

Transient Changes in Oil Quality under Frying: Effect of β -Carotene and BHT

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β -carotene is a carotenoid, known as one of the main sources of provitamin A and its low diet assumption is one of the major deficiencies among children in Africa and Asia. Even when incorporated into oils, it degrades at high temperatures, which leads to the loss of its antioxidant properties, affecting the nutritional quality and shelf life of the oil.

The objectives of this research were (1) to study the degradation kinetics of β -carotene in selected commercial cooking oils at frying temperatures (180°C) over time (up to 30 min); and (2) to investigate the effect of the addition of the synthetic antioxidant BHT (butylated hydroxytoluene) on preventing the degradation of β -carotene during frying.

Five different oils (olive oil, extra virgin olive oil, sunflower oil, rapeseed oil and coconut oil) were analysed, and degradation of β -carotene was monitored based on a spectrophotometric method. The oxidative state of the oils was assessed by measuring the acidity and peroxide values. Finally, the fatty acid profile of sunflower and coconut oil was evaluated through GC-FID. The findings revealed that BHT does not significantly protect β -carotene from thermal degradation in all oils. Notably, β -carotene degraded faster in coconut oil, which is rich in saturated fatty acids, compared to sunflower oil, which contains a higher proportion of polyunsaturated fatty acids. So, the fatty acid composition of the oil plays a crucial role in the degradation process.

1. Introduction

The Food and Agriculture Organization of the United Nations (FAO) used data on food composition analysis to create the World Food Survey. The primary energy source is represented by macronutrients or carbohydrates, lipids and proteins. Edible oils contain special lipid components, of which triglycerides are the main and significant ones. The degree of health benefits of different oils has been a highly debated area of research (Xiang et al., 2024).

Global production of vegetable oils has increased by 240%, reaching over 219 million tons worldwide in 2023 (USDA). The major oils are olive oil, coconut oil, cotton-seed oil, peanut oil, palm kernel oil, sunflower oil, rapeseed oil and soybean oil, whose global production ($\times 100,000$ tons) in 2022 was 28.21, 35.9, 50.10, 64.99, 88.26, 200.61, 316.52 and 618.75, respectively (USDA, 2025).

Edible oils are an important component of food processing which significantly modify the raw materials and ingredients of the recipes, increasing the caloric content, and promoting the sensory, taste and texture of finished products (Xiang et al., 2024).

One of the most used and oldest methods in the world for preparing food is frying which is carried out at high temperatures. Due to the high temperatures that occur in the frying process, a series of complex reactions such as hydrolysis, oxidation and polymerization occur. Furthermore, hydroperoxides are formed which are important oxidation products formed during frying. The latter decomposes to give secondary products such as esters, aldehydes, alcohols, ketones, lactones and hydrocarbons. Due to the formation of these secondary products, there is a loss of nutritional value and the formation of compounds that influence flavor. To prevent and slow down these reactions, synthetic antioxidants such as BHT, the subject of this study, are used (Cui et al., 2017).

In some vegetable oils there is a pigment that gives an orange color, β -carotene. It is a molecule that degrades in the presence of light and heat; therefore, it is lost in the oil refining processes. β -carotene is widely used as a natural colorant and preservative in the food industry (Yilmaz et al., 2017), since it has antioxidant properties that protect against damage caused by other molecules such as free radicals (Elik et al., 2020). β -carotene can be consumed to make up for vitamin A deficiency as it is a pro-vitamin. Vitamin A deficiency is a major public health problem worldwide, particularly in Asia and Africa. Along with zinc and iron deficiencies, it is one of the three most chronic nutritional deficiencies. Globally, an estimated 250 million preschool children are vitamin A deficient (Chen et al., 2021; Kumar et al., 2024;).

The objectives of this research were (1) to study the degradation kinetics of β -carotene in selected commercially available cooking oils at frying temperatures (180°C) over time (up to 30 min); and (2) to investigate the effect of BHT on preventing the degradation of β -carotene under those conditions. Five commercial cooking oils were tested including olive oil, extra virgin olive oil, rapeseed oil, sunflower oil and coconut oil at frying condition. Analysis of β -carotene as well as determination of acid and peroxide values and free fatty acid profiles of the treated oils were carried out.

2. Materials and methods

2.1 Experimental plan

Stock solutions of β -carotene and BHT were prepared as follows: 10 μ g of β -carotene were dissolved in each of the cooking oils (100 mL) under stirring with the help of a magnetic bar and away from the natural light using aluminum foil to cover the flask. The same protocol was followed to prepare the BHT stock solution but in this case 2 g of BHT were dissolved in 100 mL of oil. This amount of BHT was used because it is the maximum amount of this antioxidant reported in the regulation (EC) No 1333/2008.

Samples of cooking oil (90 mL) were transferred into Duran bottle (200 mL) and added with either 10 mL of β -carotene stock solution or 1 mL of BHT stock solution to get final concentration of BHT and β -carotene in the oil samples 0.02% and 0.01%, respectively. Three different samples were prepared for each treatment for each cooking oil.

To simulate frying conditions, 90 mL of oil were transferred to a beaker (100 mL) and placed on a heating plate with the temperature set at 180 °C. This temperature was chosen because 180°C is a classic frying temperature used for cooking and frying. Once the temperature of 180°C was reached, 10 mL of β -carotene stock solution were added to the oil monitoring that the temperature remained constant at 180 °C (a thermometer was used to check and record the temperature immediately after the addition of the stock solution and throughout the duration of the experiment). The same procedure was followed when adding 1 mL from the BHT stock solution. After adding the stock solution, samples were taken from the oil at various time intervals (0, 5, 10, 20, 25, 30 min). The temperature of 180°C and the selected times were chosen because they are values observed during deep-fat frying (Totani et al., 2013; Kumar et al., 2024). Each sample was immediately placed in an ice bath to bring the temperature down and prevent the degradation of β -carotene. The experiments were performed in triplicate for each oil for each of the treatments.

2.2 Analyses

A spectrophotometer (JENWAY 6315 Spectrophotometer) was used to measure the levels of β -carotene in the samples. To do this, the spectrophotometer was calibrated by initially measuring the absorbance of the blank, hexane. Subsequently, samples containing known amounts of β -carotene or BHT (0.2 mL of each sample) were mixed with 3.8 mL of hexane, and the absorbance was measured at 450 nm. The obtained results were used to construct the calibration curve.

Analysis of the acid value of the oil sample preparations was done using the ISO 660:2020. Treated oil (1 g) was weighed into a 50 mL flask containing 5 mL of alcohol:diethyl ether (1:1), added with two drops of phenolphthalein and then titrated with 0.1 N KOH. The acid value is expressed as mg_{KOH}/g.

The peroxide value (PV) analysis was performed and expressed as meq_{O₂}/kg according to the modified AOCS Cd 8b-90 method. Treated oil (1 g) was dissolved in a mixture of acetic acid and chloroform (3:2 V:V). After adding potassium iodide (KI), the solution was kept in the dark for 5 min. Then, 15 mL of distilled water were added and stirred to prevent phase separation. The developed iodine was titrated with Na₂S₂O₃ · 5H₂O 0.01 N, using 1% starch as an indicator.

Analysis of the free fatty acids in the treated oils was carried by Gas Chromatography – Flame Ionization Detection using an Agilent Technologies 7890B GC System. Treated oil (0.1 g) was carefully weighed into a test tube, then 5 mL of hexane was added to the oil and mixed well to dissolve the oil. Then, 1.0 mL of methanolic potassium hydroxide solution (2 M) was added to each tube and the tube was shaken for 30 sec to obtain a clear solution. For each sample, 0.5 mL of the fatty acid methyl esters (FAMES) solution contained in the top layer of hexane were transferred to a vial and diluted to a total volume of 1.0 mL. The FAMES samples (1 μ L)

were injected into a gas chromatograph equipped with a flame ionization detector. The column used was BPX-70 Capillary Column (12 m × 0.25 mm, 0.25 µm film thickness). The flow rate of the carrier gas (helium) was 1.0 mL/min. The injector and detector temperatures were 220 and 250°C, respectively. The column oven temperature was programmed with an initial temperature of 120°C and then increased to 170°C at a rate of 10°C/min, and maintained for 5 min. The identities of FAMEs were confirmed by conducting gas chromatography under conditions identical to those for authentic FAMEs (CRM47885, Supelco 37 Component FAME Mix).

All the results obtained were performed in triplicate and reported in this report as mean values ± standard deviation of the technical and analytical replicates performed. The influence of β-carotene and BHT on the various quality characteristics of the oils was assessed by two-way analysis of variance (ANOVA) using XLSTAT Statistics Software 2024.2.2 (Lumivero, Denver, CO, USA). In the case of significant influence, the means were discriminated using Tukey's post-hoc test, at a 95% confidence level.

2.4 Degradation of β-carotene

One of the objectives of this research was to investigate the degradation of β-carotene in oils enriched with β-carotene at frying temperatures of 180°C and whether BHT offered any protection. The evolution of β-carotene content following heat exposure of oils with and without the antioxidant BHT was then carried out, was elaborated according to a first-order kinetics model Eq(1) in order to deduce the degradation rate constant.

$$\ln\left(\frac{C_0}{C_t}\right) = kt \quad (1)$$

Where C_t and C_0 are β-carotene concentration at the beginning and at time t of a given frying experiment, k is the first order isothermal rate constant (h^{-1}).

3. Results and discussion

3.1 Efficiency of BHT on β-carotene degradation in five different oils

Table 1 shows the β-carotene content over frying time, in the presence or absence of the antioxidant BHT, in different oils.

Table 1: Evolution of β-carotene content as µg/mL during frying of different oils with or without BHT addition and values of degradation rate constants (k) for a first order degradation kinetics model. O: Olive oil; EVO: Extra Virgin Olive Oil; S: Sunflower oil; R: Rapeseed oil; C: Coconut oil. Values are reported as means ± s.d. Same letters in the same column indicate means not statistically different.

Time (min)	β-carotene concentration (µg/mL)				
	O	EVO	S	R	C
0-βc	0.505 ± 0.041 ^a	0.564 ± 0.034 ^a	0.502 ± 0.018 ^a	0.514 ± 0.006 ^a	0.475 ± 0.048 ^b
0-βc-BHT	0.526 ± 0.028 ^a	0.575 ± 0.024 ^a	0.508 ± 0.016 ^a	0.490 ± 0.003 ^b	0.545 ± 0.020 ^b
5-βc	0.436 ± 0.047 ^b	0.500 ± 0.022 ^b	0.427 ± 0.021 ^b	0.459 ± 0.007 ^c	0.251 ± 0.048 ^c
5-βc-BHT	0.453 ± 0.043 ^b	0.502 ± 0.007 ^b	0.422 ± 0.020 ^b	0.436 ± 0.007 ^d	0.266 ± 0.013 ^c
10-βc	0.359 ± 0.012 ^c	0.416 ± 0.016 ^c	0.380 ± 0.020 ^c	0.399 ± 0.008 ^e	0.132 ± 0.016 ^d
10-βc-BHT	0.377 ± 0.033 ^c	0.435 ± 0.010 ^c	0.387 ± 0.016 ^c	0.391 ± 0.016 ^e	0.143 ± 0.011 ^d
15-βc	0.297 ± 0.022 ^d	0.344 ± 0.007 ^c	0.339 ± 0.010 ^d	0.344 ± 0.011 ^f	0.064 ± 0.016 ^{ef}
15-βc-BHT	0.278 ± 0.016 ^d	0.371 ± 0.024 ^d	0.344 ± 0.013 ^d	0.357 ± 0.007 ^f	0.092 ± 0.005 ^e
20-βc	0.175 ± 0.030 ^e	0.280 ± 0.020 ^d	0.27 ± 0.008 ^e	0.313 ± 0.015 ^g	0.031 ± 0.012 ^{gh}
20-βc-BHT	0.245 ± 0.024 ^d	0.322 ± 0.005 ^e	0.304 ± 0.005 ^f	0.303 ± 0.016 ^g	0.058 ± 0.005 ^{ef}
25-βc	0.147 ± 0.036 ^{ef}	0.227 ± 0.011 ^e	0.238 ± 0.022 ^g	0.266 ± 0.015 ^h	0.019 ± 0.010 ^g
25-βc-BHT	0.168 ± 0.020 ^{de}	0.275 ± 0.011 ^f	0.27 ± 0.003 ^f	0.259 ± 0.017 ^h	0.044 ± 0.000 ^{gh}
30-βc	0.113 ± 0.027 ^f	0.184 ± 0.015 ^f	0.219 ± 0.030 ^g	0.224 ± 0.013 ⁱ	0.011 ± 0.006 ^g
30-βc-BHT	0.138 ± 0.019 ^{de}	0.212 ± 0.011 ^{gf}	0.225 ± 0.013 ^g	0.238 ± 0.015 ⁱ	0.018 ± 0.003 ^g
K h ⁻¹ -βc	2.88	2.16	1.8	1.44	7.56
K h ⁻¹ -βc-BHT	2.52	1.8	1.44	1.44	6.84
R ² -βc	0.985	0.996	0.997	0.998	0.999
R ² -βc-BHT	0.993	0.996	0.997	0.997	0.994

For all oils, regardless of the presence of BHT, a significant decrease ($p < 0.05$) in β-carotene levels is observed. However, statistically significant differences are observed in olive oil and sunflower oil at 20 and 30 min., and in EVO at 20, 25, and 30 min. This behavior shows that, overall, BHT does not have a protective effect against β-carotene degradation. This phenomenon, as reported by Zeb et al. (2013), is due to the low resistance of BHT to temperatures above 140°C. The behavior of liquid oils is very similar, with the kinetics constant k values ranging from 1.44 h^{-1} for rapeseed oil to 2.88 h^{-1} for olive oil. Coconut oil, however, shows a completely different

behavior, with k equal to 7.56 h^{-1} in the absence of BHT and 6.84 h^{-1} in the presence of BHT. These values indicate that β -carotene degrades much faster in coconut oil than in liquid oils. This different behavior and the resulting degradation of β -carotene are due to the different composition of the oils. Coconut oil is made up of 96% saturated fatty acids, while only 4% consists of monounsaturated fatty acids. Sunflower oil, on the other hand, is composed of 86.8% polyunsaturated fatty acids, with the remainder split between monounsaturated and saturated (O'Brien, 2009). β -carotene degrades with heat mainly due to the instability of its conjugated double bonds.

Based on the first results, two oils were selected for further analysis due to their very different behaviors: coconut oil and sunflower oil. It was hypothesized that the behavior of these two oils was different because their fatty acid compositions were different, therefore analysis of the acid value and peroxide value were conducted to help explain this. As shown in Table 2, acid values showed no significant differences up to 15 min. but became statistically different at 20, 25, and 30 min. Notably, coconut oil samples enriched with β -carotene and BHT had lower values at 25 and 30 min. Peroxide levels increased in all samples at 180°C , with a significant rise at 30 min. After 15 min., statistical differences emerged: pure coconut oil had similar peroxide levels to BHT-enriched oil, while coconut oil with β -carotene had lower levels compared to the other groups.

Table 2: Acid value and peroxide value of coconut oil during frying at 180°C , as it is or with addition of β -carotene &/or BHT. Values are reported as means \pm s.d. Same letters in the same column indicate means not statistically different.

Min	Acid Value $\text{mg}_{\text{KOH}}/\text{g}$				Peroxide Value $\text{meq}_{\text{O}_2}/\text{kg}$			
	C	C-BHT	C- βc	C- βc -BHT	C	C-BHT	C- βc	C- βc -BHT
0	0.15 \pm 0.04 ^{ab}	0.15 \pm 0.01 ^a	0.14 \pm 0.01 ^a	0.14 \pm 0.01 ^a	86.67 \pm 15.28 ^a	50.00 \pm 11.55 ^a	46.67 \pm 5.77 ^a	50.00 \pm 10.00 ^a
5	0.16 \pm 0.05 ^{ab}	0.16 \pm 0.02 ^{ab}	0.14 \pm 0.01 ^a	0.14 \pm 0.01 ^a	103.33 \pm 20.82 ^b	63.33 \pm 15.28 ^b	56.67 \pm 5.77 ^{ab}	56.67 \pm 15.28 ^{ab}
10	0.18 \pm 0.02 ^{abc}	0.20 \pm 0.02 ^{bcd}	0.18 \pm 0.04 ^{abc}	0.14 \pm 0.01 ^a	106.67 \pm 26.46 ^b	80.00 \pm 15.28 ^c	66.67 \pm 5.77 ^{ab}	66.67 \pm 15.28 ^{ab}
15	0.23 \pm 0.04 ^{def}	0.21 \pm 0.01 ^{cd}	0.20 \pm 0.02 ^{bcd}	0.16 \pm 0.02 ^{ab}	116.67 \pm 26.46 ^c	93.33 \pm 15.28 ^c	80.00 \pm 10.00 ^{ab}	80.00 \pm 20.00 ^b
20	0.23 \pm 0.04 ^{def}	0.21 \pm 0.01 ^{cd}	0.23 \pm 0.02 ^{be}	0.20 \pm 0.02 ^{bcd}	130.00 \pm 15.28 ^d	123.33 \pm 10.00 ^d	93.33 \pm 15.28 ^b	93.33 \pm 25.17 ^b
25	0.26 \pm 0.02 ^{efg}	0.28 \pm 0.01 ^{gh}	0.26 \pm 0.02 ^{fgh}	0.20 \pm 0.03 ^{bcd}	143.33 \pm 25.17 ^e	140.00 \pm 15.28 ^e	106.67 \pm 20.82 ^c	113.33 \pm 25.17 ^c
30	0.31 \pm 0.04 ^h	0.29 \pm 0.01 ^{gh}	0.26 \pm 0.02 ^{fgh}	0.23 \pm 0.02 ^{de}	180.00 \pm 15.28 ^f	150.00 \pm 10.00 ^f	120.00 \pm 22.55 ^c	130.00 \pm 20.82 ^c

Table 3: Acid value and peroxide value of sunflower oil during frying at 180°C , as it is or with addition of β -carotene &/or BHT. Values are reported as means \pm s.d.

Min	Acid Value $\text{mg}_{\text{KOH}}/\text{g}$				Peroxide Value $\text{meq}_{\text{O}_2}/\text{kg}$			
	S	S-BHT	S- βc	S- βc -BHT	S	S-BHT	S- βc	S- βc -BHT
0	0.26 \pm 0.01 ^{ab}	0.25 \pm 0.01 ^{ab}	0.25 \pm 0.01 ^a	0.25 \pm 0.01 ^{ab}	56.67 \pm 5.77 ^{ab}	40.00 \pm 0.01 ^{de}	43.33 \pm 5.77 ^{ab}	36.67 \pm 5.77 ^a
5	0.26 \pm 0.01 ^{abc}	0.27 \pm 0.01 ^{bc}	0.26 \pm 0.01 ^{abc}	0.27 \pm 0.01 ^{cd}	63.33 \pm 5.77 ^b	43.33 \pm 5.77 ^{ef}	46.67 \pm 5.77 ^{bc}	46.67 \pm 5.77 ^{bc}
10	0.28 \pm 0.01 ^{fg}	0.27 \pm 0.01 ^{de}	0.28 \pm 0.01 ^{fg}	0.28 \pm 0.01 ^{ef}	63.33 \pm 5.77 ^{bc}	46.67 \pm 5.77 ^{ef}	50.00 \pm 0.01 ^{cd}	46.67 \pm 5.77 ^{bc}
15	0.29 \pm 0.01 ^{gh}	0.29 \pm 0.01 ^g	0.29 \pm 0.01 ^g	0.29 \pm 0.01 ^{gh}	73.33 \pm 5.77 ^{bc}	50.00 \pm 0.01 ^{gh}	60.00 \pm 0.01 ^e	50.00 \pm 5.77 ^{cd}
20	0.30 \pm 0.01 ^{hi}	0.31 \pm 0.01 ^{ij}	0.31 \pm 0.01 ^{ij}	0.31 \pm 0.01 ^{ij}	73.33 \pm 5.77 ^{cd}	50.00 \pm 0.01 ^{gh}	70.00 \pm 0.01 ^{fg}	50.00 \pm 0.01 ^{cd}
25	0.32 \pm 0.01 ^{jk}	0.32 \pm 0.01 ^{kl}	0.32 \pm 0.01 ^{lm}	0.32 \pm 0.01 ^{kl}	80.00 \pm 0.01 ^{cd}	50.00 \pm 0.01 ^{hi}	70.00 \pm 0.01 ^{fg}	56.67 \pm 0.01 ^{de}
30	0.33 \pm 0.01 ^l	0.34 \pm 0.01 ^m	0.34 \pm 0.01 ^{lm}	0.34 \pm 0.01 ^m	86.67 \pm 5.77 ^e	60.00 \pm 0.01 ^{fg}	70.00 \pm 0.01 ^{fg}	60.00 \pm 0.01 ^d

There was a statistically significant increase in the acid value in sunflower oil for all samples at a frying temperature of 180°C as shown in Table 3. For this oil, unlike coconut oil, there was no statistically significant difference between the samples to which antioxidants were added. The peroxide values for the samples enriched with β -carotene and those enriched with both β -carotene and BHT show similar values, and close to those of the oil with no antioxidants. The samples of pure oil and oil enriched with only BHT show no statistically significant differences, and the values are higher than those of the samples enriched with β -carotene. This is due to the low heat resistance of BHT, as it degrades at temperatures above 140°C , losing its antioxidant effect.

3.2 Influence of β -carotene and BHT on the free fatty composition

Figure 1 shows that coconut oil exposed to 180°C presents an increase in the percentage of saturated fatty acids (SFA) and a decrease in mono (MUFA) and polyunsaturated fatty acids (PUFA) compared to the initial conditions at room temperature. Samples containing only coconut oil and those enriched with BHT show the same behavior, with an increase in SFA and a decrease in MUFA and PUFA. When coconut oil is enriched with β -carotene or with both β -carotene and BHT, the same trend of increased SFA and decreased MUFA and PUFA is observed, but in these cases, the changes are minimal and statistically not significant, like the conditions of crude oil. This suggests that BHT has no significant effect, as it loses its antioxidant activity at high temperatures.

In contrast, β -carotene, being an antioxidant, shows a significant protective effect against thermal oxidation by oxidizing itself.

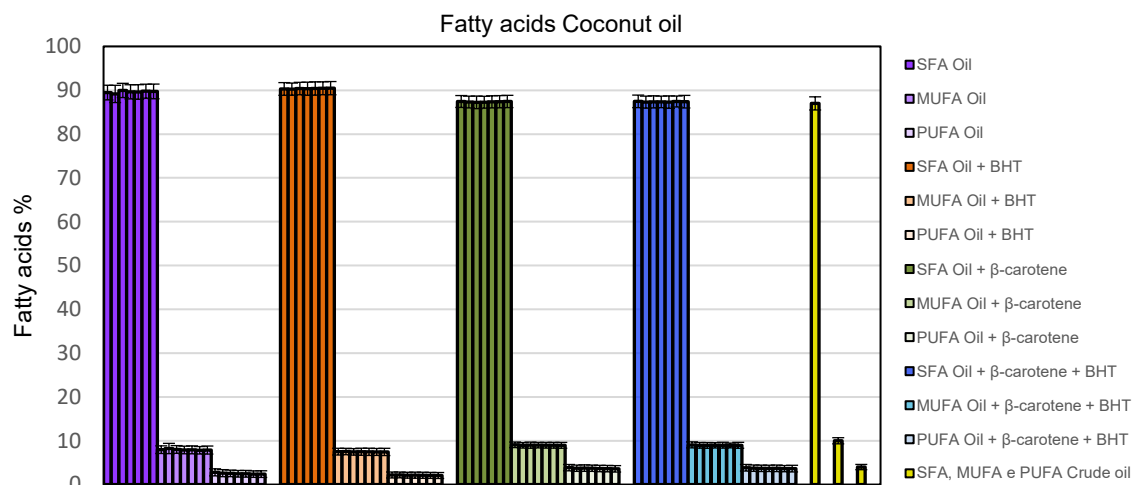


Figure 1 Comparative protective activity of BHT at concentration of 0.02% and of β -carotene at concentration of 0.01% on the changes in fatty acid composition in coconut oil. SFA means saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid.

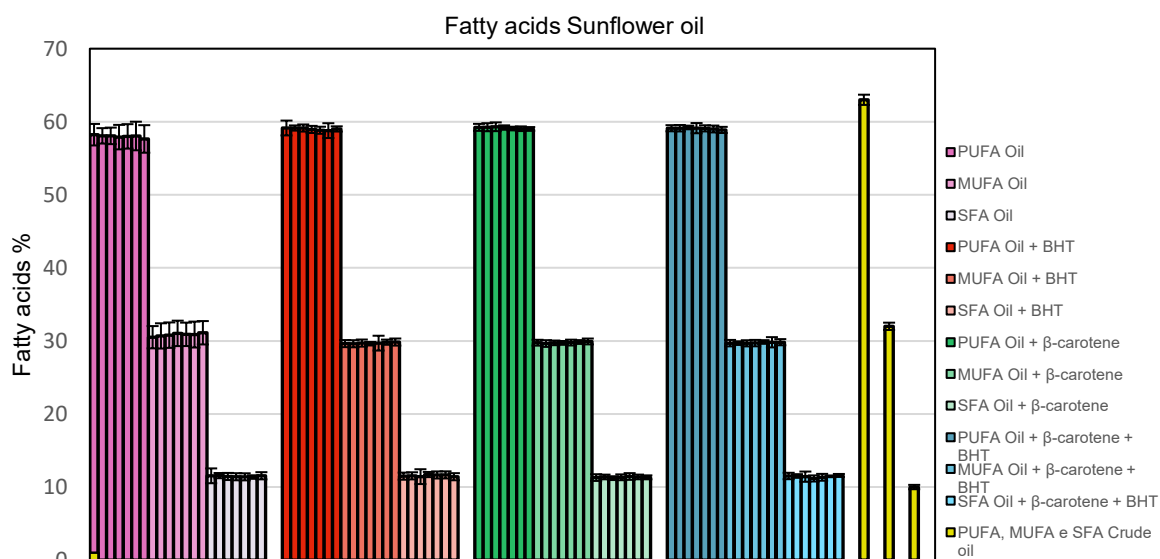


Figure 2: Comparative protective activity of BHT at concentration of 0.02% and of β -carotene at concentration of 0.01% on the changes in fatty acid composition in sunflower oil. SFA means saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid.

Figure 2 reports the values of fatty acids of sunflower oil at room temperature and the values of fatty acids of sunflower oil at frying temperature of 180°C. The percentage of saturated fatty acids SFA increased after exposure of sunflower oil samples to high temperature. However, the values of MUFA and PUFA decreased compared to the starting conditions of the oil at room temperature and decreased after 30 min. of heat exposure. Analysing the SFA, MUFA and PUFA values of the different treatments, it was found no significant statistical difference, therefore in sunflower oil there is no antioxidant effect given by β -carotene and BHT. The antioxidant BHT was added to test its effectiveness in preventing β -carotene degradation, but it proved ineffective due to high temperatures, as previously reported by Yehye et al. (2015). β -carotene, although an antioxidant, also degrades rapidly, thus losing its antioxidant properties due to its chemical structure, which is vulnerable to heat and oxidation (Donhowe and Kong, 2014). Despite this, β -carotene had a significant protective effect in coconut

oil, slowing down the oxidation of fatty acids, but not in sunflower oil. This is because sunflower oil contains polyunsaturated fatty acids, particularly linoleic acid, which are more susceptible to oxidation at high temperatures (Xiang et al., 2024).

4 Conclusions

One of the objectives of the research was to study the behavior of BHT as a protection against the degradation of β -carotene. It is possible to note that BHT at frying conditions of 180°C did not have a protective effect against the degradation of β -carotene. In addition, the degradation of β -carotene in the oils seemed dependent on the fatty acid composition of each oil as the degradation of β -carotene was observed to be faster in coconut oil, mostly composed of saturated fatty acids when compared to sunflower oil, high in polyunsaturated fatty acids, resulting in a much slower degradation of β -carotene. Another objective of the research was to study the behavior of coconut oil and sunflower oil in the presence of the antioxidants β -carotene and BHT at frying conditions at a temperature of 180°C. An increase in saturated fatty acids, and a decrease in monounsaturated and polyunsaturated fatty acids can be observed in both oils. The presence of BHT was insignificant in both oils, but it was possible to note that β -carotene had positive effects against the oxidation of the coconut oil sample.

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