



# **Physio-Chemical Properties and Oxidative Stability of Microencapsulated Silkworm Pupae Oil Enriched Mayonnaise**

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## **Authors' contributions**

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## **ABSTRACT**

This study investigates to enrich the mayonnaise with novel oil replacer which is from silkworm pupae insect. Due to their health benefits, the well-known omega-3 and omega-6 fatty acid families are the most significant polyunsaturated fatty acids (PUFA) demanded in foods. Insects are traditionally consumed as food in different parts of the world since they are both a good source of protein and rich in essential fatty acids (EPA and DHA). The product Mayonnaise was developed using Encapsulated Silkworm Pupae Oil powder (Mayo-ESOP of 10 g) and Silkworm Pupae Oil (Mayo-SPO OF 10 ML) and was compared with control mayonnaise prepared out of sunflower oil.

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The mayonnaise samples were analysed for oxidative stability through peroxide values at 0, 7, 14 and day 21 days and after preparation of physical parameters such as droplet size for surface ( $\mu\text{m}$ ), volume ( $\mu\text{m}$ ), viscosity ( $\gamma=10\text{ s}^{-1}$ ), proximate analysis, colour values ( $L^*$ ,  $a^*$  and  $b^*$ ) fatty acids profile. The study revealed that Mayo-ESOP and Mayo-SPO samples showed better oxidative stability as a result of this not affecting any quality aspects of mayonnaise samples. The proximate composition among the three variations of mayonnaise Mayo-SPO was found to have a maximum fat content of  $76.13\pm 0.01\text{g}$  per 100 g, followed by Mayo-ESOP  $72.3\pm 0.01\text{g}/100\text{ g}$  and Control  $66.21\pm 0.01\text{g}/100\text{ g}$  respectively. The fat composition indicates that silkworm pupae have a very good fatty acid profile, which includes omega 9 and omega 6. The colour values ( $L^*$ ,  $a^*$ ,  $b^*$ ) and the total colour difference represented as ( $\Delta E$  values) had no much significant difference show the yellowness colour for mayonnaise sample compared to control due to the presence of carotenoids. Overall, the colour assessment reveals that the oil was of good quality. Oxidative stability indicates the mayonnaise samples stored at a refrigerated temperature for 0, 7, 14, and 21 days had significantly difference on peroxide values due to oxidation of mayonnaise samples.

**Keywords:** *Mayonnaise; silkworm pupae oil; colour values; fatty acid profile and oxidative stability.*

## 1. INTRODUCTION

Insects are traditionally eaten as food in several different portions of the world since they are both a good source of protein and very nutritious. The practice of entomophagy in India depicts that various tribes in our nation eat roughly 255 distinct species of insects. Coleopteran Species make up the largest portion of these edible insect species, accounting for approximately 34% of all consumption, followed by Orthoptera (24%), Hemiptera (17%), and Hymenoptera (10%). Tribes have chosen insects as food based on their regional preferences, culinary preferences, seasonal availability, and traditional beliefs. All phases of development involve the consumption of insects. Roasting or boiling are the primary methods used to prepare edible insects for consumption. Insects' flavour is enhanced by the addition of spices. Entomophagy is quite widespread among the ethnic groups of people of Meghalaya and Mizoram has the fewest instances of this practice. It is necessary to shed light on entomophagy studies and to encourage entomophagy research in order to catalogue all edible insects and the manner in which they are consumed by diverse tribal people in India [1].

Silkworm (*Bombyx mori*) was consumed in China, Thailand and north-eastern India. China is the highest producer of silkworm followed by India. After the use of silkworm cocoon for reeling yarn, the pupae of the silkworm were discarded. The pupae are a waste material that is rich in many nutrients. Silkworm pupae contain 35% fat which was extracted by soxhlet extraction. Silkworm pupae oil showed 28 fatty acids; among them (12.75%) were Saturated

Fatty Acids (SFA) and 86.61% of Unsaturated Fatty Acids (USFA). Unsaturated fatty acids contain Monounsaturated fatty acids (34.97%) and Polyunsaturated fatty acids (51.64%). Around 28.35% of essential fatty acids like linoleic acid, arachidonic acid and docosahexaenoic acids were also present [2].

Consumer expectations for the manufacturing of functional foods have significantly expanded during the last few decades. Functional foods have a remarkable impact on maintaining good health, extending life, and reducing the development of chronic diseases. The well-known omega-3 and omega-6 fatty acid families are the most significant PUFA. Both of these fatty acid families share the same long-chain enzymes, and they both start out as precursors ALA for n-3 families and linoleic acid for omega-6 families end up as terminal products after a series of elongation and desaturations during metabolism. The human body cannot produce these precursor substances, but it can absorb them into longer-chain derivatives, making them necessary. Additionally, the diet must provide the body with the fatty acids it requires. Many different ailments, including autoimmune disorders, certain forms of cancer and cardiovascular disorders, have been linked to the absence of EFA in a typical diet. For daily  $\omega$ -3 supplementation, it is crucial to produce low-cost edible oil with a high concentration of  $\omega$ -3 fatty acids. Silkworm pupae oil is a suitable option in this case. A lucrative oil type called crude SPO is taken out of silkworm pupae. The primary by-product of mulberry silkworms (*Bombyx mori* L.), which are primarily utilised as fertiliser and feed, is a silkworm pupa, which is currently classified as industrial waste [3].

Insect as food has environmental benefits with respect to less special usage and H<sub>2</sub>O requirement. For example, 150 g grasshopper requires less water (3.78 L) than same amount of cattle meat which requires 3290 litres and for beef 10 times more plant materials is needed to one kilogram of insect biomass [4]. In some parts of the world, pupae are used as food. However, the spent silkworm pupae discarded after reeling the silk yarn which. they are frequently thrown away, which results in a loss of possible nutrients and a significant environmental problem [5].

For reducing lipid oxidation generally, encapsulation techniques were used extensively when enhancing food with omega-3 and omega-6 fatty acids. The purpose of this research is to study the oxidative stability and physical characteristics of mayonnaise enriched with microencapsulated silkworm pupae oil. During a storage period of up to 21 days, assessed oxidative stability of the enriched mayonnaise was along with control.

## 2. MATERIALS AND METHODS

### 2.1 Experiment Sample Procurement

Silk growing farmer's cooperative society® in the Chintamani taluk of Karnataka, India, supplied the mulberry silkworm cocoon. Silkworm cocoons for Eri and Muga were provided by the Indian state of Assam farmers who raise silkworms. By cut opening the cocoons, live silkworm pupae were separated and preserved in freezing conditions at -14 °C until they were used. The pupae of silkworms were then dried completely for 48 hours by being placed in a freeze dryer at -80 °C. For subsequent experimental examination, dried silkworm pupae were ground into a powder, sealed in HDPE, and maintained at 4°C in the refrigerator. The Silkworm Pupa Oil (SPO) and Encapsulated Silkworm Oil Powder (ESOP) were used for fortifying the mayonnaise encapsulated from silkworm pupae in the research work.

### 2.2 Preparation of Mayonnaise

The standard method and formulation for mayonnaise preparation is presented in Table 1. For the control sample, one whole egg was taken, and whisked well along with a required proportion of salt, sugar and vinegar. As the egg turns foamy, then slowly edible oil is incorporated and whisked continuously till it holds a fluffy texture. The same procedure was followed for enriched mayonnaise, where ESOP and SPO were incorporated by replacing the edible oil content and whisking continuously till it held the fluffy texture. The prepared mayonnaise was subjected to further analysis.

### 2.3 Analysis of Proximate Composition of Mayonnaise Formulation

The developed product's Nutritional composition of the mayonnaise was analysed using standard methods in the laboratory of NIFTEM, Thanjavur. Proximate composition for protein, fat, ash and crude fiber was analysed using AOAC (2006) by [6], and the carbohydrate values were computed using the Indian food composition table.

### 2.4 Analysis of Oxidative Stability of the Mayonnaise Samples

Mayonnaise samples were subjected to an oxidative stability study through peroxide values. To determine the peroxide value, 2g of oil was dissolved in 25 ml of chloroform and acetic acid (2:3 by volume ratio), 1 ml of potassium iodide solution, which was saturated and left in the dark for ten minutes. Then 30 ml of distilled water was added with 1% starch solution, and the mixture was titrated with sodium thiosulfate (0.01N) until the blue colour disappeared [7]. The samples were stored in a glass bottle for three weeks at a refrigerated temperature (4°C), and Samples were used at 0,7,14 and 21 days.

**Table 1. Formulation used for the micro-encapsulated oil powder and silkworm pupae oil**

Ingredients	Control	Mayo ESOP	Mayo SPO
Edible oil (ml)	50	40	40
Egg (no.)	1	1	1
Salt (g)	2	2	2
Sugar (g)	5	5	5
Vinegar (ml)	3	3	3
*ESOP (g)	-	10	-
*SPO (ml)	-	-	10

\*ESOP: Encapsulated oil powder \*SPO: Silkworm pupae oil

## 2.5 Analysis of Droplet Size and Apparent Viscosity

Mayonnaise samples were taken out after 21 days of storage at 25°C, and droplet size dispersion & viscosity of mayonnaise were evaluated. To measure the dispersion of droplet size persistence, 1 g of mayonnaise was liquified at a ratio of 1:5 (w/w) in sodium dodecyl sulphate (SDS) buffer (10-mM NaH<sub>2</sub>PO<sub>4</sub>) with pH 7 [8]. To prevent droplet aggregation, the solution was sonicated for 15 minutes. The results were represented in surface area with the mean diameter (µm), and volume weighed with the mean diameter (µm), and measurements were performed in triplicates.

A Brookfield Prime digital viscometer (Model DV-1) was used to analyse the viscosity of mayonnaise samples with a 100 rpm rotating speed at 25°C. The upper plate and bottom base (PP 25) both had a 2 mm gap. Up to 65 Pa, a gradient of increasing stress was applied.

## 2.6 Color Analysis

The colour of mayonnaise is considered to have an important role in determining its appearance and overall acceptability. This test's objective was to determine the extent to which the inclusion of silkworm pupae oil changed the colour of samples of mayonnaise. The colour parameters (L, a, b) of silkworm pupae powder were calculated using a Hunter colour flex model. It operates on the concept of capturing light and measuring energy from a sample that has been reflected throughout the overall visible spectrum. Each time the equipment was standardised with standards like white and black. Samples were scanned to determine the L\*, a\* and b\* where L\* indicates lightness and darkness, a\* indicates red (+a) and green (-a), b\* indicates blue (+b) and yellow (-b). [9].

## 2.7 Analysis of Fatty Acid Profile using GCMS

To study the fatty acid composition, Silkworm Pupae Oil was first transformed into FAME (Fatty acid methyl esters). A sample of 500 µl silkworm pupae oil was taken, and 2 ml of dichloro methane (DCM) and sodium methoxide were added. The sample was then vortexed for 2 min, and it was then kept in a water bath at 40 °C until it evaporated up to 95%. The sample is then cooled, and 5 ml of distilled water is added and vortexed for another 2 to 3 minutes. The top

layer of the supernatant is then collected, and the sample is injected into the GC unit. GC Column Rtx-5MS equipment 8890GC/5977B GC/MSD Agilent. Carrier gas 1ml/min with single quadrupole mass spectrometer detector used, then 2 µl sample injected with Oven temperature-110 °C and raised up to 200 °C at the rate of 10 °C/min-no hold up to 280 °C. The mass spectrometer programme was established using the NIST Version 2020 library. The electron energy was 70 eV, the source temperature was 250 °C, the inlet line temperature was 290 °C, and the entire run time was around 40 minutes [10].

## 2.8 Statistical Analysis

Using ANOVA and grouping data from the Tukey's range test, all the raw data were entered into the Minitab programme to establish the significance of the differences between mayonnaise samples. Differences between means were measured as significant at  $P \leq 0.05$  [11].

## 3. RESULTS AND DISCUSSION

### 3.1 Effect of Proximate Composition on Formulated Mayonnaise

The proximate composition of the mayonnaise was analysed using the AOAC method, and the results are presented in Table 2. Among the three variations of mayonnaise, Mayo-SPO was found to have the maximum fat content of 76.13±0.01g per 100 g, followed by Mayo-ESOP 72.3±0.01g/100 g and control showed 66.21±0.01g/100 g respectively due to addition of ESOP and SPO to mayonnaise. The fat composition indicates that silkworm pupae have a very good profile of fatty acid profile, which also includes omega 9 and omega 6 fatty acids shown in Table 5. Mayo-ESOP has a good amount of protein 6.5±0.01g/100 g, followed by Mayo-SPO 5.9±0.01g/100 g and Control 5.4±0.01g/100 g, respectively. There is no significant difference in moisture and ash content of mayonnaise variations, *i.e.*, 18.43±0.01% (Control), 17.03±0.01% (Mayo-ESOP), and 17.92±0.01% (Mayo-SPO), respectively. The ash content was found in Mayo-SPO 1.7±0.01g/100 g followed by Mayo-ESOP 1.5±0.01g/100 g and Control 1.4±0.01g/100 g, respectively. For the control 2.53±0.01g/100 g, Mayo-SPO 2.62±0.01g/100 g and Mayo-ESOP 2.78±0.01g/100 g were found to be low in carbohydrates and crude fibre present in all the

mayonnaise samples such as control ( $0.8\pm 0.01\text{g}/100\text{g}$ ) followed by Mayo-SPO ( $0.61\pm 0.01\text{g}/100\text{g}$ ) and Mayo-ESOP ( $0.6\pm 0.01\text{g}/100\text{g}$ ) respectively. The study conducted by [12] is creditable with present study.

### 3.2 Droplet Size and Viscosity of Formulated Mayonnaise

The size of the mayonnaise droplets was measured before and after 21 days storage period Table 3. represents that, as compared to the control mayonnaise prepared from sunflower oil, the mayonnaise enhanced with encapsulated silkworm pupae oil powder (Mayo-ESOP) and silkworm pupae oil (Mayo-SPO) displayed considerably ( $P < 0.05$ ) greater D (4,3) values after 21 days while it showed no much significant changes seen in the D3,2 values. At the same time, D4,3 includes data on the droplet size of the mayonnaise prepared. The D3,2 primarily represents the size of the small droplets existing in the emulsion-like substance [13]. As a result, as related to the presence of sunflower oil control, the reduction in droplet size during the production of mayonnaise was significantly reduced in the presence of Mayo-ESOP.

Likewise, [14] reported a bigger droplet size for mayonnaise enriched with Mayo-SPO when compared to the control. Nevertheless, Mayo-SPO had a larger droplet size compared to the control. This might be explained by the fact that Mayo ESOP was still present in the mayonnaise matrix, which prevented the droplet size from being reduced too much during homogenisation. On the other hand, it's possible that the control matrix, when added to the mayonnaise-based watery matrix, disintegrated, encouraging homogenisation. The droplet size of Mayo-ESOP greatly increases during storage, as evidenced by the D4,3 values in Table 3, Despite the fact that no creaming was seen. On the other hand, after 21 days of storage, Mayo-D4,3 SPOs dropped. This indicated a physical destabilisation in Mayo-ESOP caused by flocculation or coalescence, whereas possible floccules in Mayo-SPO after manufacturing vanished during storage. In mayonnaise products, these occurrences were reported by [14].

The apparent viscosity values for the mayonnaise samples were at a shear rate of  $10\text{ s}^{-1}$ . The Mayo-ESOP demonstrated a much higher apparent viscosity than the Mayo-SPO and control mayonnaise, it was found. These outcomes are consistent with previous reports of [8], who claimed that the microcapsules had

agglomeration effect in the aqueous phase was the cause of the mayonnaise samples with increased viscosity when it was enhanced with microcapsules. The Mayo-ESOP and M-SPO samples showed greater physical stability as a result of this fact was linked to an increase in viscosity. Overall, the mayonnaise fortified by the silkworm pupae oil-based enrichment system had a high level of physical stability and enhanced viscosity.

### 3.3 Colour Values of the Formulated Mayonnaise

The silkworm pupae powder colour parameters (L, a, b) were calculated using a Hunter colour flex model. It operates on the theory of absorbing light and measuring energy from a sample that has been reflected throughout the whole visible spectrum. Each time the equipment was standardised with standards like white and black. Samples were scanned to determine the L\*, a\* and b\* where L\* indicates lightness and darkness, a\* indicates red (+a) and green (-a), and b\* indicates blue (+b) and yellow (-b). The colour values assessed are tabulated in Table 4. for mayonnaise variations, which shows that the mayonnaise with L value was light in colour in all three mayonnaise variations (Control:  $84.56\pm 0.005$ , Mayo-ESOP:  $83.06\pm 0.005$  and Mayo-SPO:  $80.49\pm 0.005$ ) followed by a parameter indicated by slightly negative value (Mayo-ESOP:  $-1.29\pm 0.002$ , Mayo-SPO:  $-0.70\pm 0.005$  and control:  $-0.63\pm 0.005$ ) resulting in slightly green tinge and b parameter having positive values (Control:  $12.46\pm 0.005$ , Mayo-SPO:  $11.41\pm 0.005$  and Mayo-ESOP:  $10.82\pm 0.005$ ) indicates as the oil is yellowness in colour, this is due to the presence of carotenoids in the silkworm pupae oil [15]. The total colour values had no significant difference among ( $\Delta E$ ) for SPO and mayo ESOP. Overall, the colour assessment reveals that the mayonnaise had an acceptable colour appearance compared to the control.

### 3.4 GCMS - Fatty Acid Profile of Formulated Mayonnaise

Fatty acid profiles of three distinct types of mayonnaise are listed in Table 5, which shows that the samples are good sources of omega 9 and omega 6 fatty acids as well as unsaturated fatty acids. Linoleic acid ( $\omega$ -6) is a very good source in Mayo-SPO (48.44%), Mayo-ESOP (47.70%), and control (45.77%) samples All three samples of mayonnaise show a strong source of PUFA and MUFA. Yan et al., [16] reported that

silkworm pupae oil can be alternate source of alpha linolenic acid that having better absorption and greater digestive properties. It also contains a minimal quantity of Eicosanoic acid and Docosanoic acid. The therapeutic benefits of eicosanoic acid and docosanoic acid can be used to treat coronary heart disease [17]. Similar study done for enrichment of mayonnaise from fish oil encapsulated with zein protein shown better oxidative stability and quality of mayonnaise [8].

### 3.5 Oxidative Stability of Formulated Mayonnaise

All the mayonnaise samples stored at a refrigerated temperature for 0, 7, 14, and 21 days had significantly different peroxide values

( $P < 0.05$ ), which are depicted in Fig. 1. The rancidity of the mayonnaise samples was shown by the progressive rise in peroxide values from initial values 1.13 mEq/kg on day 0 to 3.91 mEq/kg on day 21 of oil during storage due to oxidation of oil content in samples, but the stability was high in Mayo ESOP and Mayo SPO as it contained high amounts of antioxidants and also got better protection from encapsulation of oil as well as it was stored in refrigerated conditions had more oxidative stability for the longer storage period. Mayonnaise samples have good keeps quality when it is stored in refrigerators, and their peroxide readings are less than 10meq/kg oil that can be considered as safer to consume. Similar results were reported for fish oil enriched mayonnaise reported by [8].

**Table 2. Proximate composition of formulated mayonnaise (Mayo-ESOP and Mayo-SPO) compared with the control sample**

Proximate composition	Mayo Control	Mayo ESOP	Mayo SPO
Protein (g)	5.4±0.01 <sup>c</sup>	6.5±0.01 <sup>a</sup>	5.9±0.01 <sup>b</sup>
Fat(g)	66.21±0.01 <sup>c</sup>	72.3±0.01 <sup>b</sup>	76.13±0.01 <sup>a</sup>
Moisture (%)	18.43±0.01 <sup>a</sup>	17.03±0.01 <sup>c</sup>	17.92±0.01 <sup>b</sup>
Ash(g)	1.4±0.01 <sup>c</sup>	1.5±0.01 <sup>b</sup>	1.7±0.01 <sup>a</sup>
Carbohydrate (g)	2.53±0.01 <sup>b</sup>	2.78±0.01 <sup>a</sup>	2.62±0.01 <sup>c</sup>
Crude fiber(g)	0.8±0.01 <sup>a</sup>	0.6±0.01 <sup>b</sup>	0.61±0.01 <sup>b</sup>

Different superscripts denote statistically significant differences ( $P < 0.05$ ) across various mayonnaise formulations

**Table 3. Droplet size and viscosity of different mayonnaise samples**

Sample	Surface D [3,2] (µm)		Volume D [4,3] (µm)		Apparent Viscosity ( $\gamma = 10 \text{ s}^{-1}$ ), Pa.s	
	Initial (0 <sup>th</sup> day)	day 21	Initial (0 <sup>th</sup> day)	day 21	Initial (0 <sup>th</sup> day)	day 21
Control	1.0 ± 0.005 <sup>a</sup>	0.5 ± 0.01 <sup>c</sup>	35.5 ± 0.02 <sup>a</sup>	43.4 ± 0.01 <sup>a</sup>	2.6 ± 0.3 <sup>c</sup>	2.0 ± 0.01 <sup>c</sup>
Mayo-EOP	1.0 ± 0.005 <sup>a</sup>	0.7 ± 0.02 <sup>a</sup>	27.7 ± 0.01 <sup>c</sup>	35.6 ± 0.02 <sup>b</sup>	4.8 ± 0.1 <sup>a</sup>	5.1 ± 0.05 <sup>a</sup>
Mayo-SPO	1.0 ± 0.005 <sup>a</sup>	0.6 ± 0.01 <sup>b</sup>	30.2 ± 0.005 <sup>b</sup>	30.7 ± 0.01 <sup>c</sup>	3.7 ± 0.3 <sup>b</sup>	3.4 ± 0.02 <sup>b</sup>

Different superscripts denote statistically significant differences ( $P < 0.05$ ) across various mayonnaise formulations

**Table 4. Colour values of formulated mayonnaise (Mayo-ESOP and Mayo-SPO) and control samples**

Variations	Colour values			ΔE
	L*	a*	b*	
Mayo Control	84.56±0.005 <sup>a</sup>	-0.63±0.005 <sup>c</sup>	12.46±0.005 <sup>a</sup>	0.91±0.01 <sup>a</sup>
Mayo SPO	83.06±0.005 <sup>b</sup>	-0.70±0.005 <sup>b</sup>	11.41±0.005 <sup>b</sup>	0.77±0.005 <sup>b</sup>
Mayo ESOP	80.49±0.005 <sup>c</sup>	-1.29±0.002 <sup>a</sup>	10.82±0.005 <sup>c</sup>	0.73±0.01 <sup>c</sup>

Different superscripts denote statistically significant differences ( $P < 0.05$ ) across various mayonnaise formulations

**Table 5. Fatty acid profile of formulated mayonnaise (Mayo-ESOP and Mayo-SPO) and control samples**

Sl. No.	Fatty acid compounds	Formula	Control	Mayo ESOP	Mayo SPO
1.	Palmitoleic acid	C <sub>16</sub> H <sub>30</sub> O <sub>2</sub>	0.28	0.37	0.39
2.	Palmitic acid	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	9.99	9.85	9.09
3.	Linoleic acid (ω-6)	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	45.77	47.70	48.44
4.	Oleic acid (ω-9)	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	38.07	35.36	35.27
5.	Methyl stearate	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>	5.38	5.83	5.73
6.	Eicosanoic acid	C <sub>21</sub> H <sub>40</sub> O <sub>3</sub>	ND	0.26	0.29
7.	Docosanoic acid	C <sub>23</sub> H <sub>46</sub> O <sub>2</sub>	0.51	0.63	0.79

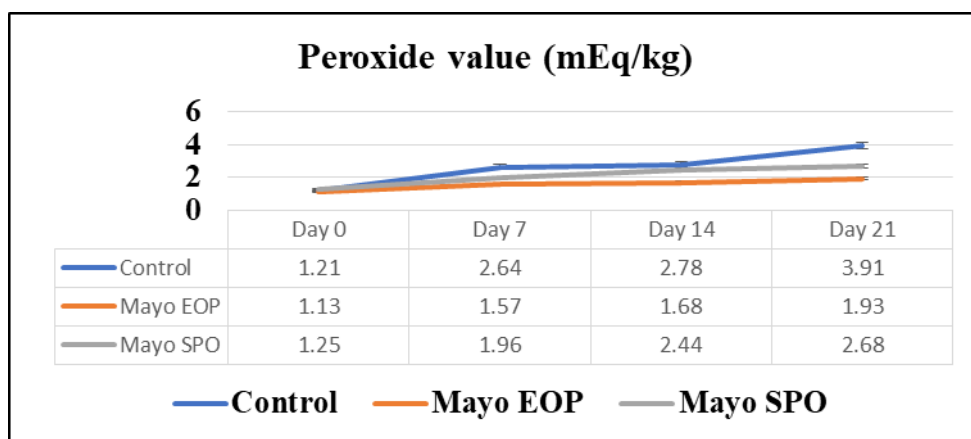


Fig. 1. Peroxide value mayonnaise variations

#### 4. CONCLUSION

The mayonnaise formulated using silkworm pupae oil and silkworm pupae oil encapsulated powder showed better quality characteristics in the oxidative stability and physicochemical properties compared to Control mayonnaise prepared out of sunflower oil. Further studies have to be conducted using silkworm pupae oil and protein-based value-added products in the form of convenience foods to meet the growing demands of the population and also to enhance the nutritional value of foods.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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