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EXTRACTION AND PHYSICOCHEMICAL ANALYSIS OF OIL FROM 4 GENETICALLY MODIFIED VARIETIES OF SOYBEANS IN NIGERIA.

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ABSTRACT

The extraction and physicochemical analysis of soybean oils extracted from four newly genetically modified soybean varieties obtained from Institute for Agricultural Research, Zaria, Nigeria were carried out in order to ascertain their qualities. The physicochemica

Introduction

Soybean (glycine max L. Merril) is one of the most important seed legumes. It contributes about 25% of the global edible oil. Oil gotten from vegetable seeds or nut seeds by solvent extraction or mechanical pressing are termed as crude vegetable oils. Soybean oil is a vegetable oil extracted from soybean seeds. It is also one of the most widely consumed cooking oils. Different physical and chemical parameters of edible oil were used to qualitatively compare the properties of the oil. Parameters including pH, peroxide value, acid value, iodine value and saponification value *were* used to assess the quality and goodness of the oil. (Farhoosh, 2008).

l parameters determined includes their colours, densities (D), saponification values (SV), peroxide values (PV), acid values (AV), free fatty acids (FFA) and iodine values (IV). The oils were extracted from each Variety by solvent extraction method using soxhlet apparatus and N-hexane as extracting solvent. The results of the analysis obtained, showed that the colours of the oils were pale yellow. The highest density was seen in TGX-1951-3F soybean oil (0.926 Kg/L) and lowest in TGX-1955-10E soybean oil (0.901 Kg/L). On the other hand, pH Values were observed to be between 7.2 to 7.4. The highest and lowest saponification values were obtained at 120.62 mgKOH/g for TGX-1448-2E, TGX-1904-2E and TGX-1951-3F respectively and 122.02 mgKOH/g for TGX-1955-10E variety. It was observed that TGX-1955-10E showed 0.5 meKOH/kg while the other three varieties had 0.4 meKOH/kg as peroxide values. TGX-1448-2E and TGX-1955-10E had the highest and the lowest free fatty acid values of 15.61 and 13.05% respectively. It was also observed that TGX-1955-10E variety recorded the highest iodine value of 25.63gI_2/g oil while the remaining varieties recorded same iodine values of 24.75gI_2/g oil. The highest acid value was observed in TGX-1448-2E variety at 15.61mgKOH/g and the lowest in TGX-1955-10E 13.05 mgKOH/g. The study and results obtained revealed the good attributes of the four varieties of soyabean as edible oil and the results compares favourably with standard parameters for edible oils.

Keywords: Density; peroxide value; acid value; iodine value and saponification value.

Soybean oil as one of the most important edible oils, are vital constituents of our daily diet which provides energy, essential fatty acids and serves as a carrier of fat soluble vitamins (Zahir, 2014). About

10% or fewer of calories consumed daily should be from saturated fat and 20-35% of total daily monounsaturated fats. Small amounts of saturated fats are common in diets, meta-analyses found a significant correlation between high consumption of saturated fats and blood LDL concentration, a risk factor for cardiovascular diseases.

Atmospheric oxygen reacts with lipid and other organic compounds of oil which causes structural degradation in the oil and leads to loss of quality and could be harmful to human health. (Bhattacharya, 2008).

Various physical and chemical parameters of edible oils were used to evaluate the compositional quality of the oils (Ceriani, 2008). Therefore, it is essential to determine the quality of oil to avoid the use of abused oil. These physicochemical parameters including pH value, viscosity value, peroxide value, acid value, iodine value and saponification value were also used to monitor the quality and functionality of the oil (Farhoosh, 2008). A good edible oil that can be used for cooking purpose must be within the approved range of the physicochemical parameters.

Evaluation of these parameters will help in determining the safety of oils especially for human consumption and industrial application, etc. Oils with Low density are highly preferable for consumption (Jinfeng, 2011). Oils having higher moisture content are preferably used for food texturing and frying. It is also used industrially in cosmetics, oil paints, soap and detergent manufacturing, (SON,2000).

The higher the saponification value, the shorter the average chain length of the fatty acids and the lower the average molecular weight of the fatty acids and vice versa (Birnin-Yauri, 2011).

From peroxide value, the quality and stability of oils can be indicated by measuring the rancidity reactions that occurred during storage. Increase in peroxide values indicates the rancidity of oils due to relatively higher oxidation in oils (AOCS, 2016). Higher acid value indicates that triglycerides of oil are converted into fatty acids and glycerol which cause rancidity of oils (Zahir, 2014).

Cooking oils must have lower acid value else the oil can affect human health. Iodine value measures the degree of unsaturation in a fat or vegetable oil (Ekwu and Nwagu, 2004). The higher the unsaturation, the more the possibility of the oils to go to rancid (AOCS, 2016).

Several researchers had studied the physicochemical parameters to determine the quality and functionality of soybean oil (Jinfeng, 2011), (AOCS, 1993). These parameters can be used to identify the characteristics of the four genetically modified soybean oil varieties available in Nigerian markets.

MATERIALS AND METHODS

Sample Collection

The four genetically modified soybean varieties were obtained on 18th/07/2019, from the Institute for Agricultural Research (IAR), Samaru, Zaria, Kaduna State.

Extraction process

Oil was extracted from each variety by solvent extraction method using soxhlet apparatus. 1kg of each of the varieties were pounded into powder. The powdered form was each inserted into the round bottom flask and 2 litres of n-hexane solvent was added to it. The extraction process lasted for two hours for each of the varieties. All solvent and reagents used in this work were of analytical grade. All measurement were conducted three times and the average was reported.

Density Measurement

Densities of oil samples were measured using a Relative Density (R.D) bottle with a capacity of 10 ml according to the formula:

$$\text{Density } (\rho) = \frac{\text{Mass of the oil sample (M)}}{\text{Volume of the R.D bottle (V)}} \text{ g/ml.}$$

Saponification Value

The saponification value was determined by placing (2g) of the oil each into a 250ml conical flask and 25ml of 0.5M ethanol potassium hydroxide solution was added and a reflux condenser was attached. The contents was reflux for about 30 minutes on a water bath while swirling until it simmered. The mixture was titrated against 0.5M HCl using phenolphthalein as indicator while still hot. A blank determination was also carried out under the same conditions and the saponification value calculated as shown below;

$$S.V = \frac{(B-S) \times 28.05}{W}$$

Where;
B=titre value of blank
S=titre value of sample
W= weight of oil.
(British Standards Institute 1995).

Peroxide Value

The peroxide value (PV) is a measure of the concentration of substances that oxidize potassium iodide into iodine. 4g of the oil each was placed in 250ml conical flask and 30ml glacial acetic acid/ chloroform (3:2 V/V) was added. The content was shaken until they dissolved. 1ml of saturated potassium iodide was added followed by the addition of 0.5ml starch indicator. This was titrated with 0.1N NaS_2O_3 until the dark blue color disappears. Blank determination was also carried out and the peroxide value calculated as;

$$PV\ (meq/kg/oil) = \frac{(S-B) \times 1000 \times N}{W}$$

where;
S= titre value of oil sample
B=titre value of blank.
N= normality of stadium thiosulphate solution
W= weight of oil sample.

(British standard institute no. 684).

Acid Value

The acid value of oils was determined by titre metric method according to AOAC (1984). 5g of oils each were poured into 250 ml conical flask. Then 25 ml of neutral ethyl alcohol was added to it and boiled on a water bath. Phenolphthalein indicator solution (1-2 drop) was added and the mixture while hot was titrated against with standard potassium hydroxide solution with shaking. The end point was noted as the first pink colour which persist for 30 seconds. Acid value was calculated and expressed in mg of KOH per gm of Oil.

$$A.V = \frac{V \times N \times 56.1}{W}$$

Acid value =W
V= Volume of standard KOH solution in ml
N=Normality of standard KOH solution.

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W=Weight of oil sample in grams.

Iodine Value

The iodine value of oil was determined according to (AOCS, 2016). 0.25g oil sample was taken into a conical flask and dissolved with 10 ml CCl_4. 30 ml hanus solution was added and the mixture was allowed to stand for about 45 minutes in the dark with occasional shaking. 10 ml 10% KI solution and 100 ml distilled water were added to washed down any free iodine on the stopper. The iodine was titrated with previously standardized $Na_2S_2O_3$ solution which was added gradually with constant shaking until yellow solution turned almost colourless. Few drops of starch indicator was each added and titration was continued until blue colour disappeared. Bottle was shaken violently so that any iodine remaining in solution with the CCl_4 might be taken up by the KI solution. The volume of $Na_2S_2O_3$ solution required for the experiment was noted. A blank experiment was conducted along with the sample. Percent weight of iodine absorbed by the oil sample was calculated by the following formula:

1ml 1N $Na_2S_2O_3$ = 0.127 g I2
(B-A) × N × 0.127 × 100
Iodine value = W
B = ml of 0.1N $Na_2S_2O_3$ required by blank
A = ml of 0.1N $Na_2S_2O_3$ required by oil sample
N = Normality of $Na_2S_2O_3$
W = Weight of oil in g.

Free Fatty Acid.

A 5g of the oil was weighted into a conical flask containing 10ml of neutralised 95% ethanol. Three (3) drops of phenolphthalein was then added. The resultant mixture was titrated against 1.0M NaOH. The process was repeated for three times and the average titre value was recorded. The percentage free fatty acid was calculated using the expression below;

Percentage free fatty acid (% oleic) =

$$\frac{V \times N \times 2.77}{S}$$

Where;

V=volume of NaOH,

S=sample weight and 2.77 is the conversion factor for oleic acid.

RESULTS AND DISCUSSION

The qualities of these four (4) genetically modified soybean oils varieties were analysed by evaluating their physicochemical properties such as density, pH values, viscosity values, peroxide values, acid values, iodine values, free fatty acids and saponification values. Results are as presented in Table 1. These physico- chemical properties are very important parameters in order to design an advanced technological process.

Density

Oils with lower density values are highly recommended for consumers. The results tabulated in Table 1 show that at room temperature of 25°C highest and lowest values of the densities are 0.923 g/ml and 0.901 g/ml for TGX-1951-3F and TGX-1955-10E soybean oil respectively. This is because the π bonds that make the bonding more rigid and rotation between C-C bonds becomes more strenuous (SON, 2000).

Saponification Value

Saponification value provides the information on the average chain length and hence the molecular weight of the fatty acids in the oils. The shorter the average chain length of the fatty acids, the higher the saponification value and the lower the average molecular weight of the fatty acids and vice-versa (Ekwu and Nwagu, 2004). The Saponification value obtained for the oil varieties in Table 1 showed 122.02 mgKOH/g for TGX-1955-10E and 120.62 mgKOH/g for TGX-1448-2E, TGX-1904-2E and TGX-1951-3F respectively. The lower saponification values suggests that the number of ester bonds is less. This may imply that the fat molecules did not interact with each other (Firestone, 1994).

Peroxide Value

Peroxide value is used as a measure of the extent to which rancidity reactions have occurred during storage. The quality and stability of fats and oils can be indicated by using the peroxide value (AOCS, 1993). In the study, TGX-1448-2E/TGX-1904-2E and TGX-1951-3F soybean oils showed the lower peroxide values of 0.4 meq O2/kg oil which indicates a relatively good quality of these oils varieties. On the other hand, TGX-1955-10E oil showed a little bit higher value of 0.5 meq O2/kg oil. There is a very slight increase in peroxide value indicating the rancidity of oils due to relative higher oxidation in oils

Acid Value

Acid value of oils indicates the amount of free fatty acid present in the oil. It determines the purity of oils. The higher the values, the lower the possibility of the oils to be used as cooking purpose. It was observed that higher and lower acid values obtained for TGX-1448-2E oil and TGX-1955-10E oil were 15.61 and 13.05, respectively. Higher values indicates that triglycerides of oil are converted into fatty acids and glycerol which cause rancidity of the oil (Zahir, 2014).

Iodine Value

Iodine value (IV) measures the degree of unsaturation in a vegetable oil. It determines the stability of oils to oxidation and allows the overall unsaturation of the fat to be determined qualitatively (Zhou, 2010). It was observed that higher and lower iodine values obtained for TGX-1955-10E and TGX-1448-2E/TGX-1904-2E/TGX-1951-3F soybean oil were 25.63 and 24.75g respectively. The lower iodine value of the oil indicates that about 95% of fatty acid in the oil was saturated so they have very low C=C double bond which have low iodine number, that is minimum unsaturation whereas higher the values indicate the higher unsaturation of the oils (Marinova, 2012). The lower the values the better the oxidative storage stability. The oxidative and chemical changes in oils during storage are characterized by an increase in free fatty acid contents and a decrease in the total unsaturation of oils (Perkin,1992).

FREE FATTY ACID.

The number of milligrams of potassium hydroxide required to neutralise the fatty acid resulting from complete hydrolysis of one gram of oil was determined.

TGX-1448-2E and TGX-1955-10E had the highest and the lowest free fatty acid value of 15.61 and 13.05 respectively. while TGX-1904-2E and TGX-1951-3F had 14.74% respectively.

PH value of the Oil

The PH values indicates the acidity or the alkalinity of the oils. TGX-1448-2E, TGX-1904-2E and TGX-1951-3F showed the PH values of 7.2 each, while TGX-1955-10E showed a ph value of 7.4. These values showed that the oils were neither acidic nor basic but rather neutral which is a very good attribute of an edible oils.

Table 1. Physicochemical properties of some genetically modified soybean oils available in Nigeria

SAMPLE IDENTITY.	ACID VALUE (mgKOH/g)	pH	DENSITY Kg/L	IODINE VALUE (gI_2/100g oil)	PEROXIDE VALUE (meq O2/kg oil)	SAPONIFICATION VALUE (mg KOH/g)	Percent free fatty acid (%)
TGX-1448-2E	15.61	7.2	0.913	24.75	0.4	120.62	15.61
TGX-1904-2E	14.74	7.2	0.912	24.75	0,4	120.62	14.74
TGX-1951-3F	14.74	7.2	0.926	24.75	0.4	120.62	14.74
TGX-1955-10E	13.05	7.4	0.901	25.63	0.5	122.02	13.05

CONCLUSION

The qualities and properties of oils extracted from newly genetically modified soybean varieties produced in Nigeria were evaluated through this study using different parameters. Results obtained indicated that when the peroxide value is high, the iodine value is also high and vice versa. It also reveals that oils variety with low acid value tend to have high saponification value and a high peroxide value which are good quality attributes of ideal edible oils.

RECOMMENDATION

Oils extracted from these soyabeans varieties can be recommended for human consumption either with or without processing.

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