Characterization of fatty acids and nutrient composition of SAU Perilla-1 [(*Perilla frutescens* (L.) Britton] seeds grown with agro climatic conditions in Bangladesh

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ABSTRACT

SAU Perilla-1 (Golden perilla BD) is a newly edible oil seed crop variety introduced by Sher-e-Bangla Agricultural University in Bangladesh. Its seed oil is a rich source of unsaturated fatty acid (91%), of which more than 50% is α-linolenic acid (type of omega-3 fatty acid). The estimated ratio of saturated, monounsaturated and polyunsaturated fatty acids was found 1: 2.26: 8.95. Moreover, the α-linolenic acid was detected (50.52%) as the most dominating polyunsaturated fatty acid, which was 4-four times higher than monounsaturated fatty acids in the oil of the crop variety. Fatty acid analysis of oil revealed a ratio (1:2.22) of Omega 6 to Omega 3 fatty acids, which lies within a healthy range as documented by the global scientific community. Compared to other plant seed oils, SAU Perilla-1 oil consists of Linoleic acid (Omega 6 fatty acid, 22.71%) – a component associated with obesity, which is far below the regular oils from soybean, sunflower and corn. Therefore, our findings indicated that SAU Perilla-1 seed is one of the best edible sources of plant oils rich in essential fatty acids conducive to human health.

Key Words: *Perilla frutescens (L.) Britton, α-linolenic acid and polyunsaturated fatty acid*

I. Introduction

SAU Perilla-1 (Golden Perilla BD) is a newly introduced oil seed crop variety in Bangladesh since 2020. The crop, generally known as Perilla (*Perilla frutescens* (L.) Britton), is a self-pollinated and photosensitive shrub plant under the Lamiaceae family and is recommended as an annual oil yielding crop variety in Bangladesh. Although this variety is native to the Republic of Korea, the species is also widely distributed and cultivated in Southeast and East Asia (Makino, 1961; Asif, 2011) the USA and the European Nations (Nitta et al., 2003) of the globe. No variety of Perilla been cultivated before the

introduction of SAU Perilla-1 (Golden Perilla BD) in Bangladesh. Even wild races (s) of this variety have not been found reported in any places before 2020 in Bangladesh. However, the crop is suitable for cultivation in Kharif II season (Mid July to Mid November) and is well-fitted with the ongoing year round cropping sequence practised in Bangladesh. The crop is shown up with blooming during the last week of September to the first week of October when the day durations are observed shortening in the country (BMD, 2019). The matured seeds worth 1.30–1.50 t/ha could easily be harvested by 100–110 days before starting preparation for subsequent winter cropping, provided the seedlings (~30days old) being transplanted by the 10–15th day of August.

Perilla has also been cultivated as a commercial oil seed crop native to Japan and Korea (Choi et al., 1980; Brenner, 1993). Similarly, the crop has also been introduced for commercial cultivation in Europe, Russia and the USA (Nitta et al., 2003) due to high quality edible oil with elevated levels of unsaturated fatty acids (α-linolenic, Linoleic and Oleic acids) dominated by the α-linolenic acid (Omega-3) (Park et al., 1981). Perilla oil has been an increased interest due to being rich in α-linolenic acid, favouring the health benefits to human health (Kim et al., 2004; Kim et al., 2007; Rao et al., 2008). It is demonstrated that the α-linolenic acid, which is rich in perilla seed, has reduced cardiovascular risk (Zhang et al., 2014). Besides, omega-3 fatty acid reduces inflammation and may help lower the risk of chronic diseases such as cancer and arthritis. The symptomatic indications of Omega-3 fatty acid insufficiency are associated with drowsiness, loss of memory, skin dryness, heart diseases, distress and peripheral arterial disease causing defective blood circulation (Vanschacky et al., 2000).

Traditionally oil crops are grown and harvested in Robi season (winter season) in Bangladesh, conferring severe competition for available land with other crops like boro rice, wheat, pulses and vegetables. In addition, the country has to spend a considerable amount of foreign exchange for importing edible oils and oil seeds to meet the increasing oil demand of the nation (Miah et al., 2017). The values of imported edible oils and oil seeds were USD1574 million and USD 354 million in 2014-2015, respectively (Bangladesh Bank, 2016). Moreover, some research reports demonstrated that the fatty acids profiles and seed oil content rely on the perilla species, the climatic condition of crop places grown and the duration needed to get maturity of seeds (Siriamornpun et al., 2006; Youfang et al., 2012). The composition of fatty acids, seed yield and seed oil content differs in plant species and other factors like seed maturity and the agro climatic environments of the crop grown (Lee et al., 1986; Brenner, 1993; Lee and Ohnishi, 2001; Siriamornpun et al., 2006). As such, the present study was undertaken to focus on the quality attributes of seed oil of SAU Perilla-1, which have never been grown in Bangladesh, to attract the crop among the modern scientific societies, environmentalists regarding conservation and popularization of this new variety as an edible oil yielding crop for minimizing oil import volume consumed by the people's republic of Bangladesh.

II. Materials and Methods
Collection of seeds
SAU Perilla-1 plant (Figure 01) was grown in Kharif II season at the Agronomy research field of Sher-e-Bangla Agricultural University, Bangladesh, located at latitude 23.7717^0 N and longitude 90.3752^0 E. At maturity stage, all the leaves turn into light green to yellowish in colour and drop off onto the ground, facilitating organic matter to the soil. However, SAU Perilla-1 seeds (Figure 02) were collected from mature plants. The original station and pure line numbers were SAU-AGRO 35 and SAU Perilla-1 (Golden perilla BD), respectively. The analyzed seeds were obtained from the generation of seeds sown in July, 2019 on sandy loam soil and were harvested between the last week of October to the 1st week of November. The collected seeds were dried in the sun for 3 to 4 days and packed in an air-tied plastic container kept in a room temperature.

Preparation of seeds and extraction of oil
To begin the process, physical impurities were cleaned and air-dried to reduce the moisture content of the seed samples. The next step was to crack the size of the seeds down and then they were flattened to form flakes, which facilitated increasing the surface area for easier extraction of oil. Later on, food-grade n-Hexane (for solvent extraction) was fed to extract oil from the flakes and refluxed using Soxhlet apparatus. The refluxed mixture was filtered to remove all the visible flakes and residues. In addition to that, the solvent of the filtrate was removed by rotary evaporation. Finally, the obtained oil from seed samples was calculated on a dry weight basis. During the oil extraction and solvent

evaporation process, degradation of all the samples was protected from light. All the measurements were performed trice of each sample and the data were recorded as mean and the standard deviations (±) were calculated as the value of n=3.

Figure 01. SAU Perilla-1 plant grown in Kharif II season at SAU farm, Dhaka 1207.

Figure 02. Matured seeds harvested from SAU Perilla-1 grown in Kharif II season at SAU farm.

Analysis of fatty acid composition
a) Preparation of fatty acid methyl ester (FAME)
The relative concentrations of fatty acids were measured from oil samples as of their corresponding methyl esters according to the IUPAC method (1979) with minor modifications. However, 5-7 drops of oil were taken in 15 mL test tube. After that, 3 mL of 0.5 M Sodium methoxide (prepared by mixing metallic sodium in methanol) was added. The resultant solutions were digested by stirring in a boiling water bath for about 15 minutes. After cooling at room temperature, 1 mL of petroleum ether (b.p 40-60^{0}C) was added, followed by 10 mL deionized water. The mixture was allowed to settle for 5-6 minutes. The clear and distinct upper layer of methyl ester in petroleum ether was separated carefully in a capped vial used for analysis. Standard solutions of fatty acids were prepared externally by dissolving 200mg of each of the authentic standards in their respective methyl ester form separately in 10 mL petroleum ether under b.p 40-60^{0}C in a series of screw-capped test tubes for 20,000ppm stock solutions. However, the aliquots of 1μL FAME were injected and the peaks of fatty acid were recorded for their respective retention time and areas by the data processor unit of a Gas Chromatography (GC).

b) The condition of gas chromatography-flame ionized detector (GC-FID) used
The composition of fatty acids was analyzed using a gas chromatograph (Shimadzu GC-148, Japan) equipped with flame ionization detector. A fused silica capillary column (FAMEWAX, Cross bond polyethylene glycol, 15m x 0.25mm x 0.25μm film thickness, Restek; Pennsylvania, USA) was used to separate the fatty acid samples being tested carried by nitrogen as a carrier gas at a constant flow rate of 2.0mL/min. The split-less injection technique was applied to pass 1μL volume of each sample through an injector holding temperature of 250^{0}C. The initial oven temperature was 150^{0}C, which was maintained for 5 minutes. The temperature increased rate was 8^{0}C/min to 190^{0}C and then escalated at a rate of 2^{0}C/min up to 200^{0}C, which was held for 10 minutes. The fatty acids as identified using respective fatty acid methyl ester standards (FAME mix) and presented as relative percentage done by the automated GC software (Class GC-10, version-2.00).

Nutrient analysis of the seeds
The seeds were pulverized and the samples were considered for nutrient composition determination. In the case of protein content analysis, it was quantified by the Kjeldahl method. The iron and carbohydrate were estimated by Atomic Absorption Spectrometer (AAS) method.

2309

III. Results and Discussion

The proximate analyses of SAU Perilla-1 seed are presented in Table 01. The seed oil content of the crop variety was found 39% (approximate). The chemical properties and the fatty acid (FA) composition of seed oil were analyzed and presented in Table 02 and Table 03, respectively. From the samples, 07 fatty acids were identified under saturated, monounsaturated and polyunsaturated categories showing the estimated values of 8.18, 18.47 and 73.23% of their total FA identified, respectively (Table 03). The fatty acid composition in the oil samples was dominated by three unsaturated fatty acids, which were α-linolenic acid (Polyunsaturated Omega-3, C18:3 n-3), linoleic acid (Polyunsaturated Omega-6, C18:2 n-6) and oleic acid (Monounsaturated Omega-9 fatty acid, C18:1) accounted by 50.52%, 22.71% and 18.47% respectively. The total unsaturated FA, in particular, accounted for over 91% (Table 03). The Palmitic acid (C16:0; 6.78%) was the major saturated fatty acid with a small amount of Stearic acid (C18:0, 1.30%), trace amount of Palmitoleic acid (C16:1; 0.08%) and Arachidic acid (C20:0; 0.02%) detected. There was no erucic acid found in the oil samples derived from SAU Perilla-1 variety under study. There were two polyunsaturated FA, namely α-Linolenic acid (Omega 3 FA) and Linoleic acid (Omega 6 FA) detected in oil samples of the crop (Table 03).

Table 01. Proximate analysis of SAU Perilla-1 seed

Sl. no.	Test parameters	Test values
1	Oil content (%)	38.0 ± 1.25
2	Total Carbohydrate (%)	25.28 ± 1.45
3	Protein (%)	26.00 ± 0.65
4	Iron (mg/100g)	7.62 ± 0.86
5	Moisture (%)	0.20 ± 0.01

Values are estimated as means ±SD of three replicated analysis

In addition, the study also revealed that crop seeds contained 26% protein, 25.28% carbohydrate and 0.20% moisture (Table 01). Besides, it contained 7.62mg (in 100g of oil) iron essential for human health. WHO/FAO has outlined quality standards of various edible vegetable oils containing fatty acids composition, antioxidants, micronutrients and other physico-chemical parameters. Their guidelines demonstrate the maximum allowable quality parameters for edible oils, including free fatty acid value (0.6 mg potassium hydroxide/g oil) and peroxide value (10 mill-equivalents oxygen/kg oil). However, the peroxide value of SAU Perilla-1 seed fresh oil that we found is 9.09 milliequivalent oxygen/kg and the free acid value of this oil found 0.28 mg potassium hydroxide/g (Table 02). In this regard, SAU Perilla-1 oil meets the WHO and FAO standards for qualifying as a quality edible oil for maintaining good health and could quickly be grown and harvested under agro climatic conditions in Bangladesh.

Table 02. Chemical properties of SAU Perilla-1 seed oil

Sl. no	Test parameters	Results
1	Free Fatty Acid as oleic (FFA%),%	0.28
2	Peroxide Value (PV), meq O_2/Kg	9.09
3	Iodine Value (IV), (Hanus method)	187
4	Saponification Value (as KOH) (SV) (mg/g)	175
5	Unsaponifiable Matter (mg/g)	2.06
6	Color by Lovibond Tintometer (1 inch cell) (Y+5R)	Y=40, R=4
7	Relative Density at $20/20^0$C (By Digital Density meter)	0.92

The average ratio among the saturated, monounsaturated and polyunsaturated fatty acids was 1: 2.26: 8.95 (Table 04), which could be matched with those reported as 1:1.50:7.50; 1:1.19:7.76 by Kim and Choi (2004) and Siriamornpun et al. (2006). The study also revealed that the ratio of saturated and unsaturated fatty acids was 1:11.21, which was reported 1:9 in a study published by Ding et al., 2012. Polyunsaturated FA was found about 4-four times higher than monounsaturated FA (Table 04). Oleic acid (C18:1; ω - 9) is only the monounsaturated FA dominated (18.47%) in the oil sample of SAU Perilla-1 (Table 03). However, growing scientific evidence has shown that Oleic acid is an important agent for lowering the low-density lipoprotein (LDL), bad cholesterol found in blood plasma (Chung et al., 2013). The most dominated PUFA identified in this study was α-linolenic acid (ω – 3), which was quantified by 50.52% of the total fatty acids, and it was calculated 55.09% of the total unsaturated

fatty acids (TUFA) and ~69% of the polyunsaturated fatty acids (PUFA), in the seed oil of SAU Perilla-1 variety (Table 03). As of the report, this high proportion of α-linolenic acid (18:3) in the oil showed as a superior quality oil seed crop over rapeseed, sesame, soybean, olive, corn, linseed, etc published by Rafalowski and his colleague (2008). The PUFA have beneficiary effects on maintaining good health and also prevents chronic diseases by modulating lipid profiles on normal levels (Mori et al., 2000; Lauritzen et al., 2000), cardiovascular (Calder, 2004; Balk and Lichtenstein, 2017) and also help to acquire normal immune functions by balancing Omega – 3 and Omega – 6 FA (Asif, 2011).

Table 03. The fatty acid composition of SAU Perilla-1 seed oil

Sl. no	Test parameters	% Composition	
1.	**Total saturated fatty acids**	**8.18**	
	i) Palmitoleic acid	0.08	
	ii) Palmitic acid	6.78	**8.18**
	iii) Stearic acid	1.30	
	iv) Arachidic acid	0.02	
2.	**Total unsaturated fatty acids (TUFA)**	**91.70**	
	a) Monounsaturated fatty acids (**Oleic acid; ω - 9**)	18.47	**91.70**
	b) Polyunsaturated fatty acids (**PUFA**)	73.23	
	i) α-Linolenic acid (**ω - 3**)55.09% of TUFA and ~69% of PUFA	50.52	**73.23**
	ii) Linoleic Acid (**ω - 6**)	22.71	
3.	**Erucic acid**	None detected	
4.	Behenic acid	None detected	
5.	Lignoceric acid	None detected	

Table 04. Calculated ratios of the fatty acid detected in the SAU Perilla-1 samples

Sl. no.	Fatty acid parameters for ratio calculation	Calculated ratio of test values
1.	Saturated**:** Monounsaturated**:** Polyunsaturated	**1 : 2.26: 8.95** (8.18 : 18.47 : 73.23)
2.	Saturated**:** Unsaturated	**1 : 11.21** (8.18 : 91.70)
3.	Monounsaturated**:** Polyunsaturated	**1 : 3.96** (18.47 : 73.23)
4.	Omega - 6 (**ω-6**) **:** Omega - 3 (**ω-3**)	**1:2.22** (22.71:50.52)

In respect of PUFAs, many health issues are dependent on the balance between Omega - 3 and Omega - 6 FA. Omega - 6 is necessary for normal immune function and blood clotting, but too much of this FA may stimulate abnormal clotting and proactive immune functions (Asif, 2011). Chronic degenerative diseases are believed to be originated due to the imbalance ratio of Omega - 6 to Omega - 3 FAs in our diet. However, the healthy ratio of these FAs ranges are reported 1:1 to 4:1 (Mattson and Grundy, 1985; Renaud, 2002) which is found 1:2.22 in SAU Perilla-1, indicating that the obtained oil from this variety holds a healthy ratio of polyunsaturated fatty acids with properties to improve the ω-6 to ω-3 ratio in our diet. Today, more than 85% of the total dietary polyunsaturated fatty acid in a developed country is ω-6 polyunsaturated fatty acid, mainly linoleic acid, a precursor of arachidonic acid, whereas the consumption of ω-3 polyunsaturated fatty acid has declined (Simopoulos, 2002). Since the consumption of ω-6 has been associated with childhood obesity, concerns have been raised (Ailhaud et al., 2006). The content of the ω-6 (linoleic acid) in SAU Perilla-1 was detected 22.71% only (Table 03), which was far below those seed oils regularly used like sunflower (≈55%), corn (≈49%), and soybean (≈46%) and was comparable to those in olive oil (≈11%) and tea oil (≈20%) (Sahari et al., 2004; Rafalowski et al., 2008). So, it will be a great opportunity and choice for us to downscale the high ratio between ω-6 and ω-3 fatty acids by substituting an amount of regular edible oil with SAU Perilla-1 in our diet since the Omega fatty acids (ω-3; ω-6; and ω-9) are essential fatty acids, which are not synthesized in the human body. Therefore, it must be received from some other sources like Perilla seed oil abundant in those FAs. Our results indicate that SAU Perilla-1 oil is one of the best oil composed of essential fatty acids conducive to human health. Moreover, it also showed that SAU Perilla-1 is a good source of protein (26%) (Table 01) comparable by quality to some other oil seed crops regarding proteins.

Table 05. Selective agrometeorological indices (average values) prevailed during the growing season (Kharif–II) of SAU Perilla-1 in Dhaka, Bangladesh

Months	Rainfall (mm)	Relative Humidity (%)	Day duration (hr.)	Sunshine hours	Temperature (°C)	
					Day	Night
July	373.1	83	13:26:08	131	31.4	26.2
August	316.5	82	12:56:17	141	31.6	26.3
September	300.4	83	12:16:59	152	31.8	25.9
October	172.3	78	11:35:55	229	31.6	23.8
November	34.4	73	11:00:20	238	29.6	19.2

Source: 1. Bangladesh Meteorological Department, 2019
2. Worldwide weather forecasts and climate information, 2019

IV. Conclusion

SAU Perilla-1 (Golden Perilla BD) cultivated in Bangladesh can be a good source of essential polyunsaturated fatty acids needed by the human body. Our results indicate that SAU Perilla-1 oil is one of the best oil enriched in Omega fatty acids. Moreover, those who are vegetarian or dislike the taste of fish as an allergy or are unable to tolerate fish oil due to reflux can prefer this oil as an alternative source of omega-3 fatty acid. SAU Perilla-1 oil can be promoted as a good supplement in nutritional food for both vegetarian and non-vegetarian persons. Therefore, commercial cultivation of SAU Perilla-1 in Kharif II season could have been served not only as an additional member-crop in minimizing deficit of the national quality edible oil seed production but also saving money for importing edible oils in Bangladesh.

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2313

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