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Evaluating the Protective Effects of Vitamin C Against Intestinal Damage Induced by Repeatedly Heated Cooking Oils

Ahmed Abdallah[1], Mohamed Alaraby[2], Ahmed Badr[2], Tito N. Habib[1,*]

[1] *Molecular Genetics Lab., Zoology Department, Faculty of Science, Sohag University, Sohag 82524, Egypt.*
[2] *Department of Zoology, Faculty of Science, Sohag University, Sohag 82524, Egypt.*
[*]*Email: titohabib99@science.sohag.edu.eg*

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Abstract: The widespread use of repeatedly heated cooking oils, especially in developing regions, poses significant health risks, including oxidative stress-induced intestinal damage. This study investigates the histological and protective effects of vitamin C against the harmful impact of thermally oxidized oils on intestinal health. Two oil types, clean oil, and repeatedly heated cooking oil (RHC), were tested with and without vitamin C supplementation. Rats were divided into four groups receiving diets containing clean oil, RHC oil, or combinations with vitamin C. Histological analysis of hematoxylin and eosin-stained intestinal tissues was performed. The clean oil group exhibited normal villous and crypt architecture with minimal vacuolar degeneration. In contrast, the RHC oil group displayed severe necrotic changes, epithelial disruption, and glandular degeneration. Cellular damage, including epithelial necrosis and pyknotic nuclei, was prominent. Vitamin C supplementation provided partial protection, preserving villous structure, and reducing cellular damage, yet vacuolar degeneration and goblet cell hyperplasia persisted. These adaptive changes suggest tissue responses to oxidative stress but may also indicate early precancerous alterations. Findings confirm that repeated oil heating severely compromises intestinal health by inducing epithelial necrosis, glandular degeneration, and metaplasia. While vitamin C preserved some tissue architecture, its protective effects were insufficient to counteract oxidative damage fully. Public health interventions should aim to reduce reliance on reused cooking oils and promote antioxidant-rich diets to mitigate oxidative stress-related damage.
Keywords: Repeatedly heated oils, vitamin C, intestinal health, oxidative stress, histological analysis, antioxidants.

1. Introduction

Edible oils are indispensable to the human diet, supplying essential nutrients such as fatty acids, vitamins, carotenoids, sterols, and antioxidants (*e.g.*, tocopherols and polyphenols) [1]. As a concentrated energy source, oils provide 9 kcal per gram more than twice the energy yield of carbohydrates and proteins making them vital for meeting the body's energy demands [2]. Additionally, oils supply essential fatty acids, including linoleic acid (omega-6) and alpha-linolenic acid (omega-3), which the human body cannot synthesize. These fatty acids are fundamental for maintaining cell membrane integrity, promoting brain development, and regulating inflammatory responses [3].

Minimally processed oils, such as extra virgin olive oil, are rich in natural antioxidants, which protect cells from oxidative stress and may reduce the risk of chronic diseases, particularly cardiovascular disorders [4]. However, prolonged heating of oils generates harmful compounds such as trans fats, free radicals, aldehydes, and acrolein, which are linked to an increased risk of cardiovascular disease, cancer, respiratory ailments, neurodegenerative conditions, and systemic inflammation [5]. Repeated heating also depletes oils of essential nutrients, including polyunsaturated fatty acids, vitamins, and antioxidants, thereby significantly reducing their nutritional value [6].

The digestive system, responsible for processing and absorbing nutrients from food, is susceptible to damage caused by harmful dietary components. Foods subjected to excessive processing or repeated heating can exacerbate digestive disorders, leading to impaired nutrient absorption, inflammation, and a range of gastrointestinal issues. Histopathological analysis has revealed structural and functional alterations in the digestive tract, including inflammation, epithelial damage, and ulceration, which contribute to disease progression [7]. Notably, colorectal and stomach cancers rank among the leading causes of cancer-related mortality worldwide [8]. Factors such as oxidative stress, microvascular alterations, and chronic inflammation within the gastrointestinal tract play a critical role in the development of such conditions [9].

Vitamin C is a vital nutrient with multifaceted roles in maintaining health. It acts as a powerful antioxidant, enhances collagen synthesis, supports immune function, and improves iron absorption [10]. Additionally, it protects cells from oxidative damage, reduces inflammation, promotes wound healing, and prevents chronic diseases [11].

In this study, we aim to investigate the histopathological changes induced by the continuous consumption of repeatedly heated oils and evaluate the protective effects of vitamin C in mitigating these impairments.

2. Material and Methods

The study utilized 24 male Wistar albino rats, aged 6–8 weeks, with body weights ranging from 180 to 200 g. The animals were housed under standard laboratory conditions, maintained on a 12-hour light-dark cycle, and provided with standard rat chow and water ad libitum. The rats were kept in the Animal House of the Department of Zoology, Faculty of Science, Sohag University. Before the experiment, a one-week acclimatization period was observed. Ethical approval for the study was obtained from the Committee for the Scientific Research Ethics (**CSRE**) of Sohag University (Approval No. **CSRE-16-24**; http://www.sohag.edu.eg).

To minimize stress, all animals were handled gently throughout the experiment. Daily monitoring ensured prompt identification of signs of illness or distress. Animals exhibiting severe or persistent symptoms were euthanized promptly to prevent suffering. The rats were randomly divided into four groups (n = 6 per group) as follows:

Group 1: Standard diet + Vitamin C (**Vit. C** group).
Group 2: Standard diet + Clean Oil (**Clean Oil** group).
Group 3: Standard diet + Repeatedly Heated Cooking Oil (**RHC Oil** group).
Group 4**:** Standard diet + Repeatedly Heated Cooking Oil + Vitamin C (**RHC Oil** + **Vit. C** group).

Experimental Procedures

Vitamin C was dissolved in distilled water and administered orally via gavage at a dose of 100 mg/kg/day. The diets of the experimental groups included 15% of the respective heated or unheated cooking oils. Exposure to these diets lasted for 8 weeks.

Histological Investigations

At the end of the treatment period, all rats were sacrificed in a manner that preserved organ integrity, avoiding the use of chemical anesthetics [12]. Dissection was performed immediately, and small portions of the intestine were collected for histological analysis. The tissue samples were fixed in 10% neutral buffered formalin for 24 hours, dehydrated through a graded ethanol series, and embedded in paraffin wax [13].

Paraffin blocks were sectioned into 5 µm thick slices using a microtome (Leica, Feasterville, Pennsylvania). The transverse sections were carefully mounted on glass slides, deparaffinized in xylene, and rehydrated through a descending ethanol gradient. For staining, the tissue sections were immersed in hematoxylin for 5 minutes, rinsed in running tap water for 5 minutes, and counterstained with eosin for 2 minutes [14].

After staining, the sections were dehydrated through an ascending ethanol gradient, cleared in xylene, and mounted with coverslips using a DPX mounting medium [15]. The stained tissue slides were examined under a light microscope (Leica DM750, Feasterville, Pennsylvania) at 400× magnification for histopathological evaluation [16].

3. Results and Discussion:

3.1. Results

Histopathological effects on intestinal tissue

To evaluate the impact of repeatedly heated oils on intestinal health, histological analyses were performed on intestinal tissue sections from rats fed a standard diet supplemented with these oils. Additionally, the potential protective effects of **Vit. C** co-administration was assessed. Rats treated with clean oils and vitamin C served as the control group to establish baseline tissue integrity.

In the **Vit. C-treated** group (Figs. 1 & 2), the intestinal mucosa remained intact, displaying normal villous architecture with well-preserved muscularis and serosal layers. The villi exhibited their characteristic morphology, lined with simple columnar epithelium interspersed with goblet cells, reflecting optimal mucosal health and functionality. The intestinal glands and crypts of Lieberkühn were well-formed and showed no signs of degeneration, highlighting Vitamin C's protective role in preserving cellular integrity and mitigating oxidative stress.

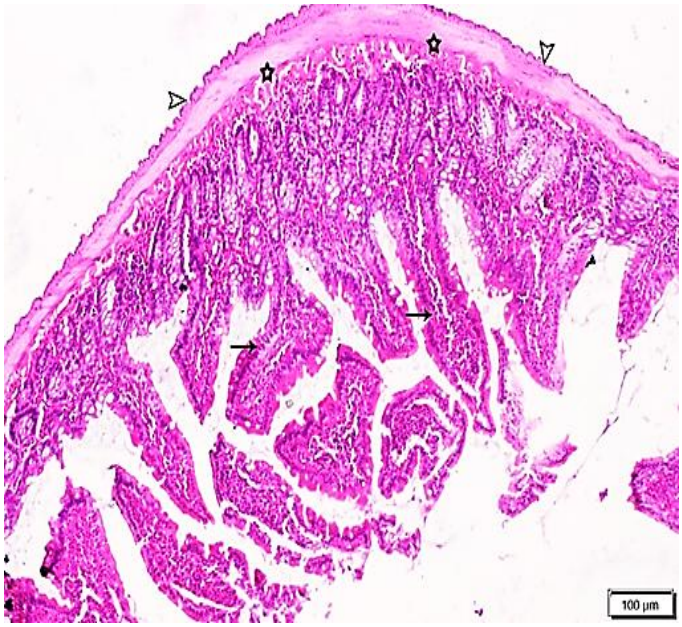

Fig. 1: Representative photomicrograph of a transverse section (T.S.) in intestinal tissue from the **Vit. C-treated** group stained with hematoxylin and eosin (**H&E**). The image illustrates well-preserved intestinal mucosa with normal villi (arrows), an intact muscular layer (stars), and a well-maintained serosal layer (arrowheads).

The **Clean Oil** group (Figs. 3 & 4) also demonstrated generally normal villous and crypt architecture. Healthy villi and well-defined crypts of Lieberkühn were evident, suggesting minimal adverse effects from clean oil consumption. However, mild vacuolar degeneration was observed within the intestinal glands, potentially indicating early signs of cellular stress or subtle tissue alterations associated with dietary oil exposure. These findings suggest that clean oil consumption has limited effects on intestinal tissue, Vitamin C supplementation may further support mucosal integrity and cellular health.

In contrast, the **RHC Oil-treated group** (Fig. 5) exhibited significant necrotic changes within the intestinal glands. These were marked by pyknotic nuclei, a classic indicator of cellular degeneration. The epithelial lining showed partial disruption, providing evidence of structural degradation in the mucosa due to prolonged exposure to reused **RHC Oil**.

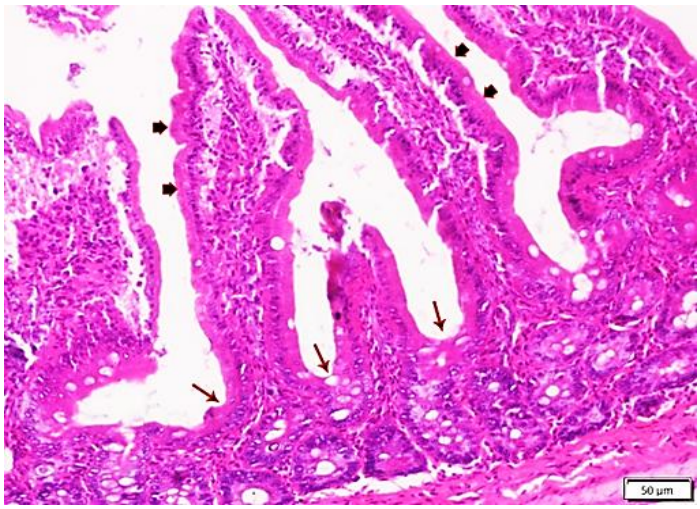

Fig. 2: Representative photomicrograph of intestinal tissue from the **Vit. C-treated group** stained with **H&E**. The T.S. section highlights normal villi lined with simple columnar epithelium interspersed with goblet cells (arrowheads) and healthy intestinal glands (arrows), reflecting optimal tissue integrity.

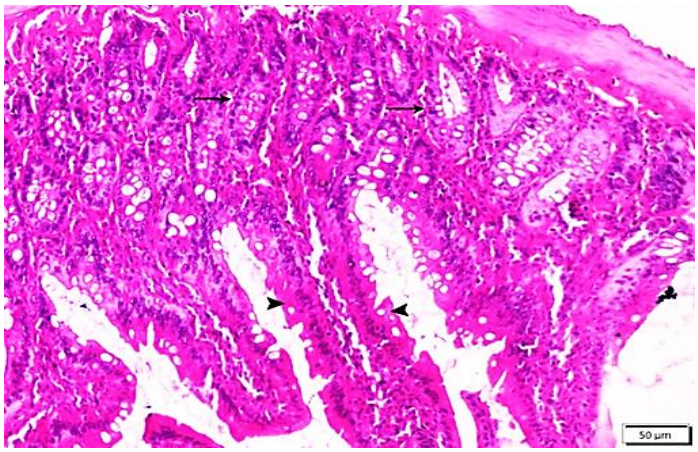

Fig. 3: A photomicrograph of a T.S. of intestinal tissue from the **Clean Oil group** stained with **H&E**. The intestinal mucosa shows normal villi (arrowheads) and well-structured crypts of Lieberkühn (arrows), indicating minimal tissue alteration.

The most pronounced changes were observed in the **RHC Oil + Vit. C group** (**Figs. 6 & 7**). Goblet cell hyperplasia was prominent, accompanied by metaplasia, where normal enterocytes transformed into goblet cells. Additionally, the intestinal tissue displayed marked vacuolar degeneration within the intestinal glands. The hyperplastic response might represent a protective adaptation to counteract the damage induced by reused **RHC Oil**. However, despite these adaptive changes, significant vacuolar degeneration persisted, underscoring Vitamin C's limited ability to mitigate the adverse effects caused by repeated exposure to reused oil.

3.2. Discussion

In various regions worldwide, particularly in Egypt, there is a heavy reliance on inexpensive and readily available fast foods, often prepared with repeatedly heated oils. Popular Egyptian staples such as falafel (*Ta'ameya*), fried potatoes, and eggplants form the basis of widely consumed sandwiches. Unfortunately, due to economic constraints, work, or study commitments, many individuals, especially the younger population, frequently resort to street food rather than home-cooked meals. Street food is commonly prepared in small, unregulated establishments that often fail to meet food safety standards. A prevalent practice in such establishments is the reuse of old oil, subjecting it to repeated heating rather than replacing it with fresh oil.

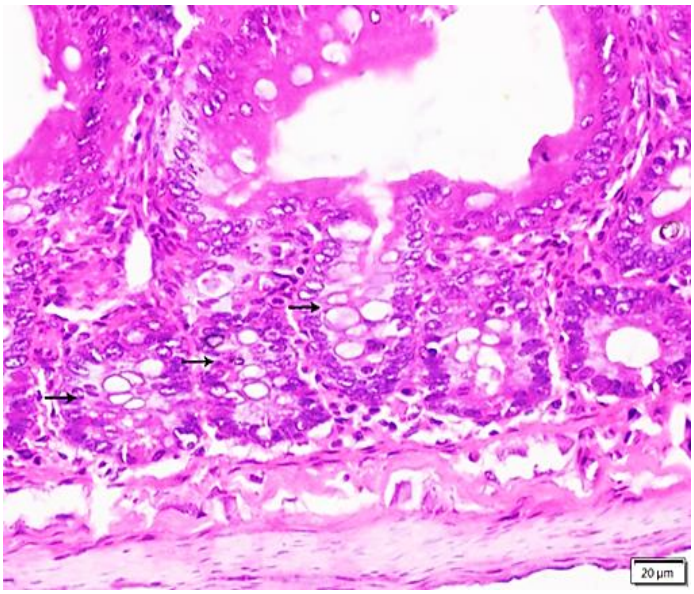

Fig. 4: A photomicrograph of intestinal tissue from the **Clean Oil group** stained with **H&E**. The T.S. section reveals intestinal glands with mild vacuolar degeneration (arrows), suggesting early signs of cellular stress.

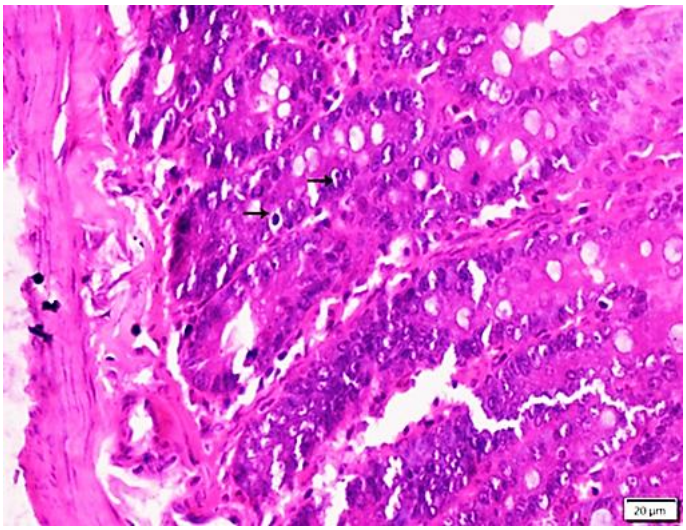

Fig. 5: Representative photomicrograph of intestinal tissue from the **RHC Oil-treated group** stained with **H&E**. The T.S. section demonstrates intestinal glands with marked necrotic changes, including pyknotic nuclei (arrows), indicating significant cellular degeneration.

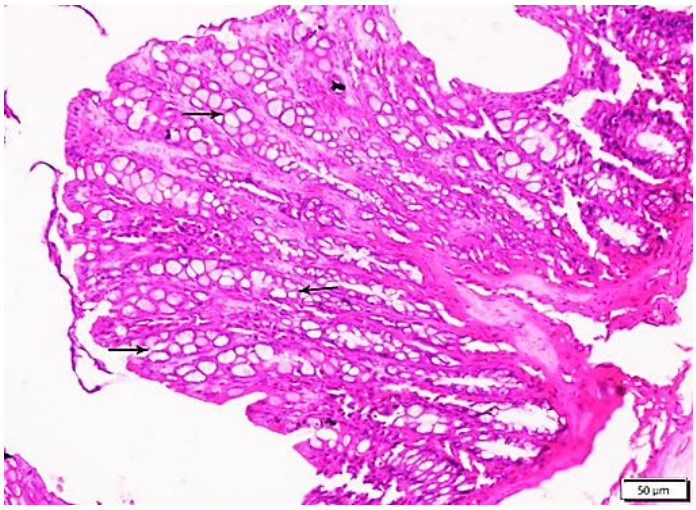

Fig. 6: A photomicrograph of intestinal tissue from the **RHC Oil + Vit. C group** stained with **H&E**. The T.S. section shows pronounced goblet cell hyperplasia (arrows), characterized by metaplasia of enterocytes into goblet cells as an adaptive response to tissue damage.

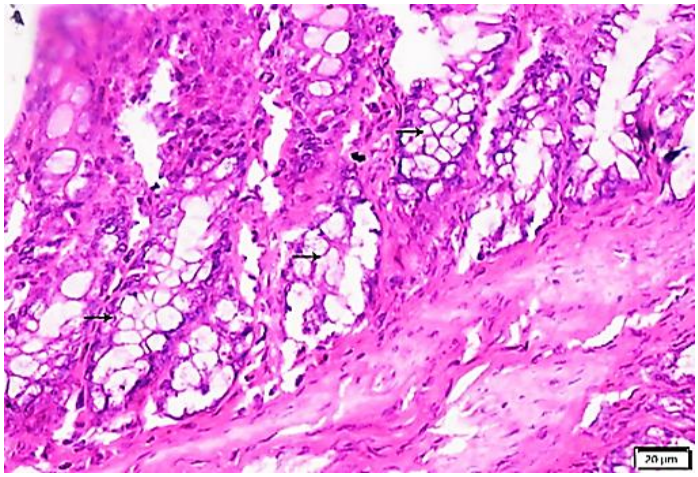

Fig. 7: Representative photomicrograph of a T.S. in intestinal tissue from the RHC Oil + Vit. C group stained with H&E. The intestinal glands exhibit marked vacuolar degeneration (arrows), highlighting persistent cellular damage despite Vitamin C supplementation.

Frying, a cooking method in which food is immersed in oil at temperatures ranging from 150 ºC to 190 ºC, facilitates direct interaction between oil, food, and air [17]. Repeatedly heating oils, however, leads to the formation of harmful compounds, including polycyclic aromatic hydrocarbons, some of which are well-documented carcinogens [18].

Globally, colorectal cancer ranks as the third most common cancer after lung and breast cancer, with its prevalence closely tied to dietary habits and lifestyle factors [19]. In Egypt, colorectal cancer predominantly affects the younger population, often presenting at advanced stages with no significant link to hereditary factors or infections like bilharziasis [20]. Previous studies have established a strong association between the frequent consumption of repeatedly heated oils and an elevated risk of various cancers, including colorectal cancer [21].

This study investigated the histological abnormalities induced by repeatedly heated oils on intestinal tissues. Vitamin C, an antioxidant, was evaluated for its protective role to examine whether these effects were mediated by oxidative stress. Oxidative stress contributes to human diseases such as atherosclerosis and cancer by inducing tissue damage [22].

The small intestine, being the primary site for nutrient absorption and exposure to dietary contaminants, plays a crucial role in maintaining overall health [23]. Disruptions to the intestinal epithelium integrity and function can have profound adverse effects on the body [24].

In this study, the administration of vitamin C showed no detrimental impact on intestinal tissue integrity, with mucosal structures remaining intact and villous architecture preserved. These observations align with prior research emphasizing the role of antioxidants in maintaining cellular integrity and mitigating oxidative stress-induced damage [25].

The healthy appearance of intestinal glands and crypts in the **Vit. C-treated** group underscores its potential in safeguarding mucosal health under non-oxidative stress conditions. In the **Clean Oil** group, the intestinal structure remained largely intact, with well-defined crypts and villi observed. However, mild vacuolar degeneration was noted within the intestinal glands, potentially reflecting early cellular stress.

Previous research has indicated that prolonged consumption of oil-rich diets in mice can alter gut microbiota and contribute to conditions such as colitis, underscoring the potential adverse effects of a high-fat diet [26].

Our histological findings revealed severe damage in the intestinal tissues of rats exposed to repeatedly heated cooking oils. The presence of epithelial necrosis, glandular degeneration, and crypt disruption highlights the harmful effects of lipid peroxidation products that accumulate in overheated oils. These findings are consistent with previous research demonstrating that oxidized lipids and toxic aldehydes in reused oils induce intestinal inflammation and barrier dysfunction, leading to increased permeability and chronic tissue damage [19].

The histological deterioration observed in the **RHC group** aligns with studies showing that repeatedly heated oils promote oxidative stress, increasing lipid peroxidation and free radical formation, which contributes to mucosal erosion and villous atrophy [20]. Furthermore, the presence of goblet cell hyperplasia and metaplasia in some groups may indicate a protective response to chronic irritation; however, such adaptations are also considered precancerous changes, increasing the risk of dysplasia and tumorigenesis [27].

In the **RHC Oil + Vit. C** group, while vitamin C supplementation demonstrated partial protective effects, it could not completely prevent tissue damage. Persistent vacuolar degeneration within intestinal glands, along with goblet cell hyperplasia and enterocyte metaplasia, suggests an adaptive response to prolonged exposure to degraded oil compounds. Goblet cell hyperplasia likely acts as a protective mechanism to enhance mucosal defense; however, metaplasia of enterocytes into goblet cells is a known precancerous alteration, associated with an increased risk of dysplasia and gastric cancer **[27, 28]**.

Oxidative stress is a key contributor to cellular injury caused

by thermally degraded oils. One of the major mechanisms involved is lipid peroxidation, which generates reactive oxygen species (**ROS**) and leads to oxidative **DNA** damage. While this study focused primarily on histological changes, future research should incorporate biochemical assessments of oxidative stress markers such as malondialdehyde **(MDA)**, a marker of lipid peroxidation, and 8-hydroxydeoxyguanosine **(8-OHdG)**, a biomarker of **DNA** oxidative damage [24]. These markers would provide deeper insights into the oxidative mechanisms underlying the observed histopathological changes.

Moreover, studies have shown that dietary antioxidants can counteract lipid peroxidation and mitigate oxidative stress-related damage. While vitamin C demonstrated partial protective effects, it was not sufficient to fully prevent tissue degeneration. This suggests that other antioxidants, such as vitamin E, polyphenols, and flavonoids, may offer superior protective benefits due to their lipophilic nature, allowing them to integrate into cell membranes and inhibit lipid oxidation more effectively [23].

Vitamin C plays a crucial role in neutralizing free radicals and reducing oxidative damage**.** In this study, vitamin C supplementation preserved villous structure and epithelial integrity, **s**upporting its role as an antioxidant. However, its limited ability to prevent vacuolar degeneration and metaplastic changes suggests that hydrophilic antioxidants alone may not be sufficient to counteract the lipophilic oxidative byproducts of thermally degraded oils [29].

Collectively, the findings highlight the detrimental effects of repeatedly heated oils on intestinal health, manifested through epithelial necrosis, glandular degeneration, and cellular metaplasia. Although dietary antioxidants such as vitamin C offer some degree of protection, they fall short of fully countering the histopathological damage caused by thermally oxidized oils. This underscores the urgent need for public health interventions promoting safer cooking practices and increased awareness of the health risks associated with reused oils.

A more comprehensive antioxidant strategy, incorporating vitamin E (a lipid-soluble antioxidant), curcumin, and resveratrol, may be more effective in preventing intestinal damage caused by oxidized lipids. Future research should investigate the synergistic effects of combining vitamin C with other lipid-soluble antioxidants to enhance cellular protection.

4. Conclusion

This study underscores the harmful impacts of repeatedly heated oils on intestinal health, particularly in regions where their use is prevalent due to economic and cultural factors. Histological analysis revealed significant damage to intestinal tissues, including epithelial necrosis, glandular degeneration, and cellular metaplasia. These findings highlight the risks associated with thermally oxidized oils, such as structural disruption of the mucosa and potential precancerous changes, emphasizing their contribution to long-term health complications.

While vitamin C supplementation demonstrated partial protective effects, including the preservation of villous architecture and cellular integrity in certain cases, it was insufficient to entirely mitigate the damage caused by repeated oil heating. Adaptive responses, such as goblet cell hyperplasia and enterocyte metaplasia, suggest the tissue's attempt to counteract damage but raise concerns due to their association with precancerous conditions.

The data collectively highlight the need for public health strategies to reduce the consumption of repeatedly heated oils. These strategies could include promoting the use of fresh oils, educating the public about the risks of reused oils, and incorporating antioxidants like vitamin C into diets to minimize oxidative stress. However, antioxidants alone cannot fully prevent the detrimental effects, reinforcing the importance of safer cooking practices and stricter food safety regulations. These findings highlight the need for further exploration of protective strategies to counteract the damage associated with thermally degraded oils.

Future research should focus on biochemical validation of oxidative stress markers**,** comparative analysis of alternative antioxidants, and long-term studies to establish more effective protective strategies against dietary oxidative stress. Comprehensive studies addressing the molecular mechanisms underlying tissue damage and adaptive responses will provide deeper insights into mitigating the health risks associated with these oils and improving dietary recommendations globally.

Credit authorship contribution statement:

The authors confirm their contribution to the paper as follows: study conception and design, M. A., A. B., and T. H.; data collection. A. A., M. A., and T. H.; results analysis and interpretation, M. A., and T. H.; draft manuscript preparation, A. B., and M. A., and T. H.; All authors reviewed the results and approved the final version of the manuscript.

Data availability statement

The data used to support the findings of this study are available from the corresponding author upon request.

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.

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References

[1] A. Mazzocchi, V. De Cosmi, P. Risé, G. P. Milani, S. Turolo, M. L. Syrén, A. Sala, & C. Agostoni, *Frontiers in Physiology*, 12 (2021): 659551.S.
[2] S.B. Heymsfield, S.A. Shapses, *New England Journal of Medicine*, 390(14) (2024): 1299-1310.
[3] M.U. Rehman, F. Khan, K. Niaz, Academic Press, 2021.
[4] Y. Lu, J. Zhao, Q. Xin, R. Yuan, Y. Miao, M. Yang, H. Mo, K. Chen, W. Cong, *Food Science and Human Wellness*, 13(2) (2024): 529-540.
[5] R.P. Venkata, R. Subramanyam, *Toxicology Reports*, 3 (2016): 636-643.

[6] R. Loganathan, A.H.A. Tarmizi, S.R. Vethakkan, K.T. Teng, A *Malaysian Journal of Analytical Sciences*, 26(6) (2022): 1378-1393.
[7] C. Maykel, L. Gammie, J. deLeyer-*Tiarks, Overview of digestive system disorders*, in: M.M. Perfect, C.A. Riccio, M.A. Bray (Eds.), 2nd ed., American Psychological Association, Academic Press, 2023.
[8] Y. Feng, H. Jin, K. Guo, H.S. Wasan, S. Ruan, C. Chen, C, *Frontiers in Oncology*, 11 (2021): 647179.
[9] T.A. Ullman, S.H. Itzkowitz, *Gastroenterology*, 140(6) (2011): 1807-1816.
[10] A.C. Carr, S. Maggini, *Nutrients*, 9(11) (2017): 1211.
[11] S. Chambial, S. Dwivedi, K.K. Shukla, P.J. John, P. Sharma, *Indian Journal of Clinical Biochemistry*, 28 (2013): 314-328.
[12] J. D. Bancroft & M. Gamble, *Theory and practice of histological techniques* (6th ed.). Elsevier Health Sciences. 2008.
[13] J. A. Kiernan, *Histological and histochemical methods: Theory and practice* (4th ed.). Scion Publishing. 2008.
[14] A. H. Fischer, K. A. Jacobson, J. Rose, & R. Zeller, *Cold Spring Harbor Protocols*, (5) (2008), pdb.prot4986.
[15] K. S. Suvarna, C. Layton, & J. D. Bancroft, *Bancroft's theory and practice of histological techniques* (8th ed.). Elsevier. 2018.
[16] C. F. A. Culling, R. T. Allison, & W. T. Barr, *Cellular pathology technique* (4th ed.). Butterworth-Heinemann. 2013.
[17] İ. İlter, Ö. Altay, Ö. Köprüalan, F.K. Ertekin, S.M. Jafari, An, *High-Temperature Processing of Food Products*, Academic Press, 2023.
[18] A.M. GadAllah, M.A. Mohamed, M.N. Azab, *Toxicology Reports*, 11 (2023): 233-240.
[19] K. Ganesan, K. Sukalingam, B. Xu, *Critical Reviews in Food Science and Nutrition*, 59(3) (2019): 488-505.
[20] E.M. Mahfouz, R.R. Sadek, W.M. Abdel-Latief, F.A.H. Mosallem, E.E. Hassan, *Central European Journal of Public Health*, 22(4) (2014): p.215.
[21] A.A. Abou-Zeid, W. Khafagy, D.M. Marzouk, A. Alaa, I. Mostafa, A.M. Ela, *Diseases of the Colon & Rectum*, 45(9) (2002): 1255-1260.
[22] V.K. Nambiar, V. Mudliar, I. Salababa, Analysis of Oral Cancer Carcinogens in Repeatedly Heated Cooking Oils, *Heliyon,* 2025.
[23] S.J. Padayatty, A. Katz, Y. Wang, P. Eck, O. Kwon, J.H. Lee, S. Chen, C. Corpe, A. Dutta, S.K. Dutta, M. Levine, *Journal of the American College of Nutrition*, 22(1) (2003): 18-35.
[24] G.D. de Barboza, S. Guizzardi, L. Moine, N.T. de Talamoni, *World Journal of Gastroenterology*, 23(16) (2017): p.2841.
[25] A.S. Londero, M.R. Arana, V.G. Perdomo, G.N. Tocchetti, F. Zecchinati, C.I. Ghanem, M.L. Ruiz, J.P. Rigalli, A.D. Mottino, F. García, S.S.M. Villanueva, *The Journal of Nutritional Biochemistry*, 40 (2017): 178-186.
[26] G. Lam, V. Apostolopoulos, A. Zulli, K. Nurgali, *Current Medicinal Chemistry,* 22(17) (2015):2100-2109.
[27] D.S. Jencks, J.D. Adam, M.L. Borum, J.M. Koh, S. Stephen, D.B. Doman, *Gastroenterology & Hepatology*, 14(2) (2018): p.92.
[28] S.K. Ku, M.M. Ruhaifi, S.S. Fatin, M. Saffana, K.T. Anna, S. Das, J. Kamsiah, *La Clinica Terapeutica*, 165(4) (2014): 217-221.
[29] T. Agura, S. Shin, H. Jo, S. Jeong, H. Ahn, S.Y. Pang, J. Lee, J.H. Park, Y. Kim, J.S. Kang, *Anat Cell Biol*., 30;57(3) (2024): 408-418.