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Abstract:

Gluten free (GF) protein enriched cookies with reduced calories were developed using sweet potato, rice, sorghum and corn flour. The level of sweet potato flour was standardized and found acceptable up to 55% with corn, sorghum and rice flour at the ratio of 1:3:5 respectively. High protein and low calorie gluten free cookies were developed by replacing sweet potato flour with whey protein concentrate (WPC) at 0, 5, 10 and 15% levels and sucrose with sucralose at 0, 30, 50, 70 and 100%. It's physical, proximate and sensorial characteristics were considered and it was investigated that the thickness and hardness of GF cookies increased; weight, diameter, spread ratio and spread factor decreased with increasing levels of WPC while there was raise in protein and crude fiber content and reduction in fat, carbohydrate and calorie contents. The GF cookies with 15% and 70% replacement of WPC and sucralose respectively scored highest for the entire sensory characteristic.

Keywords:

Calories; Cookies; Gluten Free; Protein; Sucralose; Whey Protein Concentrate

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Introduction

Cookies are widely consumed because of longer shelf life and crisp texture.^{1, 2} These are made traditionally using creamery methods from white wheat flour with different addition of taste enhancing ingredients like chocolate, butter, nuts, and flavors etc.³ But, consumers are demanding for nutritious cookies which could be prepared by replacing wheat flour with other flour having nutritive value. This could be prepared by replacing refined wheat flour with non wheat flour will be considered as functional cookies and various researchers developed functional cookies using alternative flour to wheat flour such as buckwheat, cassava, millets flour, pulses, quinoa flour etc.^{4, 5, 6} Some consumers are gluten sensitive, researchers developed gluten free cookies from rice, corn, buckwheat and potato flours.^{6, 7} India is recognized as major producer of biscuit in the world and the bakery industry has been predictable to develop annually at the rate of 15-17%.⁸ Bakery products have huge number of consumers as liked by kids as well as adults, so it is best suitable choice to fulfill the nutritional requirement of consumers.

Celiac disease (CD) is also known as gluten sensitive enteropathy is an autoimmune disorder, caused due to consumption of protein called gluten, found mainly in wheat, barley and rye. It

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affects to 1-2% of the world population.^{9, 10} Consumption of gluten in diet of celiac patients leads to damage of villous atrophy of small intestine which causes severe stomach pain, bloating diarrhea, weight loss. Micro nutrient deficiency observed in these patients due to failure of absorption by small intestine.¹¹ CD has reached worldwide particularly, in Europe and the USA and in India; overall prevalence of CD is about 1%, symptoms with dysentery in tertiary care hospitals ¹². The major prevention of celiac symptoms is stick to the gluten free diet and this is only remedy for CD. Ingestion of food contaminated with traces of gluten leads to celiac disease.^{13, 14, 15} Developments of gluten free products were attempted by many researchers and it was found that, gluten is essential component for dough development in case of preparation of bakery products. Preparation of gluten free bakery products is considered as major challenge to researchers.

Sweet potato (*Ipomoea batatas* L.) is world's important fourth crop after rice, corn and cassava as a potential source of carbohydrates and consumed in main meal food or in place of rice in most of the country's.¹⁶ It is also known as food security or famine relief crop but under exploited. In association with its nutritive value, sweet potato lacks in fat and cholesterol.¹⁷ It is source of carbohydrates but also contain vitamin C, vitamin E, vitamin B1, vitamin B2, niacin, vitamin B5, folic acid and minerals like potassium, calcium, zinc, magnesium and having high quantities of iron^{18,19} as well as dietary fiber. It was found by many investigators that a replacement of 10 - 15% of wheat flour was found desirable in food preparations.^{20, 21} The incorporation of orange-fleshed sweet potato in food products like buns, chapattis, and other bakery products increased total carotenoids contents.²² When sweet potato flour incorporated to wheat flour helps to improve nutritional importance with respect to fiber and carotenoids which also reduce the gluten level in flour and prevent from celiac disease.²³

Whey protein concentrate (WPC) is extensively used as a functional component, in order to improve the protein content, exclusive of increase in energy value from fat.²⁴ It is reported that, it have more biological value and amino acid score.²⁵ WPC is added to different food preparations as a best source of protein in order to prevent malnutrition and obesity.^{26, 27, 28, 29} Singh *et al.*,²⁸ reported that, WPC is an effective ingredient in cake preparation as an egg solids replacer useful for people allergic to egg. WPC is having 70% proteins and utilized for the preparation of protein rich foods and in ice cream, yoghurt, and soft drink etc.^{26, 27} It was reported that, whey ingredients also help to increase batter viscosity and crumb structure of muffins and provide tender texture in biscuits by holding moisture for longer time.³⁰

Currently, in baking industry different products such as sugar free, fat free, low calorie, zero cholesterol and rich in fiber and protein are liked by health-conscious consumers. Now a day, there are different artificial sweeteners which are non toxic, non caloric and sweeter than sucrose used in place of sugar in preparation of food. Sucralose which is considered as GRAS in many countries has received the approval of the Food and Agriculture Organization/World Health Organization.³¹ Sucralose is non toxic and non caloric sweeteners which is 600 times sweeter than sucrose and broadly used in different countries in food and beverages. It is also having significant role in baking industry due to its sugar like taste and stability. It provides good taste and reduce calorie in food products.³²

In present investigation, an attempt was carried out to develop protein enriched and low calorie gluten free cookies suitable for celiac patients using sweet potato flour as major ingredient with sorghum, rice and corn flour. Sweet potato is underutilized crop and there is need to explore its use by developing processed products.

Materials and Methods

Materials

Sweet potato tubers having three month maturity were received from ICAR- Central Tuber Crops Research Institute, Kerala. Tubers were converted to flour for developed of gluten free cookies by washing, peeling and slicing (5mm thickness) and sun drying for 36h. Dried slices were ground to fine flour (particle size of 0.177mm). It was packed in LDPE at room temperature to carry out further research.

Rice flour (*Oryza sativa*), sorghum (*Sorghum bicolor*) flour, corn flour (*Zea mays*), sucrose, sucralose, fat and baking powder were procured from the local market of Aurangabad. Edible guar gum of food grade (supplied by M/S Lucis Colloids., Mumbai) was also added as it helps to improve textural properties of gluten free cookies.

Methods

The experimental plan of present investigation was to study the effects of fortification of WPC and sucralose on physical and chemical properties of gluten free cookies. GF cookies were standardized using sweet potato flour at the rate of 45%, 50% and 55% with corn, sorghum and rice flour at the ratio of 1:3:5 respectively. Based on experimental trials, GF cookies containing 55% sweet potato flour, 05% corn flour, 15% sorghum flour and 25% rice flour was found

acceptable. Protein enriched and low calorie gluten free cookies were developed by substituting sweet potato flour with WPC and sucrose with sucralose from standardized sample.

Preparation of Cookies

GF cookies were formulated which includes 55% sweet potato flour with corn, sorghum and rice flour at the ratio of 1:3:5 respectively. The ingredients (g) were added in development of GF cookies were GF composite flour blend-100g, powdered sugar-32g, fat-40g, baking powder-1g and guar gum-0.5g. GF cookies high in protein having low energy value were optimized by replacing sweet potato flour with 0, 5, 10 and 15 % (w/w) whey protein concentrate (WPC) and best one selected based on evaluation of sensorial properties. In the WPC standardized gluten free cookies the sucrose was substituted with 0, 30, 50, 70 and 100% sucralose. GF cookies were evaluated for physical, proximate and sensorial properties. These GF cookies were packed in low density polyethylene bags and stored at room temperature for further analysis.

Physical Properties

The physical properties of GF cookies were evaluated to determine spread ratio (SR) by using formula as diameter/ thickness and spread factor (SF) was determined using the formula: -

(Spread factor of control/ Spread factor of experimental)×100.³³ The diameter and thickness of cookies was measured using vernier caliper. The hardness of gluten free cookies was measured in terms of Newton using Universal Texturometer. Hardness of samples was determined by breaking test. It was expressed in terms of Newton (N). The size of probe used was 5mm. Parameters were set as; cross head speed: 50 mm/min, maximum load cell force: 1 kg and compression: 75%. Pre test speed-10mm/sec, Test speed-1mm/sec.

Proximate Characterization

The proximate parameters such as moisture content, ash, crude fat, protein and crude fiber were evaluated as per standard methods (AOAC 2000).³⁴ Total carbohydrates content of the samples were determined as total carbohydrates by subtract the percentage of others from 100. Total calories were calculated by multiplying protein, carbohydrates and fat content by the factor 4, 4 and 9 respectively. The values were expressed in kcal.

Sensory Characteristics

Sensory evaluation of samples was done by group of 50 panel members using 9 point hedonic scale. It includes characteristics like appearance, color, aroma, taste, texture and overall acceptability for all cookies samples.³⁵

Statistical Analysis

Results were expressed as mean of triplicate analyses. A one way analysis of variance and Duncan's test were used to establish the significance of differences among the mean values at the 0.05 significance level. The statistical analyses were performed using SPSS 16.0 software.

Results and Discussion

Physical Properties

The physical properties of gluten free cookies developed by replacing sweet potato flour with 0-15% WPC are given in Table 1. It was found, the diameter of GF cookies reduced significantly from 46.83 to 45.27 mm with rising levels of WPC. Whereas, thickness were found significantly increased from 8.43 to 10.03 mm. As the level of WPC increased in GF cookies, the spread ratio and spread factor decreased significantly from 5.55 to 4.51 and 100 to 81.16% respectively. Singh *et al.*, ³⁶ reported that, spread ratio of cookies decreased as the level of legume increased in

wheat cookies. This might be due to the change in dough behavior properties.

Table 1 represents the physical properties of GF cookies developed via substituting sucrose with 0-100% sucralose. It was investigated that, the diameter of GF cookies decreased significantly from 45.27 to 42.03 mm as the level of sucralose increased. Similar trends were reported by Singh *et al.*,³⁷ But there was no significant difference found in thickness of GF cookies. The SR and SF of gluten free cookies decreased significantly from 4.51 to 4.11 and 100 to 91.08% respectively with increasing level of sucralose.

Table 1: Effects of different levels of WPC and sucralose on physical parameters of gluten free cookies

Physical Levels of WPC (%)			Levels of Sucralose (%)						
parameters	0	5	10	15	0	30	50	70	100
Weight (g)	10.75 ± 0.01 ^a	10.71 ± 0.01 ^b	10.64 ± 0.03 ^c	10.55 ± 0.01 ^d	10.55 ± 0.01 ^a	10.27 ± 0.01 ^b	9.81 ± 0.01 ^c	9.80 ± 0.01 ^c	9.75 ± 0.01 ^d
D(mm)	46.83 ±	45.63 ±	45.47 ±	45.27 ±	45.27 ±	44.43 ±	43.03 ±	42.23 ±	42.03 ±

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	0.01 ^a	0.01 ^b	0.01 ^c	0.01 ^d	0.01 ^a	0.01 ^b	0.01 ^c	0.01 ^d	0.06 ^e
T (mm)	8.43 ± 0.01 ^d	9.03 ± 0.01 ^c	9.63 ± 0.01 ^b	10.03 ± 0.01 ^a	10.03 ± 0.01 ^b	10.23 ± 0.06 ^a	10.23 ± 0.06 ^a	10.23 ± 0.06 ^a	10.23 ± 0.06 ^a
SR (D/T)	5.55 ± 0 ^a	5.05 ± 0 ^b	4.72 ± 0 ^c	4.51 ± 0 ^d	4.51 ± 0 ^a	4.34 ± 0.02 ^b	4.21 ± 0.02 ^c	4.13 ± 0.02 ^d	4.11 ± 0.02 ^d
SF (%)	100 ± 0 ^a	90.86 ± 0.05 ^b	84.90 ± 0.04 ^c	81.16 ± 0.04 ^d	100 ± 0 ^a	96.28 ± 0.53 ^b	93.24 ± 0.51 ^c	91.51 ± 0.50 ^d	91.08 ± 0.39 ^d
Hardness (N)	17.19 ± 0.01 ^d	18.16 ± 0.01 ^c	20.49 ± 0.01 ^b	22.26 ± 0.01 ^a	22.26 ± 0.01 ^a	21.23 ± 0.01 ^b	20.71 ± 0.01 ^c	20.19 ± 0.01 ^d	19.21 ± 0.01 ^e

The data presented as mean \pm SD of three independent analysis. Standard deviation with same superscripts in a row are not significantly different (*p < 0.05).

Reduction in SR and SF was found because of decreasing level of sucrose in GF cookies. Moreover, spreading action was found because of the presence of sugar crystals for melting, as the availability of sugar crystals was reduced which leads to decrease in spread ratio. Also it was investigated that replacement of sucrose with sucralose caused reduction of shortening required in samples preparation. Related results were reported by Abboud *et al.*, ³⁸ who state that the spread ratio of cookies also depends on the quantity of fat used in preparation. The hardness of GF cookies was found significantly decreased from 22.26 to 19.21 N with increased per cent of sucralose. This change in textural properties of developed GF cookies found due to decreased amount of sucrose and fat in the development of samples. Hardness of GF cookies was found decreased may be due to the lack of gluten and competition of sugar and flour for water. Singh *et al.*, ³⁹ as well investigated analogous findings during the standardization of sugar and fat levels in biscuits containing 20% de fatted soy flour.

Proximate Characterization

The proximate characterization of GF samples such as moisture, ash, crude fat, crude protein, crude fiber, carbohydrate and calories are mentioned in Table 2. As the per cent of WPC increases in GF cookies, there was significantly increase in proteins content from 4.92 to 12.38% and decrease in fat and carbohydrate content from 21.53 to 19.83% and 62.84 to 55.08% respectively.

Davameters	Levels of WPC (%)		5)	Levels of Sucralose (%)					
Parameters	0	5	10	15	0	30	50	70	100
Moisture (%)	3.53 ±	4.23 ±	4.53 ±	4.83 ±	4.83 ±	4.63 ±	4.53 ±	4.03 ±	3.83 ±
MOISLUI e (%)	0.06 ^d	0.06 ^c	0.06 ^b	0.06 ^a	0.06 ^a	0.06 ^b	0.06 ^b	0.06 ^c	0.06 ^d
Ash (%)	1.23 ±	1.33 ±	1.53 ±	1.63 ±	1.63 ±	1.63 ±	1.63 ±	1.63 ±	1.63 ±
ASII (//)	0.06 ^b	0.06 ^b	0.06 ^a	0.06 ^a	0.06 ^a	0.06 ^a	0.06 ^a	0.06 ^a	0.06 ^a
	21.53	20.63	20.03±	19.83	19.83	19.23	17.73	15.73	12.33
Crude fat (%)	±	±	0.06 ^C	±	±	±	±	±	±
	0.06 ^a	0.06 ^b	0.06	0.06 ^d	0.06 ^a	0.06 ^b	0.06 ^c	0.06 ^d	0.06 ^e
	4.92 ±	7.62 ±	9.92 ±	12.38	12.38	12.91	13.37	14.17	14.25
Protein (%)	0.01 ^d	0.01 ^c	0.01 ^b	±	±	±	±	±	±
	0.01	0.01	0.01	0.01 ^a	0.01 ^e	0.01 ^d	0.01 ^c	0.01 ^b	0.01 ^a
Crude fiber	5.93 ±	6.03 ±	6.13 ±	6.23 ±	6.23 ±	6.23 ±	6.33 ±	6.43 ±	6.53 ±
(%)	0.06 ^c	0.06b ^c	0.06 ^{ab}	0.06 ^a	0.06 ^c	0.06 ^c	0.06b ^c	0.06 ^{ab}	0.06 ^a
Carbohydrates	62.84	60.14	57.84	55.08	55.08	55.35	56.39	57.99	61.41
(%)	±	±	±	±	±	±	±	±	±
(70)	0.24 ^a	0.24 ^b	0.24 ^c	0.24 ^d	0.24 ^d	0.24 ^d	0.24 ^c	0.24 ^b	0.24 ^a
Calories	464.87	456.77	451.37	448.37	448.37	446.17	438.67	430.27	413.67
(kcal/100g)	±	±	±	±	±	±	±	±	±
(Real/100g)	0.4 ^a	0.4 ^b	0.4 ^c	0.4 ^d	0.4 ^a	0.4 ^b	0.4 ^c	0.4 ^d	0.4 ^e

Table 2: Effects of different levels of WPC and sucralose on proximate composition of gluten free cookies

The data presented as mean \pm SD of three independent analyses. Standard deviation with same superscripts in a row are not significantly different (*p < 0.05).

Protein content of WPC fortified GF cookies was significantly superior to that of control. Comparable outcome were reported by Munaza *et al.*,⁴⁰ in WPC fortified biscuits. Sathe *et al.*,⁴¹ reported that substitution of wheat flour with soy flour in crackers leads to increase in protein

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content. They also observed that the high protein level was related with the water binding capacity of soy flour. There was somewhat increase in ash content of GF cookies fortified with WPC might be due to the fortification of WPC in GF cookies. Gallagher *et al.* ⁴² also reported the biscuits fortified with whey protein concentrate and casein found high in ash content. Also, slightly increase in crude fiber in GF cookies; it might be due to the presence of sorghum flour in composite flour of GF cookies.

The water content was raised from 3.53 to 4.83% and found higher WPC fortified GF cookies due to high moisture retention capacity of sweet potato flour and WPC. Whereas, the increased level of WPC in samples leads to significantly decrease in fat and carbohydrate content of GF samples. Similarly, Singh and Mohamed⁴³ also found reduce in carbohydrate content in soy protein fortified bakery products.

The data in the Table 2 also represents that there is significantly rise in crude fiber, protein and carbohydrate contents of GF samples as the level of sucralose increased up to 100%. The protein, crude fiber and carbohydrate content of GF cookies increased from 12.38 to 14.25%, 6.23 to 6.53% and 55.08 to 61.41% respectively, with growing percentage of sucralose. This increase in nutritional composition in GF cookies could be due to the presence of higher amount of WPC and also due to the decrease in amount of sucrose and shortening in the development of GF samples. Moreover, there was significantly decline in fat content from 19.83 to 12.33% and this may be due to the reduction in sucrose and shortening content in the samples. Related findings were reported by Singh *et al.*, ³⁹ The incorporation of WPC and sucralose in GF cookies had large effects on the per cent of protein and calorie contents of GF cookies.

Changes in protein and calories content of GF cookies is represented in Table 2. It reveals that, on replacement of sweet potato flour with 15% WPC and sugar with 0 to 100% sucralose leads to significantly increase in protein content from 4.92 to 12.38% and 12.38 to 14.25% respectively. The rest of the nutritional components were also significantly (P>0.05) altered but mostly there is decrease in crude fat content.

Effect on protein and calories content of standardized GF cookies

The GF cookies containing 15% WPC and 70% sucralose having maximum score of overall acceptability were evaluated for per cent raise in protein content and decline in energy content. The data are given in Table 3. The GF cookies with 15% whey protein concentrate provide 12.38% protein content as compare to control it was only 4.92%. Whereas the protein content of formulated GF sample having 15% WPC and 70% sucralose was found 14.17%. The improve in protein content of over standardized GF sample was 151.62% over the control sample prepared with 15% WPC and 188% over the control sample developed with 15% WPC and 70% sucralose. Singh *et al.*,⁴⁴ investigated that the protein content of for cookies was found to be decreased from 464.87 to 430.27 kcal/100 g with the replacement of sweet potato flour with WPC and sucrose with sucralose in the GF cookies. Thus the per cent decline in the energy value of the above standardized GF sample containing 15% WPC and 70% sucralose was 7.52%. Onweluzo and lwezu⁴⁵ mentioned increase in energy value of biscuits developed using mixture of wheat-soy flour and

cassava-soy flour. Table 3: Effects of WPC and sucralose incorporation on protein and calorie content of gluten

Table 3:	trects of wP	c and sucralose	incorporation	on protein	and calori	e content of
free cool	ies for fasting	g purpose				

Cookies	Protein (%)	Increase in protein (%)	Calorific value (kcal/100g)	Decrease in calorie (%)
Control	4.92	-	464.87	-
WPC (15%)	12.38	151.62	448.37	3.55
Sucralose (70%)	14.17	188.0	430.27	7.52

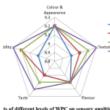


Figure 1: Effects of different levels of WPC on sensory qualities of GF cookies

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Figure 2: Effects of different levels of sucralose on sensory qualities of GF cookies

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Sensory Evaluation

The records regarding sensorial properties of GF cookies fortified with WPC and sucralose are given in Fig. 1 and Fig. 2 respectively. The data revealed in Fig.1 that GF cookies with 15% WPC obtained highest scores of texture (9.3), taste (9.3) and overall acceptability (9.3) as compared to control and other samples. It was found that, the score of color & appearance (9.2) and flavor (9.1) were similar to cookies having 15% WPC. Based on panel score, it was investigated that, the GF cookies with 15% WPC was found most acceptable with regards to overall acceptability. Based on sensorial score of WPC fortified GF samples, the GF cookies containing 15% WPC was found

overall acceptable, considered as standardized and used for further evaluation. Sathe *et al.*,⁴¹ documented parallel findings that, protein rich crackers could be prepared by substituting

refined wheat flour with soy and groundnut flours at 15% level. Singh *et al.*,³⁷ also state that WPC might be fortified up to 15% level in GF cookies without disturbing their overall quality. The effect of different level of sucralose on sensory qualities of GF cookies is presented in Fig 2. The higher sensory score was found for GF cookies containing 70% sucralose and 30% sucrose.

Conclusion

Gluten free protein enriched and low calorie cookies suitable for celiac patient can be prepared by substituting sweet potato flour with 15% whey protein concentrate (WPC) and sugar with 70% sucralose in the formulation without changing their overall quality parameters. The fortification of 15% WPC and 70% sucralose in GF cookies resulted in 188% rise in proteins while 7.52% decline in calories of the GF cookies.

Conflict of Interest

There is no conflict of interest.

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