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EXTRACTION, CHARACTERIZATION AND ANTIOXIDANT STUDIES FROM GRAPE PEEL

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Abstract
This research is aimed at extracting, characterizing and investigating the antioxidant potential of grape peels in solving the problem associated with lipid oxidation in food. Grape peel sample was collected and oven dried at 60℃ for 48 hours, after which it was grinded and sieved to a particle size of 500 µm. Extracts were estimated from grape peel using methanol as solvent in a soxhlet extractor at different solid - solvent ratio, temperature and extraction time. The optimum conditions of the solid-solvent ratio, temperature and time obtained for the grape peel extract were 10: 200, 55℃ and 180 minutes respectively. The grape peel extract obtained at the optimum conditions was then characterized using Atomic Absorption Spectrophotometer to identify both the major and minor elements present. The elemental analysis of the grape peel extract confirmed potassium as the major element by percentage composition of 90.1. In assessing the effect of the grape peel extract on the mixture of cow fat and groundnut oil sample, oxidation stability of the oil sample with and without the extract were analysed using Rancimat test. The oxidation stability of the oil mixture samples mixed with and without the grape peel extracts were found to be 569 minutes and 67 minutes respectively. This clearly showed that the grape peel extract is effective in increasing the possible time by which oxidation process can take place within the oil sample from 67 to 569 minutes. In addition, peroxide, pH and free fatty acid tests of the oil mixture with and without grape peel extract were carried out and antioxidant effectiveness of the grape peel extract calculated. The results obtained clearly confirmed the potential ability of grape peel as an antioxidant agent.
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Keywords : Extraction, Grape peel, Solvent, Fat and Oil, Oxidation, Antioxidant

1. Introduction

Fruits and vegetables are the major sources of natural antioxidants and contain different types of antioxidant compounds such as carotenoids, lutein, vitamin C, vitamin E, and lycopene (Khonkarn *et al;* 2010). Antioxidant has ability to scavenge free radicals and exhibits antimutagenic, anticarcinogenic, antiglycemic, anticholesterol, anti-inflammatory and antimicrobial properties (Friedman and Levin; 2009). They can be used in food industry to prevent oxidation of food containing high amounts of fat and oil (Andrich *et al;* 2003).

The active component of an antioxidant is phenolic compounds and were found in various fruits and vegetables. Agricultural wastes such as peels of mango has been found to be rich sources of antioxidant phenolic compounds (Pushp *et al;* 2011, Ajila *et al;* 2007). Grape fruit is a subtropical citrus tree known for its sour to semi-sweet fruit. It is an excellent source of vitamin C, a vitamin that helps to support the immune system. The rich pink and red colours of grapefruit are due to lycopene, a carotenoids phytonutrient with highest capacity to help

fight oxygen free radicals (Jian *et al;* 2007). Grape is the one of the most cultivated fruit in Nigeria and its major byproducts after processing are peels and seeds.

Oxidation is among the main severe problems encountered in the food industry as its occurrence often leads to a declining effect on food quality. The main food qualities affected by lipid oxidation include reduction in nutritional quality, increased toxicity, development of off-odour, and distorted texture and colour (Friedman and Levin; 2009). Oxidation of oils occurs during the processing and is one of the major changes evident in the distribution and final preparation of foodstuffs. And as such, stabilizing oil against oxidation by the usage of antioxidants is an effective and most efficient way of hindering the oxidation of lipids, thereby protecting oils from the damages cause by free radicals (Sima and Esmaeil, 2013).

The problem of waste and environmental pollution of most fruits is continually increasing and there are needs for the utilization of these wastes by proper conversion into useful products in order to ensure proper waste management. For example, the possibility of grape peels being processed into an antioxidant in order to reduce lipid oxidation in oil that have been so challenging in the food industry. This research ensures that the grape peels that polluted the environment is judiciously handled or

Plate I: Fresh grape peel Sample

channelled to a great resource generation. In addition, this research work will boost and complement the excellent efforts in meeting targets of the rising demand for antioxidants in the food industry. (Rashmi and Disha, 2011). This research work is aimed at the extraction, characterization and antioxidant studies of oven dried grape peels using soxhlet apparatus at different time, temperature and solid - solvent ratio and optimum condition established.

The grape peel extract was then characterized using Atomic Absorption Spectrophotometer for elemental analysis. The effect of the grape peel extract on lipid was then carried out using Rancimat method for oxidation stability index and peroxide value test, acid value test and pH performed.

2. Methodology

2.1 *Materials and Methods*

The grape sample used in this work was sourced from Vunchi in Lavun Local Government of Niger State. As shown in plates 1 and 2, the grape samples were washed, peeled, weighed and oven dried for 48 hours at 60°C, then after its moisture content determined. The dried grape peel was milled by a combination of small mortar and pestle, and grinder into a mesh size of 0.5 mm. The grinded grape peel was enclosed in a filter paper for extraction.

Plate II: Dried grape peel Sample

2.2 Extraction of Sample

One variable at a time method was used for the solvent extraction of grinded grape peel using soxhlet extraction
with methanol as the extracting solvent. The grape peel samples at different solid - solvent ratio (10:30, 10:50, 10:100, 10:150, and 10:200) were extracted at temperature range and extraction range of 45 to 60 and 20 to 200 minutes (Dirisu, 2015).

2.3 Determination of Elements present in the Grape Peel Extract

Atomic Absorption Spectrophotometer analysis was used to evaluate the elemental composition of the grape peel extract (Sykorova *et al;* 2009). This analysis is important in determining the composition of the grape peel extract and comparison made with extract obtained from antioxidant rich fruits.

2.4 Preparation of Oil Sample

In order to ascertain the antioxidant potential of the grape peel extract, oil sample was prepared by mixing unsaturated groundnut seed oil with saturated cow fat in the ratio of 4:1 respectively.

2.5 Evaluation of the Oxidation Stability of the Grape Peel Extract

The sample of the oil with extract was made by mixing the oil mixture and extract in the ratio of 8:1. An evaluation of the oxidative stability of the oil mixture mixed with and without grape peel extract was carried out using the Rancimat test. The oxidative stability was determined in 743 Rancimat apparatus from Metrohm according to ISO 6886:1997, utilizing a sample of 2.5 g. All samples were heated to 110 °C with an air flow of 10 L/h. (Farhooshi, and Moosavi, 2007), The oxidation stability times were printed automatically by apparatus software.

2.6 Evaluation of Lipid Oxidation of Grape Peel Extract

Lipid oxidation test was carried out on the oil sample mixed with and without grape peel extract in order to evaluate the antioxidant potential of the grape peel extract. 5 ml of orange peel extract was added to 40 ml of oil sample prepared in section 2.4. The pH, peroxide value and acid value tests were used to analyze antioxidant effectiveness of the orange peel extract on a mixture of groundnut seed oil and cow fat. The peroxide value and acid value tests were carried out according to the standard procedures stated by Etti *et al;* (2012), John (2010) and Fereidoon and Ying (2003). The percentage of antioxidant effectiveness was calculated using the method described by Adegoke and Gopalakrishna (1998) from the equation (1)

$$AE\ (\%) = \frac{(PVC - PVT)}{PVC} \qquad \text{---------- (1)}$$

where AE is the antioxidant effectiveness, PVC is the peroxide value of control sample and PVT is the peroxide value of test sample.

3. Results and Discussion

3.1 Effect of solid-solvent on extraction yield of grape peel

Effects of different solid- solvent ratios were investigated to obtain the optimum solid-solvent ratio on the extraction of grape peel. As shown in Table 1, the extraction yield of grape peel increased with extraction solvent-solid ratio from 10:30 to 10:200. However, the percentage increase in the yield from 10:150 to 10:200 solid-solvent ratio can be considered negligible when compare with those obtained before 10:150 solid-solvent ratio. Therefore, solid-solvent ratio of 10:150 is considered to be the optimum for the extraction of grape peel.

Table 1 Analysis of Solid - Solvent Ratio and Yield of Grape Peel Extract at 50°C and 60 minute

Solid - Solvent Ratio (g/ml)	Yield (%)
10:30	8.26
10:50	10.31
10:100	11.73
10:150	19.35
10:200	20.28

Table 2: Effect of Extraction Time on the Yield of Grape Peel Extract at 50 ℃ and Solid-Solvent Ratio of 20:200

Time (minutes)	Yield (%)
20	16.70
40	19.72
60	25.20
80	28.37
100	33.30
120	35.77
140	37.44
160	39.20
180	44.46
200	46.83

Table 3: Effect of Temperature on the Yield of Grape Peel Extract at 60 minute and Solid-Solvent Ratio of 20:200

Temperature (℃)	Yield (%)
45	21.16
50	17.76
55	21.40
60	21.12

3.2 Effect of Time on Extraction Yield of Grape Peel

In order to obtain the optimum time in the extraction of grape peel, the effects of different extraction time were investigated and the result plotted in Table 2. The extraction yield of grape peel increased with extraction time from 20 to 200 minutes. However, the yield percentage at 180 minutes and 200 minutes are 44.46 % and 46.8 % respectively. The percentage increment between 160 to 180 minutes and 180 to 200 minutes were found to be 5% and 2 % respectively. Therefore, the percentage increment after 180 minutes is observed to be negligible and the optimum time for the extraction of grape peel is 180 minutes

3.3 Effect of Temperature on Extraction Yield of Grape Peel

Effects of different extraction temperatures on extraction yield was determined and the results shown in Table 3. Temperature greatly influenced the extraction yield as at 45°C, just about 21.16% extract was realized. However, there is a sharp decrease in the extraction yield as temperature increases from 45°C to 50°C. In addition, an increase in temperature to 55°C resulted in an increase in the percent yield and a decrease as temperature increase to 60°C. An optimum temperature of 45°C is selected as the best temperature of extraction as the yield at this temperature is considered the highest when compared with the yields at other extraction temperatures.

3.4 Atomic Absorption Spectrophotometer Analysis of Grape Peel Extract

Atomic Absorption Spectrophotometer analysis was performed in order to find out the major and minor elements present in the grape peel extract sample and the result obtained presented in Table 4. Potassium (K) is the major element contained in the antioxidant as its composition is found to be 90.1 %. However, elements such as Sodium (Na), Calcium (Ca) and Phosphorus (P) are classified as intermediate and elements such as Copper (Cu), iron (Fe), Lead (Pb) and Manganese (Mn) were found to be the minor elements based on their percentage composition. This result is in agreement with the research work of Gaitry *et al;* (2013), Borang, (2010); Sykorova *et al.,* (2009). While they reported that K and Ca had the highest percentage composition in the extract, the AAS analysis obtained in this work confirmed Potassium as the only major element present in the grape peel extract.

Table 4: AAS Analysis of grape peel extract

Elements Present	Na	K	Ca	P	Cu	Fe	Mn	Pb
Grape Peel Extract (ppm)	8.6	196.0	2.6	6.28	0.3	0.1	0.01	0.1

Table 5: Oxidation Stability of oil mixture with and without Extract

Oil sample without Extract	67 minutes
Oil sample with Extract	569 minutes

3.5 Evaluation of Oxidation Stability of Oil Mixture without Extract and with Extract

The oxidation stability tests of the oil mixture with and without the grape peel extract were carried out using Rancimat method and the results obtained presented in Table 5. The oxidation stability of the oil mixture samples without the grape peel extracts was found to be 67 minutes. This clearly showed that the oil mixture can resist oxidation for just 67 minutes. However, addition of the grape seed extract to the oil mixture prolongs the stability of the oil mixture from 67 minutes to 569 minutes. That is, the time when either a level of detectable rancidity or sudden change in the rate of oxidation occurring is hereby increasing by 849 %. An important finding of this present study is that, the addition of grape peel extract was equally effective in improving the oxidation stability of an oil mixture from 67 minutes to 569 minutes.

3.6 Effect of Grape Peel Extract on Oil Mixture for a Period of 50 Days

3.6.1 Analysis of the Peroxide Value Test

At the initial stages of lipid oxidation, peroxides are the by products formed and therefore, its values can be used as a measure of how lipid oxidation occurs. In investigating the effect of grape peel extract on the mixture of groundnut seed oil with cow fat, there is a decrease in the peroxide value of the sample without orange peel extract on comparison with the sample mixed with grape peel extract. For example, peroxide value of samples without and with grape peel extract decreases from 3.1 to 2.7 meq.g/kg of oil at day 3. As shown in Table 6, the same pattern is observed when comparing the peroxide values of samples without and with orange peel extract for a period of 15, 35 and 50 days. This means, lipid oxidation was observed to be slower in oil mixture sample mixed with grape peel extract. Therefore, there is an active component(s) in the grape peel that is responsible for the anti- oxidization of the oil sample. The result obtained in this research work is in agreement with the study of Etti *et al;* (2012) on the use of antioxidants to minimize rancidity in mayonnaise and their results confirmed that samples with Aframomum danielli extracts had antioxidant effects on comparison to samples without Aframomum danielli extracts. As shown in Table 3, the antioxidant effectiveness at day 3 is 22.58 % and increases to 25%, 27.3 and 30 % at days 15, 35 and 50 respectively. This means, lipid oxidation is smallest at the initial stage and increased with increase in days of storage.

3.6.2 Analysis of Free Fatty Acid Test

The result of changes in the values of free fatty acid of oil samples with and without the grape peel extract was tabulated in Table 7. At day 3, there is a decrease in the values of free fatty acid of oil sample without grape peel extracts on comparison to that of sample with grape peel extract. The same trend was o bserved for samples at the end of 15, 35 and 50 days. This showed that addition of grape peel extract to the oil sample reduces the formation of free fatty acid and indirectly rancidity. This result further affirmed that grape peel extracts was able to reduce rancidity and thereby used as an antioxidant. The result obtained in this research work is in agreement with the work of Etti *et al;* (2012), who observed that rancidity is usually accompanied by free fatty acid formation

Table 6: Effect of Peroxide Value Test on Oil Sample for a Period of 50 Days

Sample	Day 3	Day 15	Day 35	Day 50
Oil sample with grape peel extract (meq.g/kg of oil)	2.4	2.7	3.2	3.5
Oil sample without grape peel extract (meq.g/kg of oil)	3.1	3.6	4.0	5.0
Antioxidant effectiveness (%)	22.58	25	27.3	30

Table 7: Effect of free fatty acid test on oil sample for a Period of 50 Days

Sample	Day 3	Day 15	Day 35	Day 50
Oil sample with grape peel extract (meq.g/kg of oil)	2.4	2.7	3.2	3.5
Oil sample without grape peel extract (meq.g/kg of oil)	2.62	3.10	3.70	4.50

Table 8: Effect of pH test on oil sample for a Period of 50 Days

Sample	Day 3	Day 15	Day 35	Day 50
Oil sample with grape peel extract	6.83	6.45	5.20	4.40
Oil sample without grape peel extract	6.96	6.56	6.20	5.60

3.6.3 Analysis of pH test

As shown in Table 8, there is a decrease in pH of the oil sample without grape peel extract on comparison with that of the sample with grape peel extract and this reduction moved towards acidic region. The high pH of the oil sample without grape peel extract confirmed the absence of antioxidant to reduce the activities of enzymes and microorganisms. However, the difference in the pH value of samples without and with extract increases with increase in days. This result confirms the presence of antioxidants in grape peel as its extract was found to inhibit the activities of enzymes and microorganisms.

4. Conclusion

Antioxidant was extracted from an oven dried grape peel at different solid-solvent ratio, extraction temperature and time using solvent extraction method. The optimum solid-solvent ratio, extraction temperature and time established were 10:200, 45ºC and 180 minutes respectively. Evaluation of the antioxidant potential of grape peel extract was established based on its ability to preserve a mixture of groundnut seed oil and cow fat from deterioration for 50 days. The Rancimat analysis of the oil samples with and without grape peel extracts showed the oxidation stability of 67 minutes and 569 minutes respectively. The antioxidant effectiveness of the grape peel extract on the oil sample stand between the range of 22.58% and 30% and this clearly showed that grape peel extracts has an antioxidant potential and capable of preventing lipid oxidation and prolongs the shelf life of fat and oil food stuffs.

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