



**PHYSICOCHEMICAL PROPERTIES AND FATTY ACID PROFILE OF
COCONUT OIL IN AN IN VITRO GASTROINTESTINAL SYSTEM**

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ABSTRACT

This study investigated the physicochemical changes of cold-pressed, unrefined coconut oil during digestion using a semi-dynamic in vitro gastrointestinal system that simulates the mouth, gastric, and intestinal phases. Key parameters-including free fatty acidity, peroxide value, refractive index, iodine value, fatty acid composition, and sterol profile-were analyzed, and statistical differences were evaluated by one-way ANOVA. Digestion led to significant alterations in several indices, notably a marked increase in free fatty acids in the intestinal phase ($P<0.05$), indicating extensive lipase-mediated hydrolysis. Peroxide value, iodine number, and refractive index also changed across phases, while fatty acid and sterol profiles exhibited phase-dependent shifts. These findings demonstrate that coconut oil undergoes significant physicochemical transformations during digestion, particularly under the influence of intestinal enzymatic activity. The study highlights the importance of gastrointestinal simulation when assessing the nutritional behavior and stability of dietary oils.

Keywords: Coconut oil, fatty acid, sterol, in vitro gastrointestinal system, food, nutrition

**IN VITRO GASTROİNTESTİNAL SİSTEMDE HİNDİSTAN CEVİZİ YAĞININ
FİZİKOKİMYASAL ÖZELLİKLERİ VE YAĞ ASİDİ PROFİLİ**

ÖZ

Bu çalışmada, ağız, mide ve bağırsak evrelerini simüle eden yarı dinamik bir in vitro gastrointestinal sistem kullanarak soğuk preslenmiş, rafine edilmemiş Hindistan cevizi yağından sindirim sırasındaki fizikokimyasal değişimleri araştırılmıştır. Serbest yağ asitliği, peroksit değeri, kırılma indisi, iyot değeri, yağ asidi bileşimi ve sterol profili gibi temel parametreler analiz edilmiş; istatistiksel farklar tek yönlü ANOVA ile değerlendirilmiştir. Sindirim, özellikle bağırsak evresinde serbest yağ asitlerinde anlamlı bir artışa ($P<0.05$) yol açarak belirgin lipaz aracılı hidrolizi ortaya koymuştur. Peroksit değeri, iyot sayısı ve kırılma indisi de sindirim evreleri arasında değişim gösterirken, yağ asidi ve sterol profilleri fazla bağlı farklılıklar sergilemiştir. Bulgular, Hindistan cevizi yağından özellikle bağırsak enzimatik aktivitesi altında önemli fizikokimyasal dönüşümlere uğradığını göstermektedir. Çalışma, diyet yağlarının beslenme davranışını ve stabilitesini değerlendirirken gastrointestinal simülasyon modellerinin önemini vurgulamaktadır.

Anahtar Kelimeler: Hindistan cevizi yağı, yağ asidi, sterol, in vitro gastrointestinal sistem, gıda, beslenme



INTRODUCTION

Coconut oil, derived from the fruit of the *Cocos nucifera* tree of the Arecaceae family, has been utilized for thousands of years in tropical regions. In recent years, it has attracted growing scientific interest due to findings highlighting its positive health effects (Yalçın and Özgen Özka, 2022). One hundred grams of coconut contains 3.33 grams of protein, 15.23 grams of carbohydrates, and 33.49 grams of fat. One hundred grams of coconut also contains 9 grams of dietary fiber (USDA, 2018). The oil obtained from fresh and mature coconuts, without purification, bleaching, deodorization, and without altering the oil's natural properties, is defined as virgin coconut oil. This method helps preserve the nutritional value of bioactive substances that are lost during the production of coconut oil (Agarwal, 2017). The main phenolic acids found in virgin coconut oil are caffeic acid, syringic acid, p-coumaric acid, vanillic acid, and ferulic acid. It also contains high amounts of flavonoids, especially flavanones and dihydroflavonols, as well as vitamins A and E. The melting point of coconut oil, which can be found in both solid and liquid forms, is 23-26°C. It is colorless, resistant to spoilage, and has a special aroma (Marina et al., 2009).

Medium-chain fatty acids, which make up the basic structure of coconut oil, are triglycerides with fatty acid chains of 6-12 carbon atoms. This distinguishes coconut oil from animal-based fats (Cardoso et al., 2015; Boateng et al., 2016). Digestion of fats usually occurs in the small intestine with the help of pancreatic lipase and bile acids. Medium-chain fatty acids, unlike other long-chain fatty acids, are absorbed from the gastric mucosa and transported to the liver via portal circulation (Boateng et al., 2016). They are also transported to the mitochondria independently of carnitine. In liver and muscle cells, they reach the mitochondria and undergo oxidation there, independently of the carnitine acyltransferase enzyme. Medium-chain fatty acids are metabolized in the liver to provide energy and are not stored in adipose tissue (Lu et al., 2020).

Coconut oil is unique in its slightly sweet taste, pleasant odor, high resistance to spoilage, easy digestibility, and absorbability (Shahidi and Senanayake, 2006). The various health benefits of coconut oil include antiviral, antibacterial, antifungal, antiparasitic, anti-inflammatory, antithrombotic, cardioprotective, hepatoprotective, anti-dermatophytic, anti-

diabetic, hypolipidemic, anti-cholecystitis, anti-oxidant, and anti-carcinogenic activities (Salian and Shetty, 2018). Energy from saturated fat should not exceed 10% of total daily calorie intake. In line with this recommendation, saturated fat intake should be kept below 200 calories in a 2,000-calorie daily diet. One tablespoon (14 grams) of coconut oil contains approximately 117 calories and 13.9 grams of fat, of which 11.6 grams are saturated fat. This amount corresponds to approximately 105 calories. Therefore, coconut oil consumption should be limited to no more than two tablespoons (28 grams) per day. It is essential not to exceed this limit to ensure that total saturated fat intake remains within recommended levels (USDA, 2020). It is recommended that medium-chain triglycerides (MCTs) be consumed in amounts of 5-25 grams per day to see their potential positive effects on satiety, postprandial energy expenditure, and body composition (Clegg, 2017). One study of obese individuals reported that consuming 10 grams of MCTs before lunch resulted in a reduction in the amount of food consumed at lunch (St-Onge et al., 2014).

In recent years, there has been a significant increase in research on the physicochemical degradation processes of food matrices, the colloidal structures formed, and the bioaccessibility, transformation, and absorption of bioactive compounds in the human gastrointestinal tract. While most studies conducted to date have focused on understanding the physicochemical properties of foods before consumption, research on the physicochemical basis of the behavior of these components in the human gastrointestinal tract has recently gained prominence (McClements and Xiao, 2012; Bornhorst et al., 2016).

In this direction, in vitro gastrointestinal systems are widely preferred to evaluate the behavior of food components during the digestive process. Clinical studies conducted on humans are often limited in practice due to technical difficulties, high costs, and ethical limitations, and their reproducibility is also low, partly due to inter-individual differences (Li et al., 2020). Similarly, complex physiological processes encountered in digestive system studies conducted on humans and/or animals make it technically difficult to conduct experiments, increase costs, and narrow the field of application due to ethical restrictions (Comak Göçer et al., 2016). For these reasons, researchers are turning to the design and use of reliable in vitro models that can mimic the human gastrointestinal system, considering

parameters such as digestion time and pH and covering both the stomach and intestinal stages, including the action of digestive enzymes. In vitro models offer an effective alternative to human and animal models thanks to their flexibility, accuracy, and high reproducibility (Lucas-González et al., 2018). Depending on the purpose of the research, the in vitro digestion models developed can be configured to include sections for the mouth, stomach, and small intestine. Each stage is performed using synthetic digestive fluids applied at body temperature for specific periods, and the pH of the environment is maintained at a constant level with the addition of appropriate buffer solutions (Minekus et al., 2014). In this way, in vitro gastrointestinal system established in laboratory environments allow both the evaluation of existing products and the testing of the effectiveness and stability of newly developed products.

This study aimed to determine the changes in the physicochemical structure of coconut oil during its passage through the in vitro gastrointestinal system.

MATERIAL AND METHODS

Material

Cold-pressed, unrefined coconut oil was supplied by a commercial company. As it remains solid at room temperature, the oil was gently heated to its melting point (25°C) prior to use in the analyses. Following the incorporation of coconut oil into the semi-dynamic in vitro gastrointestinal system model, samples were collected at 0 and 2 minutes to represent the oral phase, and at 0 and 120 minutes to represent the initial and final stages of the gastric and small intestinal phases, respectively. The collected samples were stored at -20°C until the analyses were performed, which included free fatty acidity, peroxide number, iodine number, fatty acid composition, and sterol composition analyses.

Methods

Establishment of in vitro gastrointestinal model

The semi-dynamic gastrointestinal system employed in this study was developed to overcome key limitations of traditional static in vitro protocols, which apply fixed pH, enzyme concentrations, bile salt levels, and mixing conditions throughout digestion. In contrast to fully static systems that oversimplify gastrointestinal physiology, the semi-dynamic approach used here incorporates phase-specific adjustments in pH, enzyme activity, and bile

salt concentration to better mimic the temporal transitions occurring between the mouth, gastric, and small intestinal compartments (Minekus et al., 2014; Li et al., 2020). Although it does not include the continuous secretion, peristaltic forces, or flow regulation characteristic of advanced multicomponent dynamic models such as TIM systems (Lucas-González et al., 2018; Singh et al., 2022), it offers improved physiological relevance while maintaining the practicality, reproducibility, and lower cost associated with static methods (Minekus et al., 2014; Sensoy, 2021). This intermediate configuration provides a more realistic representation of lipid digestion processes, particularly regarding substrate-enzyme interactions, emulsification behavior, and pH-dependent hydrolysis, and has been widely applied to complement in vivo and microbiota-focused investigations (Habib et al., 2021).

A semi-dynamic in vitro gastrointestinal system was formed from three sections to represent the mouth, stomach, and small intestine regions. The transit time through the in vitro gastrointestinal system was controlled using a double-jacketed reaction vessel, with 2 minutes in the mouth region, 2 hours in the stomach region, and 2 hours in the small intestine region. Peristaltic pumps with adjustable speeds were used to control the flow of simulated digestive system secretions. In addition, to maintain a constant pH in the stomach and small intestine sections, instantaneous pH monitoring was performed, and pH balance was achieved using 1 M sodium hydroxide (NaOH) and 1 M hydrochloric acid (HCl) as needed. Instantaneous temperature and pH monitoring were performed.

Saliva secretion was prepared by adding 2 g/L α -amylase enzyme (EC 3.2.1.1), 1 g/L mucin, 25 mL of 0.3 M CaCl_2 , and 975 mL of water. Simulated salivary secretion was added to the mouth environment at a rate of 5 mL/min, corresponding to 0.05 mL/g sample. The recommended contact time with the enzyme is 2 minutes at 37°C for all reagents. Stomach buffer solution was prepared with 2.2 g/L KCl, 6.2 g/L NaCl, 1.2 g/L NaHCO_3 , 0.22 g/L CaCl_2 . To simulate stomach secretion, 3700 ppm/L porcine pepsin enzyme (EC 3.4.23.1) and 23 g/L mucin were dissolved in a stomach buffer solution. Simulated stomach secretion was added to the reactor, representing the stomach, at a rate of 0.25 mL/min, equivalent to 0.05 mL of stomach secretion per gram of sample. After the sample was separated from the mouth environment and passed into the

reactor representing the stomach environment, 0.2 M HCl was added at a flow rate of 3.5 mL/min until the pH reached 2.5. After the pH was gradually decreased to 2.5 within 1 hour, the flow rate of HCl acid was adjusted to 0.9 mL/min to maintain a constant pH of 2.5 for 1 hour. A small intestine buffer solution was prepared with 0.6 g/L KCl, 5.0 g/L NaCl, and 0.25 g/L CaCl₂. To simulate small intestinal secretions, 1 g/L porcine pancreatin (EC 3.4.21.4), 0.6 g/L porcine lipase (EC 3.1.1.3), and 12 g/L bile salts were added to the intestinal buffer solution; bile salts were subsequently adjusted to a physiologically relevant final concentration of 10 mM in the intestinal phase. Simulated small intestine secretion was added to the reactor, representing the small intestine, at a rate of 0.25 mL/min, resulting in 0.05 mL of small intestine secretion per gram of sample. After the entire sample from the stomach environment was passed into the reactor, representing the small intestine environment, 15-20 minutes. 1 M NaOH was added at a rate of 0.65 mL/min to increase the pH to 6.5 gradually.

The pH was maintained at 6.5 throughout the digestion process. The temperature settings, digestion times, and the composition, concentrations, and flow rates of saliva, gastric, and small intestinal secretions, as well as the buffer solutions used throughout the digestion process, were determined using the *in vitro* gastrointestinal system model protocol developed by Minekus et al. (2014) which provides standardized conditions for simulated digestion (Minekus et al., 2014; Çomak Göçer, 2016).

Alfa-amylase, mucin, bile salt, pancreatin, and pepsin used in the study were obtained from Sigma-Aldrich (USA), CaCl₂, NaCl, NaHCO₃, and NaOH were obtained from AFG Bioscience (USA), KCl was obtained from TEKKİM (Türkiye), and HCl was obtained from Honeywell (Germany).

Free fatty acidity analysis

Free fatty acidity is expressed as the weight in mg of potassium hydroxide (KOH) required for the neutralization of 1 g of oil. According to the AOCS Official Method Ca5a-40, free fatty acidity as a percentage was determined by NaOH titration. Calculations were made according to the NaOH consumption (Karabulut et al., 2005).

Free fatty acids (FFA) %: $V \times N \times M \times 100/m$

V: Amount of NaOH consumed in titration (mL)

N: Normality of NaOH used in titration

M (in oleic acid): 28.2 constant value

m: Weight of weighed sample (g)

Peroxide number analysis

Peroxide number is the measure of the amount of active oxygen in oils and is the amount of peroxide oxygen in 1 kg of oil in milliequivalent grams (EEC, 1991). For peroxide analysis, 10 mL of acetic acid/isooctane (3:2) mixture was added to 0.3 g of oil and mixed in a vortex mixer. Then, 0.5 mL of saturated potassium iodide solution was added to the mixture. After waiting for one minute, 10 mL of pure water was added to the mixture and titrated with 0.002 N sodium thiosulfate until the color became clear. In the titration process, a 0.5% starch solution was utilized as an indicator (Tontul, 2011). The peroxide number was calculated as milliequivalents per kilogram (meq/kg) of oil, according to the formula specified in the method below.

Peroxide Value: $(V1 - V0)/m \times N \times 1000$

V0: Sodium thiosulfate consumption in the blank experiment (mL)

V1: Sodium thiosulfate consumption in the sample experiment (mL)

N: Normality of sodium thiosulfate solution

m: Amount of sample to be tested (g)

Refractive index analysis

The refractive indexes of the samples were determined with an Abbe refractometer. For this purpose, a coconut oil sample was placed on the prism of the refractometer, and a reading was made at 40°C (TSE, 1970).

Iodine number analysis

The iodine number is the expression of the amount of iodine that 100 g of oil can bind in grams. This value provides information about the unsaturated fatty acids in coconut oil. The iodine values of coconut oil and its fractions were determined according to the AOAC-Wijs method (AOAC, 1990).

Total polar matter analysis

A polar matter measuring device (PCE-FOT 10, Istanbul/Türkiye) was used to determine the total polar matter content (Çöl, 2023).

Fatty acid composition analysis

Lipids were extracted using the Folch et al. (1957) method, and the organic phase was evaporated under nitrogen prior to analysis. Coconut oil samples were then esterified according to 12966-2 (ISO 12966-2, 2017) to obtain fatty acid methyl esters (FAMEs). The FAMEs were analyzed by gas chromatography equipped with a flame ionization detector (GC-FID) and a capillary column, and commercial ester mixtures were used to determine retention times (AOAC, 1990).

Sterol composition analysis

First, 2N KOH and 1000 ppm 5- α -cholestane-3- β -ol were prepared as the internal standard for sterol analysis of the samples. Then, 5 mL of KOH and 1 mL of internal standard 5- α -cholestane-3- β -ol were added to the samples. The obtained solution was kept in a water bath at 80°C for 1 hour, with stirring every 15 minutes. Finally, 5 mL of water was added and allowed to cool to room temperature. After the cooling process, 5 mL of hexane was added and mixed. In the resulting phase separation, the upper phase was transferred to a separate container, and nitrogen gas was used to evaporate the hexane. After this process, 5 mL water was added to the other phase and mixed in a vortex mixer. In this process, water remained at the bottom, and the process was repeated three times by adding 5 mL of hexane. Then, the hexane was evaporated until it reached 10 mL, and the prepared sample was transferred into 10 mL volumetric flasks. Finally, 500 μ L of the previously prepared 10 mL volumetric flask was taken, 250 μ L of silylation solution was added, and then 250 μ L of pyridine was added. The resulting mixture was mixed and then kept in a 60°C oven for 15 min before being analyzed by gas chromatography (Lechner et al., 1999).

Statistical analysis

In the study, three replications and two parallel analyses were performed on the samples taken from each stage. Data were first tested for normality using the Shapiro-Wilk test. Statistical

differences between groups were then evaluated using one-way ANOVA, and Tukey's post-hoc test was applied when significant differences were detected. All statistical analyses were performed using IBM SPSS Statistics version 26, with the significance level set at $\alpha=0.05$.

RESULTS AND DISCUSSION

Free fatty acidity

FFA are released as a result of the hydrolysis of triglycerides. These FFA can be oxidized spontaneously or under the catalyzation of oxidative enzymes such as lipoxygenase, depending on the environmental conditions. Oxidation occurs especially in unsaturated fatty acids, such as oleic acid, linoleic acid, and linolenic acid, which are primarily found in oils or fats. In the hydrolysis reaction, fat is broken down into FFA and glycerol (Saikhwan et al., 2016). As shown in Table 1, the free fatty acid results of coconut oil samples taken from the in vitro gastrointestinal system model's mouth, stomach, and small intestine stages were found to be 0.68-27.73%. As a result of the variance analysis, the effect of the digestion time in the in vitro gastrointestinal system stage on the free fatty acidity amounts of samples taken from the mouth (0 min-2 min) and stomach (0 min) stages of the digestive system was not statistically significant.

On the other hand, the digestion time within the in vitro gastrointestinal system had a statistically significant effect ($P<0.05$) on the free fatty acid content of coconut oil samples collected from the gastric (120th min) and small intestinal (120th min) phases. Accordingly, coconut oil samples taken from different digestion stages comply with the specified limit values and meet the good consumption standard. Satheeshan et al. (2019) conducted a study to analyze whether there is a change in the quality parameters of hot-extracted virgin coconut oil (HVCO), cold-extracted virgin coconut oil (CVCO) and coconut oil (CO) among different production methods. The free fatty acidity values obtained for different oil samples ranged between 0.23% and 0.57%. Natalia et al. (2019) found that the free fatty acid content of edible coconut oil changed by 0.21%, while crude coconut oil had a free fatty acid content of 0.17%. Thus, it was evaluated that crude coconut oil was generally of good quality.

Table 1. Results of free fatty acidity, peroxide number, refractive index and iodine number of coconut oil samples taken from the in vitro gastrointestinal system model mouth, stomach and small intestine stages

| GIS | Free Fatty Acidity (%) Mean ± SD | Peroxide Number (meq O ₂ /kg) Mean ± SD | Refractive Index (ND40) Mean ± SD | Iodine Number Mean ± SD |
|--|-------------------------------------|---|--------------------------------------|----------------------------|
| Mouth (0 th min.) | 0.76±0.02 ^d | 0.65±0.04 ^b | 1.4475±0.0002 ^a | 3.84±0.12 ^c |
| Mouth (2 nd min.) | 0.68±0.05 ^d | 0.66±0.03 ^b | 1.4458±0.0003 ^{bc} | 4.77±0.31 ^d |
| Stomach (0 th min.) | 0.70±0.03 ^d | 0.66±0.03 ^b | 1.4461±0.0001 ^b | 5.65±0.45 ^c |
| Stomach (120 th min.) | 1.62±0.02 ^b | 0.78±0.06 ^a | 1.4454±0.0002 ^{bc} | 7.38±0.20 ^b |
| Small Intestine (0 th min.) | 0.86±0.06 ^c | 0.76±0.04 ^{ab} | 1.4454±0.0002 ^c | 4.56±0.28 ^{dc} |
| Small Intestine (120 th min.) | 27.73±1.03 ^a | 0.75±0.04 ^{ab} | 1.3574±0.0005 ^d | 9.45±0.34 ^a |

Different lowercase letters in the superscript on the same line represent statistically significant differences between the mean ± SD of samples at the mouth, stomach and small intestine stages ($P<0.05$)

Xiang et al. (2023) stated in their study on the digestion of various fats that the release of FFA during intestinal digestion was higher than during stomach digestion. Since the increase in FFA is due to the hydrolysis of triacylglycerols by lipase, the increase in FFA indicates the degree of lipid hydrolysis during in vitro gastrointestinal system. In the human body, the small intestine is where lipids are broken down and digested. Therefore, while the increase in FFAs is less during mouth and stomach digestion, lipid digestion accelerates after the structural phospholipid interface in lipids is broken down in bile. This explains the increase in free fatty acidity during the first 30 min of intestinal digestion. The lipase enzyme in the pancreas binds more easily to MUFA than to PUFA. In general, fats rich in saturated fatty acids increase FFA at a faster rate than fats rich in unsaturated fatty acids (Wang et al., 2022). When the free fatty acid values were compared with the literature, it was found that they were in accordance.

The free fatty acid value we found at the end of the 120th minute in the intestinal phase reached the highest value. In this context, it was observed that the free fatty acid content of coconut oil increased during digestion, with a particularly notable rise in the small intestine between 0 and 120 minutes. It is thought that the increase in free fatty acid levels occurs due to the activation of the pancreatic lipase enzyme, which becomes active after the coconut oil reaches the small intestine.

Peroxide number

The peroxide value is one of the most critical indicators used to assess the extent of oxidative deterioration in oils. Unsaturated fatty acids can bind oxygen to their double bonds, forming peroxides. Oils containing unsaturated fatty acids can be oxidized by oxygen, resulting in the formation of a peroxide compound. A high peroxide value indicates that the oil has undergone significant oxidative damage and is likely to become rancid in a short period of time. High peroxide numbers may be due to oxidation in some fatty acids, mainly unsaturated fatty acids. A low peroxide value indicates good oil quality, as an increase in peroxide content accelerates oxidative rancidity (Parkinson and Ciccarelli, 2016; Naseri et al., 2018). Hydroperoxides are formed as primary products during oxidation under the influence of various factors, including heat, light, moisture, and metals.

In contrast, hydroperoxides are transformed into secondary oxidation products as oxidation progresses. Therefore, although the peroxide number is a general indicator of lipid oxidation, its use is limited to the early stages of oxidation (Crapiste et al., 1999). The peroxide value has become an indicator for measuring the early stages of oxidation in oils. One of the first products formed by the oxidation of oil is hydroperoxide (Satheeshan et al., 2019). As presented in Table 1, the peroxide values of cold-pressed, unrefined coconut oil

samples collected from the mouth, stomach, and small intestine stages of the in vitro gastrointestinal model ranged between 0.65 and 0.78 meq O₂/kg. Among the cold-pressed coconut oil samples taken from different stages, it was determined that the highest peroxide value was reached in the stomach phase at 120 minutes. As a result of the variance analysis, the effect of the digestion time in the in vitro gastrointestinal system stage from the mouth (0. min-2. min) and stomach (0. min) stages of the digestive system was not significant at the level of $P<0.05$. On the other hand, the effect of the in vitro gastrointestinal system time on the peroxide amounts of coconut oils taken from the stomach (120. min) and small intestine (120. min) stages of the digestive system was significant at the level of $P<0.05$. Although peroxide values differed significantly across digestion phases, the absolute changes (0.65-0.78 meq O₂/kg) were minimal and within the expected analytical variability of the titrimetric method. Thus, the effect size is small, and these differences should be interpreted cautiously, as they may reflect analytical uncertainty rather than meaningful oxidative change. Natalia et al. (2019) stated in their study, which examined the quality of CCO and VCO, that the peroxide numbers of VCO and CCO were 0.54 and 0.68, respectively, indicating good quality. Both had acceptable peroxide numbers for consumption. Satheeshan et al. (2019), in their study to analyze whether there is a change in the quality parameters of the oil between different production methods [hot extracted virgin coconut oil (HVCO), cold extracted CVCO, and CO], found that CVCO (0.40) and HVCO (0.86) had the lowest peroxide values. This result showed that VCO samples did not undergo significant oxidation during processing. Peroxide values were found below the limits in the circular at every stage of the in vitro gastrointestinal system sections. Kayahan and Tekin (2006) stated that the oils smell after the peroxide number is 5 meq O₂/kg, and the oil becomes unusable after 10 meq O₂/kg. Accordingly, the low peroxide values of the oil samples at all stages during digestion indicate that these samples are relatively stable against oxidative deterioration. The increase in the peroxide value during the stomach phase can be attributed to the low pH at this stage, the presence of digestive enzymes, and an increase in oxidative stress. The decrease in the effectiveness of natural antioxidant components during the digestive process, combined with the fact that medium-chain fatty acids, which are abundant in coconut oil, are more susceptible to oxidation, may also have contributed to this increase.

Determination of refractive index

The refractive index is a parameter that indicates a substance's ability to refract light. The refractive index of oils is a characteristic feature of each oil, and it has been observed that each oil has its unique value. The refractive index is high in unsaturated fatty acids and their esters. As the fatty acid chain length increases, the refractive index decreases. The refractive index of fatty acid esters containing conjugated double bonds is also high. Therefore, this value was high in drying oils. The refractive index has been preferred for the identification of oils or the determination of their purity. It can also be used to monitor hydrogenation (Awuchi and Gonzaga, 2018).

The refractive indices of the coconut oil samples were measured at 40°C. Among the cold-pressed, unrefined coconut oil samples collected from different stages of digestion, the lowest refractive index was observed in the small intestinal phase at 120 minutes. It was found that the refractive index values of the cold-pressed, unrefined coconut oil samples taken from the in vitro gastrointestinal system model mouth, stomach, and small intestine stages varied between 1.3574 (small intestine 120th min) and 1.4475 (mouth 0th min). The effect of the digestion time in the in vitro gastrointestinal system stage on the refractive index values of coconut oils taken from different stages of the digestive system was found to be significant at the level of $P<0.05$. In other words, the refractive index values of coconut oils statistically varied according to digestion times, depending on the digestion stages. The decrease in refractive index observed at 120 min in the small intestinal phase may be attributed to pancreatic lipase-mediated hydrolysis into free fatty acids, monoacylglycerols and glycerol, as well as their incorporation into mixed micelles with bile salts in an aqueous environment. These structural and compositional changes in the oil-digestive fluid mixture can alter the optical density of the system and may result in lower measured refractive index values compared with pure coconut oil.

In a study conducted by Satheeshan et al. (2019) to analyze whether there is a change in the quality parameters of oil, including HVCO, CVCO, and CO, among different production methods, it was found that the refractive index of coconut oil was 1.4488. In the case of HVCO and CVCO, the values were 1.4487 and 1.4483, respectively. Similar results (1.4480) were reported in a study conducted by Srivastava et al., (2016) on the quantitative and qualitative analysis of bioactive components

present in crude coconut oil. In the study investigating the processability of cold-pressed virgin coconut oil obtained from full-fat coconut pieces as a functional oil, the refractive index was found to be 1.44 ± 0.05 (Aramugam et al., 2014). To extract, characterize, and evaluate the storage stability of pure VCO produced using cold-press and hot-press processes, Ajogun et al. (2020) found that the refractive index values for virgin coconut oil obtained by cold-pressing and virgin coconut oil obtained by hot pressing were both 1.4475. This value corresponds to the Codex standard range of 1.448 - 1.450 (CODEX, 2009), the Indian Standard of 1.448-1.449 (Gopala et al., 2010), and the Asia Pacific Coconut Community standard of 1.448-1.449 (APCC, 2009). The refractive index results we obtained from different digestion stages agree with the literature.

Iodine number

Iodine number is an accepted parameter to express the degree of unsaturation, which is generally the number of carbon-carbon double bonds in oils or fats. In addition, it has been reported that the higher the amount of unsaturation, the more iodine is absorbed; therefore, the higher the iodine value, the greater the degree of unsaturation. Iodine values above 100 are classified as drying, while those below are classified as non-drying (Ikya et al., 2012). The iodine value is a measure of the degree of unsaturation in the oil (Lechner et al., 1999).

As shown in Table 1, the iodine values of cold-pressed, unrefined coconut oil samples obtained from the mouth, stomach, and small intestine stages of the *in vitro* gastrointestinal system ranged from 3.84 to 9.45. The effect of the digestion time in the *in vitro* gastrointestinal system stage on the iodine amounts of coconut oils taken from different stages of the digestive system was found to be significant at the $P < 0.05$ level. Statistically, the iodine content of coconut oil varied with digestion time across different stages of the gastrointestinal model.

Aramugam et al. (2014) found the iodine value to be 7.6 ± 0.03 in their study on the processability of cold-pressed virgin coconut oil obtained from full-fat coconut pieces, which was used as a functional oil. Satheeshan et al. (2019) conducted a study to analyze whether different production methods affected the quality parameters of HVCO, CVCO, and coconut oil CO. The iodine values were found to be 8.02 for HVCO, 8.77 for CVCO, and 6.75 for CO, respectively. Although the oils were classified as non-drying, cold-processed oil had

higher iodine values compared to HVCO and CO oil. It was thought that the increase in iodine value was due to slightly more unsaturated fatty acids. In a study examining the thermal stability of virgin coconut oil heated at 190°C during 40 days of storage by comparing it with extra virgin olive oil, the initial iodine values of extra virgin olive oil and virgin coconut oil were found to be 81.87 ± 0.81 and 11.09 ± 0.69 , respectively. The iodine values of extra virgin olive oil and virgin coconut oil decreased significantly ($P < 0.05$) to 72.13 ± 0.77 and 3.26 ± 0.11 , respectively, after 40 days of storage (Lu and Tan, 2009). This decrease in iodine values indicated a reduction in the number of double bonds in the oils. The maximum decline in iodine values of both oils was achieved with heat treatment, as deep frying accelerates oxidation in the oils (Naz et al., 2005).

Fatty acid composition

The identity of an oil is primarily determined by its fatty acid composition. The measurement of fatty acids in edible oils is crucial for nutritional purposes (Wang et al., 2022). Table 2 and Figure 1 show that coconut oil samples obtained from different stages of digestion were particularly rich in saturated fatty acids, with lauric acid being the most abundant, ranging between 44.30% and 57.65%. Overall, lauric, myristic, caprylic, capric, and palmitic acids were identified as the predominant fatty acids in the coconut oil samples. Variance analysis indicated that digestion time within the *in vitro* gastrointestinal system had a statistically significant effect ($P < 0.05$) on the levels of all fatty acids-except arachidonic acid-in coconut oil samples across different digestive stages. These findings suggest that the fatty acid composition of the samples varied significantly depending on both the duration and phase of digestion.

As shown in Table 2, the fatty acid composition of coconut oil includes 44.30-57.65% lauric acid, 12.54-18.64% myristic acid, 7.91-13.15% caprylic acid, 6.12-8.33% capric acid, 4.88-10.06% palmitic acid, 3.14-6.31% oleic acid, 1.63-5.66% stearic acid, and 0.63-2.95% linoleic acid. According to the circular, the fatty acid composition is stated as 45.10-53.20% lauric acid, 16.80-21.00% myristic acid, 7.50-10.20% palmitic acid, 5.00-10.00% oleic acid, 5.00-8.00% capric acid, 4.60-10.00% caprylic acid, 2.00-4.00% stearic acid, 1.00-2.50% linoleic acid. When the results are evaluated in light of the communiqué, it is observed that all fatty acids vary depending on the sampled stage of the *in vitro* gastrointestinal system and generally fall within the acceptable ranges specified in the regulation (Figure 2).

Table 2. Fatty acid composition results (%) of coconut oil samples taken from the in vitro gastrointestinal system model mouth, stomach and small intestine stages

| Coconut Oil | In Vitro Gastrointestinal System Model | | | | | |
|----------------------|--|------------------------------|--------------------------------|----------------------------------|--|--|
| Fatty Acids (%) | Mouth (0 th min.) | Mouth (2 nd min.) | Stomach (0 th min.) | Stomach (120 th min.) | Small Intestine (0 th min.) | Small Intestine (120 th min.) |
| Caproic acid C6:0 | 1.29±0.03 ^a | 0.76±0.06 ^{bc} | 0.65±0.09 ^c | 0.79±0.09 ^{bc} | 0.93±0.13 ^b | 0.93±0.09 ^b |
| Caprylic acid C8:0 | 13.15±0.22 ^a | 9.30±0.27 ^{bc} | 7.91±0.47 ^d | 8.56±0.50 ^{cd} | 10.35±0.50 ^b | 8.91 ± 0.51 ^{cd} |
| Capric acid C10:0 | 8.33±0.07 ^a | 6.82±0.32 ^{bc} | 6.12±0.26 ^c | 6.29±0.19 ^c | 7.34±0.34 ^b | 6.57±0.65 ^{bc} |
| Lauric acid C12:0 | 50.23±1.12 ^b | 50.53±1.25 ^b | 49.13±0.87 ^b | 44.30±0.97 ^c | 50.82±1.05 ^b | 57.65±1.48 ^a |
| Myristic acid C14:0 | 15.06±1.04 ^b | 17.49±0.51 ^a | 18.64±1.14 ^a | 16.80±0.59 ^{ab} | 16.85±0.54 ^{ab} | 12.54±0.66 ^c |
| Palmitic acid C16:0 | 5.96±0.50 ^{cd} | 7.47±0.41 ^{bc} | 8.54±0.91 ^{ab} | 10.06±1.03 ^a | 6.94±0.30 ^{bc} | 4.88±0.26 ^d |
| Stearic acid C18:0 | 2.16±0.08 ^d | 2.79±0.17 ^c | 3.37±0.24 ^b | 5.66±0.28 ^a | 2.44±0.15 ^{cd} | 1.63±0.05 ^e |
| Oleic acid C18:1 | 3.14±0.55 ^b | 3.98±0.86 ^b | 4.58±0.64 ^b | 6.31±0.81 ^a | 3.35±0.19 ^b | 3.25±0.25 ^b |
| Linoleic acid C18:2 | 0.63±0.07 ^c | 0.75±0.18 ^{bc} | 0.92±0.08 ^{bc} | 1.02±0.09 ^b | 0.84±0.06 ^{bc} | 2.95±0.12 ^a |
| Arachidic acid C20:0 | 0.06±0.07 ^a | 0.07±0.07 ^a | 0.09±0.05 ^a | 0.12±0.10 ^a | 0.06±0.01 ^a | 0.10±0.05 ^a |
| Σ Saturated F.A. | 96.21±2.84 ^a | 95.25±1.92 ^a | 94.45± 1.50 ^a | 92.60± 2.00 ^a | 95.72±0.39 ^a | 93.21±1.90 ^a |
| Σ Unsaturated F.A. | 3.79±0.51 ^c | 4.75±1.00 ^c | 5.55± 0.72 ^{bc} | 7.40± 0.81 ^a | 4.28±0.17 ^c | 6.79±0.26 ^{ab} |

Different lowercase letters in the superscript on the same line represent statistically significant differences between the mean ± SD of samples at the mouth, stomach and small intestine stages ($P<0.05$)

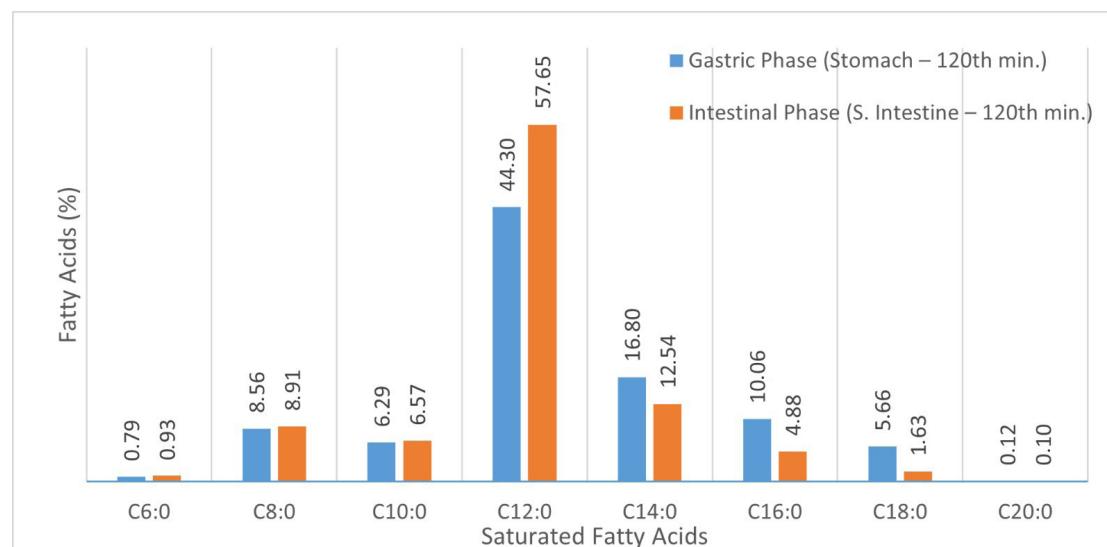


Figure 1. Changes in saturated fatty acid composition during in vitro gastrointestinal system of cold-pressed virgin coconut oil samples

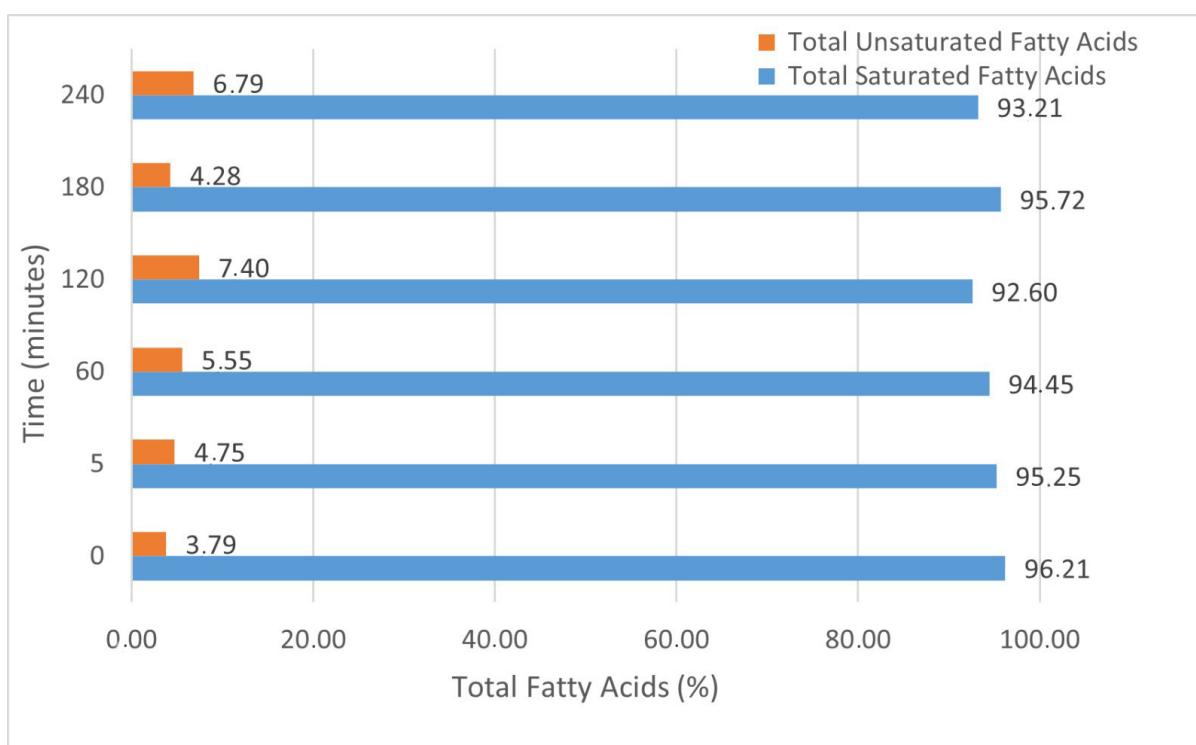


Figure 2. Changes in total saturated and total unsaturated fatty acid composition during in vitro gastrointestinal system of coconut oil samples

Yee (2011) obtained virgin coconut oil using the cold pressing method and examined its physicochemical properties. The yield of the obtained oil was determined to be 21%, and the fatty acid composition was as follows: 33.75% lauric acid, 23.19% myristic acid, 20.83% caprylic acid, 18.32% capric acid, and 3.75% caproic acid. According to the results of this study, when the values of fatty acid compositions were examined during the digestion process, it was determined that lauric acid remained high while myristic, capric, and caprylic acid were low. Arlee et al. (2013) investigated the chemical components and antioxidant substances in coconut oils obtained by fermentation and cold pressing methods from three coconut hybrid varieties (WAT: West African Tall, MYD: Malayan Yellow Dwarf, and THT: Thai Tall) Sawi Hybrid No. 1 (Malayan Yellow Dwarf x West African Tall: MYD x WAT), Chumphon Hybrid No. 60 (Thai Tall: THT x WAT), and Chumphon Hybrid No. 2 (MYD x THT). In the study, the fatty acids of the cold-pressed coconut oil of the WAT variety were 0.37% caproic acid, 6.67% caprylic acid, 5.58% capric acid, 48.18% lauric acid, 20.40% myristic acid, 8.78% palmitic acid, 3.71% stearic acid,

5.75% oleic acid, 1.10% linoleic acid, respectively. They found the fatty acid composition of MYD type cold pressed coconut oil as 0.40% caproic acid, 5.70% caprylic acid, 4.91% capric acid, 45.26% lauric acid, 20.86% myristic acid, 10.77% palmitic acid, 3.84% stearic acid, 6.80% oleic acid, 1.33% linoleic acid; the fatty acid composition of THT type cold pressed coconut oil as 0.40% caproic acid, 5.70% caprylic acid, 4.91% capric acid, 45.26% lauric acid, 20.86% myristic acid, 10.77% palmitic acid, 3.84% stearic acid, 6.80% oleic acid, 1.33% linoleic acid. When compared with this study, it was observed that the fatty acid compositions differed according to the types of coconut and the oil obtained during the digestion process in our study was 0.40% caproic acid, 5.70% caprylic acid, 4.91% capric acid, 45.26% lauric acid, 20.86% myristic acid, 10.77% palmitic acid, 3.84% stearic acid, 6.80% oleic acid, 1.33% linoleic acid. When we compared the values of the acids, it was observed that the values we found were partially higher. Idu et al. (2018) investigated the physical and chemical properties, antioxidant activity, and phytonutrient composition of cold and hot-pressed coconut oils. When they looked at the fatty acid composition,

they found lauric acid (49.29% and 47.83%), myristic acid (17.01% and 16.33%), palmitic acid (9.53% and 9.94%), capric acid (6.39% and 7.72%) and caprylic acid (5.45% and 5.77%). According to these results, it was determined that the different pressing processes caused changes in fatty acids, and the caprylic acid value of coconut oil obtained from all digestion stages was higher in our study compared to the values reported in previous studies (Ananth et al., 2019). Investigated the antioxidant capacity, tocopherol, and fatty acid profiles of six different traditional cold-pressed seed oils in a study conducted in South India. In the study, when fatty acid components were examined, it was found that cold-pressed coconut oils contained the highest amount of saturated fatty acids compared to other oil samples, including lauric acid (29.57%) and myristic acid (23.89%). On the other hand, caprylic acid, nonanoic acid, capric acid, undecanoic acid, and tridecanoic acids were found only in coconut oil. According to this study, the myristic acid value was found to be higher than that of coconut oils obtained from different digestion stages in our study. In contrast, the lauric acid value was found to be quite low. In general, the fatty acid compositions of different seed oils obtained from cold press are quite different. Pereira et al. (2023) evaluated the in vitro gastrointestinal system of lipids rich in medium-chain fatty acids (MCFAs) using dynamic and static protocols in their study, and caprylic and capric acids released during stomach digestion of MCT accounted for 61-63% and 63% of the total esterified fatty acid, respectively. They found that it varied between 36% and 38%. Lauric acid was the most representative

free fatty acid released during stomach digestion of coconut oil samples (31-54%). It was also observed in the study that the stomach digestion stage played an important role in the lipolysis of medium-chain fatty acids and that lipase activity limited the amount of MCFA released during gastrointestinal digestion, causing incomplete lipid hydrolysis (Pereira et al., 2023). Overall, the fatty acid composition results obtained in this study were found to be in close agreement with the values reported in the literature. Therefore, it is evident that coconut oil has a balanced composition in terms of fatty acid content, rich in lauric acid, as well as myristic, caprylic, capric, and palmitic acids, which are predominantly saturated fatty acids during the digestion process. Additionally, it is nutritionally important due to its balanced fatty acid composition.

Sterol composition

As seen in Table 3, when the sterol composition of coconut oil samples taken from the in vitro gastrointestinal system model mouth, stomach, and small intestine stages was examined, 50.61-52.16% β -sitosterol, 16.57-18.40% Δ -5-avenasterol, 15.26-16.91% Stigmasterol, 8.40-8.96% Campesterol, 1.05-3.77% Cholesterol, 0.56-0.67% Δ -7-stigmastenol, 0.69-0.85%, Δ -7-avenasterol was detected. In contrast, Brassicasterol Erythrodiol and Uvaol (among total sterols) could not be detected. According to the results, the sterol profile of the coconut oil samples showed partial conformity with the limits outlined in the Turkish Food Codex Communiqué.

Table 3. Sterol composition results of coconut oil samples taken from the in vitro gastrointestinal system model mouth, stomach, and small intestine stages (%)

| Coconut Oil Sterol Composition (%) | In Vitro Gastrointestinal System Model | | | | | |
|------------------------------------|--|------------------------------|--------------------------------|----------------------------------|--|--|
| | Mouth (0 th min.) | Mouth (2 nd min.) | Stomach (0 th min.) | Stomach (120 th min.) | Small Intestine (0 th min.) | Small Intestine (120 th min.) |
| β -sitosterol | 50.61 | 50.73 | 52.16 | 51.20 | 52.02 | 51.34 |
| Campesterol | 8.40 | 8.96 | 8.86 | 8.78 | 8.67 | 8.53 |
| Δ -5-avenasterol | 17.40 | 18.40 | 18.22 | 17.56 | 16.84 | 16.57 |
| Δ -7-stigmastenol | 0.56 | 0.64 | 0.61 | 0.59 | 0.67 | 0.60 |
| Cholesterol | 3.58 | 1.05 | 1.65 | 2.11 | 2.05 | 3.77 |
| Stigmasterol | 16.30 | 16.91 | 15.26 | 16.85 | 16.86 | 16.37 |
| Δ -7-avenasterol | 0.72 | 0.84 | 0.84 | 0.71 | 0.85 | 0.69 |

Idu et al. (2018) investigated the physicochemical properties, antioxidant activity, and phytonutrient composition of cold- and hot-pressed coconut oils. Both oils were dissolved in a non-polar solvent at room temperature. The phytosterol content of hot-pressed oil was found to be higher (102.79 mg/100 g), but the main phytosterols in both oils were observed to be β -sitosterol, Stigmasterol, and Δ -5-avenasterol. While hot-pressed oil had slightly better physicochemical properties and phytonutrient composition, cold-pressed oil was found to have better antioxidant properties. In a study, the sterol composition of cold-pressed coconut oil (as a percentage of total sterols) was found to be 45.23% β -sitosterol, 25.64% Δ -5-avenasterol, 15.33% Stigmasterol, 9.87% Campesterol, 1.24% Δ -7 Stigmasterol, 0.95% Δ -7-avenasterol, 1.74% other sterols, and the total sterol amount was found to be 1152 mg/kg (Çelebi, 2023).

CONCLUSION

This study investigated the behavior of coconut oil during digestion by evaluating changes in its physicochemical properties and bioactive components (such as fatty acid and sterol composition) throughout the *in vitro* gastrointestinal system. The findings revealed that parameters including free fatty acidity, peroxide value, refractive index, iodine value, fatty acid profile, and sterol composition underwent alterations during digestion. Notably, a significant increase in free fatty acidity was observed in the small intestine, primarily due to the action of lipase. These results suggest that coconut oil undergoes notable physicochemical transformations during digestion, particularly in the small intestine where enzymatic activity is most pronounced. The increase in free fatty acidity and the variability in sterol and fatty acid profiles highlight the dynamic nature of lipid digestion and the importance of simulating gastrointestinal conditions when evaluating the nutritional and functional behavior of dietary fats. Future studies involving peptide identification, enzymatic hydrolysis kinetics, and bioaccessibility assessments could further enhance our understanding of coconut oil's behavior and potential health effects during digestion.

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

AUTHOR CONTRIBUTIONS

Merve İnce-Palamutoğlu: investigation, methodology, conceptualization, data curation, funding acquisition, writing – original draft; Sabire Duman: methodology, conceptualization, data curation, investigation, writing – original draft; Recep Palamutoğlu: investigation, methodology, data curation, writing – review & editing; Erman Duman: investigation, methodology, data curation, writing – review & editing; All authors read and approved the final manuscript.

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