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An Innovative Approach for the Detection of High Boiler Adulterants in Sandalwood and Cedarwood Essential Oils

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Owing to the important uses of essential oils, its adulteration is a serious issue of concern. Among the adulterants, the high volatiles can be detected through GC and GC/MS. However, the detection of subtle high boiler adulterants is extremely difficult, and requires development of novel techniques to overcome the challenges faced by the essential oil industry. In current study, the thermogravimetric analysis (TGA) was validated as an innovative approach for quantitative estimation of adulteration in essential oils taking sandalwood and cedarwood oils as case study. The low−cost vegetable oils like castor oil, coconut oil, and synthetic polymer like polyethylene glycol-400 (PEG-400) were used as high boiler adulterants. The physical parameters like specific gravity and refractive index of pure and adulterated oil samples were analyzed followed by their TGA analysis. The physical parameters of adulterated samples did not show significant variation from that of pure essential oils, thus need alternate analytical techniques to overcome this issue. The TGA of pure essential oil was volatized in single−stage around 200–260°C, whereas the high boiler adulterants such as vegetable oils and synthetic PEG-400 majorly volatized in the range 300–500°C and 260–400°C, respectively. The adulterated samples exhibited mostly two-stage weight loss pattern, which was quantitatively estimated with high accuracy by this technique. Therefore, the TGA analysis can be used as a novel technique for rapid and precise detection of high boiler adulterants in essential oils like sandalwood and cedarwood due to difference in their volatile behaviour.

Keywords: Adulteration, Castor oil, Coconut oil, Polyethylene glycol 400, Thermogravimetric analysis

Introduction

The heartwood of *Santalum album* and *Cedrus deodara* trees impart high value essential oil commonly called sandalwood and cedarwood essential oil, respectively. India is major country in regards to the trading of these woods. Sandalwood is listed amongst most expensive essential oil. The trading of heartwoods of these high value trees are protected by various acts and orders from the government agencies. These essential oils are well−known for their pleasant aroma and bioactivities rendering them highly useful for perfumery, pharmaceutical, aromatherapy, flavour and fragrance industries. The sandalwood essential oil has also been approved by various agencies including the US-FDA for use in food and beverages.[1,2] The increased consumer inclination towards natural products has further led to substantial surge in the trading of essential oils. The major constituents in cedarwood essential oil obtained from *Cedrus deodara* are α-himachalene, β-himachalene, γ-himachalene, (*E*)-α-atlantone, (*Z*)-γ-atlantone, (*E*)-γ-atlantone, and (*Z*)-α-atlantone. The perfumery grade cedarwood oil is reported to be enriched in (*E*)-α-atlantone content, which displayed significant anti-microbial activities.[3] The sandalwood essential oil contained (*Z*)-α-santalol and (*Z*)-β-santalol as major bioactive molecules (>75%) responsible for the pleasant aroma.[4] These essential oils are isolated from woody biomass in prolong hydro/steam distillation process, and solely comprised of sesquiterpenoids.[5]

The decline of forest covering area and over−exploration of woody biomass create gap of demand-supply issue. These high demand sandalwood and cedarwood are slow growing plants, thus create acute shortage issues, which leads to adulteration by illegal traders. Till date, GC-FID and GC/MS is the authentic analytical tool available for quality

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evaluation of essential oils including the sandalwood and cedarwood. Sometimes physico−chemical properties complimented the GC-FID and GC/MS analyses. The International standard organization recommends the use of GC-FID and GC/MS techniques for the evaluation of authenticity of essential oils.[5,6] The adulterant being used if volatile, can be directly identified through these techniques, or the reduced amount of major constituents can indicate the presence of adulteration. However, non-volatile or high boiler constituents such as polyethylene glycol (PEG), castor oil or coconut oil when used for the adulteration of sandalwood or cedarwood oil, cannot be detected by GC-FID or GC/MS. Vegetable oils being readily available at reasonable prices are frequently used as adulterants in essential oils. Recently, some techniques such as near-infrared spectroscopy, high-performance thin layer chromatography, and FT-IR have been reported for the detection of adulteration in sandalwood oil.[2,4,7,8] However, these techniques can still not be applied for rapid and precise detection of all types of adulterants in essential oils, and thus require alternate analytical approaches to deal with this issue. The vegetable oils or PEG are recommended in nutraceutical and pharmaceutical applications. These essential oils and vegetable oils are of comparable densities and can also not be detected through gas−liquid chromatographic (GLC) techniques. It is known that essential oils are composed of terpenoids with volatile nature. In contrary, the vegetable oils are triacylglycerides of non-volatile properties. But, the essential oils and vegetable oils are non−polar to semi−polar in nature, thus compatible in mixing with a fix ratio.

Due to proper miscibility and non-detection through GLC technique, nowadays the illegal traders are using easily available vegetable oils or low cost synthetic polymers (PEG-400) as adulterants in cedarwood and sandalwood essential oils. Essential oils are widely used in different industries including aromatherapy, pharmaceutical, fragrance and flavor. The adulteration of essential oil falls among the acute issues being faced by these industries and therefore calls off for immediate attention. Therefore, our group used thermogravimetric analytical (TGA) technique for repurposing to detect the above high boiling adulterants in sandalwood and cedarwood essential oils. TGA can turn out to be a rapid and precise technique to be used at industrial scale. The most common easily available and low cost vegetable oils such as castor, coconut, and soybean are noticed to be used for adulteration purpose. As a case study, the varying proportions of coconut oil, castor oil and PEG-400 have been used as adulterants. The specific gravity and refractive index (RI) of adulterated samples were also measured for comparative analysis. To the best of our knowledge, this is the first time that TGA has been applied for the detection of adulteration in pure sandalwood and cedarwood essential oils.

Materials and Methods

Materials

The woodchips of *Cedrus deodara* used for the extraction of cedarwood essential oil were obtained from Aum Aromatic Private Limited, Mandi, Himachal Pradesh. The woodchips of *Santalum album* were obtained from Mysore, India. The coconut oil, castor oil and PEG-400 used for the experiments were procured commercially.

Methods

Extraction of Cedarwood and Sandalwood Oils

The finely powdered woodchips of *Cedrus deodara* and *Santalum album* were subjected to hydro-distillation in a Clevenger-type apparatus for the isolation of essential oils. The collected essential oils were dehydrated over anhydrous Na_2SO_4, and the moisture-free cedarwood and sandalwood oil thus obtained were stored in refrigerator prior to analysis. The chemical compositions of the extracted cedarwood and sandalwood oil were determined by GC-FID and GC/MS techniques. Clarus 680 gas chromatography system coupled with Perkin Elmer SQ8C (Waltham, USA) was used for GC-FID analysis. The column used was: Elite-5 (30 m, 0.25 mm, 0.25 µm). The temperature of oven was increased from 60° to 240°C at 3°C/min, hold time of 6 min at 240°C. The injector and detector temperatures were maintained at 250°C and 260°C, respectively. GC/MS analyses were carried out under similar conditions. Helium carrier flow: 1 mL/min, split ratio: 1:100, mass range (m/z): 50 to 500 amu, EI: 70 eV, inter-scan delay: 0.01 sec, scan time: 0.8 sec, ion source temperature: 280°C and inlet line temperature: 280°C were other parameters followed through the analysis. Compounds were identified by using their mass spectra, and compared with software based Wiley and NIST libraries. Further, correct isomers were confirmed by matching the calculated relative retention indices with literature report.[9,10]

Preparation of Adulterated Samples

The preparation of adulterated samples for analysis was performed by mixing coconut oil, castor oil and PEG-400 in cedarwood and sandalwood essential oil in varying proportions. To ensure thorough mixing, the mixture was vortexed for five to ten min. The pure coconut oil, sandalwood oil, cedarwood oil, castor oil, and PEG-400 were used as control.

Physico–chemical Properties

The RI of essential oil determines its impact on light passing through it. It is defined as the ratio of sine of angle of incidence to the sine of angle of refraction of a luminous ray passing through the essential oil. RI of pure and adulterated essential oil samples were measured using ATAGO (Rx-7000α)-Refractometer. The specific gravity was recorded using Density/Specific Gravity Meter DA-500. Flash point was measured by using the flash point tester miniflash FPH vision (Grabner instruments) using D6450 test method.

TGA Evaluation

The thermogravimetric analyses of adulterated samples were performed with Mettler-Toledo TGA/DSC 1 Star system. To carry out the analysis, about 5 mg of pure and adulterated samples were taken in the silica crucible and scanned from 50 to 600°C at 20°C/min, under 40 mL/min flow rate of nitrogen as purge gas.

Results and Discussion

Physico-chemical Properties

The vegetable oils (castor and coconut) and PEG-400 are colorless, slightly viscous liquids with densities close to the cedarwood and sandalwood essential oils. Therefore, these adulterants are properly mixed with the cedarwood and sandalwood essential oils upto 50% without any physical distinction. The specific gravity of essential oil can be defined as the ratio of density of oil to the density of reference, and is usually considered as an important parameter to evaluate the authenticity of essential oils.[11] The specific gravity of pure and adulterated oils were determined and are presented in Fig. 1A. The specific gravity of pure sandalwood and cedarwood essential oil determined at 20°C was 0.975 and 0.934 g/cm^3, respectively. These values fall within the range prescribed for high quality oil.[12–14] The specific gravity of adulterated cedarwood samples came out to be in the range 0.934 to 0.93 g/cm^3. Similarly, the specific gravity of adulterated sandalwood samples was approximately 0.975 to 0.971 g/cm^3. The measurement of specific gravity, in this case, did not indicate the presence of adulterants in pure essential oils. Therefore, it is imperative to develop alternate techniques for the detection of adulteration in these essential oils.

The measurement of RI is frequently used for the indication of adulteration in essential oils. For

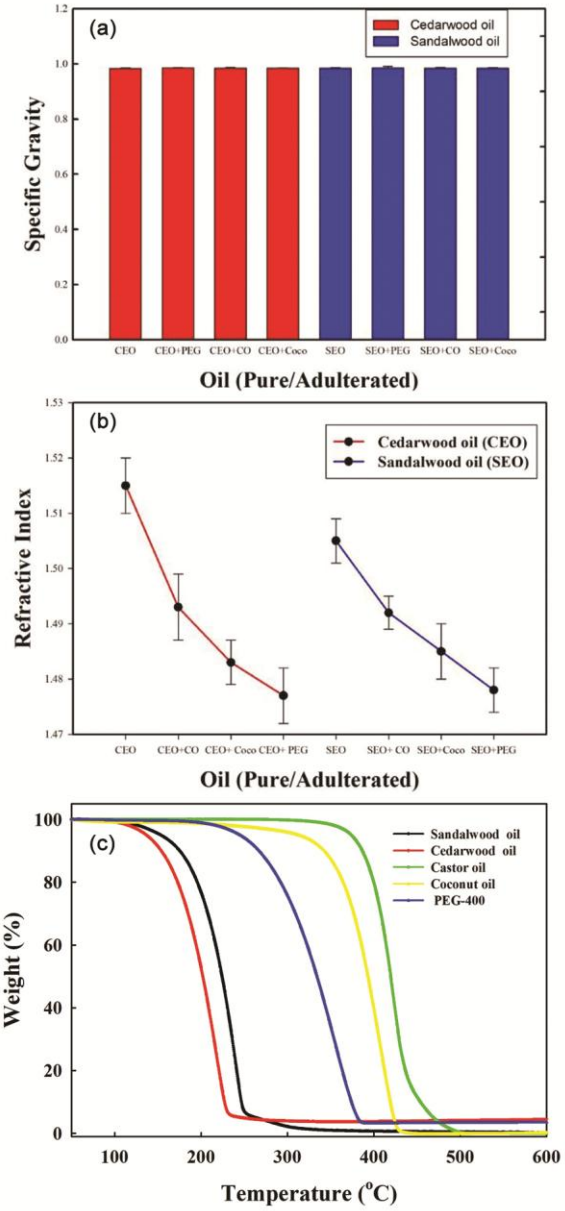

Fig. 1 — Pure and adulterated sandalwood and cedarwood essential oil: (A) specific gravity, (B) Refractive index, (C) TGA of essential oil, vegetable oil, and PEG-400 (CO = Castor Oil, Coco = Coconut Oil, PEG-400 = Polyethylene glycol-400)

example, the presence of ethanol, paraffin oil and cottonseed oil as adulterants in the essential oils of different spices including fennel oil can be indicated by measurement of RI values. However, the detection limit is quite high, for example, for ethanol critical value of detection lies in the range 10–20%.[15] It is also reported that RI measurement is not advanced or suffice for the detection of all types of adulterants.[16] In present study, the RI of pure and adulterated samples were measured and are presented in Fig. 1B. The RI of pure sandalwood and cedarwood oil was 1.505 ± 0.004, and 1.511 ± 0.005, respectively. These values are similar to those reported in literature, indicating the purity of the extracted essential oils.[13,14] The adulterated samples used for the measurement of RI were prepared by mixing 50% of adulterants (castor oil, coconut oil, and PEG 400). The RI of adulterated sandalwood oil was in the range of 1.478–1.492, and that of cedarwood oil was in between 1.477–1.493. It was observed that the RI was decreased on adulteration of pure oils, although the variation was not very high even for higher level of adulteration analyzed. The reduced RI values of sandalwood and cedarwood essential oil can therefore be taken as a preliminary indication of adulteration to be verified by more advanced detection techniques.

Chemical Composition of Cedarwood and Sandalwood Oil

The major compounds present in sandalwood and cedarwood oil were detected by GC-FID and GC/MS analysis. For sandalwood oil, the santalol isomers alone accounted for 82% of the composition with highest proportion of (*Z*)-α-santalol (50.3%), followed by (*Z*)-β-santalol (25.6%), and (*E*)-β-santalol (5.9%). Some other compounds identified were α-santalene (1.1%), β-santalene (1.6%) and (*E*)-α-bergamotol (3.9%). The santalol content of the extracted sandalwood essential oil was in good agreement with the specifications provided by International standard Organization (ISO) for sandalwood oil, which recommends the range of 41–55% and 16–27% for α-santalol and β-santalol, respectively.[12] The Indian specification (IS) for authentic sandalwood oil is similar to the international values.[17] However, the Australian standard specifies much lower content of α-santalol (25%) and β-santalol (20%).[18] The Australian sandalwood contains some amount of farnesol which is usually not found in Indian sandalwood oil. Farnesol has been mentioned as an irritant by EU Cosmetic regulation.[19,20] Therefore, the superiority of Indian sandalwood oil over Australian variety can be established which frequently leads to the adulteration for fetching higher profit.

The cedarwood oil was comprised of sesquiterpene hydrocarbons (20.6%) and oxygenated sesquiterpenoids (73.5%). More than thirty compounds accounting for approximately 94% of the composition of the essential oil were identified. The major constituents identified were atlantones (29.3%), himachalene epoxides (15.7%) and himachalene (6.1%). Some other constituents present in notable concentrations were α-dehydro-ar-himachalene (3.9%), (*E*)-α-bisabolene (3.8%), longiborneol (2.2%), himachalol (3.2%), 11-α-H-himachal-4-en-1-β-ol (6.5%), (*E,E)*-farnesol (3.7%) and dihydroxycyclo himachalene (2.4%). Chowdhary and co-workers have also reported himachalenes and atlantones as the major constituents of essential oil from woodchips of *Cedrus deodara*. However, in their findings high amounts of himachalenes in compared to atlantones were reported,[21] whereas in current study atlantones comprised the major percentages (29.3%). Atlantones are reported to enhance the perfumery potential and bioactivities of cedarwood oil.[3]

TGA as New Tool for Detection of Adulterants

The adulterated samples were prepared by mixing 5%, 10%, 30% and 50% of respective adulterants (castor oil, coconut oil, and PEG-400) in sandalwood and cedarwood essential oils. The pure essential oils and adulterants were used as control. The quality control analysis usually recommended for essential oils involve determination of their physical parameters and chemical composition by GC-FID and GC/MS.[5,6] However, for precise quantitative estimation of high-boiler adulterants, the TGA technique was validated in current study. In TGA, the exact loss in weight was measured with respect to the raising of temperature. The TGA patterns for all the controls used in current study are presented in Fig. 1C. The TGA of controls (pure essential oils and adulterants) reveal a single stage volatization pattern. The observed volatization ranges for sandalwood and cedarwood oils are below 300°C. The volatization is initiated around 115°C and it was completed around 255°C. The cedarwood essential oil exhibits early trend of volatization within the range of 115–250°C. On the other hand, for sandalwood the volatization process is completed around 260°C. The TGA patterns of vegetable oils have shown major volatization after 300°C. The castor oil is volatized in between 340–500°C, whereas the coconut oil is

volatized at 260–450℃ (Fig. 1C). For pure PEG-400, the volatization is started from 260℃ and the complete weight loss is observed at 390℃.

Detection of Adulteration in Sandalwood Oil

The pure sandalwood oil was adulterated with 5%, 10%, 30% and 50% of vegetable oil adulterants (castor oil and coconut oil). The TGA of the resulting adulterated samples are presented in Fig. 2. The results reveal a two−stage volatization pattern of weight loss for all the adulterated samples in comparison to the single stage volatization observed for pure essential oil. When castor oil is used as adulterant in sandalwood oil, the entire volatization range can be divided into three stages for clear interpretation of the data (Fig. 2A). First stage, (60–260℃), represents the volatization of sandalwood oil as established from the TGA pattern for pure sandalwood oil. Stage 2 is the region between 260–340℃ where no significant volatization is observed. Stage 3 is the region between 340–500℃, where the castor oil is completely volatized. After that, no further weight loss was observed and base line almost comes to zero level. For quantitative estimation, the first stage represents loss of weight of 95%, 90%, 70%, and 50%, which corresponds to 5%, 10%, 30% and 50% adulterated samples, respectively (Fig. 2A). It can be clearly predicted that the loss of sandalwood essential oil occurs in the first stage. In quantitative figures, the percentage of volatization at third stage is accounted to 5%, 10%, 30%, and 50% (approx.) of the adulterated sample. This loss of weight represents the volatization of castor oil. The representative TGA validation of sample composed of 70% of sandalwood essential oil and 30% castor oil is given in Fig. 3A. The adulteration of sandalwood oil using coconut oil is analyzed considering two-stage volatization pattern. First stage is the region between 60 to 260℃, where sandalwood essential oil is volatized, followed by coconut oil volatization from 260–445℃. After that, there is no more weight loss and base line came to almost zero level (Fig. 2B). As discussed above, using this technique the exact amount of coconut oil present as adulterant can be measured. The representative TGA validation of sample composed of 70% of sandalwood essential oil and 30% coconut oil is given in Fig. 3B

The TGA pattern of sandalwood oil adulterated (5%, 10%, 30% and 50%) in PEG-400 exhibited two-stage volatization pattern (Fig. 2C). The first stage represents the sandalwood essential oil volatization (60–260℃). The second stage (260–390℃) represents the volatization of PEG-400. Thereafter, none of the sample was left in the crucible, hence base line comes to zero level. Similar to the case of vegetable oils, the quantity of PEG-400 is measured and a representative curve of sample comprised of 70% of sandalwood essential oil and 30% PEG-400 given in Fig. 3C.

Detection of Adulteration in Cedarwood Oil

The pure cedarwood oil was adulterated using 5%, 10%, 30% and 50% of castor oil. The TGA of the resulting adulterated samples was performed and the graphical representation of results obtained is depicted in Fig. 4A. The TGA pattern of cedarwood essential oil adulterated with castor oil was similar to that of sandalwood. The TGA technique can be successfully implied for precise qualitative and quantitative detection of castor oil as adulterants in cedarwood essential oil samples. The TGA pattern obtained can be divided into 3 stages. The weight loss observed during stage-1 (115–250℃) can be attributed to the amount of cedarwood essential oil present in the sample analyzed on the basis of TGA pattern observed for pure cedarwood essential oil. Stage 2 represents region of constant weight where none of the component of adulterated sample showed volatization. The temperature zone representing stage 2 falls within range 250–340℃. Finally, stage 3

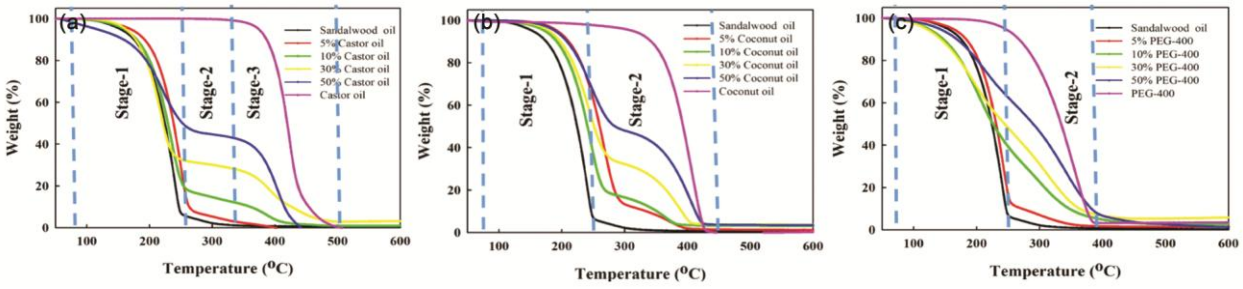

Fig. 2 — Comparison of TGA patterns of sandalwood oil: pure, and adulterated using 5%, 10%, 30% and 50% of (A) Castor Oil, (B) Coconut Oil, and (C) PEG-400

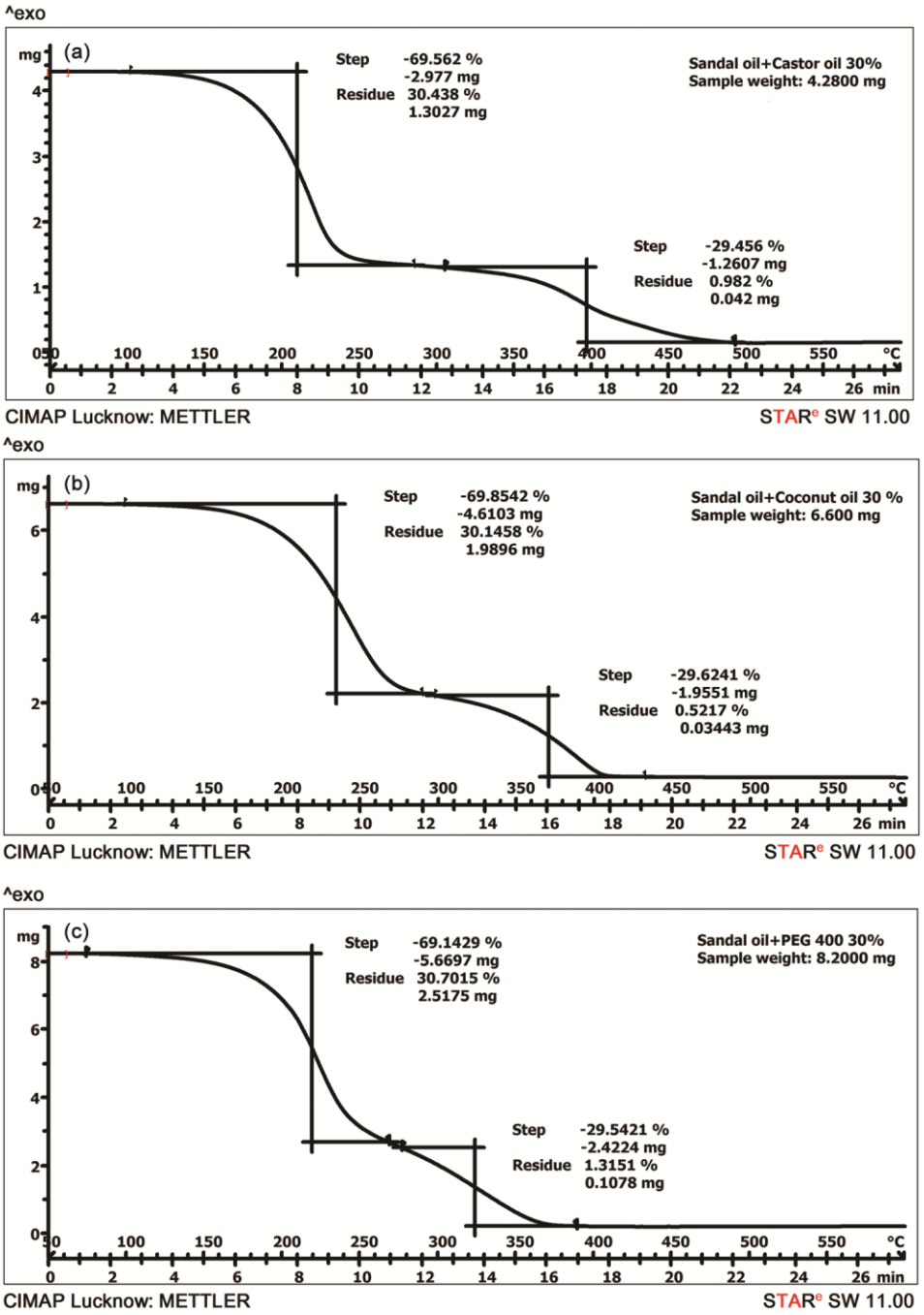

Fig. 3 — The TGA pattern for adulterated sample comprising of 70% sandalwood oil and 30% of (A) Castor Oil, (B) Coconut Oil, and (C) PEG-400

predicts the amount of adulterant present in the sample. The weight loss in the range 340–500°C represents the amount of castor oil which on precise measurement came out to be 5%, 10%, 30% and 50% respectively. Beyond stage-3 no further volatization occurred giving base line equivalent to zero level. A representative TGA pattern obtained for adulterated sample containing 30% of castor oil in 70% cedarwood oil is presented in Fig. 5A. The interpretation of coconut oil as adulterant can also be done in similar way with TGA pattern being divided into 3 stages corresponding to volatization of cedarwood oil, followed by constant weight zone and subsequent coconut oil volatization (Fig. 4B). These

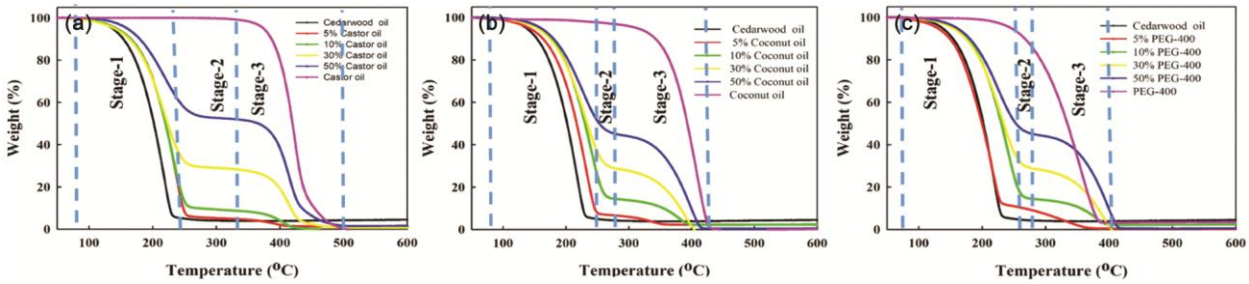

Fig. 4 — Comparison of TGA patterns of cedarwood oil: pure, and adulterated using 5%, 10%, 30% and 50% of (A) Castor Oil, (B) Coconut Oil, and (C) PEG-400

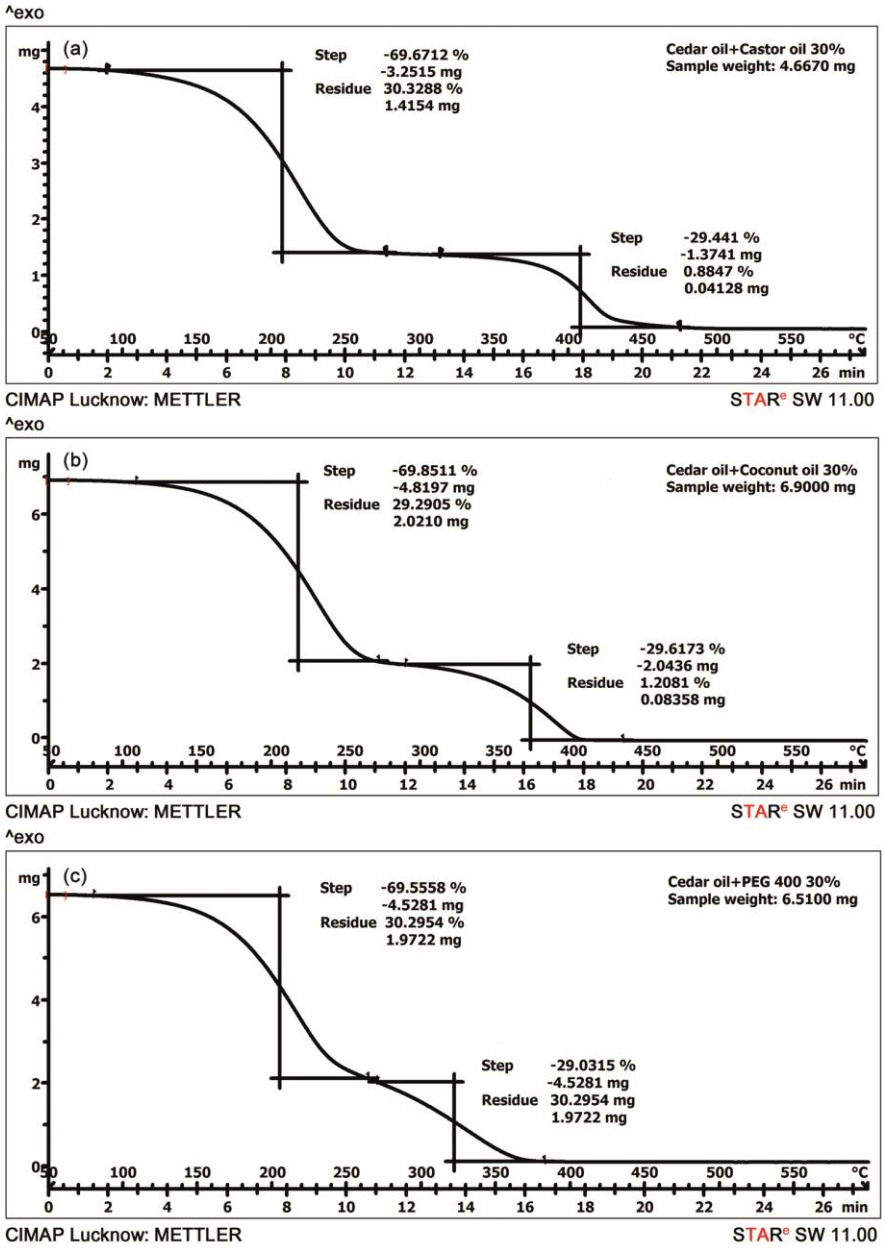

Fig. 5 — The representative TGA pattern for adulterated sample comprising of 70% cedarwood oil and 30% of (A) Castor Oil, (B) Coconut Oil, and (C) PEG−400

stages correspond to temperature zone of 115–250°C, 250–260°C and 260–445°C respectively. The weight loss occurring in stage-3 represents the amount of adulterant present in the sample. A representative pattern obtained on TGA analysis of 70% cedarwood oil adulterated using 30% coconut oil is provided in Fig. 5B.

The adulteration of cedarwood oil using PEG-400 can also be detected by observance of multi-stage dissociation pattern during TGA analysis. The entire pattern can again be divided into 3 stages (Fig. 4C) corresponding to volatization due to cedarwood oil (115–250°C),no weight loss zone (250–260°C), volatization of PEG−400 during stage−3 (260–390°C) followed by no further weight loss due to exhaustion of entire sample. The TGA pattern of cedarwood oil adulterated using 30% PEG−400 is shown in Fig. 5C.

Interpretation of Adulterants as per Physico-chemical and TGA Data

Coconut oil is mainly composed of lauric acid (C12:0), which is more than 50% of the fatty acids composition. The average molecular weight is about 740 with density (0.91 g/cm^3), refractive index (1.45 nD) and flash point (213°C). Hence, it is well mixed in these essential oils, and the physical properties of adulterated oil are very close to the pure essential oil values. Similarly, castor oil is mainly composed of ricinoleic acid (C18:1, OH), which is more than 85% of the fatty acids composition. The average molecular weight is about 950 with density (0.96 g/cm^3) and refractive index (1.47 nD) and flash point (258°C). Therefore it is also well mixed in these essential oils, and the physical properties of adulterated oil are indistinguishable as compared to the pure essential oil. The chemical composition and flash point were in complete agreement with the volatilization behavior of the coconut adulterated sample, which showed early degradation pattern as compared to the castor oil. This TGA technique is qualitatively detecting the adulterant, and same is quantified through the software (Figs 3B, 5B). These woody essential oils mostly contain sesquiterpenoids with average mass <230, so the volatization temperature is found to be around 230°C. On the other hand, that of the vegetable oils was detected at 350°C.

The synthetic polymer PEG-400 is derived from ethylene glycol. The average molecular weight is about 400 with density 1.3 g/cm^3, refractive index (1.46 nD), and flash point (212°C). Due to glycolic linkage, it is easily mixed in these essential oils, and the physical properties of adulterated oil are very close to the pure essential oil. Hence, there was two clear-cut volatization pattern obtained, which was qualitatively and quantitatively estimated as discussed above.

Conclusions

The present study proposes the use of TGA as a novel process for the detection of adulteration in essential oils taking sandalwood and cedarwood oil as case study. The adulterated oil sample can be differentiated qualitatively from the pure one by observance of multi−stage volatization pattern in the TGA. This TGA technique for the detection of high boiler adulterants in essential oil is also suitable for quantitative estimation of any vegetable oil. The respective volatization range for the pure oil and adulterant can be used for precise quantitative estimation of adulterant present in the sample. Sandalwood oil and cedarwood oil both dissociate in the range 200–260°C, castor and coconut oil mainly degrade after 300°C, whereas PEG−400 after 260°C, allowing precise qualitative and quantitative detection of these vegetable oils when present as adulterant through TGA. Therefore, the appearance of multi−stage volatization pattern in the TGA of any oil sample can indicate presence of adulterants and for most of the high−boiler adulterants both qualitative and quantitative detection is possible through this technique.

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