2017), who found that the
339 texture characteristics of pork sausages with canola oil added were lower than those

of the control group. As a result, it can be concluded that adding plant-based oil to pork patties without any pre-treatment leads to high cooking loss and negatively affects the tissue characteristics. Overall, RSO16 appears to show the best texture profile, as it does not exhibit any significant differences compared to PC. Additionally, hydrophilic binders such as carrageenan have been reported to aid in the emulsification of plant-based and meat products (Choi et al., 2019). Therefore, we suggest using a hydrophilic binder when utilizing vegetable oil to improve the texture of meat products.

TMC

The results of measuring the total microbial count (TMC) of pork patties manufactured with varying levels of RSO added on each storage day are shown in Fig 1. An increasing level of RSO on each storage day was shown to be associated with a tendency of decreasing TMC. Among the 7-day samples, NC exhibited the highest value, while the RSO24 showed the lowest values ($p<0.05$). Radish seeds contain health-promoting compounds such as sulforaphene and glucosinolates, which are found in cruciferous vegetables (Gutiérrez and Perez, 2004). Sulforaphene is a bioactive compound with anticancer effects that induces cell apoptosis and which has a similar chemical structure to sulforaphane isolated from broccoli seeds (Lim et al., 2020). Glucosinolates are organic compounds that are known for their powerful antimicrobial and anticancer properties. According to Mendiratta et al. (2013), adding radish to lamb nuggets resulted in lower microbial counts compared to the control group. This finding is consistent with a report by Lay et al. (2005) that

362 suggests that domestic radish seeds possess strong antimicrobial properties. RSO
363 has been shown to be effective as a natural antimicrobial agent in food products.

364 **VBN**

365 The results of measuring the volatile basic nitrogen (VBN) of pork patties
366 manufactured with varying levels of RSO added on each storage day are shown in
367 Fig 2. On day 1 of storage, PC exhibited the lowest value (p<0.05), and there was no
368 significant difference observed among the NC and treatment groups (p>0.05).
369 However, at day 3 and day 7, RSO16 and RSO24 showed significantly lower values
370 than NC (p<0.05). There was also a tendency for a slight decrease in VBN values as
371 the level of RSO increased. VBN content increases during storage due to the
372 breakdown of proteins into amino acids through bacterial action, thus indicating that
373 there is a close relationship between VBN content and bacterial proliferation (Kruk et
374 al., 2011). Therefore, the inhibition of protein spoilage by RSO is attributed to the
375 tocopherol compounds present in RSO, which inhibit bacterial growth. However,
376 domestic food hygiene regulations mandate that the VBN content be kept below 20
377 mg% for raw and packaged meat (KFDA, 2009). It is therefore inferred that the shelf
378 life of pork patties with RSO addition is within 7 days.

379 **TBARS and PV**

380 The results of measuring the thiobarbituric acid reactive substances TBARS and PV
381 of pork patties manufactured with varying levels of RSO added on each storage day
382 are shown in Fig. 3 and Fig. 4. In the case of TBARS, at day 1 and day 3, RSO24
383 showed values that were significantly lower than those in any of the other treatment

384 groups, while at day 7, PC, RSO16, and RSO24 exhibited the lowest values
385 (p<0.05).
386 For PV, there was no significant difference observed between PC and RSO24 on
387 days 1 and 7 (p>0.05). Both TBARS and PV showed a tendency of decreasing with
388 an increasing level of RSO. This is consistent with the study by Kazlauskienė (2021),
389 which reported that RSO has strong antioxidant activity. Plant-based oils contain
390 large amounts of phenolic compounds. RSO contains approximately 599.15 mg/kg of
391 tocopherol (Uluata & Ozdemir, 2012). Tocopherol, also known as vitamin E, is a
392 natural antioxidant that has phenolic hydroxyl groups that are capable of scavenging
393 free radicals. It has the ability to bind to proteins and macromolecules and exhibits
394 antioxidant activity (Manessis, 2020). The U.S. Food and Drug Administration (FDA)
395 has included tocopherol in its list of Generally Recognized as Safe (GRAS)
396 substances after evaluating its safety. Therefore, adding RSO to meat products is
397 expected to inhibit fat oxidation and effectively act as an antioxidant.
398 **Sensory evaluation**
399 Table 8 presents the results of the sensory evaluation of pork patties prepared with
400 different levels of RSO. In terms of color, PC showed significantly lower values
401 (p<0.05). This is consistent with the lower lightness (L*) observed in PC, which can
402 be attributed to the addition of dark-colored tocopherol. For flavor, the treatment
403 groups with RSO added showed significantly higher values compared to the control
404 group (p>0.05). In terms of smell, RSO4 and RSO8 showed significantly higher
405 values compared to the control group (p>0.05). These results are consistent with the

findings of Muguerza et al. (2002), who observed higher ratings for flavor and smell in low-fat sausages that had been supplemented with olive oil. In juiciness, treatment groups with RSO added were significantly lower than PC (p<0.05). There was also a tendency of decreasing juiciness as the amount of RSO added increased. This can be attributed to the decreasing moisture content and increasing cooking loss with increasing levels of added RSO. Texture showed no significant difference among all treatment groups (p>0.05). In terms of overall preference, the treatment groups showed significantly higher values compared to the control group (p<0.05), and RSO16 with 1.6% RSO showed the highest preference. Overall, the addition of RSO was found to enhance the taste and aroma of the patties, thus having a positive impact on overall preference.

Conclusion

In this study, we confirmed the antioxidant activity of radish seed oil (RSO) and analyzed its effects on the quality and storage characteristics of pork patties. The fatty acid composition of RSO showed a high content of unsaturated fatty acids, with 9.53% saturated fatty acids, 67.10% monounsaturated fatty acids, and 23.38% polyunsaturated fatty acids. The peroxide value of RSO was 2.75 meq/kg, which did not exceed the Codex standard value, and its DPPH radical scavenging activity was 75.46%, thus indicating antioxidant activity. The addition of RSO to pork patties resulted in decreases in both moisture and protein content, while the fat content increased. As the amount of RSO added increased, the WHC tended to decrease, while CL showed an increasing trend. In terms of TPA, hardness, springiness,

gumminess, and chewiness, the patties with RSO24 showed lower values compared to the positive control. With increasing RSO addition, TMC, VBN, TBARS, and PV values decreased, while RSO24 exhibited the most positive storage characteristics. The sensory evaluation results indicated that RSO16 showed the highest overall preference. In conclusion, adding a large amount of RSO may negatively affect the quality characteristics of patties, but it appears to have a positive impact on their storage properties. Ultimately, a 1.6% addition of RSO does not compromise the quality characteristics of the patties while improving their storage properties.

References

Al-Sheddy I, Fung DY, Kastner C. 1995. Microbiology of fresh and restructured lamb meat: A review. Critical reviews in microbiology 21:31-52.

AOAC. 2012. Official methods of analysis of AOAC International. 19th ed. AOAC International, Gaithersburg, MD, USA. p.931

Avato P, Argentieri M. 2015. Brassicaceae: A rich source of health improving phytochemicals. Phytochemistry reviews 14:1019-1033.

Banihani SA. 2017. Radish (raphanus sativus) and diabetes. Nutrients 9:1014.

Banon S, Díaz P, Rodríguez M, Garrido MD, Price A. 2007. Ascorbate, green tea and grape seed extracts increase the shelf life of low sulphite beef patties. Meat science 77:626-633.

Bloukas J, Paneras E. 1993. Substituting olive oil for pork backfat affects quality of low-fat frankfurters. Journal of Food Science 58:705-709.

Carlson DG, Daxenbichler M, Vanetten C, Hill C, Williams P. 1985. Glucosinolates in radish cultivars. Journal of the American Society for Horticultural Science 110:634-638.

Choi Y-J, Lee S-H, Lee K-S, Choi G-W, Lee K-S, Jung I-C, Shim D-W. 2019. Quality characteristics of low-fat plant oil emulsion pork patties. Journal of Life Science 29:1351-1357.
Dzudie T, Kouebou C, Essia-Ngang J, Mbofung C. 2004. Lipid sources and essential oils effects on quality and stability of beef patties. Journal of food engineering 65:67-72.
Godfray HCJ, Aveyard P, Garnett T, Hall JW, Key TJ, Lorimer J, Pierrehumbert RT, Scarborough P, Springmann M, Jebb SA. 2018. Meat consumption, health, and the environment. Science 361:eaam5324.
Gutiérrez RMP, Perez RL. 2004. Raphanus sativus (radish): Their chemistry and biology. The scientific world journal 4:811.
Jeong JY. 2016. Alternative curing technology in meat products. 축산식품과학과 산업 5:77-84.
Katsube T, Tabata H, Ohta Y, Yamasaki Y, Anuurad E, Shiwaku K, Yamane Y. 2004. Screening for antioxidant activity in edible plant products: Comparison of low-density lipoprotein oxidation assay, dpph radical scavenging assay, and folin− ciocalteu assay. Journal of agricultural and food chemistry 52:2391-2396.
Kazlauskienė D, Kasparavičienė G, Nenortienė P, Marksa M, Jukilaitytė J, Velžienė S, Ževžikovas A. 2021. Determination of fatty acid composition and antioxidant activity in vegetable oils. Chemija 32.
Kim MS, Kim D-S, Cho J-J, Hong SJ, Boo CG, Shin E-C. 2019. Oxidative stability, physicochemical, and sensory characteristics of vegetable oils at their induction periods.
Korea Food and Drug Administeration. 2009. Food code. pp. 212-251. Moonyoungsa, Seoul, Korea.
Korea Food and Drug Administeration. 2023. Food code pp. 250. Moonyoungsa, Seoul, Korea
Kruk ZA, Yun H, Rutley DL, Lee EJ, Kim YJ, Jo C. 2011. The effect of high pressure on microbial population, meat quality and sensory characteristics of chicken breast fillet. Food control 22:6-12.
Kyari M. 2008. Extraction and characterization of seed oil. International Agrophysics 22:139-142.

Lay F, Anderson M. 2005. Defensins-components of the innate immune system in plants. Current protein and peptide science 6:85-101.
Lim S, Ahn J-C, Lee EJ, Kim J. 2020. Antiproliferation effect of sulforaphene isolated from radish (raphanus sativus l.) seeds on a549 cells. Applied Biological Chemistry 63:1-8.
López-López I, Cofrades S, Cañeque V, Díaz MT, López O, Jiménez-Colmenero F. 2011. Effect of cooking on the chemical composition of low-salt, low-fat wakame/olive oil added beef patties with special reference to fatty acid content. Meat Science 89:27-34.
Luruena-Martınez M, Vivar-Quintana A, Revilla I. 2004. Effect of locust bean/xanthan gum addition and replacement of pork fat with olive oil on the quality characteristics of low-fat frankfurters. Meat science 68:383-389.
Manessis G, Kalogianni AI, Lazou T, Moschovas M, Bossis I, Gelasakis AI. 2020. Plant-derived natural antioxidants in meat and meat products. Antioxidants 9:1215.
Marinova G, Batchvarov V. 2011. Evaluation of the methods for determination of the free radical scavenging activity by dpph. Bulgarian Journal of Agricultural Science 17:11-24.
Matulis RJ, Mckeith FK, Sutherland JW, Brewer MS. 1995. Sensory characteristics of frankfurters as affected by fat, salt, and ph. Journal of Food Science 60:42-47.
Mendiratta S, Shinde A, Mane B. 2013. Effect of added vegetable (carrot, radish and capsicum) as functional ingredients in mutton nuggets. Journal of Meat Science and Technology 1:71-76.
Monteiro G, Souza X, Costa D, Faria P, Vicente J. 2017. Partial substitution of pork fat with canola oil in toscana sausage. Innovative Food Science & Emerging Technologies 44:2-8.
Paneras E, Bloukas J. 1994. Vegetable oils replace pork backfat for low-fat frankfurters. Journal of Food Science 59:725-728.
Park J-C, Jeong J-Y, Lee E-S, Choi J-H, Choi Y-S, Yu L-H, Paik H-D, Kim C-J. 2005. Effects of replaced plant oils on the quality properties in low-fat hamburger patties. Korean Journal of Food Science and Technology 37:412-417.

Parker TD, Adams D, Zhou K, Harris M, Yu L. 2003. Fatty acid composition and oxidative stability of cold-pressed edible seed oils. Journal of food science 68:1240-1243.

Ratanapariyanuch K, Clancy J, Emami S, Cutler J, Reaney MJ. 2013. Physical, chemical, and lubricant properties of brassicaceae oil. European Journal of Lipid Science and Technology 115:1005-1012.

Shan B, Cai YZ, Brooks JD, Corke H. 2009. Antibacterial and antioxidant effects of five spice and herb extracts as natural preservatives of raw pork. Journal of the Science of Food and Agriculture 89:1879-1885.

Uluata S, Øzdemir N. 2012. Antioxidant activities and oxidative stabilities of some unconventional oilseeds. Journal of the American Oil Chemists' Society 89:551-559.

Waheed A, Hamid F, Madiha B, Seemab A, Naveed A, Nadia K, Sohail A, Saqib M, Hina G. 2019. Gc-ms analysis of chemical components seed oil of raphanus sativus l. MOJ Toxicol 5:112-118.

Wendlinger C, Hammann S, Vetter W. 2014. Various concentrations of erucic acid in mustard oil and mustard. Food chemistry 153:393-397.

WHO, 2022, Guidelines for Drinking-Water Quality. World Health Organization. Geneva. 4: 438-444

Zayas JF, Zayas JF. 1997. Water holding capacity of proteins. Functionality of proteins in food:76-133.

Zhao G, Ren Y, Ma H. 2017. Extraction and characterization of radish seed oils using different methods. Tropical Journal of Pharmaceutical Research 16:165-169.

Tables and Figures

Table 1. Formulation of pork patties with various ratio of radish seed oil

Ingredients (%)		Radish seed oil (%)					
		0 (PC)	0 (NC)	0.4 (RSO4)	0.8 (RSO8)	1.6 (RSO16)	2.4 (RSO24)
Main	Meat	90	90	90	90	90	90
	Ice	10	10	10	10	10	10
Additives	Salt	1.5	1.5	1.5	1.5	1.5	1.5
	Tocopherol	0.1	-	-	-	-	-
	Sodium nitrite	0.01	-	-	-	-	-

PC (positive control), pork patty with tocopherol and sodium nitrite; NC (negative control), pork patty without RSO; RSO4, pork patty with 0.4% RSO; RSO8, pork patty with 0.8% RSO; RSO16, pork patty with 1.6% RSO; RSO24, pork patty with 2.4% RSO.

567 **Table 2. Fatty acids composition of radish seed oil**

Fatty acids	Radish seed oil (%)
C14:0 (Myristic acid)	0.09±0.00
C16:0 (Palmitic acid)	5.39±0.01
C16:1 (Palmitoleic acid)	0.22±0.00
C18:0 (Stearic acid)	2.04±0.00
C18:1 (Oleic acid, ω-9)	46.13±0.02
C18:2 (Linoleic acid, ω-6)	13.16±0.00
C18:3 (Linolenic acid, ω-3)	10.22±0.00
C20:0 (Arachidic acid)	1.13±0.00
C22:0 (Behenic acid)	0.89±0.00
C22:1 (Erucic acid, ω-9)	20.75±0.01
ΣSaturated	9.53±0.01
ΣMonounsaturated	67.10±0.01
ΣPolyunsaturated	23.38±0.00

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569

570 **Table 3. Antioxidant test and oxidative stability test of radish seed oil**

Traits	PV (meq/kg)	DPPH (%)
Radish seed oil	2.75±0.15	75.46±3.7

571

572 **Table 4. Proximate compositions of pork patties formulated with various amounts of radish seed oil**

Traits (%)	Treatments					
	PC	NC	RSO4	RSO8	RSO16	RSO24
Moisture	72.95±0.01^{b}	75.10±0.59^{a}	72.74±0.36^{b}	71.95±0.48^{c}	71.61±0.58^{cd}	70.95±0.17^{d}
Protein	15.69±0.92^{b}	17.20±1.07^{a}	18.55±0.54^{a}	18.47±0.59^{a}	17.65±0.61^{a}	15.70±0.62^{b}
Fat	10.43±0.94^{b}	7.43±1.07^{d}	7.97±0.53^{d}	8.60±0.54^{cd}	9.67±0.64^{bc}	12.50±0.65^{a}
Ash	0.94±0.05	0.95±0.00	0.95±0.03	0.94±0.06	0.94±0.04	0.96±0.04

573 PC (positive control), pork patty with 0.1% tocopherol and 0.01% sodium nitrite; NC (negative control), pork patty without RSO; RSO4, pork patty with 0.4% RSO; RSO8, pork patty with 0.8%
574 RSO; RSO16, pork patty with 1.6% RSO; RSO24, pork patty with 2.4% RSO.
575 $^{a-d}$ Least square means with different letters within the same row are significantly different (p<0.05).

576 **Table 5. pH, water holding capacity (WHC) and cooking loss (CL) of pork patties formulated with various levels of radish seed oil**

Traits	Treatments					
	PC	NC	RSO4	RSO8	RSO16	RSO24
pH	6.15±0.02^{a}	6.14±0.02^{ab}	6.12±0.02^{ab}	6.11±0.02^{b}	6.11±0.01^{b}	6.06±0.03^{c}
WHC (%)	64.76±0.68^{a}	63.66±1.32^{ab}	63.10±0.88^{ab}	63.02±0.44^{ab}	62.88±0.54^{b}	62.25±1.39^{b}
CL (%)	19.84±1.02^{c}	21.93±0.93^{cb}	19.93±1.58^{c}	20.01±1.47^{c}	23.32±1.49^{ab}	25.06±1.56^{a}

577 PC (positive control), pork patty with 0.1% tocopherol and 0.01% sodium nitrite; NC (negative control), pork patty without RSO; RSO4, pork patty with 0.4% RSO; RSO8, pork patty with 0.8%
578 RSO; RSO16, pork patty with 1.6% RSO; RSO24, pork patty with 2.4% RSO.
579 $^{a-c}$ Least square means with different letters within the same row are significantly different (p<0.05).
580

581 **Table 6. Color of pork patties formulated with various levels of radish seed oil**

Traits	Treatments					
	PC	NC	RSO4	RSO8	RSO16	RSO24
CIE L*	48.08±1.00^{c}	55.20±0.97^{b}	57.41±0.68^{a}	56.12±0.45^{b}	57.90±0.39^{a}	57.56±0.41^{a}
CIE a*	4.57±0.31^{c}	8.34±0.59^{a}	8.10±0.54^{ab}	7.57±0.19^{ab}	7.52±0.39^{ab}	7.46±0.44^{b}
CIE b*	13.43±0.29^{ab}	15.73±0.72^{a}	14.15±0.64^{c}	14.86±0.06^{b}	15.03±0.18^{ab}	15.27±0.23^{ab}

582 PC (positive control), pork patty with 0.1% tocopherol and 0.01% sodium nitrite; NC (negative control), pork patty without RSO; RSO4, pork patty with 0.4% RSO; RSO8, pork patty with 0.8%
583 RSO; RSO16, pork patty with 1.6% RSO; RSO24, pork patty with 2.4% RSO.
584 $^{a-c}$ Least square means with different letters within the same row are significantly different (p<0.05).
585

586 **Table 7. TPA of pork patties formulated with various levels of radish seed oil**

Traits	Treatments					
	PC	NC	RSO4	RSO8	RSO16	RSO24
Hardness (kg)	2.06±0.02^{b}	2.51±0.03^{a}	2.44±0.03^{a}	2.39±0.04 ^{a}	2.06±0.09 ^{b}	1.78±0.08^{c}
Cohesiveness (%)	0.87±0.01^{b}	0.91±0.03^{a}	0.87±0.01^{b}	0.87±0.02^{ab}	0.87±0.01^{ab}	0.86±0.02^{b}
Springiness (%)	0.63±0.01^{b}	0.61±0.01^{bc}	0.71±0.03^{a}	0.63±0.02^{b}	0.62±0.0^{bc}	0.58±0.01^{c}
Gumminess (kg)	1.78±0.04^{c}	2.28±0.05^{a}	2.12±0.04^{b}	2.08±0.02^{b}	1.80±0.07^{c}	1.54±0.07^{d}
Chewiness (kg)	1.13±0.04^{c}	1.39±0.05^{b}	1.50±0.08^{a}	1.32±0.03^{b}	1.11±0.04^{c}	0.90±0.03^{d}

587 PC (positive control), pork patty with 0.1% tocopherol and 0.01% sodium nitrite; NC (negative control), pork patty without RSO; RSO4, pork patty with 0.4% RSO; RSO8, pork patty with 0.8%
588 RSO; RSO16, pork patty with 1.6% RSO; RSO24, pork patty with 2.4% RSO.
589 $^{a-d}$ Least square means with different letters within the same row are significantly different (p<0.05).
590

591 **Table 8. Sensory evaluation of pork patties formulated with various levels of radish seed oil**

Traits	Treatments					
	PC	NC	RSO4	RSO8	RSO16	RSO24
Color	3.60±0.55^{b}	5.20±1.10^{a}	5.60±1.14^{a}	5.80±0.84^{a}	5.60±1.10^{a}	5.20±1.10^{a}
Flavor	3.00±1.00^{c}	4.20±0.84^{bc}	6.40±1.95^{a}	6.60±1.52^{a}	6.60±1.52^{a}	6.00±1.22^{b}
Smell	2.60±0.89^{c}	2.80±1.64^{c}	5.20±1.30^{ab}	5.60±1.82^{a}	3.40±1.14^{bc}	3.40±1.14^{bc}
Juiciness	6.90±1.00^{a}	6.40±0.55^{ab}	5.60±0.55^{b}	4.20±0.89^{c}	4.20±0.50^{c}	3.70±0.55^{c}
Texture	5.60±1.34	5.00±1.00	5.20±0.84	5.00±0.71	4.80±0.84	4.80±0.71
Overall preference	4.60±0.89^{b}	4.60±0.55^{b}	6.60±1.34^{a}	6.60±1.14^{a}	6.80±0.84^{a}	6.00±0.71^{a}

592 PC (positive control), pork patty with 0.1% tocopherol and 0.01% sodium nitrite; NC (negative control), pork patty without RSO; RSO4, pork patty with 0.4% RSO; RSO8, pork patty with 0.8%
593 RSO; RSO16, pork patty with 1.6% RSO; RSO24, pork patty with 2.4% RSO.
594 $^{a-c}$ Least square means with different letters within the same row are significantly different (p<0.05).

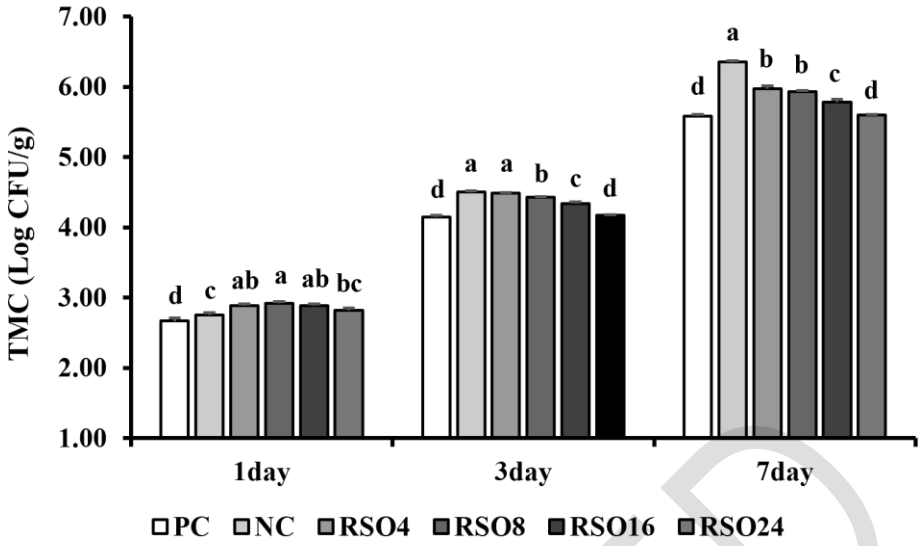

Fig1. Total microbial count (TMC, Log CFU/g) of pork patties formulated with various levels of radish seed oil

PC (positive control), pork patty with 0.1% tocopherol and 0.01% sodium nitrite; NC (negative control), pork patty without RSO; RSO4, pork patty with 0.4% RSO; RSO8, pork patty with 0.8% RSO; RSO16, pork patty with 1.6% RSO; RSO24, pork patty with 2.4% RSO.

[a–d] Least square means with different letters within the same day are significantly different ($p<0.05$).

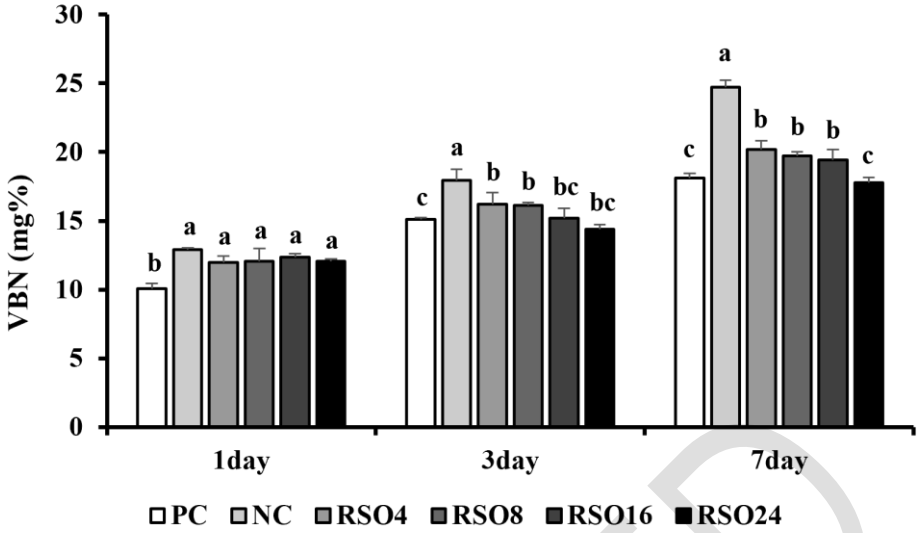

Fig 2. Volatile basic nitrogen (VBN, mg%) of pork patties formulated with various levels of radish seed oil

PC (positive control), pork patty with 0.1% tocopherol and 0.01% sodium nitrite; NC (negative control), pork patty without RSO; RSO4, pork patty with 0.4% RSO; RSO8, pork patty with 0.8% RSO; RSO16, pork patty with 1.6% RSO; RSO24, pork patty with 2.4% RSO.

[a–c] Least square means with different letters within the same day are significantly different ($p<0.05$).

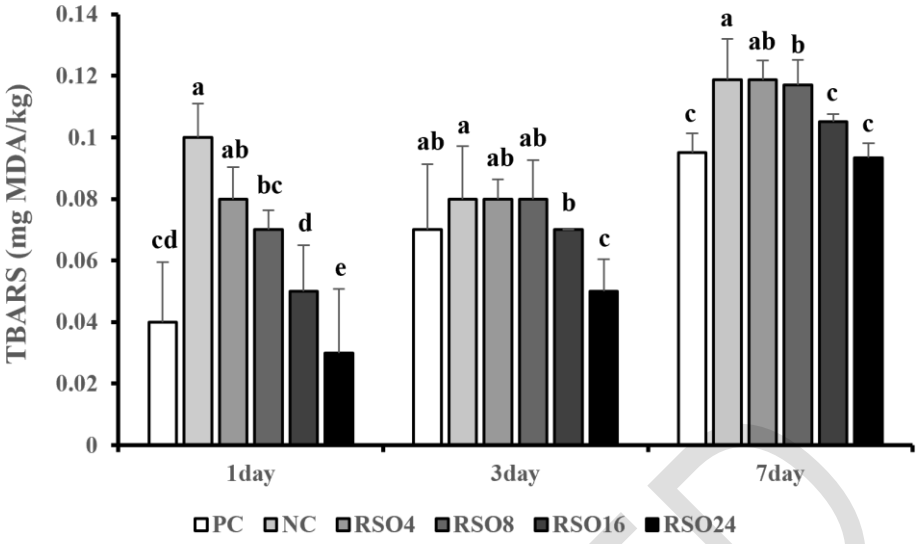

Fig 3. Thiobarbituric Acid Reactive Substances (TBARS, mg MDA/kg) of pork patties formulated with various levels of radish seed oil

PC (positive control), pork patty with 0.1% tocopherol and 0.01% sodium nitrite; NC (negative control), pork patty without RSO; RSO4, pork patty with 0.4% RSO; RSO8, pork patty with 0.8% RSO; RSO16, pork patty with 1.6% RSO; RSO24, pork patty with 2.4% RSO.

[a–e] Least square means with different letters within the same day are significantly different ($p<0.05$).

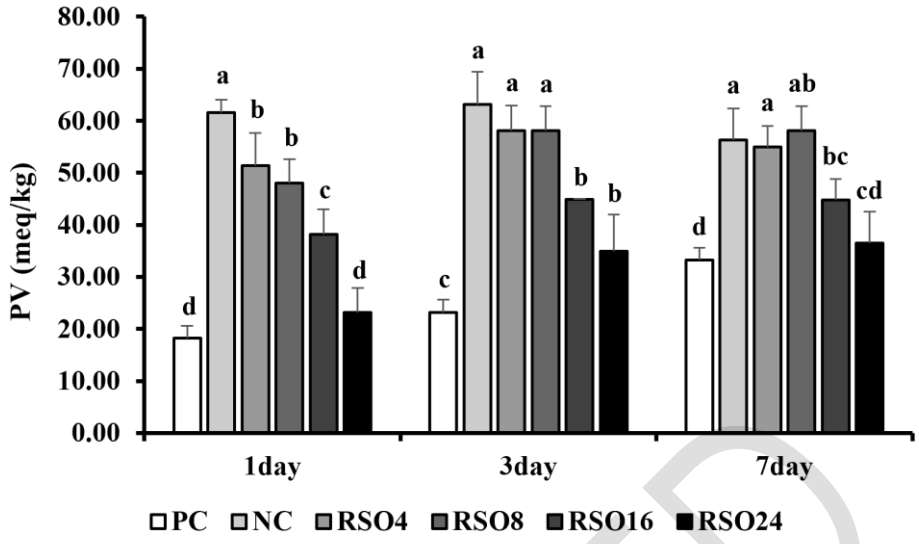

Fig 4. Peroxide value (PV, meq/kg) of pork patties formulated with various levels of radish seed oil

PC (positive control), pork patty with 0.1% tocopherol and 0.01% sodium nitrite; NC (negative control), pork patty without RSO; RSO4, pork patty with 0.4% RSO; RSO8, pork patty with 0.8% RSO; RSO16, pork patty with 1.6% RSO; RSO24, pork patty with 2.4% RSO.

[a–d] Least square means with different letters within the same day are significantly different ($p<0.05$).