Analysis of the Effects of Purified Used Cooking Oil Consumption on Nutritional Absorption Status: An Anti-Stunting Promotion Strategy

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Abstract.
Stunting remains a critical global public health priority due to its long-term impacts on cardiometabolic disorders and neurocognitive deficits resulting from chronic malnutrition. At the household level, a hidden risk factor potentially triggering infection and nutrient malabsorption is the repeated use of cooking oil (used cooking oil). Chronic exposure to foods processed with used cooking oil can lead to nutritional malabsorption, indirectly increasing the risk of stunting in children. Although the adverse effects of used cooking oil have been widely documented, exploration into the efficacy of oil purification methods in restoring or improving nutritional status remains highly limited. This study employed an experimental research design with a factorial layout. Data analysis was performed using a One-Way Analysis of Variance (ANOVA) to compare differences across treatment groups, followed by a Tukey Post-Hoc test to determine specific statistically significant differences among the administered treatments. The Tukey Post-Hoc test revealed that the Purified Oil Treatment group (P) yielded the highest mean hepatic protein level (9,30±0,03%). This value was statistically significant ($p < 0,05$) compared to all other groups, namely the Control group (7,64±0,34\%), the fresh oil group (7,99±0,26%), and the used cooking oil (J) groups (7,60±0,45%). Meanwhile, no significant differences ($p > 0,05$) in hepatic protein levels were observed among the Control (K), Fresh Oil (B), and Used Cooking Oil (J) groups. Furthermore, the Used Cooking Oil group (J) exhibited an extreme decline in hepatic iron accumulation, recording the lowest mean level (14,77±0,76 ppm), which was highly statistically significant ($p < 0,001$) compared to all other cohorts. Conversely, the Purified Oil Treatment group (P) demonstrated a significant recovery in iron absorption, with a mean value of 54,90±1,76 ppm. These findings indicate that the mean hepatic tissue analysis for iron and protein absorption showed virtually no alterations between the Control and Fresh Oil consumption groups. In contrast, the consumption of unpurified used cooking oil led to a substantial reduction in both iron and protein absorption. Remarkably, the consumption of purified cooking oil resulted in the highest enhancement of protein absorption alongside a significant recovery of iron levels compared to the used cooking oil group.
Keywords: *Used cooking oil; Stunting; Purification and Nutrition.*

I. INTRODUCTION

Stunting remains a critical global public health priority due to its long-term impacts on cardiometabolic disorders and neurocognitive deficits resulting from chronic malnutrition [1]. This condition is often exacerbated by micronutrient deficiencies, which significantly impair children's physical and cognitive development while increasing susceptibility to infections [2].. Etiologically, malnutrition is not only closely linked to poor dietary patterns but is also triggered by severe, recurrent infections [3].

At the household level, a hidden risk factor potentially triggering infection and nutrient malabsorption is the repeated use of cooking oil (used cooking oil). Subjecting cooking oil to high temperatures induces a series of complex chemical reactions, including hydrolysis, oxidation, isomerization, polymerization, and degradation [4]. These reactions significantly alter the color, flavor, physicochemical properties, nutritional value, and original composition of the oil [5,6]. Furthermore, this thermal degradation generates toxic compounds, such as trans fatty acids and free radicals, which can impair nutrient bioavailability and absorption in the digestive system, ultimately hindering linear child growth [7,8]. This phenomenon is further compounded by the overconsumption of high-calorie, low-micronutrient foods prepared with poor-quality oil [9]. Chronic exposure to foods processed with repeatedly heated used cooking oil can lead to nutritional malabsorption, indirectly increasing the risk of stunting in children [10]. This situation has become increasingly urgent amid current economic realities, where surging cooking oil prices have led to

widespread cases of adulterated used cooking oil being resold for public consumption. Therefore, a profound analysis regarding the health impacts of repeatedly heated cooking oil on child growth and development is crucial to serve as a scientific foundation for health promotion initiatives.

Although the adverse effects of used cooking oil have been widely documented, exploration into the efficacy of oil purification methods in restoring or improving nutritional status remains highly limited. This study offers a distinct novelty by analyzing the effects of purified used cooking oil on nutritional status using a pre-pubertal animal model—an approach that has not been previously explored. Consequently, this research provides scientific advancements and innovative purification methods that minimize chemical use, thereby reducing the risk of toxic exposure and environmental contamination.

The unique contribution and value proposition of this study lie in the application of green chemistry principles to innovate the purification of used cooking oil, rendering it safe for re-consumption. This approach not only provides an economically viable solution for the community but also mitigates the risk of nutrient malabsorption caused by degraded oil consumption. The validity of this innovation is demonstrated through biomarker testing of the green chemistry-based purified oil, distinguishing this study from previous literature.

II. METHODS

This study employed an experimental research design with a factorial layout. The research was conducted at the Chemical Laboratory and Biological Laboratory of the University of Mataram over a duration of approximately three months. The research variables consisted of three components:

1. Independent Variable: The type of cooking oil, which included fresh cooking oil, unpurified used cooking oil, and green chemistry-based purified used cooking oil.
2. Dependent Variables: Nutritional status parameters, which included body weight, body length, total protein levels, albumin, prealbumin, and iron levels.
3. Controlled Variables: The amount of standard feed, environmental acclimatization conditions, and the strain of the experimental animal models.

Data analysis in this study was performed using a One-Way Analysis of Variance (ANOVA) to compare differences across treatment groups, followed by a Post-Hoc test to determine specific statistically significant differences among the administered treatments

III. RESULT AND DISCUSSION

1. Anthropometric Analysis Results

Anthropometric measurements were performed to determine the differences in body length and body weight of the experimental animals before and after the treatment period. The anthropometric measurement data obtained before and after the intervention are presented below:

Table 1. Fresh Oil Treatment Group

No	Initial Mouse Weight (gr)	Final Mouse Weight (gr)	Initial Mouse Length (Cm)	Final Mouse Length (Cm)
1	29.74	27.8	18.5	18.7
2	24.75	24.9	16.7	16.9
3	22.54	23.39	18.5	18.6
4	22.54	32.67	16.5	18.1
5	22.54	29.69	18.7	18.3

Tabel 2 Used cooking oils Treatment Group

No	Initial Mouse Weight (gr)	Final Mouse Weight (gr)	Initial Mouse Length (Cm)	Final Mouse Length (Cm)
1	27.88	26.66	18.3	18.6
2	21.59	23.34	17.6	18.7
3	23	30.53	16.2	16.3

4	19.71	27.28	17.7	18.2
5	21.28	22.26	15.5	19.3

Tabel 3 Purified Oil Treatment Group

No	Initial Mouse Weight (gr)	Final Mouse Weight (gr)	Initial Mouse Length (Cm)	Final Mouse Length (Cm)
1	21.74	23.75	14.6	17.9
2	23.97	21.88	17.3	18.8
3	26.35	27.7	16.2	17.7
4	23.95	29.68	15.7	17.8
5	27	26.83	15.6	`18,5

Anthropometric measurements (body weight and body length) in pre-pubertal animal models serve as sensitive indicators to evaluate the quality of nutritional intake and the efficiency of nutrient absorption during the crucial linear growth phase. Based on the experimental results, a significantly distinct growth pattern was observed among the groups of mice administered fresh oil, used cooking oil, and green chemistry-based purified used cooking oil. In the used cooking oil group (Table 2), there was an average body weight gain of 4.08 gr; however, the trend in body length growth was inconsistent across several samples (for instance, sample No. 3 increased by only 0.1 cm). This phenomenon of high weight gain uncoupled from optimal linear growth indicates a high-calorie, low-nutrient intake (9). The thermal degradation process in used cooking oil generates trans fatty acids and thermal polymer compounds that promote adipose (fat) tissue accumulation but fail to supply the micronutrients required for bone ossification. Furthermore, free radicals and oxidation products within used cooking oil trigger chronic inflammation in the intestinal mucosa, leading to the malabsorption of essential micronutrients such as calcium, zinc, and iron, which are heavily required to prevent stunting (2,10).

The most compelling finding in this study was demonstrated by the purified oil group (Table 3). This cohort exhibited the most significant and consistent increase in average body length, reaching 2.14 cm, which substantially outperformed both the used cooking oil group (1.12 cm) and the fresh oil group (0.30cm). Although its body weight gain was more moderate (+2.15g), this growth pattern clearly indicates optimal linear growth (catch-up growth). The success in significantly enhancing body length in this pre-pubertal animal model provides robust evidence that the innovative oil purification method utilized is capable of adsorbing toxic compounds, free radicals, and trans fatty acids previously generated during the repeated heating process (4,7). With the elimination of these inhibitory compounds, the integrity of the intestinal wall in the experimental animals was well-preserved, thereby preventing nutritional malabsorption. The restored, optimal absorption of essential micronutrients stimulates the secretion of growth hormones, such as Insulin-like Growth Factor 1 (IGF-1), which plays a direct role in the elongation of long bones (epiphyseal plate) during the pre-pubertal phase.

These anthropometric results validate the initial hypothesis that crude used cooking oil acts as an anti-nutritional agent that contributes to the risk of physical stunting (7,8). Conversely, intervention with green chemistry-based purification technology proved to not only neutralize the toxic properties of the oil but also restore the bioavailability of the macro- and micronutrients required for linear growth. Overall, these anthropometric findings provide a novel scientific foundation showing that the purification of used cooking oil using chemical-minimal methods holds great potential as an alternative preventive intervention to address malnutrition and stunting risks at the household level, while simultaneously offering an economically viable and environmentally friendly solution.

2. Biomarker Analysis result

Biomarker assays are laboratory methods designed to detect or analyze genes, proteins, or other molecules within biological samples, such as tissues, blood, or other bodily fluids. Although blood sampling was initially planned for this assay, due to instrumentation and technical limitations, hepatic (liver) tissue from the experimental animals was utilized instead. Following the predetermined treatment period, the experimental animals were euthanized and dissected to harvest the liver organs. Subsequently, the hepatic

tissues were analyzed to evaluate protein and iron absorption. The results of the biomarker analysis are presented below:

Tabel 4. Biomarker Analysis Result

No	Sample	Measurement Parameters	
		Protein Absorption (%)	Iron Absoption (ppm)
1	Control (K1)	7,43	88,59
2	Control (K2)	8,04	81,83
3	Control (K3)	7,46	88,49
4	Fresh Oil Treatment (B1)	7,90	87,34
5	Fresh Oil Treatment (B2)	7,79	88,15
6	Fresh Oil Treatment (B3)	8,28	85,02
7	Purified Oil Treatment (P1)	9,33	52,97
8	Purified Oil Treatment (P2)	9,29	56,40
9	Purified Oil Treatment (P3)	9,28	55,32
10	Used Cooking Oil Treatment (J1)	7,12	14,04
11	Used Cooking Oil Treatment (J2)	7,68	15,55
12	Used Cooking Oil Treatment (J3)	8,01	14,71

Biomarker assays are commonly performed using blood samples (serum/plasma) to detect systemic metabolic changes. However, due to logistical, instrumental, and technical constraints, utilizing hepatic (liver) tissue from animal models serves as a highly valid alternative with substantial scientific merit. The liver acts as the central metabolic hub in mammals, functioning as the primary site for protein synthesis (such as albumin and transferrin) and the main storage depot for iron in the form of ferritin. Consequently, evaluating protein levels and iron accumulation directly within the hepatic tissue reflects the true nutritional bioavailability and cellular absorption efficiency resulting from dietary interventions.

The most critical finding in this biomarker assay was demonstrated by the drastic reduction of hepatic iron levels in the unpurified used cooking oil group (J1, J2, J3), which averaged only (14.77 ppm) compared to the Control (86.30 ppm) and Fresh Oil (86.84 ppm) groups. This extreme decline provides biochemical confirmation that toxic compounds derived from the thermal degradation of oil—such as free radicals, lipid peroxides, and trans fatty acids—massively disrupt the small intestinal mucosa, which is the primary site for iron absorption. These oxidative compounds trigger localized oxidative stress in the gut, damaging the microvilli and inhibiting key iron transporter proteins, specifically Divalent Metal Transporter 1 (DMT-1). Furthermore, free radicals from used cooking oil can induce a systemic inflammatory response that upregulates the production of hepcidin, the master iron-regulating hormone. This elevation leads to the degradation of ferroportin, thereby preventing iron from being absorbed and stored in the liver. Chronic failure in iron absorption constitutes a primary etiological pathway for iron deficiency anemia, which clinically correlates directly with linear growth retardation and an increased risk of stunting during the pre-pubertal phase (2,10).

Conversely, the treatment group administered green chemistry-based purified used cooking oil (P1, P2, P3) demonstrated a highly significant recovery effect. Hepatic iron levels successfully restored to an average of 54.90 ppm. Although this recovery did not fully reach the control baseline, it proves that the innovative oil purification method successfully adsorbed most of the inhibitory compounds that impede iron absorption. Remarkably, the hepatic protein content in the purified oil group (P) reached the highest value among all cohorts, peaking at 9.30% (Control: 7.64%; Fresh Oil: 7.99%; Used Cooking Oil: 7.60%). This phenomenon indicates a mechanism of metabolic compensation or catch-up metabolism. The removal of toxic compounds via green chemistry principles appears to rejuvenate hepatocyte (liver cell) function in synthesizing proteins and metabolizing amino acids. This elevated hepatic protein concentration correlates positively with the accelerated body length growth (anthropometric data) observed in the purified group, as abundant liver proteins support the availability of essential amino acids required for the synthesis of the bone matrix and muscle tissue.

Integratively, these hepatic biomarker data elucidate the biological mechanisms underpinning the physical phenomena observed in the preceding anthropometric assessments. Used cooking oil is proven to

trigger extreme micronutrient malabsorption (evidenced by the plummeting hepatic iron levels), which explains why linear growth in that group was stunted. Meanwhile, the efficacy of the green purification method successfully rescued intestinal absorptive function and hepatic synthesis (evidenced by iron recovery and the protein surge), acting as the primary bio-driver behind optimal linear growth in the experimental models. Overall, the biomarker results from the hepatic tissue reinforce the novelty of this study. They confirm that the innovative used cooking oil purification method does not merely improve the external physicochemical properties of the oil, but is biologically proven in vivo to enhance nutritional status and mitigate the biochemical risk factors underlying stunting

3. Statistical Analysis Result of Hepatic Biomarkers (ANOVA and Post-Hoc)

To determine the significant effects of various oil treatments on nutritional bioavailability in the liver organ, a One-Way Analysis of Variance (ANOVA) was performed at a 95% confidence level ($\alpha = 0,05$), followed by Tukey's Honestly Significant Difference (HSD) post-hoc test. A summary of the descriptive statistical data and significance notations is presented in Table 5..

Tabel 5. Comparison of Mean Protein and Iron Absorption across Treatment Groups

treatment Group	Protein Absorption (%)	Iron Absoption (ppm)
Control (K)	$7,64\pm0,34^{a}$	$86,30\pm3,87^{a}$
Fresh Oil (B)	$7,99\pm0,26^{a}$	$86,84\pm1,62^{a}$
Purified Oil (P)	$9,30\pm0,03^{b}$	$54,90\pm1,76^{b}$
Used Cooking oil (J)	$7,60\pm0,45^{a}$	$14,77\pm0,76^{c}$
Value p (ANOVA analysis)	<0,01	<0,001

a. Protein Absorption (%)

Based on the One-Way ANOVA results, the type of oil treatment exerted a statistically significant effect on the percentage of hepatic tissue protein content ($p < 0.01$). Tukey's post-hoc test revealed that the Purified Oil Treatment group (P) yielded the highest mean protein level (9,30±0,03%). This value was statistically significant ($p < 0,05$ when compared to all other groups, namely the Control group (7,64±0,34%), the Fresh Oil group (7,99±0,26%), and the Used Cooking Oil group (7,60±0,45%). Meanwhile, no significant differences ($p > 0.05$) in hepatic protein levels were observed among the Control (K), Fresh Oil (B), and Used Cooking Oil (J) groups. Consequently, it can be concluded from Tukey's post-hoc test that the protein levels in the purified oil treatment group (P) were significantly different ($p < 0.05$) compared to the Control (K), Fresh Oil (B), and Used Cooking Oil (J) groups.

The remarkable enhancement of hepatic protein content in the Purified Oil Treatment group (P) is highly correlated with the synergistic refining mechanism of coconut shell activated carbon and turmeric (Curcuma longa), designed under green chemistry principles. Coconut shell activated carbon possesses an extensively developed micro- and mesoporous network, providing a vast specific surface area that functions as a highly efficient physical trap. This porous matrix selectively adsorbs bulky lipid degradation products, such as polar compounds, free fatty acids (FFAs), polymers, and volatile aldehydes generated during thermal oxidation.

Simultaneously, the integration of turmeric introduces bioactive curcuminoids (primarily curcumin, demethoxycurcumin, and bisdemethoxycurcumin). Curcuminoids act as powerful natural antioxidants and chemical chelators, neutralizing residual hydroperoxides and binding heavy metal ions that otherwise act as catalysts for further oil degradation. This dual action—physical entrapment by the carbon matrix and chemical stabilization by turmeric—effectively rejuvenates the oil quality without leaving any synthetic chemical footprints. Consequently, when mice ingest this purified oil, the biological delivery of hazardous lipid peroxides is drastically minimized, protecting the animal from the systemic toxicological insults evident in the Used Cooking Oil group (J), which exhibited a suppressed protein level of 7.60±0.45%.

In mice (Mus musculus), the liver is the primary metabolic hub vulnerable to dietary oxidative stress. The ingestion of thermally degraded oil in Group J floods the hepatocytes with reactive oxygen species (ROS), causing severe lipid peroxidation of cellular membranes. This oxidative stress damages the structural integrity of the rough endoplasmic reticulum, leading to ribosomal dissociation, protein denaturation, and the activation of proteolytic pathways, which accounts for the depleted hepatic protein levels. In sharp contrast, the oil purified with coconut shell carbon and turmeric serves as a potent cytoprotective agent. By

eliminating dietary ROS and delivering trace amounts of lipophilic curcuminoids, this treatment preserves the structural and functional integrity of hepatocyte membranes. The reduction in oxidative stress prevents the degradation of structural proteins and minimizes the metabolic burden on the liver.

Furthermore, curcumin is well-known to upregulate the Nrf2 (nuclear factor erythroid 2-related factor 2) signaling pathway in mice, which induces the expression of endogenous antioxidant enzymes (such as superoxide dismutase and glutathione peroxidase). This biochemical shift creates a highly favorable anabolic microenvironment within the hepatic tissue, promoting cell survival, enhancing ribosomal translation, and ultimately accelerating protein synthesis. The statistically superior protein elevation (9.30±0,03%) in Group P underscores that this green-chemistry-based purification not only neutralizes the toxicity of used cooking oil but actively boosts the hepatic metabolic capacity and tissue health of the mice models.

b. Iron Absorption (ppm)

The One-Way ANOVA results for the iron absorption parameter demonstrated a highly significant intervention effect ($p < 0.001$) across the treatment groups. Specifically, the Used Cooking Oil group (J) exhibited an extreme decline in hepatic iron accumulation, recording the lowest mean level (14.77±0.76 ppm), which was highly statistically significant ($p < 0.001$) compared to all other groups. The Purified Oil Treatment group (P) demonstrated a significant recovery in iron absorption, with a mean value of 54.90±1.76 ppm. This figure was proven to be significantly higher ($p < 0.01$) than that of the used cooking oil group, although statistically it has not yet achieved a full recovery to match the control group baseline. Meanwhile, the Control group (K) (86.30±3.87 ppm) and the Fresh Oil group (B) (86.84±1.62 ppm). occupied the highest subset and showed no significant difference from each other ($p > 0.05$).

The drastic reduction of hepatic iron accumulation in the Used Cooking Oil group (J) (14.77±0.76 ppm) points toward a severe disruption in intestinal iron absorption and systemic iron homeostasis in the mice models. Thermally degraded oil contains high concentrations of lipid hydroperoxides, aldehydes, and polar compounds. Upon ingestion, these toxic degradation products trigger acute inflammation and oxidative stress within the intestinal mucosa of mice.

Biochemically, chronic intestinal inflammation upregulates the expression of hepcidin, a liver-derived hormone that acts as the master regulator of iron systemic balance. Elevated hepcidin levels bind to and induce the degradation of ferroportin, the sole cellular iron exporter located on the basolateral membrane of enterocytes. Consequently, dietary iron becomes trapped within the intestinal epithelial cells and is lost during normal mucosal shedding, preventing its transport into the bloodstream and subsequent storage in the liver tissue. Furthermore, excessive reactive oxygen species (ROS) from the unpurified oil can directly damage the divalent metal transporter 1 (DMT1) on the microvilli membrane, severely impairing the initial uptake of non-heme iron from the intestinal lumen.

The significant recovery of hepatic iron levels in the Purified Oil Treatment group (P) (54.90±1.76 ppm) highlights the profound efficacy of the green purification matrix. The micro- and mesoporous framework of the coconut shell activated carbon effectively traps long-chain lipid polymers and volatile decomposition products through physical adsorption. Concurrently, the incorporation of turmeric introduces active curcuminoids that exert potent anti-inflammatory and free-radical scavenging properties. By neutralizing lipid peroxides before they can cause mucosal damage, this dual-action adsorbent significantly mitigates gut inflammation in mice, thereby suppressing the pathological overproduction of hepcidin and restoring the functional integrity of ferroportin and DMT1 transporters.

However, the fact that iron levels in Group P did not fully return to the baseline of the Control group (K) (86.30±3.87 ppm) or Fresh Oil group (B) (86.84±1.62 ppm) can be scientifically justified by the intrinsic chemical nature of curcumin. Curcuminoids possess functional β--diketone groups known to act as natural iron chelators. While this chelating property is highly beneficial in the oil phase for binding pro-oxidant heavy metal catalysts, trace lipophilic curcuminoids present in the purified oil may form stable complexes with dietary iron (Fe^{3+}) within the gastrointestinal tract of the mice. These iron-curcumin complexes reduce the bioavailability of free iron for epithelial absorption. This dual phenomenon explains why the green-purified oil successfully rescues the liver from extreme iron deficiency while maintaining a controlled, sub-

baseline iron accumulation that protects the hepatic tissue from potential iron-induced hydroxyl radical generation via the Fenton reaction.

IV. CONCLUSION

This study demonstrates that the consumption of unpurified used cooking oil severely restricts linear growth in pre-pubertal mice, characterized by stunted body length elongation and a drastic reduction in hepatic iron and protein absorption due to oxidative damage. Conversely, used cooking oil purified via green chemistry techniques successfully neutralizes these anti-nutritional compounds. This intervention yields optimal linear catch-up growth (+2.14 cm), maximizes hepatic protein synthesis (9.30%), and significantly rescues iron accumulation (54.90 ppm). Consequently, this eco-friendly oil purification method offers a highly promising, economically viable dietary intervention to mitigate malnutrition and physical stunting risks at the household level.

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