

PROXIMATE ANALYSIS OF CHOCOLATES AVAILABLE IN INDIAN MARKET

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ABSTRACT

Chocolate is a unique flavoured food used in our society for more than 2000 years. From the fresh aroma to the brownish colour and the melting sensation that is felt in mouth after consumption makes it most likely food that is passed on, on occasion, loved by kids and making it one of the most major product of food industry. This research tries to put a glimpse on positive impacts as well as toxic impact of chocolate consumption by estimating various parameters about nutrients such as protein, fat, sugar, caffeine and antioxidant contents. Certain other parameters like pH and conductance are also observed. The chocolate samples used in this research are three of types that is dark, milk and white. These are collected from a variety of sources like retail market, home-made brands, cacao chocolate, organic chocolate and lastly pure form of ingredients (cocoa powder, cacao powder and cocoa butter /Theobroma oil). Results are obtained for all 14 samples, individual result of nutrient parameter will help the consumer to understand the parameter better and focus on comparison of one particular parameter in variety of chocolate. This results from the research states that dark chocolate is the most preferable and nutrient rich as compared to other chocolates. Cacao chocolate and organic chocolates can at times be a more preferable due to their nutrient value. Therefore, it is important to choose chocolates wisely as health should not be compromised because of taste.

Keywords: Chocolates, Proteins, Fats, Antioxidant activity, Sugar

1. INTRODUCTION

Chocolate is a peculiar flavoured confectionary used in our society continuously, history of chocolate spans more than 2000 years [1]. From the intense aroma, to the appealing dark colour, to smooth texture with its melting sensation after consumption makes it one of the most likely food that is passed on by people, loved by kids and making it a major

product of food industry [2]. Each manufacturer uses a secret formula that combines raw product and various other ingredients to forms this unique confectionary [3]. Raw ingredients include roasted cocoa seeds powder and cocoa butter, both these ingredients are derived from *Theobroma cacao* tree seeds [4, 5]. Processed ingredients include preservatives, solidifiers, emulsifiers and additional flavoured ingredients include wafer, nuts, dehydrate

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fruits etc [6]. *Theobroma cacao* is a tropical tree whose name translates as 'food of Gods' in Greek language [6]. There are different types of chocolates available in the market majorly of four basic types namely dark, milk, white and chocolate with extras (e.g. nuts, wafer, fruit etc). Dark chocolate comprises of cocoa powder (65 to 90%) and cocoa butter (35 to 10%). Milk chocolate comprises of cocoa powder (50%) and cocoa butter (50%). White chocolate only comprises of cocoa butter as primitive ingredients, along with them preservative, solidifiers and emulsifiers are used [7, 8]. In India, the consumption rate of chocolate was recorded to be 220 thousand tonnes in the year 2016 out of which 70% of chocolates were consumed by children, 43% by young/adults and about 16% by the remaining population; the consumption rate of chocolate increases by 13.9% every year [9, 10, 11, 12]. Chocolate industry is large scale industry across the world. In India, it comprises of two major multinational companies including Cadbury and Nestle which are approved by directorate of cashew nuts and cocoa development (DCCD) and food safety and standard authority of India (FSSAI). Annual increase in the growth rate of cocoa plants is 10 to 12 %. This industry jumped from 125-million-dollar industry in 1990 to 450-million-dollar industry in 2005. Other small-scale industries include homemade chocolate brands, organic chocolate brands and one industry that widely uses chocolate is baking industry [9, 10, 11, 12, 32]. Chocolate is known for having both its positive

and negative effects in terms of health. Nutritional composition of chocolate comprises of antioxidant, proteins, caffeine, sugar, fats, minerals, phytochemical etc [8].

Presence of protein in chocolate regulates hunger level, food cravings, metabolic activities and maintains blood pressure [13, 22, 23, 24]. Chocolate comprise of both saturated and unsaturated fatty acids. Saturated fatty acids increase bad cholesterol (i.e. low-density lipoprotein-LDL) which clogs arteries. Unsaturated fatty acids increase good cholesterol (i.e. high-density lipoprotein-HDL) which escort the LDL to liver where it is broken and finally removed from body [14, 15, 16, 22, 23, 24]. The major content of chocolate is cocoa, which is rich source of antioxidant than other foods. This includes flavonoids, catechin and procyanidins, which inhibit the free radical effect and upregulate antioxidant defences necessary in body. These defences are linked to anti-inflammatory effects, insulin resistance, decreasing the risk of diabetes, protection from nerve injury, inflammation and UV radiation [17, 18, 22, 23, 24]. The soluble carbohydrates present in chocolates, which comprise of certain negative effects like increase blood fat level and sugar level [19, 22, 23, 24]. Caffeine present in chocolate stimulates central nervous as it blocks adenosine receptors which results into prevention of drowsiness and sleepiness. Its negative effect includes acid reflux and even bone loss [20, 21, 22, 23, 24].

2. MATERIALS AND METHOD

2.1. Sample collection

Total 14 samples were collected, out of which 6 were collected from retail market namely Lindt 90, Amul dark chocolate, Bournville, Dairy milk, Galaxy, Milky bar, 3 were from a homemade chocolate brand namely homemade dark chocolate, homemade milk chocolate, homemade white chocolate, 1 sample was an organic chocolate namely Pascati 72% dark chocolate, 1 sample was a cacao chocolate namely Chocolike 75% cacao dark chocolate and 3 pure ingredients were collected namely cocoa powder, cocoa butter/ Theobroma oil and cacao powder. All samples collected were in pure bar form which did not contain any extra addition to chocolates like wafer, nuts, fruits, etc. Also, the manufacturing month of all chocolate samples was same.

2.2. Sample name and number allotted

S1- Cocoa powder, S2- Cacao powder, S3- Lindt 90, S4-Amul dark chocolate, S5- Bournville, S6- Homemade dark chocolate, S7- Pascati 72% dark chocolate, S8- Dairy milk, S9- Galaxy, S10- Homemade milk chocolate, S11- Milky bar, S12- Homemade white chocolate, S13- Chocolike 75% cacao chocolate, S14- Cocoa butter/ Theobroma oil.

2.3. Measurement of pH

The pH of samples was done by the use of potentiometer. In a beaker, 1 gram of sample was dissolved in 25 ml of distilled water. Potentiometer

was used to check the pH of the entire sample [29, 30, 33]. The readings were observed.

2.4. Measurement of conductance

The measurement of conductance was performed using conductometer. In a beaker, 1 gram of chocolate sample was dissolved in 25 ml of distilled water. Conductometer was used to check the conductance of all the samples. The readings were observed [29, 30, 33].

2.5. Quantification of protein content

The protein content of chocolate samples was determined according to Igor magalhaes da V.M, *et al*, [13] with slight modification, by using Folin Lowry method. Bovine serum albumin (BSA) was used as standard. Aliquots of 1 ml of each chocolate (melted) were prepared. 5ml of alkaline copper sulphate reagent was made and incubated for 10 minutes at room temperature. In it 0.5ml of Folin-Ciocalteu reagent was added and the mixtures were incubated in dark conditions for 30 minutes. After 30 minutes, reading of the solutions at 660 nm using the colorimeter was done. Results were observed.

2.6. Measurement of fat content

Estimation of fat content was done according to Simoneau, *et al*, [15] with slight modifications using petroleum ether as a solvent. 1 gram of sample was taken in a beaker, and dissolved by providing heat in 25ml of water, it was added to separating funnel along with pet ether (20ml), constant shaking was

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provided and it was allowed to stand for 30 mins, this procedure was repeated thrice to extract fats present in samples. The readings were observed

2.7. Measurement of sugar content

The sugar content was estimated by Titrimetric method according to Lewis H. W., *et al*, [25] with slight modifications. Sample was centrifuged and the supernatant solution is taken in a volumetric flask and the volume is made up to 100 ml before estimation of sugar. 5ml of melted sample was taken in a sugar heating tube and to it 5 ml of A ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) and B (NaHCO_3 , Na_2CO_3 and KIO) mixture was added and a control set was made without any sample. The mixture was kept at 95°C water-bath for about 20 mins. After colour change from reddish and brick-red precipitation, the tubes were placed in ice cold water to cool. At room temperature, 5 ml of Solution C (KI and $\text{K}_2\text{Cr}_2\text{O}_4$) and 5 ml of Solution D (H_2SO_4) were added and thoroughly mixed. The content of each tube was then titrated with 0.01N Sodium thiosulphate solution using starch indicator. Thus, blank reading and sample reading was obtained and analysed.

2.8. Measurement of caffeine content

Extraction of caffeine content was performed by carbon tetrachloride method according to Bhawani S., *et al*, [21] with slight modifications. In the separating funnel 5ml of sample was added with distilled water. To it 1ml of sodium carbonate solution was added followed by 20ml of carbon tetrachloride. The funnel was inverted thrice and

venting of the funnel was done after each inversion. Addition of another 20 ml of carbon tetrachloride to aqueous solution in separating funnel was done and the extraction procedure was repeated twice.

2.9. Antioxidant activity

The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method was used according to David L., *et al*, [17], with slight modifications using Ascorbic acid as standard for antioxidant activity. 2ml of DPPH reagent was added to the sample solutions. The solution was incubated in dark to prevent any photo-oxidation or auto-oxidation for 30 minutes. Reading of the solution mixtures was done at 517 nm. This procedure was repeated for all samples. Percentage inhibition was calculated using formula:

Inhibition percentage = $\frac{[(\text{absorbance of control} - \text{absorbance of sample}) / \text{absorbance of control}] \times 100}{1}$

3. RESULTS AND DISCUSSION

3.1. Measurement of pH:

The highest value observed was of sample S5 which was 6.93 and average value observed was 5.25 and lowest value observed was of sample S1 which was 2.0 (Figure 1). The optimal amount of pH in chocolate should be ranging from 5.5 to 7.0; a chocolate which does not fit in this optimal range should be avoided [4]. Acidic pH can indicate presence of high amount fats and sugar [4]. The acidity is directly proportional to sugar and fat content, this could be because of presence of high

quantity of oils and emulsifier in chocolate. This acidity can cause short term harm like acid reflex, fatigue and long-term effects like imbalance in intestine and poor digestive system [8]. Consuming chocolate milk immediately after exercise and again at 2-hour post exercise can be optimal for exercise recovery and also may attenuate indices of muscle damage [26].

3.2. Measurement of conductance:

The highest value observed was of sample S1 which was 12.50, average value observed was 1.78 and lowest value observed was of sample S7 and S11 which was 0.2 (Figure 2). Electric conductivity of chocolate is dependent upon ionic content, moisture mobility, physical structure and production process [7]. As conductivity is directly related to moisture it can affect the moisture contain of chocolate and also the physical structure depending upon various conditions provided to chocolates for example temperature and pressure [7]. If favourable conditions are not provided, disturbed physical structure of chocolate can be observe and increased moisture contain which increases the risk of fungal and bacterial growth will be observed, hence it is suggested to store chocolate in favourable conditions [3, 6].

3.3. Protein content:

The highest value observed was of sample S4 and S13 which was 3.0 g, average value observed was 1.92

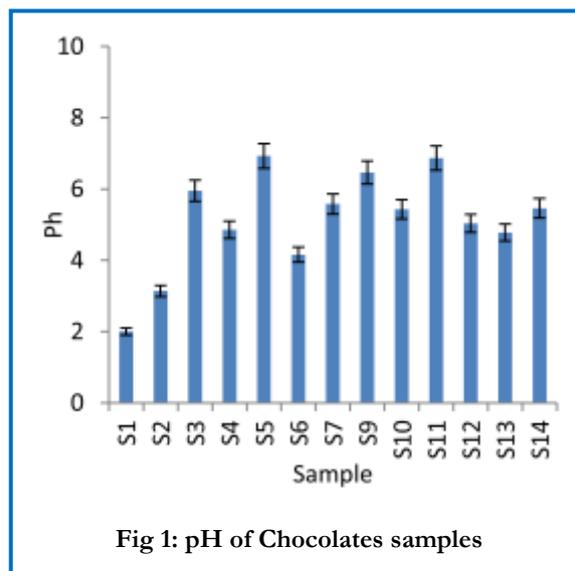


Fig 1: pH of Chocolates samples

g and lowest value observed was of sample S14 which was 0.5 g (Figure 3). According to the results, it can be observed that protein content in dark chocolate is comparatively higher than other chocolate which show moderate amount of protein. As protein is linked to increasing muscle mass and muscular strength, maintaining weight loss, lowering blood pressure, boosting metabolism, increasing fat burning, reducing cravings adequate consumption is suggested [23]. Also, moderate consumption (62.9/week) of chocolate may lower the risk of stroke [27]. Although overconsumption of protein

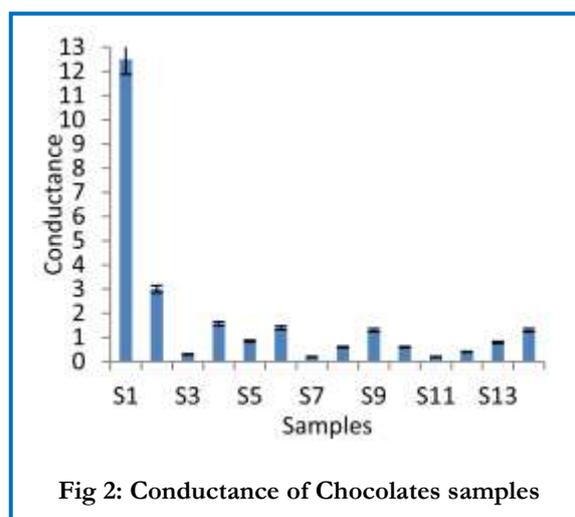


Fig 2: Conductance of Chocolates samples

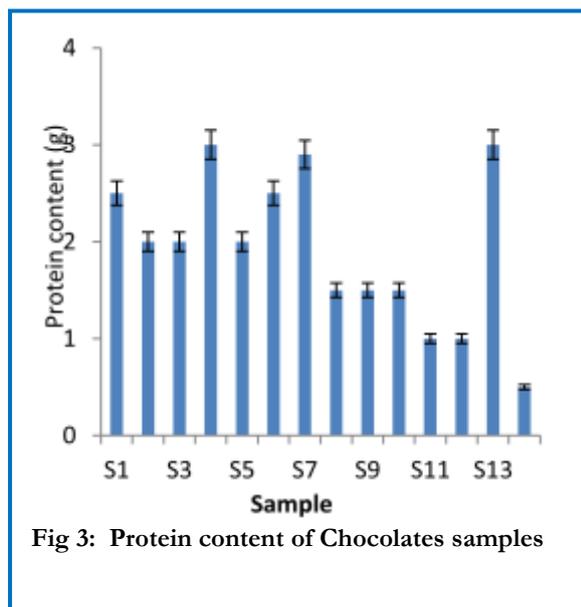


Fig 3: Protein content of Chocolates samples

may lead to variety of harmful effects like dehydration, constipation, kidney damage and increased chances of heart diseases [13, 22]. But in chocolate the highest amount was found to be 3g in 25g which if consumed in adequate amount that is not more than 100g per week would not lead to any harmful effect rather would provide positive effects on health [8].

3.4. Fat content:

The highest value observed was of sample S12 and

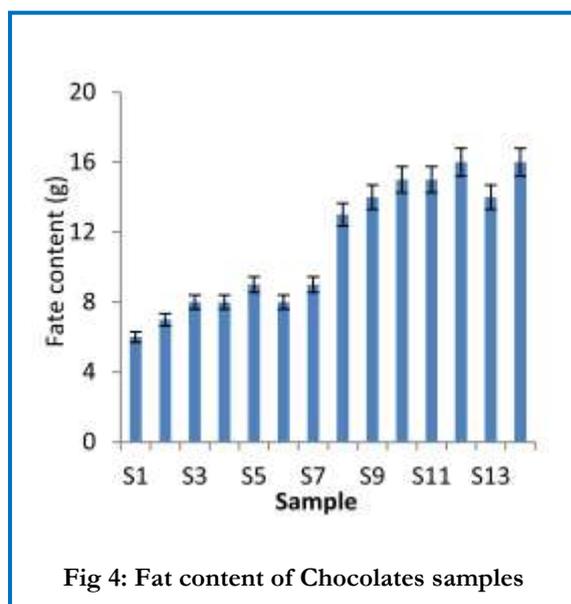


Fig 4: Fat content of Chocolates samples

S14 which was 16 g, average value observed was 11.28 g and lowest value observed was of sample S1 which was 6 g (Figure 4). Fat in chocolate comes from cocoa butter (Theobroma oil) and is made up of equal amounts of oleic acid, stearic acid and palmitic acid [31]. Stearic acid and palmitic acid are forms of saturated fat which increases bad cholesterol (i.e. low-density lipoprotein-LDL) which has shown to clog arteries, chocolate also shows presence of few unsaturated fatty acids that increase good cholesterol (i.e. high-density lipoprotein-HDL) which escort the LDL to liver where it is discarded from body [8,14]. Although stearic acid appears to have neutral effect on cholesterol specially in chocolate, but oleic and palmitic acid are linked to certain harmful conditions like obesity, chances of osteoporosis and disturbance in hormonal functions as well as liver function, hence it is suggested to consume chocolate considering the fat contents [15,16].

3.5. Sugar content:

The highest value observed was of sample S12 which was 19 g, average value observed was 12.35 g and lowest value observed was of sample S1 and S3 which was 7 g (Figure 5). Chocolate like milk and white have specifically shown increase in glucose in human body when consumed for 25 gram per day for three months, although dark chocolate when consumed 25g per day for three months did not associate with any changes [8]. Although it is indicated that sugar is linked to various harmful

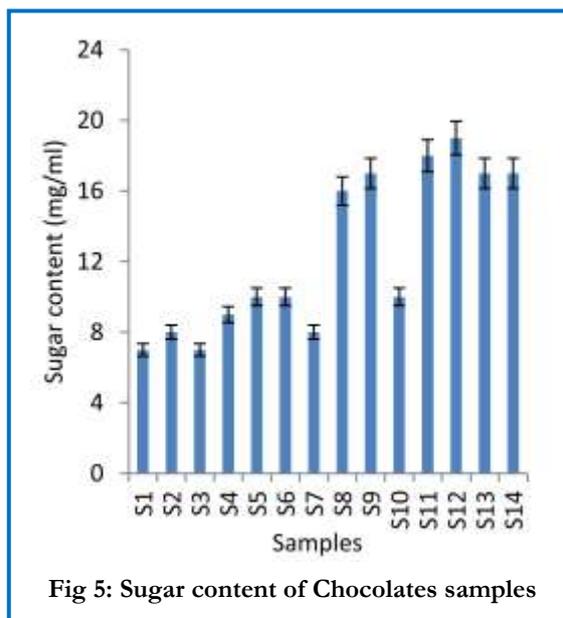
effects in body like increasing hunger and eventually leading to weight gain by causing resistance to leptin, increasing heart risk, increasing blood pressure and chances of stroke, and also causing skin problems like acne and early ageing [19, 27]. Hence it is suggested to be cautious about sugar consumption and choosing dark chocolate over milk or white chocolate [1].

3.6. Caffeine content:

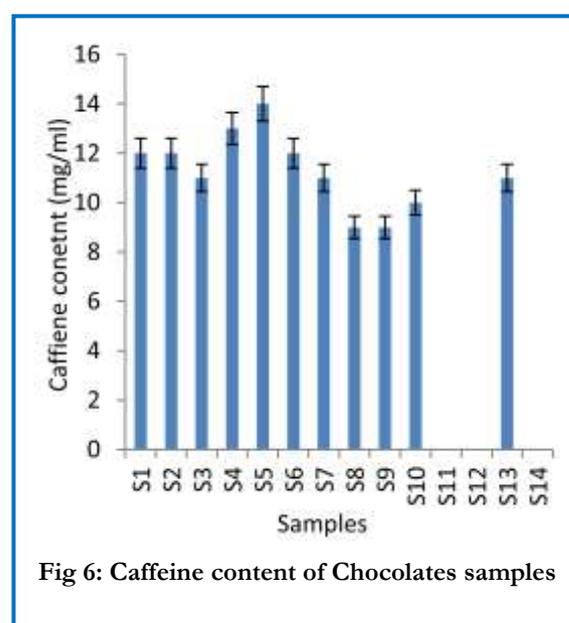
The highest value of caffeine was observed for sample S5 which was 14.0 mg, average value observed was 8.85 mg and lowest value observed was of sample S11, S12 and S14 (Figure 6). Caffeine is a methylxanthine compound that stimulates the central nervous system. Its use has been associated with enhanced cognition and improved athletic performance in few cases. It has also been used to treat drowsiness and reduce physical fatigue [21]. However, major side effects include increased anxiety, increased blood pressure, and diminished fine motor skills [20]. But it is present in very minimum amount in chocolate that is in milligram so it does not affect consumption at a primitive level [23].

3.7. Antioxidant content measured:

The percentage inhibition of samples increased with increase in concentration. The highest inhibition percentage value was observed for sample S13 which was 73.25 %, average value observed was 48.75 % and lowest value observed was of sample S14 which



showed no antioxidant activity (Figure 7). The antioxidant value is somewhat similar to that of our standard (ascorbic acid) which is 83.96 %. The antioxidant content in dark chocolate is comparatively higher than other chocolates which shows moderate amount of antioxidant activity. As antioxidant is linked to various activities like influencing insulin resistance and eventually reducing the risk of diabetes, protecting from nerve injury/



inflammation, and also protecting the skin from

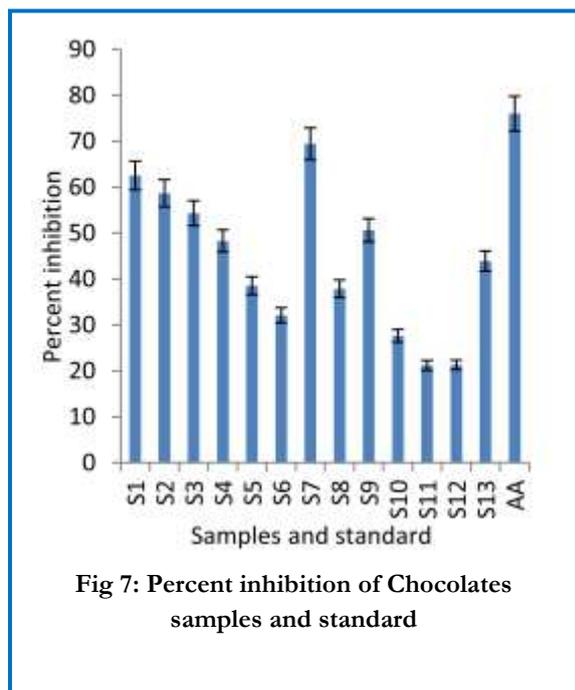


Fig 7: Percent inhibition of Chocolates samples and standard

oxidative damage from UV radiation, dark chocolate becomes a favourable choice for consumption and diet as it not only provides proteins but also antioxidants, which effects on variety of metabolic functions, cognitive function, and mood [8, 17]. Flavanols found in cocoa have been shown to increase the formation of endothelial nitric oxide which promotes vasodilation and therefore lead to blood pressure reduction [28].

4. CONCLUSION

Analysis of 14 samples was efficiently performed which were collected from variety of sources. According to the result obtained it can be concluded that dark chocolate have good amount of protein and antioxidant whereas white chocolate have more fats and sugar content. This indicates that nutrient value of chocolate is directly proportional to the amount of cocoa powder present in particular chocolate. Hence dark chocolate is the most preferable and nutrient

rich whereas white chocolate has the least nutrients and milk chocolate has moderate value of nutrients. Cacao chocolate and organic chocolates can at times be a more preferable due to their nutrient value but certain commercial dark chocolates can be as of same nutrient value when compared to cacao and organic chocolate. Also, cocoa and cacao powder in its pure form show high amount of proteins and antioxidant whereas this slightly reduces in chocolates due to addition of other ingredients like cocoa butter, emulsifiers, preservatives etc. Therefore it is important to choose chocolates wisely as health should not be compromised because of taste.

5. CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

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