

Heating Effect on Quality Characteristics of Mixed Canola Cooking Oils; Fatty Acids, Physiochemical and Statistical Analysis

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Abstract

Background

The subcontinent is famous for a variety of seasoned foods cooked in vegetable oils on high temperature. Therefore this work planned to evaluate the effect of heating on the quality of canola-based vegetable cooking oils (CBVCOs). CBVCOs were analyzed for fatty acid preparing methyl esters and physicochemical analysis of the oils.

Results

A large variation was observed in the fatty acids compositions of different CBVCOs coded as K-1 to K-5. CBVCOs were found rich in unsaturated 9-octadecadienoic acid (oleic acid C18:1) and 9,12-octadecadienoic acid (linoleic acid C18:2) along saturated Octadecanoic acid (stearic acid C18:0). Results reveals that CBVCOs are blended together in the range of 4–30% with other vegetable oils and animal fat. Quality of CBVCOs further declined after heating at 100°C, 200°C and 350°C respectively. Quality parameters of CBVCOs were changed after heating as color (510–520 nm to 570–600 nm), mass 220–237 g to 210–225 g, volume 250 mL to 239 mL, pH (6.76–6.89), specific gravity (0.87–0.92), refractive index (1.471–1.475), saponification value (0.7–2.5), un-saponifiable matter (2.4–9.8%) and acid value (1.20–5.0 mg KOH).

Conclusion

Heating exhibited a significant effect (P -value < 0.05) in pH, specific gravity and un-saponifiable matter. Large-scale variation in fatty acid composition and physicochemical parameters support to conclude that cooking at high temperatures affects the quality of CBVCOs.

1. Introduction

Edible oils (EOs) contain high energy dietary unsaturated fatty acids which not only required as human nutrition and also for the transportation of essential nutrients (El Sohaimy 2012). Oils extracted from the seeds of different vegetables are extensively used in communal and functional food items as well as in certain medications as nutraceutical components (Toscano and Maldini 2007). These oils comprise vitamins and natural antioxidants in the form of phenolic compounds. In general, these products are composed of 98% triglycerides, 2% phospholipids, and different types of hydrocarbons (Zahir, Saeed et al. 2017). Mostly, vegetable oils have a higher proportion of unsaturated fatty acids (FAs) than polyunsaturated fatty acids making them good for health (Abdulkarim, Long et al. 2007). Fatty acids vary in the number of carbon atoms as well as the position and geometry of the double bonds within the carbon chain (Sathianathan, Kannapiran et al. 2014). EOs are acknowledged as an essential source of the human and the building blocks of the body's hormones, controlling the body systems (Vaskova and Buckova 2015). The availability of healthy food is a real need of the present time and thus it is now a hot issue among the scientific community and public media (Takeoka, Full et al. 1997).

Frying is the most common process for cooking as it elicits the desired taste and quality of the food. Repetitive use and heating of oil produce undesirable components and have an adverse effect on food quality and human health. When vegetable oils are heated in the open air for long periods at high temperatures, various chemical reactions (hydrolysis, oxidation and polymerization) take place. As a result of different reactions in the oil, it produces volatile and non-volatile components, which lead to degradation (Choe and Min 2007). Vegetable oils are used for frying food in almost all the countries around the world, but their heating criteria are different. Upon sequential heating, fatty acids of vegetable oils degrade and convert into carbonyl and peroxide, which lowers the nutritional quality of the oils, and may cause colon cancer, atherosclerosis and cardiovascular issues in humans (Vaskova and Buckova 2015). Deep frying generates chemical reactions which depend on the frying conditions, quality of the oil, replacement of the oil and oxidative stability. At higher temperatures, an excess of atmospheric oxygen oxidizes the oil that causes the structural changes, which leads towards the lower oil quality and makes it risky for human health (Tomaino, Cimino et al. 2005). The use of degraded oil for cooking at high temperatures produces the following effects in humans i.e., weight and growth suppression, liver and kidney increase, thymus, and epididymis damage (Choe and Min 2007).

Research has revealed that vegetable oils heating up to 180°C changes their chemical composition and decreases their strength of quality because of decomposition of heat labile vitamin E (Marinova, Seizova et al. 2012). Thermal oxidation and autoxidation are well-defined reaction mechanisms of oil heating that affect the stability of the cooking vegetable oils. From a dietary perspective, frying oils must exhibit a high degree of oxidative stability. Vegetable oils having high amounts of saturated fatty acids comprising trans fatty acids may be detrimental for health (Aladedunye and Przybylski 2013). Monitoring of the quality of frying oils is indispensable to avoid oil consumption due to the detrimental health effects of food prepared in such oils, for maintenance of food quality and a reduction in the expenses related to the discarding of the frying oils (Abdulkarim, Long et al. 2007). More unsaturated oils oxidize more readily than less unsaturated ones, such as oils having 75–90% of oleic acid are more stable against heating than oils having 65% of linoleic acid (Caponio, Pasqualone et al. 2003). Any type of heating, conventional or microwave increases the oxidation of the oil fatty acids; and decreases the, unsaturated fatty acids with rapid rate (Mensah and Obeng 2013). From the last 20 years, edible oil production in Pakistan, from all sources, has increased 2.56 % annually, whereas, the consumption has increased 7.7%, therefore, the indigenous edible oil production has not been able to meet the demand. Pakistan imported 75% of the total crude edible oil, and the imports in the first five months of 2019-20 totaled 460 million USD and therefore, local cooking oil production has shown a rising trend.

In Pakistan, conventional crops like rapeseed, mustard, groundnut, sesame and cotton, and non-conventional crops like sunflower, soybean and safflower are grown to fulfill the cooking oil requirements. The Pakistan agriculture research council (PARC) contributes to overcoming the growing deficit of canola-based vegetable cooking oils through research on the canola varieties in Pakistan. Now, canola seed oil has a major contribution to the production of domestic canola-based vegetable cooking oils. The PARC extracted canola oil for the first time and marketed it as Mumta pure canola cooking oil, which resulted in the establishment of different industries in the country, selling canola cooking oil with different brand

names. So, for the interest of the country and to reduce the drain on foreign exchange reserves, there is a need to increase domestic oil production. Besides that, the regularization of hundreds of unregistered oil extracting units that produce low-quality edible oil products is also a herculean task. Any attempt to do so could backfire, and many of them can altogether stop operating, adding to the problem of joblessness and the shortage of oil. The province of Punjab in Pakistan is famous for its variety of food items cooked in vegetable oils at high temperatures for developing unique tastes and aromas. Oils are primarily heated for frying and roasting of a variety of food items like vegetables, meat, cereals, grams, and pulses. These edible vegetable oils are repeatedly used 3–6 times before being discarded (Ngozi, Giwa et al. 2019). While in the process of frying and cooking, heating puts extensive stress to degrade the low-quality vegetable cooking oils. A low quality of oil is because of either a poor refining process or the mixing of used cooking oils.

Canola-based vegetable cooking oils (CBVCOs). used locally have insufficient information on their oil quality attributes due to insignificant research and poor administrative legislation in Pakistan. Especially, cooking oils used for food preparations are harshly treated at high temperatures while roasting, which can change the quality attributes and usually turns them unsafe for health and for reuse. Therefore, the present study has been designed with the prime objective of fatty acid compositions and the assessment of effect of heating on the physicochemical quality characteristics of canola-based vegetable cooking oils.

2. Materials And Methods

2.1. Sample Collection and Preservation

In the present research work, five different commercially available canola-based vegetable cooking oils (CBVCOs), sold in the market as brands, were purchased and used for the research. These were, Kisan canola oil (K-1), Kousar canola oil (K-2), Talu canola oil (K-3), 121 Apna canola oil (K-4) and Sufi canola oil (K-5). All the oils were observed for their expiry date and shelf life to ensure the basic food quality. The purchased oils were brought into the research laboratory of the Department of Chemistry, University of Gujrat, Pakistan and, then preserved in the refrigerator at 4°C for subsequent analysis. All the chemicals and solvents used in this study were of analytical grade, and the absolute ethanol, methanol and glacial acetic acid were purchased from Sigma Aldrich; while, potassium hydroxide, sodium hydroxide, hydrochloric acid and sulfuric acid were from Lab Scan and Iso-octane, *n*-hexane and phenolphthalein were from Merck.

2.2. Research Design for the Evaluation of CBVCOs Quality Parameters

Four (04) equal portions of 250 mL of oils were taken for each stage of heating, and after heating the oils, they were cooled, covered with aluminum foil and stored in a refrigerator. The physiochemical properties of the oils were tested in triplicate at all the heating phases. Initially, after the procurement of the canola-

based vegetable cooking oils of different producing companies, their physicochemical properties like colors were determined on the double spectrophotometer and average maximum absorption wavelength (λ) was recorded following the method of (Popov-Raljić, Šmit et al. 2007) mass and volume calculated following the method specific gravity AOAC, Official Method 920.212 (Murthy, Manohar et al. 2014), pH via potentiometric (AOAC, Official Method 981.12) (Karim, Hijaz et al. 2010), refractive index using digital refractometer (AOAC, Official Method 921.08) (Gutiérrez, Rosada et al. 2011), specific gravity by the pycnometer method (AOAC, Official Method 920.212) (Marques, de Neto et al. 2015), saponification value (AOAC, Official Method 920.160) (Dalla Nora, Oliveira et al. 2018), acid value (IUPAC Method No 2.201) (Gecgel, Gumus et al. 2011), un-saponifiable matter by extraction with petroleum ether (AOAC, Official Method 933.08) (Nail, Ali et al. 2017), and water content (heating) were determined using the standard protocols and methodologies. After the preliminary assessments, the oils were processed for heating at 100°C, 200°C and 350°C for 8 hours on the accord of the local frying practices. The oils were heated in a shallow pan (diameter 20–30 cm) of stainless steel on the gas flame without any food; this was just to get the effect on the oils.

2.3. Preparation and Analysis of the FAMES of CBVCOs

The fatty acid (FA) compositions of the oils of all the brands as K-1, K-2, K-3, K-4 and K-5 were examined using the standard method 2.301 as applied by (Zubair, Anwar et al. 2012). For volatility, the the CBVCOs fatty acids were converted into their volatile methyl ester derivatives. An amount of 30 mg of oil samples was saponified by adding 5.0 mL of 0.5 M methanolic KOH. The whole mixture was vortexed frequently and heated for two hours at 80°C to get the monophasic layer. The samples were cooled to room temperature and then 0.3 mL each of the glacial acetic acid, hexane and distilled water were added till equilibration was reached and phase separation had taken place. In separate phases, the upper layer comprising the FAMES was transferred into the labeled sampling GC vial using a pasture pipette. After the preparation of the FAMES, the sample was injected (split less injection) into a gas chromatography-mass spectrometry (GC-MS Shimadzu GC-MS Q 2010, SHIMADZU Corporation, Japan) to analyze the fatty acid composition. The GC-MS was fitted with a polar capillary column (Rtx-5MS, film thickness – 0.25 μ m and length-30 m \times 0.32 mm ID). The oven temperature was programmed to start from 160°C for 1 min to 250°C with a ramp rate of 5°C/min and the final hold up was 10 min. Helium (He) gas, 99.9% pure, was used as the carrier, with a flow rate of 1.0 mL/min throughout the analysis. The interface, ion source and injection temperatures were 220°C, 230°C and 250°C, respectively. The mass detector was operated in electron impact (EI) mode at an ionization energy of 70 eV and m/z scan range of 30 to 500. The compound detections were made using a built-in NIST 2017 updated library in GC-MS. Triplicate injections were performed and the method was > 90% accurate and precise, with a < 4% relative standard deviation.

2.4. Statistical Analysis

Regression analysis was used to predict the required values by employing IBM SPSS Statistics application. The one-way analysis of variance (ANOVA) was estimated to determine the significant difference (5%) between the groups of canola-based vegetable cooking oils (K-1 to K-5). The Tukey HSD

test was then applied on those groups where a significant difference was observed. The data were also tested for the normality distribution by applying Shapiro-Wilk at a 0.05 level. Each experiment was performed in triplicate and the results have been reported as the mean \pm SD.

3. Results And Discussion

Five canola-based vegetable cooking oils which are being used for cooking purposes domestically, were examined for their quality attributes of fatty acids using GCMS and physiochemical parameters like color, odor, saponification value (SV), refractive index (RI), pH, acid value (AV) and un-saponifiable matter to predict the effect of heating. Fact findings are depicted in their respective tables and figures with appropriate discussion.

3.1. Fatty Acid Compositions of the CBVCOs

In the present research, commercially sold canola-based vegetable cooking oils (CBVCOs) K-1, K-2, K-3, K-4 and K-5, have been analyzed for fatty acid compositions to predict the oil composition and quality (Table 1). GC-MS analysis hence revealed the fatty acid compositions of the five different CBVCOs showing a significant variation in percent compositions of fatty acids. Analysis also revealing that CBVCOs have major proportion of canola fatty acids with small portion of other vegetable oils and animal fat. Literature reports 9-octadecenoic acid (oleic acid $C_{18:1}$) as the major fatty acid in the canola oils along with linoleic ($C_{18:2}$) and linolenic acids ($C_{18:3}$) (Omidi, Tahmasebi et al. 2010) and similarly our results also depicts 9-octadecenoic acid (oleic acid $C_{18:1}$) in the range of 40 to 57 % representing major proportion of canola fatty acids (Table 1). Beside the major fatty acids composition some oils have the presence of hexadecanoic acid ($C_{16:0}$) eicosanoic acid ($C_{20:0}$), docosanoic acid ($C_{22:0}$) and their derivatives 13-eicosanoic acid and 13-docosanoic acid in K-3, K-4 and K-5 respectively (Matthaus, Özcan et al. 2016). Overall, it is found through gas chromatography mass spectrometry analysis that CBVCOs which are available in the market have major proportion of canola oil fraction, whereas s blending of other vegetable oils extracted from vegetable seeds and algae along some animal fat was found (Figure 1). K-1 bears about 70 % canola oil fatty acids and 30 % other sources like animal fat 13% and vegetable oils 17%, K-2 represents 76.5% canola oil fatty acids, 16% other sourced oil and 7.5% animal fat composition, K-3 have almost 86% canola oil, 5.5% other vegetable oil.4.4 % algae and 4% animal fat composition, K-4 has 77.2% canola oil, 20.3% other source oil and 2.5% animal fat composition and K-5 has 97.9 % canola oil and 2.1% other source oil composition. As the composition is compared, K-5 found the best canola-based vegetable oil as it has almost 98% composition of canola oil and 2%of other vegetable oils. Overall, through analysis the principle fatty acids of CBVCOs are found unsaturated fatty acids of oleic (9-Octadecenoic acid 70-98%), linoleic (9,12-Octadecenoic acid 16-23%), linolenic (9,12,15-Octadecenoic acid 0-12%) and erucic acid (13-eicosanoic acid 0-11.1%), and the saturated fatty acids of palmitic (hexadecanoic acid 4.8-5.7%) and animal fat (1.6-8.5%). CBVCOs showed slightly lower erucic and linolenic acid, but a higher content of oleic acids and lenoleic The same value of the major fatty acid were in another research so that they did not differed significantly (Sharafi, Majidi et al. 2015). Fatty acids content showed wide variations in the species, as well as seed oil of the accessions. The nutritional

properties of CBVCOs, like other fats and oils, are dependent on its fatty acids' composition, particularly the amount of oleic, linoleic, linolenic, and erucic acids in turn has great deal of importance in terms of in human nutrition. In addition, high oleic acid oil has cholesterol lowering properties; while saturated (palmitic and stearic) fatty acids tend to raise blood cholesterol levels considerably (Miller, Zimmerman et al. 1987). At the same time, the nutritional quality of the CBVCOs is improved by increasing the dietary essential 9,12-octadecadienoic acid (C_{18:2}) contents and decreasing the 9,12,15-octadecadienoic acid (C_{18:3}) contents (Beare-Rogers, Nera et al. 1971). Thus, edible oil with high linoleic acid content is premium oil. According to results of this study, all the vegetable oils showed significant linoleic acid along linolenic acid in K-3 is also an essential fatty acid, however its presence in the oil may causes rancidity to vegetable oil. Research also revealed that CBVCOs have blending of some other vegetable oils and animal fats in the form of octadecanoic acid and cyclopentadecanoic. In respect of blending of CBVCOs 4-30% blending in different composition has been found. The blended the cooking oil had a relatively high oxidative stability and had excellent flavor and taste compared to those of the other frying oils as a result of a sensory evaluation. The results suggest that the blended oil is a good alternative oil for frying the foods.

3.2 Effect of Heating on Fatty Acids of CBVCOs

For evaluating the effect of heating on the *CBVCOs* fatty acids of K-2 was determined at all heating phases at 25, 100, 200 and 350 °C as presented in Figure-2. Oil sample was heated at different temperatures and analyzed by using the gas chromatography mass spectrometry (GC-MS) and fatty acids were identified with MS library. Results are revealing that free fatty acids are increasing on increasing the temperature which effects the triglycerides bonding to decompose thermally. It has been observed that at the normal temperature, the level of FFAs in the vegetable oil is not very high and only few FFAs were detected in GS-MS analysis which supports the earlier study (Crossley, Heyes et al. 1962). On oil heating at 100 °C, the level of FFAs start increasing of thermal degradation. In the 3rd and 4th phases FFAs increased in addition to initial phase. The level of poly unsaturated fatty acids (PUFA) such as linoleic acid decreases (from 47 to 6.13 g/100gm of oil) with increase in temperature from 25 °C to 200 °C. This decrease in the contents of linoleic acid over prolonged heating time, is due to increase in its rate of auto-oxidation with increasing heating duration (Alireza, Tan et al. 2010). Free fatty acids composition varies significantly with increase in temperature from 200 °C to 350 °C. Similarly in a research study of Dawodu et al, 2-3% increase in FFA with increasing temperature from up to 250 °C (Dawodu, Olutona et al. 2015). Cooking oils having high level of FFAs is not suitable for the human use.

3.3 Effect of Heating on Color, Mass and Volume of CBVCOs

Color is the visual indication and rapid method for investigation of oil quality. In this study during heating, there is a significant change from yellowish to darker reddish color of the oil (Table 2). The wavelength of oils increased on increasing the heating temperatures. Visually, the color of the oil first appears yellow (wavelength range of the sample 510-520nm), then on continuous heating it changed into reddish-brown (wavelength range of the sample 580-600 nm). Thus, it was observed that the darkening of the oil color

may have occurred due to the auto and thermal oxidation of phospholipids (Latha and Nasirullah 2014). The increase in the formation of ketones, dienoic acids and peroxides with increasing temperatures, results in the oil becoming darker (Tarmizi, Ismail et al. 2016). Heating affects the color of the oils and other oil properties of vegetable oils. Literature reports that aldehydes are formed during the heating of vegetable cooking oils like coconut, safflower, canola and extra virgin olive. The oils were heated at 180 °C, 210 °C and 240 °C after 6 h and the emissions were characterized by gas chromatography (Katragadda, Fullana et al. 2010). Cooking oils emit a mixture of chemicals on heating, from that the aldehydes have attracted great attention because many of them are hazardous, containing long-chains related to the fatty acids in cooking oils (Peng, Lan et al. 2017). In our research, each type of oil emitted an aroma upon heating with a strong intensity of smell which may have represented the presence of aldehydes. Evolution of oil aroma is also acceptable because of the change in the mass and volume of the oils.

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3.5 Effect of Heating on the Saponification Value of the CBVCOs

The saponification value provides very useful information and represent the optimized quality of cooking oil without the adulteration in the refined oils. Five commercial canola-based vegetable cooking oils, K-1 to K-5, were tested for their saponification values of control 25°C and heated at various temperatures

(100, 200 and 350 °C), and the results are shown in Figure 3 (a), which depicts the lower saponification value to be around 67 mg of KOH/100 g of oil at room temperature. This was increased up to 250 mg of KOH/100g of oil at the elevated cooking temperature of 350 °C. These findings are in close agreement with Odewole et al. (Odewole, Oyeniyi et al. 2016). The trend of increase in the saponification values observed in all the selected canola oils (K-1, K-5), which corresponds to increase in temperature. Highest increase in the saponification value was observed in the K-5 cooking oil in comparison to the other oils (Figure 3 (a)). Increasing trend reveals a significant change in the SV of the oils, which indicates that more free fatty acids may be producing during heating. This may be attributed to the transformation of the triglycerides to FFAs, which effect to increase the FFA content of the oils and, hence, enhanced the saponification values (Ngassapa, Nyandoro et al. 2012). According to the reported values in the literature, an optimized saponification value should be 182-193 mg of KOH/100 g of oil. The saponification value of many vegetable oils above the 200 mg/KOH indicates oil adulteration (Alfekaik and AL-Hilfi 2016). Table 3 represents the statistical description, giving the regression equations for the prediction of the saponification value by putting temperature as a factor of analysis. The one-way ANOVA was calculated based on the mean values rather than on the base values, depicting no significant variation in the saponification value ($F_{\text{calculated}} < F_{\text{critical}}$) among the groups of the cooking oils treated at different temperatures.

3.6 Effect of Heating on the Un-saponifiable Matter of the CBVCOs

The un-saponifiable matter of oil is a composition of very important naturally occurring antioxidants and vitamins that are decomposed at high temperatures. According to the codex standard, normally the value of an un-saponifiable matter in vegetable oil must be equal or less than 20%. Initially, at room temperature, the maximum un-saponifiable matter was observed in K-3 which was 10.10 g/100 g of oil; whereas, the lowest was in K-1, *i.e.*, 5.20 g/100 g of the oil. After treatment at various high temperatures the amounts of the un-saponifiable matter were reduced to 5.60 g/100 g in K-3 and 2.40 g/100 g in K-5 (Figure 3 (b)). The results revealed a significant effect of heating on the amount of un-saponifiable matter that lowered the quality of the cooking oil. In (Table 4), the regression analysis for the un-saponifiable matter was calculated by putting temperature as a factor of analysis. The one-way ANOVA was calculated on the 5% confidence level and a significant difference between these groups (K-1, K-5) was found, therefore, the null hypothesis has been rejected. It may be stated that the mean values of the un-saponifiable matter were different. Un-saponifiable matter comprises various antioxidants, therefore, this part provides the stability to the cooking oil. Herchi and his coworkers also reported a steady decrease of un-saponifiable content upon the heating of flaxseed hull oil (Herchi, AMMAR et al. 2016). Similarly, Gertz et al. (Gertz, Aladedunye et al. 2014) observed that the refined oils were less stable at elevated temperatures as compared to non-refined oils, and there was a co-relationship between the oxidative stability and un-saponifiable matter of the vegetable oils. Tavakoli and coworkers observed that the major proportion of un-saponifiable matter was linked to the tocopherols, with demonstrating healthful effects as well as antioxidant activity. The content of the un-saponifiable matter in the vegetable cooking oils, usually ranged from 0.5% to 2.5% and the values in olive and *Pistacia khinjuk* fruit oil were 1.2% and

1.5%, respectively. Similarly, in addition to the prevention of diseases and biological activities in living organisms, polyphenols also provide desirable antioxidative properties in vegetable cooking oils (Tavakoli, Brewer et al. 2017). In our assessment (Figure 3 (b)) of the cooking oils' un-saponifiable matter values, an apparent decrease was observed up to 50% in all the canola -based vegetable cooking oils.

3.7 Effect of Heating on the Specific Gravity of CBVCOs

With an increase in the temperature of canola-based vegetable cooking oils from room temperature to 350 °C, a drastic effect of heating on lowering the specific gravity was observed. The effect is shown in Figure 3 (c), with a major change from 0.95 to 0.90 in K-3. These results of our research are in close agreement with the findings of Negash et al. (Negash, Amare et al. 2019), who reported the mean specific gravity for imported and locally made oils in the range of 0.807-0.823. The statistical description based on the regression calculation and analysis of variance in one way at a 5% significance level revealed that there was a significant difference between these groups (K-1, K-5), and, hence, the null hypothesis has been rejected (Table 5). The prediction revealed that the decrease in the mean value of the specific gravity inferred poor refining of the canola-based vegetable cooking oils, showing deviation from the standards developed by the WHO/FAO (Alimentarius 1999). The decrease in the specific gravity of the canola-based vegetable cooking oils was due to the breakdown of the oil components and a decrease in the density of the oils. Idun-Acquah and his coworkers investigated that the mean specific gravity of the frying oil decreased from 0.899 to 0.887 upon heating at 180 °C during the frying of food (Idun-Acquah, Obeng et al. 2016). The initial values for the specific gravity of canola-based vegetable cooking oils are in agreement with the results of (Bello, Akindele et al. 2011, Aremu, Ibrahim et al. 2015) who reported the specific gravity of canola oils is 0.920, and (Olaleye, Eke et al. 2018) noticed, that the 0.918 specific gravity for groundnut and 0.939 for neem seed was safe to use.

3.8 Effect of Heating on the Refractive Index of CBVCOs

The initial and post-treated canola-based vegetable cooking oils were tested for their refractive index (RI) values and they were observed to be from 1.470 to 1.475 (Figure 3 (d)) which is in close agreement with Bello et al. (Bello, Akindele et al. 2011) for *T. occidentalis* oil (1.460); In previous research reports of (Shahidi and Ambigaipalan 2005, Falade, Adekunle et al. 2008, Anwar, Kamal et al. 2016) the refractive index values of conventional oils as soybean (1.466- 1.470) and palm kernel (1.449- 1.451) and for corn oil (1.467), olive oil (1.465) and virgin oil (1.468). The refractive indices of the oils remained almost constant as the values were the indication of their degrees of saturation and had slightly increased (0.005) due to the changes in color. There was a slight deviation in the refractive indices that varies from 1.470 to 1.475 in the canola oils upon being heated at high temperatures. Conclusively, the analysis of variance (ANOVA) in one way was applied on the RI values of different oils and no significant difference in their refractive indices was found based on the null hypothesis (Table 6). Godswill et al., reported slight changes in the refractive index of palm oil from the initial 1.4653 to 1.4655, sunflower oils from 1.4722 to 1.4725 and sesame oil from 1.4722 to 1.4723 after repeated frying (Godswill, Amagwula et al. 2018). The refractive index ranging from 0.1470-0.1475 of the canola oils possibly contained unsaturation in the

fatty acid sequencing as our research got similar values (Pereira, Pontes et al. 2008). In another similar study, the refractive indices of the canola cooking oils were slightly increased from 1.4713 to 1.4740 upon heating, which supported our results changing from 1.470 to 1.475 (Ali, Nouruddeen et al. 2013). The values of the refractive index of Canarium oil were 1.47 and 1.45, extracted by both mechanical pressing and solvent extraction, respectively (Rahman 2018). The refractive index values determined for peanut and corn oil were 1.46 and 1.47, respectively. These values, found in our research, are in good agreement with the values reported by (Salaheldeen 2019). An increase in oil darkening, turbidity and viscosity could presumably be responsible for an increase in the refractive index value of some vegetable oils by 0.0007 (Goburdhun and Jhurree 1995).

3.9 Effect of Heating on the pH of CBVCOs

The pH of a cooking quality vegetable oil is normally kept neutral and usually ranges from 6.9 to 6.7. An oil's pH decreases upon increasing the temperature; because a higher temperature converts the triglycerides of vegetable oils into FFAs. Figure 3 (e) indicates a clear decrease in the pH after heating the canola-based vegetable cooking oils. Overall, the highest pH (6.9-6.8) was noted in K-1 and the lowest pH value (6.7) was in K-2. Overall, a trend of the pH decreasing was observed in the cooking oils. Possibly, the formation of FFAs upon thermal treatment are important dynamics of vegetable oils which may be related to the pH decrease. All the samples have shown declines in their pH values relative to the controlled canola-based vegetable cooking oils and this may be attributed to the formation of FFAs upon the heating of the fresh oils. Upon comparing our results with other research studies, it was revealed that our results are in agreement with Yusuf et al. where the pH of the groundnut oil decreased from 6.97 to 6.89 upon increases in the heating temperature from 70 °C to 100 °C (Yusuf, Olaniyan et al. 2014). The thermal process begins the hydrolysis of oil triglycerides, changing them into FFAs and glycerol, which influences the pH-parameter. Fresh vegetable oils are weak basic liquids with pH values more than 7 which decline, upon heating, into an acidic form because of the hydrolysis of the triglycerides (Fakhri and Qadir 2011). Table 7 is a statistical comparison of the calculated and Tabulated F value and $F_{\text{calculated}} > F_{\text{critical}}$ (0.09 > 3.48); hence, this reveals that there was no significant difference between the tested groups of oils K-1, K-5.

3.10 Effect of Heating on the Acid Value of CBVCOs

The acid values (AV) of canola-based vegetable cooking oils increased upon increasing the temperature from 25 °C to 350 °C (Figure 3 (f)). The results of our finding are in close agreement with the acid values of flaxseed oil, i.e., from 1.5 mg KOH g⁻¹ of oil to 2.9 mg KOH g⁻¹ of oil upon being heating from room temperature to 110 °C as reported by Herchi and his coworkers (Herchi, AMMAR et al. 2016). Alajtal et al reported 4.49 and 5.05 (mg KOH/g) acid values for the other cooking oils before and after passing through the frying process (Alajtal, Sherami et al. 2018). The results are in support of our research results of the AVs; however, both of the oils were different in their KOH requirements. Comparison of $F_{\text{calculated}} > F_{\text{critical}}$ (0.09 > 3.48) was made using the null hypothesis under the one-way ANOVA. It was found that there was a significant difference between the AVs of the canola-based vegetable cooking oils (Table 8).

Acid values reveal the number of FFAs and indicate the suitability of the oil for degradation and the level of oxidative deterioration. Fatty acids are normally found in the form of triglycerides, but they disrupt into FFAs during processing at elevated temperatures. This may be attributed to hydrolytic and lipolysis activities increasing which decomposes the glycerides present in the oil as the operating temperature rises. An increasing acid value is a sign of oil deterioration that, in turn, is caused by the degradation of the chemical bonds in the oils at elevated temperatures. Elevated FFAs in diets should be avoided, as this can weaken the capacity of the liver to store sugar (Dawodu, Olutona et al. 2015) and also lead to coronary heart diseases. Bello et al. reported that the acid values of 0.00 to 3.00 mg KOH/g of oil are desirable for oil to be used for food preparation. It suggests that vegetable cooking oils heated up to 200 °C are safe to use (Bello, Akindele et al. 2011).

3.11 Statistical Analysis

Data of different oil quality parameters were tested for the normality of distribution using the Shapiro-Wilk at a significance level of 0.05 (Table 3-8). Standards of statistical analysis reveals that if significance value of Shapiro-Wilk is greater than 0.05, then it can be stated that the data is not significant and normally distributed. It is evident and clearly reported in earlier statistical studies that non-significant results do not need further analysis particularly with tests like Tukey, Pearson, Duncan multiple, etc. The Tukey HSD (Honestly Significant Differences) test is used for finding the significant differences among groups. That's why the Tukey HSD test is applied to only significant values in a preliminary ANOVA for study groups of un-saponifiable matter and a pH value having $p < 0.05$ (Table S1 & Table S2). So, the analysis revealed each pair in respect to its significance. If the observed significance values were less than 0.05, then it could be stated that there was a significant difference among the pairs. The analysis illustrates that groups as (K-1, K-5), (K-2, K-3), (K-3, K-2), (K-3, K-4), (K-4-K-2) (K-4-K-3) and (K-5-K-1) in pairs had no significant differences which cannot be observed by the simple ANOVA. Whereas, all other pairs had significant results. Having a non-significant difference meant that there is no considerable impact from the heating on that physiochemical parameter and vice versa. Similarly, for the pH value, when tested as an independent variable using the Tukey HSD test by putting a 0.05 level of significance, it was observed that the (K-1-K-3), (K-1-K-4), (K-1, K-5), (K-3, K-1), (K-3, K-4), (K-3, K-5), (K-4, K-1), (K-5-K-3), (K-4, K-5), (K-5, K-1), (K-5, K-3) and (K-5, K-4) pairs had no significant difference, which was not observed by the simple ANOVA. It is now clear to express that all the other pairs of the pH values had significant results.

4. Conclusions

Present study was designed primarily to find purity in quality of different refined canola cooking oils by analyzing their fatty acids composition and secondly, the variation in the quality with the effect of heating on the. Five canola-based vegetable oils that are most commonly used in cooking across the country in households and restaurants were taken into consideration for this research. Further to confirm the extent of composition change in oils, GC-MS analysis of all the oils was performed that revealed two fold information, on the one side it was found the these canola based vegetable oils has large scale

variation in fatty acids and secondly these are not pure canola oils but have blending of other vegetable oils and some animal fat. Analysis revealed that producers are blending up to 30% with canola vegetable cooking oils to achieve desirable flavor, an important factor in the sale of cooking oils. Quality parameters of the canola-based vegetable cooking oils were assessed initially after purchase and later after heating at different temperatures. Considering the objective of physiochemical and statistical analysis, it is found from the results of analysis that variations taking place and heating effects the cooking oil quality by vitiating the physiochemical parameters. The results apparently differ from Codex Alimentarius Commission Standards of rapeseed (Canola) oil (Alimentarius 2001). In variations, the color wavelength was changed (510–600 nm) representing darkening in color, mass, volume, un-saponifiable matter and pH were reduced whereas acid value, refractive index, specific gravity increased of the CBVCOs upon heating at high temperatures. ANOVA predicted significant variations in the un-saponifiable matter (the composition of phenolic, antioxidants and vitamins) and the pH of the oils. Based on the research finding, it may be concluded that canola oils studied in the research are not pure quality and heating effect their quality attributes, therefore cooking should not be performed at high temperatures in such canola oils. Additionally, the oil should not be repeatedly used. Further, it may also be suggested that oils should be used once, but later they may be converted into some value-added products to get economic sustainability.

Abbreviations

CBVCOs -canola based vegetable cooking oils, FAMES- Fatty acid methyl esters GC-MS- Gas chromatography-Mass spectrometry, EI- Electron Impact QP-Quadrupole, EOs-Edible Oils, USD- United States Dollars, PARC- Pakistan agriculture research council, AOAC- Association of Official Analytical Chemists, IUPAC-International union of pure and applied chemistry, ID- Internal Diameter, ANOVA- analysis of variance, HSD-honestly significant difference, SD- Standard Deviation, SV-Saponification value, RI-Refractive Index, AV-Acid Value.

Declaration

Ethics approval and consent to participate

Not Applicable.

Consent for publication

Not Applicable

Availability of data and materials

The authors confirm that the data supporting the findings of this study are available within the article [and/or] its supplementary materials

Competing interests

“The authors declare no conflict of interest.”

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Authors' contributions

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Tables

Table 1. Fatty acids (% Composition) of CBVCOs (K-1 to K-5) analyzed by GC-MS

Fatty acid	FA Groups	Source	K-1	K-2	K-3	K-4	K-5
9-Octadecenoic acid	MUFA	Canola	50.0	40.4	45.3	47.6	57.2
Hexadecanoic acid	SFA	Canola	-	12.7	5.2	5.7	4.8
9,12-octadecadienoic acid	MUFA	Canola	16.0	23.4	-	-	20.1
Eicosanoic acid	SFA	Canola	-	-	5.1	10.5	5.4
7-hexadecenoic acid	MUFA	Other VO	-	-	4.2	0.6	2.1
Pentadecanoic acid	SFA	Animal fat	4.9	7.5	-	2.5	-
13-Docosanoic acid	MUFA	Canola	-	-	3.1	2.5	-
Docosanoic acid	SFA	Canola	-	-	4.1	-	2.2
9-hexadecenoic acid	MUFA	Other VO	-	12.1	1.3	-	-
Cyclopropane octanoic acid	SFA	Animal Fat	8.7	-	1.6	-	-
Cyclo pentane tri decanoic acid	SFA	Other VO	-	3.9	-	2.0	-
13-eicosanoic acid	MUFA	Canola	-	-	11.3	-	-
Oxiraneoctanoic acid	SFA	Other VO	6.7	-	-	8.9	-
6-octadecanoic acid	MUFA	Canola	-	-	-	5.6	-
10-octadecanoic acid	MUFA	Canola	-	-	-	5.4	-
Cyclo propanepentanoic acid	SFA	Other VO	-	-	-	3.4	-
9,12,15-octadecatrienoic acid	PUFA	Canola	-	-	12.0	-	-
8,11-octadecadenoic acid	PUFA	Algae	-	-	4.4	-	-
7-hexadecenoic acid	MUFA	Other VO	9.8	-	-	-	-
Heneicosanoic acid	SFA	Other VO	-	-	-	2.2	-
Cyclo pentane-undecanoic acid	SFA	Other VO	-	-	-	1.8	-
Cyclo propanetridecanoic acid	SFA	Other VO	-	-	-	1.3	-
10-Octadecenoic acid	MUFA	Canola	-	-	-	-	8.2
7,10-hexadecenoic acid	PUFA	Canola	3.9	-	-	-	-
Octadecanoic acid	SFA	Animal Fat	2.4	-	-	-	-

Table 2. Effect of Heating on Color, Mass and Volume of CBVCOs.

CBVCOs	Property	25 °C	100 °C/ 8hrs	200 °C/ 8hrs	350 °C/ 8hrs
K-1	Color (nm)	515	525	550	595
	Mass (g)	222.5	222.06	215.00	212.50
	Volume (mL)	250	246	244	242
K-2	Color (nm)	510	520	555	590
	Mass (g)	220.00	219.57	215.00	210.00
	Volume (mL)	250	248	246	242
K-3	Color (nm)	515	520	545	570
	Mass (g)	237.50	236.99	227.50	225.00
	Volume (mL)	250	243	241	239
K-4	Color (nm)	520	530	560	600
	Mass (g)	225.00	224.64	215.00	212.50
	Volume (mL)	250	248	241	240
K-5	Color (nm)	515	520	545	570
	Mass (g)	230.00	229.54	222.50	217.50
	Volume (mL)	250	246	243	239

Table 3. ANOVA depicting effect of heating on saponification value (mg KOH) of CBVCOs.

CBVCOs	Mean ± SD	Df	Significant value			
K-1	140.63 ± 91.29	4	0.314			
K-2	99.26 ± 71.14	4	0.455			
K-3	117.52 ± 68.54	4	0.543			
K-4	110.30 ± 65.10	4	0.644			
K-5	155.05 ± 107.34	4	0.230			
Source of variation	SS	Df	MS	F	P-value	F _{crit}
Between Groups	7958.38	4	1989.60	0.30	0.88	3.47
Within Groups	70523.09	10	7052.31			
Total	78481.48	14				

$F > F_{crit}$ or $P\text{-value} < 0.05$: Significant and $F < F_{crit}$ or $P\text{-value} > 0.05$: Non-significant.

Table 4. ANOVA depicting effect of heating on un-saponifiable matter of CBVCOs.

CBVCOs,	Mean ± SD	Df	Significant value			
K-1	4.42 ± 0.694	4	0.630			
K-2	6.70 ± 2.12	4	0.426			
K-3	8.03 ± 2.03	4	0.737			
K-4	6.92 ± 2.67	4	0.764			
K-5	3.82 ± 1.41	4	0.817			
Source of variation	SS	Df	MS	F	P-value	F _{crit}
Between Groups	31.67	4	7.92	3.59	0.05	3.48
Within Groups	22.07	10	2.21			
Total	53.75	14				

$F > F_{crit}$ or $P\text{-value} < 0.05$: Significant and $F < F_{crit}$ or $P\text{-value} > 0.05$: Non-significant.

Table 5. ANOVA depicting effect of heating on specific gravity of CBVCOs.

CBVCOs	Mean \pm SD			Df	Significant value	
K-1	0.87 \pm 0.017			4	0.850	
K-2	0.86 \pm 0.017			4	0.850	
K-3	0.92 \pm 0.022			4	0.577	
K-4	0.87 \pm 0.024			4	0.488	
K-5	0.89 \pm 0.021			4	0.995	
Source of Variation	SS	Df	MS	F	P-value	F _{crit}
Between Groups	0.006	4	0.002	6.77	0.007	3.47
Within Groups	0.002	10	0.000			
Total	0.008	14				

$F > F_{crit}$ or $P\text{-value} < 0.05$: Significant and $F < F_{crit}$ or $P\text{-value} > 0.05$: Non-significant.

Table 6. ANOVA depicting effect of heating on refractive index of CBVCOs.

CBVCOs	Mean \pm SD			Df	Significant value	
K-1	1.47175 \pm 0.002217			4	0.103	
K-2	1.47225 \pm 0.000957			4	0.272	
K-3	1.47300 \pm 0.001414			4	0.161	
K-4	1.47275 \pm 0.001708			4	0.850	
K-5	1.47275 \pm 0.000500			4	0.001	
Source of variation	SS	Df	MS	F	P-value	F _{crit}
Between Groups	9.73333E-06	4	2.4333E-06	2.03	0.166	3.48
Within Groups	1.2E-05	10	1.2E-06			
Total	2.17333E-05	14				

$F > F_{crit}$ or $P\text{-value} < 0.05$: Significant and $F < F_{crit}$ or $P\text{-value} > 0.05$: Non-significant.

Table 7. ANOVA depicting effect of heating on pH of CBVCOs.

CBVCOs	Mean \pm SD	Df	Significant value			
K-1	6.86 \pm .039	4	0.414			
K-2	6.78 \pm .014	4	0.161			
K-3	6.84 \pm .032	4	0.071			
K-4	6.86 \pm .018	4	0.714			
K-5	6.87 \pm .014	4	0.161			
Source of variation	SS	Df	MS	F	P-value	F _{crit}
Between Groups	0.014	4	0.004	5.83	0.011	3.48
Within Groups	0.006	10	0.001			
Total	0.021	14				

$F > F_{crit}$ or $P\text{-value} < 0.05$: Significant and $F < F_{crit}$ or $P\text{-value} > 0.05$: Non-significant.

Table 8. ANOVA depicting effect of heating on acid value (mg KOH g⁻¹) of CBVCOs.

CBVCOs	Mean \pm SD	Df	Significant value			
K-1	2.7225 \pm 1.52727	4	0.671			
K-2	2.3725 \pm 1.01477	4	0.993			
K-3	2.8500 \pm 1.57641	4	0.682			
K-4	2.7575 \pm 1.56112	4	0.644			
K-5	2.7100 \pm 1.52724	4	0.664			
Source of variation	SS	Df	MS	F	P-value	F _{crit}
Between Groups	0.663	4	0.1659	0.093	0.982	3.478
Within Groups	17.686	10	1.768			
Total	18.350	14				

$F > F_{crit}$ or $P\text{-value} < 0.05$: Significant and $F < F_{crit}$ or $P\text{-value} > 0.05$: Non-significant.

Figures

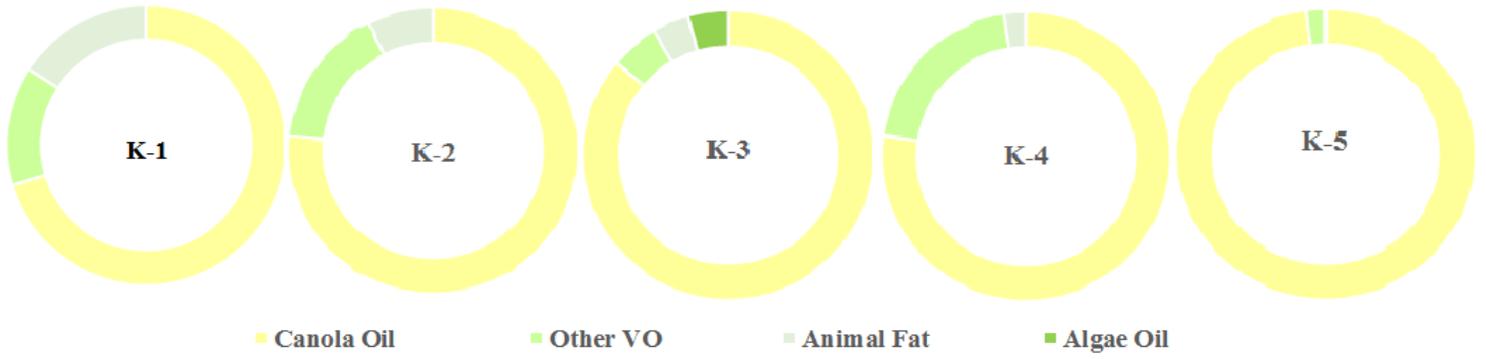


Figure 1

Different CBVCOs comprising variations of different % composition

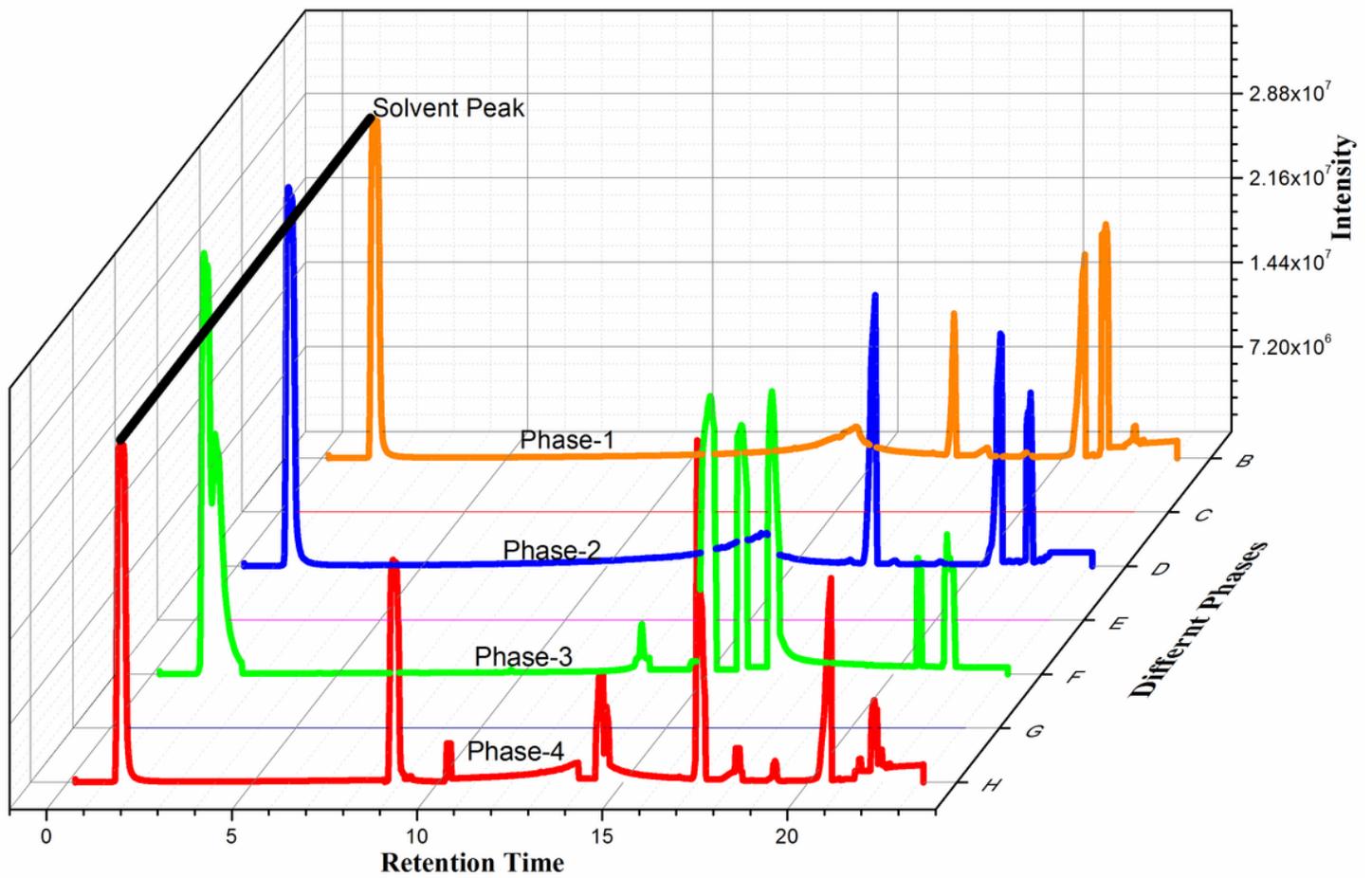


Figure 2

Effect of heating on the CBVCO Sample K-2 at four different temperature Phases

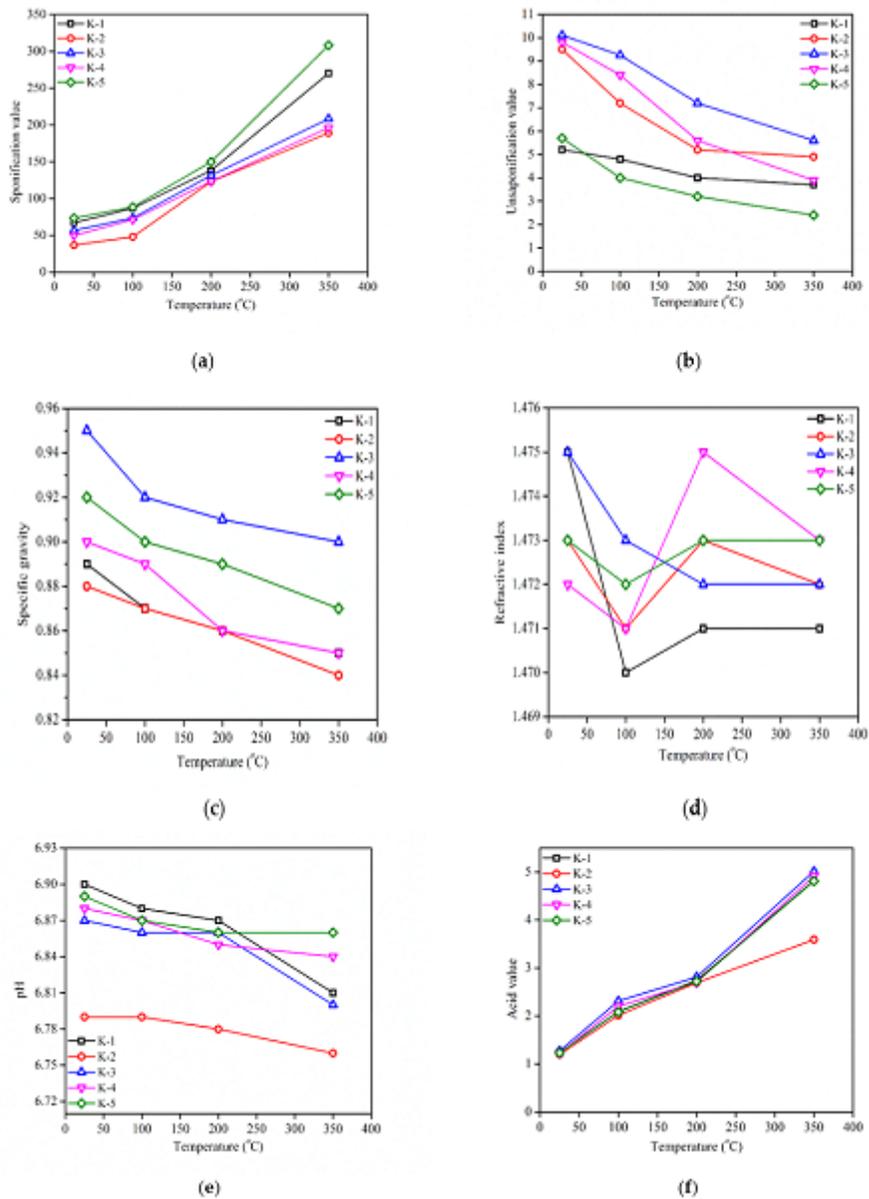


Figure 3

The effect of heating on quality parameters (a) saponification value, (b) un-saponifiable matter, (c) specific gravity (d) refractive index, (e) pH, (f) acid value of CBVCOs.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [floatimage1.png](#)
- [SUPPLEMENTARYDATA.docx](#)