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Physicochemical Changes of Reused Cooking Oils Used to Prepare Potato Chips, Chicken, and Beef

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ABSTRACT

The physicochemical characteristics of cooking oils can be altered by deep-frying techniques, which lowers the oil's quality. In this study three different food types – potato chips, chicken and beef – were deep-fried using reused soya bean or sunflower cooking oils, and the physicochemical characteristics of the reused oil were measured after each round of fry-ing. The study further compared the impact of reused cooking oil on the growth of *Aspergillus flavus* fungal colonies. By the 8th turn of frying the mean viscosity difference of beef re-used cooking oil viscosity was 42.83 +/- 0.98 cSt, significantly different from the potato chips reused cooking oil at 21.62 +/- 1.26 cSt. For both reused soya bean and sunflower oil samples, the mean difference of AV and PV generally increased. This change in the AV and PV values was influenced by the type of food being prepared as well as the frequency of re-peated use. Furthermore, media supplemented with reused oil leftover from beef frying cycles had significantly increased fungal growth in comparison to fungi grown on fresh oil supplemented media. Therefore, appropriate guidelines are required to monitor the quality of reused cooking oil and avoid health risks associated with using poor quality oils to pre-pare food for consumption.

Keywords: Acid value, *Aspergillus flavus*, Peroxide value, Reused cooking oil

INTRODUCTION

Deep frying food is a popular method for food preparation in many parts of the world. Households, public gatherings and restaurants are some of the common places where deep-fried foods prepared by reusing excess vegetable oil are served (Awuchi et al., 2018). Many physicochemical changes take place during frying such as starch gelatinization characterized by swelling of starch granules; protein denaturation; browning and crust formation, which develops as a result of drying on the surface of the fried product. These physical and chemical changes lead to structural transformations at both the macro and micro level of the edible oils (Tolulope et al., 2022).

The method of deep-frying used in most households and fast-food restaurants involve the process of immersing food in cooking oil at high temperatures of 175 °C to 190 °C (Dangal et al., 2024). The oil into which the food is immersed acts like a heat transferring compound. The process has a

preserving action caused by thermal destruction of microorganisms, enzymes, and reduction of water activity on the surface of the food (Oke et al., 2017). Fat absorption and lipid exchanges are the main physical changes involved (Dangal et al., 2024). These changes in food and oil depend on various factors such as the characteristics of the food, oil type, surface/volume ratio of the oil, rate of air incorporation into the oil, length of immersion, and the kind of material the frying container is made of (Oke et al., 2017). Chemical changes include interactions between the food and oxidized lipids and the alteration of fatty acids arrangement on the glycerol backbone of the triglyceride molecule which can change the viscosity of the oils (Urigacha, 2020). Acid value (AV) is used to measure the amount of fatty acids which have been liberated from triglycerides found in oil. This can happen as a result of lipase action however other factors such as heat, light, exposure to air, moisture and microorganisms can accelerate the hydrolysis of the triglycerides (Urigacha, 2020; Valle et al., 2024). The peroxide value (PV) of edible oils is an indicator of freshness and measures the primary oxidation of hydroxyl groups of unsaturated fatty acids into hydroperoxides and peroxides. These are very unstable and decompose to produce secondary oxidation products. The cooking process also promotes polymerization, oxidation, cyclization and degradation of the oils eventually resulting in the production of volatile organic compounds (Ganesan et al., 2019). In addition, under severe thermal oxidative conditions the fatty acid configuration is transformed from the cis to trans isomeric state. Trans fats exist as solids at room and human body temperature similar to saturated fat and are linked to detrimental health outcomes (Tsuzuki et al., 2010).

Oil deterioration can also occur during the storage time because oil samples are highly sensitive to oxidation when exposed to light. Extended exposure to sunlight can cause oil to undergo accelerated oxidation due to a reaction of the long chain hydrocarbon with oxygen in the presence of ultraviolet (UV) radiation resulting in the formation of harmful aldehydes (Freis and Vemulapalli, 2025). These absorb visible light to appear yellow at low concentrations and brown at higher concentrations after extended exposure to sunlight and their linear increase during frying could make them a good quality evaluation index for reused oil (Cairns and Forbes, 2020; Liu et al., 2022). In addition, oil samples are susceptible to microbial contamination either due to the oils been stored in close proximity to peanuts, rice or fruits like bananas or due to poor refining and packaging from the manufacturing company (Al-harethi et al., 2016). *Aspegillus flavus* is an example of an important microbial contaminant usually associated with peanuts and cereals but can also be present in oil (Li et al., 2014). *A. flavus* is capable of degrading oil by hydrolyzing triglycerides due to its lipase enzymes (Osuoha et al., 2020).

The commonly used polyunsaturated cooking oils in Zambia are made from sunflower or soya bean. The aim of this research was to evaluate the chemical changes occurring in the quality of locally available soybean and sunflower oils under deep-frying heating at 120 -150 °C (for potato chips), 170-200 °C (for beef and chicken) and during storage conditions at room temperature (25 °C). Our results suggest that repeated reheating of oils at high temperature increases AV and PV with each reuse. In addition to these results, the reused oils were found to be much thicker and more viscous compared to the fresh oil samples. Furthermore, our results showed an increased growth rate of *A. flavus* on media supplemented with reused cooking oil.

MATERIALS AND METHODS

Sunflower and soya bean cooking oils were procured from commercial suppliers in Lusaka, Zambia. Beef, chicken and irish potatoes were also procured from local retail outlets. Reagents and solvents were obtained from Sigma-Aldrich. Fungal cultures were isolated from groundnut samples procured from an open market in Lusaka. The fungal isolates were cultured on potato dextrose agar (PDA) and water agar culture media enriched with reused cooking oil.

Sample preparation and processing

Three hundred gram-samples of chicken were first boiled for five minutes and seasoned with Royco spice. Excess water was evaporated by heating for three to five minutes before deep-frying the pieces in the cooking oil for 8 minutes at temperature ranges between 170°C to 200 °C. Three hundred-gram samples of beef were boiled for five minutes and seasoned with spices before deep-frying in vegetable oil for 8 minutes. Frying temperatures used in the processing of chicken were adopted for beef using a deep-fryer (Philips Deep Fryer - HD6103: Cool wall). Three hundred grams of potato tubers were peeled, cut into small sizes, washed and rinsed in distilled water and excess moisture was removed on blotting paper. Thereafter they were deep-fried in the cooking oil for 8 minutes. The deep-frying process was repeated successively for eight turns whilst reusing the same cooking oil samples on each of the food samples. 250 mL of each oil samples were collected from each of the six deep-fried oil samples for analysis in triplicate.

Determination of PV

The American Oil Chemists' Society (AOCS) Official Method Cd 8–53 (Zhang et al., 2021) was utilized to determine the PV of the recycled cooking oils. Briefly, 10-12 g of oil samples were weighed and dissolved in 30 mL acetic acid: chloroform (3:2, v/v) mixture with occasional shaking. Samples were then treated with 1 mL of 10 % potassium iodide solution, and iodine was titrated with a solution of 0.01 N sodium thiosulfate in the presence of 1 % starch solution. The PV expressed as the milli-equivalent of active oxygen per kg of oil ($meqO_2/kg$), was calculated using equation (1):

$$\left(PV\,\frac{meqO_2}{kg}\,sample\right) = V \times N \times \frac{1000}{Sample} W\,(g) \qquad (1)$$

Where V = volume (mL) of sodium thiosulfate consumed, N = normality of sodium thiosulfate and W = weight of oil sample in grams.

Determination of the AV

The American Oil Chemists' Society (AOCS) Official Methods Cd 3d-63 (Firestone, 2017) was utilized to determine the AV changes in the oil samples. 10 g of oil samples from the deep-frying experiments was transferred into 250 mL conical flasks to which 50 mL of 99 % ethanol was added. The mixture was heated for 5 minutes and then cooled for a minute before adding 1mL of phenolphthalein indicator. The mixture was afterwards titrated with 0.1 N NaOH and the end points were indicated by the appearance of a pink color. The acid values were then calculated with the use of equation (*2*):

$$\left(AV\,\frac{KOH}{g}\,oil\right) = 56.1 \times V \times \frac{N}{W} \qquad (2)$$

Where, V = volume of standard KOH solution in mL, N = normality of standard KOH solution, W = weight of oil sample in grams.

Determination of the viscosity

The standard test for viscosity by Dip-Type Viscosity Cups was used to determine the oil sample's viscosity values according to the ASTM D4212-16 (Standard Test Method for Viscosity by Dip-Type Viscosity Cups) standard. These tests were conducted with the use of a Zahn cup (orifice diameter 1.98 mm/0.08* and Zahn range 33.5-80 cSt). 300 mL oil sample was strained into a 500 mL beaker and the oil sample was stirred thoroughly. The Zahn cup was then carefully dipped into the oil sample and the temperature was recorded. Subsequently, the Zahn cup was later on completely lifted out of the oil filled beaker and the time for the appearance of the first "break" in the flow was recorded. These steps were repeated until consistent results were obtained. The viscosity was then expressed in Zahn seconds and thereafter into Centistokes (cSt) using equation 3 for Zahn Cup #1;

$$V = 1.1(t - 29) \qquad (3)$$

Were V= viscosity, t= time in seconds

Isolation and growth rate of A. *flavus*

One kilogram of raw groundnuts was procured from an open-air market in Lusaka city and three sets of the 20 groundnut-samples were sampled from the total stock. The groundnuts were washed in distilled water and sterilized in 3.5 % v/v sodium hypochlorite solution. The groundnut samples were transferred onto sterile petri dishes with sterile moist filter paper to allow for development of mycelium. *A. flavus* was isolated and inoculated on Potato Dextrose Agar (PDA). The inoculated fungus was then left to grow under observation for 72 hours.

***A. flavus* growth rate analysis**

Oil enriched water agar culture media was prepared by taking 8 g of agar-agar powder, and suspending it into a 750 mL storage jar containing 0.5 L of distilled water and heated while stirring consistently. The culture media were autoclaved at 121 °C for 15 minutes at about 15 pounds per square inch. The prepared water agar culture media was allowed to cool and aseptically transferred into each plate, covered in a lamina flow chamber. Once the agar had solidified, the lids of the petri dishes were replaced. The plates were stored in a refrigerator at 5 ºC for 24 hours after which 50, 200, 500, 1000 and 2000 µl (0.05, 0.2, 0.1, 1 and 2 %) of either fresh or reused (beef) soya oil was spread on each of the petri dishes respectively. 10 cm cubes of *A. flavus* mycelia growing on one of the PDA media Petri dishes after 3 days were sub-cultured onto each of the oil enriched water agar media. Petri dishes were incubated at 25 ºC and the fungal growth was measured in terms of their colony diameters over a 72-hour period.

Statistical analysis

All statistical analysis was done using Graph pad prism version 9 software. The average values of repeated experiments were expressed as mean and standard deviations. For comparison between more than 2 groups, data was analyzed by two-way analysis of variance and post hoc analysis was done using Turkey's multiple comparison test (unless indicated otherwise). For comparison between 2 groups, data was analyzed using the unpaired t-test. Significance was considered if the p value was 0.05 or lower.

RESULTS

For a period of 8 days, triplicate titrations of oil samples treated by repeated deep-frying cycles were conducted to determine the viscosity, PV, and AV values. The growth rate of *A. flavus* on oil enriched water agar plates was also studies using the beef reused oil samples.

Changes in physical appearance and viscosity of reused oil samples

The first set of questions aimed to study the changes in the physical appearance and viscosity of the reused oil samples. Figure 1 shows that there was a clear trend in increased browning of the food samples and thickening of the oil with each increase in the turn of frying. The viscosity of the reused potato chip soyabean cooking oil increased from 16.32 ± 0.32 cSt to 37.94 ± 1.26 cSt whereas the viscosity of the reused beef soya bean cooking oil increased from 16.32 ± 0.32 cSt to 59.15 ± 0.98 cSt (supplementary table 1). The differences in the mean viscosities presented in table 1 were compared using an unpaired t-test. After the 5th and 8th turn of frying the increase in viscosity in the beef reused cooking oil was significantly more as compared to that of the cooking oil used to prepare the potato chips.

Changes in AV and PV of the reused cooking oil samples

The next set of experiments compared the AV and PV of the cooking oil samples after each turn of frying. Generally, the difference in AV and PV values after each turn of frying increases relative to the unused cooking oil for each food type used (figure 2). AV increased from 0.22 to 1.01, 1.31 and 1.03 mg KOH/g oil for the soya bean oil reused to prepare chicken, beef and potato chips samples respectively (Supplementary table 2). Similarly, AV increased from 0.18 to 0.97, 1.12 and 0.91 mg KOH/g oil for the sunflower oil reused to prepare chicken, beef and potato chips samples respectively (supplementary table 2). A two-way ANOVA was used to perform pairwise comparisons between the differences in mean AV after each turn of frying relative to the AV of unused oil. Results of the pairwise comparisons are shown in table 2. After the 5th turn of frying the 95 % confidence limit of difference was significant for all pairwise comparisons of the sunflower oil samples but not significant for the soya bean oil.

However, after the 8th turn of frying the 95 % confidence limit of the mean difference was significant for potato versus beef and chicken versus beef comparisons for both soya bean oil and sunflower oil reused oil samples. PV increased from 1.70 to 6.37, 8.13 and 5.43 mEq O_2/kg for the soya bean oil reused to prepare chicken, beef and potato chips samples respectively. Similarly, PV increased from 1.27 to 6.77, 7.47 and 5.83 O_2/kg for the sunflower oil reused to prepare chicken, beef and potato chips samples respectively (Supplementary table 3). Pairwise comparisons between the differences in PV after each turn of frying relative to the PV of unused oil are reported in table 3.

Interestingly after just 1 turn of frying significant differences could be seen in the PV values between most pairs for both soya bean and sunflower reused oils. Furthermore table 4 shows that for both soya bean oil and sunflower oil the source of variations in the AV and PV between the pairs was coming from both the food type and the turn of frying with P values ranging from less than 0.0001 to 0.0059.

Effect of reused oil on the growth of *A. flavus*

In the final part of this study, the growth rate of *A. flavus* on media containing reused cooking oil was investigated. Table 5 compares the diameter of the *A. flavus* colonies on media containing fresh or reused beef oil at different dilutions over a period of 72 hours. From this data we can see that increasing the amount of the beef reused oil in the media resulted in increased colony diameter. Comparison of the fungal diameters using an unpaired t-test showed that after 48 hours there was significant difference in the colony diameters of the

colonies grown on media containing 1 – 2 % beef reused oil in comparison to the colonies grown using the fresh oil (at $p \leq 0.05$). However, after 72 hours, the difference in diameter between the fresh oil and beef reused oil was significant even when the colonies were grown on agar containing only 0.05 % of the beef reused oil.

DISCUSSION

The physical and chemical properties of soya bean and sunflower cooking oil samples treated by repeated deep-frying cycles were analyzed. Our results suggest that reheated oils gradually change in terms of their chemical and physical properties, as depicted by the increased viscosity, browning of the food samples and the cooking oil as well as changes in AV and PV. According to FAO/WHO recommendations the standard permissible AV in edible oils is 0.6 mg KOH/g oil (Alimentarius, 2019; Tesfaye and Mengistie, 2016). For both soya bean oil and sunflower oil, the process of reusing the oils resulted in AV that was beyond the permissible limits after the 3rd turn of frying. AV is generally used as a measure of how edible an oil sample is hence these results suggest soya bean and sunflower oil that have been reused for deep frying more than 3 times should be used with caution. PV of edible oils is an indicator of freshness and oils with higher PV are considered unstable and more likely to become rancid. FAO/WHO recommend refined oils should have a PV of up to 10 mEq/Kg (Alimentarius, 2019). In this study the PV of both soya bean oil and sunflower oil was within the permissible range even after the 8th turn of frying for all food samples. However, it should be pointed out the beef reused oil samples showed the greatest increase in PV hence care should be taken when cooking oil used to fry beef is reused.

Our results show that both the number of times the cooking oil is reused and the type of food being prepared contribute to the variation in the chemical properties. These observed changes in the physical and chemical characteristics of the reused oils are consistent with previous studies. Dodoo et al (2022) showed that PV and AV values were elevated in reheated sunflower and soybean after three cycles of deep frying yam fries (Dodoo et al., 2022). Nduka et al. (2021) compared the effect of prolonged heating on different edible oils, they show an increase in viscosity, PV and free fatty acid for soybean oil after prolonged heating while also pointing out that the stability of the different oil types under heat treatment varies (Nduka et al., 2021). In a separate study, Nawaz et al. (2023) also show a frying cycle dependent exponential increase in saponification, PV and AV of canola oil and ghee used to fry fish. The repeated heating of the oils reduced their antioxidant properties and increased the oxidative stress of the canola oil and ghee (Nawaz et al., 2023). This is consistent with Yilmaz et al (2023) who compared oxidative changes in 10 different types of reheated cooking oils and their results showed a general increase in PV for all the oil after 10 cycles of frying (Yılmaz et al., 2023). Taken together these studies corroborate well with our findings. The increased AV of reheated oils can be attributed to the thermal hydrolysis of triglycerides to produce fatty acids which increases as the number of deep-frying cycles increases (Dodoo et al., 2022; Li et al., 2016). On the other-hand the higher PV in the reheated oils is due to increased thermal oxidation and a contributing factor to this could be the loss of anti-oxidants such as α-tocopherol due to the frying process (Rajoriya and Bigoniya, 2024). Hence there is need to provide guide lines on the safe reuse of cooking oil taking into account the type of oil and the different food matrices been prepared.

Our study also showed that reused oils can support the growth of microorganism. After 72 hours significant growth in *A. flavus* was observed in plates containing beef reused oil even at 0.05 % of added oil in comparison to unused oil. This could possibly be due to increased availability of carbohydrates, fats and other complex compounds formed in the reused oil which supported the growth of the fungi (Lamb, 2017).

Frying can lead to an increase in the moisture content of the cooking oil as water from the food evaporates as steam and some of the vapor can condense into the oil. This increased water content can not only support microbial growth but can also accelerate lipid hydrolysis and oxidation by enhancing enzyme activity, although the additional moisture can also dilute reactive oxygen species hence also having an inhibitory effect on oxidation (Pooja and Sukhneet, 2020). However repeated heating can cause a significant reduction in the moisture as reported by Dodoo et al (2022) who observed a reduction of moisture in repeatedly heated oils used to deep fry yam fries (Dodoo et al., 2022). In this study the moisture content of the food or reused oils was not determined however this can be explored in future studies as this can provide a comparative analysis of moisture loss of different food during frying and how this can be associated to the deterioration of the reused oils.

It is a widely held view that heating kills the harmful microbes however, aflatoxins which are fungal metabolites decompose at high temperatures in the range of 237 °C to 306 °C (Guo et al., 2021). This implies that utilizing *A. flavus* contaminated oil in the culinary process is likely to intoxicate food samples due to the presence of the aflatoxins. There is need for more research that looks at assessing levels of microbial toxins in reheated cooking oils.

Using animal models research has shown that chronic consumption of repeatedly heated oils is associated with detrimental health effects such as increased fat accumulation in liver tissue, oxidative stress, serum triglyceride, total cholesterol and high density lipoprotein as well as a reduction in leukocyte and hemoglobin levels (Ambreen et al., 2020; Perumalla Venkata and Subramanyam, 2016; Seema et al., 2023). Reheating oil increased the levels of the harmful polycyclic aromatic hydrocarbons benzo[a]pyrene, benzo[a] anthracene and benzo[b]fluoranthene suggesting increased carcer risk associated with reused oils (An et al., 2017). Rajendran et al. (2022) provides a comprehensive review of publications reporting on tumorigenic and genotoxic effects of reused cooking oils (Rajendran et al., 2022). More investigations are needed to fully understand the health risks of repeatedly reheated oils however research efforts have also looked at developing methods that can reduce levels of harmful chemicals in the reheated oils. Suhaimi et al. (2022) describe a smart filtering system based on activated charcoal as a solution for road side traders who regularly reuse cooking oil to prepare street food (Suhaimi et al., 2022).

CONCLUSION

The study contributes to our understanding of how repeated heating affects the quality of cooking oil that is used to prepare different food types. Overall, these results show that the chemical properties of reheated oil progressively change after each turn of frying thereby reducing the quality of the reused oil. Sunflower oil is comprised of 64.85 % linoleic acid and 19.49 % oleic acid whereas soya bean oil is comprised of 54.67 % linoleic acid and 22.24 % oleic acid (Cherif and Slama, 2022). Deep fat frying changes the profile of fatty acids hence future work can explore the changes in fatty acid

profile and volatile organic compounds of the reused oil when different food matrices are used and how these may be associated with microbial growth as well as the production of toxic microbial metabolites. Such studies are needed to open our minds to the knowledge of the effect of repeated reheating of cooking oil on food quality. Lastly there is need to increase public awareness on the need to monitor the quality of reused oils and support innovations that would provide low-cost methods for both monitoring and purifying of reused oils.

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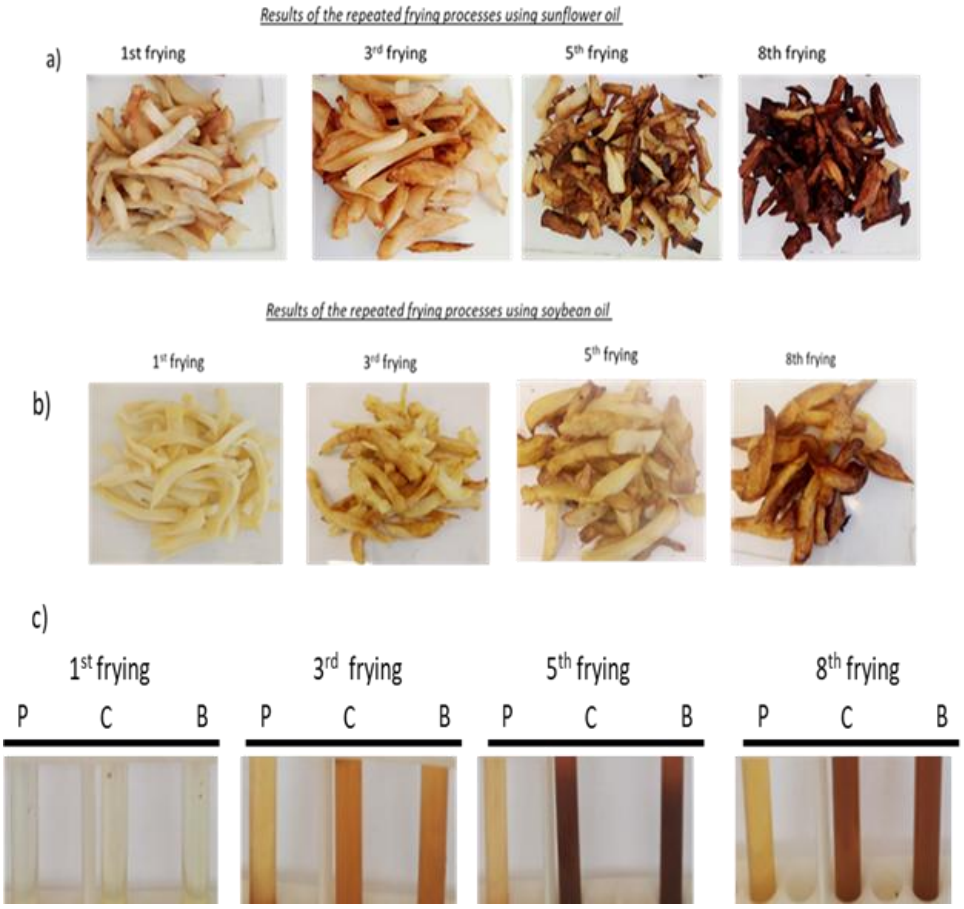

Figure 1: Potato chips were fried in sunflower or soya bean oil. Similar frying times (8 min) and temperatures (120 -130 °C) where used in this process. Representative images show the intensity of browning of potato chips after the 1st, 3rd and 5th turns of frying in: a) sunflower oil b) soya bean oil. c) Shows the change in physical appearance of the soya cooking oils used to prepare potato chips (P), chicken (C) and beef (B) during the frying turns.

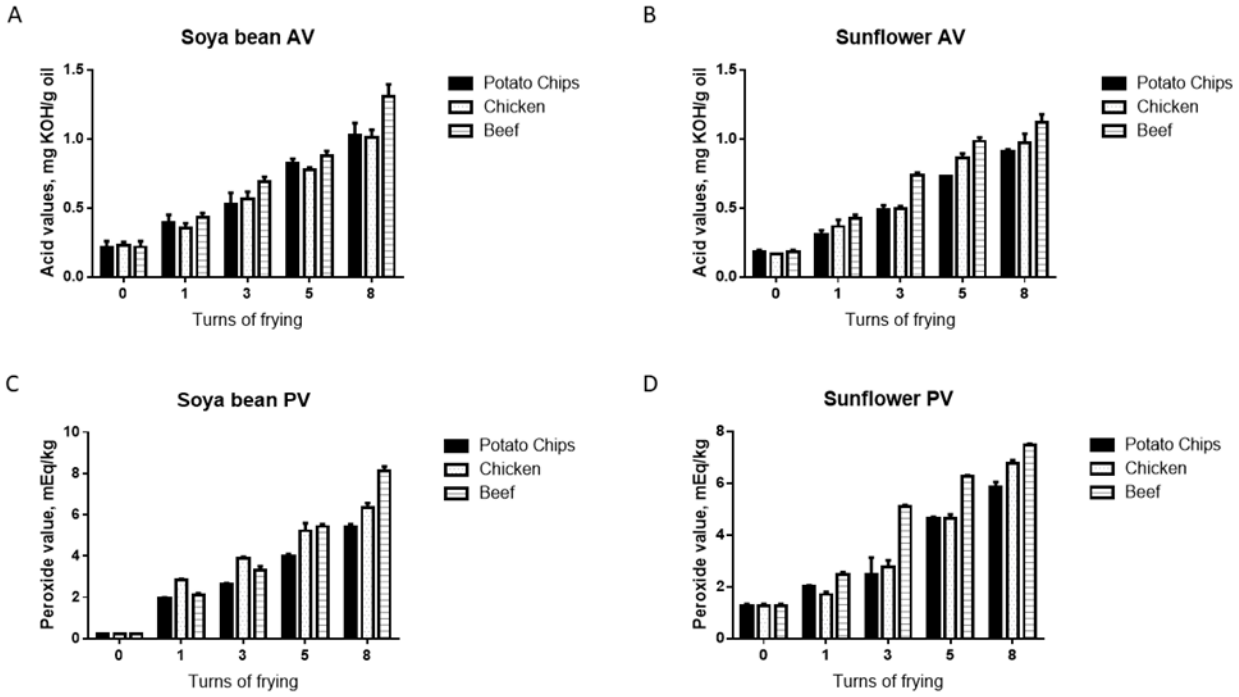

Figure 2. Potato chips were fried in soya bean or sunflower cooking oil. Similar frying times (8 min) and temperatures (120 -130 °C) where used in this process. A. soya bean oil AV values. B. Sunflower oil AV values. C. Soya bean oil PV values. D. Sunflower oil PV values. Figures show mean ± SD, n= 3.

Table 1. Comparison of difference in mean values in the viscosity of reused soya bean oil samples using the unpaired t-test.

Turns of frying	ΔViscosity (cSt)		P-Value	Significant
	Potato Chips mean ± SD	Beef mean ± SD		
1	1.83 ± 0.67	1.24 ± 0.57	0.306	No
3	6.49 ± 0.74	8.00 ± 0.23	0.028	No
5	16.23 ± 0.43	29.08 ± 0.18	< 0.0001	Yes
8	21.62 ± 1.26	42.83 ± 0.98	< 0.0001	Yes

Table 2. Pairwise comparison of differences in mean AV of reused soya bean and sunflower reused oil samples (n=3)

Pairwise comparison	Parameter: AV					
	Soya bean Oil			Sunflower Oil		
	Δ means	95 % CL of Difference	Sig.	Δ means	95 % CL of Difference	Sig.
Turn of frying No. 1						
Potato Vs. Chicken	0.04	-0.08 to 0.15	No	-0.06	-0.13 to 0.02	No
Potato Vs. Beef	-0.04	-0.15 to 0.08	No	-0.11	-0.18 to -0.04	Yes
Chicken Vs. Beef	-0.07	-0.19 to 0.04	No	-0.06	-0.13 to 0.02	No
Turn of frying No. 3						
Potato Vs. Chicken	-0.04	-0.15 to 0.08	No	-0.01	-0.08 to 0.06	No
Potato Vs. Beef	-0.17	-0.28 to -0.06	Yes	-0.25	-0.32 to -0.18	Yes
Chicken Vs. Beef	-0.13	-0.24 to -0.02	Yes	-0.24	-0.31 to -0.17	Yes
Turn of frying No. 5						
Potato Vs. Chicken	0.05	-0.07 to 0.16	No	-0.13	-0.20 to -0.06	Yes
Potato Vs. Beef	-0.06	-0.17 to 0.06	No	-0.25	-0.33 to -0.18	Yes
Chicken Vs. Beef	-0.10	-0.22 to 0.01	No	-0.12	-0.19 to -0.05	Yes
Turn of frying No. 8						
Potato Vs. Chicken	0.02	-0.09 to 0.13	No	-0.07	-0.14 to 0.01	No
Potato Vs. Beef	-0.28	-0.39 to -0.17	Yes	-0.21	-0.29 to -0.14	Yes
Chicken vs. Beef	-0.30	-0.41 to -0.19	Yes	-0.15	-0.22 to -0.08	Yes

Table 3. Pairwise comparison of differences in mean PV values of reused soya bean and sunflower reused oil samples (n=3)

Pairwise comparison	Parameter: PV					
	Soya bean Oil			Sunflower Oil		
	Δ means	95 % CL of Difference	Sig.	Δ means	95 % CL of Difference	Sig.
Turn of frying No. 1						
Potato Vs. Chicken	-0.90	-1.24 to -0.56	Yes	0.32	-0.13 to 0.76	No
Potato Vs. Beef	-0.17	-0.50 to 0.17	No	-0.47	-0.91 to -0.02	Yes
Chicken Vs. Beef	0.73	0.40 to 1.07	Yes	-0.78	-1.23 to -0.34	Yes
Turn of frying No.						
Potato Vs. Chicken	-1.25	-1.59 to -0.91	Yes	-0.28	-0.73 to 0.16	No
Potato Vs. Beef	-0.67	-1.01 to -0.33	Yes	-2.62	-3.06 to -2.17	Yes
Chicken Vs. Beef	0.58	0.25 to 0.92	Yes	-2.33	-2.78 to -1.89	Yes
Turn of frying No. 5						
Potato Vs. Chicken	-1.22	-1.56 to -0.88	Yes	0.02	-0.43 to 0.46	No
Potato Vs. Beef	-1.43	-1.77 to -1.10	Yes	-1.60	-2.05 to -1.15	Yes
Chicken Vs. Beef	-0.22	-0.55 to 0.12	No	-1.62	-2.06 to -1.17	Yes
Turn of frying No. 8						
Potato Vs. Chicken	-0.93	-1.27 to -0.60	Yes	-0.93	-1.38 to -0.49	Yes
Potato Vs. Beef	-2.70	-3.04 to -2.36	Yes	-1.63	-2.08 to -1.19	Yes
Chicken Vs. Beef	-1.77	-2.10 to -1.43	Yes	-0.70	-1.14 to -0.25	Yes

Table 4. Source of variation in AV and PV values

Parameter	Cooking oil type	Source of variation			
		No. of Frying		Food	
		P value	Summary	P value	Summary
Acid value	Soya bean oil	< 0.0001	Yes	0.0059	Yes
	Sunflower oil	< 0.0001	Yes	< 0.0001	Yes
Peroxide Value	Soya bean oil	< 0.0001	Yes	< 0.0001	Yes
	Sunflower oil	< 0.0001	Yes	< 0.0001	Yes

Table 5. Changes in the colony diameter of *A. flavus* grown on media containing fresh or beef reused oil (n=2)

Treatment (% oil added)	Colony diameter after 48 hours (cm)		P value	Colony diameter after 72 hours (cm)		P value
	Fresh oil mean ± SD	Beef reused oil mean ± SD		Fresh oil mean ± SD	Beef reused oil mean ± SD	
0.05	1.150 ± 0.212	1.450 ± 0.212	0.292	1.450 ± 0.212	2.250 ± 0.071	0.037
0.20	1.450 ± 0.071	1.450 ± 0.071	1.000	1.600 ± 0.000	3.200 ± 0.283	0.015
0.50	1.750 ± 0.071	1.800 ± 0.283	0.831	1.850 ± 0.071	3.400 ± 0.283	0.017
1.00	1.550 ± 0.212	2.400 ± 0.141	0.042	1.850 ± 0.071	4.300 ± 0.283	0.007
2.00	1.600 ± 0.141	5.200 ± 0.424	0.008	1.950 ± 0.071	7.450 ± 0.495	0.004