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Nutritional and Phytochemical evaluation of high fibrecookies using beet root and Pumpkin seeds powder

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Abstract
All essential nutrients can be provided to body from fruits and vegetables. Essential nutrients include minerals , vitamins and fiber. Beetroot (*Beta vulgaris)* is rich in minerals like sodium, potassium, magnesium and also have good antioxidant properties. While pumpkin (*Cucurbitapepo)* is source of vitamins, minerals and beta carotene. This study was done to enhance nutritional quality of cookies by making three different variations. The chemical composition of prepared cookies is Moisture(3.77%,3.81%,3.89%), Crude protein (4.42%,4.27%,4.52%), crude fat (20.033%,20.10%,20.13%), Carbohydrate (68.94%,71.12%,70.24%). Obtained results showed that the addition of beetroot and pumpkin seeds powder result in increase of polyphenols, antioxidant activity.

Keywords: nutrients, beetroot and pumpkin seeds powder, antioxidants, total phenols

1. Introduction
Bakery goods have been around for thousands of years. The art of baking was developed early during the Roman Empire. The bakery industry is one of the largest organized food industries all over the world and in particular biscuits and cookies are one of the most popular products because of their convenience, ready to eat nature, and long shelf life [1].Nutritional cookies provide a way to have a treat and gain vital nutrients, too. While they aren't as nutritious as unprocessed foods like fruits and vegetables, they provide more nutritional benefits than many other cookies — especially if you make them yourself or choose prepared cookies carefully.

Recently, increasing consumer demand for healthier foods has triggered the development of cookies made with natural ingredients exhibiting functional properties and providing specific health benefits beyond those to be gained from traditional nutrients [1].A healthy diet is a diet that helps to maintain or improve overall health. A healthy diet provides the body with essential nutrition: fluid, macronutrients, micronutrients, and adequate calories.

The beetroot is the taproot portion of a beet plant, usually known in North America as the beet, and also known as the table beet, garden beet, sugar beet, red beet, or golden beet. Packed with essential nutrients, beetroots are a great source of fiber, folate (vitamin B9), manganese, potassium, iron, and vitamin C. Beetroots and beetroot juice have been associated with numerous health benefits, including improved blood flow, lower blood pressure, and increased exercise performance.

Beetroots are rich in other valuable compounds such as carotenoids [2], glycine betainesaponins, betacyanins, folates , betanin, polyphenols and flavonoids [1]. The fresh beetroots are exposed to spoilage due to their high moisture content and needs preservation. One of the preservation methods ensuring microbial safety of biological products is drying and dehydration [3]. Dried beetroots can be consumed directly in the form of chips as a substitute to traditional snacks [b], or after easy preparation as a component of instant food [5].

The different species of pumpkin seeds exerts the different components and biological activities [6], Many researchers studied the bioactive compositions of pumpkin seeds oil that grown in the different areas of the world. Due to the differences among the species and/or varieties of *Cucurbita spp*., the yield of fatty acids, sterols or phytoestrogens and tocopherols was quite similar to those of each other and belong to the three major components of pumpkin seeds that have been focused by many studies. However, the minor components of pumpkin seeds such as protein, mineral, terpenicalcohol, and fiber also could not be ignored, because they have played role in the synergistic positive effects of pumpkin seeds [7]. Some technologies are applied to isolate the higher yield of oil from crude pumpkin seeds. Although several studies reported that crude pumpkin seeds extract itself exhibited the broad-spectrum pharmacological effects through *in vitro*, *in vivo* and human trial. *Cucurbitapepo* L. is the most popular pumpkin species to be a focus of interest of researches in the 1world. Recent studies have shown that *Cucurbitapepo* species is rich in polyunsaturated fatty acids such as palmitic acid, stearic acid, oleic acid and linoleic acid, vitamin E like α-tocopherols, γ-tocopherols and carotenoid, phytoestrogens and phytosterols such as daidzein, genistein, secoisolariciresinol, and the trace components. Among those of total percentage of ingredients in pumpkin seeds, unsaturated fatty acids showed the hugest components ranging up to 80%. This value is relatively higher than those reported for peanut seeds oil and soybean seeds oil [8]. Decreasing the moisture content of fresh foods to make them less perishable is a simple way to preserve these foods. . As a result, value-added foods or functional foods with higher level of dietary fiber and antioxidant have been developed, especially in bakery products such as cookies. The incorporation of composite flour into

traditional wheat based food products provided additional nutrients from non-wheat material and improved the nutritional value of the products [1]. The utilization of beetroot powder with sunflower seeds flour in bakery products has not been studied extensively. Therefore, the research was designed to evaluate the effect of substitution of wheat flour with different levels of beetroot powder on the physico-chemical and sensory properties of the cookies.

Materials and Methods

Sample collection and preparation

The sample of beetroot pomaceand pumpkin seeds were procured from the local market of Gwalior (M.P). After the sample collection, Beetrootpomace and seeds were dried in hot air oven at low temperature (50 C). The dried samples were secured in airtight jars to prevent degradation.

In the recipe beetroot pomaceand pumpkin seeds powder was used with flour (20%beetroot pomace powder and 15% pumpkin seeds in formulation I, 15% beetroot pomace and 20% beetroot pomace in formulation II, 15% beetroot pomace and 15 % pumpkin seeds in formulation III) in a standard cookie recipe. In the procedure sugar, egg white, butter was creamed in a hand mixture. The measured amount of flour and other ingredients with beetroot pomace powder was mixed with the liquid ingredients properly. The firm dough was then rolled and cut with a cookie cutter. Prepared cookies were then transferred to the greased trey and baked at 150°C for 15 min. The baked cookies were removed from baking oven cooled to room temperature prior to analysis.

Table 1: Composition

Ingredients	Control	Variation 1	Variation 2	Variation 3
Flour (g)	100	85	75	65
Beetroot powder (%)	-	10	15	20
Pumpkin seed powder (%)	-	5	10	15
Butter (g)	20	20	20	20
Sugar (g)	25	25	25	25
Baking powder (g)	1	1	1	1
Beaten whole egg (No's)	1	1	1	1

Proximate Analysis

Proximate analysis was conducted on the control cookies and cookies prepared from the substituted flour. according to AACC standard methods, moisture content was carried out based on AACC method(44-19.01) [9].The hot air oven was set at a temperature of 135°C.The crude fat content was determined by Soxhlet extraction method described in AACC (2000), method [30-25.01] [10]. Crude fiber analysis was conducted based on AACC method [32-10-01] [11].The crude protein content was determined by the method described in AACC method (46-10.01) [12].The ash content was determined by the method described in AACC (2000), Method no. 08-01. [13], Carbohydrate content was calculated using the following equation.

% Carbohydrate = 100% - (Moisture + Crude fat + Ash + crude protein) %

For the analysis the samples were ground in to fine particles. The analysis was carried out in triplicates for all samples and expressed in a dry basis.

Analysis of Bioactive compounds

Sample preparation for Analysis

Sample (1 g) was mixed with 100 ml methanol 70% (v/v) in conical flask (250 ml) wrapped with aluminum foil. The mixture was then shaken in an orbital shaker for 2 hrs. at 160 rpm . Next, the mixture was poured into centrifuge tubes (wrapped with aluminum foil) and centrifuged in a centrifuge at 2500 rpm for 30 minutes to get a clear solution. The extract obtained was used for total phenolic content (TPC), total antioxidant value (phospho-molybdenum method) and other bioactive component analysis. For vitamin c analysis the sample was mixed with 100 ml water, and then the above mentioned procedure has been repeated for vitamin C estimation.

Total phenolic content

Total phenolic contents in extract were determined by the folin-ciocalteu reagent method. 1 ml. of the aqueous extract of leaves was mixed with 5 ml.folin-ciocalteu reagent``(CDH) (diluted with water 1/10 v/v) and 4 ml.(7.5%) of sodium carbonate. The solution was allowed to stand for 30 min. at 20°C.Absorbance of the sample and standard were measured at 765 nm. by using spectrophotometer(ELICO Double Beam, SL164, UV-VIS Spectroscopy). The total phenolic content in plant extract in gallic acid equivalents (GLE) was calculated using the following equation [14].

$$C = (c \times V) / m$$

Where,

C= total content of phenolic compounds mg/gm plant extract

c= the concentration of gallic acid established from the calibration curve (mg/ml)

V= the volume of extract in ml.

m= weight of crude plant extract in gm.

Total Anthocyanin Content

Anthocyanins were extracted from samples by homogenizing the sample of cookies with acidified 70 % ethanol solution. The mixture was centrifuged after 10 min. The pH was adjusted by HCl. The mixture was brought to the volume of 25 ml and the absorbance was measured 515 nm, using ELICO Double Beam, SL164 UV-VIS Spectrophotometer [15].

Anthocyanin (mg per100g)= ,

absorbance at 515nm x volume of extraction solution x100/ wt of sample (gm)x 98.2

Total Flavonoid Content

The flavonoids content was measured following a spectrophotometric method (ELICO Double Beam, SL164 UV-VIS Spectrophotometer) .The sample extract were appropriately diluted with distilled water. Initially, 5% sodium nitrite solution was added to each test tube; after five minutes, (0.3 ml.)10% AlCl3 solution was added and then at 6thminutes 1.0 M NaOH was added. Finally, water was then added to the test tube and mixed well. Absorbance of resulting pink-colored solution was read at 510 nm against the blank (distilled water) [15]. The concentration of flavonoid was read on calibration line, the content of flavonoid was expressed in terms of quercetin (mg/100g).

The total antioxidant capacity

The total antioxidant capacity was evaluated by the phospho-molybdenum methodaccording to the procedure described by [16]. A 0.3 ml of extract was combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate).The tubes containing the reaction solution were incubated in a water bath at 95°C for 90 min. Then, the absorbance of the solution was measured at 695 nm using a UV-VIS spectrophotometer (ELICO Double Beam, SL164 UV-VIS Spectrophotometer) against blank after cooling to room temperature. Methanol (0.3 ml) in the place of extract was used as the blank. The total antioxidant activity is expressed as the percent inhibition of the phosphor-molybdenum complex. The calibration curve was prepared by mixing ascorbic acid with methanol.

$$\text{Total antioxidant capacity (\%)} = [(\text{Abs. of control} - \text{Abs. of sample})/(\text{Abs. of control}] \times 100$$

Sensory evaluation of Cookies

The sensory analysis was carried out using 10 members including student and staff members. The final sample has been analyzed on the basis of color, taste, appearance and sweetness. The sample was packed in a transparent package and presented with specific code. Water has been provided to every member to neutralize the previous effect of sample. For sample analysis 9- Point Hedonic scale showing least acceptable to most acceptable on selected parameters (9 is Excellent and 1 for Very poor).

Results and Discussion

Nutritional analysis

The nutritional analysis shown in Table: II,Moisture content of regular cookies which were taken as control is (2.5-3%) [17], and the moisture content of cookies containing beetroot and pumpkin seeds powder varies from 3.77-3.89%. Crude protein content of control was 4.32% whereas crude protein content of beetroot and pumpkin seeds powder containing cookies at three different variations increase from 4.27-4.52%. Ash content of cookies containing beetroot and pumpkin seeds powder of three different variations increased from 1.28-1.29% which was higher than that of control. Crude fiber content of three variations was significantly increased from 3.33-4.24% with addition of beetroot and pumpkin seeds powder up to 10 and 5 % , 15 and 10 %, 20 and 15 % respectively. Crude fat and carbohydrate contents were decreased from 20.13-20.03 %, 70.24-68.94 % with addition of beetroot and pumpkin seeds powder. The difference in moisture content between samples might be due to high fiber content in beetroot. More hydroxyl group of cellulose in fiber were able to bind with free water molecule through hydrogen bonding and therefore resulting in greater water holding capacity [17].

Table 2: Nutritional evaluation of substituted flour and cookies

Types of flour	Composition %					
	Moisture	Ash	Crude fiber	Crude fat	Crude protein	Carbohydrate
Beetroot powder	87.4	1.4	1.9	0.31	1.35	7.64
Pumpkin seed powder	5.7	3.83	18	33.2	25	19.65
Control(C)	1.041	0.973	0.211	20.36	4.32	73.08
Variation 1	3.77	1.29	3.33	20.033	4.42	68.94
Variation 2	3.81	1.28	3.45	20.10	4.27	71.12
Variation 3	3.89	1.28	4.24	20.13	4.52	70.24

Analysis of Bioactive compounds

The total polyphenolic content calculated as mg Gallic acid equivalent (GAE) of cookies in aqueous solution. The total phenol content ranging from160.00 (Variation:I),175.25 (Variation: II),181.10 (Variation:III)mg GAE/100gm. The results of total antioxidant capacity can be observed inTable:III (observations in percent inhibition),showed a significantly lower for control cookies compared to the substituted cookies.Anthocyanins water-soluble compounds having a great interest in nutrition and medicine because of their potent antioxidant capacity.As mentioned in the Table 3, the flavonoid content in different formulations is 179.5 (Variation:I) ,180.2 (Variation:2) ,182.4(Variation:3). The total antioxidant (% inhibition) was detected in different formulations is 80.63(Variation:1) ,82.50 (Variation:2) ,82.71(Variation:3), which can be due to the presence of poyphenols present in the beetroot and pumpkin seed.

Table 3: Analysis of Bioactive compounds

Types of flour	Total Phenolic content mg GAE/gm	Flavonoid content mg /gm	Anthocyanin content mg/100gm	Total antioxidant % inhibition Phospho-molybedenum (ascorbic acid)
Control (C)	105.00	0.5	00	10.80
Beetroot flour	77.81	290.66	85.32	57.63
Pumpkin seed powder	57.81	21.50	00	199.23
Variation 1	160.00	179.5	24.41	80.63
Variation 2	175.25	180.2	24.30	82.50
Variation 3	181.10	182.4	23.14	82.71

Sensory Analysis

Cookies supplemented by different levels of substitutions of rose hip and hibiscus powder were sensory evaluated and compared with control biscuits 100% wheat flour. Data indicated that the percent score of cookies containing15 and 10 % beetroot and pumpkin seed (Variation II) were found to be the most acceptable (over all acceptability).

Table 4: Sensory analysis of cookies

Formulations of Cookies	Sensory parameters				
	Color	Texture	Appearance	Taste	Overall acceptability
Control (C)	7.6	5.2	7.2	5.8	6.0
Variation 1	7.2	6.8	7.1	7.4	7.0
Variation 2	7.8	7.4	8.0	6.2	8.0
Variation 3	7.2	6.9	7.1	6.7	6.8

Conclusion

Numerous studies have been carried out in order to replace wheat flour made from fruit residue, food waste in preparation of bakery products such as cookies and biscuits, due to the new consumption trends and eating habits. Recently, increasing consumer demand for healthier foods has triggered the development of cookies made with natural ingredients exhibiting functional properties and providing specific health benefits beyond those to be gained from traditional nutrients The analysis revealed that the increment in substitution in flour has increased the nutritive value of cookies when compared to control cookies. The substitution with beetroot and pumpkin seed powder has a significant role in enhancing the TPC content, Total antioxidant capacity and other bioactive compounds in cookies. Cookies prepared from Variation 2 (beetroot and pumpkin seed) was more acceptable than other formulations on the basis of sensory evaluation. Substitution with Rose beetroot and pumpkin seed could bring much potential health benefits to the consumers by adding nutritional as well as therapeutic properties.

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