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Research Paper

Characterization of Home-Made and Industrially Produced Niger Seed Oils by Fluorescence Spectroscopy

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Abstract

Home-made and industrially produced Niger seed oils were characterized by a fluorescence spectrometer to analyze the impact of the industrial process on the physical and chemical properties of the oil. The excitation wavelength was varied from 350 nm–380 nm with an incremental step of 10 nm, and the slit width was kept at 5 nm. Experimental results indicated that the major components in oil samples (home-made, unrefined, and refined industrially produced) were Vitamin E, Poly-unsaturated fatty acid, and Chlorophyll. Emission spectra recorded in the range of band between 400 nm – 500 nm were related to Poly-unsaturated fatty acids; those spectra recorded in the range of band between 500 nm-550 nm with a peak around 548 nm belong to vitamins E; and the band of emission wavelength between 650 nm – 725 nm were due to Chlorophyll. It was also observed that the refined oil had a very small vitamin E peak. This could be associated with the heating process of refining, as vitamin E is heat-unstable. Moreover, the emergence of new peaks in the spectra of the refined oil between 400 nm – 450 nm was due to fat-soluble vitamins and lipids that emerge as a result of oxidative reaction during the heating process. Moreover, an increase in excitation wavelength also resulted in a blue shift of the emission spectra. This was because of the presence of more than one fluorophore (luminophore) in the molecule. The calculated quantum yield of the fluorescence of the sample/un-purified was 0.01042, which is a 10% reduction compared to the purified oil. An increase in pH values also resulted in a decrease in fluorescence intensity and vice-versa.

1. Introduction

Vegetable oils are the main source of fatty acids, which are predominantly triglycerides (95% - 98%); the remaining (2% -5%) consists of complex mixtures of minor compounds in a wide range of chemical classes (Romaniuk et al., 2004). The proportion of saturated and unsaturated fatty acids has an important role in the behaviors of vegetable oils. The degradation of vegetable oil quality is essentially caused by unsaturated fatty acid oxidation, which is a complex phenomenon that generates mainly hydroperoxides but also volatile compounds through a three-phase process of initiation, propagation and termination (Laguerre et al., 2007).

Niger seed (*Guizotia abyssinica*) is one of the most important edible oil crops in Ethiopia, providing 50% to 60% of the indigenous edible oils (Dutta et al., 1994). The use of Niger seeds as a source of edible oil through home-level manufacturing has been practiced since ancient times. The lightly roasted seed is first pounded by adding hot water and then transferring the pounded

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mash into an earthen pot which is rotated to let the oil migrate to the top and separated by decanting the oil.

Oxidized lipids not only result in objectionable flavors and odors, loss of colour and nutrient value but also generate potentially toxic compounds which may be detrimental to the health of consumers (Luzia et al., 1997). One of the most effective ways of retarding lipid oxidation and hence increasing the shelf life of oils and oil products is to incorporate antioxidants which may be defined as substances that when present at low concentration compared with those of oxidizable substrates significantly delay or prevent the oxidation of that substrate. In doing so, antioxidants slow down the rate at which oxidation occurs. The application of natural antioxidants as food additives was also raised because of their potential health benefits. It was reported that natural antioxidants decrease the risks of heart disease more than their counter-synthetic antioxidants (Shaker, 2007).

The addition of antioxidants and bleaching to reduce oxidation and loss of colour and taste requires a great deal of knowledge of food chemistry and the usage of sophisticated equipment (Guimet et al., 2004). In addition, a controlled environment and state-of-the-art laboratory are essential. Most of the methods are based on the extraction of neutral lipids with hexane or petroleum ether, and the extracted components are estimated gravimetrically. The method requires a triplicate grinding/extraction, making it lengthy and very detailed for the analyst (Barthet and Daun, 2004).

In this study, the researcher was interested in the photo-physical analysis of oil samples to investigate the impact of the industrial process on the antioxidant property, its quantum yield, and nutrient contents of oil using a recently developed food characterization technique, which uses the fluorescence property of a sample under investigation. The method is fast, cheap, non-destructive, does not use chemicals, is environmentally friendly, and can be used at all levels of manufacturing. It could also be used as a quality control technique in such a way that the abundance and presence of essential elements could be controlled or monitored online while the process of manufacturing is going on. Hazardous pollutants in the manufacturing process, if any, could also be identified. Thus, this method is so vital for the industrialists, the wider community, and the scientific community.

2. Materials and Methods

Mature, normal and dried Niger seeds produced at the household level were bought from the local market. The seeds were washed and roasted before undergoing the process; then. They were pounded in a wooden mortar by a pestle. Refined and unrefined Niger seed oils were purchased from the local oil manufacturer in Adama city. The fluorescence spectra of all Niger seed oil samples were characterized by using a fluorescence spectrometer (Cary eclipse-MY18490002) with the spectral range of detection ranging from 200 nm up to 900 nm. A continuous xenon arc lamp and photomultiplier were used as excitation sources and detectors, respectively.

Boiling water was added slightly and then pounded Niger seed oil into an earthen pot which was manually rotated to move the oil to the top, and the oil was separated by decantation. The decanted oil was further filtered by a sieve to avoid solid suspensions. The purified samples were stored in a glass bottle and kept at room temperature for experimentation. After different molar concentrations were prepared, emission spectra were collected in the visible region of the spectrum. The excitation wavelength ranged from 350 nm to 380 nm with an increment of 10 nm. The widths of the slits were fixed at 10 nm for excitation and 5 nm for emission monochromators. To ensure the validity of the different measurements, four scans were made at each excitation wavelength. Data analysis was done using origin-pro 8 software.

3. Results and Discussion

3.1. Analysis of home-made Niger seed oils

For excitation wavelength ranging from 350 nm to 380 nm, with an incremental step of 10 nm, the emission spectra were observed to show fluorescence peaks in the range λ_{em}: 400 nm – 725 nm. Different peaks and bands of fluorescence were observed for each excitation wavelength as illustrated in Figure 1. To differentiate the spectra, blue, red and black colours were assigned to each excitation wavelength and their corresponding emission spectra: green colour for spectra excited at λ_{ex}: 380 nm, blue colour for spectra excited at λ_{ex}: 370 nm, red colour for spectra excited at λ_{ex}: 360 nm, and black colour for spectra excited at λ_{ex}: 350 nm, respectively.

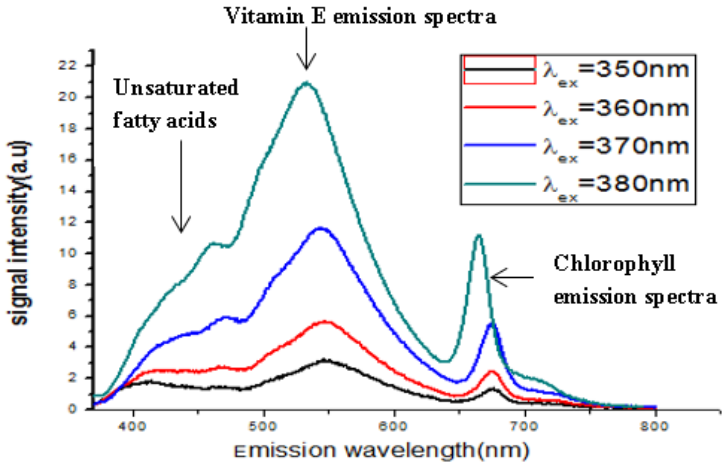

Figure 1: Fluorescence Intensity versus Wavelength of Homemade Niger Seed Oil

Comparing the result with literature values for vegetable oils and chlorophyll fluorescence (Yvon et al., 2011 and Theppawut et al., 2015), it was concluded that emission spectra recorded in the range of band between 400 nm - 500nm are related to polyunsaturated fatty acids. On the other hand, emission spectra recorded in the range of band between 500nm-550nm with a peak around 548nm was related to vitamin E, and the regions of emission wavelength band between 650nm - 725nm was due to chlorophyll present in the Niger seed oil. The resulting fluorescence spectra are indicated in Figure 1.

It was observed that as the wavelength of excitation increased from 350nm to 380nm, the emission spectra underwent a blue shift towards a shorter wavelength and resulted in higher intensity counts. This could be due to the presence of more than one fluorophore (luminophore) in the molecule or a high concentration of polyunsaturated fatty acid. The emission peaks for vitamin E also had a higher proportion than chlorophyll.

3.2 Analysis of Unrefined Niger Seed Oils

The excitation wavelength used was similar to part 3.1. The corresponding emission spectra were observed to show fluorescence peaks in the range λ_{em}: 400 nm – 725 nm. Different peaks and bands of fluorescence were observed for each excitation wavelength. To differentiate the different spectra, blue, red and black colours were assigned to each excitation wavelength and their corresponding spectra: green colour for spectra excited at λ_{ex}: 380 nm, blue colour for spectra excited at λ_{ex}: 370 nm, red colour for spectra excited at λ_{ex}: 360 nm, and black color for spectra excited at λ_{ex}: 350 nm, respectively (Figure 2). The values for fluorescence spectra in the range of bands between 400 nm – 500 nm were due to unsaturated fat and fat-soluble vitamins (Yvon et al., 2011). It was observed that the fluorescence intensity increased by increasing exciting wavelengths from 350nm-380nm and there was also a blue shift in the emission wavelength. The peak of fluorescence for vitamin E around the band 548 nm was found to be smaller compared to the homemade oil. This was expected to be the result of oxidation during the factory process. The bands around 676 nm belong to chlorophyll fluorescence (Theppawut et al., 2015).

3.3 Analysis of Refined Niger Seed Oils

A similar procedure was followed in parts 3.1 and 3.2 to characterize the refined Niger seed oil. The excitation wavelength range and fluorescence bands of the spectral region were observed to be in the same range. However, high intensity and many new bands of peaks emerged between 400 nm – 500 nm as shown in Figure 3. This could result from high-temperature oxidation that induced chemical transformation. The bands were assigned to fat-soluble vitamins, lipids, and hydroperoxides (Yvon et al., 2011). On the other hand, the peak that existed around 548 nm, in home-made oil, Figure 1, has almost disappeared. This indicated the absence of vitamin E. The bands at 676 nm have been assigned to chlorophyll as already cited above. The researcher concludes that bleaching used to make refined oils transparent and heating at very high temperatures to extract the maximum amount of oil from the seeds could result in the loss of nutrients like vitamin E, color and taste.

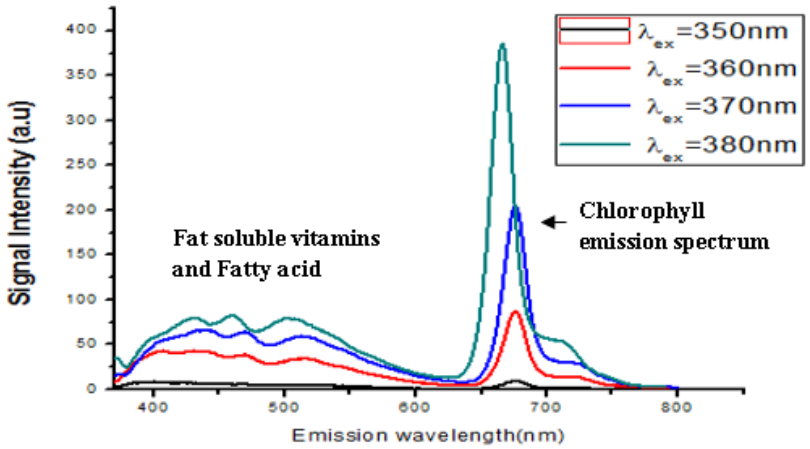

Figure 2: Fluorescence Intensity versus Wavelength of Unrefined Niger Seed Oil

3.4. Difference & similarity between homemade, refined & unrefined Niger seed oil

The major difference between homemade, unrefined, and refined Niger seed oils could be observed in Figure 4 below. The process of refining oil undergoes so many chemical processes: heating the oil at a very high temperature, chemical bleaching to make it transparent, and loss of unpleasant odor. The oxidation during the heating process resulted in the emergence of new peaks and loss of essential nutrients like vitamin E.

The unrefined oils retained many of the nutrients and antioxidants of the original seed. The only downside of unrefined oils was that it was often less stable than refined oils. They are more prone to going acidic in a shorter time. It was also observed that the relative intensities of the fat and fat-soluble vitamins for home-made oil was lower. However, both industrially produced oil samples and unrefined and refined oil samples, show similar behaviour in fat and fat-soluble vitamins. Since vitamin E is not heat-stable, prolonged time of heating and the high temperature seems to have resulted in negligible pear in both samples. Thus, industrially manufactured oils lack antioxidant properties. Homemade Niger seed oil is an unrefined oil. It differed from the industrially produced oils in that it has a higher concentration of vitamin E. Unrefined Niger seed oil is nutritionally valuable, as it contains linoleic acid (unsaturated fats), which are essential for good health. They prevent cardiovascular diseases and are precursors of structural components of plasma membranes and some metabolic regulatory compounds. The low intensity of the peaks around 400nm - 475 nm is due to their large content of monounsaturated fatty acids and phenolic antioxidants, like vitamin E, which provide more stability against oxidation.

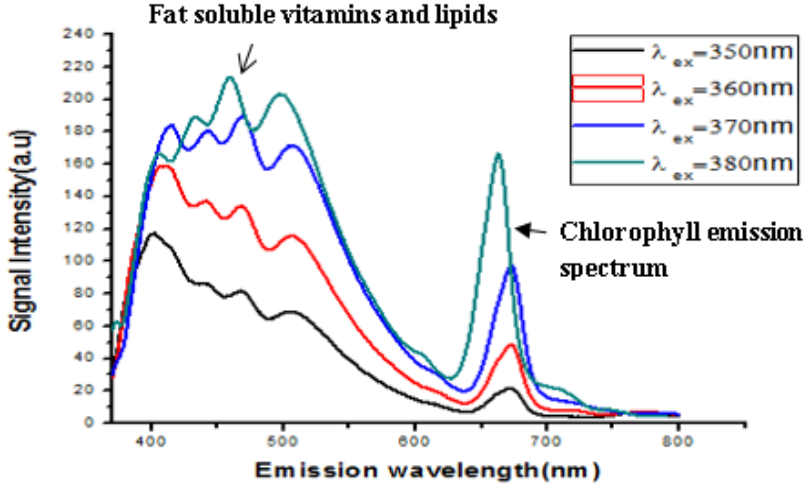

Figure 3: Fluorescence Intensity versus Wavelength of Refined Niger Seed Oil

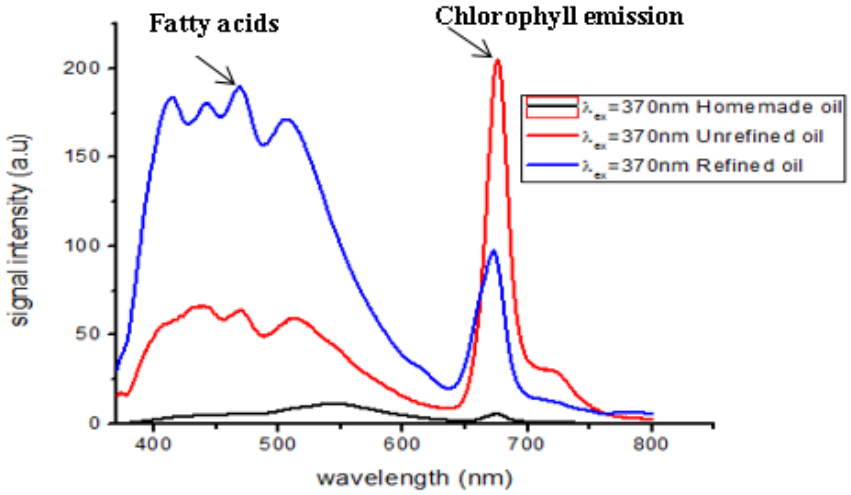

Figure 4. Comparison of Spectral Peaks for Home-Made, Unrefined and Refined Niger Seed Oils of Different Acidity (pH)

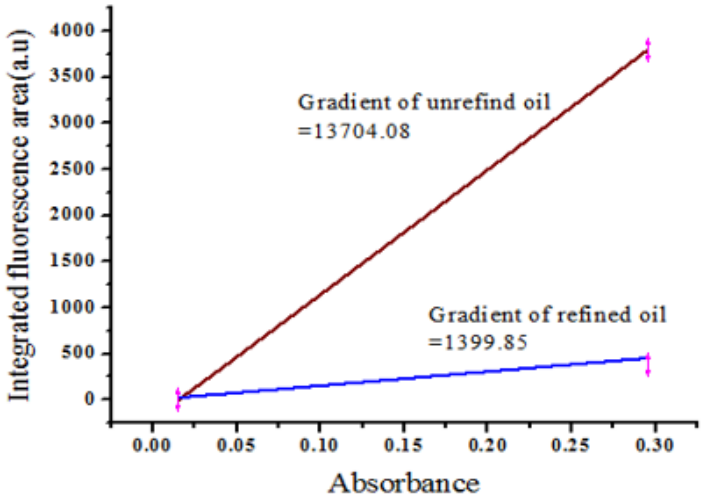

Figure 5. Integrated fluorescence intensity versus Absorbance (a.u).

On the other hand, refined oil shows a high-intensity peak around 400 nm - 475nm which is due to fatty acid oxidation formed as a result of the high-temperature heating. It was also observed that the pH values of the home-made, unrefined, and refined Niger seed oil were 5.7, 5.4, and 5.1, respectively. So, the unrefined Niger seed oil has high acidity. As fluorescence is pH sensitive, the small variations in pH values have resulted in spectral variation (Figure 4). A decrease in pH increased to a fluorescence peak.

3.5. Determination of fluorescence quantum yield (QY)

Fluorescence quantum yield (QY) for Niger seed oils was determined from the gradients of the integrated fluorescence area versus absorbance (Figure 5). Relative Fluorescence QY values were calculated employing standard equations from the literature (Williams et al., 1983) where the relative indices of refraction of the samples were closer to unity. Using the quantum yield of a reference sample (the refined oil), which was 0.102, the fluorescence quantum yield of a test sample was calculated and found to be 0.01042. The result indicated a 10% reduction in the quantum yield of the fluorescence property due to various quenching effects of impurities that existed in the unpurified oil sample.

4. Conclusion

The study has successfully distinguished the major fluorescent compounds found in the home-made and industrially produced Niger seed oils. The variations of each compound (oxidation products, vitamin E, and chlorophyll) resulted from the process of heating and purification. The study proved the possibility of using

fluorescence spectroscopy for analysis of the photo-physical properties to check the quality of vegetable oils. Emission spectra of major fluorophores found in the samples were identified. Accordingly, the major compounds found were fatty acids, vitamins, and chlorophyll. It was also observed that heating oil results in the oxidation of fatty acids that result in the emergence of fluorescence peaks of fat-soluble vitamins, but it results in loss of essential antioxidant vitamins such as vitamin E. It was also found that chlorophyll was abundant in all the sample types, but with more intensity in the industrially produced oil samples. A decrease in pH increased to a fluorescence peak. The calculated quantum yield of the fluorescence of the sample could also be calculated from the quantum yield of a reference sample and integrated fluorescence intensity versus absorbance of a test sample.

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