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Formulation & analysis of papaya fortified biscuits

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Abstract
Biscuits are popularly worldwide known for its taste, flavor and health benefits. A formulation of biscuits with *Carica papaya* pulp extract powder was prepared for enhancing multiple health benefits and also for increasing beneficial usage of biscuits as nutraceutical, especially seeing the enzyme action i.e. papain. The Chemical composition analysis where it was tested for different parameters like ash and moisture analysis, protein, carbohydrate content and antioxidant profile (DPPH, contents of flavonoids of control and fortified biscuits). Sensory Analysis was conducted by applying hedonic scale test by applying the method of 9 point hedonic scale. In analysis the result appeared that the antioxidant activity was significantly improved. This was clearly demonstrated by the fact that increases in flavonoid contents can also be observed in the fortified cookies. Hence, it was tested that by fortifying biscuits with papaya powder or juice could enhance the nutritional quality of biscuits without getting the parameters such as rheological, sensorial and antioxidant properties being affected along with receiving the benefits of digestive enzyme papain acting as a functional food.

Keywords: rheology, sensory, biscuits, textural characteristics, chemical tests

1. Introduction
Biscuits constitute a major component of human snacks in most part of the world. (Hasker *et al*. 2016) [1]. It is an unleavened crusty baked, sweet edible item made from wheat flour, shortening (hydrogenated fat) & sugar, and is normally produced light by mixing with baking powder (cereal flour mixed with sodium carbonate, sodium bi-phosphate) (Lakshmi M *et al*.) [2]. Wheat flour is the major constituent and acts as the basic ingredient for biscuit production because of its gluten proteins, which are found to be absent in flour of other cereals. (Ortolan and Steel *et al*.) [3]. Gluten protein supports to provide elasticity to the dough during the process of baking and provides high organoleptic quality of the end product. (Wang *et al*. 2017) [4].
The Baking industry holds a major portion in food industries and it has been growing at an average rate of 15% during the past 3 years. Therefore, various cereals based preparations, blending and mixing with different compounds to improve the nutritional standard of Indian diet is very important (Bassey, FI *et al*. 2013) [5]. Supplementary foods should be such, if taken in small quantity, could provide the necessary amount of nutrients. They should be made in the form of ready to eat snacks, drinks, etc. Biscuit has been good for a long time as a processed food (Ahmad S and Ahmed M, 2014) [6]. The primary objective is to develop healthy and nutritional biscuits fortified with papaya pulp powder. Papaya is an ingredient for different uses, all over the world and has been utilized in a variety of products after processing such as fruit juices, jams, and ice cream, dried fruit. The chemical compositions of papaya fruit are water 88.8%, carbohydrate 9.8%, fiber 0.8%, protein 0.6%, ash 0.6% and fat 0.1%. From 100 grams of papaya high amount of calories approximately 39 calories can be achieved. Papaya is a very good source of vitamin A. (Sujatha Y and Bera S, 2014) [7]

2. Materials and Methods
2.1 Reagents
All analytical grade reagents and solvents used were procured from Fluka (Fluka, Switzerland) and Sigma-Aldrich (Sigma- Aldrich, St. Louis, MO, USA) and purchased from Rankem (RFCL Ltd, India).

2.2 Main Ingredients
All the ingredients- The papayas, Atta (whole wheat flour) and Maida (refined flour) were procured from the local market of North Delhi (India). Fresh papaya was washed, peeled and grated. The grated papaya pieces were kept in the hot air oven dryer. Drying was carried out overnight at 50°C.

After the papaya pieces were dried to snapping stage, drying was complete. The papaya pieces were ground in a mixer–grinder and the powder papaya obtained was sealed in polyethylene (PE) bags.
The papaya powder was stored in the refrigerator along with atta and maida until usage.

2.3 Formulation of Biscuits
Biscuits were prepared in two different formulations. In one formulation papaya pulp powder was used and a control was also prepared in which no papaya was added. The formulation of biscuits was also done by adding papaya pulp powder and atta and a small amount of maida flour to add gluten to the mixture.

Table 1: Different concentration for formulation of biscuits

Biscuits code	Atta	Maida	Papaya pulp powder
C1	300	90	10
C2	300	100	-nil-

2.4 Determination of nutritional constituents
For nutritional analysis the formulated bars were crushed in mortar and pestle.
Moisture and total ash content were determined by AACC methods (AACC, 2000) [8]. The protein content was determined by the IS: 7219: 1973 standard. The total dietary fibre content was determined by the methods of (Lee *et al*. 1992 [9] and Prosky *et al*.1998) [10].

2.5 Estimation of Vitamin A and C Content
2, 6-Dichlorophenol indophenol method is used to estimate the total vitamin C content. (Ranganna, 1999) [11] Estimation of vitamin A content was done by the method of FRAC/SOP/INST/097 adopted by FICCI Research & Analysis Centre.

2.6 Phytochemical Analysis
2.6.1 Estimation of Total Phenolics Content
Estimation of total phenolic content was done by using the Folin Ciocalteu Assay (Siddiqui *et al*. 2017) [13].

2.6.2 Determination of Total Flavonoids
Total flavonoids were estimated using aluminum chloride colorimetric method. (Chang *et al*. 2002) [14]

2.7 Antioxidant Activity
DPPH radical scavenging Assay
DPPH radical scavenging activity was analyzed by the method of Blois, 1958 [15] with significant modification as described in paper.

2.8 Papain Activity test
Papain activity was measured using the method followed by (Trivedi *et al*, 2013) [16]

2.9 Microbial Analysis
Total plate count agar Media was prepared and autoclaved at 121° C for 15 mins at 5 psi. Meanwhile 10gm sample was crushed and dissolved in 90ml saline water. Further dilutions were made 10^{-9} concentration. Further 1ml of sample from the concentration of 10^{-3} and $10^{-6}$was plated and incubated in bacteriological incubator for 24 hours at 37 ℃. There after results were recorded according the number of microbial colonies appeared on the plate. (FSSAI Manual. 2015) [17].

3. Results and Discussion
3.1 Phytochemical estimation of papaya pulp powder
The phytochemical compounds that are found by testing of methanolic extract of papaya pulp powder fortified biscuits revealed the presence of phenolics, antioxidants and flavonoids.

3.2 Total phenolic content
The Total phenolic content was estimated colorimetrically by Folin Ciocalteu (FC) method and the result was expressed in terms of Gallic Acid Equivalent (GAE) which can be observed in table 2.
The determination of total phenolic contents was calculated by using the linear equation based on the calibration curve of Gallic acid:
$y = 0.0172x - 0.0404$, $R^2 = 0.9904$
Where y is the absorbance of sample and x is the amount of Gallic Acid Equivalent in µg.

Table 2: Total phenolic content of the Biscuits

Sample	GAE equivalents (µg GAE/mg sample)
Biscuit sample with papaya pulp powder	1.705

3.3 Total Flavonoid Content
By using the aluminium chloride method the results of total flavonoids were estimatedand it was expressed in terms of Catechin equivalents (CE) which is shown in table 3.
By using the following linear equation the total flavonoid content of the extract was calculated $y = 0.0045x - 0.0138$, $R^2 = 0.9976$
Table: Cathechin equivalent for biscuits made from papaya pulp (µg CE mg sample)

Table 3: Total flavonoid content of the biscuits

Sample	CE equivalents (µg CE/ mg sample)
Biscuit sample with papaya pulp powder	1.017

3.4 Determination of antibacterial activity
The total microbial count was found to be too less to count. From this we can say that if the biscuits are stored in proper packaging and away from moisture it could be safe for consumption after 15 days.

3.5 Determination of Vitamin Content
The results found after the vitamin content estimation, test was done are shown below:

Table 4: Total vitamin content of the Biscuits

Parameters	Results	Test method
Vitamin C mg/100g	5.88	IS:5838:1970
Vitamin A mcg/100g	235.26	FRAC/SOP/INST/097

3.6 Results of Protein Content
The total protein content in the biscuit sample was estimated by IS: 7219:1973 method and was estimated to be 6.96 g/100g.

3.7 Determination of Dietary Fibre
The dietary fiber in food sample was estimated by AOAC 985.29 method and was found to be 3.35g/100g.

3.8 Antioxidant Activity
The antioxidant activity was done by DPPH radical scavenging activity.

Table 5: Total antioxidant inhibition

Sample	% Inhibition
Papaya pulp fortified biscuits	90.8471

Papaya fruit contains 84.04% of the antioxidant and in comparison it was clearly found that the biscuits are highly enriched with antioxidants after baking.

Papain Activity Test: Papain activity test showed a positive result as the liquid coagulates. This showed that the biscuit sample contains papain but in very less amount due to baking process.

3.9 Sensory Evaluation of formulating Biscuits

The sensory qualities, particularly the flavor attributes, are essential to be measured subjectively (S Ranganna, 1986) [11]. The main objective behind the sensory analysis is to check the overall acceptability of the formulated biscuits. The samples were characterized for color, flavor, crisp and general acceptability. This was done by 9 point hedonic scale method. 9 represents like extremely 1 means dislike extremely as mentioned in a paper by author Ihekoronye and Ngoddy (1985) [18].

Table 6: Sensory Analysis chart

Sample code	Crispy	Taste	General acceptability
C1	7	7	7
Control	7	8	7

4. Nutritional analysis of formulated biscuits

Papaya pulp powder was used for the formulation of biscuits. After the sensory evaluation it was found that the taste and overall acceptability of the formulated biscuits was almost same to the controlled one.

The moisture content of biscuits is very low due to which they are less susceptible to spoilage and have a longer shelf life as compared to other bakery products. As soon as it was put under the convection water evaporates easily.

The moisture content was found to be 2.26% as it contained a high amount of fat in it, the ash content was found out to be 1.1%.

Table 7: Nutritional Analysis done for biscuits

S. No.	Parameters	Results (mg/100gm)*
1.	Calcium (Ca)	24.88
2.	Iron (Fe)	35.27
3.	Potassium (K)	199.14
4.	Magnesium (Mg)	37.72
5.	Sodium (Na)	351.42
6.	Zinc (Zn)	0.90

*Method: FRAC/SOP/INST/242

During investigation it was observed that the moisture content, fat and protein content of biscuits were found to increase with fortification of the biscuits with papaya powder. The composition along with the ingredients such as papaya powder, wheat flour and refined wheat flour is presented in Table1. Inside the oven keeping the temperature of 165 °C it was baked for 15mins. The moisture content found in the Papaya powder fortified biscuits sample was 1.36% higher than the control biscuits, which was due to the addition of papaya pulp powder. The protein content of Papaya pulp fortified biscuits sample was higher than those of control biscuits. The fat content of the fortified biscuits was also higher due to the incorporation of more butter. The data indicate that the fiber content of the biscuits was high.

It was found that papaya pulp fortified biscuits have higher amount of sodium, vitamin A and iron (as shown in Table). The concept behind this is the stability of each micronutrient during processing that involved different steps, including mixing, forming and baking. However, it was found that some of the micronutrients are stable in case of fortified biscuits.

The concentration of vitamin A in the fortified biscuits was found to be 235.26 mg/100g, and it is comparatively low in sweet potato fortified wheat biscuits (Herawati D *et al*. 2015) [19].

The effect of the papain enzyme found in papaya can be retained, though in a less amount through these biscuits.

5. Conclusion

Biscuit manufacture is no longer considered a craft, but a fully-fledged technology, developed after a full understanding of the various processes involved with the help of basic principles of science and engineering. All over the world, manufacturing equipment has been replaced by new and advanced automatic machines with high outputs. In some of the foreign country biscuit manufacturing has been computerized (Aziah AN *et al*. 2012) [20]. Irrespective of rural or urban area, it is the choice of consumer and hence the biscuit is the largest consumed processed product in India (Umadevi L and Uma N, 2014) [21].

The fortification process helps food to enrich their quality along with a great value of micro and macronutrients. The main view behind this fortification process is so to gain the essential nutrients directly without consuming the raw. Wu *et al*. 2016 [22], indicated that the rich amount of polyphenols (good sources of antioxidants) is found in the flesh and peel of white papaya, which is found to be effective in inhibiting the growth of melanoma cells. Red papaya is rich in nutrients such as proteins, vitamins and flavonoids, antioxidant and has radical scavenging activity (Septembre-Malaterre A *et al*. 2016) [23].

The basic objective of this study was to assist with or to evaluate total phenolic content, antioxidant capacity, flavonoid in the biscuit part fortified with semi ripe papaya. And the results showed that even after baking the nutrients can be retained to some extent.

Hope food processing technology will gain much benefit in near future through this process and help mankind to gain health benefits.

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