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Research Article

Formulation and Sensory Evaluation of Non-Dairy Synbiotic Chocolates Enriched with Almond Milk, Oats Powder and Probiotic Microorganism (*Streptococcus thermophilus* BURD PB 8)

Paromita Mukherjee[1], Souvik Tewari[2]*, Anindita Ray Chakravarti[3] and Swati Nakhale[4]

[1]*Researcher, Department of Food and Nutrition, Swami Vivekananda University, Barrackpore, W.B., India*

[2]*Assistant Professor, Department of Food and Nutrition, Swami Vivekananda University, Barrackpore, W.B., India*

[3]*Assistant Professor and Head, Department of Food and Nutrition, Maharani Kasiswari College, University of Calcutta, Kolkata, W.B., India*

[4]*Principal, Indian Institute of Food Science and Technology (IIFST), Aurangabad, Maharashtra, India*

***Corresponding Author:** Souvik Tewari, Assistant Professor, Department of Food and Nutrition, Swami Vivekananda University, Barrackpore, W.B., India.

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Abstract

This study is to develop Non-dairy Synbiotic Chocolates as an alternative to conventional Chocolate by using a probiotic microorganism (*Streptococcus thermophilus* BURD PB 8) in combination with varying percentages of oat powder, almond milk, beetroot powder and stevia powder. Additionally, the ratio of oat powder, almond milk, beetroot powder and stevia powder were optimized based on sensory evaluations such as color, flavor/taste, texture, and overall acceptability. A panel of 50 tasters with extensive backgrounds in the Food Science field evaluated the organoleptic qualities of commercially produced control Chocolate (T_0) and experimental Non-dairy Synbiotic Chocolates (T_1, T_2 and T_3). The sensory evaluation was place over the course of three days. A 9-point hedonic scale was used to score each attribute, with 9 representing the highest rating and 1 representing the lowest. Following an overall acceptability score, T_2 treatment had a higher score (8.10 ± 0.06^c) than the T1 score (7.10 ± 0.11^b) and T3 score (6.00 ± 0.02^d).

Keywords: Synbiotic Chocolates; Sensory Evaluation; Hedonic Scale; Probiotics; Acceptability; *Streptococcus thermophilus*; Oats; Beetroot; Almond

Introduction

Food preferences have steadily altered in the modern day, yet some foods are becoming more and more popular due to their novel flavors, textures, and health advantages. People of all ages, from young children to elder, like eating Chocolate, a culinary product that has long been popular among all. Typically, roasted or ground cocoa seed kernels are used to make Chocolate [1]. The Chocolate can be of various forms such as solid, semi-solid, liquid, pasted and many more [2].

In general, ingredients for Chocolate include milk, sugar, cocoa butter, cocoa powder, and preservatives [3]. The readily accessible Chocolates on the market are high in fat, sugar, and preservatives; therefore, frequent consumption of them can result in a number of health issues, including elevated blood sugar, cholesterol, and triglyceride levels, obesity, and a higher risk of kidney stones.

In this study, a new type of treatment combination was used to develop a Non-dairy Synbiotic Chocolate in view of deliver a number of health benefits. Here, oats powder was used as a prebiotics, almond milk was used as the substitute for cow's milk and stevia powder was used as a substitute of common table sugar. Chocolate is incredibly popular food with a very high consumption rate globally. However, producing Synbiotic Chocolate with the ideal target content and suitable sensory characteristics is highly challenging because the addition of various functional ingredients profoundly affects their organoleptic attributes such as taste, texture, and appearance [4].

One of the critical aspects of food acceptability testing is the sensory analysis by consumers. The most commonly utilized scale to ensure acceptability of food to consumers and gather their matrices is the 9-point hedonic [5]. The attributes of the hedonic scale

are mostly two – the extreme ends are "dislike extremely" and "like extremely", whereas the middle or midpoint is "neither like nor dislike". The hedonic scales may be labeled with a verbal cue that actually represents the level of each of the two extreme concepts to avoid any confusion [6]. Therefore, the labeled hedonic scale is a simple way to gather responses from untrained consumers regarding sensory attributes to allow integration in further testing techniques [7].

In this research study, Sensory evaluation was carried out by using the hedonic scale. A panel of 50 tasters with extensive backgrounds in the food science field evaluated the organoleptic qualities of commercially produced control (T_0) and experimental Non-dairy Synbiotic Chocolates (T_1, T_2, and T_3).

Use of oats powder as a prebiotics in newly developed non-dairy synbiotic chocolate

Among all the prebiotic foods, whole oats may include β-glucan, a prebiotic polysaccharide that has been shown to improve gut health in humans by decreasing blood cholesterol and having antioxidative and anticancer properties [8]. One of the most generally and dietary commercial crops cultivated around the world is *Avena sativa*, also known as oats (Ahmad., *et al*., 2014). For several health benefits, oats could be used as a source of dietary fiber and a bulking agent in Non-dairy, no-sugar Chocolate [9]. They are rich in macronutrients and micronutrients such as proteins, carbohydrates, dietary fiber, vitamins, and minerals. Oligosaccharide beta-glucan contributes to the prebiotic index of oats. Oat phenolic chemicals include avenanthramides, along with β-glucan, a soluble fiber [10]. The most common positive effects of consuming oats are achieved mainly due to the presence of β-glucan and other bioactive compounds [8]. Several studies have reported that, which showed a significant decrease in total and Low-Density Lipoprotein (LDL) levels when consuming oat β-glucan, which, accordingly, may reduce the risk of cardiovascular diseases. In addition, the fiber content of oat has a beneficial effect on satiety and weight control [11].

Use of almond milk as a substitute of cow's milk in newly developed non-dairy synbiotic chocolate

Almond milk is prepared from soaked almonds which is low in fat content, but energy, proteins, lipids and fiber content is high in it [12]. Almond milk can be used as a substitute of cow's milk because almond milk has no lactose. Almond milk contain various minerals such as calcium, magnesium, iron, phosphorus, sodium, potassium and zinc and Vitamins include such as pyridoxine, vitamin C, thiamine, riboflavin, niacin, folic acid and vitamin E.

However, Almond milk contributes many more bioactive compounds than cow milk. Almond milk, a low-calorie, low-carb milk variant than cow milk, yet to a significant level, includes beneficial unsaturated fat, vitamin E, and calcium, when supplemented [13]. Phenolic compounds, vitamin E, unsaturated fatty acids such as oleic acid and linoleic acid, phenolic acids, and flavonoids are present in almond milk. Due to the biochemical properties like anti-inflammatory and antioxidant influences, almond milk has become highly beneficial to the consumer [14]. Almond milk is beneficial for those who have a dairy allergy or who are lactose intolerant, making it a good substitute for cow's milk. It may help to reduce the chance of cardiovascular disease and to boost lipid profiles because of the unsaturated fats [15]. The vitamin E content of almond milk demonstrates antioxidant features in the battle against cells' oxidative stress and immune support. Unsweetened almond milk, which has a low GI, may also be consumed by people with diabetes or blood sugar levels that need to be maintained [16].

Use of probiotic microorganism in newly developed non-dairy synbiotic chocolate

Probiotics are the beneficial microorganisms improving the intestinal microbial balance in the host when administrated into the gut in sufficient numbers. The FAO/WHO and the International Scientific Association for Probiotics and Prebiotics defined probiotics as "live microorganisms that, when administered in an adequate amount, confer a health benefit on the host". Probiotic food products have used worldwide and they are gaining an increasing popularity day by day Current trends in the consumption of probiotics are associated with increased levels of health-consciousness and the availability of probiotics in the form of dietary supplements, here some of the names listed below used as the probiotic strains for food product development [17].

In this study *Streptococcus thermophilus* BURD PB 8 was used as a probiotic culture. This new probiotic strain *Streptococcus thermophilus* BURD PB 8 (Accession number: MN121741) was isolated and identified from local curd sample from Purba Bardhaman district of West Bengal, India [18].

Use of beetroots powder as a natural coloring agent in newly developed non-dairy synbiotic chocolate

In this newly developed Non-dairy Synbiotic Chocolate, the natural coloring agent used for the attractive coloration. Beetroot which is a popular root vegetable with health properties is being used as coloring agent. It has been a root vegetable that holds all the bioactive constituents. Beetroot is an antistress flavor utilized in the form of natural color and antioxidant in low-sugar Non-dairy Chocolate formulation [19]. Beetroot can be considered a source

of carbs, fiber, and vitamins and can supply minerals like potassium, manganese, and folate. Beetroot contains Betalains that can be used in potential to have a prebiotic effect [20]. Among the bioactive compounds present in beetroot, there are phenolic compounds, nitrates, and betalains. Betalains are water-soluble antioxidants with anti-inflammatory properties; their prebiotic effects are that they have betaxanthin and betacyanin, which are reddish-violet and yellow pigments, respectively [21]. Furthermore, beetroot contains high dietary nitrates which are easily used by the body in nitric oxide, which has a vasodilatory effect. The bioactive constituents of beetroot have been linked to numerous health advantages. Beetroot nitrates enhance endothelial function, reduce blood pressure, and improve physical activity capacity [22]. Betalains anti-inflammatory and antioxidant effects may safeguard against chronic disease ranging from cancer to heart disease. High folate levels in beetroots create a well-formed fetus and reduce the risk of neurotube dysfunction and a lower possibility of neural tube defects [23].

Use of stevia as a sweetening agent in newly developed non-dairy synbiotic chocolate

The leaves of the *Stevia rebaudiana* plant are used to make stevia, a natural sweetener. More than 130–300 times sweeter than sucrose is its refined extract powder. In addition, it improves digestion, lowers body weight, stops teeth decay, and avoids diabetes [24]. Stevia (*Stevia rebaudiana*) has been used for developing Non-dairy Synbiotic Chocolate as a substitute of table sugar [25]. For each batch, amounts of stevia (3gm) were added in treatments T_1, T_2 and T_3 respectively and properly blended.

By discussing and looking over the previously mentioned review literature, almond milk can be substituted for cow milk in the treatment combinations of Synbiotic Chocolate. However, stevia can be used in place of regular table sugar. Additionally, Beetroot powder can be used as a coloring ingredient into the newly developed Non-dairy Synbiotic Chocolate.

Materials and Methods

This study was conducted at the department of Food and Nutrition, Swami Vivekananda University, Barrackpore, W.B., India.

The Non-dairy Synbiotic Chocolates were prepared according to standard Chocolate-making procedures, with the functional ingredients incorporated during the mixing stage. All raw materials were procured from local commercial suppliers of Behala, Kolkata, W.B., India. The control Chocolate (T_0) was prepared by using cocoa butter, cocoa liquor, whole milk powder, sugar and soy lecithin [26]. The quantities of different ingredients for the preparation of control Chocolate are tabulated in table 1. The flow diagram for the preparation of control Chocolate is given in figure 1. In case of experimental Non-dairy Synbiotic Chocolate preparation, oats powder, almond milk, beetroots powder, stevia powder and probiotic microorganisms (*Streptococcus thermophilus* BURD PB 8) were used. The newly developed Non-dairy Synbiotic Chocolate formulations were tempered, molded and stored at 4°C until sensory evaluation. The quantities of different ingredients for the preparation of experimental Chocolates are tabulated in table 2. The flow diagram for the preparation of control Chocolate is given in figure 2.

Treatments (Control Chocolate)	**Cacoa powder** (%)	**Cacoa liquor** (%)	**Whole milk powder** (%)	**Sugar Powder** (%)	**Soy lecithin** (gm)
T_0	20	12.4	25.4	43	0.5

Table 1: Treatment combination of Control Chocolate (T_0) [26].

Treatments (Experimental Chocolate)	**Almond milk** (mL)	**Oats Powder** (gm)	**Beetroots Powder** (gm)	**Probiotics (*Streptococcus thermophilus* BURD PB 8) in** percentage	**Coconut Oil** (mL)	**Stevia Powder** (gm)	**Soy lecithin** (gm)
T_1	60	30	7	1	2	0.10	0.5
T_2	65	25	5	1	4	0.50	0.5
T_3	70	20	3	1	6	1	0.5

Table 2: Treatment combination of newly developed Non-dairy Synbiotic Chocolates (T_1, T_2 and T_3).

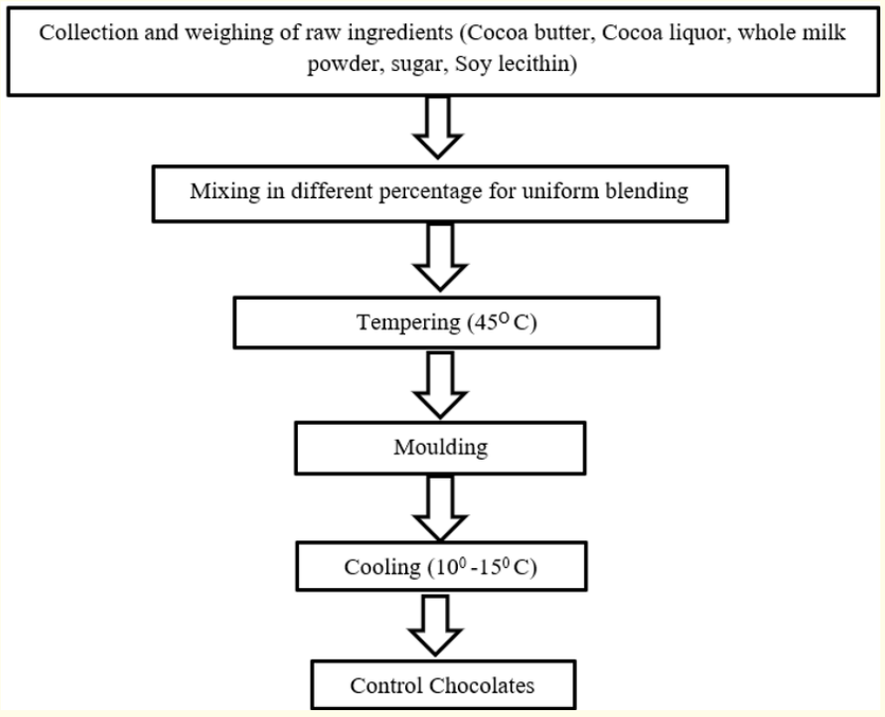

Figure 1: Flow diagram for development of control Chocolate.

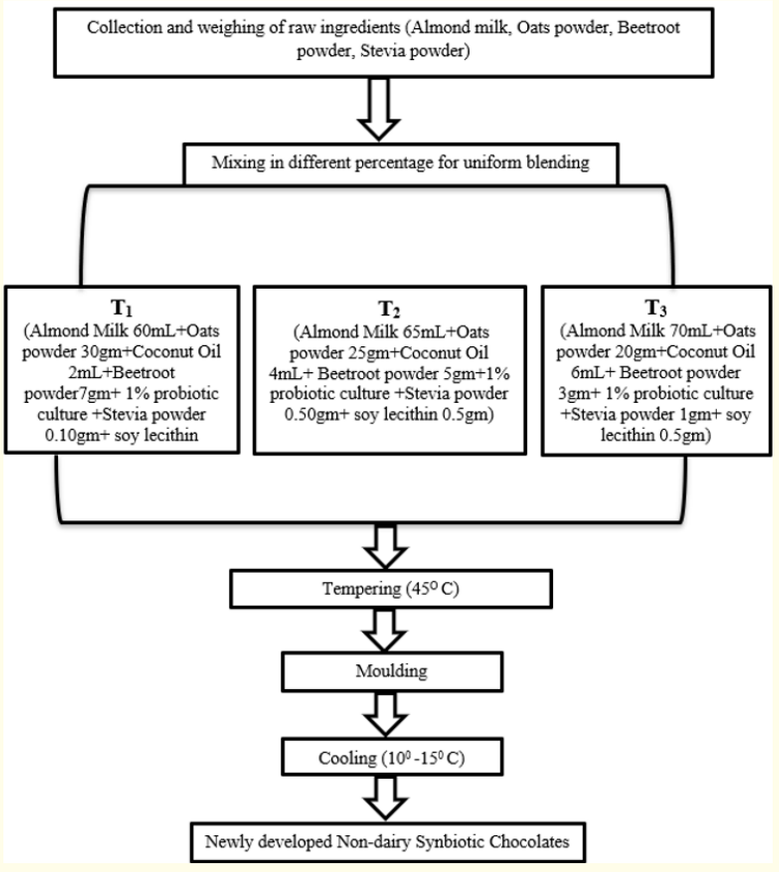

Figure 2: Flow diagram for development of Non-dairy Synbiotic Chocolates.

Sensory evaluation

For sensory evaluation fifty healthy participants aged 20 to 22 years old were recruited from Swami Vivekananda University, Barrackpore, West Bengal, India. The health status of potential participants was assessed through a screening questionnaire and basic medical examination. The questionnaire collected information on medical history, drug use, and current health issues. Blood pressure and resting heart rate were measured using standard procedures. Participants meeting the inclusion criteria were enrolled in the sensory evaluation study.

Statistical analysis:

To determine the statistical significance of the research data, One-Way Analysis of Variance (ANOVA) technique and Critical difference (C.D) were used. All values are expressed as mean and standard deviation of three parallel measurements.

Results and Discussion

Treatment combinations	Color	Flavor/Taste	Texture	Overall acceptability
T_0	7.8 ± 0.05^{a}	7.53 ± 0.12^{ac}	7.60 ± 0.02^{a}	7.66 ± 0.06^{ac}
T_1	6.46 ± 0.05^{b}	6.86 ± 0.18^{b}	7.76 ± 0.01^{b}	7.10 ± 0.11^{b}
T_2	8.06 ± 0.08^{ca}	$7.66 \pm 0.1^{0}c$	7.90 ± 0.02^{c}	8.10 ± 0.06^{c}
T_3	6.06 ± 0.26^{dbc}	5.03 ± 0.05^{d}	8.30 ± 0.02^{d}	6.00 ± 0.02^{d}

Table 3: Sensory scores for the Non-dairy Synbiotic Chocolate formulations.

All the test were performed in triplets. Different letter in the same column indicates statistical significance level of $p < 0.0001$.

This research study was carried out in the Laboratory of Food and Nutrition (Swami Vivekananda University, Barrackpore, W.B., India). The sensory evaluation data (mean scores ± standard deviation) for the four Chocolate formulations are presented in Table 1. ANOVA results for each sensory attribute are shown in Tables 2-5, along with Tukey test results indicating statistically significant differences ($p < 0.0001$) between pairs of formulations.

Under the current experiment, Non-dairy Synbiotic Chocolate was prepared by using almond milk as a substitute of cow's milk, oats powder as a prebiotic, Beetroot powder as a natural coloring agent and Stevia powder as a substitute of common table sugar. After making newly developed Synbiotic Chocolate Sensory evaluation, and consumer acceptability were assessed by 9-point hedonic scale.

The mean sensory attributes of the control and newly prepared Non-dairy Synbiotic Chocolate are presented in table 3. After sensory evaluation of color score, it was observed that T_2 treatment showed height score compare to the other treatments T_1 and T_3 (Figure 3). It was also observed that the treatment T_0 (7.8 ± 0.05^{a}) was significantly difference from T_1 (6.46 ± 0.05^{b}) and T_3(6.06 ± 0.26^{dbc}). And a significantly difference was found between T_0 (7.8 ± 0.05^{a}) and T_2 (8.06 ± 0.08^{ca}).

The newly developed Chocolate became purplish brown in color than the control Chocolate (T_0) due to use of Beetroot powder. The purplish brown color was gradually brighter due to decrease the percentage of Beetroot powder in formulations (Figure 4) Among all treatment combinations T_2 was much more accepted by Panelist.

After sensory analysis of flavor score, it was observed that T_2 (7.66 ± 0.10^{c}) treatment combination showed highest score than other treatment combinations. After analysis, it was also found that the treatment combination T_3 (5.03 ± 0.05^{d}) showed lowest score than other treatment combinations. The T_2 treatment was prepared by 65mL Almond milk, 25g Oats powder, 5g Beetroot powder, 4ml Coconut oil, 0.50gm Stevia powder, Soya lecithin 0.5g with 1% probiotic culture (*Streptococcus thermophilus* BURD PB 8), that was highly accepted by panelist. Significantly difference was found between T_0 (7.53 ± 0.12^{ac}) and T_1 (6.86 ± 0.18^{b}); T_0 (7.53 ± 0.12^{ac}) and T_3 (5.03 ± 0.05^{d}); T_1 (6.86 ± 0.18^{b}) and T_2 (7.66 ± 0.10^{c}); T_1 (6.86 ± 0.18^{b}) and T_3 (5.03 ± 0.05^{d}); T_2 (7.66 ± 0.10^{c}) and T_3 (5.03 ± 0.05^{d}) at $p < 0.0001$. And insignificantly difference was found between T_0 (7.53 ± 0.12^{ac}) and T_2 (7.66 ± 0.10^{c}).

After sensory analysis of texture score, it was observed that T_3 (8.30 ± 0.05^{d}) treatment combination showed highest score than other treatment combinations. After analysis, it was also found that the treatment combination T_1 (7.76 ± 0.01^{b}) showed lowest score than other experimental treatment combinations. The T_3 treat-

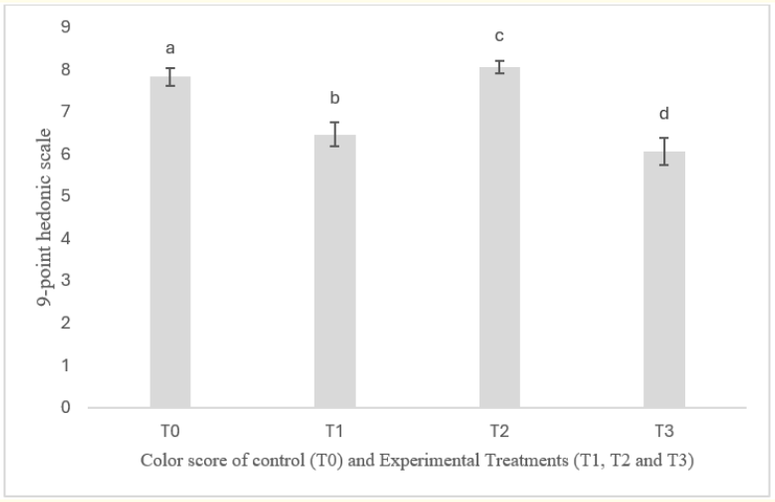

Figure 3: Graphical representation of color of control and experimental Chocolate.

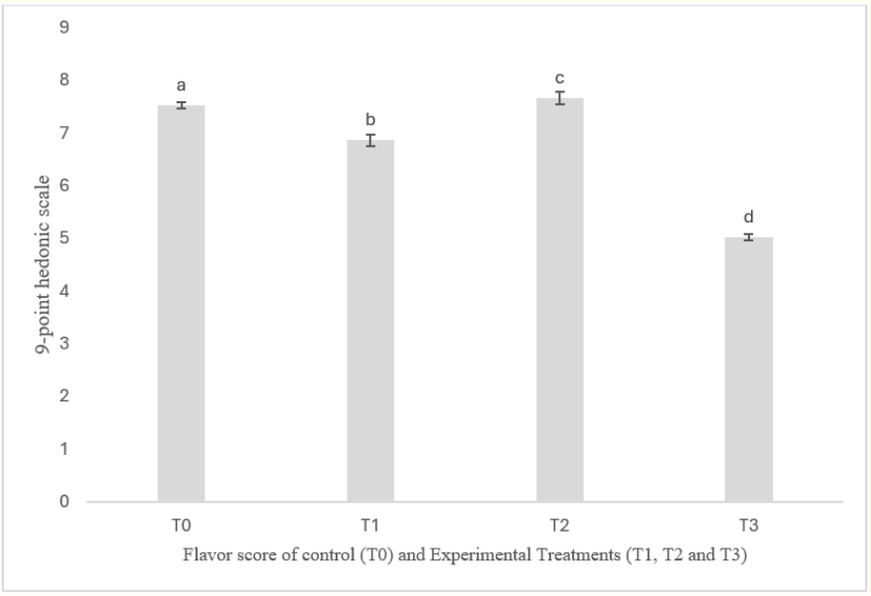

Figure 4: Graphical representation of flavor of control and experimental Chocolate.

ment was prepared by 70mL Almond milk, 20g Oats powder, 3g Beetroot powder, 6mL Coconut oil, 0.50gm Stevia powder with 1% probiotic culture (*Streptococcus thermophilus* BURD PB 8), that was highly accepted by panelist. Significantly difference was found between T_0 (7.60 ± 0.02^a) and T_1 (7.76 ± 0.01^b); T_0 (7.60 ± 0.02^a) and T_2 (7.90 ± 0.02^c); T_0 (7.60 ± 0.02^a) and T_3 (8.30 ± 0.02^d); T_1 (7.76 ± 0.01^b) and T_2 (7.90 ± 0.02^c); T_1 (7.76 ± 0.01^b) and T_3 (8.30 ± 0.02^d); T_2 (7.90 ± 0.02^c) and T_3 (8.30 ± 0.02^d) at $p < 0.0001$. As a stabilizing agent, soy lecithin was used in this chocolate preparation. It is reported by [27] that stabilizers and emulsifiers were used to improve the texture and surface appearance of the chocolates. Experimental chocolates showed better texture and surface appearance than control chocolates due to the use of oat powder. The same observation was reported in a previous published article [28]. It was found that the use of corn flour improves the texture of chocolate.

After sensory analysis of overall acceptability score, it was found that T_2 (7.90 ± 0.02^c) treatment combination showed highest score than other treatment combinations i.e. T_1 (7.76 ± 0.01^b) and T_3 (8.30 ± 0.02^d) and T_3 (8.30 ± 0.02^d) treatment combination was showed lowest score than the other treatment combination because the treatment formula that was used to prepare T_3 treatment is not accepted by panelist. Significantly difference was found

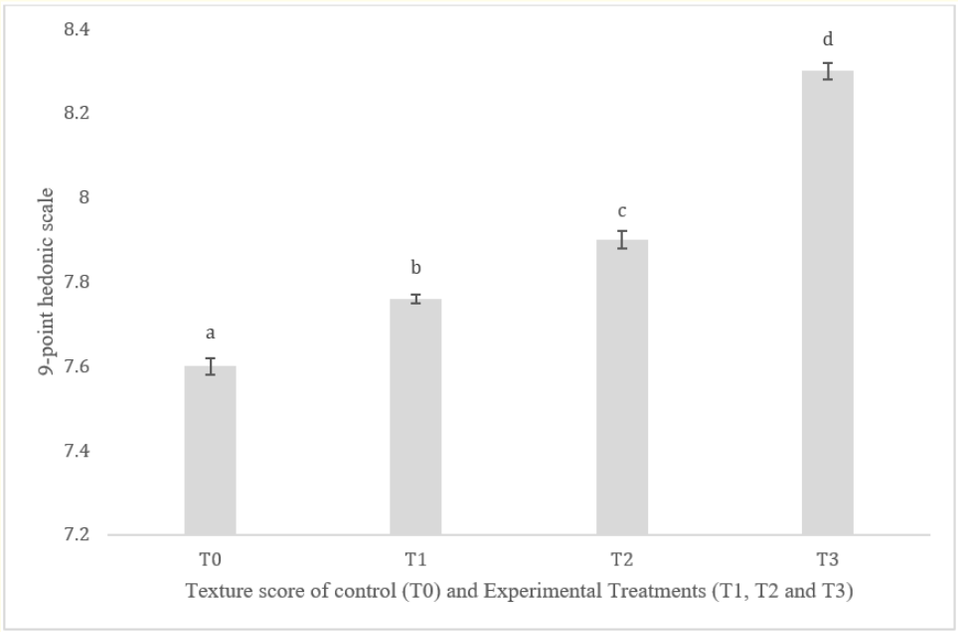

Figure 5: Graphical representation of texture of control and experimental Chocolate.

between T_0 (7.66 ± 0.06^{ac}) and T_1 (7.10 ± 0.11^{b}); T_0 (7.66 ± 0.06^{ac}) and T_3 (6.00 ± 0.02^{d}); T_1 (7.10 ± 0.11^{b}) and T_2 (8.10 ± 0.06^{c}); T_1 (7.10 ± 0.11^{b}) and T_3 (6.00 ± 0.02^{d}); T_2 (8.10 ± 0.06^{c}) and T_3 (6.00 ± 0.02^{d}) at $p < 0.0001$.

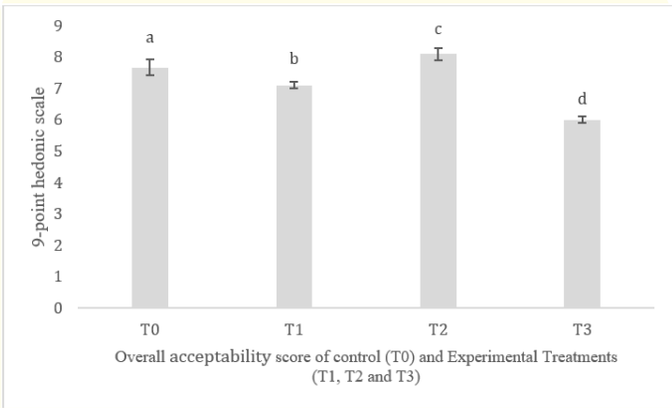

Figure 6: Graphical representation of overall acceptability of control and experimental Chocolate.

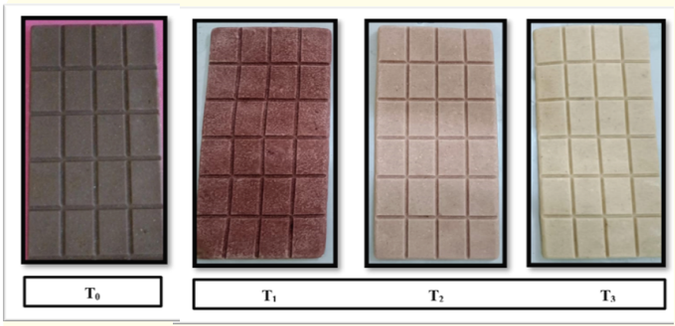

Figure 7: Pictorial view of control (T_0) and newly developed Non-dairy Synbiotic Chocolates (T_1, T_2 and T_3).

Conclusion

All consumer groups have noticed an increase in demand for dairy products. Chocolate is the most popular confectionery out of all the milk-based products. Almond milk can serve as an acceptable substitute to milk in cases where an individual is both lactose intolerant and allergic to milk products. The chocolates available on the market are highly loaded with sugar and fat. But newly developed Non-dairy Synbiotic Chocolate will be lactose- and sugar-free. So newly developed Non-dairy Synbiotic Chocolate will be much better for human health than control chocolate. After sensory evaluation, it was found that the T_2 formulation with 65 mL of almond milk, 25gm of oats powder, 4gm of beetroot powder, 4 mL of coconut oil, and 1% probiotics was highly accepted by panellists. The overall acceptability score of treatment T_2 was the highest, while the difference was not significant above the control.

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Conflicts of Interest

The authors declare no conflict of interest.

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