Food Research 8 (4) : 245 - 251 (August 2024)
Journal homepage: https://www.myfoodresearch.com

FOOD RESEARCH

RESEARCH PAPER

The influence of high oleic oils during intermittent frying on the formation of acrylamide in french fries

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Article history:
Received: 20 May 2023
Received in revised form: 8 August 2023
Accepted: 1 July 2024
Available Online: 4 August 2024

Keywords:
Frying,
Acrylamide,
Polyunsaturated fatty acid (PUFA),
Total polar compound (TPC),
Frying cycle

DOI:
https://doi.org/10.26656/fr.2017.8(4).174

Abstract

High oleic oils are often used for industrial frying considering a better nutritional value and stability to thermal oxidation than high polyunsaturated fatty acid oils. This research aimed to investigate the correlation between total polar compound (TPC) and acrylamide content during intermittent frying using high oleic oils such as super palm olein (SPO), high-oleic canola oil (HOCO) and high-oleic sunflower oil (HOSO). All oils were accessed in terms of fatty acid composition and tocopherol quantity prior to intermittent frying. The TPC of frying oil was recorded at every 8th interval of the frying cycle and french fries fried with respective oils were collected, and oil was extracted for acrylamide quantification using HPLC-MS. Pearson correlation showed a positive correlation between the total polar compound of frying oil and the acrylamide content of french fries ($p<0.01$). SPO contained the highest total tocopherol content, followed by HOCO and HOSO, accordingly. French fries fried with SPO had the lowest acrylamide content, followed by HOCO and HOSO, respectively. By contrast, SPO had the highest TPC content throughout the frying cycles, followed by HOCO and HOSO. The presence of oleic acids might not produce higher TPC, however, it produced higher acrylamide formation in the french fries. Overall, SPO is suitable for intermittent frying uses as it yielded the lowest concentration of acrylamide in french fries.

1. Introduction

Deep fat frying is the use of oils as a medium to cook food at a temperature that is higher than the boiling point of water. Frying produces desirable sensorial properties, such as flavour, crispiness and golden-brown appearance within fried food (Kim *et al.,* 2018; Asokapandian *et al.*, 2019; Chew *et al.*, 2020). However, high temperature degrades the oil faster through oxidation, hydrolysis and polymerization reactions which eventually poses a health concern for fried food consumption (Hosseini *et al.,* 2016; Hau *et al.*, 2020). Specifically, intermittent frying is the most common frying culture that have been used at household levels, which defines the process of frying batch by batch. This means the frying oil has undergone heating and cooling periodically, which accelerates the oxidation process because it increases the oxygen solubilization of frying oils (Lee Kuek *et al.*, 2020). With the same frequency of the frying cycle, intermittent frying tends to reach the TPC limit faster than continuous frying (Abd Razak *et al.*, 2021).

In general, vegetable oil used for frying should be stable under high temperature and has a high smoking point. Oils with high saturated fatty acids (SFA) or monounsaturated fatty acids (MUFA) are more resistant to oxidation and frying breakdown reactions (Hu *et al.,* 2020; Elaine *et al.*, 2022). Lots of oil industries have produced vegetable oils with high oleic contents for industrial frying uses. For instance, high-oleic sunflower oil (HOSO) and high oleic canola oil (HOCO) which contain high polyunsaturated fatty acids (PUFA) in nature, are modified to contain high MUFA (oleic acid) (Zambelli, 2021). Meanwhile, palm oil initially has a balanced composition of moderate amounts of saturated and unsaturated fatty acids. Super palm olein, is the type of palm oil obtained by further fractionation of palm oil to obtain the oil with a higher unsaturation content. Thus, it contains higher monounsaturated fatty acids than saturated fatty acids, which claim to have good stability and visual clarity. Besides fatty acid composition, the

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eISSN: 2550-2166
Published by Rynnye Lyan Resources

antioxidant content in frying oils also affects the oxidative stability of frying oils. Tocopherol is a common example of a naturally occurring lipid antioxidant in vegetable oils, which plays a major role in preventing the loss of unsaturated fatty acids (Liu *et al.*, 2022) and anti-polymerization activity (Kmiecik *et al.*, 2019).

In assessing the quality of frying oils, total polar compounds are good indicators as oils tend to produce more polar compounds during degradation (ben Hammouda *et al.*, 2019). The TPC elevated the level of free radicals in HepG2 cells and induced lipid accumulation in a dose-dependent manner, contributing to detrimental health influence (Ju *et al.*, 2019). According to the TPC legislation standard, good quality frying oil should not have TPC > 25% (Abdalla *et al.*, 2021). Meanwhile, acrylamide is part of the fried food concerns because it forms easily through the Maillard reaction (Koszucka *et al.*, 2020). Maillard reaction occurs when there is asparagine protein and carbonyl group subjected to a heating process at a temperature above 120°C. Acrylamide can be found in foods such as fried foods, roasted foods, and baked foods. (Koszucka *et al.*, 2020; Abd Razak *et al.*, 2021). According to European Commission regulation, the benchmark level of acrylamide in ready-to-eat french fries is 500 µg/kg (Koszucka *et al.*, 2020). There are numerous studies reported the acrylamide content of french fries during frying (Abd Razak *et al.*, 2021).

As both TPC and acrylamide are part of the health concerns of fried food, the investigation of both indicators is important. In this study, the fatty acid composition and tocopherol contents of frying oils (high oleic sunflower oil, high oleic canola oils, and super palm olein) were investigated. This paper aims to compare the effect of different high oleic oils on the formation rate of total polar compounds of frying oils and acrylamide content in french fries throughout the frying cycles until reaching their frying limit. Lastly, the correlation between the total polar compound of frying oil and acrylamide content in french fries fried throughout frying cycles was analysed.

2. Materials and methods

2.1 Materials

Super palm olein (SPO), high-oleic sunflower oil (HOSO), and high-oleic canola oil (HOCO) and shoestring french fries (Kawan, Malaysia) were purchased from local market (Cheras, Malaysia). Acrylamide D-3 and acrylamide analytical standards were purchased from Sigma-Aldrich, Germany.

2.2 Sample collection processes

All oil samples (SPO, HOSO, HOCO) used were not added with antioxidants. The shoestring french fries with a length × width × thickness of 6 cm × 0.8 cm × 0.8 cm. Firstly, ~3.4 L fresh oil sample was filled into the pot and heated to 180±2°C. Once the temperature of the oil reached 180°C, approximately 50 g of french fries were fried for 3.5 mins. Once the 1st frying cycle ended, frying oil was allowed to rest at a consistent interval of 10 mins before proceeding to the next frying cycle (Elaine *et al.*, 2022). The next frying cycle starts once the oil temperature reaches 180°C, a total of 56 consecutive frying processes were conducted, and no oil replenishment was done throughout intermittent frying. The total polar compound of oil was recorded at every interval of the 8th frying cycle. On the other hand, french fries were collected at every 8th frying cycle and allowed to cool at room temperature. The fries sample was packed and stored at -18°C for acrylamide analysis.

2.3 Fatty acid profile

The fatty acid profile in the oil sample was analysed according to the previously established method of Chew *et al.* (2018). The fatty acid methyl esters (FAME) of oil samples were prepared by using 50 mg of oil and followed by adding 950 µL of n-hexane and 50 µL of sodium methoxide in methanol (30 mL/100 mL). The top layer (1 µL) was injected into a gas chromatography (Agilent Technologies 7890A, USA) coupled with a flame ionization detector and a polar capillary column BPX70 (0.32 mm internal diameter, 30 m length and 0.25 µm film thickness; SGE International Pty. Ltd., Victoria, Australia), with a split ratio of 50:1. Oven temperature was programmed from 115 to 180°C at 8°C/min and hold for 17 min and then programmed to 250°C at 20°C/min. Individual fatty acids were identified by comparing the retention times with a certified fatty acid methyl esters (FAME) mix (Sigma-Aldrich, St. Louis, USA), which was analysed under the same operating conditions. The fatty acid composition was expressed as a relative area percentage.

2.4 Determination of tocopherol contents

High performance liquid chromatography (Agilent Technologies 1200 Series, USA) was used to analyse the tocopherol and tocotrienol contents, with a Cosmosil 5PFP packed column (5 µm × 250 mm× 4.6 mm) (Nacalai Tesque, Inc., Japan). The oil sample (0.5 g) was dissolved with 1 mL of isopropanol and the method of determination followed to the previously established method (Chew *et al.*, 2018).

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2.5 Acrylamide extraction from french fries

The 2.5 g sample was mixed with 10 mL ultrapure water, followed by adding 5 mL hexane into the sample-water mixture and vortexed for 5 mins. The solution was centrifuged at 9000 rpm for 5 mins at 4°C. Approximately 4 mL of the transparent layer was pipetted and evaporated to 1 mL of extract. The 900 µL extract was mixed with 100 µL of 2.5 ppm acrylamide-d3 solution. The supernatant solution was filtered through 0.45 µm with a syringe membrane filter. Then the filtered solution was subjected to LC-MS analysis.

2.6 Acrylamide analysis by HPLC-MS

LC-MS condition was done with the HPLC system coupled to a 6460 MS (Agilent Technologies, Inc., CA, USA) detector. The ionization and separation of ions were performed with an AJS-ESI (Agilent Jet Stream Electrospray Ionization) interface and triple quadrupole, respectively. The analytical separation was performed on a Zorbax Eclipse XDB-C18 (150 × 4.6 mm, 5 µm) column with mobile phase A (100% water) 97.5% and B (100% methanol: acetonitrile (1:1) 2.5%. Elution was carried out at 30°C with a flow rate of 0.2 mL/min.

Data acquisition was performed in selected ion monitoring (SIM) mode using the interface parameters of drying gas (N, 100 psi g) flow of 4 l/min, nebulizer pressure of 45 psi g, drying gas temperature of 250°C, vaporizer temperature of 425°C, a capillary voltage of 3 kV, corona current of 4 IA, fragmentor voltage of 55 eV. Ions monitored were m/z 72 and 55 for the quantification of acrylamide in the samples.

2.7 Statistical analysis

All prepared sample was analysed in triplicate (n=3) and the results were interpreted as mean ± standard deviation (SD). All data collected were analysed with a one-way-analysis of variation (ANOVA) and linear regression analysis, the significance value was set at $p<0.05$ using the statistical software Minitab®16.

3. Results and discussion

3.1 Fatty acid composition

Table 1 shows the fatty acid profiles of super palm olein (SPO), high-oleic sunflower oil (HOSO) and high-oleic canola oil (HOCO). In this study, all frying oils contained the highest percentage of oleic acid (C18:1) among other fatty acids ($p<0.05$). Specifically, HOSO contained the highest amount of oleic acid (C18:1) accounting for 86.14%, followed by HOCO (79.10%) and SPO (46.88%), respectively. Another distinctive difference among all frying oils was that SPO contains significantly highest percentage of 33.35% palmitic acid (C16:O), 3.68% stearic acid (C18:0), 13.87% linoleic acid (C18:2). The balanced proportion of saturated and unsaturated fatty acid composition makes palm olein having better frying performance. this is because with high unsaturated fatty acids alone, is susceptible to oxidation (Abd Razak *et al.*, 2021). Overall, high proportions of monounsaturated fatty acid (MUFA) make these three oils suitable to be used as frying oil.

Table 1. Fatty acid profile of high oleic oils.

Fatty acid (%)	SPO	HOSO	HOCO
C12:0	$0.51±0.01^{gA}$	-	-
C14:0	$1.09±0.09^{eA}$	-	-
C16:0	$33.35±0.02^{bA}$	$3.67±0.00^{cC}$	$4.11±0.01^{dB}$
C18:0	$3.68±0.03^{dA}$	$2.85±0.06^{dB}$	$1.53±0.00^{eC}$
C18:1	$46.88±0.24^{aC}$	$86.14±0.03^{aA}$	$79.10±0.10^{aB}$
C18:2	$13.87±0.09^{cA}$	$6.10±0.07^{bC}$	$11.84±0.05^{bB}$
C18:3	$0.62±0.00^{fB}$	-	$2.91±0.02^{cA}$
C20:0	-	$1.24±0.03^{eA}$	$0.51±0.00^{fB}$

Values are presented as mean±SD. Values with different lowercase superscripts within the same column are statistically significantly different ($p<0.05$). Values with different uppercase superscripts within the same row are statistically significantly different ($p<0.05$).

3.2 Tocopherol content

Tocopherol acts as an antioxidant in oils, and plays an important role in preventing the loss of unsaturated fatty acids, with α-tocopherol exhibiting the highest antioxidant activity during frying (Liu *et al.*, 2022). High levels of tocopherol content in oil have a positive effect on the reduction of acrylamide levels in the frying oil and french fries. Table 2 shows the total tocopherols in SPO, HOSO and HOCO. Significantly, SPO contained the highest amounts of β- and γ-tocopherols, followed by α-tocopherol and δ-tocopherol. HOSO and HOCO had similar trends as SPO in terms of percentages of tocopherols but at lower amounts. HOSO contained the highest α-tocopherol, followed by HOCO and SPO, respectively. Meanwhile, the total of β- and γ-tocopherols, SPO had the highest value of 1386 mg/kg, followed by significantly lower amounts of HOCO and

Table 2. Tocopherol content.

Tocopherol (mg/kg)	SPO	HOSO	HOCO
α tocopherol	$228.11±0.43^{bC}$	$486.1±0.98^{bA}$	$431.08±1.91^{bB}$
β+γ tocopherols	$1386.00±0.79^{aA}$	$513.77±2.13^{aC}$	$801.81±4.72^{aB}$
δ tocopherol	$69.03±0.46^{cA}$	$27.62±1.30^{cB}$	$5.67±0.12^{cC}$
Total	1683.14	1007.49	1238.56

Values are presented as mean±SD. Values with different lowercase superscripts within the same column are statistically significantly different ($p<0.05$). Values with different uppercase superscripts within the same row are statistically significantly different ($p<0.05$).

https://doi.org/10.26656/fr.2017.8(4).174

HOSO by 801.81 mg/kg and 513.77 mg/kg, accordingly. SPO had the greatest advantage of having 69.03 mg/kg of δ-tocopherol, while HOSO and HOCO had 27.62 and 5.67 mg/kg, respectively. Among all types of tocopherols, δ-tocopherol was the most stable compound, as it degraded at the slowest rate (Kmiecik *et al.*, 2019).

3.2 Total polar compounds of frying oils

The total polar compounds (TPC) of all oil samples have increased gradually with prolonged frying time. There were significant differences in the total polar compound between different frying cycles of super palm olein (SPO), high-oleic canola oil (HOCO), and high-oleic sunflower oil (HOSO), respectively. It can be observed that both HOCO and HOSO had the lowest TPC content, at which the testometer could not detect the presence of TPC. Xu *et al.* (2020) have also reported a lower TPC content of high oleic oils than generally refined oils. By contrast, SPO had the highest TPC of 1.83% during 1st frying cycle. Consecutively, the TPC of SPO remained the highest as compared to HOCO and HOSO throughout the whole frying cycle. It may probably be due to the higher content of diglycerides that are typically present in palm oil and SPO (6-8%) (Hu *et al.*, 2020; Abd Razak *et al.*, 2021). Moreover, according to tocopherol quantification, SPO has the highest tocopherol contents than other oils. Tocopherol degrades during the early stage of frying, which causes the carbonyl value and total polar compounds to increase significantly (Liu *et al.*, 2022).

HOCO had the second-highest TPC content after SPO throughout the frying cycle. Meanwhile, HOSO had the lowest TPC content throughout the frying cycle which might be explained by the initial lower TPC value. Ahmad *et al.* (2021) reported that palm oil had an overall higher TPC content than sunflower oil and soybean oil throughout the frying cycle. Specifically, HOSO only started to show a significant increase in TPC at the 16th frying cycle with a value of 0.17%, which was still considerably low. The low TPC value in high oleic oils might be due to its monounsaturated fatty acid content (oleic acid) present dominantly in the composition.

Overall, SPO had a linear increase of TPC content from the 1st to 56th frying cycle, evaluated from the linear significant difference small letter script in Figure 1. Meanwhile, HOCO had two plateau moments in terms of TPC. Overall, the TPC of super palm olein, high-oleic canola oil, and high-oleic sunflower oil fell within the range of 25% TPC after the 56th frying cycle, indicating that they were still safe to be used.

3.3 Acrylamide analysis of french fries fried at different frying cycles

There were significant differences in the acrylamide content between different frying cycles of french fries fried by SPO, HOCO and HOSO, respectively. Based on Figure 2, french fries fried with SPO had the lowest acrylamide content throughout the frying cycle ($p<0.05$). Meanwhile, HOSO had the highest acrylamide concentration throughout frying cycles. Lee Kuek *et al.* (2020) also reported palm oil to have the lowest acrylamide concentration as compared to soybean oil and sunflower oil. However, the results showed palm oil had high acrylamide content of 447 μg/kg at 1st frying cycle and did not show significant differences with the increase of the frying cycle. Furthermore, the acrylamide content of fries fried with sunflower oil, soybean oil, and red palm olein were fluctuating throughout the frying cycles.

Compared to Figure 2 of this study, the acrylamide content in french fries was increased with frying cycles. French fries fried by SPO had a significant increase in acrylamide concentration at every 8th frying cycle except for the 48th and 56th frying cycles. Meanwhile, french fries fried by HOCO and HOSO showed slightly different increasing acrylamide concentrations. HOCO had 8th and 16th frying cycles showed an insignificant

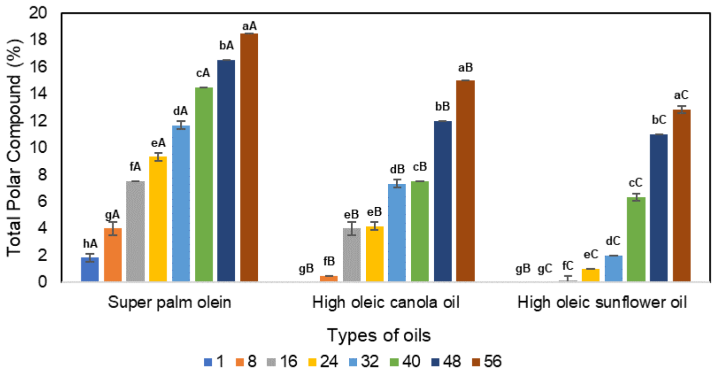

Figure 1. Total polar compounds of frying oils. Bars with different lowercase superscripts are statistically significantly different ($p<0.05$) compared to the different frying cycles on one type of oil. Bars with different uppercase superscripts are statistically significantly different ($p<0.05$) compared to the different types of oil in the same frying cycle.

https://doi.org/10.26656/fr.2017.8(4).174

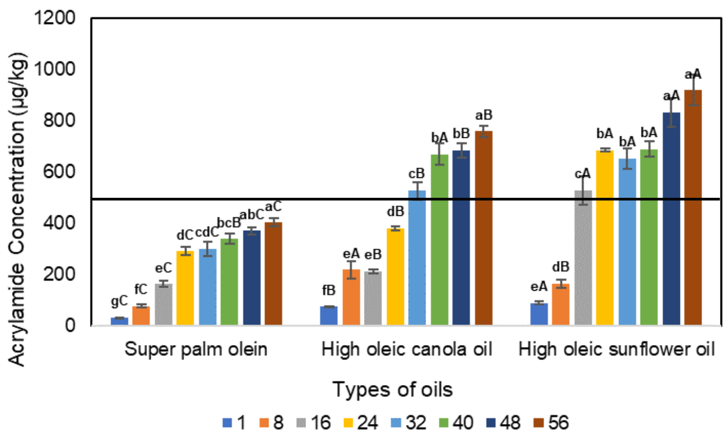

Figure 2. Acrylamide content of french fries fried at different frying cycles. Bars with different lowercase superscripts are statistically significantly different (p<0.05) compared to the different frying cycles on one type of oil. Bars with different uppercase superscripts are statistically significantly different (p<0.05) compared to the different types of oil in the same frying cycle. The permissible limit of acrylamide content in food based on EU regulation 2017 is represented by the black solid line.

difference, and a similar case happened between 40th and 48th frying cycles too. On the other hand, the HOSO 24th to 40th frying cycle showed insignificant differences, followed by the 48th and 56th frying cycles. At the 40th frying cycle, HOCO and HOSO showed insignificant differences in acrylamide content, which showed the similarity of acrylamide content using high-oleic oils.

Overall, the content of acrylamide in French fries fried by SPO fell within the range of 500 µg/kg after the 56th frying cycle, indicating they were still safe to be consumed. Meanwhile, french fries fried by HOCO and HOSO reached 500 µg/kg after the 32nd and 16th frying cycles, respectively. This can confirm that the oils containing high levels of unsaturated fatty acids resulted in higher levels of acrylamide in fries. This is due to the susceptibility of carbonyl groups in oleic acid to break down at a higher temperature, thus accelerating the Maillard reaction, and producing higher acrylamide content (Daniali *et al.*, 2016).

3.4 Correlation analysis between total polar compounds of frying oil and acrylamide content of french fries

Across frying cycles, the TPC results showed that SPO had the highest TPC value throughout the frying cycle. However, the acrylamide content in the french fries showed that SPO indeed had the lowest acrylamide content as compared to HOSO and HOCO. Thus, a correlation between acrylamide content in the total polar compound of frying oil and the acrylamide content of french fries was analyzed. The high ratio of polyunsaturated fatty acids in frying oils should have a positive effect on acrylamide formation (Daniali *et al.*, 2016). Based on the correlation analysis in Table 3, a highly significant positive linear relationship was found between the total polar compounds in SPO ($r = 0.899$; $p<0.01$), HOCO ($r = 0.997$; $p<0.01$), and HOSO ($r = 0.888$; $p<0.01$) and acrylamide levels in french fries. Lee Kuek *et al.* (2020) reported a positive correlation between acrylamide content in fries with p-anisidine and free fatty acids, but not with peroxide value and total oxidation status. Ahmad *et al.* (2021) have also reported a weak relationship between lipid oxidation and hydrolysis on the acrylamide concentration. Overall, there was a positive correlation between the total polar compounds of frying oil and acrylamide content in french fries during intermittent frying throughout the frying cycles.

Table 3. The Pearson correlation between acrylamide content and total polar compounds (TPC).

Oil	Correlation	P value
Super palm olein	+ (r = 0.899)	0.00
High-oleic canola oil	+ (r = 0.977)	0.00
High-oleic sunflower oil	+ (r = 0.888)	0.00

4. Conclusion

Overall, all frying oils (super palm olein (SPO), high oleic canola oil (HOCO), and high oleic sunflower oil (HOSO)) used in this study contained high oleic acids, with significant amounts of tocopherol contents. Specifically, SPO contained the highest tocopherol content, followed by HOCO and HOSO, respectively. SPO had the highest value of total polar compounds (TPC), followed by high-oleic canola oil (HOCO) and high-oleic sunflower oil (HOSO). The overall high TPC value of SPO was attributed to the tocopherol content that degrades into carbonyl compounds during intermittent frying. Nevertheless, french fries fried with HOSO had the highest value of acrylamide content in every frying cycle, followed by HOCO and SPO. A strong positive correlation and contribution were found between TPC and acrylamide formation. High oleic oils can be used for intermittent frying; however, the degree of unsaturation still contributes to the acrylamide content

https://doi.org/10.26656/fr.2017.8(4).174

in fried foods. This study concluded frying oil with balance fatty acid composition of saturated and monounsaturated fatty acids, with significant antioxidant content is suitable for intermittent frying. Nevertheless, frying oil should not be used consecutively in french fries to reduce acrylamide formation in french fries.

Conflict of interests

The authors declare there is no financial or non-financial conflict of interest related to this work.

Acknowledgements

The authors would like to thank the research funds by the Malaysia Palm Oil Council (IND-FAS-2022/001) is gratefully acknowledged.

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