Compatibility of Unsaturated Fat Ratios in My City's Market Samples with Literature Standards: An Analytical Study

Word count : 3995

Research Question: "*How does different vegetable oils which are extracted from the vegetable (avocado, olive, maize, sunflower, and sesame) with a Soxhlet extraction method varies in terms of unsaturated fat ratio which is determined by iodine number with Wijs Method?*

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1. INTRODUCTION

My dietitian suggested that I adjust my diet and talked about the positive health effects of consuming unsaturated oils. This made me think about the health consequences and composition of fats in other foods and, I decided to do a study about food oils unsaturated ratios of fats. Therefore, I read some articles to determine the method for my study on the unsaturated fat ratio of food oils. (Samanta, S., Saha, S., & Dutta, D. (2023). Wijs, potassium iodate, and AOCS official method to determine the iodine value (IV) of fat and oil, and Srigley, C. T., & Mossoba, M. M. (2017) ,(Current analytical techniques for food lipids. In U. G. Spizzirri & G. Cirillo (Eds.), Food safety: Innovative analytical tools for safety assessment (pp. 33–64)).

Fats play a critical role in the structure of cell membranes, hormone production, various biochemical processes and as a source of energy (Ravnskov et al., 2014). Their structural differences determine their health effects and, in this context, unsaturated fatty acids, which are chemically more active and beneficial due to their double bonds, stand out (Roche, 1999). They also contain essential fatty acids that cannot be produced by the body (Twining et al., 2016).

Extensive research has shown that unsaturated fats have benefits such as heart health, cholesterol regulation, inflammation prevention, and brain function support (Lunn & Theobald, 2006). They are preferred as they help lowering the risk of heart disease and improve overall health with a balanced diet (Elluch et al., 2011; Khan et al., 2015).

Olive, sesame, avocado, sunflower, and maize oils are important for weight management and metabolic health by increasing metabolic rate and promoting fat burning (Lunn & Theobald, 2006). Avocado oil increases satiety, sesame oil improves insulin sensitivity, and sunflower and maize oils limit fat storage. Diets containing these oils have the potential to protect heart health, control weight, and improve overall metabolic health (Roche, 1999).

1.1. UNSATURATED FAT RATIO

Fats are classified as single (MUFA) or multiple (PUFA) depending on the double bond change in the carbon chain, which affects the fluid retention and health benefits. (Lunn and Theobald, 2006). From a nutritional standpoint, the unsaturated fat ratio represents a crucial element in the evaluation of the quality of dietary fats.

The identification of unsaturated fatty acids is important for the health effects of foods and their evaluation as dietary products. For effectively extracting fats from foods by repeated solvent circulation the Soxhlet method is commonly preferred. (López-Bascón & De Castro, 2020). The unsaturation rate of the pure oil obtained is measured by the iodine number; a higher iodine number indicates that it contains more unsaturated bonds (Knothe, 2002).

The experiment compared the unsaturated fat ratio and iodine number values in the literature with oils extracted from avocado, olive, maize, sunflower, and sesame that I bought from a market in my city to determine if they satisfied food quality standards.

1.2. EXTRACTION OF FATS WITH THE SOXHLET METHOD

Extracting oil from the sample is the initial step in measuring the ratio of unsaturated fatty acids in oils. For this, the Soxhlet extraction method is frequently accomplished. (Wang et al., 2010). The technique is based on the ongoing solvent extraction of oil from a solid substrate. (e.g. plant tissue or seeds). The idea behind the Soxhlet device is that the solvent vaporizes, enters the sample, is collected in a condenser, and then re-evaporates. (Hawthorne et al., 2000).

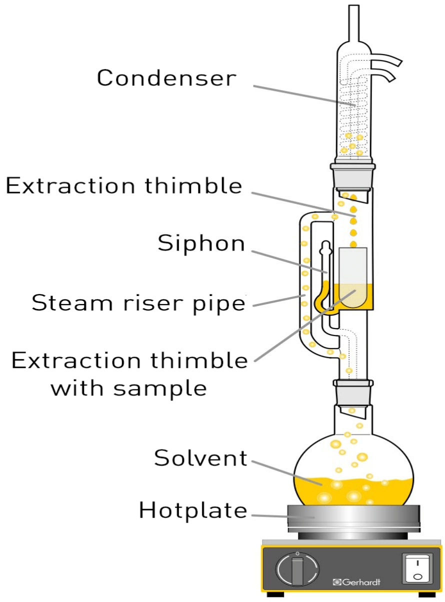

Figure 1. Soxhlet apparatus and its main components (Adapted from Gerhardt, n.d.)

The soxhlet method minimizes solvent waste and increases extraction efficiency by continuous recycling (Wang et al., 2010). It prevents oil degradation and ensuring that the solid is completely saturated with solvent. The oil obtained is then used for titration.

1.3. DETERMINATION OF IODINE NUMBER BY TITRATION

The iodine number is a basic technique used to measure the proportion of unsaturated fatty acids in the extracted oil (Toscano et al., 2012). Since unsaturated fatty acids contain double bonds, these bonds can be saturated by reacting with halogens such as iodine (Saka, 2012). Figure 2 illustrates the chemical reactions involved in determining the iodine number of a biodiesel sample using the Wijs method.

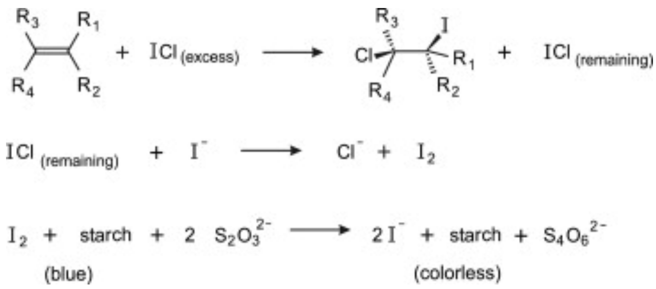

Figure 2. Reactions used to calculate a biodiesel's iodine number using the Wijs technique.

Firstly, the oil carbon sample is divided in an organic solvent such as tetrachloride. Then, excess iodine or iodine monochloride is added to the sample; in the meantime, it reacts with unsaturated bonds in the oil. At the end of the reaction, the remaining iodine is titrated with sodium thiosulfate, the solutions become completely colorless. Higher iodine numbers indicate that the oil has more unsaturated bond properties and therefore is more reactive. (Toscano et al., 2012).

2. THEORETICAL VALUES AND EXPERIMENT

2.1. THEORETICAL VALUES

Table 1 , represents the scientific literature of unsaturated fat ratios and iodine numbers of various plants. According to EU Regulation 1047/2012, If unsaturated fat makes up at least 70% of the fatty acids, the product has a high unsaturated fat ratio and can be used in diets (EUR-Lex, n.d.)

Table 1. Unsaturated fat ratio and iodine number of the plants used in the experiment in the literature

Type of Plant	Unsaturated Fat Ratio	Iodine index
Avocado	70 – 80 % (Dreher & Davenport, 2013)	75 – 95 (AMD Oil Sales, 2024)
Olive	70 – 80 % (Carapelli, n.d.)	74 – 94 (Gupta & Kanwar, 1994)
Maize	80 – 90 % (Dupont et al., 1990; Stępień et al., 2024)	107 – 135 (Onu & Mbohwa, 2021)
Sunflower	80 – 90 % (Akkaya, 2018)	125 – 136 (Gupta & Kanwar, 1994)
Sesame	80 – 85 % (Parsaeian et al., 2020)	104 – 120 (Onu & Mbohwa, 2021)

2.2. VARIABLES

Independent Variables: The type of vegetable used to test different vegetables' oil (avocado, olive, maize, sunflower and sesame)

Dependent Variables: Unsaturated Fat ratio by determining iodine values of oils

Table 2. Controlled Variables

Type of Variable	Variable	Method of Controlling / Measuring Variable
Controlled Variables	Constant temperature	The titration is carried out at a constant temperature by a calibrated thermostat at 25°C. Therefore, fluctuations in the reaction rate is prevented and ensured that iodine absorption accurately reflects the degree of unsaturation.
	Constant time intervals	The iodine solution is subjected to a constant reaction with unsaturated fats for 30 minutes. This avoids timing variability, reflects iodine value accurately.
	The same mass of each type of oil used in	In the experiment, 5 grams of sample from each plant was used for fair comparison. Therefore, the iodine index measured by titration accurately reflects the unsaturated fat ratio and allows reliable comparison between different oils.
	Apparatus	For consistency and accuracy, all oils are analyzed with the same equipment (burette, pipette, conical flask, etc.) and the same concentration of chemicals. This ensures that differences in iodine index are due to the unsaturation ratio of the oils and not to changes in equipment or chemicals.
	Titration procedure standardization	Reliability of the titration is provided by the end point being detected by a distinct color change and by slow titrant addition. Moreover, repeated titration of the samples under controlled conditions (temperature, illumination) minimized human error.
	Molarity of solutions	Titration is done with constant molarity solutions, which prevents reaction rate fluctuations between iodine and unsaturated relations.
	Number of drops of indicator	In titration, fixed number of indicator drops are used for accuracy and consistency with providing reliable results.

2.3. APPARATUS

Determination of fat ratio by using Soxhlet method

Materials:

- Soxhlet extraction apparatus
- Extraction flask
- Extraction thimbles
- Analytical balance
- Oven
- Desiccator
- Solvent bottle

Chemicals:

- 150 cm^3 Hexane (extraction solvent)
- Cold Water (used for cooling the condenser)

Determination of iodine number by using Wijs method

Materials:

- Titration flask (± 1.0 cm^3)
- Burette (± 1.0 cm^3)
- Pipette (± 0.5 cm^3)
- Erlenmeyer flask (± 1.0 cm^3)
- Glass stirring rod
- Thermometer (± 0.5 °C)
- Electronic scale (± 1.0 cm^3)
- Distilled water
- Wash bottle

Chemicals:

- 0.1 mol dm^{-3} Carbon Tetrachloride (CCl_4)
- 0.1 mol dm^{-3} Iodine Monochloride Solution (ICl)
- 2 drops of starch indicator
- 0.1 mol dm^{-3} Sodium Thiosulfate ($Na_2S_2O_3$)
- 100 g dm^{-3} Potassium Iodide (KI)

- Glacial Acetic Acid
- 5 x5 g of the samples (avocado, olive, maize, sunflower, sesame)
- Distilled water

2.4. METHODOLOGY

2.4.1. Soxhlet Method

1) Dry the samples, whose oil contents were to be determined, in an oven at 105°C until constant mass
2) Homogenise the dried sample by fine grinding
3) Weight them to a precision of 5g.
4) The weighed sample was wetted with hexane and placed in the cartridge using a small piece of cotton buffer.
5) Place the cartridge in the extractor.
6) Add 150 cm^3 of hexane to the flask
7) Connect the flask with extractor and cooler
8) Adjust the temperature so that the hexane can coil slowly and the back distillation rate is at least three drops per minute
9) Continue extraction for 8 hours, then stop.
10) Most of the hexane was recovered by distillation; care was taken not to burn the oil in the oil flask.
11) Place the glass bottle in a 105 °C oven to remove the remaining oil.
12) After the oil is removed, cool the bottle in a desiccator for 2 hours.
13) Weigh to the nearest 1 mg.
14) Most of the hexane in the flask was recovered by distillation. During this process, care was taken not to burn the oil collected in the oil balloon.
15) Place the glass flask in an oven at 105 °C to remove the remaining oil
16) Cool the flask in a desiccator for two hours
17) Weight it to a sensitivity of 1 mg
18) Place the flask in an oven at 105 °C for 10 minutes, , cool and weigh it again. It was ensured that the difference between the two weighing was less than 10 mg.
19) Record the final weight of the balloon, after express the amount of fat in the balloon as %.
20) Repat process for 5 times for each plant sample, totalling 25 times.

Figure 3. Soxhlet extractor procedure

2.4.2. Wijs Method

1) Approximately 0.1 gram of oil sample was weighed with an electronic scale
2) Place the sample in a 500 cm ^{3} iodine determination flask. Meanwhile, ensure that the flask was dry and clean.
3) Add 20 cm^3 from the CCl_4 to dissolve the sample
4) Stir the flask until it dissolves completely
5) Add exactly 25 cm^3 (0.1 mol dm^{-3}) of Wijs solution ICl to the oil sample by 25 cm^3 pipette
6) Close the bottle tightly and stir well
7) When Wijs solution was added, ICl reacted with the unsaturated bonds of the oil. Therefore, extra care was taken to guarantee that the sample and solution were in complete contact.
8) Place the flask in a dark place and kept it for 1 hour. During this time, unsaturated bonds reacted with ICl to form free iodine.
9) Upon completion of the waiting period, 20 cm^3 of 10% KI solution was added to the flask to release free iodine by 25 cm^3 pipette.
10) Add 150 cm^3 of distilled water to the flask and mixed well by a flask.
11) Add 2 drops of starch indicator to the solution to start the titration process. The starch produced a blue colour, indicating the presence of iodine.
12) Titrate the solution with 0.1 mol dm^{-3} of $Na_2S_2O_3$ solution by burette
13) Stir the flask continuously, until the blue colour disappears completely.
14) Carry out a 'Blank Titration' without using any oil sample and $Na_2S_2O_3$.
15) Perform the blank titration by adding KI and water to the same Wijs solution. This allows to obtain accurate results by controlling systematic errors and spontaneous consumption of reagents.

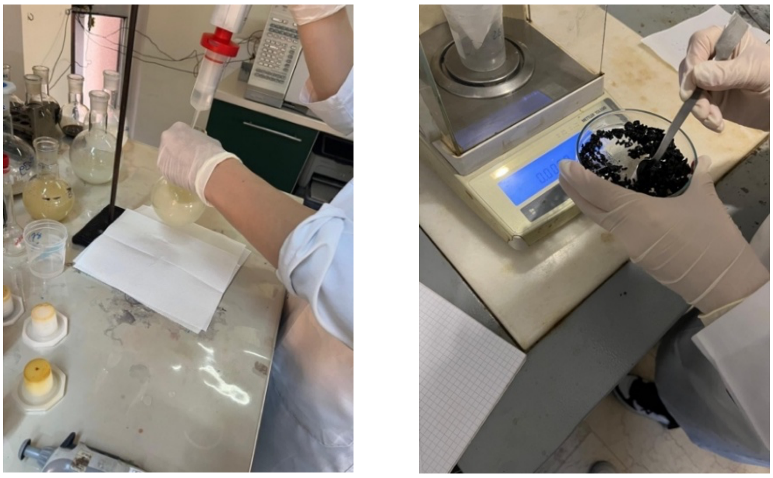

Figure 4. Wijs titration method

2.4.3. Risk Assessment

Table 3. Risk Assessment

• *Carbon Tetrachloride (CCl_4)*: Highly toxic, can damage liver and kidneys; inhalation or skin contact can cause serious health problems. Although not flammable, prolonged exposure may cause cancer.
• *Iodine Monochloride Solution (ICl):* May cause severe irritation to skin and eyes; inhalation of vapours may irritate respiratory tract. It is a strong oxidiser and can produce dangerous reactions with reagents.
• *Starch Indicator:* Relatively safe, but ingestion or contact with eyes may cause mild irritation.
• *Sodium Thiosulfate ($Na_2S_2O_3$):* Generally, of low toxicity, but inhalation in powder form or contact with high concentrations may cause irritation.
• *Potassium Iodide (KI)*: Excessive exposure may cause thyroid disorders. Inhalation in powder form may irritate the respiratory tract.
• *Glacial Acetic Acid:* Strong acid; may cause severe burns to skin and eyes. Inhalation of vapours may cause irritation of the respiratory tract.
• *Hexane (Extraction Solvent):* Highly flammable, inhalation of vapours may result in depression of the central nervous system, and extended exposure may lead to neurotoxicity.

2.4.4. Environmental Considerations

❖ The Wijs and Soxhlet methods for determining iodine value in oils have minimal impact on the environment. In addition, the resulting chemical waste is collected in designated waste containers.

2.4.5 Ethical Considerations

- The Wijs and Soxhlet methods experiment did not involve organisms, so there weren't major ethical concerns related to human experimentation.

3. DATA AND ANALYSIS

The iodine value indicates the unsaturation rate of the oil and therefore the amount of double bonds in fatty acids.

The iodine value was calculated by comparing the amounts of sodium thiosulphate used for both the blank and the sample. The formula used is:

$$Iodine\ Index\ (g/\ 100\ g) = \frac{(V_1 - V_2)x\ N\ x\ 12.69}{m}$$

In formula:

V_1: Volume of iodine solution blank titration (cm^3)

V_2: Volume of iodine solution after titration (cm^3)

N: Normality of sodium thiosulphate solution (mol dm^{-3})

m: Mass of the oil sample (g)

12.69: Amount of iodine in grams in 1 dm^{-3} 0.1 mol dm^{-3} iodine solution (g/cm^3)

3.1 Raw Data

Table 4. Raw data from Wijs method

		Types of Oil Sample				
		Sunflower	**Olive**	**Maize**	**Sesame**	**Avocado**
Volume of 0.1 M $Na_2S_2O_3$ used for sample ±0.001cm^3	**Trial 1**	16.605	16.300	17.450	17.275	15.600
	Trial 2	19.067	16.745	10.884	15.425	15.015
	Trial 3	17.240	10.752	12.773	14.216	16.025
	Trial4	18.217	15.348	11.133	16.922	15.780
	Trail 5	17.793	13.072	14.573	16.012	16.240
Weight of the lipid sample ± 0.0001g	**Trial 1**	0.1958	0.1720	0.0786	0.1319	0.2078
	Trial 2	0.1525	0.1586	0.1256	0.1589	0.2058
	Trial 3	0.1963	0.2536	0.1025	0.1556	0.2125
	Trial 4	0.1582	0.1886	0.1173	0.1444	0.2197
	Trial 5	0.1896	0.2066	0.0903	0.1489	0.1947

Table 5. Average volume of iodine solution of blank titration.

	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Avg.
Volume of iodine solution in blank titration $\pm 0.001 cm^3$	31.005	29.289	27.990	32.306	30.035	*30.125*

Example of iodine calculation for sunflower oil:

$$Sample\ 1: Iodine\ Index\ (g/100g) = \frac{(30.125 - 16.605)\ x\ 0.1009\ x\ 12.69}{0.1958} = 88.41$$

Example of percentage uncertainty calculation for sunflower oil

$$30.125 \pm 0.001 - 16.605 \pm 0.001 = 13.520 \pm 0.002$$

$$\frac{0.002}{13.520} \times 100 = 0.015\%$$

$$\frac{0.0001}{0.1958} \times 100 = 0.051\%$$

$$0.015\% + 0.051\% = 0.066\%$$

3.2 Processed Data

Table 6. Iodine numbers of the plant samples tested

	IODINE NUMBER				
Tested plant	**Expt. 1**	**Expt. 2**	**Expt. 3**	**Expt. 4**	**Expt. 5**
Sunflower	88.41 ±0.066%	92.85±0.084%	84.05±0.066%	96.38±0.080%	83.28±0.069%
Olive	102.90±0.073%	108.00 ±0.078%	97.81±0.050%	100.30±0.067%	105.70±0.060%
Maize	206.50±0.017%	196.20±0.090%	216.80±0.11%	207.300 ±0.10%	220.40±0.12%
Sesame	124.70±0.091%	118.50±0.077%	130.90±0.077%	117.10±0.084%	121.40±0.081%
Avocado	89.50±0.062%	94.01±0.062%	84.96 ±0.061%	83,60±0.059%	91.31±0.066%

Average uncertainty $= \frac{Max\ value - Min\ value}{2\sqrt{Number\ of\ trials}}$

Percent Average uncertainty: $\frac{average\ uncertainty}{\text{Average number of iodine numbers}} \times 100$

Example percent average uncertainty calculation for sunflower oil**:** $\frac{96.38 - 83.28}{2\sqrt{5}}$ =2.93

$$\frac{2.93}{89.00} \times 100 = 3.29\%$$

Table 7. Average iodine numbers of the 5 plants sample tested

	IODINE NUMBER		
Tested plant	**Average number of iodine numbers**	**Standard Deviation**	**Percent Average Uncertainty**
Sunflower	89.00	5.04	3.29%
Olive	102.94	3.65	2.21%
Maize	209.44	8.52	2.58%
Sesame	122.52	4.93	2.52%
Avocado	88.68	3.88	2.63%

Based on the measurements of plants, distinct patterns in their descriptive statistics are observed. Sunflower have a mean value 89.0 with a relatively small standard deviation of 5.04, indicating consistent values across the samples. Olive displays a slightly higher mean of 102.94, with moderate variability with the lowest standard deviation of 3.65. In contrast, maize exhibited the highest mean value at 209.44 and the largest spread among all seed types, as evidenced by a standard deviation of 8.52. Sesame has mean value 122.52 with a standard deviation of 4.93, while avocado values were the most consistent, is shown by standard deviation of 3.88 and an average value of 88.68. Overall, these statistics highlight that while maize values are more dispersed, avocado, olive and sunflower show more uniformity in their measurements. The graphs of the results are shown in Figure 5, with error bars, and Figure 6 shows average iodine values for each plant type.

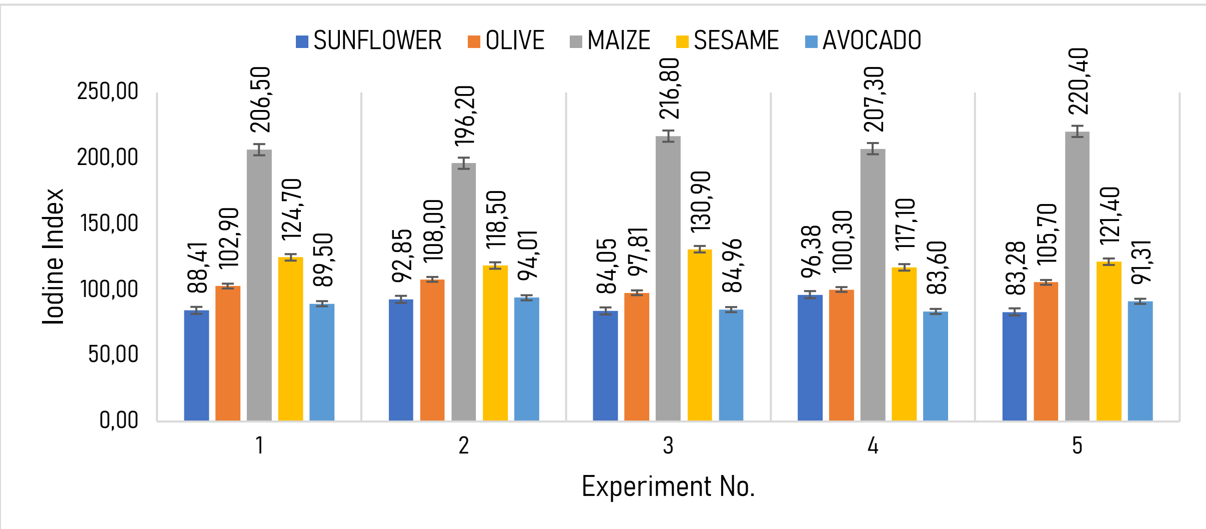

Figure 5. Iodine numbers of samples according to 5 different experimental results

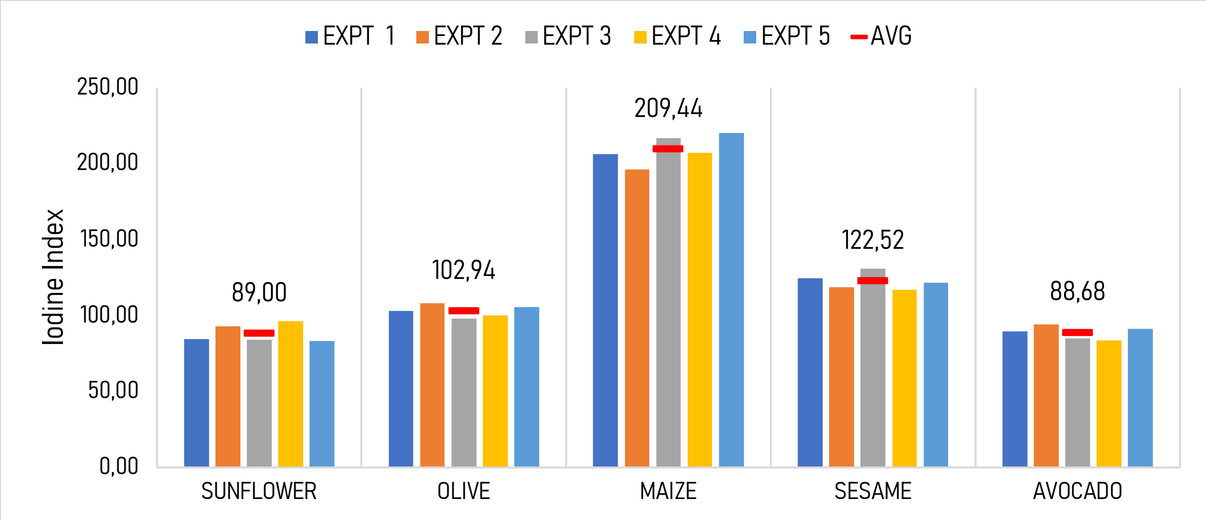

Figure 6. Average iodine numbers for each sample group

3.3 UNSATURATED FAT RATIO DATA

The Soxhlet method is employed to obtain the fat ratios of the samples. The formula to calculate fat ratio of each plant by Soxhlet extractor,

$$Fat\ ratio\ (\%) = \frac{(m_2 - m_1)}{m_s}\ x\ 100$$

where,

m_1: Mass at last weighing

m_2: Mass at first weighing

m_s: Weighed sample quantity

Fat ratio calculation of sunflower in trial 1:

$$Fat\ ratio\ (\%) = \frac{(73.7042 - 72.6299)}{3.2955} = 32.6\%$$

Table 8. Fat ratios of the samples as a result of Soxhlet method test

	FAT RATIO					
Tested plant	**Expt. 1**	**Expt. 2**	**Expt. 3**	**Expt. 4**	**Expt. 5**	**Avg.**
Sunflower	32.6%	33.6%	31.3%	34.5%	33.1%	*33.02%*
Olive	20.1%	22.2%	18.8%	22.6%	21.1%	*20.96%*
Maize	4.6%	5.2%	3.9%	4.4%	3.5%	*4.32%*
Sesame	53.4%	51.8%	48.4%	54.3%	54.8%	*52.54%*
Avocado	11.7%	11.9%	12.4%	13.4%	12.8%	*12.44%*

The iodine number is a parameter indicating the degree of unsaturation of the fat and measures the capacity of double bonds in fat molecules to react with iodine. Therefore, a high iodine number indicates that the fat contains more unsaturated fatty acids. Although there is no simplified formula for finding the unsaturated fat content using iodine number, the following empirical formula can be used to roughly estimate the unsaturated fat content of vegetable oil without performing unsaturated fat content analysis (Shadidi, 2005).

Theoretically, there is no upper limit to the iodine number of a vegetable oil. However, in practice, vegetable oils with the highest iodine count are usually around 200-220 (Wikipedia, 2024). Maize is a moderately unsaturated vegetable oil and its iodine number is usually in the range 103-130 (Onu & Mbohwa, 2021). Therefore, it is highly unlikely that maize has an iodine number of 210. Therefore, the iodine number of the maize sample may have been incorrect due to various measurement errors that occurred during the experiment. The iodine number of the maize sample will not be taken into account when calculating the unsaturated fat content.

$$Unsaturated\ Fat\ Ratio\ (\%) = \frac{Iodine\ Number\ \times\ 280}{253.8 \times 1.5} \times 100$$

- The average molecular weight of fatty acids is ~280 g/mol,
- The average number of double bonds per molecule is ~1.5 (a typical value for many vegetable oils)
- 253.8: Molecular weight of iodine (I_2).

The formula can be simplified as follows:

$$Unsaturated\ Fat\ Ratio\ (\%) \approx Iodine\ Number \times 0.735$$

The calculation of the unsaturated fat ratio (%) of sunflower is shown below for the result of experiment 1.

$$Unsaturated\ Fat\ Ratio(\%) = 88.41 \times 0.735 = 64.98\%$$

Table 9. Unsaturated fat ratios of the samples as a result of calculations

	UNSATURATED FAT RATIO					
Tested plant	**Expt. 1**	**Expt. 2**	**Expt. 3**	**Expt. 4**	**Expt. 5**	**Avg.**
Sunflower	64.97%	68.21%	61.74%	70.85%	61.23%	*65.40%*
Olive	75.63%	79.38%	71.88%	73.72%	77.69%	*75.66%*
Sesame	91.65%	87.10%	96.21%	86.07%	89.23%	*90.05%*
Avocado	65.78%	69.09%	62.48%	61.45%	67.11%	*65.18%*

4. RESULTS

The analysis of the unsaturated fat ratios for five different seed types reveals notable differences in their distributions. Sunflower seeds maintain a mean unsaturated fat ratio of 65.40%, with moderate variability across the samples. Olives exhibit a higher average of 75.66%, with slightly lower variability than sunflower seeds. Sesame seeds demonstrate the highest mean of 90.05%, with noticeable variability. In contrast, avocados have a lower average of 65.18%, with consistent results across samples. These findings highlight that while maize and sesame have the highest unsaturated fat levels, avocados and sunflower seeds display lower values, indicating diverse fat composition characteristics among the tested plant types. The graphical representations of these results are shown in Figure 7, which includes error bars, and Figure 8, illustrating the average values for each plant type in the test group.

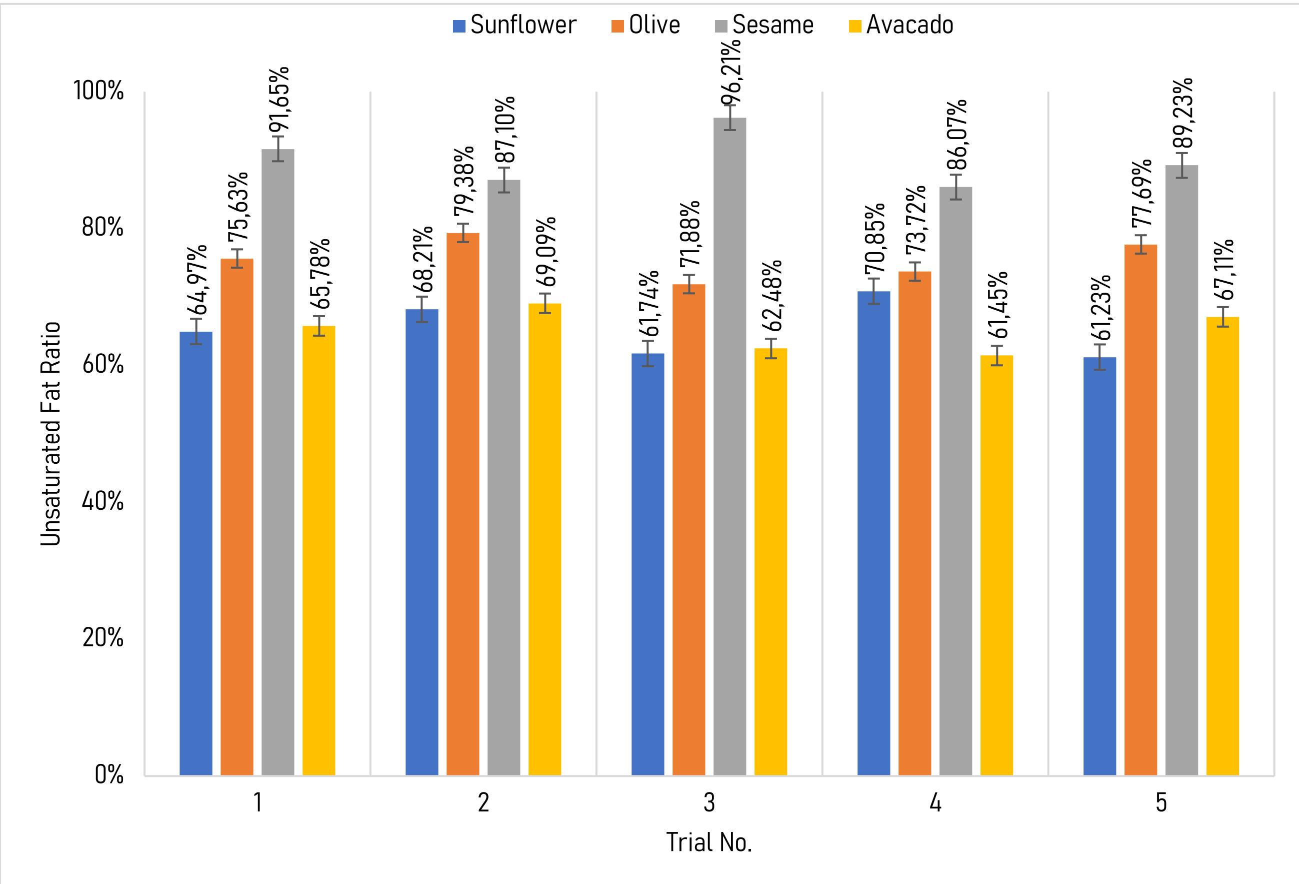

Figure 7. Unsaturated fat ratios of samples based on iodine numbers and fat ratios

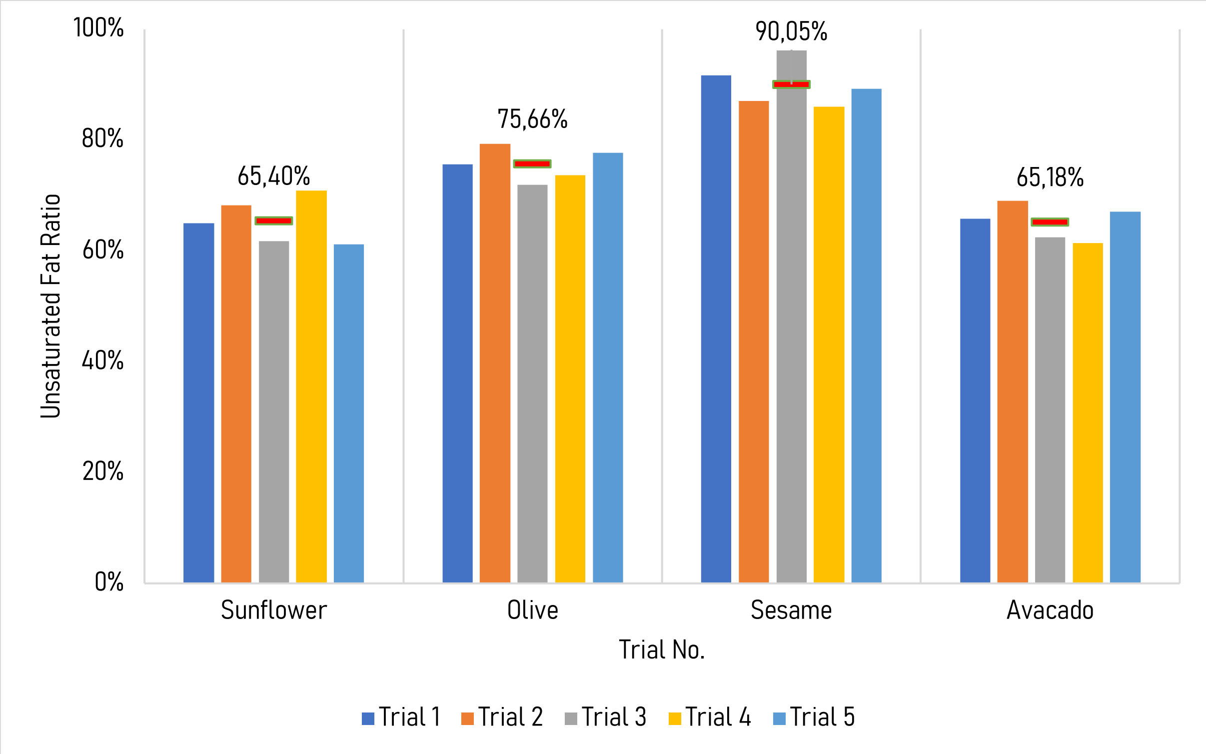

Figure 8. Average unsaturated fat ratios for each sample group

In the study, the unsaturated fat ratios of plant species were compared with the 70% reference value determined in the EU regulation 1047/2012. If the average unsaturated fat ratio is above 70%, the plant can be considered as a dietary product. Correspondingly, the average unsaturated fat ratio of sunflower seeds was measured as 65.40%; 75.66% in olive; 90.05% in sesame, and 65.18% in avocado. This situation shows that sesame and olive in particular have high nutritional value, while sunflower and avocado are inadequate as a dietary product. Figure 9 presents the average unsaturated fat ratios of the samples specifically.

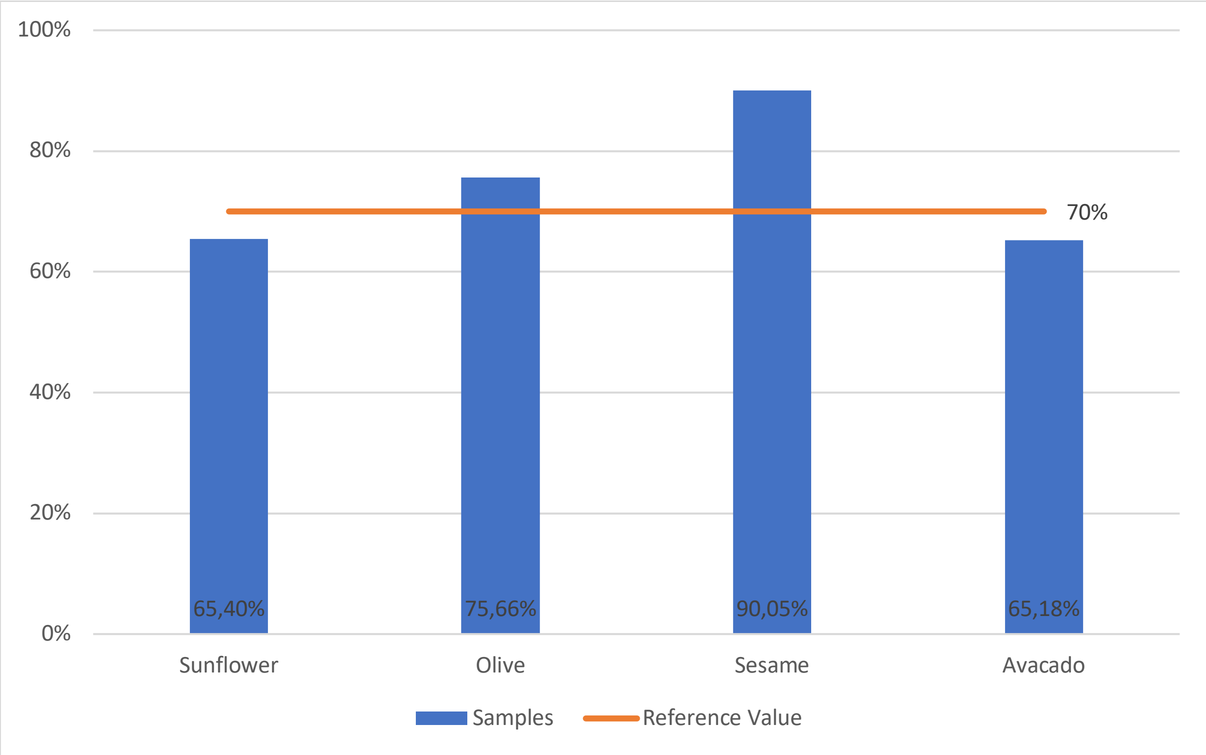

Figure 9. Average unsaturated fat values of the samples and reference unsaturated fat value according to (EU) No 1047/2012)

5. CONCLUSION

The iodine number indicates the amount of iodine that can be bound in 100 grams of oil in grams, which is directly related to the number of double bonds the oil contains. Since unsaturated fatty acids, contain carbon-carbon double bonds, iodine molecules bind these bonds. Therefore, higher the iodine number of an oil, the more unsaturated fat it contains.

In this study, the unsaturated fat and iodine ratios of five plants purchased from a market in my city were determined and compared with the values reported in the literature. The findings show that each plant has a different unsaturated fat ratio, with reference to at least 70% of fatty acids coming from unsaturated fat, and some plants are more suitable as a dietary product.

Based on the oil ratio of the sunflower sample obtained by Soxhlet method and the iodine index obtained by Wijs method, the average unsaturated fat ratio was determined as 65.40%. This value was below the reference rate of 80-90% showing a significant difference. This result suggests that sunflower is not able to be assessed as healthy food source that can be used as a dietary product because the unsaturated fat ratio of sunflower is below the recommended reference value of 70% (EU) No 1047/2012). Although the reference value of sunflower in the literature tells us that sunflower is a good product as a dietary product, it can be noted that the quality of this sunflower does not have a sufficient value in terms of unsaturated fat ratio as stated in the literature.

The average unsaturated fat ratio of the olive sample was 75.66%. This value was within the range of 70-80% given for olive in the literature and did not reveal statistically significant variation. In addition, since the unsaturated fat ratio of olives is between 70-80%, which is the reference value for recommendation as dietary products compared to other plants, it can be considered as a healthy food source that can be used as a dietary product. Furthermore, since the quality of olives is in line with the unsaturated fat ratio given in the literature, it can be concluded that our olive sample has sufficient quality.

The mean unsaturated fat ratio of the avocado sample was 65.18%. This value was above the 70-80% range typically cited for avocado in the literature, with a significant difference. It can be posited that this product represents a healthy food source that may be incorporated into a diet. The unsaturated fat ratio of avocado is significantly higher than 70%, the reference value that is recommended for dietary products. Nevertheless, the literature value demonstrates that this avocado's unsaturated fat ratio qualifies it as low quality.

In particular, the sesame sample, with an unsaturated fat ratio of 90.05%, exceeds the reference value of 80-85% reported in the literature. Additionally, it is well above the 70% threshold for recommendation as a dietary product. This suggests that the sesame sourced from a supermarket in my city meets or even surpasses expected quality standards, reinforcing its potential benefits as a dietary ingredient.

In laboratory tests, the iodine index of maize was found to be 209.44. Maize is a moderately unsaturated vegetable oil and the reference iodine number in the literature is usually in the range of 103-130 (Onu & Mbohwa, 2021). Therefore, the iodine number of maize was considered unlikely to be 210 and the unsaturated fat ratio was not calculated as the iodine number of the maize sample may be inaccurate due to various measurement errors that occurred during the experiment.

Upon analysis of the iodine index results, it was observed that the reference value for sunflower was 125-136, whereas the sample average was found to be 88.18. Similarly, the reference value for olive was between 74-94. Similarly, the mean value for sesame was 122.52, which is also higher than the reference value that is between 104-112. The results demonstrated that the values obtained for sunflower oil, olive, maize, sesame and avocado exhibited a notable divergence from the iodine number reference values documented in the existing literature. The mean value for maize was 209.44, which is significantly higher than the reference value, resulted from errors ,in titration. The literature value for avocado is given as 75-95, while the average iodine number value in our samples was 88.68, which was found to be statistically significant. Only the iodine number of avocado samples were found to be statistically consistent with the iodine number in the literature.

6. DISCUSSION AND EVALUATION

6.1. STRENGTH

a) The experiment confirmed that high iodine index is proportional to high unsaturated fat ratio.

b) Repeating the experiment 25 times, 5 times for each of 5 different product types, was an important factor to increase the reliability of the results.

c) In order to minimise systematic errors, the experimental environment was meticulously arranged and care was taken to increase the accuracy of the data obtained.

d) Automatic and semi-automatic experimental systems were used to increase the consistency of the experimental conditions by performing the heating and mixing processes with maximum precision.

e) As a result of a detailed review of the literature, reference values for unsaturated fatty acid and iodine numbers were determined, which strengthened the scientific basis of the study.

6.2. WEAKNESS AND LIMITATIONS

Table 10. Weakness and limitations

Limitation	Source of Error	Type of Error	Effect on Results	Suggestions for Improvement
Low number of samples (5) used for statistical analysis.	Small sample size.	Random Error	Inadequate sample size may lead to unreliable statistical conclusions and increase variability.	Increase the sample size to meet statistical requirements for robust analysis.
Potential presence of impurities affecting the reaction with iodine in the Wijs method.	Contamination in samples or reagents.	Systematic Error	Over- or underestimation of the unsaturated fat ratio due to interference by impurities.	Ensure high-purity reagents and implement strict sample handling protocols.

Human error during titration, including over/under titration, seen in maize oil.	Operator variability and manual handling of the process.	Random Error	Variability in titration endpoints led to inconsistent results in maize, that is detected in the unsaturated fat ratio calculation process.	To increase accuracy and decrease human error, use automated titration devices.
Variability in iodine value due to environmental factors (e.g., temperature, humidity).	Environmental fluctuations during the analysis.	Systematic / Random Error	May cause inconsistencies in the reaction, affecting the reproducibility of results.	Perform titration in a controlled environment with stable temperature and humidity.
Limited ability of the Wijs method to differentiate between types of unsaturated fats.	Intrinsic limitation of the chemical method used.	Systematic Error	Cannot provide detailed information about the specific types or distribution of unsaturated fatty acids present.	Combine Wijs method with complementary techniques like GC for detailed profiling.

7. FURTHER INVESTIGATION

To expand the study, maize oil can be re-titrate and same experiment can be applied to determine the unsaturated fat ratios in coconut, palm, trans and walnut oils. Thus, dietary diversity is increased by obtaining summary information about healthy and avoidable fats.

Modern methods such as HPLC can be used to precisely measure the unsaturated fat content. (Kanwal & Musharraf, 2024). This technique separates the prepared fat sample with a liquid mobile phase and a stationary phase column; different fatty acids are eluted and quantified by detection in the chromatogram. Thus, the accuracy of the results is increased for nutritional research and quality control (Shantha & Napolitano, 1992).

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9. APPENDIX

The formula to calculate iodine index of each plant is,

$$Iodine\ Index\ (g/100g) = \frac{(V_1 - V_2)\ x\ N\ x\ 12.69}{m}$$

where,

V_1: Volume of 0.1 M $Na_2S_2O_3$ used for blank in mL

V_2: Volume of 0.1 M $Na_2S_2O_3$ used for sample in mL

N: Normality of $Na_2S_2O_3$

12.69: grams of iodine in 1 L of 0.1 M Iodine solution

m: Weight of the lipid sample in g

Iodin number calculation of sunflower

$$Sample\ 1: Iodine\ Index\ (g/100g) = \frac{(30.125 - 16.605)\ x\ 0.1009\ x\ 12.69}{0.1958} = 84.4$$

$$Sample\ 2: Iodine\ Index\ (g/100g) = \frac{(30.125 - 19.067)\ x\ 0.1009\ x\ 12.69}{0.1525} = 92.8$$

$$Sample\ 3: Iodine\ Index\ (g/100g) = \frac{(30.125 - 17.240)\ x\ 0.1009\ x\ 12.69}{0.1963} = 84.0$$

$$Sample\ 4: Iodine\ Index\ (g/100g) = \frac{(30.125 - 18.217)\ x\ 0.1009\ x\ 12.69}{0.1582} = 96.4$$

$$Sample\ 5: Iodine\ Index\ (g/100g) = \frac{(30.125 - 17.793)\ x\ 0.1009\ x\ 12.69}{0.1896} = 83.3$$

Iodin number calculation of olive

$$Sample\ 1: Iodine\ Index\ (g/100g) = \frac{(30.125 - 16.300)\ x\ 0.1009\ x\ 12.69}{0.1720} = 102.9$$

$$Sample\ 2: Iodine\ Index\ (g/100g) = \frac{(30.125 - 16.745)\ x\ 0.1009\ x\ 12.69}{0.1586} = 108.0$$

$$Sample\ 3: Iodine\ Index\ (g/100g) = \frac{(30.125 - 10.752)\ x\ 0.1009\ x\ 12.69}{0.2536} = 97.8$$

$$Sample\ 4: Iodine\ Index\ (g/100g) = \frac{(30.125 - 15.348)\ x\ 0.1009\ x\ 12.69}{0.1886} = 100.3$$

$$Sample\ 5: Iodine\ Index\ (g/100g) = \frac{(30.125 - 13.072)\ x\ 0.1009\ x\ 12.69}{0.2066} = 105.7$$

Iodin number calculation of maize

$$Sample\ 1: Iodine\ Index\ (g/100g) = \frac{(30.125 - 17.450)\ x\ 0.1009\ x\ 12.69}{0.0786} = 206.5$$

$$Sample\ 2: Iodine\ Index\ (g/100g) = \frac{(30.125 - 10.884)\ x\ 0.1009\ x\ 12.69}{0.1256} = 196.2$$

$$Sample\ 3: Iodine\ Index\ (g/100g) = \frac{(30.125 - 12.773)\ x\ 0.1009\ x\ 12.69}{0.1025} = 216.8$$

$$Sample\ 4: Iodine\ Index\ (g/100g) = \frac{(30.125 - 11.133)\ x\ 0.1009\ x\ 12.69}{0.1173} = 207.3$$

$$Sample\ 5: Iodine\ Index\ (g/100g) = \frac{(30.125 - 14.573)\ x\ 0.1009\ x\ 12.69}{0.0903} = 220.4$$

Iodin number calculation of sesame

$$Sample\ 1: Iodine\ Index\ (g/100g) = \frac{(30.125 - 17.275)\ x\ 0.1009\ x\ 12.69}{0.1319} = 124.7$$

$$Sample\ 2: Iodine\ Index\ (g/100g) = \frac{(30.125 - 15.425)\ x\ 0.1009\ x\ 12.69}{0.1589} = 118.5$$

$$Sample\ 3: Iodine\ Index\ (g/100g) = \frac{(30.125 - 14.216)\ x\ 0.1009\ x\ 12.69}{0.1556} = 130.9$$

$$Sample\ 4: Iodine\ Index\ (g/100g) = \frac{(30.125 - 16.922)\ x\ 0.1009\ x\ 12.69}{0.1444} = 117.1$$

$$Sample\ 5: Iodine\ Index\ (g/100g) = \frac{(30.125 - 16.012)\ x\ 0.1009\ x\ 12.69}{0.1489} = 121.4$$

Iodin number calculation of avocado

$$Sample\ 1: Iodine\ Index\ (g/100g) = \frac{(30.125 - 15.600)\ x\ 0.1009\ x\ 12.69}{0.2078} = 89.5$$

$$Sample\ 2: Iodine\ Index\ (g/100g) = \frac{(30.125 - 15.015)\ x\ 0.1009\ x\ 12.69}{0.2058} = 94.0$$

$$Sample\ 3: Iodine\ Index\ (g/100g) = \frac{(30.125 - 16.025)\ x\ 0.1009\ x\ 12.69}{0.2125} = 85.0$$

$$Sample\ 4: Iodine\ Index\ (g/100g) = \frac{(30.125 - 15.780)\ x\ 0.1009\ x\ 12.69}{0.2197} = 83.6$$

$$Sample\ 5: Iodine\ Index\ (g/100g) = \frac{(30.125 - 16.240)\ x\ 0.1009\ x\ 12.69}{0.1947} = 91.3$$