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Formulation Of Rosa Nutballs And Evaluation Of Its Proximate Principles, Phytochemical Components, And Antioxidant Property

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ABSTRACT

As the world is grappling with COVID-19, consumers resort to improve their immunity by ingesting immune-enhancing foods like health beverages, soups, snacks, and savories. This impetus has led to innovation in the snack market a healthy and delicious product. In the present study, Rosa nut balls were formulated and tested for their antioxidant activity. Rosa nut balls made of nuts and rose petal is a good source of energy, protein, and phytochemicals as well. They can be consumed as snacks that provide a load of nutrients. They are relished by people of all ages due to their irresistible taste. Rosa damascena, known as Damask rose is the most famous ornamental flower with medicinal properties. Toffee made of nuts covered with rose petals was analyzed for proximate principles. The antioxidant power of rose petals was estimated by DPPH and FRAP. The total carbohydrate, protein and fat per toffee of 25 gms were found to be 14.98 gm,1.95gm, and 5.025 gm. The antioxidant activity was determined by DPPH and Ferric reducing power. Percent of inhibition at 120 (µg/mL) was 52.59 and 50.85 respectively.

Key words: Rosa damascene, phytochemicals, therapeutics, anti-diabetic, antimicrobial

Introduction

Snack foods are usually defined as energy-dense, nutrient-poor foods that are taken in between meals to lessen hunger pans. Sometimes they also offer nutrients like sugar, sodium, and saturated fat in very high quantity that is detrimental to health [1]. Eating a “snack food” has led to an overall perception as negative dietary practice whereas when developed with proper knowledge and care, snacks can act as supplementary food or a booster food for people of all age groups [2]

Snack bars are consumed by people who want an instant source of energy due to a lack of time for an adequate meal and to refresh them. Such bars are also frequently called energy bars, food bars, protein bars, fruit bars, cereal bars, granola bars, nut bars, or sports bars. They are ready-to-eat confections that are not only rich in energy but also in proteins [3]

Pasteli is the first-ever snack made with a focus on nutritional content. Pasteli is made out of honey, sesame and nuts. It was used by Greeks to improve the protein content of food and is believed to have healing properties by the ancient Greeks; therefore, incorporated them into Pastéli, a functional food [4]

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In consideration of the urbanized lifestyle, it can be predicted that the snack industry will broaden its spectrum not only to provide nutrient-rich products but also to furnish disease-specific formulations in the mere future

With the above background, Rosa nutballs, a nutritious snack ball was prepared using a mixture of nuts and rose petals. The nutballs are loaded with protein, carbohydrate, fat, and phytochemicals. The ingredients used for making the rosa balls included white chocolate, walnuts, peanuts, almonds, and rose petals. Rosa damascena petals are known for their medicinal properties. The petals are a good source of polyphenols. Both the nutrients and the phytochemicals present in nutball make it a functional food and nutraceutical. The objective of the study is to formulate rosa nut balls and analyze the proximate principles.

Material and Methods

The study was conducted in a food processing laboratory of a private University. Edible rose petals of the species Rosa damascena were purchased from a traditional medicine shop. The phytochemical screening was done to detect the presence of phytochemicals. Quantitative analysis was done to estimate the amount of total phenol and flavonoid. FRAP assay and DPPH were done to demonstrate the antioxidant activity. Formulation of rosa nut ball was carried out step by step and analyzed for sensory quality. The proximate principles were analyzed in a laboratory to find out the quantity of macronutrients present.

Phytochemical assay

The different qualitative phytochemical tests were performed for establishing the profile of the aqueous extract for its chemical composition. The following tests were performed on the extracts to detect various phytoconstituents present in them.

1. Detection of alkaloids

To the extract, a few drops of concentrated hydrochloric acid were added to the sides of the test tube, followed by a few drops of saturated picric acid solution. Yellow precipitate indicates the test as positive for alkaloids.

2. Detection of phenol

To the extract, 200µl of neutral ferric chloride solution was added and shaken

well. Dark green or Violet Purple or dark brown colour indicates the presence of phenol.

3. Detection of glycosides

To the extract, a Sodium nitroprusside solution few drops and 3-5 drops of Pyridine were added. Blood red colour indicates the presence of glycosides.

4. Detection of terpenoids

Salkowski test :

The extract was added to 2 ml of chloroform. Concentrated H2S04 (3ml) was carefully added. A reddish-brown ring coloration of the interface indicates the presence of terpenoids.

5. Detection of flavonoids

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To the extract, a few drops of 1% NaOH solution, were added. A yellow colour formation within short period is positive for flavonoids.

6. Detection of tannins

To the extract, few drops of lead acetate solution was added and observed for brownish green or a blue-black coloration

7. Detection of saponins

Foam test

The extract was diluted with 5mL distilled water. The suspension was shaken in a graduated cyclinder for 15 min. A 2cm layer of foam indicated the presence of saponins.

Quantitative estimation

Total phenolic content

The total phenolic content (TPC) was estimated using the Folin-Ciocalteu reagent. Briefly, 0.5 mL of three different extracts were mixed with 0.5 mL of distilled water and added to2.5 mL of diluted Folin's reagent, followed by the addition of 2 mL of 7.5% (w/v) sodium carbonate. After incubation in the dark, their absorbance was determined by a spectrophotometer at 750 nm. All measurements were repeated three times and the concentration of total phenolic content was expressed as µg of gallic acid equivalent per gram of the extract

Total flavonoid content

The total flavonoid content of rose petals extract was estimated by the aluminium chloride method. 250 µl of the three different fractions of crude extracts was added to 1.25 ml of deionized water and 75 µl of 5% NaNO2 (w/v). After 5 min, 150 µl of 10% AlCl3 and 0.5 ml of 1 M NaOH (w/v) were added. The volume was completed to 2.5ml with deionized water and measured at 510 nm. All measurements were repeated three times the concentration of total flavonoid content was expressed as µg of quercetin equivalent per gram

Evaluation of antioxidant activity

DPPH˙ radical scavenging activity:

The radical scavenging capacity of the ethanol Rose petals extract was measured based on DPPH (1, 1- diphenyl 2-picrylhydrazyl) radical scavenging activity. One mL of 0.1 mM DPPH solution in methanol was mixed with 1 mL of various concentrations (20-120 µg/mL) of rose petals extract sample. The mixture was then allowed to stand for 30 min incubation in dark. One mL methanol mixed with 1 mL DPPH solution was used as the control. The decrease in absorbance was measured at 517 nm. Ascorbic acid was used as the standard reference. The percentage of inhibition was calculated as % of DPPH˙ radical inhibition = Control –Sample × 100 Control

Ferric (Fe 3+) reducing power assay

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The reducing power of ethanol Rose petals extract was determined by the potassium ferricyanide method. One mL of extract with different concentrations (20 - 120 µg/mL) was mixed with 1 mL of 1% (w/v) potassium ferricyanide [K3Fe (CN)6] solution and 1 mL of phosphate buffer (0.2 M, pH 6.6). The mixtures were then incubated at 50°C in a water bath for 20 min. Five hundred µL of 10% (w/v) trichloroacetic acid solution was added to each mixture followed by 1 mL of 0.1% (w/v) freshly prepared FeCl3 solution was added and shaken well. The absorbance was measured at 700 nm and ascorbic acid was used as the standard reference. The percentage of inhibition was calculated using the following formula: % of Fe3+ reduction = Sample– Control ×100 Sample

Proximate analysis

Proximate Analysis stands for a method, which determines the values of the macronutrients in food samples. In general, those values are being declared as the nutritional value of food products, but they are also being determined during the production process.

The nutrient content of rosa toffee with regard to carbohydrate, protein, fat, fiber, and energy has been assessed by the following procedure.

Method of Estimation of Carbohydrate - Anthrone method (Yemm and Willis 1954)

Protein content - Lowry method (Lowry et al., 1951)

Fat - Soxhlet method (Folch et al.,1957)

Ash content - AOAC 1990

Fibre content – AOAC method 1999.

Results and discussion

Qualitative and quantitative phytochemical analysis

The rose petals were qualitatively assessed for their phytochemical compounds followed by a quantitative estimation of anthocyanin. The antioxidant activity of the rose petals was evaluated by FRAP and DPPH assay. The proximate principles showed that rosa balls contain a sufficient amount of macronutrients along with phytochemical anthocyanin.

The screening for the phytochemicals by trease and evans method indicated the presence of phytochemicals (table 1) flavonoids, tannins, phenols, alkaloids, and terpenoids. Saponin was absent in the rose petal extract.

A study by (Singh &Patel, 2021) [5]concluded that plant petal extracts contain many secondary metabolites such as flavonoids, polyphenols, steroids, and terpenoids. Nowak et al., 2013 stated that in the methanolic extract, four phenolic acids were determined, including large amounts of gallic acid (9.55 mg to 1 g of dry extract) followed by smaller amounts of protocatechuic, gentisic and pcoumaric acids. LC-ESI-MS/MS analysis of flavonoids revealed the presence of nine flavonoid glycosides, primarily quercetin (0.32– 1.12 µg mg−1) and kaempferol derivatives (0.05–0.14 µg mg−1).[6]

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The total phenolic content (table 2) of fresh Rosa damascena species was 276 ± 0.03 µg/mg. TPC of rose petals differs with rosa species. The TPC in different species varied from 25.13 to 52.01 mg GAE/g DW. The highest values of TPC were observed in R. canina species and the lowest values in R. webbiana species.[7] determined that the TPC in 12 cultivars of edible flowers was from 253 to 528 mg GAE/100 g FW. In another study, the TPC of Rosa hips ranged from 55 to 122 mg GAE/g DW.

Antioxidant assays

The antioxidant activity of the phenolic compounds was attributed to its redox properties, which allow them to act as reducing agents, hydrogen donators, singlet oxygen quenchers, and have also metal chelating properties.[8]

FRAP ASSAY

Table 3 shows the antioxidant activity of the fresh rose petals. Ferric reducing antioxidant power was about 24.56 %, 24.89 %, 28.63 %, 33.33 % and 47.72 % and 50.85 % respectively at 0.2, 0.4, 0.6, 0.8, 1.0 and 1.20 concentration. On an average basis, water extract was found most potent in terms of its antioxidant potential and it was found to have rutin (182.45 µg g^{-1}) as the most abundant phenolic compounds. Water extract also exhibited maximum TAA and FRAP (ferric reducing activating power) with $AU_{0.5}$ values of 116.0 and 18.5 µg, respectively. Total phenolic and flavonoid contents were found maximum in methanol and ethyl acetate fractions, respectively . [9]

DPPH ASSAY

Oxidative stress is critical for chronic human diseases such as cancer. Roses were proposed to display diverse biological activities and possess strong antioxidant activity from *R. brunonii*, *R. bourboniana*, and *R. damascene*.[10] Water or methanolic extractions are suitable for enhancing antiradical activity in the extracts tested. The free radical scavenging activity of methanol extract can be attributed to the presence of polyphenols and flavonoids as this fraction contains the maximum amount of these secondary metabolites. [11]

Table 4 shows the antioxidant activity of the fresh rose petals by DPPH assay. Antioxidant activity was about 3.89 %, 19.80 %, 29.22 %, 32.46 %, 44.80 % and 52.59 % respectively at 0.2, 0.4, 0.6, 0.8, 1.0 and 1.20 concentration. As demonstrated by the DPPH assay 100 µg/ml of rose petal extract exhibited over 44 % radical scavenging activity; this was comparable to that of 10 µg/ml of vitamin C

Proximate analysis

The proximate value of the toffee was assessed for 100 gms (table 5). Carbohydrate was 52.25 gms while the protein content was found to be 7.95. Toffee contains a very good amount of proteins and it can be consumed by people of all ages. Protein is essential for the syntheis of cells as well as repairing the damaged cells, for the synthesis of hormones and enzymes. Fat content was found to be 16.10 gms of which most of the fat were unsaturated fatty acids. Nuts are good sources of unsaturated fatty acids. Thus when a person consumes a

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toffee of 25 – 30 gm, he can easily get 100 calories along with a good source of protein, fat, and fiber. Phytochemicals present it helps in delaying age-related diseases, bolster immunity and promote good health. [12]

Carbohydrates were the most abundant macronutrients, followed by proteins and ash. Fructose, glucose and sucrose were identified in all the rose cultivars. Rose petals gave the highest content of organic acids, mainly due to the presence of malic and quinic acids, respectively. Polyunsaturated fatty acids predominated over saturated fatty acids, mainly due to the contribution of linoleic acid .[13]

Sensory evaluation of Rosa toffee

Five healthy adults were chosen as panel members to score the sensory attributes of the toffee. The size of the toffee was 30 mg. The participants were instructed to taste the sample and immediately provide the sensory rating based on a five-point hedonic scorecard for evaluation. The five-point hedonic scale (table 6) was scored as 1-poor, 2- fair, 3- good, 4-very good and 5 – excellent. Individual scores were given to appearance, colour, texture, flavour and taste.

The sensory evaluation received a mean score higher than four on a total score of five for all the sensory attributes indicating that the product was good and was acceptable for consumption. The lowest score was rated for taste (4.27±0.69) and the highest score was rated for the colour of the supplement. Polyphenols contribute to the sensory and nutritional qualities of toffee. The higher sensory rating of the rosa toffee can be attributed to the presence of phenolics. It is indicated that the main phytochemicals in the rose petals were flavonoids, terpenes, tannins, and polyphenols.

Conclusion

The rosa balls made out of rose petals and nuts are a good source of protein polyunsaturated fatty acid and fiber. It has a mouth lingering taste and flavor. The phytochemicals add to their functional value. The fibre content improves the probiotic property of the toffee. Thus rosa toffees can be consumed as a snack by people of all ages. It can act as a supplement food by enhancing nutrient intake.

Acknowledgment

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Conflict of Interest

The author has no conflict of interest.

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Tables

Qualitative screening of Phytochemicals

Table :1

Phytochemicals	**Presence/absence**
Flavonoids	+++
Tannins	+
Saponins	_-
Phenols	+++
Alkaloids	++
Terpenoids	+++

+++ - abundance, ++ - moderate, + - presence – absence

Table 2 : Quantitative estimation of rose petals

Phytochemcial	Quantity
Total phenol content - µg/mg of gallic acid	276 ± 0.03
Flavonoid - µg/mg of Quaricitine	13.2 ± 0.01

Table 3: Ferric reducing antioxidant power

S.No	**Concentration µg/ml**	**Absorbance**	**% Reduction Fe 3 + reduction**
1	**20**	**0.228**	**24.56**
2	**40**	**0.229**	**24.89**
3	**60**	**0.241**	**28.63**
4	**80**	**0.258**	**33.33**
5	**100**	**0.329**	**47.72**
6	**120**	**0.350**	**50.85**

Table 4 : DPPH assay

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S.No	Concentration µg/ml	Absorbance	% Reduction DPPH radical
1	20	0.296	3.89
2	40	0.247	19.80
3	60	0.218	29.22
4	80	0.208	32.46
5	100	0.170	44.80
6	120	0.146	52.59

Table 5: Proximate principles

S.No	Nutritional value	Results
1	Protein	7.95 g/100 g
2	Carbohydrates	52.25 g/100 g
3	Total fat	16.10 g/100 g
4	Energy	421 k/cal
5	Fibre	3.7 g/100 g
6.	Moisture	11.17 g/100 g
7	Ash	0.83 g/100 g
II	**Antioxidants**	
8	BHA	LOQ 10.0
9	BHT	LOQ 10.0
10	TBHQ	LOQ 10.0

LOQ – Limit of quantification g/100 g

Table 6: Sensory evaluation

Sensory attributes	Minimum Hedonic score	Maximum Hedonic score	Sensory hedonic rating	
			Mean	SD
Appearance	1	5	4.50	0.51
Colour	1	5	4.87	0.35
Texture	1	5	4.77	0.43
Flavour	1	5	4.43	0.57
Taste	1	5	4.27	0.69

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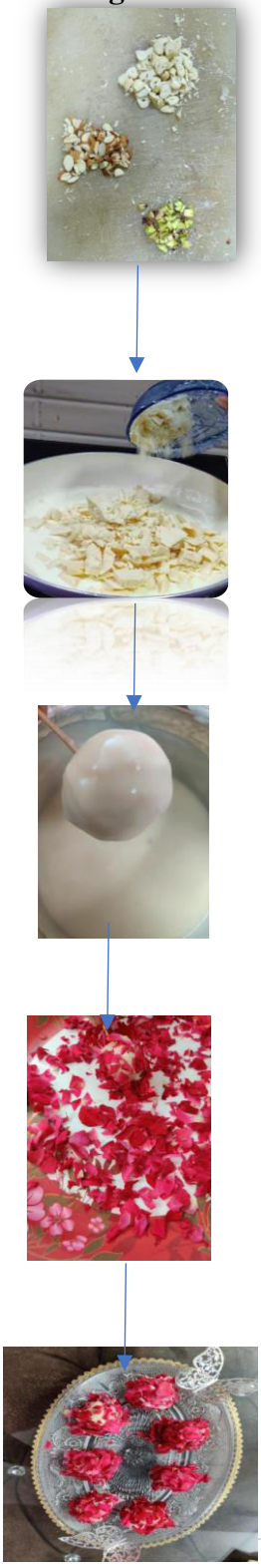
Overall Acceptability	1	5	4.57	0.30

Figure - making of rosa toffee



1. Crushing of nuts after roasting
2. Mixing in white chocolate
3. Spreading it on rose petals
4. Serve it cool.