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Meat crust as a novel food ingredient to regulate lipid peroxidation and oxidative stress

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ABSTRACT

Pan Fry (PF) is a common heating treatment however, there is limited data on meat oxidation after PF using direct contact with an uncoated iron pan. After PF, a crust is formed, and in this study, we aim to evaluate the potential anti-oxidation and anti-lipid peroxidation capacity of such crust. Ground beef and turkey meat were heat treated using PF or microwave. Lipid peroxidation was evaluated using malondialdehyde accumulation. PF meat generated lower lipid peroxidation levels versus microwave-heated meat. Iron PF has decreased lipid peroxidation versus Teflon pan heating. The crust significantly lowered lipid peroxidation and possessed millard reaction products (MRPs), strong reducing abilities, iodine removal capacity, and some iron chelation capacity. We demonstrated that the crust substantially decreases lipid peroxidation levels in various systems and can be used as a novel seminatural antioxidant ingredient, which may lead to extended shelf life and protects various food products.

1. Introduction

Oxidative stress due to lipid peroxidation products in foods and postprandial oxidative stress is defined as an elevated susceptibility toward oxidative distress after the consumption of a meal rich in lipids (Kanner et al., 2012, 2017). Lipid peroxidation can cause quality deterioration in food products especially in meat (Lorenzo and Gómez, 2012; Min and Ahn, 2005). This notorious process starts immediately after slaughtering due to the disruption of blood flow (Chaijan and Panpipat, 2017). Hence, in all the distinct stages of handling, food processing, and storing a careful approach must be applied (Hui, 2006).

Dry heat cooking such as pan-frying (PF) is a common and easy heat treatment to process meat. The temperature can rise up to 300 °*C* and it is considered to be a common domestic cooking process (Cernela et al., 2014). The effect of PF has been examined in meats and was shown an increase in lipid peroxidation (Abdel-Naeem et al., 2021; Nuora et al., 2015). However, to the best of our knowledge, there is limited data regarding meat oxidation after direct PF at moderate temperatures on an uncoated iron pan. Furthermore, most of the experiments examining PF cooking have used oil as a medium mediator, and this affects both the overall contact between the iron pan and the meat and overall, the lipid content (van Hecke and de Smet, 2021). Moreover, the data regarding the changes in meat oxidation after PF following incubation at relevant gastrointestinal (GI) pHs and digestion is insufficient.

The initiation of the chemical, non-enzymatic browning maillard reaction products (MRPs) involving free amino and carbonyl groups occurs through the storage and thermal processing of foods (Wang et al., 2011). In this process, various compounds of the maillard reaction products (MRPs) such as Amadori rearrangement products (ARPs) and melanoidins are being formed. It was reported that these compounds possess antioxidant abilities due to various mechanisms including scavenging of reactive oxygen species (Feng et al., 2022; Nooshkam et al., 2019; Shakoor et al., 2022). Due to the extensive production of the MRPs, it was even suggested that they can be used as antioxidants to decrease the oxidation occurring in food containing lipids (Nooshkam et al., 2019).

During PF, the external layer of the meat, which is in direct contact with the pan, and as a result, a crust is formed. There is no scientific data that examines the chemical and anti-oxidation capacity of such heat-generated crust produced. Due to the increasing need of the food

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industry to replace synthetic antioxidants in the attempt to inhibit lipid peroxidation, there is a growing interest in examining various antioxidant components (Lorenzo et al., 2017) We hypothesize that heat-generated crust is an efficient semi-natural antioxidant that can protect against lipid peroxidation.

In the current study, we compared two heat treatments (iron PF and microwave heating) on the lipid peroxidation process in turkey and beef rib eye steak (Entrecote). In addition, we used intestinal and gastric pH and tested the effect of the pH following incubation at 37 °C on lipid peroxidation in the heated meat. We found increased oxidizability of meat at intestinal pH 6 versus gastric pH 3. Moreover, PF-treated meat was found to be significantly less susceptible to the accumulation of lipid peroxidation products compared to MW-treated meat due to the crust layer and its antioxidant abilities and high amounts of MRPs formation.

2. Materials and methods

2.1. Materials

Turkey and Entrecote were bought at 2 different commercial stores in Israel, on separate occasions. Hydrochloric acid (HCl) 37%, sodium chloride, and chloroform were purchased from Bio-Lab Ltd. (Jerusalem, Israel). Sodium hydroxide and toluene were purchased from Frutarom (Haifa, Israel). 2-Thiobarbituric acid (TBA), methanol, ascorbic acid, ferene, Iron (III) chloride anhydrous, and catechin were purchased from Sigma-Aldrich (St. Louis, MO) and Trichloroacetic acid (TCA) was bought from Merck (Darmstadt, Germany). Potato starch was bought from BDH (BDH limited Poole, England) and Meth-Perp 2 from Alltech (Alltech Associates Inc.) Potassium iodide was purchased from J.T. Baker (J.T. Baker, USA). To simulate the gastric pH, we used simulated gastric fluid (SGF). The fluid was freshly prepared according to the United States Pharmacopeia (Rockville, MD; 2000) without pepsin. The SGF contained NaCl (200 mg), and HCl (700 μL of 37%) that were added to Double-distilled water (DDW, 100 ml). The intestine relevant-pH fluid (IRF) contained 100 ml of DDW and NaCl (200 mg) and this fluid was adjusted to pH 6 with 1 M HCl.

2.2. Meat initial characteristics tests

2.2.1. Fat percentage determination

The fat determination was carried out using the Folch method (Sündermann et al., 2016). 5 g of ground turkey or entrecote were homogenized at a 1:3 ratio with DDW (w/v). 1 ml of the homogenate was diluted 1:5 with Chloroform-methanol (2:1) along with 1 ml of water. The lower organic phase was collected in pre-weighed test tubes, left to dry completely, followed by weighing the tubes to estimate the mass of extracted fat. The results were presented as percentage (%) of the fat content.

2.2.2. Moisture percentage quantification

Ground turkey or entrecote at quantity of 5 g were placed in an oven for 18 h at 105 °C. The dried meats were weighed after 18 h, and the resulting weight differences were presented as moisture (water content) (%).

2.2.3. Initial levels of lipid peroxidation

The grounded meats were homogenized (Polytron PT 3000) with three portions of DDW. The homogenate was mixed immediately with 12% TCA at a 1:1 ratio and centrifuged (Thermo Fisher, Heraeus Megafuge 16 R) for 10 min at 10,000 RPM at 4 °C. Lipid peroxidation was determined by measuring TBA reactive substances (TBARS) which were expressed and calculated as malondialdehyde (MDA) levels. The supernatant after the centrifuge was mixed with TBA (10 mM) at a 1:1 ratio, and the samples were heated in a boiling water bath for 15 min. Then, the absorbance of the samples were measured at 532 nm (Shimadzu UV-1700, PharmaSpec), and the MDA was calculated according to 1 μmol/L = 0.156 absorbance and expressed as nmol/g meat.

2.3. Sample preparation for heating treatments

After purchase, the ground turkey or entrecote was weighed to 5 ± 0.05 g and kept at −80 °C until use. The cuts were shaped at the same thickness (~1.5 cm). For the preparation of the PF meat, the pan (3 mm thickness) was warmed on an electric stove (Rotel AG, Switzerland) to 180 °C. The cuts were placed on the pan without oil and fried for 3 min exactly per side. The internal temperature of the cuts after heating achieved 70 °C and the cuts' middle color changed to a mild brown. For the MW treatments, the meat cuts were heated (Dow, South Korea, at 800 W) for 1 min heating, and they developed to a light brown color.

2.4. Lipid peroxidation determination in the heated meats

After the preparation, the meat cuts were homogenized with three portions of SGF\IRF. Later on, the homogenate was adjusted either to pH 3.0 or pH 6.0 and was incubated in a shaking bath at 37 °C for 120 min. During the incubation, homogenates were sampled at 4-time points (0, 30, 60, and 120 min) followed by estimation of the lipid peroxidation level by measuring TBARS as described earlier, and the results were expressed as MDA (nmol/g meat).

2.5. Crust extricating, MRPs and anti-oxidant assays

2.5.1. Crust extricating and MRPs assays

After PF (see Section 2.3) the entrecote crust was pulled out from the whole patty and was divided into the crust layer that was chopped into small pieces, and the middle of the beef patty (MB). The MB will be used as a comparison factor to the crust. To have a better understanding of the crust characteristics, we performed two assays to evaluate the various MRPs being formed. The first assay estimated the protein carbonylation (PCO), and was performed as previously described (Colombo et al., 2016). The second MRPs assay was a quantification of the advanced glycation end products (AGEs) using a commercial competitive ELISA kit (OxiSelect™ STA-817, Cell Biolabs, Inc. San Diego, CA, USA) according to manufacturer instructions. In both experiments, we compared the entrecote crust to the MB.

2.5.2. Crust reducing quantification assays

The first anti-oxidant assay was the crust's ability to reduce ferrous into ferric. Relying upon the ferene-based iron assay (Abbasi et al., 2021), we tested the reducing capacity of the crust. After applying the treatments (see Supplementary 1 - reducing capacity), 5 mM of ferene was added into samples followed by centrifuging at 5000 RPM for 5 min. The supernatants were filtered utilizing a PVDF 0.22 μM filter and the samples' absorbance were measured at 595 nm. Using a calibration curve, the results were expressed as μM of ferrous.

Iodine to iodide reducing assay: the reducing capacity was evaluated by a redox titration performed as follows: after adding the various treatments (see Supplementary 1 - redox titration), the tubes were centrifuged at 10,000 RPM for 5 min. The supernatants were filtered and transferred to a tube containing 0.5% potato starch and water (creating a ratio of 1:6:40 between the starch, sample, and water, respectively). Tri-iodide solution (0.5 mM) was utilized for the sample's titrations and the final titration point was appointed as soon as the blue color of the starch-iodine complex appeared. The results were expressed as nmol of KI_3.

2.5.3. Crust chelation quantification assay

We tested the iron chelation capacity of meat components. After receiving defined treatments (see Supplementary 1- chelation capacity), the tubes with samples were centrifuged at 5000 RPM for 5 min. The supernatant was filtered, followed by addition of 100 μM of ascorbic acid (AA), vigorous vortexing, and finally by the addition of 5 mM of

ferene. The sample tubes absorbance was read at 595 nm and the results were expressed as μM of ferrous using a specific calibration curve.

2.6. Washed meat (membranes) residue system

The washed meat residue system was performed as follows: fresh entrecote meat was homogenized with four portions of pH 6.5 acetate buffer 0.05 M. The liquid was centrifuged at 14,000 RPM at 4 °*C* for 30 min. Then, the supernatant was discarded, while the residue was weighed and supplemented with the acetate buffer (4 times the residue weight). After 30 min this process was repeated and, overall, was conducted 3 times to produce the membrane phase (MP). The same process was performed for turkey meat. The entrecote MP (0.5 g) was homogenized with IRF, followed by crust/MB (100 mg) both produced from entrecote after PF. Catechin (400 μM) was added as a positive control. Later on, 10 μM of $FeCl_3$ and 100 μM of AA were supplemented to each tube, the final volume was adjusted to 5 ml, and the MDA levels were quantified via TBARS within 60 min.

The dose-dependent effect of the crust on lipid peroxidation was determined using the following crust quantities: 100, 75, 50, and 10 mg. A turkey MP was employed in this assay, due to its fatty acid composition (van Hecke and de Smet, 2021), and the same protocol as we described previously, was conducted here. The turkey MP (0.5 g) was homogenized with the IRF and the crust in various amounts was added. An addition of 10 μM of $FeCl_3$, 100 μM of AA followed by adjusting to a final volume of 5 ml and accompanied by the determination of the MDA levels within 60 min.

2.7. Fatty acid composition analysis

The total lipids were extracted from the meats after heating, following the Folch method (Sündermann et al., 2016). After grounding 1 g of meat with 3 ml of DDW, the samples were diluted to a 1:10 ratio with DDW. 1 ml of that homogenate was mixed with four volumes of Chloroform-methanol 2:1. The lower layer was collected after centrifuged for 10 min at 800 RPM. The samples were evaporated under a flow of nitrogen, and for the further drying of the samples, 200 μl of tert-butyl was added to each one, followed by incubation at - 20 °C for approximately 4 h and Lyophilization. To evaluate the fatty acid profile, 100 μl of toluene, 20 μl of methanol, and 40 μl of Meth-Prep II (sodium methoxide) were added and the samples were subjected to the Gas Chromatography (GC). Chromatographic analysis was performed employing a GC apparatus (Agilent Technologies, Santa Clara, CA, USA) equipped with a fused-silica capillary column (60 m × 0.25 mm ID, DB-23, Agilent Technologies) under the following conditions: the oven temperature was programmed to rise from 130 °C to 170 °C at a rate of 27 °C/min, from 170 °C to 215 °C at a rate of 2 °C/min, held at 215 °C for 8 min, from 215 °C to 250 °C at a rate of 40 °C/min, held at 250 °C for 5 min. The run time was 37.9 min. The carrier gas was Helium at 2.21 ml/min and the Flame ionization detector temperature was 270 °C, whereas the injector temperature was 280 °C. To enable maximal detector response, air and hydrogen flows were adjusted. Peak identification was based on the relative retention times of two external standards. The area of each fatty acid peak was recorded using ChemStation software (Agilent Technologies) and the content of each individual fatty acid was defined as a percentage of total fatty acids within each sample.

2.8. Surface meat analysis via electronic microscope

To assess the impact of the different heating methods on the meat surface (MW and PF), an analysis was performed by scanning electronic microscope (SEM Jeol IT 100, working conditions: 20kv WD10mm). The samples were prepared as previously described (Fischer et al., 2012). Briefly, after fixation with Glutaraldehyde 4% in phosphate buffer, the samples were dehydrated in increasing ethanol concentrations. When the samples were at 100% ethanol, they were subjected to drying in a Critical Point Dryer (K850 Quorum). After the drying, the samples were mounted on metal stubs and coated with gold (Q150T ES Quorum).

2.9. Statistical analysis

The results were expressed as mean ± standard deviation (SD). The data were analyzed by analysis of variance with one-way analysis of variance post hoc Tukey−Kramer HSD test, Student's t-test, and Dunnett's methods test using JMP Pro 16.0.0 (SAS Institute, Inc.) software. The statistical tests were performed on the results obtained either: immediately, after 60 min, or on the differences (Delta Δ), between the baseline and after 120 min. Differences were considered significant at $p \leq 0.05$ and were indicated by different letters.

3. Results

3.1. Initial characteristics of the raw meat

The fat percentages in entrecote meat were higher than those from turkey meat. The moisture levels were 72.3 ± 0.54% and 76.6 ± 0.24% in entrecote and turkey, respectively. The initial levels of MDA in turkey was 7.69 ± 0.08 nmol/g meat and 4.68 ± 0.17 nmol/g meat in entrecote meat (Table 1.)

3.2. Change in lipid peroxidation at different pHs following MW or PF treatments

The MDA accumulation in turkey after MW incubated at intestinal pH 6 was significantly higher compared to turkey after MW and PF in gastric pH 3 ($p < 0.0001$). The MDA levels in turkey after PF at pH 6 were significantly lower than the levels found in the MW-treated turkey at pH 6 ($p = 0.01$) (Fig. 1A).

The entrecote meat MDA levels were extensively higher after MW heating followed by exposure to pH 6 compared to all other entrecote treatments ($p < 0.0001$). Similarly to turkey, a mild decrease with time was observed in entrecote meats placed in gastric pH 3 after both MW and PF (Fig. 1B).

To study the meat oxidation on an uncoated iron pan Vs. Teflon we evaluated the differences in MDA accumulation in turkey meat after PF using different pans using turkey meat due to it high oxidizability. Meat oxidation on an uncoated iron pan was extensively lower compared to the Teflon pan after 2 h ($p < 0.0001$) (Fig. 1C).

3.3. Crust MRPs content and antioxidant assays

3.3.1. MRPs levels in the crust

To assess the MRPs on meat proteins, the PCO content was evaluated. Carbonyls are necessary for schiff base formation in Amadori rearrangement products. We have used the method with 2,4-dinitrophenylhydrazine (DNPH) (Colombo et al., 2016). The levels of PCO in the crust were substantially higher compared to the quantity found in the middle of the beef patty per mg of total protein ($p \leq 0.05$) (Fig. 2A). In addition to PCO, we evaluated AGEs using a commercial competitive ELISA kit. As was found in the PCO content, the crust had significantly higher AGEs levels versus the MB ($p \leq 0.05$) (Fig. 2B).

Table 1
Initial parameters of the raw meat's.

Meat Type	Initial parameter		
	Fat (%)	**Moisture (%)**	**Level of lipid peroxidation as MDA (nmol/g meat)**
Entrecote	24	72.3 ± 0.54	4.68 ± 0.17
Turkey	17.3 ± 1.88	76.6 ± 0.24	7.69 ± 0.08

Data are presented as mean ± SD of triplicates. *mean of duplicate.

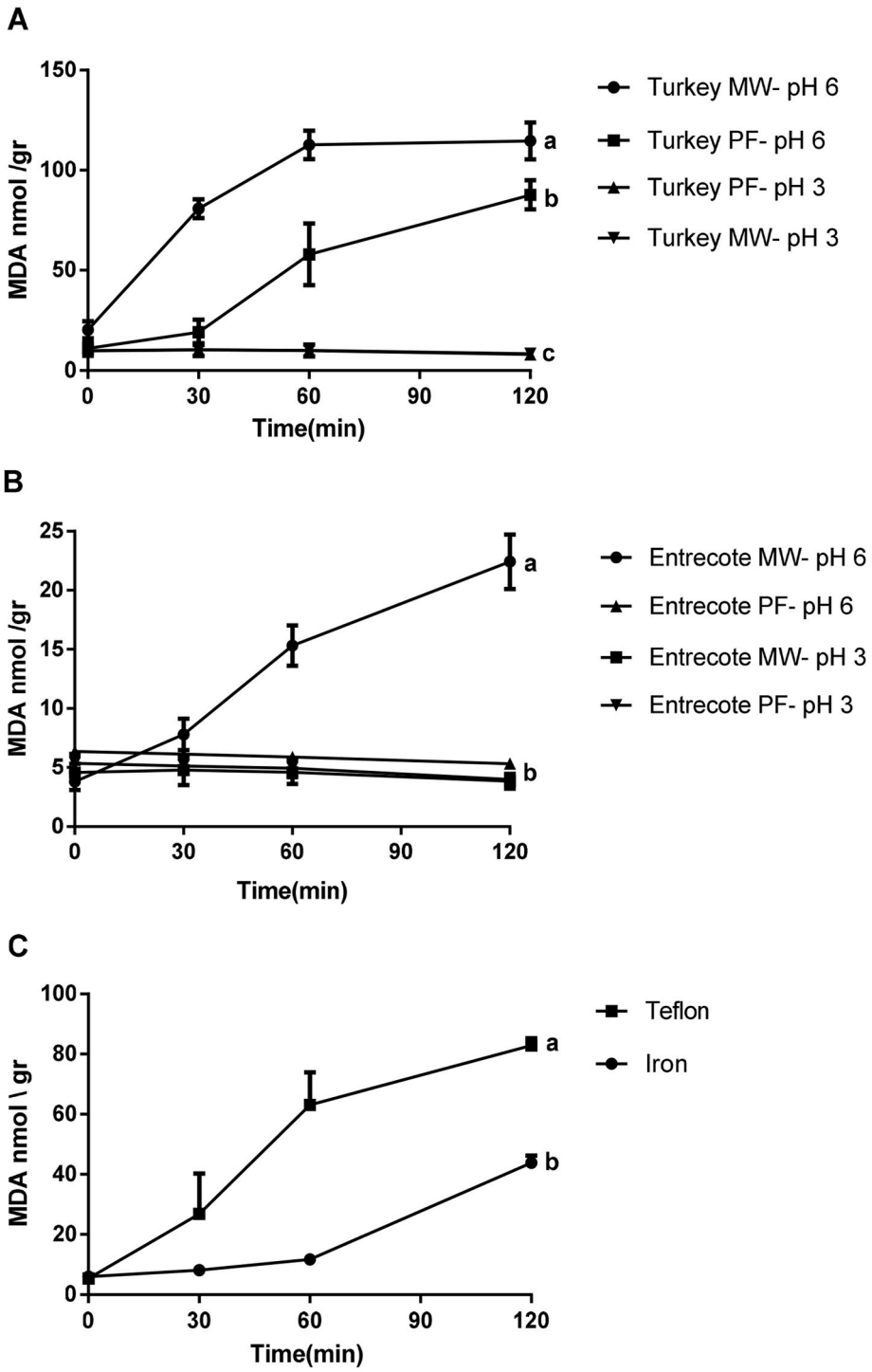

Fig. 1. MDA accumulation in turkey (A) and entrecote (B) after PF and MW followed by exposure to pH 6 (Intestine) and pH 3 (Gastric). MDA quantity in turkey post thermal heat treatments (Iron versus Teflon) (C). For A and B each experiment was repeated 4 times with triplicates (n = 12); for C experiments were repeated twice with triplicates (n = 6). Differences were considered significant at $p \leq 0.05$. The statistical tests in graphs A and B were made using the delta change between 0 time and 120 min within each sample and a comparison of mean values of the change between treatments was done using Student's t-test. In graph C, statistical tests were done on the MDA levels after 120 min utilizing the Tukey–Kramer HSD test.

3.3.2. Crust-reducing abilities

To understand the mechanism by which PF-treated meat is less sensitive to peroxidation compared to MW-treated meat, we evaluated the reducing capacity for antioxidant potential of the crust based on the ferene-ferrous complex formation from ferric. As a reference, the samples containing iron with an excess of AA, produce the highest ferene-ferrous complex among all of the treatments. The addition of 100 mg of entrecote crust had significantly higher reducing abilities compared to 100 mg of the MB and iron (Fe^{+3}) alone ($p = 0.04$) (Fig. 2C). The second assay was a redox titration with a KI_3 solution. Between the samples containing crust/MB with AA versus AA alone, there was a statistically significant elevation in the amount of KI_3 solution needed for the titration ($p \leq 0.05$) (Fig. 2D).

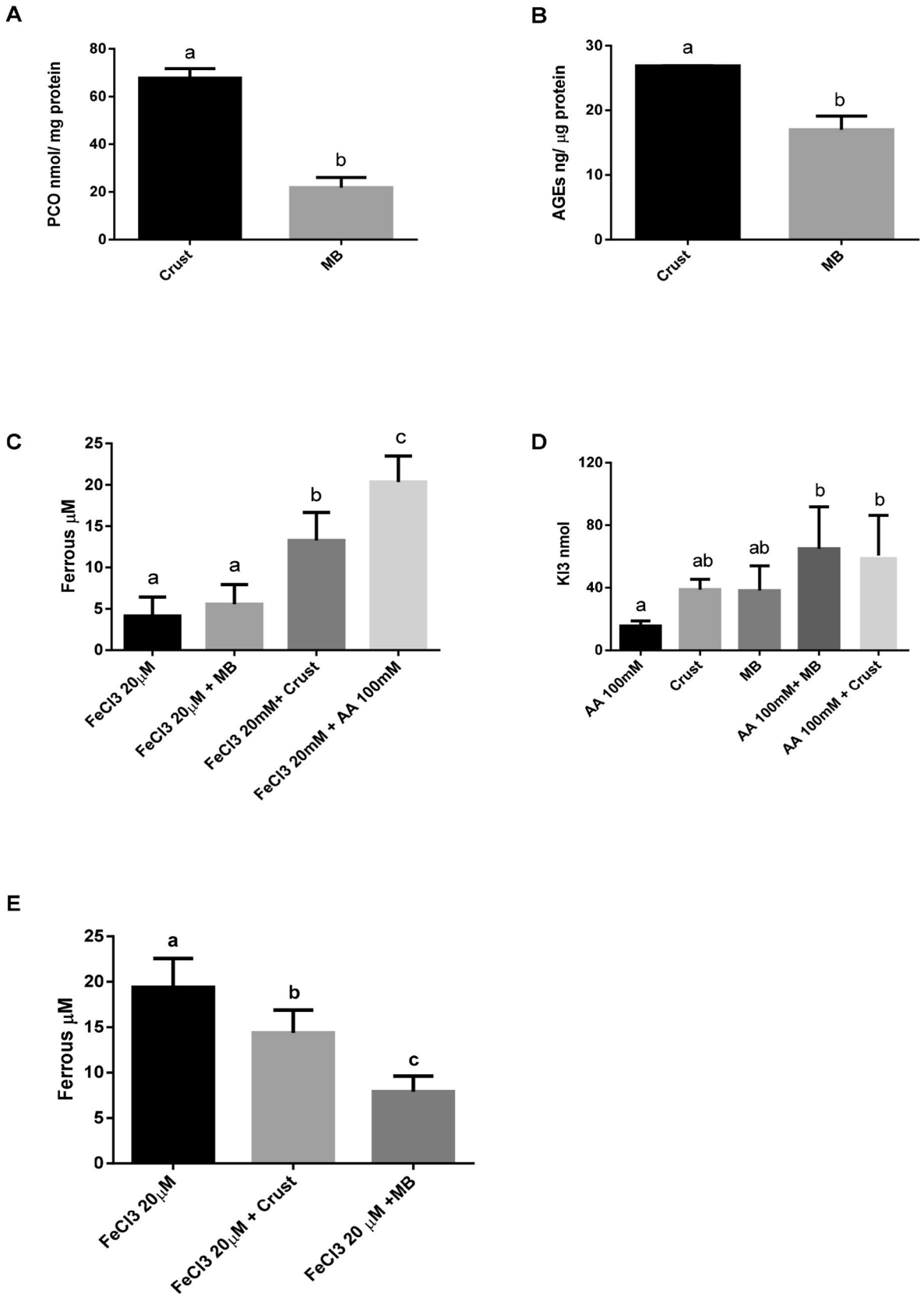

Fig. 2. MRPs content and redox properties of the crust. Protein carbonyls (PCO) content in the crust and MB presented as nmol per mg of protein (A). The AGEs quantity in the crust layer versus the MB are presented as ng per μg of protein (B). The reducing capacity of 100 mg of crust/MB entrecote meat with $FeCl_3$ compared to $FeCl_3$ with or without AA (C). A redox titration with a tri-iodide solution of 100 mg of crust/MB entrecote meat with or without AA and compared to AA alone (D). Iron chelation capacity in the presence of 100 mg of crust/MB entrecote meat with $FeCl_3$ versus $FeCl_3$ alone (E). The statistical analysis utilized for graphs A and B was the Tukey−Kramer HSD test ($n = 8$), and the Student's t-test ($n = 4$) for graphs C, D, and E. Differences were considered significant at $p = 0.05$ and indicated by different letters.

3.3.3. Crust-iron chelation ability

The samples containing 100 mg of MB with the iron have led to the highest chelation capacity compared to all other treatments. The addition of the crust produced a significantly lower ferene-ferrous complex formed versus iron (ferric ions) alone ($p = 0.02$) (Fig. 2E).

3.4. Washed meat residue system-membrane oxidation assay

Utilizing isolated meat membranes (washed meat) we have demonstrated that the addition of crust inhibited the pro-oxidant effect of AA/iron and has led to significantly lower lipid peroxidation levels compared to only iron and AA ($p < 0.0001$). The addition of the MB has led to the highest MDA accumulation after 60 min. The crust and catechin had the lowest MDA levels and there was no statistical difference between the two ($p = 0.19$) (Fig. 3A).

Examining the dose-dependent effect of the crust on turkey MP, the highest MDA levels were observed in the control samples (MP with iron and AA) and the samples containing 10 mg of crust. There was a significant difference between the last two and the samples who have been treated with 75 and 50 mg of crust ($p < 0.03$). The addition of 100 mg has led to the lowest MDA accumulation and was significant versus all treatments ($p < 0.0001$) (Fig. 3B).

3.5. Changes in fatty acid composition between the meats

An analysis was performed to assess the fatty acid profile in raw meat and after the different heat treatments. Neither in turkey nor in entrecote there were significant changes in the percentages of SFA, MUFA, and PUFA composition, between any one of the treatments in comparison to raw meat (Table 2).

Table 2
Fatty acids analysis in Raw meats and, after heat treatments.

Fatty acids %/Treatments	SFA%	MUFA%	PUFA%
Raw turkey	41.7 ± 0.7	32.8 ± 2.8	25.1 ± 3.4
PF turkey	41.5 ± 1.0	31.3 ± 0.6	27 ± 0.4
MW turkey	43.6 ± 1.8	31.5 ± 2.3	24.7 ± 0.3
Raw entrecote	54.1 ± 2.2	37.9 ± 2.1	7.7 ± 0.1
PF entrecote	52.4 ± 6.8	41.4 ± 4.6	6.2 ± 2.3
MW entrecote	48.0 ± 0.9	46.2 ± 3.1	6.1 ± 3.55

Data presented as mean ± SD of two duplicates (n = 2).

3.6. The differences in meats surface via electronic microscope images

Since we observed that the crust formation is responsible for antioxidant capacities due to its chemical properties, we also evaluated the physical effect of the crust on surface porosity. Turkey after MW showed a meat surface with meaningful damage and an extensive number of pores compared to turkey after PF which possesses a typical solid crust (Fig. 4A and C, respectively). Post-enlargement of the images shows the depth and shape of the pores and the effects MW heat has compared to PF heat (Fig. 4B and D, respectively).

Entrecote meat after MW did show damage but on a lower magnitude compared to MW of turkey meat (Fig. 5E and F). Like turkey, entrecote post-PF had a common crust produced during cooking and kept a solid homogenous surface (Fig. 5G and H).

4. Discussion

PF is a common and straightforward way to process meat which has been examined for the amount of lipid peroxidation products

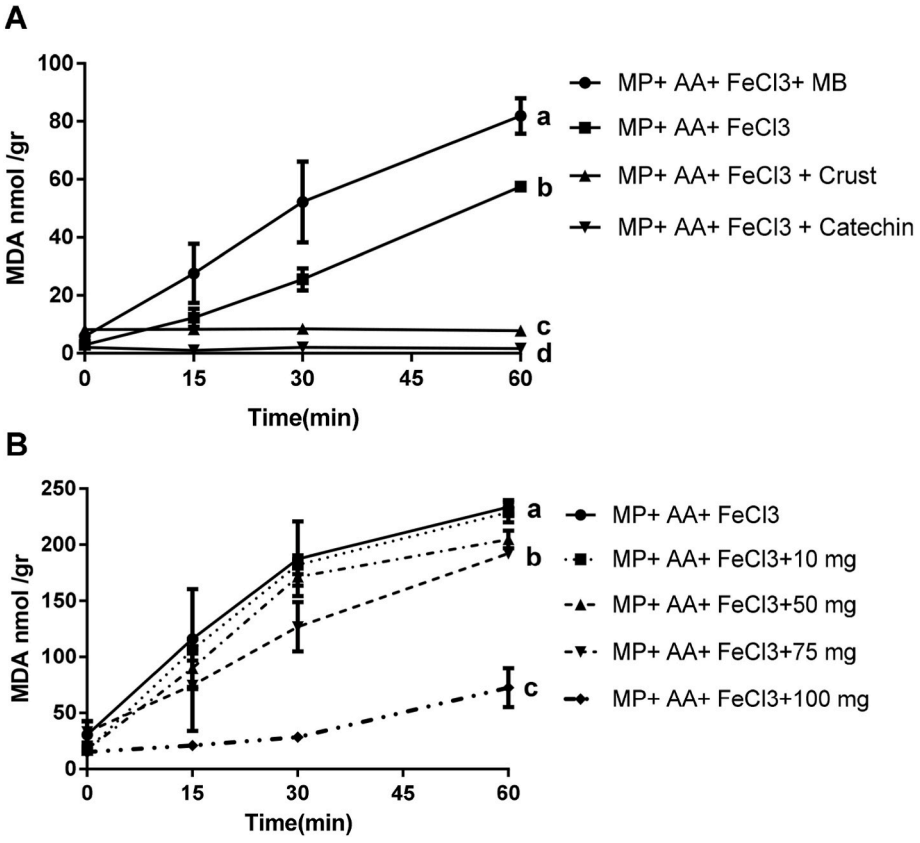

Fig. 3. Evaluation of antioxidant properties and a dose-dependent effect of the crust. MDA accumulation within 60 min in entrecote membrane phase (MP) with/without catechin (400 μM), crust, and middle of the beef patty (MB) (both 100 mg) (A). Dose-dependent antioxidant effect of crust: MDA accumulation within 60 min in turkey MP with various amounts of crust (B). Experiments were repeated twice with duplicates (n = 4). Differences were considered significant at $p \leq 0.05$. The statistical tests compared the difference in MDA after 60 min of treatments using the Tukey–Kramer HSD test for graph A and the Student's t-test for graph B.

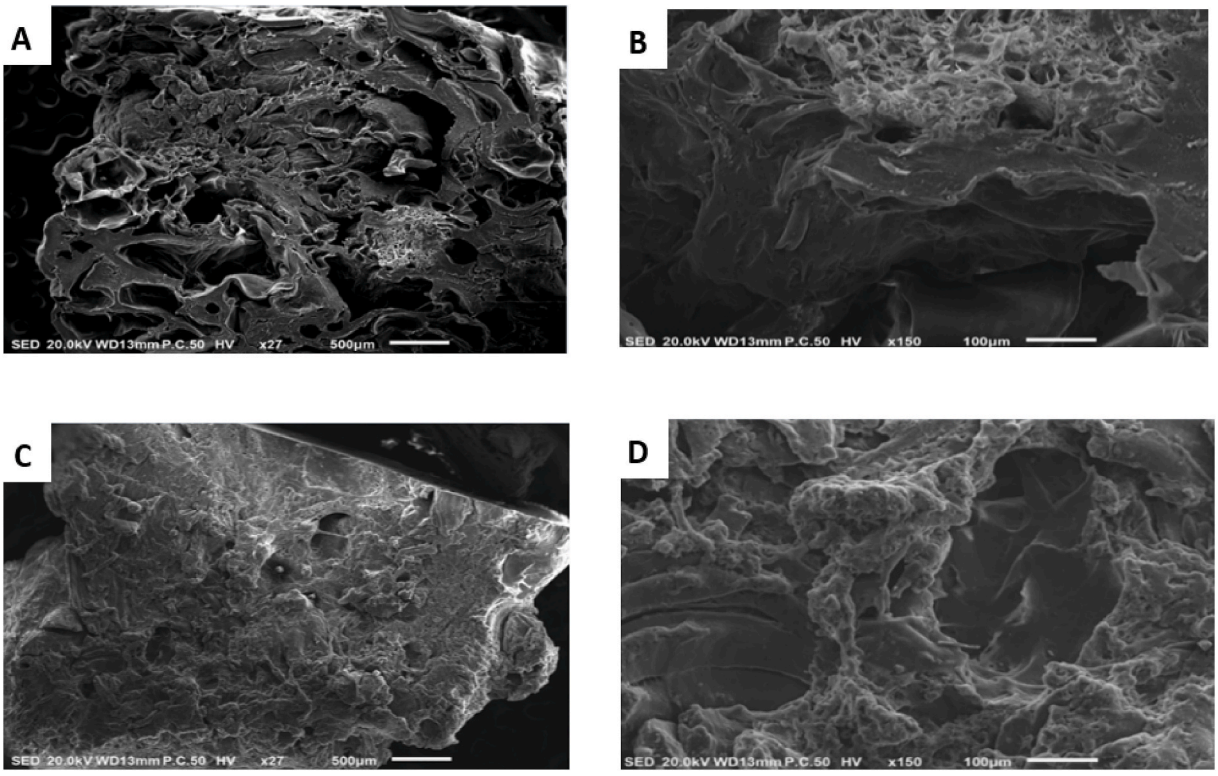

Fig. 4. Meat surface images of turkey meat after MW (A, B) and after PF (C, D).

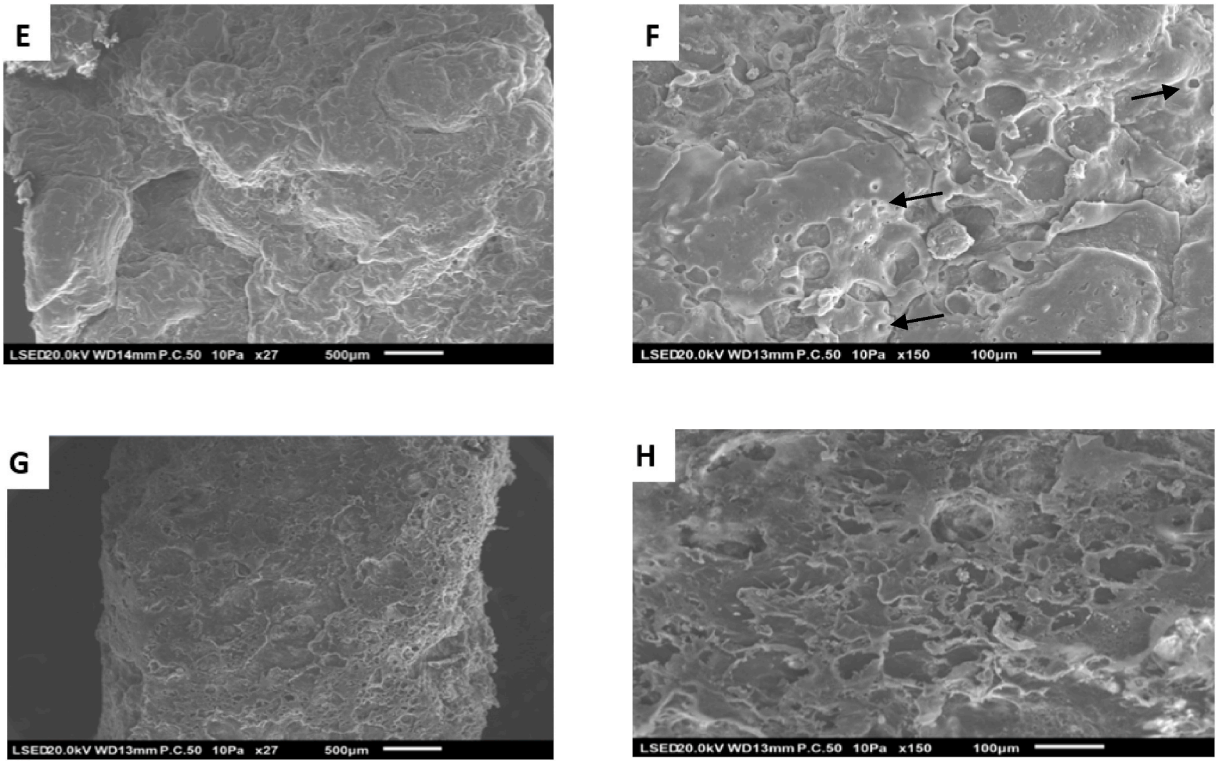

Fig. 5. Meat surface images of entrecote meat after MW (E, F) and after PF (G, H).

accumulating after heating (Abdel-Naeem et al., 2021; Nuora et al., 2015). However, there is limited data regarding meat oxidation after PF on an uncoated iron pan and without adding dietary oils. Additionally, the knowledge of meat oxidation following PF at relevant GI pHs is insufficient.

We found that the meats accumulated more MDA levels in the intestine-pH 6 compared to gastric-pH 3. The differences in lipid peroxidation between the different pHs could be explained by myoglobin

activity as peroxidase in various pHs. The myoglobin acts as a catalyzer in the breakdown of lipid hydroperoxides and was found to be highly effective at lower pHs, like in the gastric fluid, compared to higher pH levels, such as found in the intestine (Lapidot et al., 2005a).

Therefore, fresh and minimally oxidized meat under the stomach conditions could promote the activation of myoglobin antioxidant effect and even prevent downstream oxidation in the low-oxygen environment of the intestine. However, under conditions of regular processing and elevated levels of hydroperoxides in the meat, the stomach pH can potentiate more oxidation as we published previously (Lapidot et al., 2005b).

Teflon and iron-coated pans frying are common practices that have been used in experiments to evaluate various parameters of processed food (Abdel-Naeem et al., 2021; Oz et al., 2017). However, we did not find a comparative study that assesses lipid peroxidation levels after employing those pans for frying. We have showed that the use of an uncoated iron pan generated significantly lower MDA levels versus a Teflon pan in turkey meat after 120 min. This difference can be attributed to the direct contact that the meat has with the iron pan as the common heat-generated crust been formed. As we found, this crust possesses antioxidant abilities that reduce substantially the MDA levels.

During the thermal processing of foods, the Maillard reaction occurs. There are many MRPs being formed and some of these products are attributed antioxidant abilities (Feng et al., 2022; Nooshkam et al., 2019; Shakoor et al., 2022). Due to the MRPs capacity to serve as antioxidant agents and the growing need of the food industry to replace synthetic antioxidants (Lorenzo et al., 2017), we explored the anti-oxidation capacity of the crust layer being formed after PF.

We have shown for the first time that the crust possesses anti-oxidant capacities and was found to be highly effective in various systems. This effectiveness can be attributed to the MRPs in the crust, as was shown by the elevated levels of PCO and AGEs compared to the MB. As mentioned, those compounds such as Amadori rearrangement products and melanoidins were found to have antioxidant abilities and was even suggested to be used for reduction of lipid peroxidation in food (Nooshkam et al., 2019).

The entrecote crust was found to be able to function as a reducing agent. Entrecote crust manifested both the ability to efficiently reduce ferric ions to ferrous and had capacity to remove iodine radicals resulting in an extensively higher amount of KI_3 solution needed to react with the starch indicator. However, the crust presented a relatively low ability to act as a chelator to ferrous, as this antioxidant function is probably not a substantial factor contributing to the antioxidant capacities.

To evaluate the isolated crust antioxidant potential we used the meat membrane (washed meat) to study its capacity to prevent lipid peroxidation. In the presence of the crust, a substantial decrease in lipid peroxidation levels was observed. This suggests there is a direct antioxidant effect on the lipid membrane as a propagation suppressor of lipid peroxidation in the meat regardless of the inhibition of the initiation step of lipid peroxidation either by iron chelation or by scavenging of radicals.

MW heating was shown to cause more structural damage in diverse types of meat. It was demonstrated previously that the heat distribution in MW treatment causes more damage to the meat surface (Yarmand and Rad, 2011). The SEM images in this study also display extensive damage after MW heating compared to PF in turkey meat, and on a lower scale in entrecote meat. A visible relationship can be distinguished between the physical damage and membrane disruption occurring post heating treatment and the amount of MDA accumulation within the meats incubated at different pHs.

The effect of various cooking methods on lipid profile has been studied, showing diverse outcomes, as in some studies a more substantial effect was observed (Abdel-Naeem et al., 2021) and in others, a very mild one compared to raw meat (Sobral et al., 2020). We did not find any statistical difference in fatty acid distribution post-heat treatments compared to that of a raw meat. According to these results combined, the increase in lipid peroxidation products can be attributed to the physical damage occurred to the meat following incubation. It is worth mentioning that due to the higher heat temperature in PF meat compared to MW, more free fatty acids (FFA) can be released from triglycerides, and it was shown that FFA can have antioxidant ability (Wu et al., 2021).

From a culinary point of view, the "sealing" of the meat from all sides by PF could be a good method to decrease lipid peroxidation during cooking and digestion probably by preventing initial oxidation which affect the oxidation rate during digesting. That is achieved by the formation of the crust layer which manifested protective chemical and physical properties in the simulation of GI postprandial incubation.

5. Conclusion

The crust, as a semi-natural antioxidant, could be used in various applications in the food industry and particularly in the meat industry to reduce lipid peroxidation and possibly extend shelf life. It is worth mentioning that full characterization of the precise compounds of the crust that display this antioxidant ability is still needed along with studying the sensory effect of the crust using basic organoleptic control from a small, trained panel.

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CRediT authorship contribution statement

Eylon Asido: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Project administration. **Haim Zeigerman:** Methodology, Formal analysis, Writing – review & editing. **Michal Verman:** Methodology, Formal analysis. **Nurit Argov-Argaman:** Methodology, Formal analysis. **Joseph Kanner:** Conceptualization, Methodology, Validation, Investigation, Writing – review & editing, Project administration. **Oren Tirosh:** Conceptualization, Methodology, Validation, Investigation, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition, All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2023.100652.

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