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Comparative study of thermal treatments on stability of Moringa oil by using physicochemical analyses and FTIR spectroscopy

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Abstract

Comparative oxidative stability of Moringa oil was analysed by giving three different treatments of time, temperature, and mode of heating (microwave, oven, and deep fat frying) to check the stability and acceptability of Moringa oil with various physicochemical and sensory parameters. The results showed that FFA, AV, TOTOX, CD, CT, viscosity, density, RI, and specific gravity increased while iodine value, the content of polyphenols, phytosterols, and tocopherols decreased as three heat treatments progressed. It has, thus, been concluded that Moringa oil had shown good oxidative stability after 8 h of continuous frying, 20 min of microwave heating at P-100 as well as after 20 min of conventional oven heating at 200°C, and remained acceptable. The physicochemical and sensory evaluation showed that values obtained after 8 h of continuous Moringa oil frying were comparable with microwave heated samples at P-100 for 20 min, whereas, the oven heated samples at 200°C for 20 min were similar to microwave heated samples at P-80 for 20 min and 6 h of deep fat frying.

Abbreviation

FFA: Free fatty acid; AV: Anisidine value; CD: Conjugated Diene; CT: Conjugated Triene

Introduction

Moringa oleifera seed oil falls in the category of high-oleic oils and contains a high ratio of MUFA/SFAs (Schwingshackl *et al.,* 2015). The oil contains a high level of MUFAs up to an average of 76.74%, and dominated by oleic acid (73.58%) (Abdulkarim *et al.,* 2007). Various researchers have reported the presence of phenolic compounds and tocopherol such as flavonoids, and their nutritional and economic potentials (Rashid *et al.,* 2008; Rahman *et al.,* 2009; Ogbunugafor *et al.,* 2011). Tsaknis and Lalas (2002) have studied the stability of *M. oleifera* (Periyakulam 1) seed oil of Indian origin, extracted using different types of methods such as cold press solvent extraction during deep fat frying. Khattab *et al.* (2012) elucidated the frying effects on the physicochemical properties and frying quality of different oils, namely *M. oleifera*, groundnut oil, and also their blends.

Microwave cooking is a uniform and versatile method which can speed up cooking time, and are energy efficient in comparison to conventional heat treatment (Decareau, 1985; Albi *et al.,* 1997). Albi *et al.* (1997) investigated the effects of heating (i.e. microwave heating, electric oven heating, without heat, and microwave energy exposure) on the degradation of the quality of five different types of edible oils. Khedr and Aboneima (2016) demonstrated the effect of conventional and microwave heating of four samples (such as olive oil, cotton seed oil, supply oil, and corn oils) on physical parameters (viscosity and refractive index) of oils. Farag *et al.* (1992) performed a comparative study on refined cottonseed oil and hydrogenated palm oil's deterioration when exposed to microwave and conventional heating. Frying is an efficient, cheap, versatile, and fast process because it is a result of fast heat, mass transfer, and high temperature. Hydrolysis of oil, oxidation, and polymerisation are the main chemical reactions which usually take place in oils during this process. (Oke *et al.,* 2017).

The literature survey reveals that Moringa oil is not commonly utilised as frying oil. There are few literatures available on the conventional mode of frying using *M. oleifera* (10 repeated succession spanning of 2 h) by Tsaknis and Lalas (2002), *M. stenopetala* (5 frying/day for 5 d) by Lalas and Tsaknis (2002), and high oleic *M. oleifera* seed oil (6 h frying/day for 5 d) by Abdulkarim *et al.* (2007). However, there is no literature available on changes in physicochemical properties of Moringa oil when subjected to microwave and conventional oven heating. In the light of above gaps found in literature, the present work was therefore

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aimed to check the oxidative stability of Moringa oil on continuous frying, microwave heating as well as conventional oven heating.

Materials and methods

All raw materials (Bengal gram dal) were procured from a local market of Bathinda, Punjab, India, and Moringa oil (Periyakulam 1) were obtained from KP Aromatics, Gujarat, India.

Thermal treatment for Moringa oil

Frying treatment

Eight kg of Bengal gram dal was soaked in 25 L of water for a period of 4 - 5 h. After soaking, the dal was filtered with the help of muslin cloth and was tightly squeezed to get rid of excess water. Then, dal was fried in Moringa oil by placing it in a wire mesh basket. The frying operation cycle was optimised by checking the fried dal acceptance using different temperatures (140, 150, 160, 170, 180, and 200°C) and time intervals (2, 3, 4, 5, and 6 min) by semi-trained panellist available in FST department. Sensory evaluation of the fried dal samples was made by the panellists in terms of mouthfeel, flavour, and texture. For grading, hedonic scale (9 points) was used with the numeric score "1" representing highly disliked sample, whereas "9" for a highly liked sample. Frying performance of Moringa oil was checked by heating 4 L of oil at a temperature of 180°C in a deep fat fryer with frying cycle of 400 g/10 min (including recovery frying temperature). The dal was fried for a period of 8 h at the rate of 2.5 kg soaked dal/h. After every 2 h interval of frying, the samples were withdrawn and analysed for various physical and chemical parameters.

Microwave treatment

The microwave oven (IFB Model No.30BRC2) was used for oil heating, having input and output of 1400 and 900 W, respectively, and having 11 power levels for heating corresponding to the percentage. The low, moderate, and high-power levels of microwave treatment were selected and represented as 100% (P-100), 80% (P-80), and 60% (P-60) as the Moringa oil (100 mL oil in a 150 × 20 mm Petri plate) was placed on the rotatable surface of the oven. The samples were heated by microwave for various periods (5, 10, 15, and 20 min) at three power settings.

Oven treatment

The conventional oven (Usha OTG Model no. OTGW3619R) was used for heating Moringa oil (100 mL oil in 150 × 20 mm Petri plate) at 150, 180, and 200°C for 5, 10, 15, and 20 min. The oven radiation emitting frequency was 50 Hz. The rapid cooling of samples was performed after every heat treatment and then stored at refrigerated temperature by keeping them in sealed tubes.

Free fatty acids (FFA), peroxide value (PV), iodine value (IV), anisidine value (AV), TOTOX number, conjugated diene (CD), and triene (CT)

The free fatty acids (FFA), peroxide value (PV), iodine value (IV), and anisidine value (AV) were determined by a titrimetric method (AOAC, 1990). List *et al.* (1974) described the method which was used to measure the anisidine value. TOTOX value (AOCS, 1990; Cd 18-90) was calculated by the addition of anisidine value with two times the peroxide value (AV + 2 PV). Lipid oxidation of CT and CD were observed by measuring specific absorptivity values at 270 and 232 nm, respectively (Rohman *et al.,* 2011).

Polyphenol, tocopherol, and phytosterol contents

The polyphenols, tocopherol, and phytosterol were estimated by the method described by Edisbury *et al.* (1954), Singleton *et al.* (1999), Sabir *et al.* (2003), and Seneviratne *et al.* (2009). The 5.0 g oil sample was extracted thrice with methanol/water (80:20 v/v, 1 mL) at 40 Hz for 20 min for polyphenol extraction. Following this, the mixture was centrifuged (1,080 *g*, 10 min), and the resulting clear solution was separated. The final extract was added to Folin-Ciocalteu reagent (1:1) at 0.5 mL, and stored in an amber vial. After 3 min of incubation, 2 mL of 7% sodium bicarbonate was added, and test tubes were placed in boiling water bath for 1 min and cooled. The OD of this solution was measured at 650 nm, and the concentration was calculated using gallic acid as a standard curve ($y = 0.002x + 0.077, R^2 = 0.984$) with mg Gallic Acid Equivalents (GAE) per g extract unit.

For tocopherol estimation, 0.2 g oil was mixed for 20 min with 10 mL Tris buffer (1.5142 g in 250 mL, adjusted to pH 7.4 with HCl) and 10 mL hexanes. The 1 mL hexane layer was mixed with 3 mL of α-α' bipyridyl and $FeCl_3$ after mixing. The OD of this solution was taken at 540 nm following incubation in the dark for 5 min. The concentration of tocopherol was calculated using a standard curve ($y = 343.8x - 1.076, R^2 = 0.998$).

For phytosterol estimation, 1 g of oil was mixed with 10 mL of chloroform for 1 h, and 3 mL of this extract was mixed with 2 mL of Liberman Burchard reagent. The test tubes were kept in an ice bucket until the characteristic of green colour formed, and the absorbance were determined at 640 nm. The concentration of phytosterol was calculated using a standard curve ($y = 0.088x + 0.012, R^2 = 0.981$).

Specific gravity, refractive index (RI), viscosity, and colour

The AOCS (1990) Cd-23-93 standard method was followed to measure the refractive index. Briefly, a drop of oil was put on the dry clean lower prism of hand held pocket refractometer (ATAGO PAL-RI, Japan). The viscosity of oil samples was taken before and after heat treatment by using a Brook Field Viscometer (Model No. DV2T), using the spindle LV-61, 25 RPM at 25°C. The colour values of treated samples were analysed in Lovibond Tintometer by following Cc 13e-92 standard method (AOCS, 1990) of taking oil samples in a standard glass cell. The specific gravity of oil was determined by Cc 10c-95 standard method (AOCS, 1990).

Fourier-transform infrared spectroscopy (FTIR) and differential scanning calorimetry

Fourier transform infrared spectroscopy (FTIR, Bruker Model Tensor) was used to analyse the oil samples. FTIR spectrometer used to record the IR spectra consisted of a ZnSe beam splitter, KBr Pellet Accessories, with Attenuated Total Reflectance (ATR). The pressure device attached to the accessory was used to provide the same pressure to all the given samples. The 16 scans were generally used to average to reduce the noise. By using the OPUS software provided along with the instrument (Rohman *et al.,* 2011), all spectra were recorded at four resolutions, and analysed with the device.

About 8 - 10 mg of oil sample was placed in hermetically sealed aluminium pans. An empty aluminium pan was used as a reference. DSC runs were operated from -60 to 25°C at a scan rate of 10°C/min. Based on the measured amount of energy (heat) absorbed by a sample during a run, the DSC manufacturer's software (DSC Sirius 3500, Proteus software) were used to analyse the heat flow data and determine the onset of melting (T_o), end set melting (T_e), peak melting temperature (T_p), and enthalpy (Δ H) of the oil samples.

Statistical analysis

The data analysis for Duncan multiple comparisons and response optimisation were done using STATISTICA stat software release 8.0 package.

Results and discussion

The oxidative stability of Moringa oil was analysed by giving three different treatments of time, temperature, and mode of the heating process. For optimisation of frying cycle, the initially soaked dal was subjected to 160, 170, 180, and 200°C for 3, 4, 5, and 6 min to get the fried dal which was analysed by semi-trained panellist for all the sensory parameters. The sensory score was judged by hedonic values and the obtained result was 7.5 for colour, texture, taste, and OAA, respectively; this was in favour of the sample treated up to 180°C for 5 min. The similar pattern of optimisation was performed in the microwave (P-100, P-80, and P-60 for 5, 10, 15, and 20 min) and oven (150, 180, and 200°C for a period of 5, 10, 15, and 20 min) treatments. The results of various physico-chemical parameters of Moringa oil (PKM-1) samples after 8 h of continuous frying, microwave, and oven treatment are tabulated in Tables 1 and 2.

Changes in peroxide value (PV) and free fatty acid (FFA)

The results showed that initial Moringa oil had 3.20 meq O_2/kg PV which increased to 8.04 meq O_2/kg after 6 h, and finally decreased to 7.56 meq O_2/kg after frying for 8 h (Table 1). The reason is that the primary oxidation of oils and formation of peroxide (at double bond) is unstable in nature. It simultaneously decomposed to form secondary oxidation products such as carbonyl and aldehyde compounds. Similar trend of PV values in olive residual oils has been reported by Chatzilazarou *et al.* (2006); however, Lalas and Tsaknis (2002) and Ogunsina *et al.* (2014) reported a continuous increase in PV in *M. oleifera* and *M. stenopetala*. Abdulkarim *et al.* (2007) demonstrated that the PV of soybean, canola, palm olein, and Moringa oil initially increased, and decreased after attaining a peak value, and reported that Moringa oil is more stable towards oxidative degradation of oils. In the microwave treatment, the PV increment was 4.55, 5.59, and 7.82 meq O_2/kg after 20 min of treatment at P-60, P-80, and P-100, respectively. Further, an increase was observed at 20 min of treatment at P-60 (42.1%) and P-80 (74.6%). However, at P-100 for 20 min treatment, the PV (7.82 meq O_2/kg) was almost similar to the 8 h of conventionally fried samples (Table 1). The same trend of PV had been reported during microwave treatment in sunflower, high oleic peanut, and zero-erucic rapeseed oils. The PV in a conventional heating increased from 3.20 to 5.65 meq O2/kg (Table 1), but this increase was to a lesser extent on comparing it with microwave treatment and deep fat frying.

The present work revealed that the FFA content of raw Moringa oil was 0.79% lauric acid, which increased to 1.66% (Table 1) after 8 h of deep fat frying, and the values are within the range specified by FAO and WHO (2009) which is 2.89 ± 0.01. In addition to frying, oil samples were subjected to microwave treatment at P-60, P-80, and P-100 power levels for 20 min, and values of FFA obtained were 0.92, 1.12,

Table 1. Changes in physical properties, peroxide value (PV; meq O_2/kg fat), free fatty acid (FFA; % lauric acid), anisidine value (AV), polyphenol (µg/g), phytosterols (µg/g), tocopherol content (µg/g), conjugated dienes (CD), and trienes (CT) during deep fat frying of 8 h, microwave heating (at P-60, P-80, and P-100 for 5, 10, 15, and 20 min), and electric oven treatment at different temperatures (150, 180, and 200°C) for different exposure times (5, 10, 15, and 20 min).

Treatment	Sample Name	PV (meq O_2/kg fat)	FFA (% acid)	AV	TOTOX	IV (g I2/100 g)	Polyphenol (µg/g)	Phtyo Sterols (µg/g)	Tocopherol (µg/g)	CD	CT	Density	Specific Gravity	Viscosity (cP)	RI
Frying	0	3.20 ± 0.01^{a}	0.79 ± 0.001^{a}	1.76 ± 0.01^{a}	8.16	65.97 ± 0.14^{a}	189.51 ± 1.01^{a}	8.35 ± 0.01^{a}	34.40 ± 0.51^{a}	0.913 ± 0.001^{a}	0.210 ± 0.001^{a}	0.885 ± 0.001^{a}	0.920 ± 0.001^{a}	36.64 ± 0.01^{a}	1.4495 ± 0.0001^{a}
	2 h	5.94 ± 0.02^{b}	0.98 ± 0.002^{b}	1.98 ± 0.01^{a}	13.86	65.89 ± 0.11^{a}	174.35 ± 2.02^{b}	8.16 ± 0.02^{a}	32.26 ± 0.40^{b}	1.050 ± 0.002^{a}	0.271 ± 0.003^{a}	0.890 ± 0.001^{a}	0.922 ± 0.001^{a}	37.12 ± 0.02^{a}	1.4662 ± 0.0001^{a}
	4 h	7.95 ± 0.01^{c}	1.15 ± 0.001^{c}	2.22 ± 0.11^{b}	18.12	65.75 ± 0.19^{a}	141.24 ± 1.01^{c}	7.84 ± 0.02^{b}	30.24 ± 0.28^{c}	1.141 ± 0.003^{b}	0.320 ± 0.002^{a}	0.901 ± 0.001^{a}	0.934 ± 0.001^{a}	39.28 ± 0.01^{b}	1.4608 ± 0.0001^{a}
	6 h	8.04 ± 0.02^{d}	1.50 ± 0.002^{c}	2.45 ± 0.02^{b}	18.53	65.18 ± 0.13^{a}	133.11 ± 2.01^{d}	7.13 ± 0.03^{b}	27.91 ± 0.33^{d}	1.838 ± 0.002^{b}	0.540 ± 0.003^{b}	0.967 ± 0.001^{a}	1.002 ± 0.001^{b}	41.67 ± 0.02^{c}	1.4651 ± 0.0001^{a}
	8 h	7.56 ± 0.01^{c}	1.66 ± 0.001^{c}	2.73 ± 0.02^{b}	17.85	64.01 ± 0.12^{b}	118.26 ± 1.01^{e}	6.57 ± 0.01^{c}	22.50 ± 0.47^{e}	2.040 ± 0.001^{c}	0.591 ± 0.002^{b}	1.007 ± 0.001^{b}	1.045 ± 0.001^{b}	43.84 ± 0.01^{d}	1.4653 ± 0.0001^{a}
Microwave treatment															
P-60	5 min	3.49 ± 0.25^{a}	0.80 ± 0.02^{a}	1.81 ± 0.04^{a}	8.79	65.96 ± .019^{a}	186.28 ± 1.01^{a}	8.29 ± 0.21^{a}	34.10 ± 1.03^{a}	0.990 ± 0.012^{a}	0.229 ± 0.012^{a}	0.899 ± 0.003^{a}	0.920 ± 0.002^{a}	37.07 ± 0.91^{a}	1.4614 ± 0.002^{a}
	10 min	3.54 ± 0.33^{a}	0.82 ± 0.01^{a}	1.89 ± 0.19^{a}	8.97	65.93 ± .021^{a}	181.25 ± 1.11^{b}	8.22 ± 0.19^{a}	34.01 ± 0.91^{a}	0.981 ± 0.021^{a}	0.258 ± 0.012^{a}	0.897 ± 0.001^{a}	0.922 ± 0.001^{a}	37.18 ± 0.85^{a}	1.4641 ± 0.001^{a}
	15 min	3.50 ± 0.19^{a}	0.87 ± 0.03^{a}	2.03 ± 0.09^{a}	9.03	65.92 ± .032^{a}	179.69 ± 1.26^{c}	8.19 ± 0.30^{a}	33.63 ± 0.89^{a}	0.980 ± 0.014^{a}	0.297 ± 0.023^{a}	0.895 ± 0.002^{a}	0.924 ± 0.003^{a}	38.46 ± 1.11^{a}	1.4617 ± 0.002^{a}
	20 min	4.55 ± 0.24^{b}	0.92 ± 0.04^{b}	2.12 ± 0.11^{b}	11.22	65.92 ± 0.12^{a}	177.96 ± 1.48^{c}	8.16 ± 0.18^{a}	33.08 ± 0.87^{a}	0.980 ± 0.011^{a}	0.311 ± 0.021^{a}	0.895 ± 0.001^{a}	0.928 ± 0.001^{a}	38.56 ± 0.56^{a}	1.4617 ± 0.001^{a}
P-80	5 min	3.59 ± 0.61^{a}	0.84 ± 0.01^{a}	1.83 ± 0.12^{a}	9.01	65.88 ± 0.23^{a}	176.53 ± 1.86^{c}	8.17 ± 0.14^{a}	33.0 ± 0.79^{a}	0.989 ± 0.030^{a}	0.380 ± 0.023^{a}	0.912 ± 0.002^{a}	0.921 ± 0.002^{a}	38.90 ± 0.94^{ab}	1.4627 ± 0.001^{a}
	10 min	3.60 ± 0.18^{a}	0.91 ± 0.02^{b}	1.92 ± 0.16^{a}	9.12	65.85 ± .032^{a}	163.97 ± 1.17^{d}	8.13 ± 0.16^{a}	32.63 ± 0.69^{b}	0.990 ± 0.015^{a}	0.397 ± 0.013^{b}	0.919 ± 0.001^{a}	0.932 ± 0.001^{a}	39.41 ± 1.21^{b}	1.4629 ± 0.002^{a}
	15 min	4.62 ± 0.92^{b}	0.98 ± 0.03^{b}	1.98 ± 0.19^{a}	11.22	65.81 ± 0.41^{a}	161.82 ± 1.19^{d}	8.05 ± 0.17^{a}	32.11 ± 0.54^{b}	1.070 ± 0.012^{b}	0.411 ± 0.015^{b}	0.926 ± 0.003^{a}	0.950 ± 0.001^{a}	39.51 ± 0.97^{b}	1.4637 ± 0.001^{a}
	20 min	5.59 ± 0.85^{b}	1.12 ± 0.02^{c}	2.14 ± 0.12^{b}	13.32	65.76 ± 0.11^{a}	159.65 ± 1.35^{e}	7.99 ± 0.21^{b}	31.16 ± 0.68^{b}	1.161 ± 0.031^{b}	0.440 ± 0.017^{b}	0.934 ± 0.001^{a}	0.968 ± 0.002^{a}	41.45 ± 0.92^{c}	1.4647 ± 0.002^{a}
P-100	5 min	4.97 ± 0.76^{b}	0.89 ± 0.04^{a}	1.19 ± 0.11^{a}	11.13	65.75 ± 0.17^{a}	157.58 ± 1.21^{e}	7.88 ± 0.23^{b}	31.01 ± 0.75^{b}	1.179 ± 0.016^{b}	0.461 ± 0.013^{bc}	0.927 ± 0.002^{a}	0.971 ± 0.001^{a}	39.43 ± 1.13^{b}	1.4648 ± 0.003^{a}

	10 min	6.31 ± 0.82^{b}	0.97 ± 0.02^{b}	2.02 ± 0.09^{a}	14.64	65.68 ± 0.18^{a}	142.61 ± 1.17^{f}	7.63 ± 0.18^{b}	29.98 ± 0.68^{c}	1.308 ± 0.021^{b}	0.493 ± 0.012^{c}	0.946 ± 0.001^{a}	0.981 ± 0.001^{a}	40.89 ± 1.02^{b}	1.4644 ± 0.004^{a}
	15 min	8.03 ± 0.79^{d}	1.28 ± 0.03^{c}	2.27 ± 0.11^{b}	18.33	65.21 ± 0.17^{a}	126.99 ± 1.13^{g}	7.20 ± 0.14^{b}	27.54 ± 0.54^{d}	1.726 ± 0.032^{b}	0.514 ± 0.018^{c}	1.011 ± 0.002^{b}	1.048 ± 0.001^{b}	41.04 ± 0.84^{c}	1.4667 ± 0.001^{a}
	20 min	7.82 ± 0.69^{c}	1.68 ± 0.01^{c}	2.69 ± 0.14^{b}	18.32	64.76 ± 0.17^{b}	116.34 ± 1.69^{h}	6.49 ± 0.14^{c}	23.17 ± 0.61^{e}	2.108 ± 0.041^{c}	0.522 ± 0.015^{c}	1.288 ± 0.001^{b}	1.067 ± 0.001^{b}	43.52 ± 0.75^{d}	1.4701 ± 0.002^{a}
Hot air oven treatment															
150°C	5 min	3.21 ± 0.11^{a}	0.81 ± 0.02^{a}	1.79 ± 0.10^{a}	8.21	65.97 ± 0.15^{a}	187.87 ± 3.03^{a}	8.30 ± 0.23^{a}	34.01 ± 0.16^{a}	0.987 ± 0.012^{a}	0.269 ± 0.003^{a}	0.885 ± 0.002^{a}	0.920 ± 0.012^{a}	37.21 ± 0.69^{a}	1.4617 ± 0.0001^{a}
	10 min	3.37 ± 0.02^{a}	0.88 ± 0.01^{a}	1.83 ± 0.11^{a}	8.57	65.96 ± 0.12^{a}	186.46 ± 1.24^{a}	8.29 ± 0.24^{a}	34.00 ± 0.15^{a}	0.986 ± 0.021^{a}	0.288 ± 0.002^{a}	0.886 ± 0.001^{a}	0.920 ± 0.011^{a}	37.22 ± 0.91^{a}	1.4628 ± 0.0001^{a}
	15 min	3.53 ± 0.13^{a}	0.91 ± 0.02^{b}	1.88 ± 0.08^{a}	8.94	65.94 ± 0.11^{a}	183.29 ± 2.39^{a}	8.23 ± 0.16^{a}	33.98 ± 0.11^{a}	0.983 ± 0.011^{a}	0.297 ± 0.011^{a}	0.886 ± 0.002^{a}	0.920 ± 0.013^{a}	37.44 ± 0.58^{a}	1.4639 ± 0.0001^{a}
	20 min	3.72 ± 0.21^{a}	0.91 ± 0.03^{b}	1.99 ± 0.09^{a}	9.43	65.91 ± 0.32^{a}	182.87 ± 1.47^{b}	8.22 ± 0.20^{a}	33.96 ± 0.21^{a}	0.981 ± 0.023^{a}	0.307 ± 0.012^{a}	0.887 ± 0.011^{a}	0.920 ± 0.012^{a}	37.80 ± 0.92^{a}	1.4645 ± 0.0002^{a}
180°C	5 min	3.73 ± 0.15^{a}	0.89 ± 0.01^{b}	1.81 ± 0.07^{a}	9.27	65.91 ± 0.35^{a}	181.73 ± 2.56^{b}	8.20 ± 0.19^{a}	33.90 ± 0.25^{a}	0.988 ± 0.024^{a}	0.319 ± 0.013^{a}	0.887 ± 0.021^{a}	0.921 ± 0.001^{a}	37.40 ± 0.86^{a}	1.4620 ± 0.0001^{a}
	10 min	3.94 ± 0.05^{a}	0.97 ± 0.02^{b}	1.90 ± 0.11^{a}	9.78	65.87 ± 0.11^{a}	180.85 ± 1.69^{b}	8.13 ± 0.31^{a}	32.99 ± 0.19^{b}	0.990 ± 0.012^{a}	0.323 ± 0.014^{a}	0.887 ± 0.013^{a}	0.913 ± 0.002^{a}	37.61 ± 0.79^{a}	1.4627 ± 0.0001^{a}
	15 min	3.99 ± 0.07^{a}	1.09 ± 0.03^{c}	2.03 ± 0.13^{b}	10.01	65.84 ± 0.18^{a}	179.78 ± 2.79^{c}	8.03 ± 0.12^{a}	32.91 ± 0.18^{b}	1.027 ± 0.013^{a}	0.341 ± 0.012^{a}	0.887 ± 0.014^{a}	0.908 ± 0.010^{a}	38.03 ± 0.82^{a}	1.4647 ± 0.0001^{a}
	20 min	4.04 ± 0.14^{b}	1.29 ± 0.04^{c}	2.39 ± 0.14^{b}	10.47	65.80 ± 0.16^{a}	176.26 ± 2.01^{c}	8.02 ± 0.32^{a}	32.80 ± 0.12^{b}	1.121 ± 0.021^{b}	0.352 ± 0.011^{a}	0.889 ± 0.015^{a}	0.921 ± 0.011^{a}	41.73 ± 0.76^{c}	1.4646 ± 0.0002^{a}
200°C	5 min	4.12 ± 0.23^{b}	0.86 ± 0.01^{a}	1.90 ± 0.06^{a}	10.14	65.78 ± 0.11^{a}	171.84 ± 1.52^{d}	8.01 ± 0.24^{a}	32.18 ± 0.17^{b}	1.443 ± 0.015^{b}	0.369 ± 0.012^{a}	0.889 ± 0.002^{a}	0.921 ± 0.013^{a}	38.79 ± 0.81^{a}	1.4650 ± 0.0001^{a}
	10 min	4.72 ± 0.22^{b}	1.11 ± 0.02^{c}	1.99 ± 0.08^{a}	11.43	65.72 ± 0.14^{a}	153.91 ± 1.56^{e}	7.99 ± 0.16^{b}	31.97 ± 0.20^{b}	1.537 ± 0.016^{b}	0.377 ± 0.013^{b}	0.890 ± 0.011^{a}	0.922 ± 0.011^{a}	40.77 ± 0.69^{b}	1.4651 ± 0.0001^{a}
	15 min	5.17 ± 0.31^{b}	1.37 ± 0.03^{c}	2.11 ± 0.09^{b}	12.45	65.41 ± 0.23^{a}	143.56 ± 2.51^{f}	7.81 ± 0.04^{b}	30.81 ± 0.23^{c}	1.636 ± 0.013^{b}	0.401 ± 0.011^{b}	0.893 ± 0.013^{a}	0.924 ± 0.010^{a}	41.02 ± 0.73^{c}	1.4655 ± 0.0001^{a}
	20 min	5.65 ± 0.40^{b}	1.51 ± 0.02^{c}	2.42 ± 0.12^{b}	13.72	64.02 ± 0.14^{b}	137.54 ± 3.11^{g}	7.05 ± 0.10^{b}	25.12 ± 0.22^{e}	2.020 ± 0.012^{c}	0.481 ± 0.022^{b}	0.896 ± 0.016^{a}	0.928 ± 0.002^{a}	42.92 ± 0.81^{d}	1.4656 ± 0.0002^{a}

Values are mean ± standard deviation. Means with different superscript letters within the same column differ significantly ($p < 0.05$).

and 1.68% respectively (Table 1). During oven treatment at 150, 180, and 200°C for 20 min, the values obtained were 0.91, 1.29, and 1.51 respectively (Table 1). For the oils heated in the deep fat fryer, microwave, and electric oven, an increase in values of FFA was observed as compared to fresh oils, and this increase continued until the end of the heating period. This increment in FFA resulted from partial hydrolysis and the presence of polymeric products like carboxylic groups during frying (Tyagi and Vasishtha, 1996). A similar trend has been reported by Lalas and Tsaknis (2002) in a study of frying stability of *M. stenopetala*; and Aydinkaptan *et al.* (2017) during microwave heating of sunflower at different power levels. The increase in FFA content after 8 h of frying is almost similar to increase in FFA after 20 min of microwave treatment at P-100 power level. However, this increase is comparable to a lesser extent during oven treatment at 200°C for 20 min, which resembled a value after 6 h of deep fat frying.

The trend of PV and FFA was in favour of the results obtained from the FTIR peak range 3800 - 3200 cm^{-1} in which –OH stretching region associated with common oxidation end product formation during 08 h of continuous frying process, but the spectral examination did not visually disclose any appreciable differences between their spectral features. The initial absorbance value of oil sample was 0.03704 which increased to 0.03916 after 06 h of frying, and finally decreased to 0.02917 at 3300 cm^{-1} which was associated with hydroperoxide or FFA formation. The absorbance value of fried oil Moringa samples increased from 0.9483 to 1.1577 at intensity of 1739 cm^{-1} which showed the decomposition of hydroperoxide and formation of carbonyl compounds (a secondary oxidation product) as previously reported by Smith *et al.* (2005).

Changes in iodine value (IV)

The effect of deep fat frying, microwave, and conventional oven heating on the IV of Moringa oil was measured, and values were in the range of 65.97 to 64.01, 65.97 to 64.76, and 65.97 to 65.02 g I_2/100 g oil as shown in Tables 1 and 2, respectively. The IV before frying of oil decreased from 65.97 to 64.01 g I_2/100 g after frying (8 h). The calculated values were lower than the range specified for edible oils by FAO and WHO (2009) i.e. 80 - 106 g I_2/100 g. This decrease in IV is the result of a reduction in double bonds when the oil undergoes an oxidation or polymerisation process (Alireza *et al.,* 2010). The percent of decrease in IV after three different treatments i.e. 8 h of frying (deep fat), 20 min of microwave heating (P-100 power level), and oven heating at 200°C were 2.97, 1.83, and 1.44% respectively. The data analysis showed that oil oxidised more rapidly during deep-fat frying than microwave and oven heating.

Changes in p-anisidine value (p-AV) and total oxidation (TOTOX value)

The p-AV of fried Moringa oil samples ranged from 1.76 to 2.73 (Table 1). Similar changes in p-AV values after frying in high oleic Moringa oil has been reported by Abdulkarim *et al.* (2007). The p-AV of Moringa oil during exposure to different power levels in microwave at P-60, P-80, and P-100 for 20 min showed an increasing trend as shown in Table 1, while heating of Moringa oil samples in conventional oven for 20 min at 150, 180, and 200°C were 1.99, 2.39, and 2.42, respectively (Table 1). Meghahed *et al.* (2011) showed an increasing trend of p-AV values while demonstrating the effect of microwave heating on the formation of oxidised products of linseed oil.

The calculated TOTOX value of fresh and fried Moringa oil samples showed an increment from 8.16 to 17.85, and then decrease after 8 h of continuous frying (Table 1). Initially, hydroperoxides were formed at a frequent rate during frying process which was very unstable and break into aldehyde; apart from it, hydroperoxide of the saturated fatty acid can also produce the aldehyde at higher temperature which was the cause of increase in p-AV and changes in TOTOX value. After 20 min of exposure to microwave heating, TOTOX values were 11.22, 13.32, and 18.32 at a power level of P-60, P-80, and P-100, respectively (Table 1). However, after 20 min of oven treatment at 150, 180, and 200°C, the values were 9.43, 10.47, and 13.72, respectively (Table 1).

Changes in polyphenol, phytosterol content tocopherol contents

The Moringa oil had an initial polyphenol content of 189 mg GAE g^{-1} which continuously decreased after 8 h of frying, and the percent decrement was 37.59%. The total polyphenol content of Moringa seed oil reported in the present work is higher than that reported by Ogbungafor *et al.* (2011). The rate of polyphenol oxidation was more at higher temperature and time combinations. However, the presence of saturated fats and water in the oil can enhance the rate of polyphenol oxidation. Many factors like maturity state, sample preparation, and the analytical method used in the process could have led to the difference in the results. The percent decrement at P-60, P-80, and P-100 after 20 min of microwave treatment was 6.1, 15.79, and 38.6%, respectively (Table 1). Similar trend of reduction of polyphenol content was observed during conventional oven treatment of oil at 150, 180,

and 200°C for 20 min, and were 3.5, 7.0, and 27.4%, respectively (Table 1). The trend is similar to that reported by Albi *et al.* (1997) during their study on microwave and conventional heating effects of vegetable oils. It was clearly observed that the total polyphenol content of Moringa oil decreased as conventional frying, microwave heating, and heating through the electric oven progressed, and this decrement was more pronounced in microwave heating.

The changes in phytosterol values were measured to compare the heating effects of microwave and conventional heating, and are tabulated in Tables 1 and 2. The Moringa oil had initial phytosterol content of 8.35 µg/g, which decreased with the frying time, and the percent decrement was 37.59% after 8 h of frying. Similar trend of decrement was observed during microwave heat treatment at P-60 (2.27%), P-80 (4.3%), and P-100 (22.2%) after 20 min, and in the conventional oven treatment at 150°C (1.55%), 180°C (3.9%), and 200°C (15.56%) for 20 min, respectively. Furthermore, it was observed from our result that the amount of phytosterols in Moringa oil decreased as conventional frying, microwave heating, and heating through the electric oven progressed.

The tocopherol content of raw Moringa oil was 34.40 µg/g but decreased by 18.86 and 34.59% after 6 and 8 h of deep fat frying, respectively. The percentage decrease in total tocopherol content after 20 min at P-60, P-80, and P-100 were 3.8, 9.4, and 32.6%, respectively. In addition to this, heating in an electric oven at 150, 180, and 200°C lead to 1.27, 4.6, and 26.9% reduction of tocopherol content in comparison to fresh oil after 20 min of exposure time, respectively. Hassanein *et al.* (2003) also reported similar results of decrease in tocopherol content during microwave heating of edible oils, concluding that tocopherols were unstable in vegetable oils rich in unsaturated fatty acids, and attributed the losses in tocopherols mainly to degradation and partly to peroxidation of oils. It is evident from the results that the extent of oxidative degradation was higher in deep fat fried and microwaved than in conventionally oven treated oils.

Change in specific absorptivity (CD232 and CT270)

The specific absorptivity of raw Moringa oil sample at 232 and 270 nm was 0.913 and 0.210 $mmolL^{-1}$, which increased to 2.040 and 0.591 $mmolL^{-1}$ respectively, after 8 h of frying (Table 1). Farhoosh (2009) reported that the values for CD and CT linearly increased with frying. The results agree with that of Abdulkarim *et al.* (2007) who reported that the value of the CD and CT increased during frying because of high oleic acid in Moringa seed oil. It was observed in our analysis that the levels of conjugated trienes were lower than that of dienes in case of Moringa oil. Tsaknis and Lalas (2002) also reported similar trend of CD during frying of Moringa oil (PKM-1). The values of the CD and CT during microwave exposure of Moringa oil for 20 min at P-60, P-80, and P-100 were 0.980, 1.161, and 2.108 $mmolL^{-1}$; and 0.311, 0.440, and 0.522 $mmolL^{-1}$, respectively (Table 1). For conventional oven treatment at 150, 180, and 200°C, the values of CD and CT were 0.981, 1.121, and 2.02 and 0.307, 0.352, and 0.481, respectively, after 20 min (Table 1). Heating in the conventional oven also led to an increase in values of CD and CT, but to a somewhat lesser extent when compared to deep fat frying and microwave heating. The oxidative stability of Moringa oil is directly related to low levels of CD and CT because Moringa oil is rich in polyphenols which play a major role in inhibiting the formation of peroxides (Marina *et al.,* 2009).

Changes in physical parameters (viscosity, density, specific gravity, refractive index, and colour)

The viscosity of raw Moringa seeds oil was 36.64 cp. It was observed that the Moringa oil viscosity was lower than previously reported values by Tsaknis *et al.* (1999) (57 cp), and Salah (2006) (45.82 cp). The viscosity of Moringa oil increased from 36.64 to 43.84 cp after 8 h of deep fat frying (Table 1). The viscosity of oil depends on the nature and type of triglycerides present in them. It progressively increases in the same manner as the rate of oxidation is accelerated upon heating. The possible reason for this viscosity increase is the formation of cyclic and high molecular weight compounds as a result of cross-linking of the carbon atom present in fatty acid molecules, and due to the presence of air and moisture during heating (Tsaknis and Lalas, 2002). The rate of deterioration is directly proportional to the viscosity of the frying oil. Deterioration during oil frying is accelerated by the presence of air and moisture, which leads to an increase in the density and viscosity of oils (Zahir *et al.,* 2017). The similar increase in viscosity was reported by Lalas and Tsaknis (2002) while estimating the frying stability of Moringa oil (PKM-1) variety. The viscosities of oil samples during microwave treatment at P-60, P-80, and P-100 for 20 min were 38.56, 41.45, and 43.52 cp, respectively (Table 1). During conventional oven heating, oil samples after 20 min of heating have viscosity values of 37.80, 41.73, and 42.92 cp at temperatures of 150, 180, and 200°C, respectively (Table 1). The percent increment after 4, 6, and 8 h of deep fat frying was 7.2, 13.7, and 19.65%,

respectively. The percent increment in viscosity after 8 h of frying was comparable with the value at P-100 power level during the 20 min of microwave heating (18.7%) and of oven heating at a temperature of 200°C (17.1%).

The changes in the density of fried Moringa oil are given in Table 1. The density of Moringa oil was 0.885 mg/mL. This value is lower than previously reported by Lalas and Tsaknis (2002) of 0.909 mg/mL at 24°C. However, it is almost similar to 0.8809 mg/mL reported by Tsaknis *et al.* (1999). The density of oil after frying showed gradual increase with frying time from 0.885 to 1.007 mg/mL. The changes may be due to the presence of added mass like polymerised products of oils, carbonyl compounds, and water. Kalogianni *et al.* (2011) showed an increase in density during the repetitive frying of palm or olive oil. A similar rise in density and specific gravity due to repeated frying was reported by Tyagi and Vasishtha (1996). The density of oil samples during microwave heating at P-60, P-80, and P-100 for a period of 20 min were 0.895, 0.934, and 1.0288 mg/mL, respectively (Table 1). The density values of 0.887, 0.889, and 0.896 mg/mL were observed when oil samples were subjected to oven treatment for 20 min at temperatures of 150, 180, and 200°C, respectively (Table 1). The percent increment in values of density after 8 h of conventional frying, microwave heating at P-100, and oven treatment at 200°C for 20 min were 16.24, 13.78, and 1.24%, respectively. The results showed that a slight change in density was observed during conventional oven heating of Moringa oil.

The specific gravity of raw Moringa oil was 0.920, and a change was observed after 8 h of continuous frying i.e. 1.045 (Table 1). This value of specific gravity confirmed the use of Moringa as edible oil as it matched the specifications given by FAO and WHO (2009; 0.9 - 1.16) for edible oils. After 20 min of exposure to microwave heating, specific gravity values were 0.928, 0.968, and 1.067 at a power level of P-60, P-80, and P-100, respectively (Table 1), while after 20 min of oven treatment at 150, 180, and 200°C were 0.920, 0.921, and 0.928, respectively (Table 1). This increase in specific gravity was due to the generation of dipoles as described by Tabasum *et al.* (2012). However, Adejumo *et al.* (2013) stated that the specific gravity of Moringa seed oil decreased upon exposure to heat and temperature. The results revealed 13.47, 15.97, and 0.86% increment in specific gravity after 8 h of deep fat frying, 20 min of microwave heating (P-100) and 20 min of oven treatment (200°C), respectively, with a lower increment during oven heating of oil.

The value of the refractive index for raw Moringa oil was 1.4495, which is comparable to 1.4570 given by Basuny and Al-Marzouq (2016), and lower than the values observed by Tsaknis *et al.* (1999) and Anwar and Rashid (2007). The values of RI slightly increased and achieved a value of 1.4653 after 8 h of frying (Table 1). This gradual increase in RI is due to an increase in polymer weight of compounds. Microwave heating of Moringa oil showed a slight increase in values of RI with an increase in power levels, exposure time, and temperature. The values of RI at P-60, P-80, and P-100 after 20 min of heating were 1.4617, 1.4647, and 1.4701, respectively (Table 1). In the same manner, oven treatment of oil at 150, 180, and 200°C for 20 min also showed an increasing trend, and values observed were 1.4645, 1.4646, and 1.4656, respectively (Table 1). The percent increment in RI after 8 h of conventional frying, 20 min of microwave (P-100), and oven (200°C) heating was reported as 1.09, 1.42, and 1.11%, respectively. A similar pattern of the increase was reported by Farag *et al.* (1992) during a comparative study on cottonseed and palm oil deterioration by conventional and microwave treatment.

The initial colour values of Moringa oil was 0.2 Red (R) + 0.8 Yellow (Y) + 0.0 Blue (B) + 0.5 Neutral (N) which changed to 1.1 (R) + 18.0 (Y) + 0.0 (B) + 0.4 (N); 0.2 (R) + 0.5 (Y) + 0.0 (B) + 0.5 (N); 1.0 (R) + 0.8 (Y) + 0.0 (B) + 0.0 (N); 0.4 (R) + 2.1 (Y) + 0.0 (B) + 0.1 (N); 0.45 (R) + 1.7 (Y) + 0.0 (B) + 0.1 (N); 0.5 (R) + 2.1 (Y) + 0.0 (B) + 0.1 (N); and 0.9 (R) + 6.4 (Y) + 0.0 (B) + 0.1 (N) after 8 h of frying, P-60 (20 min), P-80 (20 min), P-100 (20 min), 150°C (20 min), 180°C (20 min), and 200°C (20 min) heating treatment, respectively.

FTIR spectra and DSC analysis of Moringa oil samples

The changes in the oxidation of fresh, fried, microwaved, and conventionally oven heated oil samples are shown in the spectra in Figures 1(a), 1(b), and 1(c), respectively. The fresh oil samples showed the absorption bands at peak ranges of 3200 - 2800 cm^{-1} (C-H alkane stretch), 1450 - 1375 cm^{-1} (CH_3 bending), 1600 - 1900 cm^{-1} (C-C alkane), 1000 - 650 cm^{-1} (alkene out of plane bend), and 1850 - 1650 cm^{-1} (C=O carbonyl stretch), which are similar to the peak ranges reported by Virbhute *et al.* (2015) in Moringa seed oil.

Zhang *et al.* (2015) reported that the absorption spectrum 966 - 968 cm^{-1} in the FTIR spectrum gives the idea of trans fatty acid formation. The absorption value increased from 0.13958 to 0.17730, 0.13958 to 0.17736, and 0.13958 to 0.15683 after 8 h

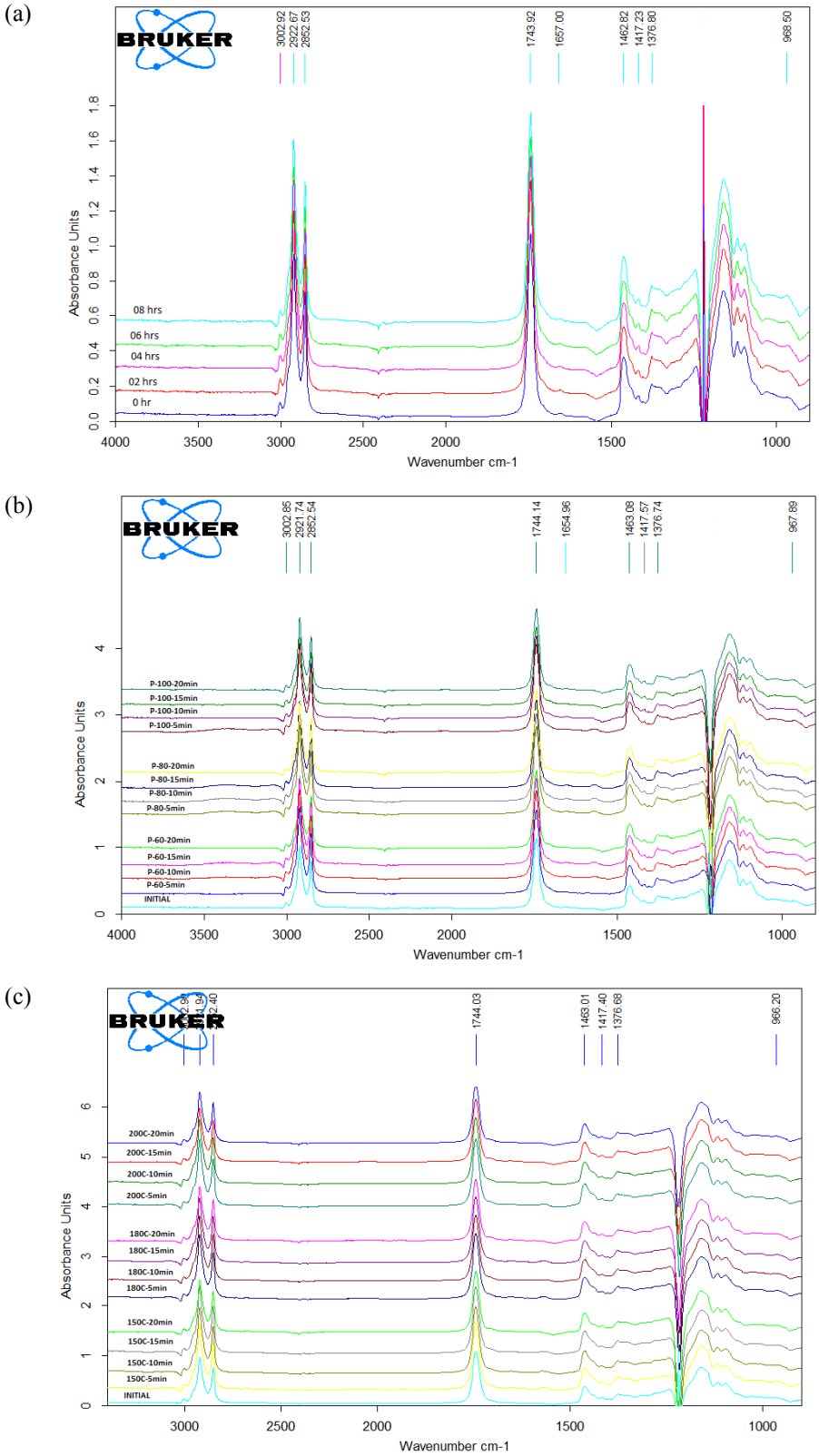

Figure 1. FTIR spectra of Moringa oil samples of (a) frying (after 0, 2, 4, 6, and 8 h), (b) microwave heating (P-60, P-80, P-100 for 5, 10, 15, and 20 min), and (c) oven heating (150, 180, and 200°C for 5, 10, 15, and 20 min).

of continuous Moringa oil frying, microwave heating for 20 min at P-100, and oven heating at 200°C for 20 min, respectively, which clearly indicated the formation of trans fatty acid (Figure 2). The fresh Moringa oil showed 0.0968 absorbance value at peak 3008 cm-1 which decreased to 0.07334 (8 h of frying), 0.06892 (microwave heating for 20 min, P-100) and 0.06336 (oven heating at 200°C, 20 min) (Figure 2). Previously, Andina *et al.* (2017) reported that peak at 3008 cm^{-1} correlated with a decrease in unsaturation

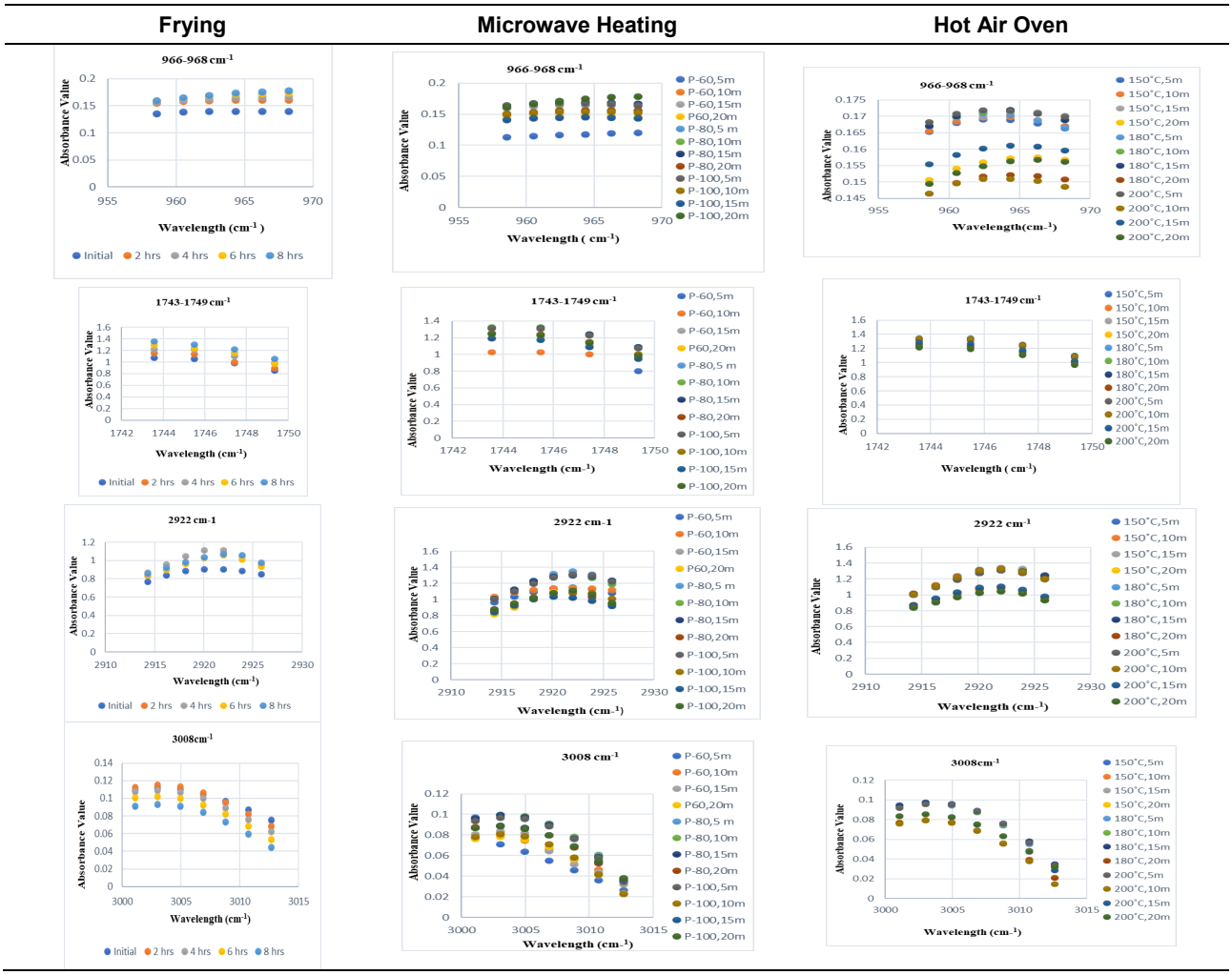

Figure 2. FTIR spectra of Moringa oil samples for frying (after 0, 2, 4, 6, and 8 h), microwave heating (P-60, P-80, and P-100 for 5, 10, 15, and 20 min), and oven heating (150, 180, and 200°C for 5, 10, 15, and 20 min).

of cis double bond. Hashim *et al.* (2017) and Sivakesava and Irudayaraj (2000) observed that the peak 2922 and 1749 cm^{-1} showed the formation of a conjugated diene and triene, respectively, in different fried oil samples. The graph shows peaks at 2922 and 1749 cm^{-1}, which indicate the increase in the absorbance value leading to the formation of CD and CT (Figure 2).

The onset, peak, end set, and ΔH of melting and crystallisation of Moringa oil at different testing conditions are shown in Table 2. The results showed that there was a shift in melting peaks from a lower temperature to higher temperature with respect to time. The thermal properties of treated oil samples were characterised by onset, endset, and peak temperature from melting and crystallisation curves. It was observed that the crystallisation peak (onset, endset, and peak) shifted towards higher side in all the three types of treated samples (fry, microwave, and hot air oven) which might be due to the increase

Table 2. Changes in onset, peak, end set, and ΔH of melting and crystallisation of Moringa oil of initial, after 8 h of frying, P-100 (20 min) (microwave treatment), and 200°C (20 min) (hot air oven treatment).

Sample Name	Melting Peak				Crystallisation Peak			
	Onset	Peak	Endset	ΔH (J/g)	Onset	Peak	Endset	ΔH (J/g)
Initial	-7.40 ± 0.13	0.70 ± 0.17	4.50 ± 0.13	47.31 ± 0.10	-34.40 ± 0.12	-31.2 ± 0.11	-28.0 ± 0.16	2.37 ± 0.09
Frying (8 h)	-12.10 ± 0.11	-7.30 ± 0.14	-2.80 ± 0.16	6.51 ± 0.15	-37.80 ± 0.14	-28.6 ± 0.19	-20.8 ± 0.12	4.71 ± 0.17
P-100, 20 min	-10.00 ± 0.12	-0.30 ± 0.11	3.30 ± 0.12	38.88 ± 0.13	-33.30 ± 0.18	-29.2 ± 0.15	-24.8 ± 0.11	5.23 ± 0.13
200°C, 20 min	-9.60 ± 0.10	-3.00 ± 0.12	-1.10 ± 0.11	0.82 ± 0.12	-29.40 ± 0.10	-24.2 ± 0.13	-22.6 ± 0.14	0.25 ± 0.11

Values are mean ± standard deviation.

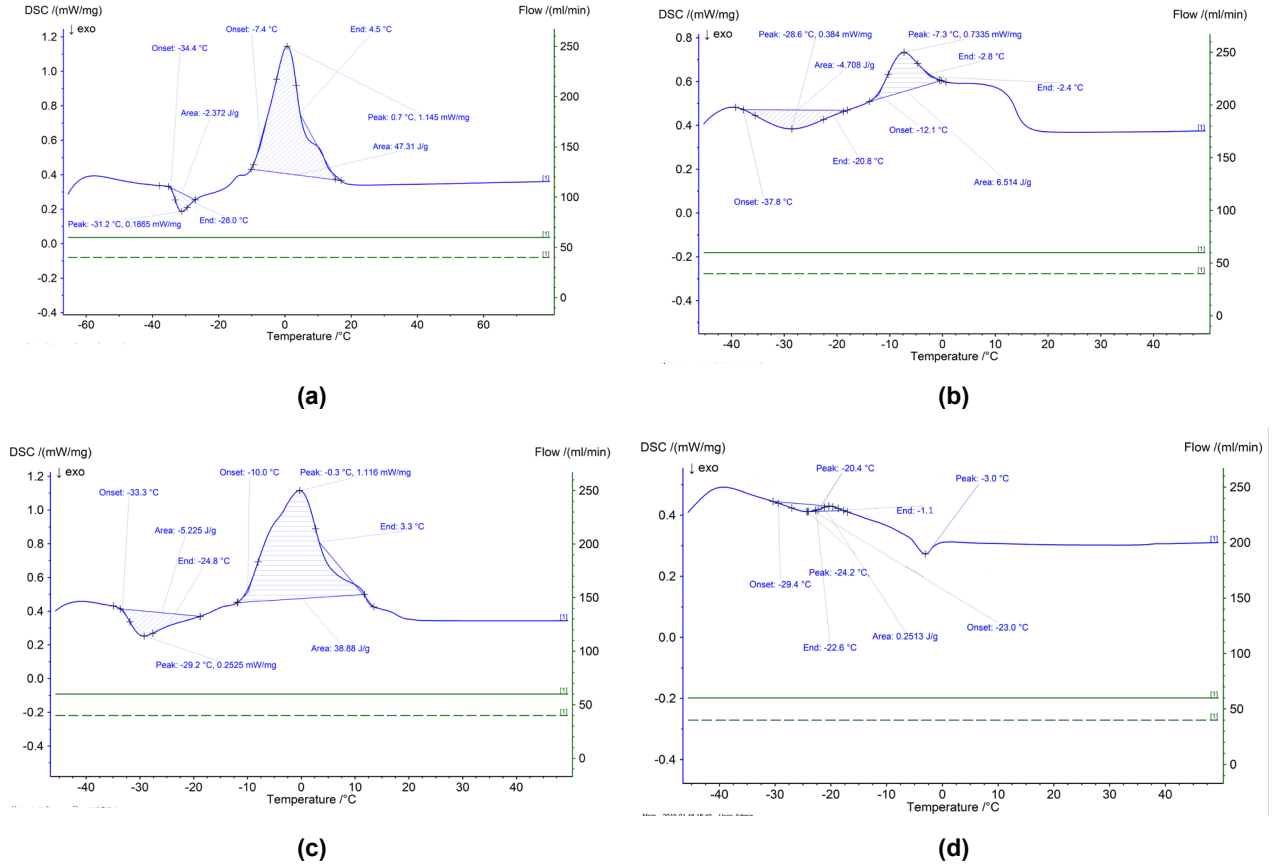

Figure 3. DSC thermograms of Moringa oil samples for (a) initial, (b) frying (after 8 h), (c) microwave heating (P-100 for 20 min), and (d) oven heating (200°C for 20 min).

in FFA or decrease in triglycerides (Figure 3). The shifting of melting peaks towards higher level in all samples might be due to the complexity of triglycerides from molten to crystallisation state by nucleation, activation crystal growth, and lattice (Srivastava *et al.,* 2017).

Conclusion

The results of physical (specific gravity, density, RI, and viscosity) and chemical analyses of Moringa oil indicate that the oil was stable and acceptable after 8 h of frying as well as at high microwave power level exposure (P-100 for 20 min), and in conventional oven heating at 200°C for 20 min. FTIR absorbance value at 3300 cm^{-1} showed hydroperoxide or FFA formation. The increase in absorbance at 1739 cm^{-1} was an indication of hydroperoxide and carbonyl compound formation. The visibility of 966 - 968 cm^{-1} peak indicated trans fatty acid formation.

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