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Physico-Chemical Characterization and Oxidative Stability Studies of Eri Silkworm Oils

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Abstract: Eri silkworm pupal oils from castor and tapioca leaf fed eri pupae were studied for oxidative stability at room temperature. The storage study of both crude and refined eri pupal oils was conducted for about six months period during which, the stability of the oils was measured for every month by determining the peroxide value, iodine value, fatty acid composition along with other physico-chemical characteristics. The peroxide value increased from 2.32 to 3.95 and 2.51 to 4.39meq/kg in crude oils of castor and tapioca leaf fed pupae respectively during six months. In refined silkworm oils, the peroxide value was observed to be in the range of 2.14 to 5.45 and 2.38 to 6.03meq/kg respectively for the oils of castor and tapioca leaf fed pupae. The increase in peroxide value is higher in refined pupal oils compared to crude oils. The iodine value (IV), free fatty acids (FFA), fatty acid composition and other physico-chemical characteristics of silkworm oils of both varieties were unchanged during storage. Silkworm oils of both varieties are known to contain phospholipids and tocopherols which could be protecting the oils against the oxidation during storage period. The results from the present study show that silkworm oils of crude and refined varieties are not affected greatly by oxidation during storage at room temperatures which suggests that the oil has a good shelf life.

Keywords: Physico chemical characterization, storage studies, eri silkworm oils and peroxide value

1. Introduction

There are very few natural sources which have a high content of alpha linolenic acid (ALA). Among such sources, seeds of flax, perilla, cameline and chia are reported to contain major amounts of ALA whereas soybean and mustard oils have smaller quantities of ALA [1]. Eri silk worm (*Samia Cynthia ricini*) oil is reported to possess a high content of ALA and also projected to be a potential alternative source for ALA. Eri silkworm feeds on leaves of castor and tapioca and it was found that the pupae fed on tapioca leaves had a higher content of ALA compared to the pupae fed on castor leaves [2]. In the eri silkworm oil, the ALA content was found to be in higher quantities compared to mulberry B*ombyx mori* silkworm oils as reported in the literature [3]. The silkworm pupal oil has wide range of applications in oleochemical and food processing industries and reports suggest that this oil works as an anti-aging agent, helps in darkening gray hair and in body weight reduction [4]. In China the use of silkworm pupae as food has been practiced since ancient times and silkworm has been recently approved as a new food source by the Ministry of Health of the Republic of China [5]. The refined chrysalis silkworm pupal oil was suggested to be used in medicine, cosmetic and food as the oil contains high amounts of unsaturated fatty acids [6]. Therefore, eri silkworm pupae represents a potential source of oil that can be harvested for use in the food and feed industry by providing additional income to the farmers who are growing silkworms [7]. Moreover, eri silkworms are very easy to harvest when compared to mulberry based silkworms in terms of moisture content while handling the pupae.

The n-3 fatty acids are essential in growth and development throughout the human life cycle and should be included in the diet. The n-3 PUFAs are reported to have a positive influence on plasma cholesterol concentrations as they decrease the levels of low density lipoprotein (LDL) cholesterol levels without affecting the HDL cholesterol levels [8]. ALA is one of the nutritionally essential polyunsaturated fatty acid which is reported to be a precursor of other long chain n-3 PUFA such as EPA and DHA by enzymatic desaturation and elongation pathway [9, 10, 11]. ALA is reported to have positive biological effects on human body like accelerating brain development in neonates, anti arrhythmic and neuroprotective [9]. A recent study suggested that the silkworm oil was toxicologically safe and nutritionally equivalent to commonly used vegetable oils such as sunflower oil with added benefits due to high content of ALA [7]. However, ALA and other unsaturated fatty acids are susceptible to oxidation and undergo autoxidation leading to formation of rancidity [12]. Unsaturated fatty acids undergo lipid oxidation involving a series of chemical reactions with reactive oxygen molecular species which causes the food spoilage and deterioration [13, 14, 15, 16].

The shelf life of the oils with PUFA is decreased due to the off-flavor compounds which also produce toxic compounds depending on the oxidized

products [17, 18]. Oxidation of oils depends on the fatty acid compositions that can show effect on the storage stability of oils [19]. However, oxidation process may get delayed or slow down in case the oils contain higher amounts of natural antioxidants [20]. Hence storage and stability of oils rich in PUFA needs to be taken care of when the oil is intended to be used for edible applications. The stability studies of eri silkworm oil was not studied so far and therefore, the present study was aimed to determine the stability of both crude and refined eripupal oils extracted from pupae fed on castor and tapioca leaves.

2. Materials and Methods

2.1. Chemicals and Silkworm Pupae

Fatty acid standard mixtures (C 4-24), reference triacylglycerols, tocopherols and tocotrienols were purchased from Sigma Chemical Co., USA. Pre-coated TLC plates (silica gel 60 F254) were purchased from Merck, Darmstadt, Germany. All other analytical grade chemicals and solvents were used for the present work are purchased from SD Fine-Chemicals, Mumbai, India. The castor leaf fed silkworm pupae collected from Shadnagar in Ranga Reddy district, Telangana State and tapioca leaf fed silkworm pupae collected from Rampachodavaram in East Godavari district, Andhra Pradesh, India were supplied by Central Silk Board, Bengaluru, India.

2.2. Drying and Extraction of Silkworm Oil

The silkworm pupae grown on castor and tapioca leaves having moisture content of 73 and 70% respectively were dried completely using vacuum hot air oven at 80-90°C followed by Soxhlet extraction using hexane as reported earlier [2]. The crude oils of castor and tapioca leaf fed silkworm oils were purified by refining according to reported protocol for vegetable oils [21, 22].

2.3. Oxidative Stability Studies

Crude and refined silkworm oils of both varieties were subjected for storage studies at ambient temperature for six months according to a reported method [23]. The silkworm oils of both the varieties of crude and refined were taken in plastic containers of 1 lit capacity. The containers were closed tightly and stored at ambient temperature 25-30°C for about six months. Samples were drawn at every month avoiding longer exposure to atmospheric air and were stored at 5°C under nitrogen atmosphere for further analysis.

2.4. Physico-chemical Characterization of Pupal Oils

Both refined and crude oils of castor and tapioca leaf fed silkworm oils were analyzed for physicochemical characteristics such as for peroxide value, iodine value, free fatty acids, unsaponifiable matter, saponification value, colour and moisture content, refractive index according to AOCS methods [24] and phosphorous content was determined by following IUPAC method [25]. The density and kinematic viscosity was determined as per ASTM methods (ASTM, D4052) [26].

2.5. Preparation of Fatty Acid Methyl Esters

Fatty acid methyl esters (FAME) of silkworm oils were prepared according to the methods described by Christie [27]. The extracted pupal oils were converted in to FAME by following acid catalyzed methylation. Oil (10-20 mg) was taken in 15 ml of 2% H_2SO_4 in methanol solvent and refluxed for about 3 hours. After complete conversion of methyl esters monitored by TLC, solvent was partially removed and the remaining mixture was extracted with ethyl acetate (2 x 20 ml) and the combined ethyl acetate layers were washed with water until neutral. Then the ethyl acetate extract was dried over anhydrous sodium sulphate and evaporated under reduced pressure on a rotary evaporator to obtain FAME.

2.6. GC Analysis

The fatty acid methyl esters analysis was performed on Agilent 6890N Series Gas Chromatograph equipped with a FID and the capillary column DB- 225 (30 m x 0.25 mm i.d. x 0.5 μm film thickness). The injector and detector temperatures were maintained at 230 and 250°C respectively. The oven temperature was programmed for 2 min at 160°C, further increased to 230°C at 5°C/min and finally maintained for 10 min at 230°C. The carrier gas, nitrogen was used at a flow rate of 1.5 ml/min. The injection volume was 1 μl, with a split ratio of 50:1.

2.7. Tocopherols and Tocotrienols by HPLC

Tocopherols and tocotrienols (tocols) were determined by using High performance liquid chromatography (HPLC) according to AOCS Official method Ce 8-89 [28]. Briefly, the method describes the separation of tocols by HPLC using fluorescence detector and the column used was a normal phase silica column LiChrosorb Si-60, dimension 250 x 4.0 mm having a mean particle size of 5 μm. The fluorescence detector was set at an excitation wavelength of 290 nm and emission wavelength at 330 nm. The isocratic mobile phase consisting of hexane and isopropyl alcohol (99.5:0.5, vol/vol) at a flow rate of 1.0 ml/min was used for elution of the components. Calibration factors were determined for each tocopherol from the chromatography of solutions of standard tocopherols. The total tocopherol and tocotrienol content was expressed as micrograms per gram (μg/g). All the analyses were carried out in triplicate and average of three values is reported.

2.8. Statistical analysis

The reported results are the means of three measurements presented as means ± standard deviations (SD) and were analyzed by a paired Student's t-test to evaluate the statistical significance.

3. Results and Discussion

3.1. Physico-chemical Characterization of Pupal Oils

The oil content in the crude silkworm oils was found to be in the range of 20 to 22%. The crude oils were refined employing different steps namely degumming, neutralization, bleaching followed by

deodorization for removal of undesirable components such as gums, FFA, odor and coloring pigments. Both castor and tapioca (crude and refined) eri silkworm oils were analyzed for different physico-chemical characteristics and the results are given in Table 1.

Table 1: Physico-chemical Characteristics of Eri Silkworm Oils

Characteristic	Castor leaf fed oil		Tapioca leaf fed oil	
	Crude	Refined	Crude	Refined
FFA (%)	5.41±0.11	0.10±0.06	6.62±0.18	0.13±0.03
Moisture (%)	0.08±0.09	0.05±0.10	0.07±0.08	0.04±0.06
Peroxide value (ppm)	2.32±0.10	2.14±0.19	2.51±0.19[a]	2.38±0.14[c]
Iodine value (g/100 g)	130.90±0.31	131.23±0.16	154.42±0.17[b]	154.01±0.69[d]
Unsaponifiable matter (%)	3.33±0.41	2.83±0.16	3.02±0.10[a]	2.55±0.14[e]
Saponification value	196.45±0.28	196.22±0.17	195.43±0.26	195.35±0.21
Phosphorous (ppm)	590.21±0.15	0.0	792.0±0.28[b]	0.0
Density (g/ml)	0.9126±0.10	0.9138±0.08	0.9152±0.11	0.9175±0.04
Specific gravity	0.9148±0.06	0.9166±0.02	0.9188±0.08	0.9197±0.04
Viscosity (cSt)	32.21±0.14	33.82±0.10	35.77±0.18	37.62±0.11
Refractive index at 40°C	1.47102±0.01	1.47109±0.0	1.47126±0.06	1.47130±0.04
Colour as Y + 5 R	45	8	48	30
α- Tocopherol (ppm)	167.05±0.92	82.88±0.58	224.05±0.78[b]	93.03±0.65[d]
γ- Tocopherol (ppm)	20.87±1.10	15.80±0.14	35.17±0.72[b]	17.15±0.35[f]

Data are means ± SD for three measurements. [a] Significantly different from castor fed crude oil: $P < 0.1$. [b] Significantly different from castor fed crude oil: $P > 0.001$. [c] Significantly different from castor fed refined oil: $P < 0.1$. [d] Significantly different from castor fed refined oil: $P > 0.001$. [e] Significantly different from castor fed refined oil: $P < 0.05$. [f] Significantly different from castor fed refined oil: $P < 0.001$.

The results indicate that crude pupal oils had higher content of free fatty acids and phosphorous content in both the varieties of oils and during the refining process, both phosphorous and free fatty acids were reduced to minimum levels as per the specifications of common refined edible oils. After refining, the colour of both the oils was found to be improved compared to the crude oils. The peroxide value was found to be reduced slightly for refined oils and the iodine value was found to be higher in tapioca based oil as expected because of higher unsaturation in the oil compared to to castor fed pupal oil. Both crude and refined silkworm oils of the two varieties were determined for tocopherol and tocotrienols content by HPLC. It was found that tapioca leaf fed silkworm oils had a higher content of both alpha and gamma tocopherols compared to castor fed silkworm oils. Alpha tocopherol is the major tocopherol in both the eri silkworm oils and the same trend was observed in earlier reports for the mulberry leaf fed silkworm oil [29]. However, there was a decrease in tocopherol content during the refining process in both the varieties of eri silkworm oils compared to the initial content.

The fatty acid compositions of eri silkworm oils were determined by GC and are shown in Table 2. The data showed that ALA was high in tapioca leaf fed pupal oil compared to castor leaf fed pupal oils which was due to the influence of host plant as previously reported [2]. ALA content was observed more in both castor (41.56%) and tapioca leaf fed eri silkworm oils (52.74%) compared to oak and mulberry based silkworm oils where the ALA content was reported to be 34.27 and 38.02% respectively. However, the total unsaturated fatty acids were observed to be more in oak silkworm oil compared to castor and tapioca leaf fed eri silkworm oils [30].

Data are means ± SD for three measurements. [a] Significantly different from castor fed crude oil: $P < 0.001$. [b] Significantly different from castor fed crude oil: $P < 0.01$. [c] Significantly different from castor fed crude oil: $P > 0.001$. [d] Significantly different from castor fed refined oil: $P > 0.001$.

3.2. Oxidative stability studies

Castor and tapioca leaf fed eri silkworm oils being a good source of ALA can be used in nutraceutical and food applications. However, these fatty acids are unstable and are susceptible to oxidation [12]. The storage of any oil and fat is necessary to maintain certain quality parameters for industrial operations and applications [31]. Hence, the storage stability study was conducted to determine the oxidative stability of the eripupal oils at 25-30°C for six months. The present study was aimed to study the primary oxidation status of the eri silkworm oils by determining the peroxide value along with other physico-chemical parameters. The physico-chemical characteristics of crude silkworm oils of castor and tapioca leaf fed were collected for every month during storage are shown in Table 3 and 4 respectively.

The results revealed that, there was a slight increase in peroxide value in both the silkworm oils during the storage period at ambient temperature. The peroxide values of the samples increased from 2.32 to 3.95 and 2.51 to 4.39 in castor and tapioca leaf fed eri silkworm oils respectively during the study period which is shown in Figure 1. The colour of the pupal oils

Table 2: Fatty Acid Composition of Castor and Tapioca Leaf fed Eri Silkworm Oils

Fatty acid	Castor fed silkworm oil		Tapioca fed silkworm oil	
	Crude	Refined	Crude	Refined
Myristic acid (14:0)	0.48±0.11	0.41±0.01	0.30±0.01	0.31±0.01
Palmitic acid (16:0)	29.43±1.73	28.25±0.21	23.51±0.14[a]	23.80±0.28[d]
Palmitoleic acid (16:1)	1.61±0.41	1.85±0.07	1.10±0.01	1.11±0.02
Stearic acid (18:0)	4.59±1.54	3.72±0.02	4.40±0.13	4.52±0.07
Oleic acid (18:1)	17.22±1.52	18.85±0.21	13.53±0.08[b]	13.50±0.14[d]
Linoleic acid (18:2)	5.10±1.13	5.85±0.21	4.42±0.45	4.15±0.07
α-Linolenic acid (18:3)	41.56±0.33	41.05±0.07	52.74±0.21[c]	52.61±0.03[d]

Table 3: Characteristics of Crude Castor Leaf fed Silkworm Pupae Oil during 6 Months Storage Study

Characteristic	Time (months)						
	0	1	2	3	4	5	6
FFA (%)	5.41±0.11	5.44±0.06	5.59±0.06	5.57±0.03	5.61±0.05	5.66±0.09	5.69±0.10
PV (ppm)	2.32±0.10	2.52±0.06	2.82±0.13	3.0±0.21	3.20±0.16	3.65±0.28	3.95±0.14
IV (g/100 g)	130.90±0.31	131.15±0.42	131.06±0.19	130.53±0.29	130.23±0.26	130.02±0.17	130.04±0.14
Unsap matter (%)	3.33±0.41	3.22±0.15	3.31±0.25	3.19±0.11	3.21±0.08	3.17±0.17	3.22±0.14
Sap value	196.45±0.28	196.98±0.19	196.38±0.11	196.43±0.06	196.22±0.09	196.35±0.10	196.42±0.11
P' Content (ppm)	590.21±0.15	590.64±0.13	590.46±0.06	590.74±0.13	590.45±0.14	590.32±0.10	590.15±0.09
Colour as Y+5R	45	45	45	45	45	45	45

Table 4: Characteristics of Crude Tapioca Leaf fed Silkworm Pupae Oil during 6 Months Storage Study

Characteristic	Time (month)						
	0	1	2	3	4	5	6
FFA (%)	6.62±0.18	6.55±0.14	6.60±0.15	6.65±0.21	6.72±0.32	6.82±0.25	6.90±0.21
PV (ppm)	2.51±0.19	2.80±0.14	3.09±0.16	3.55±0.24	3.76±0.12	4.03±0.17	4.39±0.13
IV (g/100 g)	154.42±0.17	154.79±0.22	154.24±0.12	154.02±0.17	154.0±0.07	153.94±0.23	153.59±0.29
Unsap matter (%)	3.02±0.10	3.11±0.23	2.93±0.11	3.05±0.28	3.13±0.32	2.95±0.13	2.91±0.12
Sap value	195.43±0.26	195.01±0.13	196.0±0.21	194.97±0.24	195.03±0.11	195.08±0.24	195.92±0.10
'P' Content (ppm)	792.0±0.28	792.95±0.21	791.71±0.37	792.0±0.21	790.63±0.81	791.15±1.55	790.82±0.24
Colour as Y+5R	48	47.5	48.5	48	47.5	48	48

was stable and was not changed during six months study. Also the results indicate that other parameters like unsaponifiable matter, iodine value, saponification value and phosphorous content were not altered to initial values of the study.

The fatty acid composition of crude castor and tapioca leaf fed silkworm oils collected during storage period are given in Tables 5 and 6. In castor and tapioca leaf fed silkworm oils α-linolenic acid is the major fatty acid, which was not changed during six month study at 25-30°C. The results of the study indicated that, all other fatty acids of castor and tapioca fed silkworm oils remained same during the study period. The double bonds of polyunsaturated fatty acids were not deteriorated in silkworm oils of castor and tapioca leaf fed oils, which was also confirmed by iodine value and fatty acid compositional analysis by GC method.

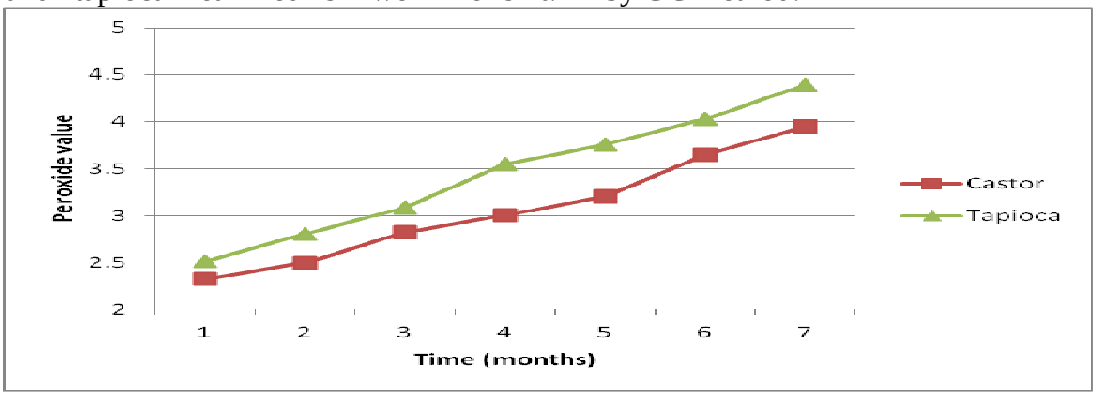

Figure 1: Change of PV of crude eri pupal oils during storage period.

Table 5: Fatty Acid Composition (wt %) of Crude Castor fed Silkworm Oil during 6 Months Storage Study

Fatty acid	**Time (month)**						
	0	**1**	**2**	**3**	**4**	**5**	**6**
Myristic acid (14:0)	0.48±0.11	0.49±0.01	0.45±0.07	0.42±0.02	0.43±0.01	0.45±0.03	0.47±0.05
Palmitic acid (16:0)	29.43±1.73	28.45±0.07	29.55±0.07	28.78±0.11	28.87±0.01	28.92±0.05	29.36±0.19
Palmitoleic acid (16:1)	1.61±0.41	1.90±0.01	1.85±0.07	1.95±0.06	1.92±0.03	1.93±0.09	1.88±0.01
Stearic acid (18:0)	4.59±1.54	3.71±0.03	3.63±0.03	3.69±0.01	3.75±0.09	3.73±0.19	3.75±0.12
Oleic acid (18:1)	17.22±1.52	19.05±0.07	18.42±0.02	18.97±0.10	19.10±0.02	18.97±0.11	18.90±0.13
Linoleic acid (18:2)	5.10±1.13	5.75±0.07	5.45±0.07	5.71±0.13	5.63±0.06	5.74±0.14	5.54±0.06
α-Linolenic acid (18:3)	41.56±0.33	40.65±0.05	40.65±0.08	40.38±0.16	40.16±0.18	40.22±0.33	40.06±0.18

Table 6: Fatty Acid Composition (wt %) of Crude Tapioca fed Silkworm Oil during 6 Months Storage Study

Fatty acid	**Time (month)**						
	0	**1**	**2**	**3**	**4**	**5**	**6**
Myristic acid (14:0)	0.30±0.01	0.30±0.01	0.30±0.01	0.29±0.01	0.30±0.01	0.39±0.13	0.30±0.01
Palmitic acid (16:0)	23.51±0.14	23.66±0.34	24.02±0.11	24.28±0.12	24.04±0.05	24.89±0.22	24.94±0.05
Palmitoleic acid (16:1)	1.10±0.01	1.11±0.01	1.11±0.01	1.18±0.02	1.13±0.04	1.19±0.09	1.12±0.01
Stearic acid (18:0)	4.40±0.13	4.45±0.22	4.55±0.21	4.51±0.01	4.58±0.03	4.50±0.03	4.64±0.03
Oleic acid (18:1)	13.53±0.08	13.58±0.03	13.55±0.07	13.38±0.17	13.59±0.29	13.32±0.19	13.43±0.15
Linoleic acid (18:2)	4.42±0.45	4.38±0.38	4.14±0.06	4.16±0.23	4.08±0.04	4.32±0.06	4.34±0.03
α-Linolenic acid (18:3)	52.74±0.21	52.40±0.01	52.26±0.05	52.19±0.16	52.11±0.01	51.36±0.12	51.19±0.13

Table 7: Characteristics of Refined Castor fed Silkworm Oil during 6 Months Storage Study

Characteristic	**Time (month)**						
	0	**1**	**2**	**3**	**4**	**5**	**6**
FFA (wt %)	0.10±0.06	0.11±0.06	0.10±0.06	0.12±0.04	0.13±0.09	0.13±0.10	0.15±0.11
PV (ppm)	2.14±0.19	2.82±0.24	3.55±0.18	4.23±0.16	4.68±0.10	5.11±0.18	5.45±0.14
Sap value	196.22±0.17	196.12±0.18	196.32±0.10	196.21±0.15	196.24±0.08	196.17±0.24	196.15±0.21
Unsap matter	2.83±0.16	2.90±0.14	2.84±0.12	2.78±0.05	2.73±0.12	2.53±0.17	2.55±0.09
IV	131.23±0.16	130.98±0.19	130.75±0.15	130.80±0.22	130.50±0.17	130.45±0.10	130.26±0.16
P' Content (ppm)	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Colour as Y+5R	8	8	8	8	8	8	8

Table 8: Characteristics of Refined Tapioca Fed Silkworm Oil during 6 Months Storage Study

Characteristic	**Time (month)**						
	0	**1**	**2**	**3**	**4**	**5**	**6**
FFA (wt %)	0.13±0.03	0.14±0.06	0.16±0.06	0.15±0.13	0.16±0.08	0.17±0.07	0.18±0.10
PV (ppm)	2.38±0.14	3.12±0.17	3.83±0.18	4.45±0.28	5.08±0.24	5.67±0.38	6.03±0.16
Sap value	195.35±0.21	194.96±0.19	195.01±0.12	195.23±0.12	195.82±0.24	196.01±0.15	195.47±0.25
Unsap matter	2.55±0.14	2.62±0.17	2.50±0.35	2.35±0.28	2.38±0.19	2.41±0.15	2.33±0.10
IV	154.01±0.69	154.06±0.34	153.83±0.24	153.22±0.17	153.05±0.28	153.0±0.21	152.65±0.14
P' Content (ppm)	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Colour as Y+5R	30	30	30	30	30	30	30

Further, the crude oil was refined and the physico chemical characteristics of refined oils of both pupal varieties collected for every month during storage are shown in Table 7 and 8.The results showed an increase

in peroxide value in both varieties of refined silkworm oils during six months which is shown as Figure 2.

The peroxide value of the samples increased from 2.14 to 5.45 and 2.38 to 6.03 meq/kg in the refined pupal oils. According to earlier report on the oxidative stability of linseed oil at room temperature, it was found that peroxide value was in the range of 2.0-6.2 during storage period for 0-7 months [32]. However, in another report on linseed oil having moisture content of 9.5%, an increase in peroxide value from 2.1 to 26.7meq/kg was observed during the storage at 28°C [33]. The eri silkworm oils of both the varieties used in this study were dried completely under reduced pressure which resulted in very low moisture contents (<0.1%) which could have helped in keeping the peroxide value at lower levels. The peroxide value is the most common parameter used to characterize the oils and fats and it is suggested that products with peroxide values between 1 and 5 meq/kg are classified to be at low oxidation state [34]. Hence, the PV in the silkworm oils is within the level of lower oxidation state even after storage for about six months. Increase of oxidation in terms of peroxide value was observed to be more in refined oils compared to crude pupal oils which could be due to synergetic effect between phospholipids and tocopherols present in silkworm

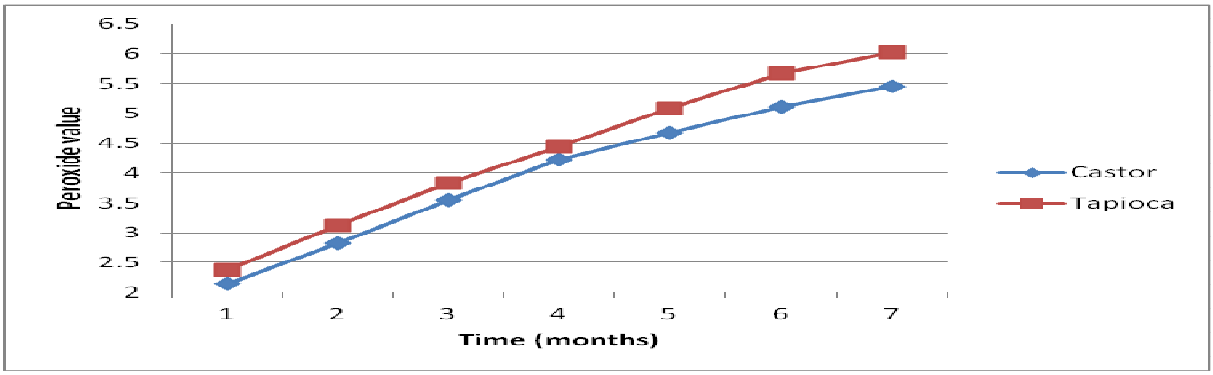

Figure 2: Change of PV of refined eri pupal oils during storage period.

Table 9: Fatty Acid Composition of Refined Castor fed Silkworm Oil during 6 Months Storage Study

Fatty acid	Time (month)						
	0	1	2	3	4	5	6
Myristic acid (14:0)	0.41±0.01	0.41±0.02	0.42±0.02	0.46±0.01	0.42±0.02	0.42±0.12	0.51±0.09
Palmitic acid (16:0)	28.25±0.21	29.07±0.18	28.62±0.16	29.20±0.36	28.68±0.25	29.13±0.60	29.38±0.70
Palmitoleic acid (16:1)	1.85±0.07	1.90±0.14	1.93±0.10	1.89±0.03	1.95±0.07	1.74±0.21	1.94±0.03
Stearic acid (18:0)	3.72±0.02	3.65±0.06	3.82±0.02	3.79±0.13	3.81±0.01	3.75±0.08	3.70±0.04
Oleic acid (18:1)	18.85±0.21	18.85±0.07	19.23±0.09	18.86±0.24	19.19±0.16	18.96±0.23	19.0±0.15
Linoleic acid (18:2)	5.85±0.21	5.57±0.10	5.75±0.08	5.59±0.29	5.74±0.08	5.94±0.20	5.44±0.22
α-Linolenic acid (18:3)	41.05±0.07	40.48±0.03	40.14±0.08	40.17±0.26	40.12±0.11	40.06±0.62	40.01±0.60

Table 10: Fatty Acid Composition of Refined Tapioca fed Silkworm Oil during 6 Months Storage Study

Fatty acid	Time (months)						
	0	1	2	3	4	5	6
Myristic acid (14:0)	0.31±0.01	0.30±0.01	0.29±0.01	0.30±0.0	0.3±0.01	0.30±0.01	0.3±0.01
Palmitic acid (16:0)	23.80±0.28	24.35±0.35	24.20±0.14	24.65±0.07	24.94±0.06	24.99±0.03	25.55±0.21
Palmitoleic acid (16:1)	1.11±0.02	1.10±0.01	1.05±0.14	1.10±0.01	1.06±0.08	1.12±0.02	1.21±0.04
Stearic acid (18:0)	4.52±0.07	4.15±0.21	4.61±0.02	4.05±0.07	4.63±0.03	4.49±0.01	4.65±0.07
Oleic acid (18:1)	13.50±0.14	13.6±0.14	13.61±0.03	13.50±0.14	13.32±0.12	13.20±0.01	12.94±0.07
Linoleic acid (18:2)	4.15±0.07	4.15±0.07	4.13±0.04	4.15±0.07	4.34±0.02	4.29±0.01	4.50±0.14
α-Linolenic acid (18:3)	52.61±0.03	52.34±0.06	52.11±0.17	52.20±0.14	51.34±0.23	51.50±0.08	50.85±0.07

oils [29]. In refined silkworm oils, tocopherol content is observed in lower amounts compared to crude oils and this could be the reason for the observed low peroxide value in crude silkworm oils compared to refined oils. Silkworm oils contain natural tocopherols, which are good source of vitamin E and are known to be having antioxidant properties in protection of oils [33]. There are literature reports on the oxidative stability of both pupae as such and its oil where the authors studied the pupal powder and the oil as antioxidative agents to protect the food products [35, 36]. A small change was observed in iodine value of refined castor and tapioca fed pupal oils during storage period. The colour of the pupal oils was not changed during six months study and there was no change in FFA, colour, unsaponifiable matter and saponification value of refined castor and

tapioca fed oils.The fatty acid composition of refined castor and tapioca leaf fed pupal oils were determined by GC and the results are given in Tables 9 and 10.

The results showed that, the ALA content in refined eri silkworm oils of both varieties was not altered drastically during six month study at 25-30°C. The ALA content in refined castor fed silkworm oil was 41.05% and after six months it was observed to 40.01%. Similarly in tapioca fed refined silkworm oil, 52.61% of ALA was observed initially and after six months, it was observed to be 50.85%. In a reported study it was found that, there was no significant change in fatty acid composition of linseed oil, soybean oil and other oils during the storage at 28 and 55°C [37]. In comparison, the present study also shows that the fatty acid composition was not influenced during the storage period as observed for linseed and soybean oils. Hence the shelf life of eri pupal oils was found to be stable at ambient temperature which widens its application in products which can be developed based on eri silkworm oil.

4. Conclusion

In the present study, castor and tapioca leaf fed eri silkworm oils were studied for oxidative stability studies at 25-30°C for six months period. Physico-chemical characteristics of both the varieties of silkworm oils were determined during storage period at every month. A slight increase was observed in peroxide value of both the varieties of silkworm oils, which was slightly more in refined silkworm oils compared to crude silkworm oils for both the varieties. Oxidation stability was found to be superior in the case of crude oils compared to refined oils which could be due to the presence of tocopherols and phospholipids in crude oils. The study suggests that the eripupal oils exhibit good storage stability.

5. References

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