Computation of Melting Point from Iodine Value of Heated, Unheated PKO and POL using Correlation and their Fatty Acid Composition

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Abstract
Utilization of PKO and POL in Nigeria is on the rise, because PKO is inexpensive and in easy reach. The melting point of fats is used to identify oils and fats related to their physical properties, such as hardness and thermal behaviour. Heated PKO and PO were insistent on iodine values, melting point and fatty acid profiles at temperatures of 100, 150 and 200°C and duration of 20, 40 and 60mins were ascertain. The fatty acid profile of PKO shows that lauric acid and oleic acid were the assertive fatty acids with a total of about 60.13% to the fatty acid pool of the oil. The fatty acid profile for palm olein showed palmitic acid ($C_{16.0}$) give a total of 36.60% and oleic acid ($C_{18.1}$) contributing 46.18%, making both palmitic acid and oleic acid the major fatty acids with a total of 84.78% of the total fatty acid profile. PKO was more persistent during heating than POL concerning iodine values. A negative correlation was observed between iodine values and melting point which was statistically not significant at P≤ 0.05 except for oils heated at 100°C for 40mins which was statistically significant at P≤ 0.05. The results indicate that the melting point of heated, unheated PO and PKO can be predicted from the measurement of iodine number with precision similar to experimental methods, on condition that the mechanism used in the determination of iodine value is adequately precise and the estimation meticulously made.

Keywords: Melting point, Iodine value, Thermal behaviour, PO, PKO, Experimental Method, computation

Introduction The Blending of vegetable oils and fats has appear as an inexpensive technique to make wholesome oils void of any chemical behaviour and which possess natural flavour and characteristics as well as nutritional value. The superiority of using blending as a means of modifying oils is that it is uncomplicated and cheap. More notably, the blending of oils work to refine and improve the nutritious and grade of the oils by blending oils into one thus, enhancing marketability (Imoisi *et al*., 2020; Abdulkarim *et al*., 2010).

Heating of oil is one of the famous and key procedure in food preparation. It is frequently employ in food preparations such as iced frizzled foods, snacks and fast foods. It gives result with enticing sensational features like crispy texture, yearn for orange-brown colour and attribute flavour (Franke and Reimerdes, 2007). In thermal stress of oil, the depth of the heated oil used for deep fat frying is about 20-200mm or greater and heated oil is recycle a number of times (Imoisi *et al*., 2020) (Xu,2000: Pokorny, 2002). The attribute of the heated oil and the commodity prepared by deep fat frying is dependent in addition on the frying state such as the temperature of the heated oil, heating time and frying oil, but also on the type of oil used in the process (Moreira *et al*., 1999).

Iodine value (IV), also called iodine absorption value or iodine index is the mass of iodine in grams that is absorb by 100grams of a chemical substance (Alimentos, 2018). The Iodine value is insistent on the fact that each double bond in a fatty acid fragment takes up two atoms of iodine when fatty acids react with iodine (Madsen *et al*, 1992). The Iodine value is expressed as the grams of iodine absorbed per 100 g of sample. Vegetable oils normally contain more unsaturated fatty acids than saturated fatty acids, and as a result, these oils have elevated iodine values than fats because there are more double bonds present in the unsaturated fatty acids (Cast, 2010; Yusof *et al.,* 2000; Frank, 2011).

Melting point is broadly used to distinguish the melting and solidification properties of oils and fats. For unblended substance, a particular melting point is a benchmark of purity. However, fats and oils are variable blend of triglycerides, having melting point ranges (Setiowaty and Cheman, 2002). The determination of melting point in refined, bleached and deodorized palm oil is a usual and uniform necessity in the manufacture of fats and oils. Melting point determination is considered

economical and robust and had been applied to different lipids melting point determinations such as cocoa butter and palm oil blends. Lipid mixtures are used to map out critical temperature index because a comprehensive scale of melting temperatures may be attained by blending lipids of different melting points (IUPAC, 1979). The short-chain fatty acids containing 2-4 carbon atoms per molecule have lower melting points and are more soluble in water, medium-chain fatty acids have carbon chain lengths from 6-12, and long-chain fatty acids are composed of 14-20 carbon atoms whereas, the lengthy chain fatty acids have elevated melting points.

The melting point is also based on the level of unsaturation (Alimentos, 2018). Unsaturated fatty acids have lower melting points compared to saturated fatty acids of similar chain lengths. Generally, lipids containing a high level of unsaturated fatty acids tend to be more fluid at room temperature than those that have a lower level of unsaturation. As the chain length of a saturated fatty acid reduces, the melting point also reduces (Imoisi *et al*., 2018). Thus, saturated fatty acids with lengthy chains have higher melting points than short-chain saturated fatty acids. In palm oil, the two most prevalent fatty acids are palmitic acid (C16:0) saturated and oleic acid (C18:1) unsaturated. The fatty acid structure of PKO is similar to that of coconut oil. The utmost fatty acids in PKO are lauric acid, C12:0, myristic acid, C14:0 and oleic acid and C18:1 (Codex, 2001).

Quality control in fats and oils processing such as the physical refining of crude palm oil involves the determination of iodine value to provide information for proactive decision-making in rectifying process faults when they occur, due to high variations in the quality of materials in the process. Quality control is done once an hour on materials input and quality control on materials fed into the deodorizer is done twice in one hour. In a situation where laboratory determination of the quality control parameter takes a long time, iodine value for example, requiring about one hour, the information will be belated as ongoing processes would have been completed and process malfunction would have led to the production of defective products which could have been corrected if the parameter determination was faster.

The study aims to use correlation to predict the value of melting point from iodine value. This could save time and money in industrial quality control because of the money and time that would otherwise be spent determining all the quality parameters in normal, routine industrial quality control procedures.

Methodology
The oils used in this analysis were refined, bleached and deodorized PO and crude PKO. Samples were gotten from PRESCO Nigeria, PLC. Benin City, Nigeria.

Blend preparation
Unheated and heated PO and PKO were used. The heated PO and PKO were heated at 100, 150 and 200°C, respectively and mixed regularly on a magnetic stirrer for 9 hours. The samples were instantly deep-frozen for physicochemical analyses.

Fatty acid composition
The fatty acid composition was done according to the method described in IUPAC, 1979.

Preparation of Fatty Acid Methyl Ester (FAME)
Relative concentrations of fatty acid (FA) from oil samples were measured as their corresponding methyl esters according to the method described in IUPAC (1979) with a minor modification. Eight drops (~50 µl each) of oil was taken in a 15 ml test tube and 8 drops of 0.5M methanolic potassium hydroxide, and 10mL of heptane were mixed gently and allowed to settle for some time. The distinct upper layer of methyl ester in heptane was separated carefully in a capped vial from a series of screw -capped test tubes (Imoisi *et al*., 2020). The fatty acid was analysed using an Agilent 6890 series gas chromatograph equipped with aliquots of 1µLinj(100:1 split). FAME was injected into GC-MS Agilent 5973 network-mass selective detector (Agilent technology- 7683 series). Autosampler, - GC system, was used with the injection temperature maintained at 230°C and 250°C, respectively. The carrier gas was helium (1ml/min). The percentage was recorded with a standard chem station data system. The MS library search report data/ NIST02.L Int was used to identify the various peaks.

Melting point (AOAC Official Method Cc 3-25)
The solidified samples were completely melted at 60°C before proceeding with the determination. The already melted samples were filtered through filter paper Whatman no. 41H to remove any impurities. Three clean capillary tubes were dipped into the oil samples. The sample rose to about 10mm high in the tubes. The samples were chilled by holding the ends of the tubes that contained the sample against a piece of ice until the fat solidified. The tubes were placed in the beaker and a refrigerator at 4-10°C for 16hrs. The tubes were removed from the refrigerator and attached with a rubber band, to the thermometer (multi-thermometer Eurolab ST9269B -50°C to +300°C) so that the lower ends of the melting point tubes were even with the bottom of the mercury bulb of the thermometer. The thermometer was suspended in 400mL of clear distilled water. The bottom of the thermometer was immersed in the water to the immersion mark (Gold et al, 2011and Imoisi *et al*., 2018). The starting bath temperature was adjusted to 8-10°C below the slip point of the sample. The heating source SI Analytics was agitated with a small stream of air and heat was applied to the bath at the rate of 1°C/min, slowing the rate to 0.5°C/min as the slip point was approached. There was continuous heating until the fat column rose in each tube. The temperature at which each column rose was observed, and the average temperature of all tubes were calculated. This was reported as a slip melting point.

Iodine value (using palm oil tester)

The iodine value was analysed by the CDR palm oil tester. All chemicals used in the determination were procured from CDR Foodlab and Sigma Chemical Co. and were used without further purification (Imoisi et al, 2020). Some fat test cuvettes were prepared before analysis. Exactly 1mL of reagent was dispensed into each cuvette and closed with its supplied cap. The filled cuvette was incubated in the incubation cells for 5 minutes. The cuvette was then inserted in the reading cell. This was done to read the blank value of all filled cuvettes. At the end of the blank reading, the caps of the incubated cuvettes were removed and care was taken not to mix up the caps. The melted oil 20µL was added to the cuvette and mixed thoroughly. The cuvette was immediately inserted into the reading cell to initiate the sample reading. At the end of the reading, the result was printed out automatically.

Results and Discussion Table 1 shows the percentage fatty acid make-up of PKO and POL. The fatty acid make-up of PKO revealed that lauric acid and oleic acid were the major fatty acids with a total of about 60.13% of the fatty acid pool of PKO. The paramount fatty acids in POL were palmitic acid ($C_{16.0}$) with a total of 36.60%, oleic acid ($C_{18.1}$) with a total of 46.18% and linoleic acid ($C_{18.2}$) contributing 8.76%. Palmitic acid, oleic acid and linoleic acid all contributed a total of 99.93% of the total fatty acids in POL. This shows that PKO contains medium-chain saturated fatty acid while POL contains long-chain unsaturated fatty acid. Caproic acid ($C_6.0$) was found in unheated PKO with a value of 0.08%, when heated it was not evident. Caprylic acid ($C_{8.0}$) was detected in unheated PKO; heating the PKO increased its value. Linolenic acid ($C_{18.3}$) was found in unheated POL, heated POL and unheated PKO. While, behenic acid ($C_{22.0}$ and $C_{24.0}$) and lignoceric acid were detected only in unheated PKO. In PKO the saturated fatty acid content increased as the heating temperature increased. Unsaturated fatty acid decreased as the heating temperature increased. Unheated POL had higher unsaturated fatty acid content than the heated ones (Imoisi et al., 2020; Siew et al., 1995). The Iodine value estimate the level of unsaturation of oils and fats as determined by several technique such as the Wijs (AOCS, 2006). Table 2 shows the iodine values of POL and PKO. The iodine value of unheated POL was 53.27g±0.00/100g and for PKO it was 13.08g±0.00/100g. When POL and PKO were heated, results in Table 2 indicate that POL was more at risk to decomposition at the beginning of heating, but as heating proceed there was a depletion in the iodine values of POL heated at 200°C for 40 and 60mins. There was inappreciable depletion in the value for PKO. The noticed reduction in iodine values of POL shows loss of unsaturation as an outcome of the heat treatment resulting upon oxidation at high temperatures. PKO was steady throughout heating since at elevated temperatures the PKO manifest no exceptional loss in iodine value (Imoisi et al., 2020; Imoisi et al., 2018; Siew et al., 1995). The results in Table 3 show that at the start of the experiment the melting point of POL was

11.50°C and PKO was 29.33°C. The allowable melting point maximum (AOCS, 2006) for POL is 19.40-23.5°C and PKO 23.0-30.0°C. The melting point result for POL was far below the AOCS value for POL, while that of PKO was within the AOCS limit. When POL and PKO were heated, there were elevation in the melting points of POL and a decline in PKO. The pattern of melting point outcome was in accordance with the trend of the fatty acid make-up of the oil samples (Table 1) which agrees with the fact that PKO contains medium-chain saturated fatty acids such as lauric acid resulting in lower iodine values and higher melting points. The melting point of POL was lower because it is mostly unsaturated as showed by elevated iodine value as seen in Table 3. In fats and oils processing such as in refining, the processing operations include degumming, bleaching, steam distillation and deodorization. Sensitive methods are required to detect slight changes in unsaturation (AOCS, 2006) such as measurement of iodine value and melting point values, which has been done in the present studies (Imoisi et al., 2018). Table 4 shows a negative correlation between iodine value and melting point. A negative correlation between two variables implies that as the value, of one is increasing; the value for the other is decreasing. Concerning the data presented, it means that as the iodine value is increasing, the melting point was decreasing. This is not surprising since iodine value measures the level of unsaturation (high iodine value corresponds to high level of unsaturation). If that is the case, one would expect the melting point to decrease with increased unsaturation. This is what is observed in this study from the data point of view; the statistical correlation was not significant at the test level. In Table 4 negative correlation was observed with a significance level higher than $P < 0.05$ except at 100°C for 40mins where a negative correlation of -0.654 was observed at a significant level of 0.017. The significance level of 0.017 is less than $P<0.05$. Therefore, the correlation is statistically significant. This means that the iodine value is a statistically significant predictor of melting point at 100°C for 40mins as one is increasing the other is decreasing. The linear prediction rule was done using the equation in Table 4 where y stands for iodine value and x stands for melting point (Imoisi et al., 2018).

Conclusion

Iodine values correlate negatively with the melting point of POL, PKO and their blends heated at 200°C, as the iodine value is increasing, the melting point was decreasing. Which was not supposed to be. Therefore, the melting point cannot be determined by computation from the quantification of iodine value with precision similar to analytical methods. Regression equations cannot be used to predict values of melting point from iodine value, and vice-versa.

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Table 1: Percentage fatty acid make-up of PO and PKO

Fatty acid	Unheated POL	Unheated PKO	POL 100°C 20	POL 100°C 40	POL 100°C 60	POL 150°C 20	POL 150°C 40	POL 150°C 60	POL 200°C 20	POL 200°C 40	POL 200°C 60	PKO 100°C 20	PKO 100°C 40	PKO 100°C 60	PKO 150°C 20	PKO 150°C 40	PKO 150°C 60	PKO 200°C 20	PKO 200°C 40	PKO 200°C 60
$C_{6.0}$		0.08										2.97	2.84	3.05	2.98	2.94	3.01	3.23	3.50	3.47
$C_{8.0}$		1.41										2.93	3.03	2.99	2.95	2.90	2.95	3.12	3.26	3.24
$C_{10.0}$		1.48		1.11																
$C_{12.0}$	0.25	20.72	1.02	1.79	0.63	0.21	0.21	0.19	0.21	0.33	0.23	45.01	44.61	44.81	45.02	43.56	45.41	46.92	46.46	48.10
$C_{14.0}$	0.72	8.28	0.91	1.19	0.81	0.59	0.62	0.64	0.64	0.71	0.65	17.26	17.29	17.44	17.28	16.79	17.42	17.19	17.59	18.17
$C_{16.0}$	36.60	7.17	36.56	36.69	37.22	37.93	37.91	37.74	38.74	38.58	39.92	9.17	9.47	9.24	9.14	10.28	9.12	8.93	9.33	9.36
$C_{16.1}$	0.36		0.23		0.23	0.20	0.21	0.15	0.15		0.20									
$C_{18.0}$	5.46	2.29	5.39	5.23	5.37	5.23	5.34	5.48	5.26	5.51	5.54	2.69	2.77	2.75	2.75	2.85	2.69	2.59	2.75	2.79
$C_{18.1}$	46.18	21.44	46.21	45.49	46.12	46.51	46.27	46.60	46.48	46.22	44.99	16.98	17.13	17.01	17.25	17.72	16.85	15.98	15.49	13.84
$C_{18.2}$	8.76	3.50	8.75	8.48	8.66	8.32	8.72	8.75	7.98	7.67	7.76	2.58	2.65	2.61	2.63	2.83	2.54	2.05	1.34	0.68
$C_{18.3}$	0.26	0.10	0.19	0.18	0.21	0.19	0.21	0.19		0.13	0.16									
$C_{20.0}$	0.41	0.29	0.38	0.37	0.38	0.37	0.36		0.39	0.40	0.38		0.12	0.09		0.13			0.13	
$C_{20.1}$		0.51	0.16	0.16	0.16	0.17	0.15	0.17	0.16	0.18	0.17									
$C_{22.0}$		0.06																		
$C_{24.0}$		0.06																		

Table 2: Changes in Iodine Values (g/100g) of POL and PKO (heated and unheated) at 100, 150 and 200 °C, respectively

Temperature	Control	100°C 20	100°C 40	100°C 60	150°C 20	150°C 40	150°C 60	200°C 20	200°C 40	200°C 60
POL (unheated)	52.37± 0.00									
POL (unblended)		54.48±0.01	54.65±0.48	52.19±0.01	57.64±0.00	56.68±0.00	50.70±0.00	50.95±0.02	44.96±0.00	42.31±0.01
PKO (unheated)	13.08±0.00									
PKO (unblended)		13.00±0.00	13.00±0.00	13.00±0.00	14.34±0.00	13.31±0.00	13.71±0.00	17.60±0.00	12.00±0.00	11.00±0.00

Mean ±SD, POL: Palm olein, PKO: Palm kernel oil

Table 3: Changes in melting point (°C) of POL and PKO (heated and unheated) at 100, 150 and 200°C, respectively

Temperature	Control	100°C 20	100°C 40	100°C 60	150°C 20	150°C 40	150°C 60	200°C 20	200°C 40	200°C 60
POL (unheated)	11.50±0.70									
POL (heated)		12.30±0.26	12.33 ±1.46	12.93±0.45	13.87±1.91	1.4651±1.23	14.87±1.76	13.05±1.00	14.37±1.18	10.93±1.20
PKO (unheated)	29.33±3.79									
PKO (heated)		27.87±0.45	24.37±0.90	24.50±0.36	24.43±1.19	24.50± 1.64	24.43±1.86	25.23±1.09	25.00±0.61	26.43±0.76

Mean ±SD, POL: Palm olein, PKO: Palm kernel oil

Table 4: Correlation of iodine values and melting points at 100,150 and 200°C

Variable	100°C (heating/ Time)	Pearson correlation (r)	Sig (2- tailed)	The rate of increase in IV (Regression equation)
IV &MP	20mins	-0.415	0.355	y= 49.162-0.777x
	40 mins	-0.654	0.017	y= 70.810-1.894x
	60 mins	-0.556	0.194	y= 80.567-2.200x
	150°C			
	20 mins	-0.647	0.116	y= 67.395-1.588x
	40 mins	-0.306	0.505	y= 51.466-0.897x
	60 mins	-0.183	0.694	y= 40.318-0.416x
	200°C			
	20 mins	-0.485	0.270	y= 52.911-0.982x
	40 mins	-0.556	0.195	y= 66.424-1.697x
	60 mins	-0.669	0.100	y= 77.015-2.298x

IV: iodine value, MP: melting point, r: Pearson correlation