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Suka Thangaraju, Research Scholar, Department of Food Engineering, National Institute of Food Technology, Entrepreneurship and Management-Thanjavur, Ministry of Food Processing Industries, Government of India, Thanjavur, Tamil Nadu, India

Dr. Venkatachalapathy Natarajan, Professor and Head, Department of Food Engineering and Chairman, Centre of Excellence for Grain Sciences, National Institute of Food Technology Entrepreneurship and Management Thanjavur (NIFTEM-T), Ministry of Food Processing Industries (MoFPI), Government of India, Pudukkottai Road, Thanjavur, Tamil Nadu, India

Corresponding Author:
Dr. Venkatachalapathy Natarajan, Professor and Head, Department of Food Engineering and Chairman, Centre of Excellence for Grain Sciences, National Institute of Food Technology Entrepreneurship and Management Thanjavur (NIFTEM-T), Ministry of Food Processing Industries (MoFPI), Government of India, Pudukkottai Road, Thanjavur, Tamil Nadu, India

Effect of lecithin on proximate composition of dark chocolate

Suka Thangaraju and Venkatachalapathy Natarajan

Abstract
The main aim of this research is to study the effect of different proportions of rice bran lecithin and soy lecithin on the proximate composition of dark chocolates. Moisture content, carbohydrate, protein, fat, ash content, and energy values were calculated. The observed data revealed no discernible difference in the chocolate's proximate composition. Increasing the cocoa content and reducing the fat content of dark chocolate leads to enhancing the health benefits. Soy lecithin is allergic to some individuals; rice bran lecithin can be the best alternative.

Keywords: Dark chocolate, soy lecithin, rice bran lecithin, proximate composition

1. Introduction
Chocolate is one of the most popular confectionary goods in the world, and dark chocolate is a mixture of 70% of solids like cocoa powder and sugar in a continuous phase of cocoa butter. Chocolate is valued not only for its unique sensory features (flavor and pleasant taste) but also for its high concentration of easily digested nutrients such as carbohydrates, protein, lipids, minerals, vitamins, as well as physiologically active substances (flavonoids and other antioxidants) (Birtea *et al.*, 2020) [2]. Moderate chocolate consumption, particularly dark chocolate, has many beneficial effects on the body, including defending the cardiovascular system, enhancing glucose tolerance, preventing cancer, and reducing obesity; it has beneficial impacts on the development of the gut microbiome, regulating immune cells involved in immunity, protection of the nervous system and neurological functions by diminishing neurological dysfunction and other age-related abnormalities, etc. (Chaudhari *et al.*, 2018) [4]. Emulsifiers have been used for a long time to alter the flow behavior of chocolate. These surface-active chemicals diminish surface tension between the dispersed and continuous phases and, in addition to rheological characteristics, impact many properties, such as moisture and temperature sensitivity, as well as tempering behavior, due to their unique molecular structure (Schantz and Rohm, 2005) [7]. Furthermore, emulsifiers may impact certain characteristics of solidified chocolate, like fat bloom, stability of fat, and oxidation.
Soy lecithin is the most commonly used emulsifier in chocolate manufacturing industries, added in the weight percentage of 0.5%. In the present scenario, soybeans remain the primary natural source of lecithin in the food sector. But some individuals may be allergic to soybean; thus, having alternative lecithin sources might be beneficial. Rice bran oil is a nutritious and healthy oil with a high concentration of bioactive components like oryzanol, tocopherols, tocotrienols, and squalene (Modupalli *et al.*, 2022; Thangaraju *et al.*, 2022) [5, 9]. And its by-product (gum/lecithin) during the degumming process can be the best alternative for soy lecithin (Thangaraju, Pulivarthi and Natarajan, 2020) [10]. Sun *et al.* (2020) [8] studied the emulsifying properties of rice bran lecithin and reported that enzymatic degummed lecithin gave better emulsifying properties. This paper aims to evaluate the nutritional properties of the formulated dark chocolate with varied percentage addition of soy lecithin and rice bran lecithin.

2. Materials and methods
2.1 Materials
Cocoa powder, cocoa butter, and sugar were obtained from the local market in Thanjavur, Tamil Nadu, India. Soy lecithin (SL) (30%) was procured from SRL chemical, India. Rice bran lecithin (RBL) was extracted after an ultrasonic-assisted enzymatic degumming process (Thangaraju *et al.*, 2022) [10].

All additional chemicals and solvents were bought from Sigma-Aldrich Chemicals, Private Limited and were of analytical grade.

2.2 Methods

2.2.1 Chocolate preparation

Chocolate was prepared according to Cassiday (2012) [3] with little modifications. The level of SL and RBL used are listed in Table 1, and the process of making chocolate is given in Fig 1.

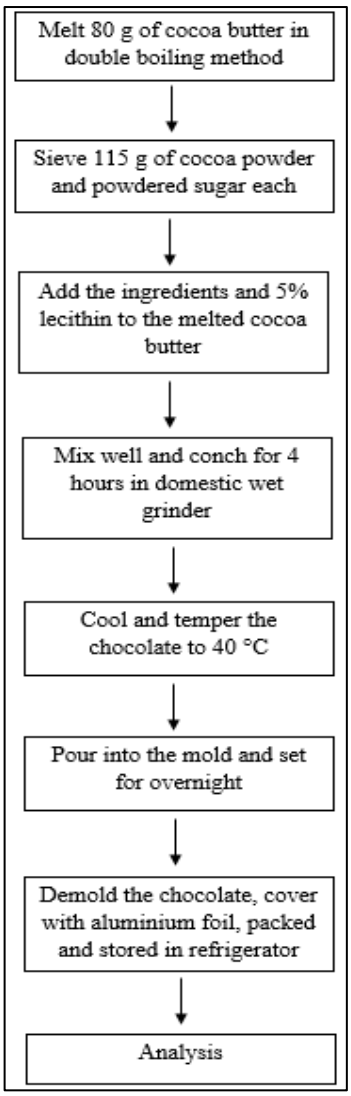

Fig 1: Flow chart for processing of dark chocolate

Table 1: Level of lecithin in the chocolate formulation

Sample No.	Rice bran lecithin (%)	Soy lecithin (%)
1	100	0
2	75	25
3	50	50
4	25	75
5	0	10

2.2.2 Analytical procedure

The moisture content of the samples was analyzed using the oven-drying method. The samples were kept at 105 °C till they reached the constant weight and moisture content of the samples were calculated using the equation (1),

$$\text{Moisture content (db \%)} = \frac{\text{initial weight(g)} - \text{final weight(g)}}{\text{final weight (g)}} \times 100 \quad (1)$$

The fat content of the samples was analyzed according to Saputro *et al.* (2019) [6] with little modifications. Approximately 5 g of samples were taken, melted, and mixed with 30 mL of petroleum ether. After vortexed for 5 seconds, the mixture was centrifuged at 9000 rpm for 10 minutes. The precipitate was exposed to the same technique twice after the supernatant was collected in the preweighed empty and dry oil flask. The supernatant was then subjected to hot air at 120 °C for 15 minutes. The fat content in the samples was calculated using equation (2),

$$\text{Fat content (\%)} = \frac{\text{weight of fat (g)}}{\text{weight of sample (g)}} \times 100 \quad (2)$$

Protein content was determined by AOAC (2000) [1] method. Briefly, 1 g of the sample was hydrolyzed with 10 mL of concentrated H2SO4, 5 g Na2SO4 and 1 g CuSO4 were added, and the contents were mixed in a digestion flask at 420 ºC for 2h. Boric acid of 4% was added to the acid tank, and the alkali tank was filled with 40% NaOH solution. The digested sample in the Kjeldahl tubes was distilled in acid and base, and the distillate was collected in a 250 ml conical flask. Two drops of mixed indicator solution were added into a conical flask containing the distilled sample. The condensate was then adjusted with 0.1 N HCl solution to turn light pink. The percent total nitrogen and crude protein were calculated using equation (3), and total nitrogen was calculated by multiplying with a conversion factor of 6.25.

$$\text{Protein (\%)} = \frac{(\text{TV} - \text{BV}) \times \text{Normality of acid} \times 14.01 \times 100}{\text{weight of the sample (g)} \times 1000} \times 6.25 \quad (3)$$

where TV – titre value, BV – Blank value.

Ash was measured in triplicate by AOAC (2000) [1]. In a previously dried and weighed porcelain crucible, 2.0 g of the sample was weighed. The crucible was heated in a furnace for 6 hours at 550 °C. Before reweighing, the crucible was cooled in a desiccator till it was at room temperature. Equation (5) was used to calculate the ash content of the sample.

$$\text{Ash (\%)} = \frac{\text{weight of crucible after ashing(g)} - \text{empty weightof crucible(g)}}{\text{weight of sample(g)}} \times 100 \quad (4)$$

The energy of the samples was analyzed using the Bomb calorimeter. A sample weight of approximately 1 g was taken and ignited in the calorimeter. The initial and final constant temperature of the water was noted down, and the energy value of each sample was calculated by using the formula (6)

$$\text{E(kcal/g)} = \frac{\text{C} \times \Delta\text{T}}{\text{w}} \quad (5)$$

where E – energy of combustion (kcal/g), C – heat capacity of the bomb calorimeter (kcal/°C), ΔT – increase in temperature (°C), and w – weight of the sample

The total carbohydrate present in the sample can be calculated using theoretical equation (7)

$$\text{Carbohydrate (\%)} = \frac{\text{Energy value} - (4 \times \text{Protein value}) - (9 \times \text{fat value})}{4} \quad (7)$$

2.3 Statistical analysis

All experiments were carried out in duplicate and independently, and the results were given as means ± standard deviations. Using SPSS 25.0 software, ANOVA with Tukey's test was used to analyze the mean value difference between samples (IBM, USA). The differences were statistically

significant at $p< 0.05$.

3. Results and Discussion

The proximate analysis was done for the formulated chocolates, and the observed results are shown in Table 2. According to the data shown, large levels of nutritional principles may be identified in the studied composition of dark chocolate, which can offer a major portion of the required nutritional demand. The moisture content in the samples was observed to be 1.2%. The carbohydrates in all the samples were the highest among the studied nutritional factors. It was also observed that no significant difference was found between the samples. Birtea *et al.* (2020) [2] have reported that carbohydrate was observed to be decreased with an increase in the concentration of cocoa mass.

The fat content of the samples was between 30% and the highest in soy lecithin-infused samples, and the rice bran lecithin (100%) infused sample had the lowest fat content. This could be due to the formulation of rice bran lecithin, composed of phospholipids and lysophospholipids, while soy lecithin is composed of only phospholipids. According to table 2, there was no significant difference between the samples ($p>0.05$). The amount of crude protein present in the sample depends on the cocoa mass or dark chocolate percentage. Crude protein increases as cocoa mass (cocoa powder and cocoa butter) increase (Birtea *et al.*, 2020) [2].

Table 2: Proximate composition of dark chocolates

Sample	Moisture	Carbohydrate %	Fat %	Protein %	Ash %	Energy Kcal
1	1.24±0.03	35.36±0.25	30.63±0.98	10.34±0.56	1.78±0.31	458.47
2	1.29±0.01	35.42±0.32	30.76±0.24	10.21±0.76	1.77±0.24	459.36
3	1.22±0.05	35.63±0.14	30.45±0.76	10.11±0.42	1.76±0.16	457.01
4	1.26±0.02	35.28±0.56	30.27±0.82	10.63±0.23	1.75±0.42	456.07
5	1.23±0.08	35.37±0.21	30.34±0.25	10.42±0.36	1.74±0.53	456.22

The amount of ash in the samples is directly proportional to the amount of minerals present. A higher percentage of ash was found in 100% rice lecithin chocolate. This could be due to the rice lecithin chocolate (100%) being observed to be more emulsified, and the presence of minerals was readily available for ashing. The previous research observed that rice lecithin was found to be more stable with lesser particle size distribution (Sun *et al.*, 2020) [8]. The chocolate's total energy value was represented in kilo calories per gram. There was no statistically significant difference between the samples.

4. Conclusions

The data shows that dark chocolates' nutritional properties are influenced by the quantity of components used in their production. After examining the proximate compositions of dark chocolates with soy lecithin and rice bran lecithin, it can be concluded that no significant differences were observed between the dark chocolate samples. Being one of the highest rice and rice bran oil producers, the by-product (gum) from the rice bran oil refining can be the best alternative for soy lecithin (which is allergic to some individuals).

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