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Process standardization for preparation of sorghum chocolate

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Abstract

Sorghum grain is a staple food in different parts of the world and in some parts of Western countries, is also used as animal feed. A combination of the increasingly important ability of sorghum crops to resist heat and drought, the limited history of the use of sorghum in Western foods, and the excellent functional properties of sorghum grain in healthy diets, suggests a greater focus on the development of new sorghum-based foods. Sorghum is rich in ash, antioxidants, crude fibre, fat, moisture, protein, total phenolic content, total flavonoids content. The roasted sorghum flour is mixed with dark chocolate, we able to sorghum popular enough along with enhanced it acceptability along various age groups. The sorghum chocolate will increase the taste, mouthfeel, nutrient properties, shelf life and medicinal properties like anti- obesity, anti diabetic, anti-cardiovascular, anti- inflammatory, antimicrobial and anticancer activity. The medicinal properties of dark chocolate helps in improve blood flow, lower blood pressure and reduce the heart diseases risk. The results revealed that it contain 2.4%ash, 34.9% antioxidants, 3.6% crude fibre, 32.2% fat, 3.8% moisture, 5.9% protein, 2.57mg total phenolic content, 1.45mg total flavonoids content.

Keywords: Standardization, preparation, sorghum, chocolate

Introduction

Sorghum bicolor (L). Moench is also known as great millet, is a grass species cultivated for its grain, which provides the food for humans and animals feed. Sorghum is produced in warmer climates, for increasing harvesting the minimum temperature should be 25degree Celsius. The morphological culture traits make it one of the currently cultivated cereals that has the best drought tolerance abilities. During the drought, it rolls its leaves to lessen water loss due to perspiration. If the drought continue then it will become dormant instead of dying. The leaves are protected by a waxy cuticle to reduce evaporate transpiration. Sorghum is the fifth most important cereal crop in the world after rice, wheat, corn and barley. It is the main cereal food for over 750 million people who are living in semi-arid tropical regions of Africa, Asia and Latin America (Kumar, 2019).

Nutritional Composition of Sorghum

The composition of sorghum grain and its parts is generally similar to that of corn, except for lower oil content. The grain contains 8 to 12% protein, 65 to 76% starch with approximately 2% fibre. The germ, a rich source of oil (28% of the germ) also has high levels of protein (19%) and ash (10%) (Hegde, 2005). Although almost all the bran is cellulose and hemicellulose, appreciable quantities of starch are deposited in the mesocarp tissue of this fraction. Bran lipid consists mostly of wax rather than oil. The composition of sorghum grain from different sources may vary because of many factors, including the nature of the hybrid, soil and climatic conditions, and manner of crop management (Chand *et al*, 2017) Older grain sorghum varieties differed considerably in kernel size and relative amounts of grain parts. With the newer hybrids and wider use of irrigation the grains are larger and are better filled with starch, and have lower protein content. The proximate composition and nutritional aspects of sorghum grain have been extensively reviewed. Lu *et al*. (2007) found that sorghum grain protein varies from 4.4 to 21.1% with a mean value of 11.4%. Sorghum grain is known for its hardness compared to other food grains. The hardness of the grain is due to higher content of protein prolamin (3.6 to 5.1%) (Mohamed *et al*, 2009). The lysine content ranges from 1.06 to 3.64%. The protein fractionation studies in sorghum indicated that the distribution of albumin-globulin, prolamin and glutelin is about 15, 26 and 44% respectively of total nitrogen. Starch is the major constituent of grain accounting for 56-75% of the total dry

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matter in the grain (Rathore *et al*, 2019). The total content of soluble sugars of sorghum grain ranged from 0.7 to 4.2% and the reducing sugars from 0.05 to 0.53%. Fat content in sorghum grain varies from 2.1 to 7.6%, crude fibre from 1.0 to 3.4% and ash from 1.3 to 3.3%. Another study on the physico-chemical characterization of sorghum accessions showed a wide variation in protein (7.99 to 17.8%), lipids (2.52 to 4.76%), starch (51.88 to 85%), and amylose (12.30 to 28.38%) content^[10]. Linoleic acid (18:2) and oleic acid (18:1) were the major fatty acid constituents of sorghum lipids (Hegde *et al*, 2005). The grain is commonly eaten with the testa which retains the majority of the nutrients. The wide range in composition of mineral and trace elements indicated that sorghum is a good source of minerals. The mineral composition however is influenced by the environmental conditions (Mohana *et al*, 2017)

The word "chocolate" is derived from the Classical word *chocolāte*. Chocolate word comes from the word "cacao" which is plant. This plant contains the high level of minerals and antioxidants. Chocolate is one of the most beloved foods that have usually creamy texture, sweet taste and brown color. It temporarily makes you feel happy. It is prepared by roasted and ground Theobromine cacao seeds which can be made in the form of a liquid or paste. The seeds of cocoa have bitter taste and used as a flavoring ingredient in many other foods. The making of chocolates is the result of long discovery and innovation.

Rationale and scope of the study

This study aimed at producing quality Chocolate from cheap and underutilized crops (Roasted Sorghum Flour). Chocolate were produced from roasted sorghum flour and dark chocolate blends using various proportions (15:85; 20:80; 25:75; 30:70 and 100% dark chocolate as control). Consumption of the Sorghum chocolate will increase the nutrient intake of the people especially children and also increase the utilization of sorghum in developing countries.

Then sensory were done through hedonic scale which was performed by different individuals. At last, Various Analysis (Nutritional, Anti-Nutritional and Antioxidant Activity) were performed and final product were kept for shelf life analysis which was performed after 5, 10 and 15 days.

Objectives of study

- Pre-treatment (Nutritional and Anti-nutritional Composition) analysis of the raw product [Sorghum].
- Formulation and optimization of Sorghum Chocolate.
- Various treatment (Nutritional, Anti-nutritional and microbial) analysis and shelf life analysis of the final product.

Material and Methods

All the below Experiments were done in Food Science and Nutrition Laboratory in Lovely Professional University, Phagwara, Punjab.

Raw Material

A raw material for preparation of sorghum Chocolate will be sorghum flour, Dark Chocolate, will be procured from the local market of Jalandhar.

Sorghum

The Sorghum used for the production of Sorghum Chocolate

is collected from agriculture store (LPU).

Chocolate

Chocolate used for the production of Sorghum Chocolate is collected from the local market, Phagwara.

Chocolate Mold

Chocolate mold used for the production of Sorghum Chocolate is collected from the local market, Jalandhar.

Reagents

All the chemicals utilized for the preparation of different reagents were of analytical grade (AR). The reagents required for analysis were freshly prepared in lovely professional university for adopting standard procedures.

Hot air oven

Hot air oven is used for drying glassware as well as sample

Weighing balance

The 0.001g to 0.1g accuracy weighing balance has been used for weighing the materials.

Blender

Mixer (230 v) was used for grinding of Sorghum Grains.

Refrigerator

For the storage of sample refrigerator was used.

Glassware

Glassware's (Borosil) were cleaned with cleaning solution and washed thoroughly through tap water and finely rinsed with distilled water and dried before use.

Microwave oven:

Microwave oven (LG) was used for chocolate melting.

Other minor equipments

- Steel Pan
- British Sieve
- Desiccator
- Spatula
- Bowl

Preparation of Sorghum Chocolate

Firstly, we do pre-treatment analysis of raw product like, (Nutritional Composition) analysis testing which include Moisture, Ash, Fat, Crude Fiber, Dietary Fiber, Protein, Resistant Starch and (Anti- Nutritional Composition) Analysis like Total Flavonoids Content and Total Tannins Content along with Antioxidant Activity. For making final product Initially, we grinded the sorghum grains in mixer grinder to make fine powder. Then by using British sieve we filtered powder having very fine particles out of it. Roasting of the Sorghum powder were carried out. After that, we took chocolate compound and Weigh the chocolate in weighing balance along with roasted sorghum powder then placed it in microwave oven with temperature 90 degree Celcius in order to melt it. After every 15 seconds we stirred our chocolate until good consistency were not achieved. We done it in order to prevent clumps.

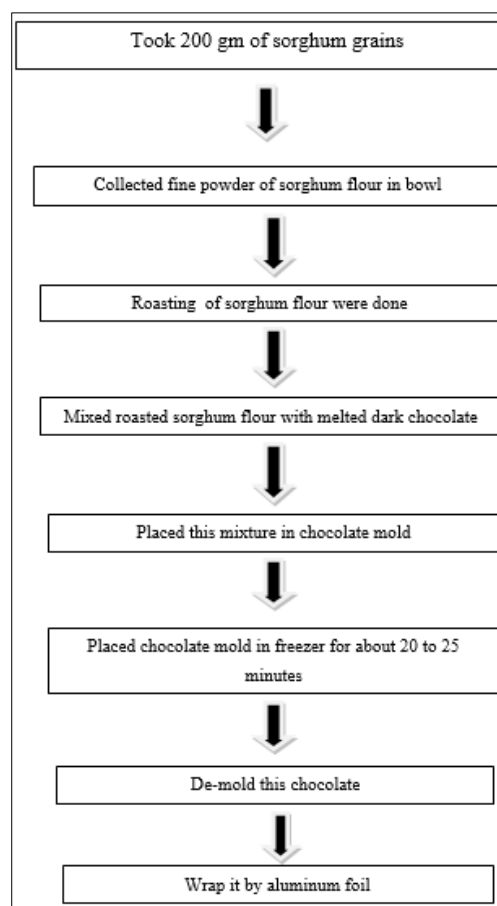
Formulation of the product were carried out according to different concentration i.e. for 10g of total product we took approx. 8.5g chocolate and rest 1.5g sorghum powder in S1,

In S2, we took 8g chocolate and 2g Sorghum powder, In S3, 7.5g Chocolate and 2.5g Sorghum Powder and 7g Chocolate and 3g Sorghum powder in S4. We prepared Control as well for comparing with our treatment samples, for this, we took only dark chocolate. Sorghum chocolate treatment with different concentration were shown in table below.

Table 1: Sorghum Chocolate treatments with different concentrations

S. No.	Treatment	Chocolate	Sorghum
1	S1	85	15
2	S2	80	20
3	S3	75	25
4	S4	70	30
5	S5	100	00

Mix the ingredients properly for few minutes until it fine consistency were made. Placed this mixture in chocolate mold. Important thing we done was to tap the chocolate mold so, by doing it there was not any kind of air bubbles or cracked left in it and we got the chocolate in proper shape and placed this chocolate mold in a freezer for about 20 to 25 minutes at a temperature of -19 degree Celsius in order to make it hard. Then carefully de-mold this chocolate and wrap it by using aluminum foil, then packed it in LDPE (Low Density Polyethylene) boxes and placed in freeze for few hours. At last, we grinded again this chocolate into fine powder for processing Nutritional Composition (Moisture, Ash, Crude Fiber, Protein, Fat, Energy), Anti-Nutritional Composition (Total Flavonoids Content, Total Tannins Content), Antioxidant Activity and Microbial Analysis.



Flow chart 1: Manufacturing Process of Sorghum Chocolate



Fig 1: Graphical Representation of Sorghum Chocolate with different concentration

Physiochemical analysis of sorghum chocolate

1) Determination of moisture

Moisture content is measured using AOAC method which is done by taking empty dish and lid are cleaned and dried in hot air oven at 105°C for about 4hrs and cool it in desiccator. Then weigh about 2g of sample and place into the dish and place the oven at 4hrs. After drying, place the sample with

dish inside desiccator to cool and weigh the sample.

$$\text{Moisture content (\%)} = \frac{(W1-W2)}{W1} \times 100$$

Where

W1= weight (g) of sample before drying.

W2= weight (g) of sample after drying.

2) Determination of ash content

Ash content of the sample can be determined by using a Muffle Furnace.

Procedure

Five grams of sample was weighed in a pre-dried silica dish. The dish was heated gently on a flame at first and then in muffle furnace at $550 \pm 20^\circ\text{C}$ till the gray ash resulted. The dish was then cooled in a desiccator and weighed. Heating, cooling and weighing steps were repeated till the difference between two successive weights was less than one milligram.

Calculations

The ash content was determined using the formula;

$$\text{Total ash percent by mass} = \frac{100 \times (M_2 - M)}{(M_1 - M)}$$

Where, M = mass in gram of the empty dish.

M_1 = mass in gram of the dish – material taken for the test

M_2 = mass in gram of the dish + ash

3) Determination of Fat content

Sample is weighed into a thimble and it is extracted using Soxhlet extractor for about 1 hour. The extract is received into the pre weighed flask with some solvent. The solvent is evaporated by placing it in a hot air oven at 48°C for about 1 hour. Place it in a desiccator to cool and then weigh it.

$$\text{Fat content (\%)} = \frac{\text{Weight of oil extracted}}{\text{Weight of sample taken}} \times 100$$

4) Determination of crude fiber

Crude fiber was estimated using AOAC method in which oven dried sample is placed inside the crucible and treated with 1.25% sulphuric acid and NaOH solution in the automatic fibre estimation machine. The residue is then placed inside muffle furnace for about 4.5 hr at 525°C . The crude fibre is calculated as,

$$\text{Crude fibre \% by wt} = \frac{W_1 - W_2}{W} \times 100$$

Where

W_1 = weight of crucible + sample before ashing

W_2 = weight of crucible + sample after ashing

W = weight of dried sample used

5) Estimation of Protein content

Protein content was estimated by the Kjeldahl method.

Kjeldahl method

The protein content of foods can be determined by numerous methods. The Kjeldahl method and the *nitrogen combustion (Dumas) method* for protein analysis are based on nitrogen determination. Both methods are official for the purposes of nutrition labelling of foods. While the Kjeldahl method has been used widely for over a hundred years, the recent availability of automated instrumentation for the Dumas method in many cases is replacing the use of the Kjeldahl method. In general, the Kjeldahl technique is separated into three primary advances. The strategy must be completed in legitimate grouping. The means digestion, distillation, and titration.

$$\text{Formula (\%)} = \frac{(b-a) \times 0.1 \times 14 \times 100}{\text{Weight of the sample}}$$

b = final reading during titration

a = initial reading

A factor is used to convert the percent N to percent crude protein. Most proteins contain 16% N, so the conversion factor is 6.38 ($100/15.65=6.38$)

% N/0.16 = % protein

The normal value for conversion of N percent crude protein is 6.25 but for dairy products like chocolate, the value is 6.46 (Eurachem. 2014).

6) Determination of DPPH

4 mg of 0.1mM DPPH is dissolved in 100ml methanol to make working solution. 200µl sample is dissolved in 2ml of working solution and incubator for 30 minutes in dark. Absorbance is read at 517nm for calculating DPPH free radical. The DPPH inhibition percentage was calculated as follow:

$$\text{DPPH inhibition \%} = \frac{A_c - A}{A_c} \times 100$$

❖ Microbiology analysis of sorghum chocolate

1. Determination of total plate count

Materials

Sorghum Chocolate sample, normal saline, petridishes, pipettes, nutrient agar.

Method

Aseptically transfer 1 g of Sorghum chocolate sample in 9 ml normal saline and mix the content homogenously and this is 1st dilution. Transfer 1 ml from 1st dilution into 9 ml normal in test tube and mix the content well to give 2nd dilution, similarly prepare 10^{-3} and 10^{-7} . Transfer 1 ml of the sample (10^{-3} and 10^{-7}) into sterile petridishes. Pour 10-15 ml (15-20 ml for 10 ml diluted sample volume) of NA into the petridishes. Mix the content well by clock and anti-clockwise rotation and allow the agar to solidify. Incubate the petridishes at 42°C for 24-28 hours. Enumerate the colonies formed and calculated by multiplying the average number of colonies per petridish with dilution factor.

Calculation

$$\text{LAB count (cfu/ml)} = \frac{\text{Number of colony count}}{\text{Serial dilution}}$$

2. Yeast and Mold

Materials

Sorghum Chocolate sample, Normal saline, Petridishes, Pipettes, Potato Dextrose Agar (PDA), 1% Tartaric acid solution.

Method

Aseptically transfer 1 g of Sorghum Chocolate sample in 9 ml normal saline and mix the content homogenously and this is 1st dilution. Transfer 1 ml from 1st dilution into 9 ml normal in test tube and mix the content well to give 2nd dilution, similarly prepare 10^{-3} , 10^{-5} and 10^{-7} . Transfer 1 ml of the sample (10^{-3} , 10^{-5} and 10^{-7}) dilution or 1 ml for 3rd, 5th or 7th

dilution into sterile petridishes. Acidify the melted and cooled (40-45 °C) PDA to pH 3.5 using 1% sterile tartaric acid solution (at 1% of the medium). Pour 10 – 15 ml (15 – 20 ml for 10 ml diluted sample volume) of the acidified PDA into petridishes. Mix the content well by clockwise and anti-clockwise rotation and allow the agar to solidify. Incubate the petridishes at 24-37 °C / 3-4 days. Enumerate the colonies formed and calculated the total yeast and mold by multiplying the average number of colonies per petridishes with dilution factor.

Calculation

$$\text{Yeast and mold (cfu/ml)} = \frac{\text{Number of colony count}}{\text{Serial dilution}}$$

Sensory analysis of sorghum chocolate

9-point hedonic scale is used for the sensory test where attributes such as Hardness, taste, flavour, bitterness and mouthfeel are considered using a 10-member panel. An overall acceptance rate is also calculated.

Table 2: Score card

Score card: Hedonic rating scale						
Name of student:.....			ID No:.....			
Name of judge:.....			Date of judging:.....			
Instruction: You are provided with a set of samples. Taste the sample and tick how much you like or dislike it. You can taste the sample more than once.						
Grade	Score	Sample No				
		Control (T ₀)	Sample 1 (T ₁)	Sample 2 (T ₂)	Sample 3(T ₃)	Sample 4 (T ₄)
Liked extremely	9					
Liked very much	8					
Liked moderately	7					
Liked slightly	6					
Neither like nor disliked	5					
Disliked slightly	4					
Disliked moderately	3					
Disliked very much	2					
Disliked extremely	1					

Results and Discussion

Experimental Results

The results obtained from the present study entitled “The Process Standardization for the preparation of Sorghum Chocolate” are as follows.

Proximate analysis of Raw Sorghum Flour

The quality of flour is influenced by the stage of pre-harvesting factors. From table-2 it was found that sorghum flour contains 10.69% of moisture in it whereas the ash content was found to be 2.20%. The protein content as observed by Kjeldahl method was found to be 11.82% along

with a crude fibre content of about 2.19% and having a fat 2.46%. Apart from this, having antioxidant of about 52% and having total flavonoids and total phenolic content of about 0.96 mg QE/g and 1.04 mg GAE/g respectively.

Chocolate & Sorghum concentration were made in the proportion of 85:15, 80:20, 75:25, 70:30 and control 100:00, in which 85:15 concentration emerges out to be an optimum one having an overall acceptability of score 8.1. At last, shelf life of final product were tested in which after 5 days, moisture increases to some extent, ash, protein, fibre and fat decreases. This trend will go same after 10 days, 15 days.

Table 3: Proximate Analysis of Raw fresh Sorghum flour

S. No.	Parameters	Fresh Sorghum	Roasted Sorghum
1	Moisture	10.69%	8.09%
2	Ash	2.20%	2.51%
3	Fat	2.46%	2.55%
4	Crude Fiber	2.19%	2.22%
5	Protein	11.82%	10.57%
6	Antioxidant Activity	52.6%	64.9%
7	Total Flavonoids Content (TFC)	0.96 mg QE/g	0.91 mg QE/g
8	Total Phenolic Content (TPC)	1.04 mg GAE/g	0.98 mg GAE/ g

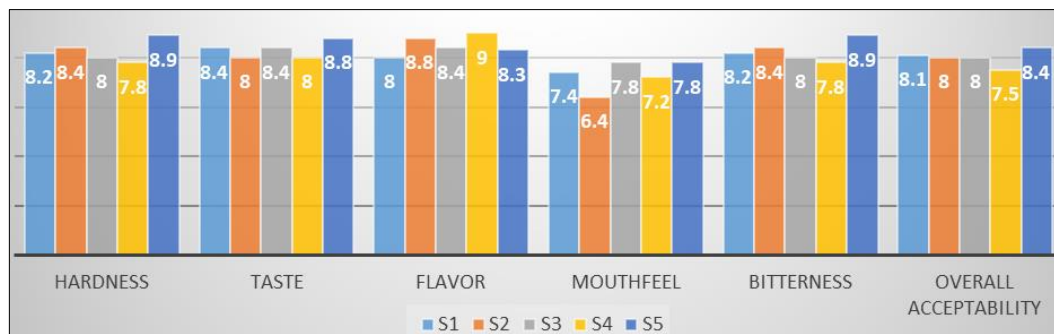
Quality Evaluation of Chocolate treated with various concentration of Sorghum Flour

Sorghum flour with different concentrations was used for chocolate preparation. Initially preliminary trails with different concentrations were performed and on the basis of

sensory evaluation an optimized value were obtained. Sensory analysis were done using hedonic scale in which product were tasted by different individuals. Which are shown in table-4 below

Table 4: Effect of different concentration on the sensorial properties of chocolate

Samples	Taste	Flavour	Mouth feel	Bitterness	Hardness	Overall acceptability
S1	8.4	8.0	7.4	8.2	8.2	8.1
S2	8.0	8.8	6.4	8.4	8.4	8.0
S3	8.4	8.4	7.8	8.0	7.8	8.0
S4	8.0	9.0	7.2	7.8	7.7	7.5
S5	8.8	8.3	8.6	8.9	7.6	8.4

**Fig 2:** Sensory attributes of Sorghum chocolate

As the concentration of sorghum was decreased from 30% to 25% to 20% to 15%, an increased level of bitterness was observed in the chocolates which might be because of the quantity and the types of polyphenols within that specific

bean used to make that chocolate. From the sensory evaluation, that shown in the table above; S1 which have the concentration of 15% Sorghum & 85% Chocolate have better overall acceptability that S2, S3 and S4.

Table 5: Storage stability of sorghum enriched chocolate

Sample	0 Day	5 Day	10 Day	15 Day
Moisture (%)	3.79 ± 0.1 ^d	2.60 ± 0.0 ^a	2.91 ± 0.1 ^b	3.2 ± 0.1 ^c
Ash (%)	2.71 ± 0.2 ^d	2.11 ± 0.1 ^c	1.80 ± 0.1 ^b	1.31 ± 0.5 ^a
Fat (%)	32.2 ± 0.01 ^d	31.50 ± 0.1 ^c	31.2 ± 0.1 ^b	31.11 ± 0.1 ^a
Protein (%)	5.91 ± 0.1 ^d	5.50 ± 0.1 ^c	5.11 ± 0.1 ^b	4.80 ± 0.1 ^a
Crude fiber	3.68 ± 0.01 ^c	3.20 ± 0.01 ^b	3.20 ± 0.01 ^b	3.11 ± 0.1 ^a
Antioxidant Activity	34.91 ± 0.01 ^d	27.61 ± 0.01 ^c	24.11 ± 0.01 ^b	20.50 ± 0.01 ^a
Total Flavonoids Content	1.44 ± 0.01 ^a	2.87 ± 0.01 ^b	2.95 ± 0.01 ^c	3.68 ± 0.1 ^d
Total Phenolic Content	2.55 ± 0.02 ^a	3.19 ± 0.01 ^b	3.96 ± 0.01 ^c	4.67 ± 0.1 ^d

Data are represented as Mean ± S.D.

In final proximate composition of sorghum chocolate is represented in table 5. The ash, antioxidants, fat, fibre, protein, moisture, total phenolic content, total flavonoids were found to be 1.3%, 20.5, 31.1%, 3.1%, 4.8% 3.2%, 4.68mg, 3.68mg respectively. Similar results were reported by ash content is 1.4% is higher than obtained analysis, antioxidants 21.9% is higher than obtained analysis, fat 30.1% which is lower than the results obtained, fibre 2.9% which is lower than the results obtained, protein 6.2% is higher than obtained analysis, moisture 2.7% which is lower than the results obtained, total phenolic content 4.92 mg it is similar with obtained results, total flavonoids is 3.2mg it is similar with obtained results. Higher temperature and longer roasting time had a detrimental effect on color and sensory acceptability of product. Roasting also extends the shelf life and safety of products by lowering the water activity, which reduces the rate of microbial growth. Roasted grains displayed enhanced crispiness and volume and improved texture because of puffing. The additional advantage was attributed to swelling and deformation of starch granules during roasting.

Conclusion

Sorghum is unpopular crop. The utilization of sorghum is lower especially in the urban areas. By blending sorghum with chocolate, it will ultimately increased the utilization of

sorghum to manifold. Through this popularity of sorghum will be going to enhanced. Through this study we may conclude that the optimization of Sorghum Chocolate with different concentration of Sorghum such as 1.5g (S1); 2g (S2); 2.5g (S3) & 3g (S4) in 8.5g, 8g, 7.5g, 7g of Chocolate. Through the sensory analysis, we found that the overall acceptability of S1 concentration is more as compared to the higher concentration of Sorghum S2, S3 and S4.

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