

## STUDY ON THE CHEMICAL ANALYSIS OF OIL AND FATTY ACID OF SOME RAPESEED VARIETIES AVAILABLE IN BANGLADESH

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### Abstract

The experiment was conducted at the Department of Biochemistry, Sher-E-Bangla Agricultural University, Dhaka and Bangladesh Agricultural Research Institute (BARI), Gazipur to investigate the chemical constituents of oils from rapeseed varieties Tori-7, SAU Sharisha-1 and Sampad, respectively. The highest (42.15%) oil content of seed was recorded in SAU Sharisha-1. On the other hand, the lowest (37.45%) oil content was recorded in the cultivar Sampad. The highest (185.13) saponification value was recorded in SAU Sharisha-1. On the other hand, the lowest (159.89) saponification value was recorded in Tori-7. The lowest (303.19) saponification equivalent was recorded in the SAU Sharisha-1, while the highest (350.88) saponification equivalent was recorded in Tori-7. The highest (0.55) acid value was recorded in Sampad, while the lowest (0.26) acid value was recorded in SAU Sharisha-1. The highest (110.00) iodine value was recorded in SAU Sharisha-1 and the lowest (104.50) iodine value was recorded in Sampad. The highest (0.75) unsaponifiable matter was recorded in Sampad, while the lowest (0.60) was recorded in Tori-7. The highest (4.38%) saturated fatty acid was recorded in SAU Sharisha-1, while the lowest (3.45%) was recorded in Tori-7. The highest (96.55%) total unsaturated fatty acid was recorded in Tori-7 and the lowest (90.43%) was recorded in SAU Sharisha-1.

**Keywords:** Oil, acid value, fatty acid, saponification, iodine value.

### Introduction

The rapeseed is one of the leading oilseed crops in Bangladesh. It is mainly self-pollinating crop, although on an average 7.5 to 30% out-crossing does occur under natural field conditions (Rakow and Woods, 1987; Abraham, 1994). In our country 1952 metric ton seeds of oilseed were produced by BADC in the year 2013-2014 (BBS, 2014). Oilseed crops play a vital role in human nutrition. It is used as a condiment, salad, green manure and fodder crop, and as a leaf and stem vegetable in the various mustard growing countries of the World. It is not only rich source of energy (about 9 kcal g<sup>-1</sup>) but also rich in fat soluble vitamins A, D, E and K. The National Nutrition Council of Bangladesh reported that recommended dietary allowance (RDA) per capita per day should be 6 g oil for a diet with 2700 kcal. In Bangladesh, sources of edible oil are rapeseed-mustard, sesame, groundnut, soybean, niger, linseed, sunflower and safflower. But rapeseed-mustard is one of the important oilseed crops of the world after soybean and palm (FAO, 2004). Rapeseed oil is widely used as cooking oil and medicinal ingredient and supplies fat in our daily diet. Bangladesh is running with acute shortage of about 70% edible oil. Annual production of edible oil is about 0.16 million tons as against the requirement of 0.5 million tons. To meet up the demand, the country has to import oil and oilseeds costing about 160 million US \$ every year (Wahhab *et al.*, 2002). Mankind derives its energy from carbohydrates, protein and fats. The fats have the highest energy per unit quantity oils and fats are concentrated sources of different types of local races, advanced lines and exotic materials of rapeseed available in our country. To utilize the variability, it is necessary to evaluate the variations in respect of genetic and phenotypic co-efficient of variation, nutrient status and other relevant parameters which have been reported by many authors (Biswas, 1989; Lebowitz, 1989; Yadava *et al.*, 1985; Nanda *et al.*, 1995; Kumar *et al.*, 1996). The nutritional quality of *Brassica* seeds of all these varieties or advanced lines are not yet analyzed. If the nutritional quality of mustard oil is known, its consumption as well as its multipurpose uses will be increased which will play a vital role in improving the nutritional status.

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Now a crucial question arises about the morphological, physiological and biochemical quality of BARI developed mustard varieties. In this experiment three varieties of *Brassica* seed viz., Tori-7 (best performing BARI released), SAU Sharisha-1 (Newly released by Prof. S. R. Bhuiyan of Sher-e-Bangla Agricultural University) and Sampad (S-S 75, BARI released) were investigated. The present research work has been undertaken in order to analyze the different physicochemical characters of Tori-7, SAU Sharisha-1 and Sampad.

## Materials and Methods

The experiment was conducted to investigate the chemical constituents of oil (percent of oil, total solid, saponification value, saponification equivalent, acid value, iodine value and unsaponifiable matter) and fatty acid of rapeseed varieties Tori-7, SAU Sharisha-1 and Sampad, respectively.

**Oil percent:** For determining the oil percent; electrical weighing balance (Mettler H 18), mortar and pestle, soxhlet apparatus and petroleum ether (40 - 60°C) were used as materials. The oil was obtained from the seeds by solvent extraction process. Sixty (60) g of *Brassica* seed were crushed by pestle in mortar and oil was extracted with petroleum ether in a soxhlet apparatus.

**Total solid:** Total solid was calculated by the amount of oil cake that was found from the extraction of oil as a solid matter.

### Saponification value

It is defined as “the milligrams of KOH required to saponify 1 g of fat” completely. Saponification value of a fat indicates the average molecular weight (average chain length) of the fatty acids constituting or comprising the fat. This number is inversely proportional to the average chain length of the fatty acids. The higher saponification value, the shorter will be the chain lengths of the fatty acids and vice versa. Saponification value was measured following the procedure described by Kafi (2002).

**Procedure:** About 2 g of sunflower oil was weighed accurately in a 250 ml round bottom flask. Twenty five (25) ml of the alcoholic KOH solution was added; reflux air condenser was attached to the flask and kept it in boiling water for 1 hr. The contents of the flask were frequently mixed. While the solution was still hot, 1 ml phenolphthalein solution was added and the excess alkali titrated against 0.5 N HCl (a) the experiment was repeated without the oil or fat to obtain the blank value (b) as 1 ml of 0.5 N HCl was equivalent to 0.02805 g of KOH, the following formula was used to calculate the saponification value:

$$\text{Saponification value} = \frac{(b - a) \times 0.02805 \times 1000}{\text{Wt in g of substance}}$$

**Saponification equivalent:** Saponification equivalent was determined using the formula:

$$\text{Saponification equivalent} = \frac{56100}{\text{Saponification value of the oil}}$$

### Determination of acid value and free fatty acid

Acid value (A.V.) of the oil was determined following the procedure as reported earlier.

**Reagents:** 95% neutralized alcohol, potassium hydroxide solution (0.05N) and phenolphthalein solution, 1% in 95% alcohol.

**Procedure:** A known weight of the oil (3 - 5 g) was taken in a conical flask and mixed with 50 ml of 95% neutralized alcohol. The mixture was heated to boiling and the content of the flask was titrated with aqueous potassium hydroxide solution until a faint pink color persisted for at least 10 seconds. The content of the flask was shaken continuously and vigorously during the titration Kafi, (2002). Acid value of the oil was calculated using the formula given below:

$$A.V. = \frac{65.1 \times N \times V}{W}$$

Where, A.V. = Acid value

N = Strength of alkali

V = Number of ml of aqueous alkali required for titration

W = Weight of the oil taken in the grams.

### Iodine value

Iodine value (I.V.) of the oil was determined by using the formula of Kafi,(2002):

$$\text{Iodine} = \frac{S \times (X - Y) \times 0.127 \times 100}{W}$$

Where, S = Strength of the sodium thiosulphate solution.

X= ml of sodium thiosulphate solution required in the blank test.

Y = ml of sodium thiosulphate required in the test experiment.

W = Weight of the oil taken in grams.

**Reagents:** Chloroform, 15% potassium iodide solution, starch indicator: Starch (1 g) was dissolved in 50 ml hot water and diluted to 100 ml with cold water, 0.1N sodium thiosulphate solution, 0.1N potassium dichromate solution, conc. hydrochloric acid, sodium bicarbonate, potassium iodide and hanus solution: Iodine (13 g) was dissolved in glacial acetic acid. Bromine (3 ml) was added to it and the solution was diluted with glacial acetic acid to 1 liter.

**Procedure:** The oil (1 - 2 g) was dissolved in 10 ml of chloroform in a dry glass stoppered bottle (500 ml). To the content of the bottle 25 ml of Hanus solution was added and the mixture was allowed to stand in the dark for exactly 30 minutes with occasional shaking. Potassium iodide solution (10 ml) was mixed to it and the mixture was shaken well. Freshly boiling cooled water (100 ml) was added to the mixture and the content of the bottle was titrated with sodium thiosulphate solution, using starch solution as indicator.

A blank experiment (without the oil) was performed exactly in the same manner as described above.

### Determination of quantity of unsaponifiable matter

Amount of unsaponifiable matter present in the oil was determined using the S. P. A. Method.

**Reagents:** Diethyl ether, acetone, alcoholic solution of potassium hydroxide (1N), aqueous solution of potassium hydroxide (0.5 N).

**Procedure:** The oil (5.001 g) mixed with 1N alcoholic potassium hydroxide solution (50 ml) and was refluxed for 45 minutes at about 70 to 80 °C with occasional swirling. The solution was then transferred into a separating funnel and rinsed it with 100 ml of water. Each extraction was made by shaking vigorously in the separating funnel and allowing the two layers to separate. The aqueous alcoholic layer at the bottom of the separating funnel was run off and the ethereal solution from the top was poured into another separating funnel containing 20 ml of water. The total ether extracts in the separating funnel was washed thrice with 20 ml water. The ethereal layer was then washed three times with 20 ml aqueous potassium hydroxide solution by shaking vigorously, each alkali wash being followed by a wash with 20 ml of water. After the aqueous alkali treatment, the solution was washed with successive quantities of water until the wash water no longer gave alkaline reaction to phenolphthalein solution. The ether extract was then transferred into a weighed flask and the ether was distilled off. The residue was dried to constant weight at 80°C. Drying was assisted by adding 2 - 3 ml of acetone to the extract when nearly all the ether was evaporated. The flask with its contents was weighed. The quantity of unsaponifiable matter present in the 100 g of oil was calculated from the following formula.

$$\text{Unsaponifiable} = \frac{\text{Weight of unsaponifiable matter}}{\text{Weight of oil taken}} \times 100$$

**Fatty acid:** Fatty acid composition of *Brassica* from three varieties was determined by Gas Liquid Chromatograph method.

## Results and Discussion

Significant differences were observed in the different components of oil in different varieties of rapeseed. The results have been presented and discussed, and possible interpretations given under the following headings:

### Oil content

The oil content among the selected cultivar varied from 37.45 to 42.15%. The highest (42.15%) oil content of seed was recorded in SAU Sharisha-1. On the other hand the lowest (37.45%) oil content was recorded in Sampad which was statistically similar (38.60%) with Tori-7 (Table 1). The present findings were fully agreed with the report revealed by Pathak *et al.* (1973). The findings of the present study corroborates with the findings of Uddin *et al.* (1995) and Azad and Hamid (2000).

### Total solid

A statistically significant variation was recorded for total solid content of different cultivar that was used in this study (Table 1). The total solid content of the *Brassica* variety varied from 51.25 to 61.34%. The highest (61.34%) total solid content of seed was recorded in Tori-7. On the other hand, the lowest (50.10%) total solid content was recorded in SAU Sharisha-1 which was statistically similar (51.25%) with cultivar Sampad (Table 1). Similar results also reported by Ahmed (1989).

**Table 1. Oil and total solid content of Tori-7, SAU Sharisha-1 and Sampad of *Brassica campestris* seeds**

Name of the variety	Oil content (g%)	Total solid (cake g%)
Tori-7	38.60 b	61.34 a
SAU Sharisha-1	42.15 a	50.10 b
Sampad	37.45 b	51.25 b
LSD <sub>(0.05)</sub>	2.975	3.215
CV(%)	3.78	2.97

In a column, means having similar letter(s) are statistically similar and those having dissimilar letter(s) differed significantly as per 0.05 level of probability.

### Saponification value

Hydrolysis of a fat or oil by alkali into glycerol and alkali salt is known as saponification and the number of mg of potassium hydroxide required to saponify one g of fat or oil is called saponification value. It is inversely proportional to the average molecular weight or chain length of the fatty acids present in the oil or fat. Different varieties showed statistically significant differences in saponification value among them. The saponification value of the oil of *Brassica* varieties varied from 159.89 to 185.13. The highest (185.13) saponification value was recorded in the variety SAU Sharisha-1. On the other hand, the lowest (159.89) saponification value was recorded in Tori-7 which was closely followed (169.42) by Sampad (Table 2). The results were similar to that as reported by Youngs (1951) and Kapur and Daubert (1949).

### Saponification equivalent

Statistically significant differences were observed in respect of saponification equivalent of different *Brassica* varieties (Table 2). The Saponification equivalent of the oil of the varieties varied from 303.19 to 350.88. The lowest (303.19) saponification equivalent was recorded in SAU Sharisha-1, while the highest (350.88) saponification equivalent was recorded in Tori-7 which was closely followed (331.13) by Sampad (Table 2). Ahmed (1989) reported that the saponification equivalent was 311.5 to 337.17 in *Brassica* seeds.

### Acid value

Acid value is the amount of potassium hydroxide requires neutralizing the free fatty acids in one g of fat/oil. As the liberation of free fatty acids in natural fat sample due to hydrolysis by lipase may be an important contributory factor for rancidity, a high acid value may indicate a higher tendency to become rancid. Statistically significant differences

were observed in respect of acid value of the oil of different *Brassica* varieties (Table 2). The acid value of the varieties varied from 0.269 to 0.550. The highest (0.550) acid value was recorded in the variety Sampad, while the lowest (0.269) acid value was recorded in SAU Sharisha-1 which was closely followed (0.367) by Tori-7 (Table 2). Acid value in *Brassica* seed was 0.833 to 2.30 recorded earlier by Ahmed (1989).

### Iodine value

Iodine value is defined as grams of iodine absorbed by 100 g of fat. It gives an estimate of the degree of unsaturation and so, of the relative amounts of unsaturated fatty acids in the triglyceride molecules of the fat. Iodine value of different *Brassica* varieties are presented in Table 1 and found that there was statistically non-significant variation among the varieties under the experiment. The iodine value of the oil of the varieties varied from 104.50 to 110.00. The highest (110.00) iodine value was recorded in SAU Sharisha-1 and the lowest (104.50) iodine value was recorded in Sampad (Table 2). Mittelbach (1991) reported similar iodine value from their study.

**Table 2. Chemical constants of oil of Tori-7, SAU Sharisha-1 and Sampad of *Brassica campestris* seeds oils**

Name of the variety	Saponification value	Saponification equivalent	Acid value	Iodine value	Unsaponifiable matter (%)
Tori-7	159.89 c	350.88 a	0.367 b	106.00 b	0.600 b
SAU Sharisha-1	185.13 a	303.19 c	0.269 c	110.00 a	0.650 b
Sampad	169.42 b	331.13 b	0.550 a	104.50 c	0.750 a
LSD <sub>(0.05)</sub>	6.065	10.10	0.019	--	0.089
CV(%)	2.77	4.54	3.94	4.63	3.12

In a column, means having similar letter(s) are statistically similar and those having dissimilar letter (s) differed significantly as per 0.05 level of probability.

### Unsaponifiable matter

The unsaponifiable matter is a fraction of fat or oil that remains insoluble after saponification of the fat sample by alkali. A statistically significant variation was recorded among the variety for unsaponifiable matter of different *Brassica* (Table 2). The unsaponifiable matter of the oil of released varieties and lines varied from 0.600 to 0.750. The highest (0.750) unsaponifiable matter was recorded in Sampad, while the lowest (0.600) unsaponifiable matter was recorded in Tori-7 which was statistically similar (0.650) with the variety SAU Sharisha-1 (Table 2). Ahmed (1989) reported that the unsaponifiable matter 0.50 to 2.00% in different varieties of *Brassica*.

### Saturated fatty acid

A statistically significant variation was recorded among the varieties for palmitic, Stearic and total saturated fatty acid (Table 3).

**Table 3. Percentage composition of fatty acid of different varieties of *Brassica* seed oil (by GLC analysis)**

Name of the variety	Fatty Acid Percentage								
	Saturated Fatty Acid			Unsaturated Fatty Acid					
	Palmitic C <sub>16:0</sub>	Stearic C <sub>18:0</sub>	Total	Oleic C <sub>18:1</sub>	Lenolic C <sub>18:2</sub>	Lenolenic C <sub>18:3</sub>	Ecosenoic C <sub>20:1</sub>	Eurcic C <sub>22:1</sub>	Total
Tori-7	3.20 a	0.25 c	3.45	10.85 c	19.52 a	15.66 a	15.16 a	35.36 c	96.55 a
SAU Sharisha-1	3.22 a	1.16 b	4.38	17.54 a	19.13 a	8.04 c	0.35 b	45.49 b	90.43 b
Sampad	2.05 b	1.77 a	3.82	14.64 b	13.89 b	12.40 b	3.90 b	51.35 a	96.14 a
LSD <sub>(0.05)</sub>	0.121	0.025	0.312	1.368	1.612	0.879	1.048	2.841	3.489
CV(%)	3.11	2.15	2.84	3.08	1.95	3.56	4.05	2.22	3.69

In a column, means having similar letter(s) are statistically similar and those having dissimilar letter(s) differed significantly as per 0.05 level of probability.

The palmitic saturated fatty acid of released varieties and lines varied from 2.05 to 3.22%. The highest (3.22%) palmitic saturated fatty acid was recorded in SAU Sharisha-1 which was statistically similar (3.20%) to Tori-7, while the lowest (2.05%) was recorded in Sampad. The palmitic saturated fatty acid of released variety and lines varied from 2.05 to 3.22%. On the other hand the stearic saturated fatty acid of released variety and lines varied from 0.25 to 1.77%. The highest (1.77%) stearic saturated fatty acid was recorded in Sampad which was closely followed (1.16%) by SAU Sharisha-1, while the lowest (0.25%) was recorded in Tori-7. Total saturated fatty acid of released variety and lines varied from 3.45 to 4.38%. The highest (4.38%) saturated fatty acid was recorded in SAU Sharisha-1, while the lowest (3.45%) was recorded in Tori-7 which was closely followed (3.82%) by Sampad. Weiss (1983) found oleic acid content of mustard was 45.4%.

### Unsaturated fatty acid

A statistically significant variation was recorded among the varieties for oleic, lenolic, lenolenic, ecosenoic, Eurcic and total unsaturated fatty acid (Table 3). The oleic unsaturated fatty acid of released varieties and lines varied from 10.85 to 17.54%. The highest (17.54%) oleic unsaturated fatty acid was recorded in SAU Sharisha-1 which was closely followed (14.64%) by sampad, while the lowest (10.85%) was recorded in Tori-7. The lenoic unsaturated fatty acid of released varieties and lines varied from 13.89 to 19.52%. The highest (19.52%)lenolic unsaturated fatty acid was recorded in Tori-7 which was statistically identical (19.13%) with SAU Sharisha-1, while the lowest (13.89%) was recorded in Sampad. The lenolenic unsaturated fatty acid of released varieties and lines varied from 8.04 to 15.66%. The highest (15.66%) lenolenic unsaturated fatty acid was recorded in Tori-7 which was closely followed (12.40%) bySampad, while the lowest (8.04%) was recorded in SAU Sharisha-1. The ecosenoic unsaturated fatty acid of released varieties and lines varied from 0.35 to 15.16%. The highest (15.66%) ecosenoic unsaturated fatty acid was recorded in Tori-7 which was closely followed (3.90%) by SAU Sharisha-1, while the lowest (0.35%) was recorded in SAU Sharisha-1. The eurcic unsaturated fatty acid of released varieties and lines varied from 35.36 to 51.35%. The highest (51.35%) eurcic unsaturated fatty acid was recorded in Sampad which was closely followed (45.49%) by SAU Sharisha-1, while the lowest (35.36%) was recorded in Tori-7. Total unsaturated fatty acid of released variety and lines varied from 90.43 to 96.55%. The highest (96.55%) total unsaturated fatty acid was recorded in Tori-7 which was statistically identical (96.14%) with Sampad, while the lowest (90.43%) was recorded in SAU Sharisha-1. Wesis (1983) found that linoleic acid and linolenic acid content of mustard seed were 0% and 9.1%.

### Conclusion

The proximate composition and some of other nutrient compositions of some *Brassica* species were studied to investigate the chemical contents of oils of varieties Tori-7, SAU Sharisha-1 and Sampad *Brassica* species. The highest oil content (42.15%) in seed was recorded in SAU Sharisha-1. On the other hand, the lowest (37.45%)oil content was recorded in Sampad. The highest total solid content 61.34% in seed was recorded in Tori-7 and the lowest (50.10%) total solid content was recorded in SAU Sharisha-1. The highest (4.38%) saturated fatty acid was observed in SAU Sharisha-1 while the highest (96.55%) total unsaturated fatty acid was observed in Tori-7 and the lowest (90.43%) was observed in SAU Sharisha-1. As the highest oil content (42.15%) in seed was recorded in SAU Sharisha-1, it was the best variety among the three varieties under tested.

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