

## Comparison of *trans* fatty acid content in commonly consumed seed oils and commercially refined counterparts

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### Abstract

Conventional seed oils and their commercially refined counterparts may contain different levels of *trans* fatty acids (TFA) due to refining and high heat treatment during deodorization. To identify and quantify TFA content in five commonly consumed marketed refined vegetable oils namely refined soybean oil (RSO), refined palm oil (RPO), mustard oil (MO), refined rice bran oil (RRBO), and refined sunflower oil (RSuO) were analyzed. To identify the potential source of TFA, seed extract and crude extract of the studied oil were also analyzed. Among the 57 analyzed samples, the highest TFA was found in RRBO 4.35±3.88% followed by RSO 3.50±1.54%. RSuO and RPO contained TFA within the recommended level of the World Health Organization whereas TFA was below the detection limit in MO. Very little TFA was observed in crude palm oil, rice bran, and soybean seed extracted oil, while no TFA was found in mustard and sunflower seed extracted oil.

**Keywords:** *Trans* fatty acids; Seed oil; Refined oil; Heat treatment; Gas Chromatography

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### Introduction

*Trans* fats also referred to as *trans* fatty acids (TFAs), can be defined as the geometric isomers of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) having at least one carbon-carbon double bond at opposite positioned hydrogens (Iqbal, 2014). MUFAs and PUFAs are suggested to be consumed in replacement of saturated fatty acids (SFA) due to their positive effects in lowering Low-density lipoprotein cholesterol (LDL) from plasma (Lada and Rudel, 2003). However, edible oils that abound with PUFA, are more susceptible to producing TFA during industrial processing like partial hydrogenation. Although some TFAs come from the bacterial *trans*-formation of unsaturated fatty acids in the rumen of ruminants (Dhaka *et al.* 2011), most of the TFAs are produced industrially through hydrogenation or refining process

(Destailats and Angers, 2005; Dhaka *et al.* 2011; Puprasit *et al.* 2020; Sarwar *et al.* 2024). The heat treatment during the cooking procedure or deep frying has also caused the translocation of hydrogen atoms attached to double bond leads the conversion of *cis* to *trans* fatty acids in oil (Bhardwaj *et al.* 2016; Tsuzuki *et al.* 2010).

TFAs engender deleterious effects on the human body by increasing insulin levels, triglycerides, and LDL and reducing high-density lipoproteins (HDL)(Katan *et al.* 1995). The structural transformation from *cis* to *trans* fatty acids influences physicochemical properties like the increment of melting point, which leads to detrimental effects on physiological properties in the human body (Oteng and Kersten, 2020). The melting points of elaidic acid (C18:1n9t),

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oleic acid (C18:1n9c), and stearic acid (C18:0) are, respectively, 42, -5, and 70 °C which suggests that just like saturated fat *trans*-fat remains solid at room temperature as well as human body temperature (Song *et al.* 2015). Therefore, TFAs have a great influence in elevating the risk of coronary heart disease as well as endangering the brain cell membrane and neurons, which ultimately undermine mental performance (Ginter and Simko, 2016). A high-TFA diet has been linked to negative alterations in the plasma lipoprotein profile, including a drop in high-density lipoproteins (HDL) and an increase in low-density lipoproteins (LDL), according to many clinical investigations (Brouwer *et al.* 2010; de Roos *et al.* 2001; Garshick *et al.* 2014). Research has indicated that TFAs may deleteriously affect atherosclerosis's inflammatory process by elevating inflammatory cytokines. Considering the noxious effect of TFA on human health, the World Health Organization (WHO) recommended limiting 2 g of industrially produced TFAs per 100 g of total fat in all foods (WHO, 2018). Edible vegetable oil, an important component of the human diet, provides the energy and fatty acids essential for the human body and increases digestion followed by absorption of fat-soluble vitamins (Zhao *et al.* 2021). The worldwide consumption of vegetable oil has increased to almost 222 million metric tons in the year 2023/24, indicating that people are more avid to consume vegetable oil as a healthier alternative than animal fat due to the abundance of unsaturated fatty acids (Shahbandeh, 2024). However, TFAs consumption is also increasing with the rise of vegetable oil consumption since a significant amount of TFA is formed during different refining stages, especially at the deodorization step (Tasan and Demirci, 2003). In a recent study in Bangladesh, Sarwar *et al.* (2024) found an elevated amount of TFAs in locally available refined branded and non-branded soybean oil. Although refining vegetable oil at high temperatures is a possible cause of producing TFA (Pipoyan *et al.* 2021; Xie *et al.* 2019), some previous studies showed the presence of TFA in non-refined seed oils (Brühl, 1996; Dixit and Das, 2012; Fritsche and Steinhart, 1998) arising the questions about the natural occurrence of TFA in oilseeds.

In Bangladesh, there are several packaged and labeled refined vegetable oils available under various brand names; of these, 39% are soybean oil, 20% are rice bran oil, 19% are sunflower oil, 18% are palm oil, and 79% of the oils that are available in the market are locally refined (GAIN and icddr, 2017). Although the scenario of TFA in soybean and palm oil of the Bangladeshi market is conspicuous in Sarwar *et al.*

(2024), the condition of other vegetable oils is prevaricating till now. Apart from that, the presence of the individual isomers of *trans* oleic, *trans* linoleic, and *trans* linolenic should be assessed in both the seed oils and their processed counterparts which was reported by multiple studies (Dixit and Das, 2012; Li *et al.* 2012; Tsuzuki *et al.* 2008). Considering the above-mentioned risk of *trans* fatty acid in human health and since it was not done before in the Bangladesh perspective to the author's best knowledge, the assessment of the TFAs content along with the individual isomers in seed has become imperative for the indigenous seed oils. The main objective of this study is to assess the presence of TFAs in solvent-extracted oils in the laboratory from locally available oilseed (soybean, sunflower, rice bran, mustard, and palm) that naturally occurred and give conclusive evidence of TFAs abundance in their refined counterparts.

## Materials and methods

### *Standard materials and reagents*

The mix standard used for fatty acid analysis was Supelco 37 component FAME mix (Supelco, Bellefonte, PA, USA) and for the *trans*-fatty acid analysis were elaidic acid (C18:1), Linoleic acid methyl ester mix (C18:2 isomers) and Linolenic acid methyl ester mix (C18:3 isomers) (Supelco, Bellefonte, PA, USA). All other used chemicals of high purity were obtained from Merck (Darmstadt, Germany).

### *Oilseed and refined oil sample collection*

The seed sample consists of sunflower seed (*Heliathus annuus*), Soybean seed (*Glycine max*), and mustard seed (*Brassica juncea*). All the seeds and rice bran were collected from the local market (New-market, Dhaka) of the capital city of the country where the seeds were sourced from various parts of Bangladesh. The crude Palm oil (CPO) samples were collected from the refinery before processing. The refined soybean oil (RSO), refined sunflower oil (RSuO), Refined rice bran oil (RRBO), refined palm oil (RPO), and mustard oil (MO) (branded and non-branded) were collected from the same market and a departmental store.

### *Oil extraction from seed*

Soybean seed extracted oil (SSEO), sunflower seed extracted oil (SuSEO), rice bran extracted oil (RBEO), and mustard seed extract oil (MSEO) were prepared by cold extraction procedure using petroleum ether. All the seeds were ground into powder and soaked in petroleum ether overnight. Oil was obtained from the dried filtration of the seed-petroleum

mixture. Temperature was strictly maintained below 80°C in every step to avoid TFA formation in the extracted oil.

#### *Effect of processing on TFAs in processed edible oils*

To understand the effect of processing on TFA in refined oil, all the extracted and crude oil samples (SSEO, SuSEO, RBEO, MSEO, and CPO) and refined samples (RSO, RSuO, RRBO, RPO, and MO) were subjected to an attenuated total reflectance Fourier transform infrared spectrophotometry (ATR-FTIR) to see the presence of *trans* fatty acid in seed and processed oil. Then the fatty acid content was analyzed by gas chromatography (GC) in processed oils and compared with their corresponding seed extract.

#### *ATR-FTIR conditions*

FTIR analyses were conducted using a Thermo Fisher Nicolet™ iS™ 5 FTIR Spectrometer from MA, USA, equipped with OMNIC software and operated in ATR mode. Spectra were obtained across the wavenumber range of 4000 to 500  $\text{cm}^{-1}$  at a 4  $\text{cm}^{-1}$  resolution, adhering to an established industrial standard. Air served as the reference background material during the measurements.

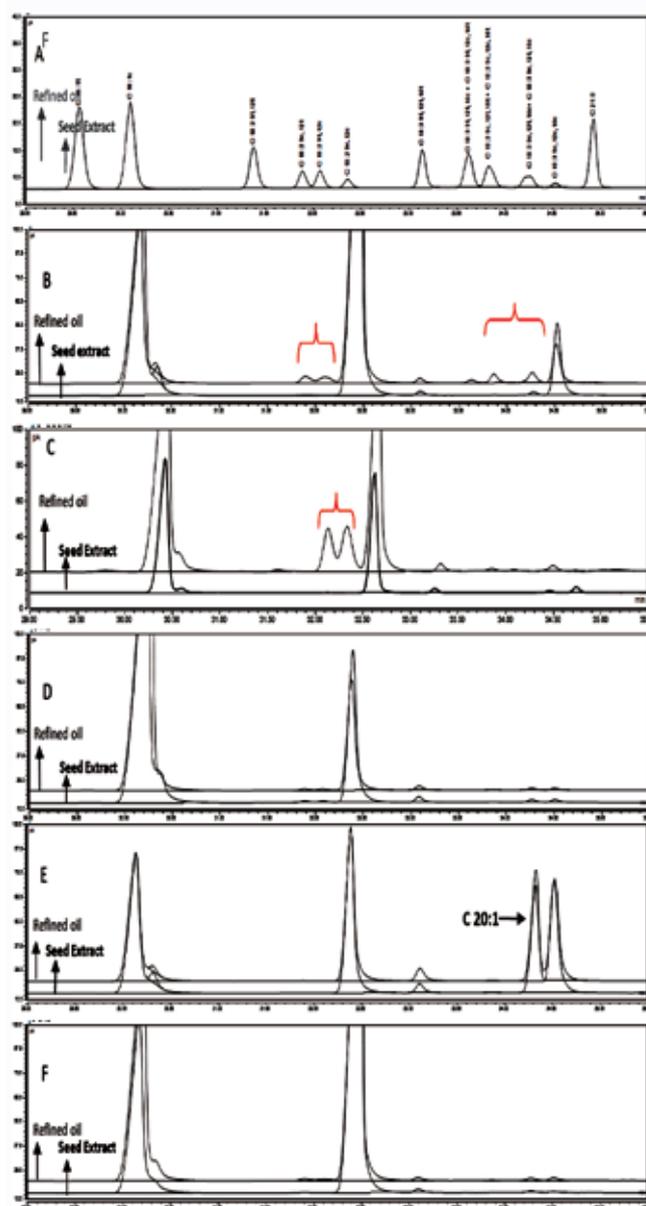
#### *FAME Preparation*

Fatty acids in oils were esterified to converted into FAMES by following ISO 12966-2:2017(E) with slight modification as described by Lisa *et al.* (2024) (Lisa *et al.* 2024). About 50 mg of the oil sample was transferred into a test tube containing 2 mL of isooctane and shaken vigorously. With the solution, 2 M methanolic KOH (0.1 mL) was added, and properly shaken for 1 minute, 2 mL saturated sodium chloride was added and kept for 2 minutes for phase separation. Then clear portion was passed through an anhydrous sodium sulfate column and collected into a 2 mL vial for GC analysis.

#### *Gas Chromatography Instrumentation*

Identification and quantification of FAMES of the oils were conducted using Thermo Fisher Scientific Gas Chromatography (Trace 1300, MA, USA) equipped with flame ionized detector (FID), autosampler and a highly polar fused silica capillary column (Rt-2560, Restek, Bellefonte, PA, USA) (100 m  $\times$  0.25 mm i.d  $\times$  0.20  $\mu\text{m}$  film thickness) was used for fatty acid methyl ester separation. The initial oven temperature was 150°C with 10 min holding time. The temperature was then increased to 200°C at a rate of 5°C/min and 10 minutes held at this temperature. After that, the temperature

increased at 10°C/min to 240°C and held for 20 minutes. Split injection mode was used for this analysis and split ratio was 20:1, injection temperature and detector temperature were 250°C. The identification and quantification of separated peaks were performed by using reference standards (Figure 1).



**Fig. 1. Standard chromatograms of 18 isomers (A) and the chromatograms of the seed extracted oil and their refined counterparts; (B) Soybean oil, (C) Rice bran oil, (D) Palm oil, (E) Mustard oil, (F) Sunflower oil**

### Statistical Analysis

All the analyses were conducted in triplicate ( $n=3$ ), and values were reported as mean  $\pm$  standard deviations (SD). Independent sample t-test was used to compare mean values and  $p < 0.05$  was considered as statistically significant. Statistical analysis was performed by using Statistical Package of Social Sciences (SPSS) version 25 (IBM, NY, USA).

### Results and discussion

#### *Analysis of TFAs in seed extract and processed oil using ATR-FTIR*

The infrared spectra of seed extract and refined oil are shown in Figure 2. The absorbance band was assigned in the mid-IR spectrum ( $500\text{-}4000\text{ cm}^{-1}$ ) as it is widely used for oil sample analysis (Jamwal *et al.* 2021). The spectrum is

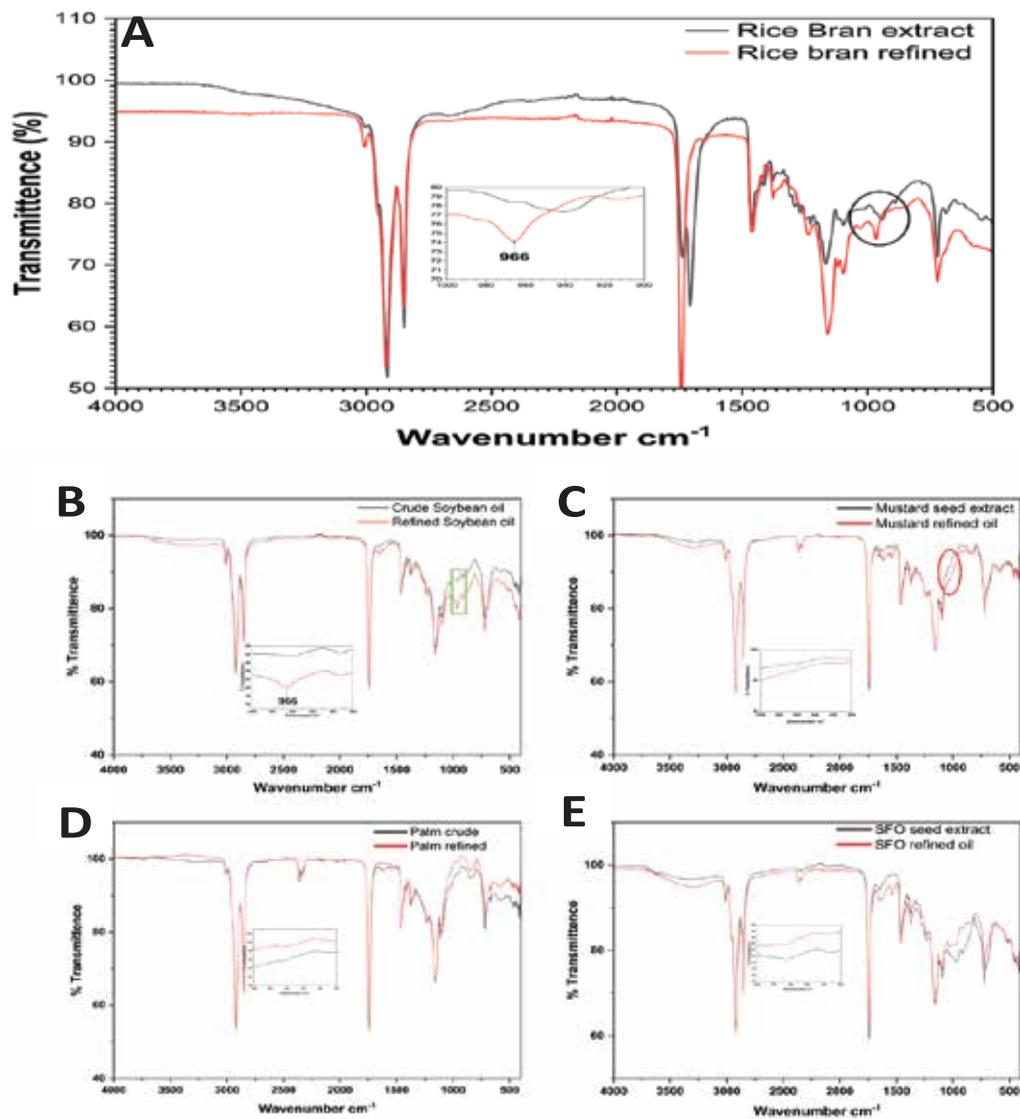
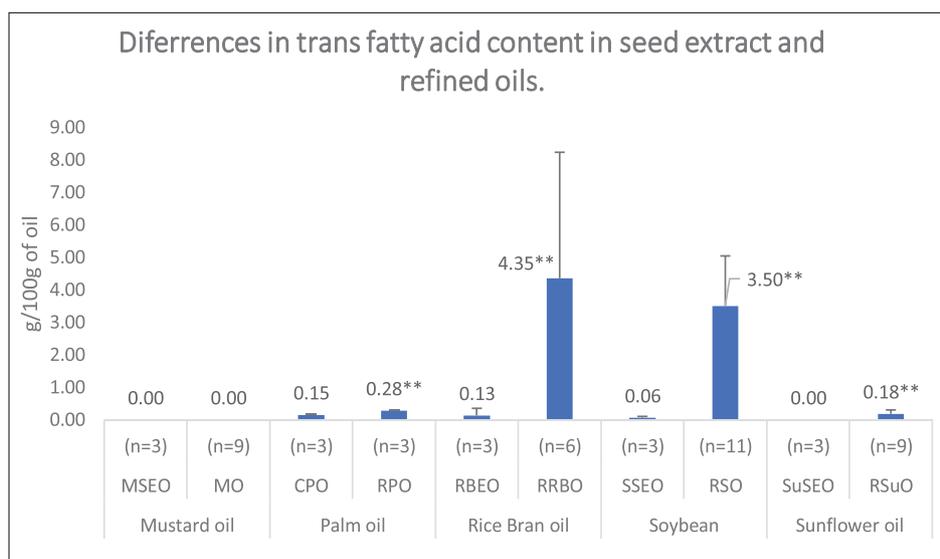


Fig. 2. Infrared spectra of seed extract and refined oil, A. Rice Bran, B. Soybean, C. Mustard oil D. Palm oil E. Sunflower oil; Prominent peak at  $966\text{ cm}^{-1}$  was observed Rice bran oil and soybean oil indicating the trans figuration of C-H group at C-C double bond



**Fig. 3. Differences in *trans* fatty acid content in seed extract and refined oils**

mainly divided into four regions and all the regions have identical peaks of corresponding seed extract and refined oil (Bunaciu *et al.* 2022; Guillén and Goicoechea, 2007; Li *et al.* 2012; Rohman and Che Man, 2012). The fingerprint region (600-1500  $\text{cm}^{-1}$ ) is unique and specific for each oil. In this region, the *trans* configuration of the unsaturated fatty acids' isomer is expected to absorb 960-970  $\text{cm}^{-1}$  (Coates, 2000). That means prominent peaks will be observed if there is any *trans* fatty acid present in the oil. The enlarged form of the spectra of this region of each refined and their corresponding seed extract was as shown in the inset of Figure 3 A. Rice Bran, B. Soybean, C. Mustard D. Palm E. Sunflower oil. A prominent peak was observed at 966  $\text{cm}^{-1}$  in RRBO (inset A) whereas no visible peak was observed in its corresponding seed extract by cold-pressed. Moreover, there were no peaks on both sides of the 966  $\text{cm}^{-1}$  peak which indicated the *trans* formed in the RRBO was non-conjugated *trans* fatty acids (Christy, 2009). In RSO (inset B) the prominent peak was at 963  $\text{cm}^{-1}$  and the seed extract had no such peaks in that band. Although Li *et al.* reported the *trans* band at 966  $\text{cm}^{-1}$  in processed soybean oil which was increased in size with the increasing heat (Li *et al.* 2012), analyzed peak of the current study is well within the range. In Sunflower oil (inset C) both processed and seed extract have no prominent peaks in the reference region which was alike to previous reported studies (Guillén and Goicoechea, 2007; Ozulku *et al.* 2017).

#### *Comparison of TFAs content in seed extract and refined oils*

All the seed samples were collected from three different sources and analyzed separately. All the seeds are fundamentally different from each other, so their fatty acid content will differ from each other. The source of oils or the technological procedure used in their refining determines the variation in their fatty acid profile (Orsavova *et al.* 2015). The fatty acid composition of SSEO, SuSEO, RBEO, MSEO, CPO, RSO, RSuO, RRBO, RPO, and MO are presented in Table 1. In the present experiment, very little amount of TFA was observed in RBEO ( $0.13 \pm 0.22$ ), SSEO ( $0.06 \pm 0.04$ ), and CPO ( $0.15 \pm 0.01$ ) while no TFA was found in MSEO and SuSEO. Among the refined oils, RRBO showed the highest TFA content having  $4.35 \pm 3.88\%$  followed by RSO with  $3.50 \pm 1.54\%$  TFAs. Refined SuO and PO contained very little amount of TFA, 0.23% and 0.28% respectively. TFA content in RRBO is about double that of the previous studies (Cui *et al.* 2017; Mishra and Sharma, 2014), but total TFA content in RSO is comparable with Hou *et al.* (2012) and Dixit and Das, (2012) who found 3.11% and 3.25% TFAs in RSO respectively. The most common TFA was non-conjugated *trans* linoleic acid (9c12t; 9t12c) found in all the refined oils except mustard oil. *Trans* linoleic acid was the main contributor to total TFAs content, and the content was  $3.63 \pm 1.81$  for RRBO,  $1.51 \pm 0.51$  for RSO, and  $0.15 \pm 0.1$  for RPO, this result is similar to the previous studies where *trans* linoleic acid was also found as

Table 1. Effect of processing techniques on TFAs in processed edible oils (g/100g of oil)<sup>a</sup>

| Fatty acids   | Mustard oil  |              |              | Palm oil      |              |                | Rice Bran oil |                 |              | Soybean        |  |  | Sunflower oil |  |
|---|--------------|--------------|--------------|---------------|--------------|----------------|---------------|-----------------|--------------|----------------|--|--|---------------|--|
|   | MSEO (n=3)   | MO (n=9)     | CPO (n=3)    | RPO (n=3)     | RBO (n=3)    | RRBO (n=6)     | SSEO (n=3)    | RSO (n=11)      | SuSEO (n=3)  | RSuO (n=9)     |  |  |               |  |
| C 16:0  | 3.29 ± 0.30  | 3.39 ± 1.86  | 0.42         | 37.96 ± 0.89  | 20.07 ± 0.86 | 19.30 ± 0.73   | 0.37          | 10.92 ± 0.30*   | 6.13 ± 0.03  | 6.15 ± 0.40    |  |  |               |  |
| C 16:1  | 0.18 ± 0.03  | 0.17 ± 0.03  | 0.14 ± 0.03  | 0.16 ± 0.01   | 0.13 ± 0.12  | 0.19 ± 0.01    | 0.12 ± 0.01   | 0.09 ± 0.02*    | nd           | 0.13 ± 0.04**  |  |  |               |  |
| C 18:0  | 1.30 ± 0.27  | 1.51 ± 0.37  | 4.42 ± 0.16  | 4.22 ± 0.17   | 2.34 ± 0.41  | 1.97 ± 0.20    | 3.48 ± 0.06   | 4.47 ± 0.19***  | 4.72 ± 0.02  | 3.41 ± 0.21*** |  |  |               |  |
| C 18:1 9c   | 22.44 ± 8.22 | 23.89 ± 8.51 | 0.58         | 43.28 ± 0.24  | 41.81 ± 1.58 | 39.93 ± 2.82   | 0.24          | 23.20 ± 0.83*** | 23.56 ± 0.06 | 35.00 ± 6.27*  |  |  |               |  |
| C 18:1 11c  | 1.00 ± 0.25  | 1.47 ± 0.44  | 1.53 ± 0.15  | 1.12 ± 0.01** | 1.13 ± 0.18  | 1.29 ± 0.10    | 1.79 ± 0.38   | 1.65 ± 0.09     | nd           | 1.03 ± 0.12*** |  |  |               |  |
| C 18:2 9t, 12t  | nd           | nd           | nd           | nd            | nd           | 0.04 ± 0.02    | nd            | nd              | nd           | nd             |  |  |               |  |
| C 18:2 9t, 12c  | nd           | nd           | 0.08 ± 0.01  | 0.01***       | 0.08 ± 0.14  | 1.79 ± 1.01    | 0.06 ± 0.04   | 0.78 ± 0.38**   | nd           | 0.12 ± 0.06**  |  |  |               |  |
| C 18:2 9c, 12t  | nd           | nd           | 0.07 ± 0.01  | 0.01***       | nd           | 1.84 ± 1.96    | nd            | 0.73 ± 0.38**   | nd           | 0.05 ± 0.07    |  |  |               |  |
| C 18:2 9c, 12c  | 13.97 ± 0.29 | 15.20 ± 3.17 | 0.94         | 10.62 ± 0.72  | 29.79 ± 1.58 | 29.81 ± 1.86   | 0.56          | 50.02 ± 1.16    | 62.97 ± 0.04 | 52.14 ± 6.35*  |  |  |               |  |
| C 20:0  | 0.71 ± 0.07  | 0.86 ± 0.11  | 0.40 ± 0.02  | 0.39 ± 0.01   | 0.86 ± 0.08  | 0.72 ± 0.07*   | 0.31 ± 0.01   | 0.39 ± 0.13     | 0.30 ± 0.02  | 0.26 ± 0.02*   |  |  |               |  |
| C 18:3 9t, 12t, 15c + 18:3 9t, 12c, 15t + 18:3 9c, 12c, 15t | nd           | nd           | nd           | nd            | nd           | 0.16 ± 0.07**  | nd            | 0.20 ± 0.17     | nd           | nd             |  |  |               |  |
| C 18:3 9t, 12c, 15c   | nd           | nd           | nd           | nd            | 0.02 ± 0.01  | 0.24 ± 0.06**  | nd            | 0.89 ± 0.30     | nd           | nd             |  |  |               |  |
| C 18:3 9c, 12c, 15c   | nd           | nd           | nd           | nd            | nd           | 0.24 ± 0.07*** | nd            | 0.90 ± 0.33     | nd           | nd             |  |  |               |  |
| C 20:1  | 10.91 ± 2.03 | 6.90 ± 2.55* | 0.18 ± 0.01  | 0.19 ± 0.01   | 0.50 ± 0.09  | 0.52 ± 0.06    | 0.22 ± 0.01   | 0.22 ± 0.01     | nd           | 0.15 ± 0.01*** |  |  |               |  |
| C 18:3 ALA  | 9.58 ± 0.90  | 8.07 ± 0.79* | 0.17 ± 0.01  | 0.20 ± 0.04   | 1.22 ± 0.26  | 0.54 ± 0.33*   | 6.08 ± 0.16   | 4.15 ± 0.83**   | nd           | 0.09 ± 0.06*   |  |  |               |  |
| C 22:0  | 0.44 ± 0.13  | 0.76 ± 0.17* | nd           | 0.01***       | 0.30 ± 0.05  | 0.24 ± 0.03    | 0.31 ± 0.02   | 0.48 ± 0.04***  | 0.87 ± 0.01  | 0.76 ± 0.05**  |  |  |               |  |
| C 22:1  | 33.23 ± 9.29 | 10.02        | nd           | nd            | nd           | nd             | nd            | nd              | nd           | nd             |  |  |               |  |
| C 24:0  | 0.20 ± 0.05  | 0.31 ± 0.07  | nd           | 0.05 ± 0.05   | 0.39 ± 0.06  | 0.35 ± 0.06    | 0.13 ± 0.01   | 0.17 ± 0.06     | 0.28 ± 0.00  | 0.27 ± 0.02    |  |  |               |  |
| Σ cis-UFAs  | 92.89 ± 0.58 | 90.80 ± 2.08 | 0.56         | 55.61 ± 1.00  | 74.58 ± 0.60 | 72.50 ± 3.36   | 0.08          | 79.46 ± 1.66**  | 86.53 ± 0.02 | 88.56 ± 0.73** |  |  |               |  |
| Σ trans-UFAs  | nd           | nd           | 0.15 ± 0.01  | 0.28 ± 0.01** | 0.13 ± 0.22  | 4.35 ± 3.88*** | 0.06 ± 0.04   | 3.50 ± 1.54***  | nd           | 0.18 ± 0.12*   |  |  |               |  |
| Σ SFAs  | 5.98 ± 0.36  | 6.89 ± 2.08  | 43.42 ± 0.57 | 43.63 ± 1.00  | 24.43 ± 1.54 | 22.90 ± 0.70   | 15.91 ± 0.21  | 16.61 ± 0.50**  | 12.30 ± 0.04 | 10.96 ± 0.51** |  |  |               |  |
| Σ MUFAs   | 68.76 ± 1.34 | 67.17 ± 4.58 | 0.39         | 44.77 ± 0.26* | 43.60 ± 1.49 | 42.00 ± 2.89   | 28.87 ± 0.65  | 25.20 ± 0.85*** | 23.56 ± 0.06 | 36.32 ± 6.37** |  |  |               |  |
| Σ PUFA  | 24.13 ± 1.06 | 23.68 ± 2.87 | 10.87 ± 0.95 | 11.12 ± 0.75  | 31.11 ± 1.97 | 34.89 ± 3.54   | 0.75          | 57.76 ± 1.28**  | 62.97 ± 0.04 | 52.42 ± 6.37*  |  |  |               |  |
| Total FAs   | 98.83 ± 0.90 | 98.74 ± 0.56 | 0.01         | 99.52 ± 0.04  | 99.13 ± 1.18 | 99.75 ± 0.21   | 0.31          | 99.56 ± 0.14    | 98.83 ± 0.02 | 99.70 ± 0.23   |  |  |               |  |

<sup>a</sup> Note: n represents the number of samples; Analysis of each sample was conducted in triplicate and values are reported as mean ± standard deviations (SD); nd = not

5 detected; \* value is significant at p < 0.05 level; \*\* value is significant at p < 0.01 level; \*\*\* value is significant at p < 0.001.

main contributor of total TFA in SO and PO (Fang *et al.* 2023; Hou *et al.* 2012; Sarwar *et al.* 2024). The other TFA found in this experiment was *trans* linolenic acid which found RRBO (0.64±0.21) and RSO (1.99±0.91). The presence of *trans* linolenic acid in RSO was also described by Hou *et al.* (2012) and Fang *et al.* (2023) on the other hand, Sarwar *et al.* 2024 did not discuss the presence of it in their study Sarwar *et al.* (2024). However, elaidic (C 18:1 9t), the most described *trans* fatty acid was not identified in any of the seed extract or refined oil samples in the present study.

In Figure 3, the comparison of *trans* fatty acids in seed oil and their refined counterparts has been shown. The figure clearly shows that the refined oils contain more *trans* fatty acids than the seed-extracted oils, while the abundance of *trans* fatty acids is maximum in RRBO followed by RSO.

CPO had the highest saturated fatty acid (SFA) content (43.86%) with a considerable portion of palmitic acid (38.10%). With a major content of 34.01% erucic acid, MSEO had the highest level of monounsaturated fatty acids (MUFA) at 66.45%. The polyunsaturated fatty acid (PUFA) content of SuSEO was highest (62.97%), whereas oleic acid was only less than one-fourth of total fatty acid (23.57%). These results were consistent with some earlier research findings (Mancini *et al.* 2015; Schwarzingler *et al.* 2022).

Palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), and linolenic acid (C18:2) are the most prominent fatty acids found in all types of oil which follow the specification mentioned in codex standard for vegetable oil (CODEX STAN 210, 1999). Furthermore, the findings of fatty acids in this investigation aligned with the findings of earlier research (Chotimarkorn and Silalai, 2008; Petraru *et al.* 2021).

Among the five studied oils, sunflower oil is imported in refined form and no treatment is applied in Bangladesh. In contrast, soybean oil and palm oil are imported as crude form, and refining and purification are performed in Bangladesh. On the other hand, rice bran oil is extracted and refined inside the country and mustard oil is extracted by mechanical pressing without any chemical treatment.

In this experiment seed oils were collected by cold extraction involving heat treatment less than 80°C, hence, very little amount of TFAs was found in seed extract indicating that naturally plant seeds contain negligible TFAs. A high level of TFAs found in RRBO and RSO was

certainly generated during the refining stages, besides, no TFA in MO and a small amount in RPO and RSuO indicate processing without heat treatment and careful refining limits the TFA content within the recommended level (Bruhl, 1996).

## Conclusion

In conclusion, it can be said that, naturally, a small amount of *trans* fatty acid is present in oil extracted from seeds by the physical procedure which increased several folds in rice bran oil and soybean oil during refining whereas, palm oil and sunflower oil showed less increment. As no heat treatment in the case of mustard oil extraction in Bangladesh, TFAs in commercial mustard oil are similar to seed extract oil. In the case of rice bran oil and soybean oil, high heat treatment is involved during refining which is considered the main cause of the TFAs formation. Hence, further study is needed to find out the potential stage where TFA is formed and heat treatment should be adjusted accordingly to control TFA in its acceptable limit.

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