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A rapid reversed-phase thin layer chromatographic detection protocol for adulteration in some edible fats and oils food formulation.

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Abstract

The problems of adulteration in the vegetable oil and fat have been the major draw backs in the food products formulation, in spite of the various adulteration detection methods in different applications that have been reported. However, the detection tools that can be fast and reliable for the routine analysis necessitated the current work. The two groups of three different samples: vegetable fat containing sample (Blue Band, Golden Penny, La Prairie Classic) and animal fat containing samples (Kell Salad Cream, Crosse & Blackwell and Nola) was used for the purity check using the reversed phased - thin layer chromatographic (RPTLC) method of analysis were developed. The average R_f ratio of 0.95 and 0.92, found for the vegetable and animal fat groups were reported, respectively. The R_f = 0.03 difference between the two groups indicated the presence of sistosterol (plant sterol) and cholesterol (animal sterol), an improvement over color detection methods to screen oils and fats to ascertain purity.

Keywords: Sistosterol, Cholesterol, Adulteration, Animal fat, Vegetable oil

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Introduction

Vegetable oils and fats (VOFs) have a big contribution in our diet as cooking or frying oil and in food products formulation. For economic profits, expensive vegetable oils and fats are usually and deliberately adulterated with less expensive oils and fats (Rani, 2015 and Wasnik *et al.*, 2019).

Issue of vegetable oils adulteration is not peculiar to a region or country. Usually, health related problems have been associated with the adulteration of VOFs in many countries. If edible and expensive oil such as olive oil is admixed with less expensive edible one; however, it has been reported that adulteration of vegetable oils caused serious health problems in some cases like Spanish olive oil syndrome due to selling non-edible rapeseed oil as an edible rapeseed oil (Jee, 2002). Adulteration is when substitution of valuable constituents in oils and fats with another less valuable constituent occurs. Sometimes adulteration is used to dilute or conceal some defects in oils and fats such as making them to appear bulkier and bigger in weight than the original but with reduced quality. The literatures have reported the adulterations of olive oil with tea tree oils and mustard oil with poisonous Argemone oil (Ntakatsane *et al.*, 2013; Ahmed *et al.*, 2020). To detect edible oils and fats adulteration, several methods have been used to check the purity of edible oil such as modified nitric acid test, sodium azide test, boudouin test, azo dye test, halphen's test,

hexabromide test, Solvent partition test and Molybdate method (Ramamurthy, *et al.*, 1967, Motghare, *et al.*, 1995, Shukla, *et al.*, 2002 and 2004, Sharma, *et al.*, 2009, Ntakatsane, 2013). It is possible to use both major and minor components as detection tool. Since each oil and fat have an especial major and minor component at a known level, their presence and levels is a detection tool. For example, brassicasterol is present in canola oil; therefore, it is possible to use brassicasterol to detect other VOFs adulteration with canola oil. Another example like phytosterols (Campesterol, Stigmasterol and Sitosterol) are present in small amount in butter, if there was high level of phytosterols in butter, it can be concluded that it has been admixed with VOFs (Clemente and Cahoon, 2009; Hassoun *et al.*, 2020).

Since the detection of adulteration is important especially in food industries, the present study hope to offer simple, fast and reliable tests for distinguishing vegetable oil and animal fat chemical constituents at known level. With refinement, this newly developed TLC method is an improvement over other detection methods to screen oils and fats to ascertain purity in industry.

Materials and Methods

Materials and Chemicals:

Ethanol, petroleum ether, acetonitrile, chloroform, methanol phosphomolybic acid, hexane (Sigma Aldrich, USA); RP-TLC (reverse-phase thin layer chromatography)

plates and TLC silica gel 60 RP-18 F256S (Merck Specialties Private Ltd. Mumbai, India); A – Blue band, B – Golden penny, C– Kell Salad Cream, D– La Prairie Classic, E – Crosse & Blackwell and F – Nola (Supermarket in Ibadan, Oyo State, Nigeria).

Extraction processes of unsaponifiable matter (USM).

Extraction process from samples.

The extraction of unsaponifiable matter (USM) for both cholesterol and beta-sitosterol from two groups of samples: vegetable fat containing samples (Blue Band, GoldenPenny, La Prairie Classic) and animal fat containing samples (Kell Salad Cream, Crosse and Blackwell Nola) were carried out using the method of Sharma, *et al.* (2009). 0.1 g of the standard cholesterol and beta-sitosterol samples were taken in a 15 mL sampling bottle, 5 mL of 5% methanolic KOH was added and the tube incubated at 90°C in a water bath for about 20 min (shaking intermittently at every 5 min). After incubation, the tube was cooled under tap water to room temperature; 5 mL of hexane and 1 mL of water were added with 4-5 min vigorously shaking. Followed by 2 min centrifugation at 2000 rpm. The upper hexane layer was removed, allowed to be evaporated to obtain dried unsaponifiable matter which was re-dissolved in chloroform and transferred to 500 µL in an eppendorf tube.

Extraction process for adulterated (mixed) samples

Vegetable fat containing samples (Blue Band, GoldenPenny, La Prairie Classic) were added to the animal fat containing samples (Kell Salad Cream, Crosse & Blackwell and Nola) at the rate of 2, 5, 7 and 10%. The extraction was similarly carried out using the aforementioned method of Sharma, *et al.* (2009)

The separation using reversed phase - thin layer chromatography (RP-TLC)

The separation conditions of standard cholesterol and sitosterol

The petroleum ether; acetonitrile; methanol (20: 40: 40 v/v) as developing solvents were added to a TLC glass chamber lined with filter paper on the three sides. After the saturation of the chamber for about 15 min, 6 µL of the 500 µL solutions in chloroform (containing the solution of standard cholesterol and beta-sitosterol) was spotted on TLC silica gel 60

RP-18F254S plate at about 1cm from the bottom at different spot and allowed to air dry. The plate was removed after three-quarters of the length of the plate had been travelled by the solvent front in the developing chamber and dried. The phosphomolybdic acid (20% solution in ethanol) was used to spray the plate and kept at 90 - 95°C for 5 min.

The separation conditions for samples

The petroleum ether; acetonitrile; methanol (20: 40: 40 v/v) as developing solvents were added to a TLC glass chamber lined with filter paper on the three sides. After the saturation of the chamber for about 15 min, 6 µL of the unsaponifiable matter solution from 500 µL solutions in chloroform (containing the solution of cholesterol and beta-sitosterol) was spotted on TLC silica gel 60 RP-18F₂₅₄S plate at about 1cm from the bottom at different spot and allowed to air dry. The plate was removed after three-quarters of the length of the plate had been travelled by the solvent front in the developing chamber and dried. The phosphomolybdic acid (20% solution in ethanol) was used to spray the plate and kept at 90 - 95°C for 5 min. The distinct bluish band position (R_f values) were compared with reference cholesterol and sitosterol standards [Jarusewez *et al.* 2005; Anupama, *et al.* 2015 and 2016].

The separation conditions for adulterated (mixed) samples

The petroleum ether; acetonitrile; methanol (20: 40: 40 v/v) as developing solvents were added to a TLC glass chamber lined with filter paper on the three sides. After the saturation of the chamber for about 15 min, 6 µL of the unsaponifiable matter solution (mixed) 500 µL solutions in chloroform (containing the solution of cholesterol and beta-sitosterol obtained from mixed samples at the rate of 2, 5, 7 and 10%) was spotted on TLC silica gel 60 RP-18F254S plate at about 1cm from the bottom at different spot and allowed to air dry. The plate was removed after three-quarters of the length of the plate had been travelled by the solvent front in the developing chamber and dried. The phosphomolybdic acid (20% solution in ethanol) was used to spray the plate and kept at 90 - 95°C for 5 min. The distinct bluish band position (R_f values) were compared with reference cholesterol and sitosterol standards (Jarusewez *et al.* 2005; Anupama, *et al.* 2015 and 2016).

Results and Discussion

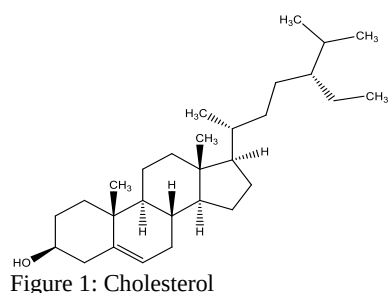


Figure 1: Cholesterol

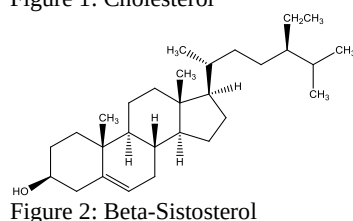


Figure 2: Beta-Sistosterol

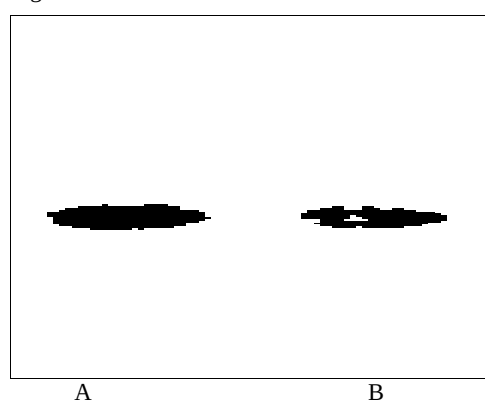


Figure 3: RP-TLC of standard cholesterol (A) and sistosterol.(B)

The Figures 1 and 2 are the structures of cholesterol and beta-Sistosterol, while Figure 3 is RP-TLC of standard cholesterol (A) and sistosterol (B). Table 1 shows the standard R_f values of cholesterol (0.92) and sistosterol (0.95) from the two TLC experiments.

Table 1: R_f values for cholesterol and sistosterol standards

Standards Sterols	R _f Values
Cholesterol	0.92
Sistosterol	0.95

Table 2 shows the R_f mean values for cholesterol and sistosterol in the six different samples. The average R_f ratio of 0.95 and 0.92, were found for the vegetable (A, B & D) and animal fat (C, E & F) containing groups were reported, respectively.

Table 2: : R_f values for cholesterol and sistosterol in samples

Samples	R _f Values
A	0.95
B	0.95
C	0.92
D	0.95
E	0.92
F	0.92

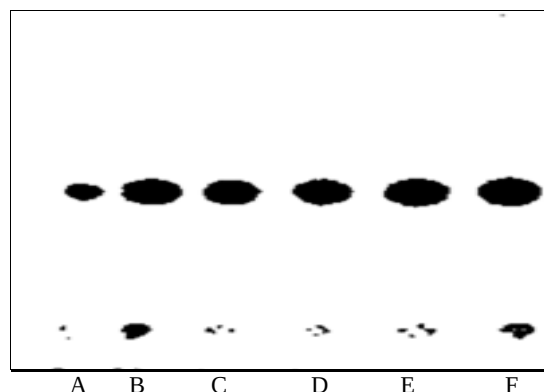


Figure 4: RP-TLC of samples (A) = Blue Band; (B) = Golden Penny; (C) = Kell Salad Cream; (D) = La Prairie Classic; (E) = Crosse and Blackwell; (F) = Nola)

The R_f = 0.03 difference between the two groups indicated the presence of sistosterol (plant sterol) and cholesterol (animal sterol) as shown in Figure 4. It show six different spots corresponding to different samples of A (Blue band), B (Golden Penny), C (Kell Sald Cream), D(La Prairie Classic), E(Crosse and Blackwell) and F(Nola). The three samples of A, B and D were plant fats containing sistosterol while samples C, E and F belong to animal fat from cholesterol. The observation from the chromatogram clearly indicated different appearances at R_f 0.92 and 0.95, furthering our argument that RP TLC as a preliminary method can be effective in determining adulteration in vegetable and animal fats. The distinct bluish band position (R_f values) were compared with reference cholesterol and sistosterol standards (Jarusewez *et al.* 2005; Anupama, *et al.*, 2015]. Figure 5 have shown the chromatogram of standard samples A (cholesterol) and B (sistosterol) with their corresponding positions of prominent bands at the R_f 0.92 and 0.95 respectively. The difference in their R_f values was an indication that the two compounds can be detected due to separation affinity in the solvent.

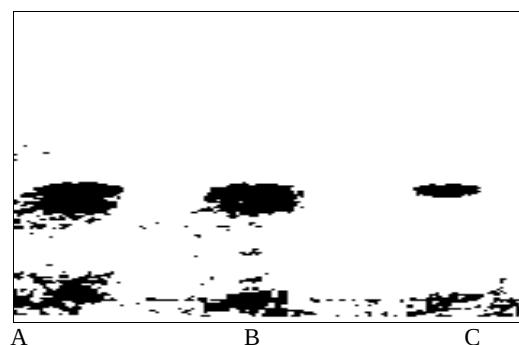


Figure 5: RP-TLC of mixed samples [A = A (Blue Band) + C (Kell Salad Cream)]; [B = B (Golden Penny) + E (Crosse and Blackwell)]; and [C= D (La Prairie Classic) + F (Nola)]

Figure 5, three spot of A, B and C were mixture of six samples. The spot A contains the mixture of A (Blue band) and C (Kell salad cream); the spot B contains the mixture of B (golden penny) and E (Crosse and Blackwell) and spot C contains the mixture of D (La Prairie Classic) and F (Nola). The R_f value of the samples were very close because of the closeness in the R_f values of cholesterol and sistosterol, which was of 0.03 difference.

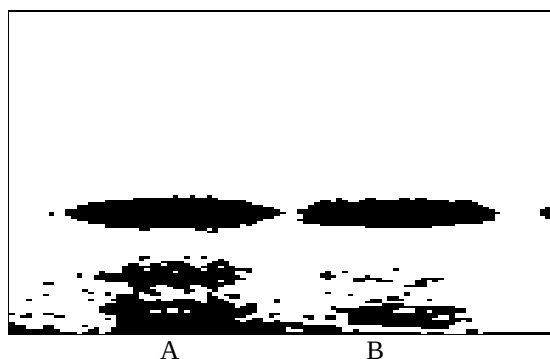


Figure 6: RP-TLC of mixtures of samples [A= A (Blue Band) + B (Golden Penny) + C (Kell Salad Cream)]; [B= D (La Prairie Classic) + E (Crosse and Blackwell) + F (Nola)].

In Figure 6, three different samples were mixed in TLC spots A and B, again to test the efficacy of separation on RP-TLC and similar results were obtained. The RP-TLC method earlier reported in the literatures (Ramamurthy, *et al.* 1967; Kamath and Mathew 1978; Msalya 2017) where 25% of adulteration was found in the coconut oil has been standardized and made simpler, reliable and more rapid in the present investigation.

Conclusions

This work was about the adulteration in edible fats of animals and plants origin using reversed phase thin layer chromatographic standardized protocol. The observation showed the same pattern of separation and it confirmed that if the RP-TLC are properly harnessed and developed,

it will serve as a better preliminary method in detection of adulteration of oils and fats from both plants and animals sources.

The protocol developed in the study is rapid, cheap and easy to perform in any laboratory assessing the level of adulteration in edible oil; this test does not require sophisticated equipment, so it can be recommended to the testing laboratories of law enforcing agencies as well as food testing laboratories.

Further chemical and instrumental methods should be developed to compliment the TLC method reported in this work.

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