Optimization of Gotu Kola Frying Temperature and Time based Secondary Metabolites and Antioxidant Activity using TOPSIS Analysis

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Abstract. Fried vegetables are appreciated for their savory flavor and crunchy texture. Gotu kola can also be consumed fried, but frying temperature and time impact its nutrients and antioxidant properties. This study aims to identify the optimal frying method to preserve the beneficial qualities of gotu kola. In this study, gotu kola frying process was conducted with three temperature variations (130°C, 145°C, 160°C) and six frying durations, namely (1, 3, 5, 7, 9 and 11 minutes). The samples were then tested for secondary metabolite content including total flavonoid content (TFC), total phenol content (TPC), and asiaticoside content (AC). The data were analyzed using DMRT to assess the impact of temperature and boiling duration on secondary metabolite content. Based on asiaticoside content, the three best methods were selected as alternatives for further study. These methods were then evaluated for antioxidant activity using DPPH and Nitric Oxide scavenging assays, followed by analysis with the TOPSIS method. The study found that frying gotu kola at 160 ºC for 1 minute preserves its secondary metabolites and antioxidant activity, suggesting that medium-temperature, short-duration frying helps retain its health benefits.

1 Introduction

Gotu kola (*Centella asiatica* (L.) Urban) is a leafy vegetable and medicinal herb known for its various health benefits. Gotu kola is rich in triterpenoids, flavonoids, alkaloids, saponins, steroids, tannins, and one of its bioactive components, asiaticoside [1]. The health benefits of gotu kola include antioxidants, anti-inflammatory, wound healing, antimicrobial memory enhancing, neuroprotective, antidiabetic effects, and cognitive function improvement as well as anxiety reduction [2].

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One of the common ways to consume gotu kola is by cooking it, including frying. Frying gotu kola aims to enchance the flavor in terms of both color and texture [3]. However, the temperature and duration of frying significantly affect the chemical content of gotu kola, affecting its health benefits. The cooking method by frying can affect the secondary metabolite compounds of flavonoids and total phenols. Studies have shown that frying vegetables can reduce flavonoid content, which suggests that cooking methods need to be optimized to maintain maximum nutritional value [4]. In addition, heat can cause partial destruction of heat sensitive nutrients. Some studies suggest that the antioxidant activity in cooking oil decreases with the length of frying time. The longer the oil is heated, the more antioxidant activity decreases [5].

The frying process of gotu kola has the potential to reduce both the levels of secondary metabolites and its antioxidant activity. Therefore, optimizing the frying method is essential to achieve the best possible results. This study aims to identify the optimal balance between frying temperature and duration that preserves beneficial compounds and antioxidant properties. Such optimization will help maximize the health benefits of gotu kola when consumed as a fried vegetable. To determine the most effective frying conditions, the Technique for Order of Preference by Similarity to Ideal Solution (TOPSIS) analysis is employed. TOPSIS is a multi-criteria decision-making method that ranks and selects alternatives based on their proximity to an ideal solution [6].

2 Materials and methods

2.1 Materials

In this study, the sample used was the herb part of gotu kola (*Centella asiatica* (L.) Urb.) from UPF Pelayanan Kesehatan Tradisional Dr. Sardjito in Tawangmangu. Gotu kola plants are cultivated in the Kebun Tanaman Obat (KTO) Toh Kuning located in Karangpandan District, Karangpandan Regency, Central Java Province.

The tools used include analytical balance (AND GF Series), hot plate (FAITHFUL SH-2 Magnetic Stirrer), spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA.), digital thermometer, and digital timer.

2.2 Sample Preparation

A fresh gotu kola sample weighing 100 grams was stirred in 1000 mL of oil for the duration and temperature before being drained (Table 1). The optimization design of the gotu kola frying method is a combination of three temperatures (T) and six frying durations (W).

Table 1. Optimization design of fresh gotu kola frying.

Temperature (ºC)	**Duration (minutes)**					
	W1 (1)	**W1 (1)**	**W1 (1)**	**W1 (1)**	**W1 (1)**	**W1 (1)**
T1 (130)	T1W1	T1W2	T1W3	T1W4	T1W5	T1W6
T2 (145)	T2W1	T2W2	T2W3	T2W4	T2W5	T2W6
T3 (160)	T3W1	T3W2	T3W3	T3W4	T3W5	T3W6

2.3 Secondary Metabolite Determination

Secondary metabolites tested in this research include total flavonoid content (TFC), total phenol content (TPC) and asiaticoside content (AC) using the spectrophotometer method.

2.3.1 Asiaticoside Content (AC)

The standard curve of asiaticoside was made in the concentration series from 10-100µg/mL. The linear regression equation is y = 0.0072 x - 0.045 with slope (a) is 0.0072, intercept (b) is -0.045 and coefficient of determination (r2) is 0.9992. The methanol extract solution of gotu kola was prepared in methanol with a concentration of 4 mg/mL. A total of 1 mL of each sample was mixed, and methanol was aliquoted from this mixture to a concentration of 4 mg/mL. The samples were then read for absorbance with a spectrophotometer at a wavelength of 278 nm [7].

2.3.2 Total Flavonoid Content (TFC)

The concentration of the extract in the methanol solution was 1,250 µg/mL. In a 1250 µL container, the reaction mixture included 100 µL of extract, 150 µL of 0.1 M AlCl3 solution (in a separate volume without AlCl3 and 150 µL of methanol), 350 µL of carbon-free distilled water, 250 µL of acetate buffer (pH 3.8), and enough methanol to fill the remaining space. Thirty minutes were spent incubating the reaction mixture at 37 degrees Celsius. The microplate was filled with the 150 µL test solution, and the absorbance was recorded at 398 nm using a spectrophotometer. Rutin serves as a benchmark through the creation of a standard curve. Various concentrations of methanol were used to dissolve quercetin, with concentrations ranging from 0 to 500 µg/mL. The quantity of flavonoids is expressed as mg of Quercetin Equivalents (mg QE)/gram of fresh sample [8].

2.3.3 Total Phenol Content (TPC)

The ultimate composition of the reaction mixture comprises 40 mg of extract, 4% methanol, 10% FCR, and 5% NaCO3. 40 µL of extract (1 mg/mL: 1 mg diluted in 1 mL of methanol using a sonicator for 15 minutes) was combined with 360 µL of dH2O and 100 mL of FCR. The solution was homogenised and permitted to rest for 2 minutes. The reaction was neutralised with 500 mL of a 10% w/v sodium carbonate solution, stirred until homogenous, and incubated for 35 minutes at 37 °C. 150 µL of the test solution was dispensed into the microplate. The absorbance was quantified using a spectrophotometer at λ 732 nm, indicating the peak absorbance of gallic acid. Gallic acid was standardised using a concentration series of 0, 2, 4, 8, 16, and 32 µg/mL. Total phenol concentration is quantified as mg of Gallic Acid Equivalent (GAE)/gram of fresh sample [8].

2.4 Optimization of gotu kola frying method

2.4.1 Data Analysis

Initial testing was carried out with Duncan's Multiple Range Test (DMRT) with a 95% confidence level using R software to determine the means of treatments that were significantly different in the optimum condition of asiaticoside content by selecting 3 candidates of gotu kola frying method. The three selected candidate methods were then

tested for antioxidant activity including DPPH scavenging activity and nitric oxide scavenging activity.

Further analysis using the TOPSIS method to determine the temperature and time in producing the optimal levels of secondary metabolites and antioxidant activity in the gotu kola frying process. The criteria used as an assessment of the quality of gotu kola, namely asiaticoside with criteria code c1; total flavonoids with criteria code c2; total phenols with criteria code c3; dpph with criteria code c4 and nitric oxide with criteria code c5. Each of the criteria will be given a weight, which is determined by the researcher based on the parameters of secondary metabolites and antioxidant activity (Table 2). TOPSIS method is a method that works by selecting the best alternative that shows maximum closeness to the positive ideal solution and maximum distance from the negative ideal solution [6].

Table 2. Criteria and Weights

Criteria	Parameters	Weight	Cost/Benefit
C1	Asiaticoside Content (AC)	5	Benefit
C2	Total Flavonoid Content (TFC)	4	Benefit
C3	Total Phenol Content (TPC)	4	Benefit
C4	DPPH IC50	3	Cost
C5	NO IC50	3	Cost

TOPSIS requires a performance rating of each Alternative on each criteria *rij* which is normalized using the formula:

$$r_{ij} = \frac{x_{ij}}{\sqrt{\sum_{i=1}^{m} x_{ij}^2}} \quad (1)$$

The positive ideal solution A+ and negative ideal solution A- can be determined based on the normalized weight rating (*yij*) as follows:

$$y_{ij} = w_j \times r_{ij} \quad (2)$$

The distance between Alternative (Ai) and the positive ideal solution (D+) is formulated as:

$$D_i^+ = \sqrt{\sum_{j=1}^{n} (v_{ij} - v_j^+)^2} \quad (3)$$

The distance between the Alternative (Ai) and the negative ideal solution (D-) is formulated as:

$$D_i^- = \sqrt{\sum_{j=1}^{n} (v_{ij} - v_j^-)^2} \quad (4)$$

The preference value for each Alternative (Vi) is given as:

$$V_i = \frac{D_i^-}{D_i^+ + D_i^-} \quad (5)$$

The larger value of Vi indicates that the Alternative is preferred.

2.4.2 Antioxidant Activity

Antioxidant activity tested in this research include DPPH scavenging activity (DPPH IC50) and nitric oxide scavenging activity (NO IC50) using the spectrophotometer method.

2.4.2.1. DPPH scavenging activity (DPPH IC50)

This test uses the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method [8]. The extract was dissolved using distilled water with five different concentrations. DPPH was dissolved in methanol p.a. with a DPPH concentration of 350 µM. Samples were prepared in 1500 µL microtubes by mixing a maximum of 200 µL sample solution, 600 µL methanol, and 200 µL DPPH, while the control consisted of 200 µL sample and 800 µL methanol. The tubes were vortexed for 10 seconds and incubated at 37°C for 35 minutes in an incubator every 10 minutes, and the tubes were inverted. The absorbance was measured with a spectrophotometer at λ 515 nm. DPPH radical capture assay was performed three times. Radical inhibition activity was calculated using the formula below:

$$DPPH\ Inhibition = \frac{(Ac - Ab) - (As - Asc)}{Ac - Ab} x100\% \quad (6)$$

where *DPPH Inhibition* is DPPH scavenging activity (%), A_b is absorbance of the blank solution, A_c is absorbance of the control solution, A_s is absorbance of the sample solution, and A_{sc} is absorbance of the control sample solution. After obtaining the % inhibition activity, the IC50 value is then sought.

2.4.2.2. Nitric oxide scavenging activity (NO IC50)

Nitric oxide radical scavenging activity testing using the Banerjee method with several modifications [9]. Sample stock solutions were made at five different concentrations. Phosphate buffer PH 7.4 was prepared by mixing NaOH and KH2PO4 solutions. NAOH was carefully weighed at 0.8 grams, distilled water was added up to 100mL, and KH2PO4 was added as much as 2.72 grams. Distilled water was then added up to 100mL. 39.1 mL of NaOH solution was added with 50 mL of KH2PO4 solution. The solution was measured for pH using a pH meter. NaOH solution can be added if the pH is too acidic until the pH reaches 7.4.

Griess reagent was made separately between sulfanilamide solution and N-Naphthylethylenediamine solution. A sulfanilamide solution is made by mixing 1 gram of sulfanilamide and 2 grams of phosphoric acid and then adding distilled water until the volume reaches 50 mL. Meanwhile, the N-Naphthylethylenediamine solution dissolves 0.1 grams of distilled water with 50 ML. A sodium nitroprusside five mM solution was created by adding Na-nitroprusside 150 mg with phosphate buffer solution 7.4, which is sufficient for a volume of 100 mL.

Activity testing was done by adding 500 mL of the test sample with 250 µL of Na-nitroprusside solution left for 30 minutes at room temperature. Next, 125 mL of Sulfanilamide solution was added and incubated at 37 ºC for 1 hour. After incubation, the solution was added 125 µL N-Naphthylethylenediamine solution and then allowed to stand for 30 minutes until the pink color appeared. The sample was then read at 546 nm wavelength.

$$NO\ Inhibition = \frac{(Ab - As)}{Ab} x100\% \quad (7)$$

where *NO Inhibition* is Nitric oxide scavenging activity (%), A_b is absorbance of the blank solution, and A_s is absorbance of the sample solution. Following the acquisition of the percentage inhibition activity, the IC50 value is ascertained.

3 Results and discussion

3.1 Secondary Metabolite

Secondary metabolites are important parameters because they are compounds that provide defense and adaptation functions in plants while acting as the main source of bioactive compounds used in traditional and modern medicine.

3.1.1 Asiaticoside Content (AC)

Based on the DMRT analysis test on the frying process, it was found that the temperature and duration of frying gotagan significantly affected the asiaticoside content (Fig. 1). Based on the analysis, the best frying method of Gotu Kola, namely T3D1, T2D1 and T1D6 proved that the smaller the frying duration, the higher the asiaticoside content. Frying duration influences the chemical composition and marker level of plants, often causing degradation of some beneficial compounds with prolonged frying, while shorter frying times may enhance of release or availability of certain bioactive marker [5]. High oil temperatures during frying can generally reduce the levels of some secondary metabolites due to thermal degradation and oxidation processes. However, some studies have reported an increase in certain secondary metabolites at high frying temperatures. This is due to the short duration of frying, so there has not been a high degradation of asiaticoside levels. Heating oil at moderate cooking temperatures (≤200°C) has minimal effects on some compounds, but temperatures above 200°C and prolonged heating can increase levels of harmful secondary metabolites such as trans fatty acids and oxidation products [10].

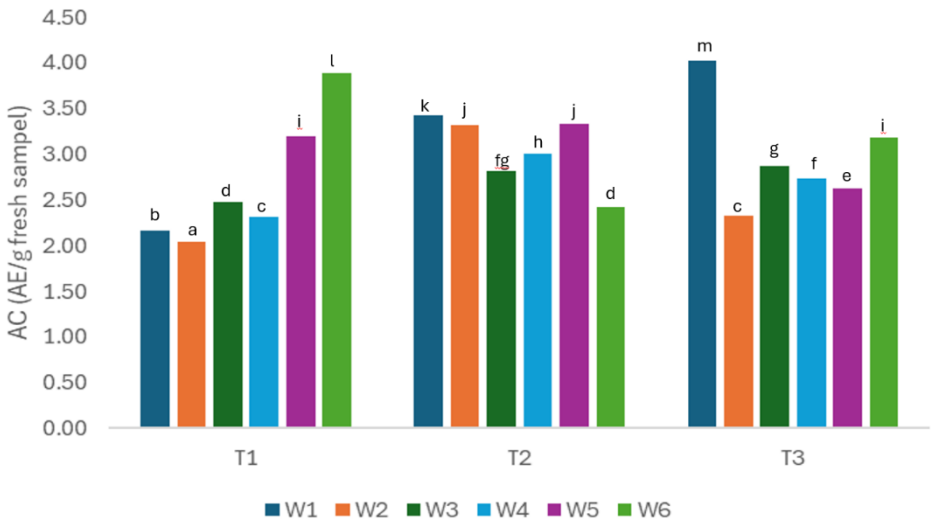

Fig. 1. Asiaticoside content (AC) during the frying process of *C. asiatica* leaves

3.1.2 Total Flavonoid Content (TFC)

The frying method, especially the temperature and duration of frying on gotu kola, had a significant effect on the total flavonoid content (Fig. 2). Total flavonoid levels at 130 ºC frying temperature were very low but increased to 145 ºC and then decreased again at 160 ºC. An initial decrease at low temperatures is due to limited release and some degradation, an increase at moderate temperatures is due to increased release from the matrix, and a final decrease at high temperatures results from thermal degradation of the flavonoids themselves.

The highest flavonoid content was obtained at the lowest frying duration of 1 minute. Short duration frying can retain total flavonoid levels mainly because short heat exposure inactivates microbes and plant enzymes that can degrade flavonoids and minimizes the time for thermal degradation and leaching to occur. In particular, short time frying has been shown to increase flavonoid retention by rapidly inactivating degradative enzymes without prolonged heat exposure that would break down flavonoids [11]. In addition, the solubility of flavonoids in oil is low, so short frying times can reduce flavonoid loss into the frying medium. Studies have also shown that most of the decrease in flavonoid content during frying occurs within the first few minutes, after which the levels stabilize, indicating that limiting the duration of frying helps to preserve flavonoids [12]. In addition, shorter frying times prevent extensive oxidation and degradation caused by heat and prolonged exposure.

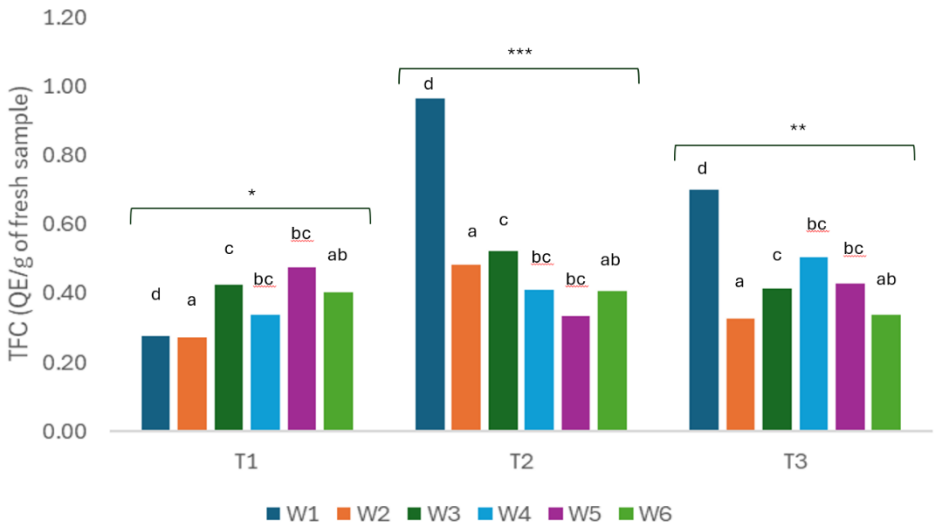

Fig. 2. Total Flavonoid Content (TFC) during the frying process of *C. asiatica* leaves

3.1.3 Total Phenol Content (TPC)

Analysis showed that both temperature and frying duration significantly affected the total phenol content of gotu kola during the frying process (Fig. 3). The frying process of gotu kola at the longest duration (11 minutes) obtained the highest total phenol content. During frying, the total phenol content in oil usually decreases due to the degradation of phenolic compounds caused by high heat and long cooking time. However, in certain studies involving frying with palm oil, total phenol levels have been observed to increase with longer frying duration. This is because palm oil contains natural antioxidants such as vitamin E (tocopherols and tocotrienols) and other minor components that can undergo oxidative transformation during frying. These transformations can produce new compounds that react

positively in the phenol test, leading to an increase in total phenol content [13]. In addition, in this study, the frying temperature was classified as moderate, below 200 degrees Celsius, so it did not degrade phenol levels. During frying, especially at moderate temperatures, some oxidative reactions and the breakdown of larger molecules may lead to the formation or release of new phenolic compounds, which temporarily increases the total phenol content in the oil [4]. Frying at lower temperatures can slow down the degradation of phenolic compounds, so these compounds can be retained for longer periods even with longer frying times. Studies have shown that low-temperature frying or heating can retain higher phenolic levels compared to high-temperature frying, where phenols degrade rapidly [14].

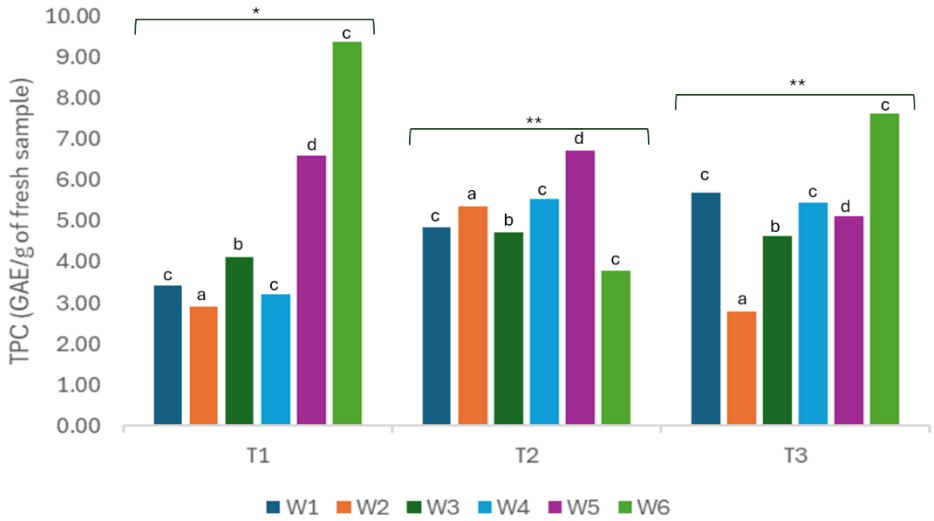

Fig. 3. Total Phenol Content (TPC) during the frying process of *C. asiatica* leaves

3.2 Optimization of gotu kola frying method

Preliminary analysis based on asiaticoside content parameter was taken 3 frying methods of gotu kola with the highest asiaticoside content using DMRT analysis. Based on DMRT analysis, the 3 methods with the highest asiaticoside content were T1W6, T2W1, and T3W1. The three samples were then tested for antioxidant activity including IC50 DPPH and IC50 NO (Table 3).

Table 3. IC50 DPPH and IC50 NO of fresh gotu kola frying.

Temperature (°C)	Sample	Antioxidant Activity	
		DPPH IC50 (µg fresh sample /mL)	NO IC50 (µg fresh sample /mL)
T1 (130)	T1W6	428.07 ± 6.97	1189.69 ± 18.75
T2 (145)	T2W1	578.10 ± 7.79	2134.21 ± 52.59
T3 (160)	T3W1	286.17 ± 3.66	1134.69 ± 29.79

Data on secondary metabolite parameters and antioxidant activity were then subjected to normalized decision matrix calculation. After calculating the normalized matrix, the next step is to multiply it with the predetermined weights (Table 4).

Table 4. Normalized and weighted normalized matrix

Ai	Normalized Matrix					Weighted Normalized Matrix				
	C1	C2	C3	C4	C5	C1	C2	C3	C4	C5
A_1	0.5927	0.3221	0.7822	0.5529	0.4416	2.9634	1.2884	3.1289	1.6588	1.3248
A_2	0.5226	0.7659	0.4036	0.7467	0.7922	2.6132	3.0636	1.6146	2.2402	2.3766
A_3	0.6128	0.5565	0.4745	0.3696	0.4212	3.0642	2.2259	1.8982	1.1089	1.2636

Then determine the matrix of positive ideal solutions and negative ideal solutions obtained by determining the maximum and minimum values of each criterion variable (Table 5).

Table 5. Positive and Negative Ideal Matrix

	C1	C2	C3	C4	C5
A+	3.06423	3.06358	3.12891	1.10895	1.26356
A-	2.61322	1.28838	1.61459	2.24020	2.37661

The next step is to determine the distance between the values of each alternative and the ideal solution matrix obtained. From the results of the distance value of the positive ideal solution and the negative ideal solution determined, then perform calculations to get the preference value of each alternative by sorting the largest value to the smallest (Table 6).

Table 6. Relative closeness to ideal solution

Alternatif	D+	D-	V
A_1	1.86216	1.96469	0.51340
A_2	2.02040	1.77520	0.46770
A_3	1.48878	1.91868	0.56308

Based on the results of calculations with the TOPSIS method, the most optimal method in the process of frying gotu kola with the assessment criteria for asiaticoside content, total flavonoid content, total fevol content, DPPH scavenging activity, and Nitric Oxide scavenging activity is obtained in Alternative 3 (A_3), namely frying at a temperature of 160 ºC with a frying duration of 1 minute. Frying at moderate temperatures for short periods of time limits the exposure of oils and bioactive foods to high heat, thereby reducing the degradation of sensitive secondary metabolites and antioxidant. Short-time frying minimizes exposure to excessive heat that can cause damage to vitamins and antioxidant compounds such as vitamins E and K contained in palm oil. Vitamin E functions as a primary antioxidant that captures free radicals, thus maintaining the stability of bioactive compounds during frying [15].

4 Conclusions

The optimal levels of secondary metabolites and antioxidant activity in the gotu kola frying process were obtained at a temperature of 160 ºC for a duration of 1 minute. Controlled frying conditions (moderate temperature and short time) help maintain the level of secondary metabolites and antioxidant activity during gotu kola frying. This reduces oxidative

degradation and preserves the functional properties of the fried gotu kola. Frying at 160 ºC for a short duration can be applied for optimal health benefits.

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