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"Exploration of Foundational and trial-level Methodology for the Formulation, characterization, and Evaluation of Nutraceutical-enriched Cookies"

[1*]Bhatt Mehul K., [2]Mandani Varshal, [2]Thumar Deep, [2]Vaghani Parth, [2]Makwana Hardik, [3]Shah Nehal

[1]Department of Pharmacognosy,
School of Pharmacy, Indrashil University, Rajpur, kadi, Gujarat, India
[2]B.Pharm,
Indubhai Patel College of Pharmacy and Research centre, Dharmaj
GTU, Gujarat, India
[3] Department of Pharmaceutical Chemistry,
School of Pharmacy, Indrashil University, Rajpur, kadi, Gujarat, India
* Corresponding author email:mkb_0999@yahoo.co.in
Telephone: +91 7984509041

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Abstract:

Nutraceutical cookies represent a novel and innovative approach to combine the pleasure of indulging in a delightful snack with the potential health benefits derived from bioactive compounds. These cookies are designed to deliver specific nutrients, functional ingredients, and bioactive components, which can contribute to overall well-being and support various aspects of human health. This abstract provides an overview of nutraceutical cookies, highlighting their formulation, key ingredients, and potential health-promoting properties. The formulation of nutraceutical cookies involves selection of ingredients (Almond, Sorghum, Stevia, Hibiscus) to incorporate functional components such as dietary fibers, vitamins, minerals, antioxidants, and plant-based bioactive compounds. These ingredients are incorporated into the cookie matrix, ensuring their stability and bioavailability during consumption. This Nutraceutical cookies offer several potential health benefits. They are gluten free so the patient with celiac diseases can eat it. The inclusion of Stevia in place of sugar from natural sources helps for the diabetic patient. Furthermore, nutraceutical cookies have been formulated to address specific health concerns, such as weight management, bone health, immune support, cognitive function, and energy enhancement. In conclusion, nutraceutical cookies represent an exciting avenue for integrating health and taste. With their carefully crafted formulations and potential health benefits, these cookies offer a convenient and enjoyable way to incorporate functional ingredients into daily diets. Further research and development efforts are needed to explore new ingredient combinations, optimize formulations, and validate the efficacy of nutraceutical cookies in improving human health.

Key words: Bioactive compounds, Almond, Sorghum, Stevia, Hibiscus, Bioavailability, Antioxidants.

Introduction:

The growing awareness of the link between diet and health has led to an increased interest in functional foods and nutraceuticals. Nutraceuticals encompass foods or food components that provide health benefits beyond basic nutrition. In this context, the present study aims to develop nutraceutical cookies that not only serve as a delicious treat but also deliver essential nutrients and bioactive compounds. By incorporating these cookies into daily diets, consumers can enhance their overall well-being and potentially reduce the risk of various diseases.

Functional Foods: Functional foods belong to a special category of foods that offer health benefits beyond their basic nutritional value. These foods contain specific bioactive compounds that positively impact human health when consumed as part of a regular diet. They include natural nutrient-rich ingredients like fruits and vegetables, as well as fortified foods enriched with vitamins, minerals, probiotics, and dietary fiber.[1][2][6]

The History of Functional Foods: The term "functional foods" originated in Japan during the early 1980s and has since expanded to encompass various related terms such as "nutraceuticals," "Vita foods," "medical foods," "probiotics," and "pharmafoods."[3][4][5] Over time, functional foods have evolved to include a diverse range of food types intentionally enhanced or enriched to provide health benefits that go beyond essential nutrients.[7]

Types of Functional Foods: Functional foods can be classified into several types based on their unique characteristics and health-promoting properties:

- Fortified Foods: These foods have essential nutrients added to enhance their nutritional value.
- Enriched Foods: Specific nutrients are restored to their original levels after processing.
- Enhanced Foods: Foods enriched with additional bioactive compounds for added health benefits.
- Altered Foods: Foods with modified components to improve their health effects.
- Non-Altered Foods: Naturally nutrient-rich foods that inherently provide health benefits.[8]

Celiac Disease: Celiac disease is a chronic digestive and immune disorder triggered by the consumption of gluten-containing foods. Gluten, a protein found in wheat, barley, and rye, causes an immune response that damages the lining of the small intestine, leading to digestive issues and impaired nutrient absorption [9].

What is Celiac Disease? Celiac disease is an autoimmune disorder primarily affecting individuals with a genetic predisposition. When individuals with celiac disease consume gluten, their immune system attacks the small intestine's lining, leading to the deterioration of villi responsible for nutrient absorption. This results in malnutrition and other health complications [10].

Types of Celiac Disease: Celiac disease can manifest in various forms:

- Classical Celiac Disease: The most common type, characterized by typical digestive symptoms.
- Non-classical Celiac Disease: Presents with symptoms unrelated to the digestive system, such as anemia, skin rash, and neurological issues.
- Silent Celiac Disease: Asymptomatic, causing damage to the small intestine without apparent signs.[11][12]

Diabetes: High blood glucose levels are a hallmark of this long-term metabolic illness. Type 1 diabetes is caused by insufficient insulin production, while Type 2 diabetes is caused by the body responding less to insulin.[13]

Origin of Diabetes: Genetic, lifestyle, and environmental factors contribute to the development of diabetes. Type 1 diabetes has a genetic basis, while Type 2 diabetes is influenced by lifestyle choices, such as diet and physical activity [14].

Symptoms of Diabetes: Diabetes symptoms vary based on type and severity, including increased thirst, frequent urination, unexplained weight loss, fatigue, and blurred vision. Untreated diabetes can lead to serious health complications. [15]

In conclusion, functional foods, celiac disease, and diabetes are significant areas of research in nutrition and health. Understanding their complexities and potential benefits is essential for promoting overall well-being and developing targeted interventions for individuals affected by these conditions. Further exploration and evidence-based studies will continue to advance our knowledge and improve public health outcomes.

Cancer is a devastating disease characterized by the uncontrolled growth and spread of abnormal cells within the body. It can manifest almost anywhere in the human body, which is composed of trillions of cells that usually grow, divide, and replace old or damaged cells in a well-organized manner. However, in cancer, this orderly process breaks down, leading to the formation of tumors, some of which can be cancerous (malignant) and others non-cancerous (benign).[16]

The origin of cancer is complex and involves various factors, including genetic mutations, environmental exposures, lifestyle choices, and unknown elements. The disease's diverse nature is reflected in over 100 different types of cancer, each named based on the organs or tissues where they originate. Common categories include carcinoma, sarcoma, leukemia, lymphoma, multiple myeloma, melanoma, brain and spinal cord tumors, neuroendocrine tumors, and carcinoid tumors.[16]

Cancerous tumors can invade nearby tissues and metastasize to distant parts of the body, making treatment challenging. In contrast, benign tumors can often be safely removed without recurrence. Some cancers, such as leukemia, do not form solid tumors but affect the blood or bone marrow.

Understanding the signs and symptoms of cancer is essential for prompt diagnosis and treatment. General symptoms include exhaustion, fever or chills, unexplained weight fluctuations, abnormalities of the skin, alterations in bowel or bladder habits, respiratory problems, trouble swallowing, chronic indigestion, muscular or joint pain, and unexplained bleeding or bruises.

In India, cancer poses a significant health burden, with an estimated 14.6 million incident cases in 2022. Lung and breast cancers top the list of prevalent cancers in males and females, respectively. Early detection and proper management are vital to improving cancer outcomes.[17]

Cancer research and awareness efforts continue to be essential in addressing this global health challenge and enhancing patient care. By understanding the different types of cancer and recognizing potential symptoms, progress can be made towards better treatment and prevention strategies, offering hope for a future with improved cancer outcomes.

Materials for preparation:

Hibiscus flower powder, Arrowroot powder, Distill Water, Almond flour, Sorghum flour, Ghee, Stevia Powder, Baking Soda, Coconut milk, Salt. [18]-[45]

Methods of preparation:

Cookies: Prepare the Composite Flour:Mix 50.31%almond flour and 12.57% sorghum flour to create the composite flour.Create the Premixing Cream:Mix 18.86% ghee and 5% stevia powder to form a smooth premixing cream.Combine Flours and Premixing Cream:Add the composite flour to the premixing cream and blend together.Sprinkle 0.62% $NaHCO_3$ (baking soda) into the mixture and incorporate it.Make the Dough:Pour 12.57% coconut milk into the mixture to form a proper dough.Let the dough rest for 30 minutes.Shape, Bake, and Store:Preheat the oven to 200°C (392°F).Roll the cookie dough into desired shapes and place them on a baking sheet.Bake the cookies for about 15 minutes until they turn golden brown.Allow the cookies to cool at room temperature and store them in an appropriate container.[46]

Hibiscus jam: Collect the hibiscus flowers and wash them with water to remove foreign matters from flowers. Dry flowers under the sun and grind it with the grinder. Take 4.47% powder of dried hibiscus and soak in 20ml water and heat it for 15 minutes. Boil it with continues stirring, after boiling filter the solution. Add 1.11% stevia powder and cool for 20 minutes. Add the solution mixture of 4.47% arrowroot powder in 20ml water. Boil it further for 7 minutes to reduce volume. Cool it and store in jar at ambient temperature.[47]

SENSORY EVALUATION

Score card method

The 10-point scorecard for sensory evaluation is a widely used method to assess the quality and characteristics of a product. Each score on the scale represents a specific level of liking or disliking for various attributes, such as appearance, aroma, flavor, texture, and overall acceptability. A score of 10 indicates that the product excels in all aspects and fully meets the desired criteria, while a score of 0 suggests that the product fails to meet any of the criteria. The scale ranges from extreme dislike (1) to extreme liking (9), with a neutral midpoint (5) for neither liking nor disliking. Evaluators use this scoring system to provide objective feedback, which helps in refining and improving the product. Additionally, comments may be given to offer more detailed insights for further enhancement. This scorecard ensures a structured and systematic approach to sensory evaluation, enabling manufacturers to better understand consumers' preferences and make informed decisions in product development and enhancement.[48]

SHELF LIFE

Hedonic scale

The 9-point Hedonic scale is utilized to assess the shelf life of a product, determining its acceptability. A score of 9 signifies the highest quality and meeting desired criteria, while a score of 0 indicates non-compliance with any criteria. This method is commonly employed in research laboratories to evaluate the acceptability of hibiscus jams and cookies. A single sample of the product was served to five individuals for sensory evaluation and overall acceptability assessment.[48]

Physical evaluation parameters of the hibiscus jam

Viscosity: Viscosity measurements were carried out using advanced equipment, LFRA Texture Analyzer, made by Brookfield Engineering. The experiments were carried out in the controlled stress mode to measure the viscosity of the jam. About 250 g of jam was put into the stationary rheometer cup. The viscosities of the products were measured at temperatures between 25 to 26°C (±1). [47]
Instrument= Brookfield Viscometer
Appearance: The appearance of the jam was judged by its colour and roughness. [48]

Spreadability: For the determination of spreadability, excess of jam (3g) was applied in between two glass slides and was compressed to uniform thickness by placing 1000g weight for 5 minutes. Thereafter weight (50g) was added to the pan and the top plate was subjected to pull with the help of string attached to the hook. The time in which the upper glass slide moves the lower plate to cover a distance of 10cm is noted. A shorter interval indicates better spreadability. [48]

Antioxidant activity of individual Hibiscus water extract:
A solution of hydrogen peroxide (40mM) was prepared in phosphate buffer (PH 7.4) The concentration of hydrogen peroxide was determined by absorption at 230nm using a spectrophotometer. Hibiscus water extract(0.1-1 mg/ml) in distilled water were added to a hydrogen peroxide solution (0.06 ml, 40mM). The absorbance of hydrogen peroxide at 230nm was determined after ten minutes against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging by the water extracts of hibiscus and a standard compound was calculated as follows. [49]

% Inhibition = [(Control - Experimental) / Control] × 100

Ferric reducing antioxidant power (FRAP) assay
Prepare a phosphate buffer solution by dissolving the appropriate amounts of sodium phosphate monobasic (NaH_2PO4) and sodium phosphate dibasic (Na_2HPO4) in distilled water to achieve the desired PH. Commonly used pH values for the phosphate buffer in the FRAP assay range from 6.0 to 7.4. Prepare the sample solution (water extract of hibiscus) containing the antioxidant of interest. Dilute the sample if necessary to achieve a suitable concentration (1,2,3,4) for the assay. In a cuvette or microplate wells, combine the FRAP reagent with the sample solution in the desired ratio. The specific ratio may vary depending on the expected antioxidant concentration in the sample.Allow the reaction mixture to incubate at a defined temperature, typically at room temperature or as specified in the assay protocol. The incubation time may range from a few minutes to half an hour. After the incubation period, measure the absorbance of the coloured complex formed during the reduction of ferric ions. Use a spectrophotometer set at the appropriate wavelength, typically around 593 nm. Construct a calibration curve using known concentrations of a standard antioxidant compound, such as Ascorbic acid with same concentration. Determine the antioxidant capacity of the sample by comparing its absorbance value to the calibration curve. Express the results as Ascorbic acid equivalents or another standard antioxidant equivalent. [49]

% Inhibition = [(Control - Experimental) / Control] × 100

Phytochemical test (Tannin)
The tannin test was conducted by dissolving 1 ml of the water extracts oh hibiscus in a solution of 10% FeCl3. A greenish-black or dark blue colour indicated the presence of tannin. [50]

Estimation of Total tannin content

The tannins were determine by Folin-Ciocalteu method. About 0.1 ml of the sample hibiscus water extract was added to volumetric flask (10ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu phenol reagent, 1 ml of 35% sodium carbonate solution and dilute to 10 ml with distilled water.The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solution of tannic acid were prepared in the same manner as described earlier in different concentration such as 1,2,3,4,5.Absorbance for test and standard solutions were measured against the blank at 700nm with an UV/ Visible spectrophotometer. The tannin content was expressed in terms of mg of tannic acid equivalents /g of dried sample. [50]

Estimation of total phenolic content

Using the Folin-Ciocaltaeu test, the total phenolic content of the dry extracts was determined. 1 ml of sample (1mg/ml) was mixed with 1 ml of Folin-Ciocaltaeu phenol reagent. After 5 minutes, 10 ml of 7% sodium carbonate solution was added to mixture followed by the addition of 13ml of deionized distilled water and mixed thoroughly. The mixture was kept in dark for 90 minutes at 23°C, after which the absorbance was read at 760nm.The total phenolic content determined from extrapolation of calibration curve which was made by preparing Gallic acid solution.The Total Phenolic content was expressed as milligrams of Gallic acid equivalents (GAE)/g of dried sample. [50]

Thin layer chromatography for Hibiscus water extract

Thin layer chromatography (TLC) was used to determine the number of phyto-constituents in the extract. This method separated the phyto-constituents in the sample based on polarity. An inert substance, such silica gel, was applied on a thin plate to serve as a stationary phase.The mobile phase, which was put inside the chamber, is a 4:1 combination of methanol (CH_3OH) and chloroform ($CHCl_3$). Sample were prepared by diluted the extracts 0.5 ml of methanol.The water extract of hibiscus was applied to the TLC plate by using a capillary. After drying, it was put on the chamber's mobile phase. Phyto-constituents containing in the sample migrated and eluted at different rates based on its polarity. After completion, spots of Phyto-constituents in the TLC plate was visualized by spraying reagent (vanillin reagent) The Phyto-constituents was identified based on the retention factor (R_f) of its spot. The spot's location in the stationary phase following elution is represented by the retention factor, a metric used to analyze the migrated sample.The R_f formula is as follows: [50]

ash content of cookies Weigh empty silica crucible with 4 decimal place. Then add 1 cookie powder Then weight silica crucible with cookie powder. Heat the crucible on the burner till the cookie colour changes to ash grey. Cool it to room temperature then wait silica crucible.
Calculation-
Ash value(%) =[weight of the ash / weight of sample] X 100
Moisture content of cookies Moisture content of cookies was determined by hot air oven method. 10g of samples were weighed in China Dish. The dishes and their contents were put in the oven.. thermostatically controlled at 105oC and heated until successive weighing showed no further loss in weight. At the end, the dishes were removed from the oven and placed in desiccators, allowed to cool and weighed.
Calculation-[51]

Moisture content(%) =[(initial weight - dry weight) / initial weight] X 100 [51]

Physical evaluation of cookies

Volume-

Using a ruler, measure the height and radius of cookies in centimetres. Find the volume using formula-Volume=$\pi r^2 H$ (where r=Radius and H=Height of Cookie).[47]

Density

Using a ruler, measure the height and radius of cookies in centimetres. Find the volume using formula- Volume=$\pi r^2 H$ (where r=Radius and H=Height of Cookie).Now, find the density by using formula- **Density = mass / volume**[47]

Weight

Weight 10 cookie individually. Then after find the average weight of 10 cookie.[47]

Radius

Using ruler measure length of cookie. Then divide it with 2 to get the radius of cookie.[47]

Height

Using ruler measure height of cookie.[47]

Results and Discussion:

Score Card method for Acceptability of hibiscus jam and cookies

Sensory evaluation

10-point scorecard for sensory evaluation is a method used to assess the quality and characteristics of a product. The highest point 7.8 and lowest 7.0. The products include factors such as appearance, aroma, flavour, texture, and overall acceptability.

For hibiscus jam

Batch:1

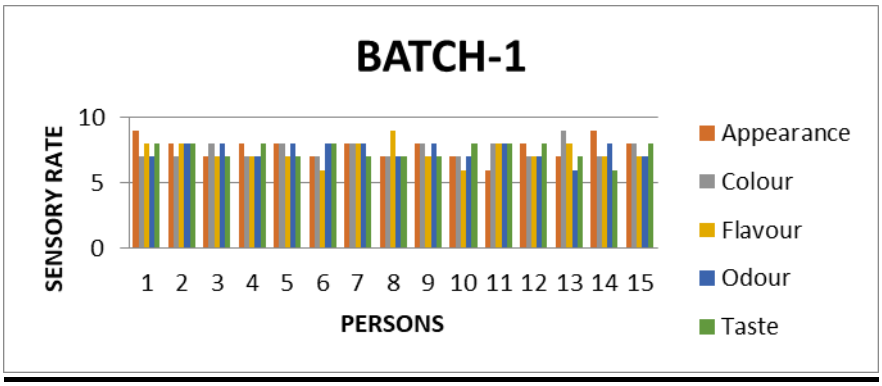

Fig. 1 sensory evaluation of jam batch-1

Batch:2

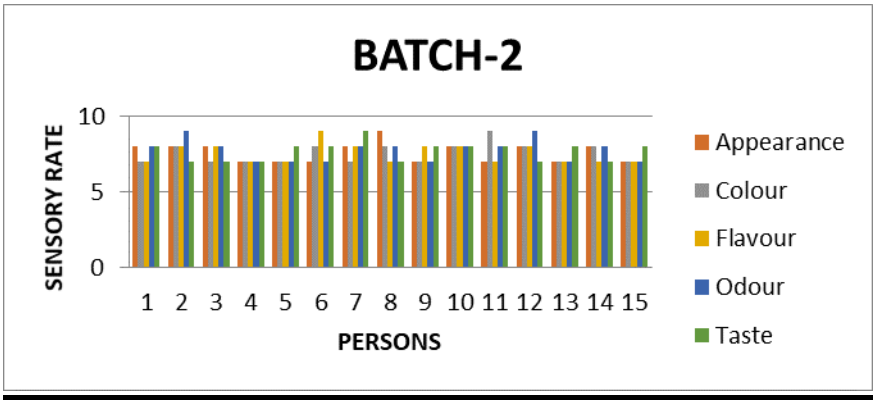

Fig. 2 sensory evaluation of jam batch-2

Batch:3

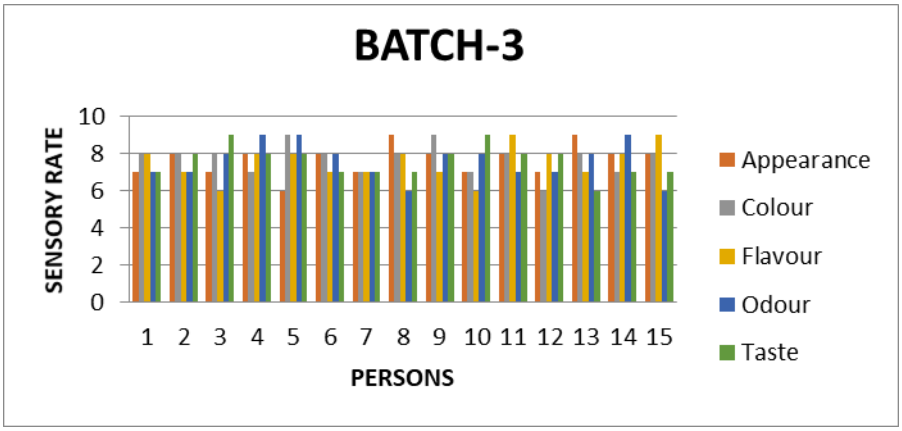

Fig. 3 sensory evaluation of jam batch-3

Sensory evaluation for cookies:

Batch:1

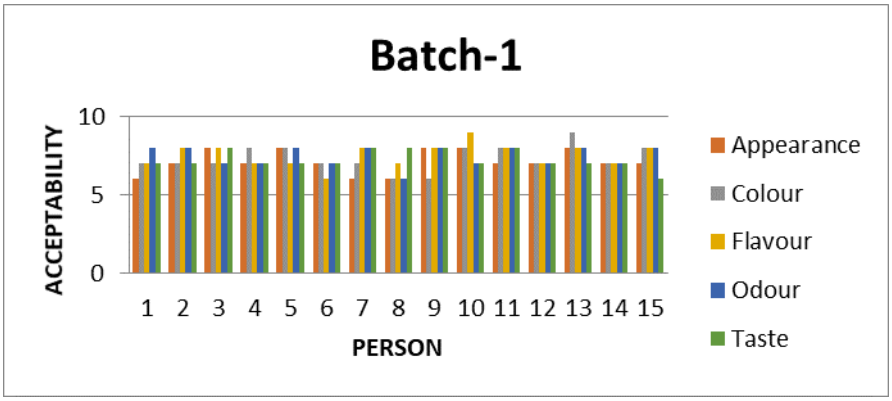

Fig. 4 sensory evaluation of cookies batch-1

Batch:2

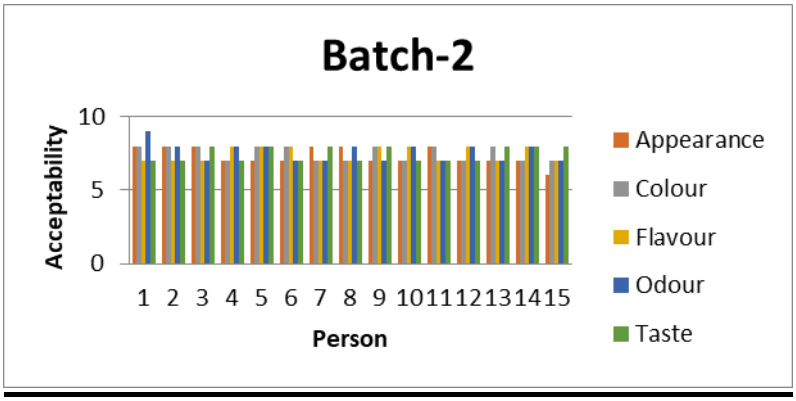

Fig. 5 sensory evaluation of cookies batch-2

Batch:3

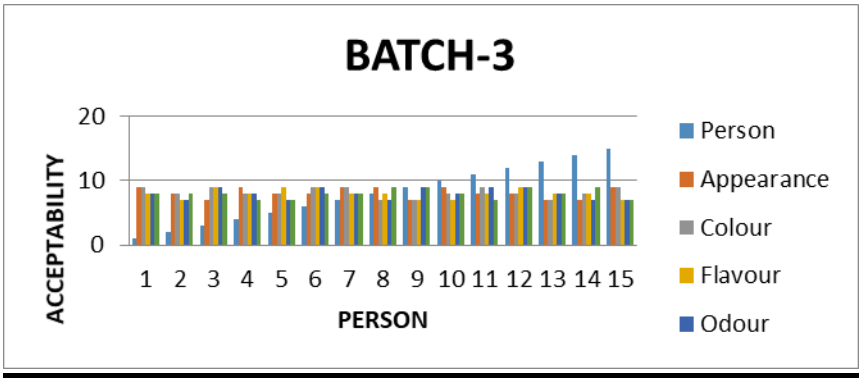

Fig. 6 sensory evaluation of cookies batch-3

Overall Acceptibility for Hibiscus jam:

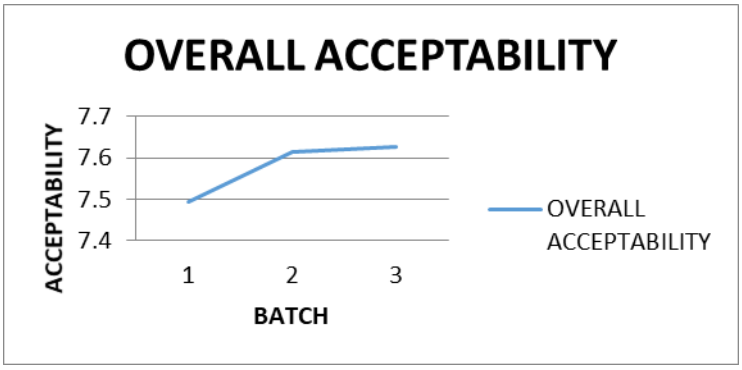

Fig. 7 overall acceptability of jam

Overall Acceptibility for cookies:

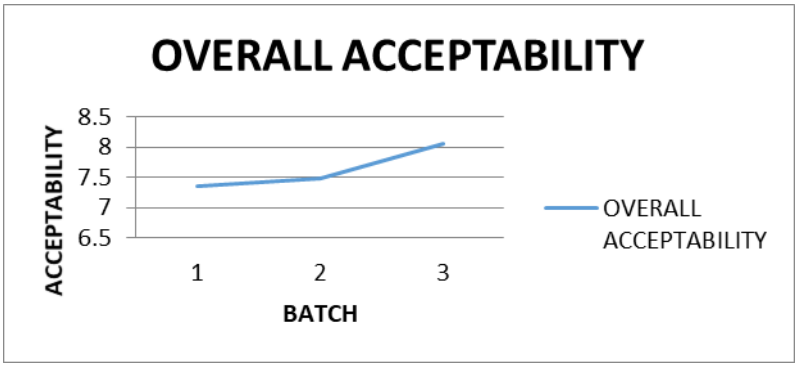

Fig. 8 overall acceptability of cookies

The overall acceptability of jam and cookies are like very much in scorecard method. Shelf life analysis of hibiscus jam and cookies analysed for shelf life evaluation by hedonic scale method.

Test the acceptability of hibiscus jams and cookies are evaluated using hedonic scale.

Hydrogen peroxide scavenging activity of *Hibiscus rosa-sinensis*

Concentration (mg/ml)	%inhibition	
	Sample (hibiscus)	Ascorbic acid
0.1	93.12%	94.02%
0.2	87.18%	92.91%
0.3	80.31%	90.37%
0.4	71.56%	84.72%
0.5	66.25%	83.75%

Table 1 % inhibition of sample and standard

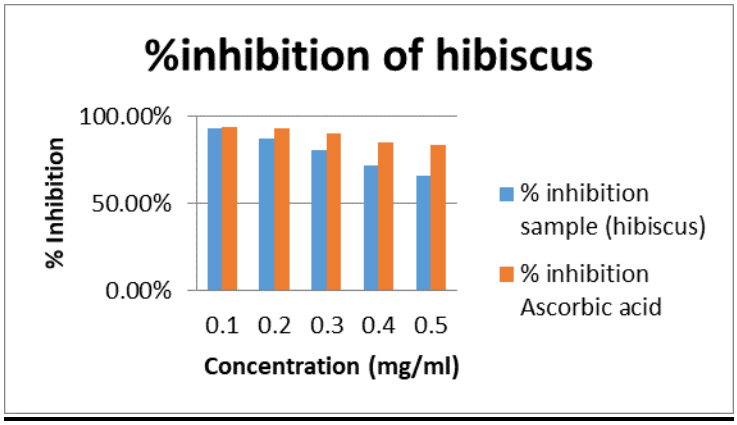

Fig. 9 % inhibition of sample and standard

Sample of *Hibiscus rosa-sinensis* inhibited the production of hydroxyl radical by 93.12% showing strong scavenging activity. However, the activity was comparable to ascorbic acid.

Ferric reducing antioxidant power (FRAP) assay for hibiscus%inhibition of hibiscus on different concentration

Concentration (mg/ml)	%inhibition	
	Sample (hibiscus)	Ascorbic acid
0.2	93.94%	94.07%
0.4	92.51%	92.77%
0.6	90.51%	90.80%
0.8	89.85%	89.96%

Table 2 % inhibition of sample and standard

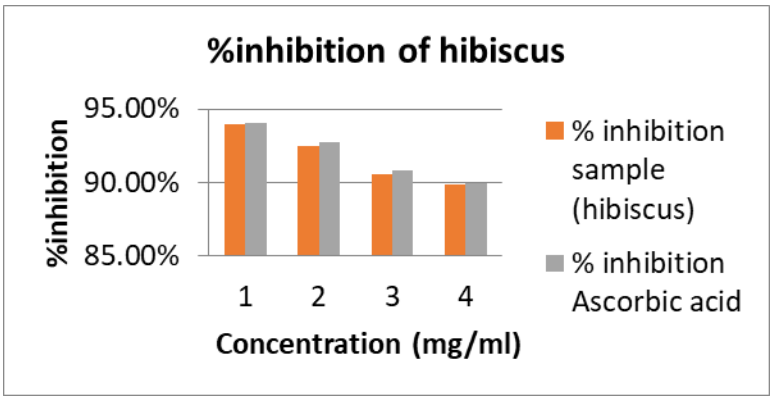

Figure 10 % inhibition of sample and standard

Sample of *Hibiscus rosa-sinensis* inhibited the free radical by 93.94% showing strong ferric reducing antioxidant power. However, the potential was comparable to ascorbic acid.

Tannin test for hibiscus water extract

When 1 ml extract dissolved in 10% $FeCl_3$, it shows Greenish-black that indicates the presence of tannin in extract.

Estimation of Total tannin content in hibiscus water extract

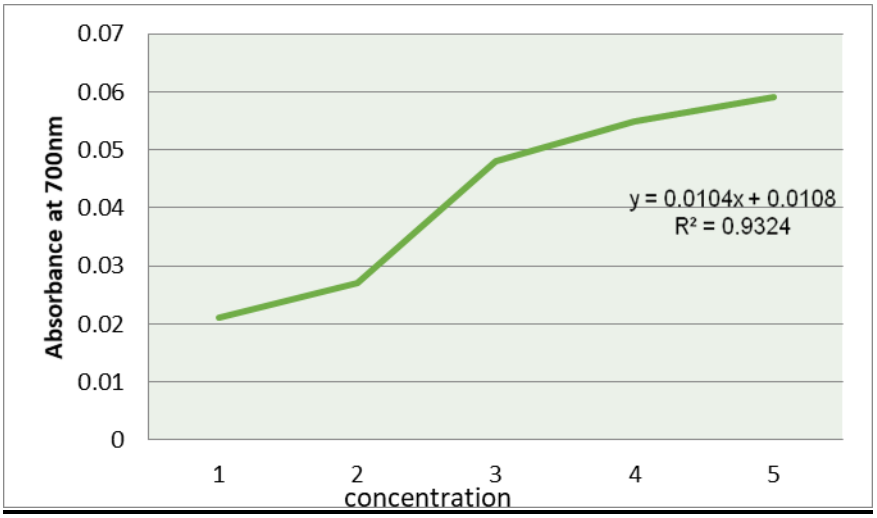

Fig. 11 total tannin content

Concentration of tannin can be calculated from absorbance graph withy = 0.0104x + 0.0108. The concentration of tannin was found to be 1.5576 mg/g.

Estimation of total phenolic content in hibiscus extract

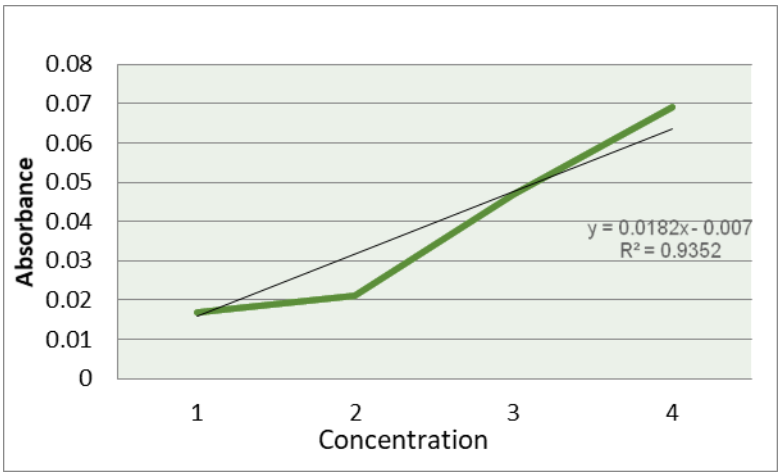

Fig. 12 total phenolic content

Concentration of phenols can be calculated from absorbance graph with y = 0.0182x - 0.007. the concentration of phenol was found to be 1.5384 mg GAE/g of extract.

Thin layer chromatography of hibiscus water extract

The TLC shows thre spot at respective R_f value 0.52, 0.58, and 0.51 respectively.

Physical evaluation of cookies

Volume	6.28 m^3

Density	1.2738 g/m^3
Weight	8 gm
Radius	2 cm
Height	0.5 cm
Thickness	0.5 cm
Spreadability	2

Table 3 physical evaluation of cookies

Ash Content of Cookies

The total ash value of cookie was found to be 73.33 %w/w. With help of the ash value we can find total inorganic mineral content present in product.

Total moisture content of Cookie

The total Moisture Content of cookie was found to be 5%.With help of the Moisture Content we can find quality and texture , self-life, packaging consideration , stability and sensory Abrribute

Conclusion:

The exploration of various flour combinations has led to the creation of hibiscus jam and gluten-free, sugar-free cookies enriched with antioxidants, offering a delightful and health-conscious treat. The presence of phytoconstituents in hibiscus flower contributes to their notable anti-oxidant properties. However, the development of hibiscus jam has revealed certain stability concerns. As result, it is evident that additional comprehensive research is imperative to overcome these challenges and facilitate the creation of a stable hibiscus jam product in the future. Incorporating hibiscus, known for its high antioxidant content, into the jam and combining it with gluten-free and sugar-free ingredients results in a delicious and guilt-free indulgence. Almonds, a gluten-free ingredient, ensure that individuals with gluten sensitivities or celiac disease can enjoy these cookies. The use of Stevia, a natural sweetener, instead of sugar makes these treats suitable for those seeking to reduce their sugar intake or follow a sugar-free lifestyle. The addition of hibiscus brings extra health benefits due to its powerful antioxidant properties, which combat oxidative stress in the body. By incorporating these hibiscus jam and cookies into our diet, we can enjoy a delightful treat while potentially reaping the benefits of antioxidants to support overall health and reduce the risk of chronic diseases. These homemade treats provide a wonderful combination of taste, dietary inclusivity, and potential health advantages, making them a guilt-free pleasure that contributes to a balanced and wholesome lifestyle. In totality, further systematic investigation is warranted to facilitate the refinement and advancement of these Nutraceutical cookies.

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