Physico-chemical analysis of newly prepared prebiotic chocolates by using Galacto Oligosaccharides (GOS)

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Abstract

In India, chocolate is very famous mood freshening sweet food product specially for children. But high consumption of chocolate may be harmful for human health, because most of the chocolates contain high fat. This research has been done to increase the health benefits of chocolate by using cocoa and Galacto Oligosaccharides (GOS). Many researchers have shown their research that GOS is very beneficial for human health such as it helps to lower down the cholesterol levels in the blood, to prevent colon cancer, and helps to improve mineral absorption in human body. Basically, GOS is a type of prebiotic e.g., food for probiotic bacteria. Prebiotics are non-digestible ingredients that benefit the host by boosting the growth and activity of one or a few bacterial species that are already present in the colon. It was also studied by many researchers that cocoa powder contains a lot of caffeine, flavanols, which are polyphenolic compounds. Flavanols, particularly epicatechin monomer and oligomer cocoa flavanols have been linked to a number of health advantages, including boosting nitric oxide synthase, enhancing blood flow and arterial flexibility, lowering blood pressure and platelet aggregation, and reducing inflammation. The main objectives of this research are to develop the formulation of prebiotic chocolate and to evaluate the physicochemical parameters of the newly prepared chocolate. After physico-chemical analysis of carbohydrate, fat, crude fibre and p^H, it was found that both the newly prepared prebiotic enriched chocolates (T_1 and T_2) were significantly different ($p \leq 0.01$) from control (T_0) and after analysis of protein content, it was also found that both the newly prepared prebiotic enriched chocolates (T_1 and T_2) were insignificantly different ($p \leq 0.01$) from control (T_0).

Keywords: Cocoa, flavanols, Galacto Oligosaccharides, prebiotics, probiotic bacteria, polyphenolic compounds.

1. INTRODUCTION:

1.1. Background and History of the chocolates

The Mayans were most likely the first people in South America to cultivate the cocoa plant, according to the history of chocolate. Chocolate, according to the Mayans, was a cocoa drink made with hot water and often flavored with cinnamon and pepper. "Drink of the Gods" was the name of the drink. Chocolate, derived from the seeds of the cocoa tree, was once thought to be the 'Food of Gods' **(Verna, 2013)**. The scientific name Theobroma cacao for the cocoa tree was derived from the Greek terms "theo" which means God and "broma" which means food, as a result of this link. T.L. **(Dillinger *et al.*, 2000)**. The term cocoa refers to the least processed and most natural form of the bean. The solid chocolate industry originated in the 19th

century, when Coenraad Johannes van Houten discovered a process for extracting cocoa butter **(Galaniha, 2018)**. Since then, the chocolate business has evolved a variety of cocoa seed processing technologies, resulting in a diverse spectrum of chocolate products. Chocolates, which began as a luxury commodity, have evolved into one of the world's most popular snacks. Cocoa trees are found in the lower levels of an evergreen rain forest in their natural habitat. Cocoa trees thrive in fairly hot weather (with a maximum annual average of 30-32 °C and a minimum annual average of 18-21 °C) and high relative humidity, which can reach 100% during the day and drop to 70-80% at night.

1.2. The scenario of the chocolate market

According to the International Cocoa Organization (ICCO), total world cocoa bean production in 2016-17 was 4,739,000 tons, with Africa accounting for the majority (3,622,000 tons) **(Montagna *et al*., 2019)**. Icco announced that world cocoa production for the 2020/21 season reached a new high of 5.024 million tonnes. According to a recent study estimate, the worldwide chocolate confectionery market is predicted to reach USD 161.1 billion in size and share.

The Indian chocolate market was estimated to be worth $ 1.9 billion in 2020. India's chocolate market is now considered one of the world's fastest-growing chocolate markets. Since then, the market for chocolate packaging, such as foil and paper, has risen as a result of this trend. India's robust economic growth over the last decade has increased per capita disposable income and supported the chocolate industry's expansion. As a result, customers are purchasing chocolate for both every day and special occasions. Another important driver of the chocolate confectionery sector is India's young population, which is a prominent confectionery consumption demographic. Lifestyle changes, westernization, the rise of the hospitality sector, and value creation are all other market factors **(Messerli, 2012)**.

1.3. Chocolate consumption in India

India has a voracious appetite for sweets. The rising desire for chocolate is proof of this. In India, the average per capita chocolate intake ranges from 100 to 200 grams. The amount of chocolate sold in India increased by 13% in 2016, according to new statistics from research firm Mintel **(Petroff, 2017)**. India is a big outlier in this pattern. In most other countries, the amount of chocolate sold has decreased as customers seek out better alternatives. The only other country where volume climbed last year was Poland, which had a 2% increase. Volume declined by up to 6% in Russia, Brazil, and China. Given India's expanding disposable income and young population's appetite for this indulgence, the chocolate confectionery market has seen consistent expansion. Chocolate has long been considered a child's treat in India. Adults, on the other hand, are now being targeted by marketers. Furthermore, 44% of Indian customers believe that sweet and sugary treats such as chocolates and cakes are healthful.

Given the high demand for chocolate in India, boosting the overall functionality of the components to increase the quality and nutritional value of the product would be good to the population's general health **(Barrientos, 2013)**. So, the goal of this research is to create prebiotic chocolate that will help consumers maintain good gut

health by encouraging the growth of beneficial *Bifidobacteria*, which can help break down food particles, absorb nutrients, and combat pathogenic organisms.

1.4. Chocolate craving

Because of its enticing sensory features, chocolate is coveted by many people all over the world. The sensory features of food products account for the majority of desires, according to a broad study of cravings **(Weingarten and Elston, 1990)**. Chocolate's chemosensory properties, such as sweetness, texture, and appealing aroma, cause consumers to have an innate need for it **(Rozin *et al*., 1991)**. Cocoa butter's propensity to melt at body temperature produces a distinctively pleasant mouth experience, which is a big draw for customers. Consumers consume chocolate despite its high fat and sugar content because of the distinct chocolate aroma. A study also reported that 75% of male and 77% of female chocolate cravers say there is no other non-chocolate alternative to their craving for chocolate **(Rozin *et al*., 1991)**. According to research, children's chocolate cravings are a multifaceted phenomenon that reflects some ambivalence about their eating habits. These craving aspects influence chocolate consumption frequency and quantity, as well as a variety of disordered eating behaviours. A gender gap in chocolate-related guilt appears to arise in childhood, possibly contributing to a higher risk of females developing exaggerated body image and weight problems **(Cartwright *et al*., 2007)**.

1.5. Beneficial components of chocolate in this research

Apart from its enticing sensory properties, chocolate has a number of health benefits that may encourage consumers to consume it **(McCrickerd and Forde, 2016).** Chocolate that is good for human health has become a hot topic in the world of health studies **(Fraga *et al*., 2019).** Chocolate is a food that is craved by children, teenagers, and adults alike. The polyphenols, fat, and sugar levels of chocolate determine its healthiness. When someone has a chocolate craving, they often overlook the nutritional value, calorie consumption, and other vital components such as sugar and fat. As a result, chocolate must be reformulated. The goal of chocolate reformulation is to make a product that is low in calories and sugar while being high in cocoa, which is high in polyphenols. Cocoa contains a lot of flavonoids and antioxidants. Chocolate is a popular food product that, despite its beneficial nutritional and health impacts, can put customers' health at danger due to its high calorie content. Sucrose replacement with low-energy prebiotic chemicals may assist to mitigate the above-mentioned hazards. Sucrose can be replaced with low-energy prebiotic substances to reduce these dangers or issues. Here As a helpful component, inulin can be added. The prebiotic component inulin is well-known. *Bifidobacterium* spp., *Bifidobacterium adolescentis*, *Bifidobacterium longum*, *Bifidobacterium pseudocatenulatum*, *Bifidobacterium bifidum*, *Faecalibacterium prausnitzii*, and *Bifidobacterium longum*, *Bifidobacterium pseudocatenulatum*, *Bifidobacterium bifidus* as a result, gut infections and constipation are prevented **(Özer *et al*., 2015)**. They've also been shown to improve mineral absorption, lower the risk of colon cancer, and boost the production of B vitamins in the host **(Ayyadurai *et al*., 2022)**.

In this research, prebiotic chocolate was made by using cocoa and Galacto Oligosaccharides. The health benefits of cocoa and prebiotic effects of Galacto Oligosaccharides as follows.

1.5.1. Cocoa

The seeds of the tropical tree *Theobroma cacao L*. are used to make cocoa beans. Cocoa comes in four varieties: Forastero, which accounts for 95% of global cocoa production and is the most frequently used; Criollo, which is rarely produced due to disease susceptibility; Trinitario, a disease-resistant hybrid of Criollo and Forastero; and Nacional, which is only grown in Ecuador **(Di *et al*., 2017)**. Dark chocolate and cocoa powder contain a lot of caffeine, flavanols, which are polyphenolic chemicals. Flavanols, particularly epicatechin monomer and oligomer Cocoa flavanols have been linked to a number of health advantages, including boosting nitric oxide synthase, enhancing blood flow and arterial flexibility, lowering blood pressure and platelet aggregation, and reducing inflammation **(Crozier *et al*., 2011)**. Cocoa includes a lot of proteins in addition to polyphenols and methylxanthines. The creation of flavor precursors is usually attributed to cocoa peptides. Albumins, globulins, prolamin, and glutelin are the four types of proteins found in cocoa beans **(Jalil and Ismail, 2018)**.

1.6 Galacto Oligosaccharides and Health Benefits

Oligosaccharides have long been recognized as a functional food additive with the potential to improve food quality. Beverages, newborn milk powders, confectionery, and dairy desserts are among the current principal uses of oligossacharides. Water-soluble oligosaccharides with a mild sweetness are ideal for foods that require a bulking agent with less sweetness (**Wang *et al*., 2023**).

Galacto Oligosaccharide (GOS) products are a mixture of galactose-based oligossacharides with glucose, galactose, and lactose with different degrees of polymerization (DP) and linkage arrangement (**Muller *et al*., 2009).** GOS has been accepted as a Generally Recognized as Safe (GRAS) product in the United States (GRAS). GOS has no substantial influence on clinical diseases, according to toxicological tests (**Anthony *et al*., 2006)**. GOS is indigestible, hence it resembles cellulose and other fibers (**Muller *et al*., 2009).**

GOS is a type of prebiotic e.g., food for probiotic bacteria. The presence of galacto-containing oligosaccharides in human milk has been linked to the growth of bifidus bacteria, which are often utilized to make yogurt, in the intestines of breast-fed newborns **(Amira *et al*., 2021)**. Prebiotics are non-digestible ingredients that benefit the host by boosting the growth and activity of one or a few bacterial species that are already present in the colon (**Louis *et al*., 2007**).

GOS is thought to have health benefits such as lowering cholesterol levels in the blood, preventing colon cancer, and improving mineral absorption **(Lim *et al*., 2005; Sauer *et al*., 2007; Ooi and Liong, 2010; van den Heuvel *et al*., 2016; Winardi-Liem, 2011; Alliet *et al*., 2007).** Furthermore, two significant research involving GOS digestion have demonstrated its chemopreventive impact in rat models.

In DMH (dimethylhydrazine)-treated

rats, benign and malignant tumors were considerably reduced when fed a 27 percent GOS diet in low, medium, and high fat diets **(Wijnands *et al.*, 1999)**. Additionally, using AOM (azoxymethane) treated rats, GOS has been demonstrated to have an anticarcinogenic impact when consumed as 20% of the diet. The multiplicity of aberrant crypt foci (ACF, a precursor to colon cancer that eventually forms colorectal polyps) was shown to be greatly reduced. However, this was the sole evidence that GOS could protect against colon cancer **(Wijnands *et al.*, 2001)**. In animal models, this research suggests that GOS is helpful against colon cancer. Human studies, on the other hand, have yet to be done.

Conventional chocolates may be detrimental to health due to its fat content without any other health benefits. So, this research aims to develop a prebiotic chocolate that is both low in fat and high in cocoa polyphenol content without sacrificing the favourable taste that consumers expect from good dark chocolate. The main objectives of this research are to develop the formulation of prebiotic chocolate and to evaluate the physicochemical parameters of the newly prepared chocolate.

3. Materials and Methods

Present Investigation was under taken to studies on "Physico-chemical analysis of prebiotic chocolate". Their search was conducted in IIFST, Aurangabad, Maharashtra, India.

The material used and Methods employed were as under.

3.1 Materials

- Cocoa beans
- Cocoa butter
- Galacto-oligosaccharides (GOS)
- Cane sugar
- Stabilizer And Emulsifiers

3.2 Process:

1. Melt the cocoa butter on a double boiler. Add powdered sugar, switch off heat and keep stirring till well combined.
2. Add cocoa powder and mix well till smooth.
3. Add Galacto-oligosaccharides (GOS) different level wise
4. Pour the chocolate mixture into the moulds and refrigerate.
5. Demould and serve.

3.3 Treatment combination:

T_0: Control chocolate (without prebiotic) Cocoa powder (50%) + cocoa butter (20%) + sugar (30%) + GOS (0%) + soya lecithin (0.5%)

T_1: Cocoa powder (40%) + cocoa butter (10%) + sugar (30%) + GOS (20%) + soya lecithin (0.5%)

T2: Cocoa powder (40%) + cocoa butter (5%) + sugar (30%) + GOS (25%) + soya lecithin (0.5%)

3.4 Physico-chemical analysis of prebiotic chocolate.

3.4.1 determination of carbohydrate

The carbohydrate percent in the sample was measured by method as per **Ranganna (1977)**.

3.4.2 Determination of protein

The protein content of prebiotic chocolate samples will be determined by method as per **Ranganna (1977)**.

3.4.3 Determination of fat

Fat content of prebiotic chocolate samples will be determined by modified Gerber method **(Kleyn *et al.*, 2001)**.

3.4.4 Determination of crude fibre

The crude fibre percent in the sample was measured by AOAC, 2000 standard method **(AOAC, 2000)**.

3.4.5 Determination of p^H

p^H of the samples was determined as per the procedure laid down in **AOAC (2000).**

4. Results and discussions

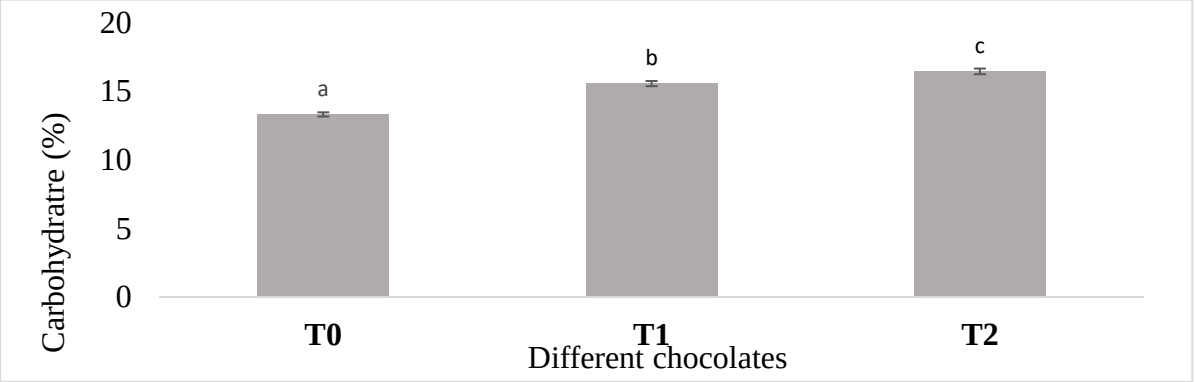
Table 1 *Physico-chemical analysis of newly prepared prebiotic chocolates*

Figure 1 showed the findings of the total carbohydrate content (%) analysis. In comparison to the control **T_0** (13.302 ± 0.1543^a), the newly prepared experimental prebiotic chocolate **T_1** (15.55 ± 0.1887^b**)** and **T_2** (16.44 ± 0.2069^c) contained more total carbohydrate content (%). Both the experimental prebiotic chocolate (**T_1** and **T_2**) and control batches met the requirements in accordance to **PFA (2006)**. The addition of GOS (0% in control vs. 20% and 25% in experimental prebiotic chocolate mix) contributed to the greater total carbohydrate content of the experimental prebiotic chocolate. According to **Figure 1**, both the newly prepared prebiotic enriched chocolates were **significantly** different ($p \leq 0.01$) from control.

Treatment combinations	**Carbohydrate (%)**	**Protein (%)**	**Fat (%)**	***crude crude fibre* (%)**	**p^H**
T0	13.302 ± 0.1543^a	2.282 ± 0.1723^a	12.438 ± 0.1756^a	3.376 ± 0.1857^a	7.28 ± 0.1626^a
T1	15.55 ± 0.1887^b	2.396 ± 0.1717^a	10.31 ± 0.157^b	4.382 ± 0.1547^b	7.622 ± 0.1461^b
T2	16.44 ± 0.2069^c	2.426 ± 0.2079^a	10.302 ± 0.1605^c	4.5 ± 0.2005^c	7.382 ± 0.1284^c

Figure: 1 *Graphical representation of carbohydrate percentage of different chocolates.*

(All the samples were evaluated in triplicate. In the similar column, different superscript alphabet showed significant difference).



The results of the analysis of protein content (percent) were given in **Figure: 2.** The newly prepared experimental prebiotic chocolate **T_1** (2.396 ± 0.1717[a]) and T2 (2.426 ± 0.2079[a]) had higher protein contents (percent) than the control **T_0** (2.282 ± 0.1723[a]). The control batches and the experimental prebiotic chocolate (**T_1** and **T_2**) both met **PFA (2006)**. The higher protein content of the experimental prebiotic chocolate was a result of the inclusion of GOS (0% in control vs. 20% and 25% in experimental prebiotic chocolate mix). According to **Figure 2**, both the newly prepared prebiotic enriched chocolates were **insignificantly** different ($p \leq 0.01$) from control.

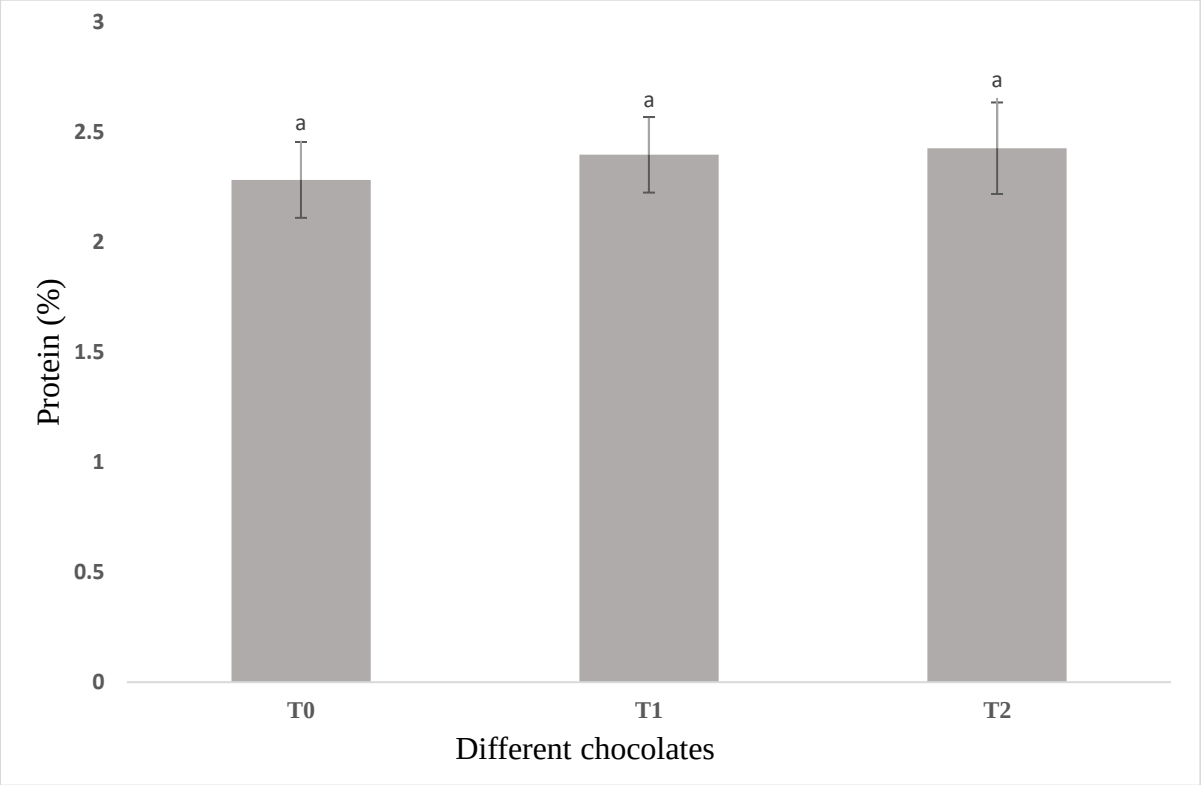


Figure: 2 *Graphical representations of protein percentage of different chocolates.*

(All the samples were evaluated in triplicate. In the similar column, different superscript alphabet showed **insignificant** difference).

Figure 3 showed the findings of the fat content (%) analysis. In comparison to the control **T_0** (12.438 ± 0.1756[a]), the newly prepared experimental prebiotic chocolate **T_1** (10.31 ± 0.157[b]), **T_2** (10.302 ± 0.1605[c]) contained lower amount fat content (%). Both the experimental prebiotic chocolate (**T_1** and **T_2**) and control batches met the requirements in accordance to **PFA (2006)**. The addition of GOS (0% in control vs. 20% and 25% in experimental prebiotic chocolate mix) contributed to the lesser amount of fat content of the experimental prebiotic chocolate. According to **Figure 3**, both the newly prepared prebiotic enriched chocolates were **significantly** different ($p \leq 0.01$) from control.

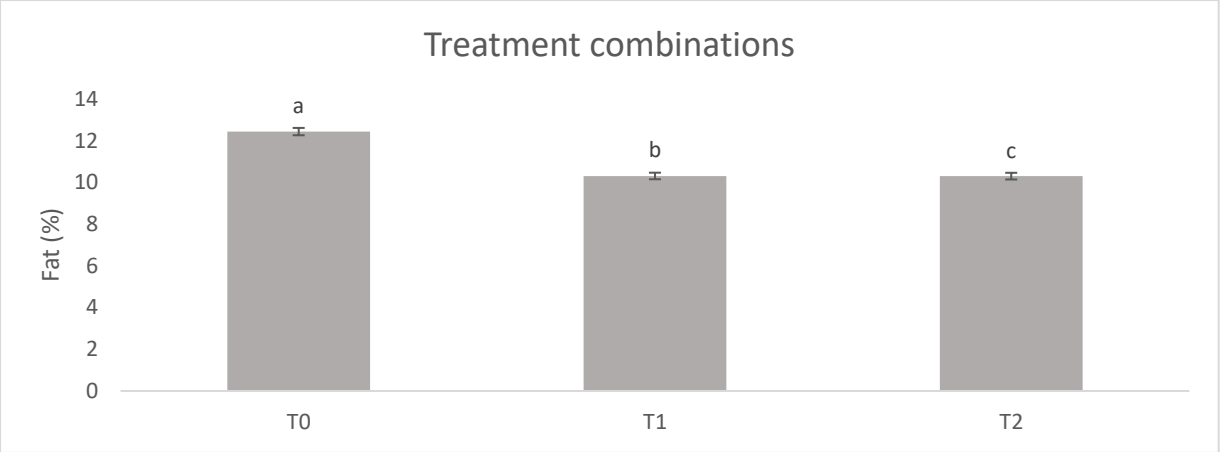


Figure: 3 *Graphical representations of mean value of fat percentage of different chocolates.*

(All the samples were evaluated in triplicate. In the similar column, different superscript alphabet showed **significant** difference).

Figure 4 showed the findings of the antioxidant content (%) analysis. In comparison to the control **T_0** (1.452 ± 0.1676[a]), the newly prepared experimental prebiotic chocolate **T_1** (2.452 ± 0.1747[b]) and **T_2** (2.554 ± 0.1952[c]) contained greater antioxidant content (%). Both the experimental prebiotic chocolate (**T_1** and **T_2**) and control batches met the requirements in accordance to **PFA (2006)**. The addition of GOS (0% in control vs. 20% and 25% in experimental prebiotic chocolate mix) contributed to the higher amount of antioxidant content of the experimental prebiotic chocolate. According to **Figure 4**, both the newly prepared prebiotic enriched chocolates were **significantly** different ($p \leq 0.01$) from control.

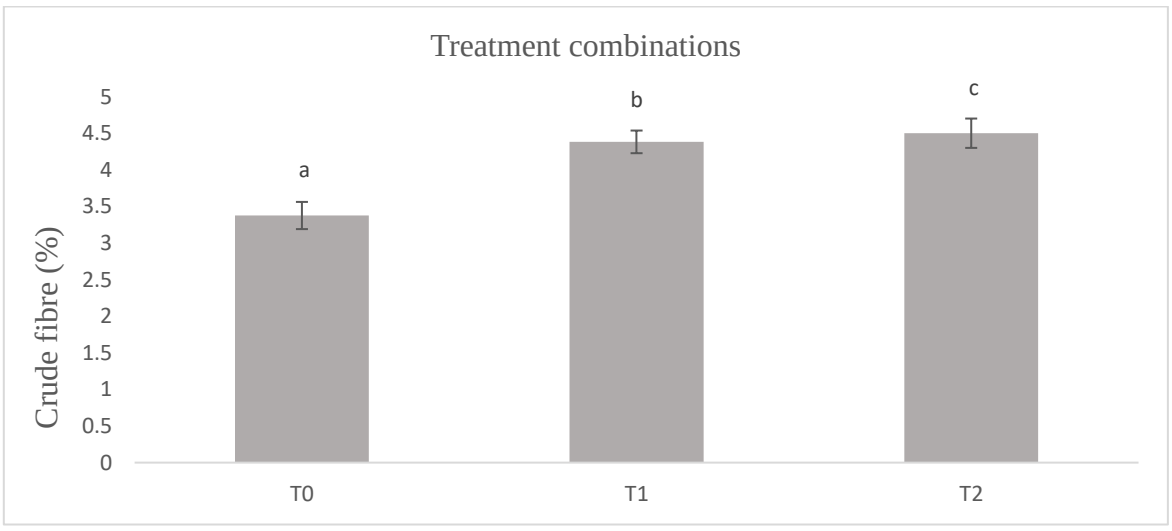


Figure: 4 *Graphical representation of mean value of crude fibre percentage of different chocolates.*

(All the samples were evaluated in triplicate. In the similar column, different superscript alphabet showed **significant** difference).

Figure 5 showed the findings of the p^H content (%) analysis. In comparison to the control **T_0** (7.28 ± 0.1626[a]), the newly prepared experimental prebiotic chocolate **T_1** (7.622 ± 0.1461[b]) and **T_2** (7.382 ± 0.1284[c]) contained greater crude fibre content (%). Both the experimental prebiotic chocolate (**T_1** and **T_2**) and control batches met the requirements in accordance to **PFA (2006)**. The addition of GOS (0% in control vs. 20% and 25% in experimental prebiotic chocolate mix) contributed to the higher amount of crude fibre content of the experimental prebiotic chocolate. According to **Figure 5**, both the newly prepared prebiotic enriched chocolates were **significantly** different ($p \leq 0.01$) from control.

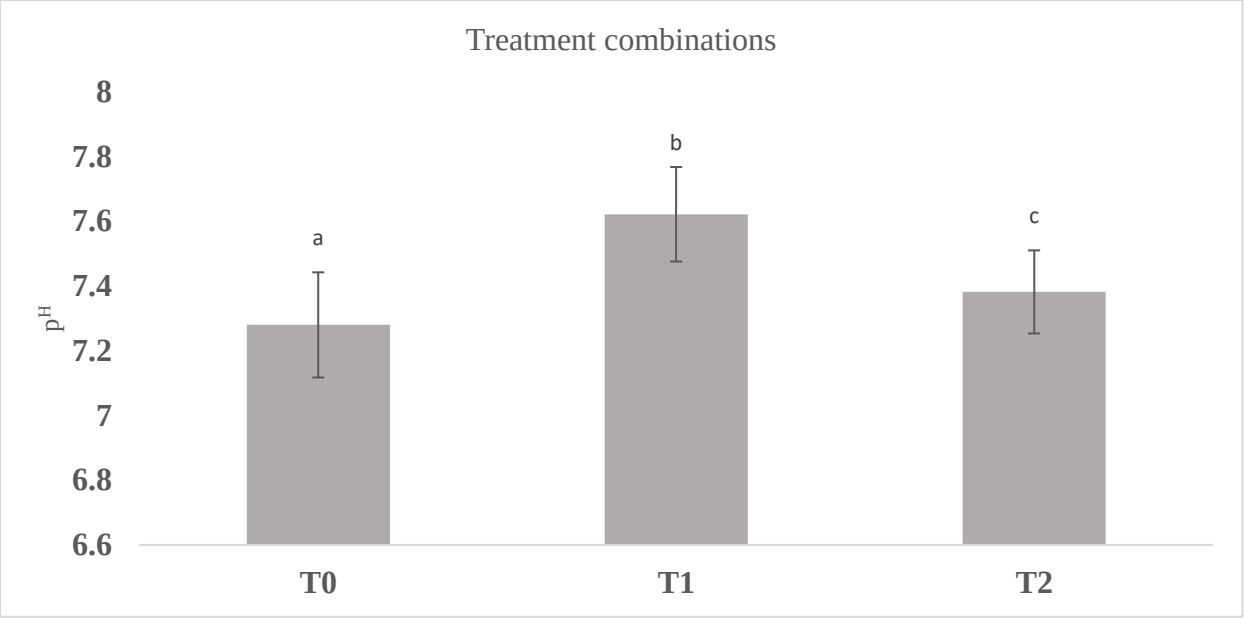


Figure: 5 *Graphical representations of mean value of pH percentage of different chocolates.*

(All the samples were evaluated in triplicate. In the similar column, different superscript alphabet showed **significant** difference).

Conclusion:

The freshly made experimental prebiotic chocolate T_1 and T_2 had higher levels of total carbohydrate content, protein, crude fibre, and p^H when compared to the control T_0. The freshly made experimental prebiotic chocolate T_1 and T_2 had reduced fat content than the control T_0. The newly prepared prebiotic enriched chocolates (T_1 and T_2) were both significantly different ($p \leq 0.01$) from the control (T_0) after physico-chemical analysis of carbohydrate, fat, crude fibre, and p^H. The newly prepared prebiotic enriched chocolates (T_1 and T_2) were both in significantly different ($p \leq 0.01$) from the control (T_0) after analysis of protein content.

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