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Development and Physico-chemical Analysis of Chocolates Incorporated with Lycium barbarum (Goji Berries) and Salvia hispanica L. (Chia) Seeds

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Abstract

The study of chocolate incorporated with goji berries and chia seeds was carried out at Parul Institute of Applied Sciences, Parul University, Vadodara. The study aimed to develop a functional food product by incorporating goji berries and chia seeds into chocolate and evaluating its sensory properties and antioxidant activity. Incorporating Lycium barbarum (Goji Berries) and Salvia hispanica L. (Chia) seeds in chocolates can enhance their nutritional value and provide potential health benefits to consumers. The immune-boosting properties of goji berries are believed to be due to their high content of antioxidants, polysaccharides, and other bioactive compounds. Three different formulations were prepared T1, T2 and T3 by using dark chocolate, goji berries, chia seeds, dates, almonds, walnuts and hazelnuts. The best composition (T3) is used for physicochemical parameters such as moisture, ash, protein, fat, carbohydrate, fiber and antioxidant activity. All chocolates were examined for sensory evaluation like appearance, aroma, mouthfeel, taste, texture and overall acceptability. The T3 chocolate formulation was chosen as the best based on sensory assessment. Selected chocolates included 3.90% moisture, 1.77% total ash, 24.74g fat, 2.27g protein, 69.32g carbohydrates, 2.36g total fiber, and 81.37% antioxidant activity. The findings demonstrate that chocolates incorporated with goji berries and chia seeds are well-regarded and excellent immunity boosters and stress relievers.

Keywords: Chocolate, Goji berry, Chia seeds, immunity-booster, antioxidant-rich

1. Introduction

Chocolate is often consumed as a confectionery treat, but it is also used in baking and cooking. It is high in calories and fat, but it also contains antioxidants and other beneficial compounds, particularly in dark chocolate with a high percentage of cocoa solids. It has a long history and cultural significance, and it is enjoyed by people all over the world. It contains several compounds that are believed to have positive effects on the body, including flavonoids, which are antioxidants that can help protect the body from damage caused by free radicals. Some studies have suggested that eating chocolate may reduce the risk of heart disease and stroke, improve cognitive function, and even boost mood. As of 2021, the global chocolate market was valued at approximately 130 billion USD and is expected to grow at a CAGR of around 4% during the forecast period of 2021-2026. The market is driven by the growing demand for premium and organic chocolates, as consumers become more health-conscious and seek out higher-quality products. In addition, the increasing popularity of dark chocolate, which is perceived to have health benefits due to its higher cocoa content, is also contributing to market growth.

Lycium barbarum also known as goji berry or wolfberry, is a small, red fruit that grows on a shrub native to China. It is a popular superfood that has been used in traditional Chinese medicine for centuries. It is a 1-2 cm long berry, with a bright orange-red ellipsoid tint with a sweet and tangy flavour [Kulczyński B., Gramza-Michałowska A et al., 2016]. Goji berries are rich in antioxidants, vitamins, and minerals, making them a nutritious addition to your diet. They are particularly high in vitamin C, beta-carotene, and zeaxanthin, which are important for eye health [Murillo AG, Hu S, Fernandez ML et al., 2019]. In addition to their nutritional benefits, goji berries are believed to have a range of health benefits. They are said to boost the immune system, protect against age-related diseases, and improve brain function. However, more research is needed to confirm these claims. Some studies suggest that goji berries may help regulate blood sugar levels and improve insulin sensitivity, which could be beneficial for people with diabetes [Vidović BB, Milinčić DD, Marčetić MD et al., 2022].

Traditional Chinese medicine used goji berries to treat various ailments, including liver disease, eye problems, and infertility [Bucheli, Peter et al., 2011]. They were also used to improve circulation, boost the immune system, and promote longevity [Skenderidis P, Leontopoulos S et al., 2022]. Some studies have suggested that goji berries may have anticancer properties, possibly due to their high antioxidant content. Goji berries are a rich source of antioxidants, which are compounds that can protect the body from damage caused by free radicals [Ma ZF, Zhang H, Teh SS et al., 2019]. Free radicals are unstable molecules that can cause oxidative stress, a process that has been linked to various diseases, including cancer, heart disease, and Alzheimer's. One of the main antioxidants found in goji berries is vitamin C. Vitamin C is a water-soluble vitamin that plays an important role in many bodily functions, including the immune system, wound healing, and the production of collagen. Vitamin C is also a potent antioxidant that can neutralize free radicals and protect against oxidative stress [Kulczyński, Bartosz & Gramza Michalowska et al., 2016].

In addition to vitamin C, goji berries contain other antioxidants, such as zeaxanthin and polysaccharides [Donno, D., Beccaro, et al., 2015]. Zeaxanthin is a type of carotenoid that is particularly beneficial for eye health. It can help protect the eyes from damage caused by ultraviolet (UV) light and blue light, which can lead to age-related macular degeneration (AMD) and other eye conditions [Mohamad Hesam Shahrajabian et al., 2018v]. Goji berries are rich in antioxidants, such as carotenoids and phenolic compounds, which help to protect cells from damage caused by free radicals. Analysis of the antioxidant activity and phenylpropanoids in various medicinal Chinese herbs has documented the presence of these constituents in Goji berry at the level of 22.7 mg (gallic acid equivalent/extract) [Guo et al., 2008]. Antioxidants may also have anti-inflammatory effects and play a role in preventing chronic diseases.

Goji berries are known for their nutritional and medicinal properties, and one of the key components contributing to these benefits is flavonoids [Jianjun Chen, ChihCheng T et al., 2017]. Flavonoids are a group of plant compounds that are widely distributed in fruits, vegetables, and herbs, and they have been linked to a range of health benefits, such as antioxidant and anti-inflammatory effects. Wang et al. [2010] isolated and estimated the levels of unique flavonoid traces. The most familiar were quercetin-3-O-rutinoside, kaempferol-3-O-rutinoside, chlorogenic acid, caffeic acids, and small amounts of caffeoylquinic acid and p-coumaric acid [Zhong et al. 2013]. These flavonoids have been shown to have potent antioxidant and anti-inflammatory properties, which can help protect the body against oxidative stress and inflammation, two key factors that contribute to the development of chronic diseases [Agradi, S., Draghi, S, et al., 2022]. Quercetin is a flavonoid that is particularly abundant in goji berries, and it has been shown to have a range of health benefits, such as improving heart health, reducing inflammation, and enhancing immune function. Kaempferol is another flavonoid found in goji berries that have been shown to have anti-inflammatory and anticancer properties [Cao GW, Yang WG, Du P et al., 1994]. Myricetin and rutin are two other flavonoids found in goji berries that have been shown to have antioxidant and anti-inflammatory effects.

Chia seeds are small, edible seeds that come from the plant Salvia hispanica, native to Mexico and Guatemala [de Falco, B., Amato et al., 2017]. They have been consumed for centuries by the Aztecs and Mayans as a staple food source, and have gained popularity in recent years due to their numerous health benefits. Chia seeds are an excellent source of nutrients, including fiber, protein, omega-3 fatty acids, calcium, magnesium, and phosphorus [Kulczyński B, Kobus-Cisowska et al., 2019]. They are also high in antioxidants and low in calories. Chia seeds can be easily incorporated into your diet by adding them to smoothies, yogurt, oatmeal, or salads. They can also be used as a vegan egg substitute by mixing them with water. Chia seeds are known for their ability to absorb liquid and create a gel-

like substance, which makes them a popular ingredient in puddings and beverages [Charles W. Weber, Howard S. Gentry et al., 2010]. They are also commonly used as a thickening agent in soups and sauces. When consumed in moderation, chia seeds can provide numerous health benefits, including supporting digestion, promoting weight management, reducing inflammation, and supporting heart and bone health [Taga, M.S., Miller et al., 1884]. However, it is important to note that chia seeds should not be consumed in excess, as they are high in fiber and can cause digestive discomfort if consumed in large amounts.

2. Materials and Methodology

The present study entitled "Development of chocolate incorporated with goji berries and chia seeds" was carried out in the Department of Food Technology, Parul Institute of Applied Sciences, Parul University, Vadodara. This section enlists the materials used and elaborates on the processing techniques, organoleptic evaluation and analytical procedures followed during the research.

2.1 Materials

A. Raw Ingredients

The raw materials required for making of chocolates were goji berries, chia seeds, almonds, walnuts, hazelnuts, dates, dark chocolate and white chocolate. Most of the ingredients were bought from the local market and some from an online store.

B. Processing Equipment

Equipment required for the preparation of chocolate are weighing balance, spatula, bowl, chocolate mould, and other utensils were obtained from the food technology lab processing lab, Department of food technology, Parul Institute of Applied Sciences, Parul University, Vadodara.

C. Product Manufacturing Process

a. **Procurement Of Raw Material**
Good quality raw ingredients should be selected. The main ingredients are goji berries, chocolate, dates, hazelnuts, almonds, chia seeds, and walnuts.

b. **Weighing Of Ingredients (per serving)**
All the ingredients should be weighed accordingly:
- Goji berries: 10 g
- Dark Chocolate: 130g
- Dates: 30 g
- Almonds: 15 g
- Walnuts: 10 g
- Hazelnut: 15 g
- Chia seeds: 5 g
- White chocolate: 25g

c. **Chopping Dried Fruits and Nuts**
Cut finely all the mentioned dried fruits and nuts.

d. **Mixing**
Add chia seeds to chopped dried fruits and nuts and mix them.

e. **Tempering of Chocolate**

Tempering chocolate requires a specific time and temperature depending upon the type we use. White milk chocolate should be melted to 45 degrees and cooled down to 26 degrees and reheated to 29 degrees. Whereas the ideal working temperature for the chocolate is 87º to 91°.

f. **Piping white chocolate**
Pipe a little white chocolate into the mould and let it freeze for 5 minutes.

g. **Adding Dried Fruits, Chia Seeds, and Nuts**
Remove the mould from the freeze and add dried fruits, chia seeds, and nuts.

h. **Piping Dark Chocolate**
Now with help of a clean piping bag, add dark chocolate to cover the mould to the top.

i. **Freezing**
Freeze the chocolate in the freezer for 10 minutes or in the refrigerator for 20 minutes.

2.2. Methods

A. Physico-chemical Analysis

Ingredients like Goji berries, chia seeds, almonds, walnuts, hazelnuts, dates, dark chocolate and white chocolate that were used to prepare chocolates were analyzed for proximate composition including moisture, ash, protein, fat, carbohydrate, fibre, energy and antioxidants as per the standard procedures given by (AOAC 2005).

a. **Moisture Content**

Moisture content was estimated by drying the empty dish and 5g of the sample was weighed and crushed in the dish. The dish was then subjected to the oven for drying at 105°C for 4hrs. It was again weighed after cooling in a desiccator until constant weight. The resultant loss in weight was estimated as moisture content.

Moisture % = Initial weight(W1) - final weight (W2) / Initial weight 1 × 100

b. **Ash content**
Ash content was determined using the (AOAC 2005) procedure. Take a pre-weighed crucible, add 5g of sample, and heat at low flame till it is completely charred (smokeless), and then cool it down. The sample was then maintained in the muffle furnace for about 4 hrs, at 550°C. Later, cool it in a desiccator and weigh it. The procedure was repeated until two consecutive weights were constant. The percent ash was calculated by comprehending the difference between the initial and final weight.

Ash %=Weight before heating - Weight after heating weight of sample × 100

c. **Determination of Protein content**: Protein content was specified by the Micro-Kjeldhal method.

- Digestion: 200mg of the defatted crushed sample was accurately weighed and a pinch of the catalyst mixture K2So4:CuSo4:H8O red (91:8.2:0.8g) was added and then it was repositioned to the digestion flask, digestion was brought out with ml of concentrated H2So4 for 2-3hrs at 45°C till the content evolves colourless.

- Neutralization and Distillation: Digested sample was diluted to 50ml in a volumetric flask and made the final volume to 50ml with distilled water. Then the 5ml of aliquot was neutralized with 30% HCL and 40% of NaOH containing 5g of sodium thiosulphate. Distillation was carried and liberated ammonia was absorbed in a 2% boric acid solution containing methyl red as an indicator.

- Titration: Titre the collected ammonia against 0.01N H_2SO_4. Titer reading was noted, Nitrogen was calculated by using the following formula, and % protein was calculated by multiplying 6.25. Simultaneously a blank sample was also run.

Crude Protein % = (Sample titre - Blank titre) × 0.0014 × 6.25 / Sample weight × 100

d. **Crude Fat**: The fat analysis of chocolate was done using Soxhlet. 5g of sample was weighed and taken in thimble The extraction cups were dried in the oven at 130°C for 15 min and took the weight of empty cups. The extraction cups were cooled and 70ml of petroleum ether was added to them. The apparatus was pre-heated and when the temperature was acquired, the extraction cups were attached to the apparatus and left for boiling for 30min, followed by increasing for 20min, and finally, recovery of solvent was done for 10 min. The recovered ether was assembled and fat retained in extraction cups was estimated.

Fat = (W2 - W1) /W x 100

e. **Determination of Carbohydrates:** The carbohydrate content was estimated by deducting the sum of the value of moisture, fat, protein, total ash, and crude fiber. Calculate the NFE by the following formula

NFE % = 100 - (CP% + CF% + CF% + TOTAL ASH%)

CP = Crude protein, CF= Crude fat, CF= Crude fiber.

2.3 Quality Control

Quality refers to the level of excellence or superiority of something, whether it's a product, service, or process. It is often measured by its ability to meet customer expectations and requirements, compliance with industry standards, and metrics such as customer satisfaction, defect rates, and reliability.

A. Raw Material Quality Control

The goal of raw material quality control is to prevent quality issues from arising during production, which can lead to waste, rework, or product recalls. It is an essential aspect of quality management and involves establishing clear specifications, sampling procedures, and testing protocols to ensure that the raw materials meet the desired quality standards. Inspections, tests, and analyses on the raw materials was done to detect any defects, impurities, or inconsistencies that could affect the quality of the finished product.

B. **Process Control**

All treatments used during processing were standardized, and materials were utilized in precise proportions. Accurate techniques for preparation were used, and the containers were verified to be in good condition. During processing. satisfactory sanitary conditions are also maintained. The goal of process control was to ensure that the production process runs smoothly and consistently, with minimal variability and waste.

C. **Inspection of the Finished Product**

The purpose of the inspection is to identify any defects or issues in the finished product that could affect its performance or safety. Some tests were performed to evaluate specific properties related to the fined product like visual inspection, functional testing, destructive testing, and non-destructive testing. Inspection of the finished product is a quality control process that involves examining the final product to ensure that it meets the desired specifications and standards.

2.4 Sensory Evaluation

Sensory evaluation of chocolates incorporated with goji berries and chia seeds was carried out using a 9-point Hedonic scale by a panel of 10 semi-trained judges. Different chocolate samples were evaluated for their colour and mouthfeel. taste. flavour. and overall acceptability.

2.5 Formulation of Goji Berry Chocolates

Optimization of Chocolate incorporated with goji berries and chia seeds formulation (goji berries: chia seeds):

For optimization of the formulation of chocolates, three different ratios of goji berries: chia seeds were evaluated I.e. 1:1, 1:1.5 and 1:0.5. the best combination i.e. 1:0.5 was analyzed for both proximate composition and sensory attributes. The chocolates were prepared using the following ingredients: dark chocolate(130g), white chocolate(25g), goji berries(10g), chia seeds(5g), almonds(15g), dates(30g), walnuts(10g) and hazelnuts(15g). The total product yield was 25 chocolates.

Figure 1: Chocolates incorporated with Goji berries and chia seeds

Table 1: Ingredients and quantity used in the production of goji berry chocolates

Ingredients	T1 (1:1)	T2 (1:1.5)	T3 (1:0.5)
Dark Chocolate	250g	200g	130g
White Chocolate	-	25g	25g
Goji Berries	10g	10g	10g
Chia Seeds	10g	15g	5g
Dates	100g	50g	30g
Almond	15g	30g	15g
Walnut	-	15g	10g
Hazelnut	-	30g	15g

3. Results and Discussions

The result obtained during the evaluation of chocolates incorporated with goji berries and chia seeds is discussed here. Chocolate mould was used to shape the tempered chocolate and dates were used as a sweetener and binding agent. The final product was analyzed for physics-chemical analysis, and sensory analysis and stored at room temperature. Research experiments undertaken to standardize the method for manufacturing of chocolates have been discussed below:

A. Proximate Analysis of Goji Berry and Chia Seed Chocolates

Quality Characteristic	Units	Results
Moisture	%	3.90
Total ash	%	1.77
Fat	g/100g	24.74
Protein	g/100g	2.27
Carbohydrate	g/100g	69.32
Sugar	g/100g	33.20
Energy	KCal/100g	501.02
Total fiber	g/100g	2.36
Antioxidant activity	%	81.37

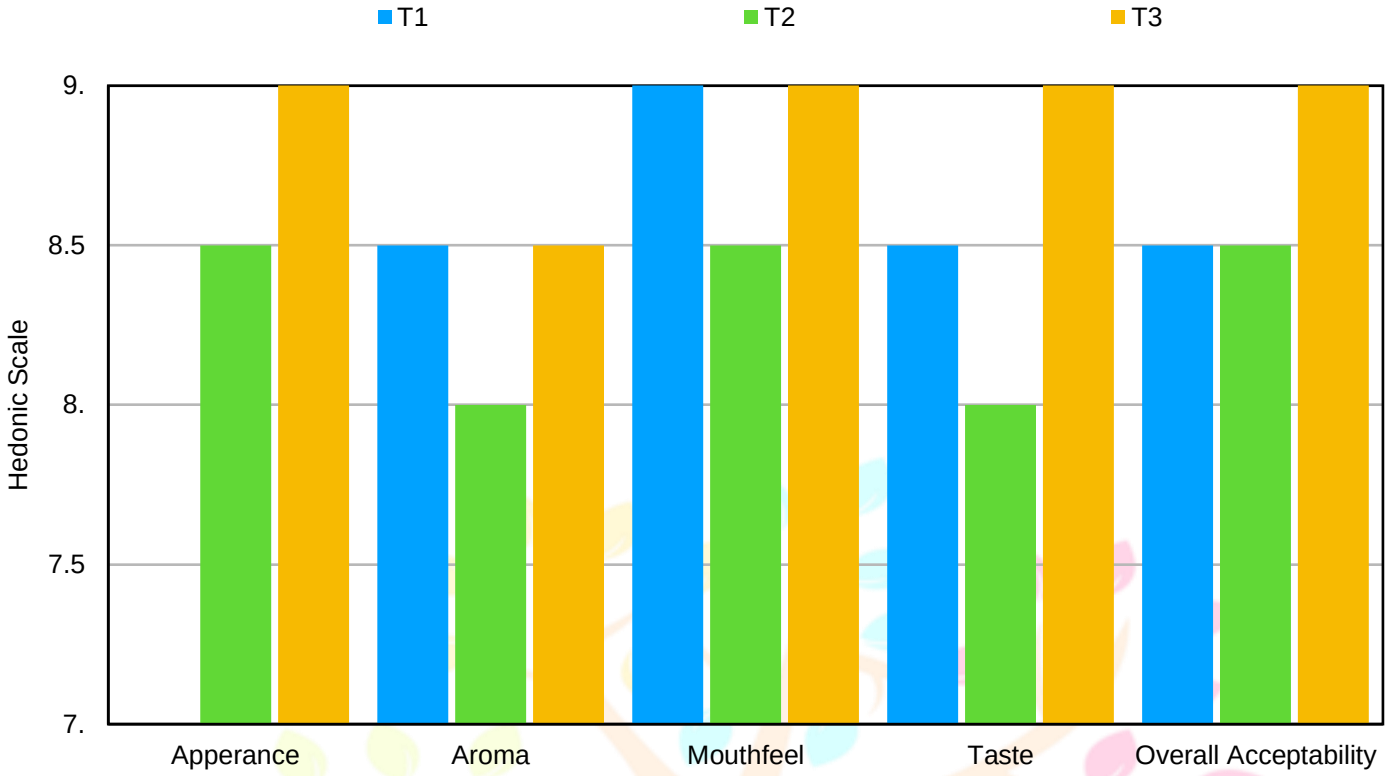
B. Sensory Evaluation Of Chocolates

A panel of semi-trained judges was responsible for the sensory evaluation of chocolates. The sensory score of chocolates incorporated with goji berries is shown below. According to the results presented, there was a significant increase in the taste and overall acceptability go goji berry chocolates. Formulation T3 has the highest score while T2 has the lowest score indicating the excess amount of chia seeds may alter the overall taste and palatability of chocolates. The overall acceptability score signifies that the T3 composition was best suited for the consumer palate.

4. Conclusion

From the above research, it can be concluded that the objectives of this study were met by incorporating goji berries and chia seeds and various other dried fruits and nuts into the chocolates. The development and evaluation of physicochemical properties and antioxidant activity were also carried out to check consumer satisfaction with the product. According to the results of all the formulations containing varied quantities of goji berries and chia seeds, T3 was found to be the best among all the different treatments. It also depicted good antioxidant activity. Selected chocolates included 3.90% moisture, 1.77% total ash, 24.74g fat, 2.27g protein, 69.32g carbohydrates, 2.36g total fiber, and 81.37% antioxidant activity. Goji berries are a good source of antioxidants, vitamins, and minerals. They are also

high in protein and fiber, making them a great addition to a healthy diet. They are rich in antioxidants, which can help



to protect the body against oxidative stress. This may be particularly important for mental health, as oxidative stress has been linked to a range of mental health disorders. Chia seeds, on the other hand, are also high in fiber and protein, as well as omega-3 fatty acids, which are important for brain and heart health. The market of chocolates is always ranking and is highly competitive. Companies are investing in product innovation and marketing to stay ahead of the competition as there has been a surge in sales of chocolate in the retail sector as consumers seek out comfort foods during times of stress and uncertainty. Combining newly popular superfoods with chocolate is an effective way to gain consumer attention and boost economic value.

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