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Formulation of multi millet cookies anf evaluate its hypoglycaemic effect in albino rats

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ABSTRACT

Multi millet cookies was standardized by incorporating with four different millet flour viz., kodo millet, little millet, foxtail millet, finger millets and wheat flour at different levels (20 - 80 per cent) and organoleptically evaluated using nine point hedonic scale. The sensory score revealed that cookies prepared from wheat flour, kodo, little, foxtail and finger millet flour at each 20 per cent level was highly acceptable. Moisture, carbohydrate, protein, fat, fibre, calcium and iron content of the standardized cookies were found to be 2.99 (%), 61.55g, 5.88g, 28.22g, 0.44 g, 51.22mg and 4.65 mg per100g respectively. Hypoglycemic effect of the multi millet cookies evaluated on wistar albino rats and the results showed that reduction in the blood glucose levels was high in the rats fed with multi millet cookies than the rats fed with control cookies. Millet thus is an amazing grain offering great opportunities for diversified utilization and value addition

Keywords : Cookies, hypoglycaemic, refined wheat flour, small millets

Millets are small seeded grains in which are classified into major and minor millets. These include many health benefits like which are low glycemic index, low glycemic load, gluten free and help in treating celiac diseases, anti cancer, antioxidant, anti cholestoremic, anti hypertensive, autherosclerosis *etc*., (Rao and Deepika, 2016). Millet crops comprise of pearl millet, sorghum (*Pennisetum glaucum*), finger millet (*Eleusine coracana*) and small millets namely foxtail millet (*Setaria italic*), proso millet (*Penicum miliaceum*), barnyard millet (*Echinochloa utilis*), kodo millet *(Paspalum setaceum)* and little millet (*Panicum sumatrense*). These are grown in arid and semi arid tracts of Asian, Africa and American countries under low rain fall (200-600 mm) where fine cereals like rice and wheat cannot be grown profitably (Ganapathy *et al.*, 2016). Millet grains, before consumption and for preparing of food, are usually processed by commonly used traditional processing techniques include decorticating/ dehulling, malting, fermentation, roasting, flaking, and grinding to improve their edible, nutritional, and sensory properties (FAO 2012).

Millets have great potential for being utilized in different food systems by virtue of their nutritional quality and economic importance. There is a wide scope of their exploitation in different food products including baked goods like breads, biscuits, cakes, cookies, breakfast cereals, muffins, pies, pancakes, snacks and extruded food stuffs. The present study with the objectives of develop and evaluation of multi millets cookies. The products were analysed for proximate composition, sensory attributes and their bioavailability evaluated in the wistar albino rats.

MATERIALS AND METHODS

Collection of the Samples

The millets such as finger millet, kodo millet, foxtail millet and little millet were procured from the Department of Millets, Tamil Nadu Agricultural University, Coimbatore and deulled by using double chamber centrifugal dehuller, cleaned, washed and sundried, milled into flour. The remaining ingredients were procured from local departmental stores.

Preparation of composite flour

Multi millet composite flour blends was prepared using whole wheat flour (20%), millet flour *viz*., kodo millet flour (20%), little millet flour (20%), foxtail millet flour (20%), finger millet flour (20%)

Standardization of cookies

Cookies were standardized using different levels of composite flour and refined wheat flour as indicated in table 1. Cookies were prepared according to the commercial procedure described by Padma and Rajendren (2016). The refined wheat flour is used as control (A). Multi millet composite flour was taken in different proportions ranging 25 per cent (B), 50 per cent (C),75 per cent (D) and 100 per cent (E). The supporting ingredients added to the multi millet flour were fat/ shorting (50gm), icing sugar (30gm), baking powder (0.5gm), corn flour (1gm) and vanilla essence (few drops). Table 3 shows the composition of ingredients for 100 gm of flour. Dough was prepared by blending all the raw materials, rolled into uniform sheet of desired size (thickness of 0.6 and 4 cm diameter) and was cut into circular shapes using cookies cutter and baked in an oven at 180°C for 15 minutes. The baked

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cookies are cooled to room temperature, packed in polyethylene containers and sealed until analysis and testing.

Organoleptic evaluation

The sensory evaluation of cookies was performed by 30 panelists asked to evaluate color, appearance, taste, flavor, texture and overall acceptability. The ratings were on a 9-point hedonic scale, ranging from 9 as like extremely to 1 as dislike extremely as outlined by Subbulakshmi *et al*. (2014). Sensory trials were replicated thrice. Among the different levels of incorporation cent per cent composite millet flour cookies (E) was highly acceptable this was taken for further analysis as T_1 and compared with refined wheat flour cookies as control (T_0).

Pasting properties

Pasting properties of starch in the whole wheat flour multi millets blends were measured on a Rapid Visco Analyser (RVA-4) by AACC (2000)

Physical properties

The Physical characteristics like height, weight and breadth of the control cookies dough (T_0) and multi millet composite cookies dough (T_1) were measured by the method of Giwa. and Victor (2010).

Table 1: Formulation of multi millet cookies

Characteristics	T_1	T_2	T_3	T_4	T_5
Composite flour (g)	-	25	50	75	100
Refined wheat flour (g)	100	75	50	25	-
Fat (g)	50	50	50	50	50
Icing sugar (g)	30	30	30	30	30
Baking powder (g)	0.5	0.5	0.5	0.5	0.5
Corn flour (g)	1.0	1.0	1.0	1.0	1.0
Vanilla essence (ml)	2	2	2	2	2

Baking properties

The standardized composite cookies were studied for their physical characteristics including diameter, thickness, spread ratio and water activity as per the AACC method 74-09, 1983. Spread ratio of cookies: the diameter (D) and thickness (T) of ten cookies were measured using a verniear calliper. Four measurements were made at different sides for thickness of the cookies and the average measurements (mm) were noted. Two measurements were made at two different sides for diameter and the average measurement was noted. Spread ratio was calculated as mean value of diameter of the cookies (mm) divided by mean value of thickness of the cookies (mm).

Proximate analysis

Multi millet cookies were estimated for moisture (Ranganna, 1995). Carbohydrates (Sadasivam and Manickam (1996), crude protein (Micro kjeldahal, Nx6.25), crude fat (solvent extraction), ash (muffle furnace - dry ash), calcium (titration), iron (colorimetric) were determined by the AOAC (2000). Crude fibre (acid and alkali) was determined by the method of Sadasivam and Manickam (1996) and for Energy was determined by Nutritive value of Indian food (ICMR, 2010).

Hypoglycaemic effect of multi millet cookies in albino rats

The study was conducted in Karpagam College of Pharmacy, Coimbatore, India. The experiments were approved by the institutional animal ethics committee, Karpagam University, Coimbatore. The wistar albino rats were housed in large spacious cages and they were fed with multi millet cookies and control cookies.The study was conducted for a period of 28 days including the period to standardize their feed intake for first three days. Rats were given free access to diet and water, daily food intake, weekly body weights and faecal bulk were recorded Diabetes mellitus was induced into wistar albino rats by single intraperitoneal injection of freshly prepared solution of Alloxan monohydrate (150 mg kg^{-1} BW) in physiological saline after overnight fasting for 12hrs and development of hyper glycaemia in rats was confirmed by plasma glucose estimation on 72hrs post alloxan injection. The rats with fasting plasma glucose level of >150mg/dL were used for this experiment. The levels of blood glucose, was measured at the start and after 28 days of feeding trial. The blood samples were collected retro-orbitally from the eye under light ether anaesthesia using capillary tubes in fresh vials containing EDTA as anticoagulant agent and serum was separated in a T_8 electric centrifuge at 2000 rpm for two minutes. Then serum samples were used for various biochemical tests.

Statistical analysis

All the data are presented as mean ± SED of two replicates. The nutritive values of bread were subjected to Analysis of variance (ANOVA) to distinguish the responses of different levels of substitution and performed using Completely Randomized Design (CRD). The levels of significant differences are reported as pd"0.05.

RESULTS AND DISCUSSION

Organoleptic evaluation

Subjective sensory characteristics refined wheat flour cookies and different levels of multi millet flour incorporated cookies are summarized in table 2. The overall acceptability scores for control and at 25, 50,75 and 100 per cent incorporation levels were 8.6±0.49 ,

8.5±0.45, 8.1±0.34 and 8.2, 8.2±0.44 respectively. The texture and taste of the cookies increased in incorporation levels of the multi millet and the flavour of the cookies showed gradual decrease in the mean score value at increased levels of millet flour incorporation. The overall acceptability score revealed that cookies prepared from whole millet flour (E) was highly acceptable when compared to the market variety. Kumar *et al.* (2006) developed the biscuits from 10 per cent soybean flour and 90 per cent of kodo millets flour.

Table 2: Organoleptic scores of cookies

Parameters	Control	Level of incorporation			
		25 %	50%	75%	100%
Colour	8.6±0.67	8.4±0.36	8.2±0.44	8.2±0.13	8.3±0.37
Appearance	8.9±0.30	8.6±0.65	8.4±0.50	8.2±0.54	8.8±0.37
Flavour	8.4±0.96	8.4±0.23	8.5±0.37	8.3±0.67	8.2±0.68
Texture	8.4±0.99	8.4±0.12	8.5±0.67	8.5±0.78	8.6±0.50
Taste	8.6±0.50	8.6±0.82	8.7±0.69	8.8±0.29	8.8±0.50
Overall Acceptability	8.6±0.49	8.5±0.45	8.1±0.34	8.4±0.31	8.2±0.44

Values are mean ± SD

Pasting properties

The water activity of control and multi millet cookies is given in table 3. The mean value of water activity was 0.46 for T_1V_1, 0.49 for T_2V_2 and T_3V_3 respectively which was lower than the control (T_0) with the value of 0.61.The peak viscosity (maximum viscosity during heating), peak time, pasting temperature, holding strength, breakdown, final viscosity (viscosity after pasting and holding), setback from peak and setback from trough (viscosity at the end of holding time) are given in table 3. The pasting temperature of T_0 was 80.13°C, which decreased to 74.72°C in multi millet flour. The peak viscosity and hold viscosity for T_0 was 2681.50 cP and 1604.50 cP, which was higher than the composite flour with values 2261.00 and 1298.00 cP. The final viscosity was higher in multi millet flour **(**3060.00 cP) compared to T_0 (3011.00 cP). From the results it was observed that the peak viscosity and final viscosity of optimized multi millet flour blends at 90°C were higher than that of corresponding holding, breakdown and set back viscosity. A similar trend was reported by Aprianita *et al.* (2009) for taro flours, Musa *et al.* (2011) for rice starch suspensions and Chaisawang and Suphantharika (2005) for cationic tapioca starch. On cooling, the viscosity of starch raised due to the high retrogradation tendency of the amylase fraction. The changes taking place during heating of starch in excess water have been studied in detail by Shinoj *et al.* (2006) and Bhattacharya (2012). Starch granules were insoluble in water, but when a starch suspension in water was heated above critical temperature, the hydrogen bond responsible for structural integrity of the granules weakened allowing the penetration of water, and hydration of the linear segments of amylopectin (Qiang, 2005).

Table 3: Pasting properties of control and multi millet flour

Treat-ments	Peak 1	Trough 1	Break down	Final Viscosity	Set back	Peak time	Pasting temp
T_0	2681.5 ± 36.06	1604.50 ± 55.86	1077.00± 19.79	3011.00± 33.94	1406.50± 21.92	6.065± 0.09	80.13± 0.59
T_1	2261.0 ± 2.82	1298.00± 11.31	916.00± 8.48	3060.00± 1.41	1762.00± 9.89	5.535± 0.09	74.72± 0.52

T_0 – Control cookies T_1 – Multi millet flour cookies

Physical properties of multi millet dough

The physical characteristics of the multi millet cookies dough is presented in table 4. The dough weight of T_0 was 160g which was lesser than T_1 with the value being 168. The weight of the cookies increased due to the high fibre content of the small millets. The height of the cookies was 0.4 cm for T_0 and 0.5 cm for T_1 and also after baking the height of the cookies was observed to raise by 0.1cm for T_0 and multi millet cookies.

Table 4: Physical properties of dough

Characteristics	T_0	T_1
Dough weight (g)	160	168
Height before baking (cm)	0.4	0.5
Height after baking (cm)	0.5	0.6
Breadth (cm)	3.5	3.6
Spread ability (cm)	0.1	0.2
Final out put (g)	161	163
No. of cookies	20	22

The breadth and spreadability of T_0 was 3.5cm and 0.1cm and that of composite dough was 3.6cm and 0.2cm. The final output and the number of cookies for T_0, and T_1 were 163g and 20, 165g respectively. The final output and number of cookies was higher for multi millet cookies than the control due to the high fibre content of the small millets.

Baking properties of multi millet

The physical characteristics of multi millet cookies are presented in table 5. The diameter and thickness of multi millet cookies was lower than the control with values of 34.71 mm and 7.09mm and that of T_0 was 41.46mm and 7.57mm respectively. The spread ratio of the multi millet cookies (5.83) was observed to be higher than the control cookies (5.47). The water activity (a_w) of the multi millet cookies was found to be lower than the control.

Table 5: Baking properties multi millet cookies

Treatments	Diameter (mm)	Thickness (mm)	Spread Ratio	Water Activity (a_w)
T_0	41.46±0.20	7.57±0.25	5.47±0.15	0.223±0.03
T_1	34.71±0.77	7.09±0.12	5.83±0.06	0.223±0.01

Proximate analysis of multi millet Cookies

The nutritive value of cookies from control and multi millet were analyzed and presented in the table 6. The cent per cent composite flour blends cookies (T_1) provides high amount of nutrients *viz*., 65.59 per cent carbohydrate, 13.15 per cent protein, 20.57 per cent fat, 1.92 per cent crude fibre, 31.42 mg calcium and 1.74 mg of iron per 100 g. compared to control (T0) contained 66.54 per cent carbohydrate,13.65 per cent protein, 20.12 per cent fat, 0.46 per cent crude fibre, 11.62 mg calcium and 15.42 mg of iron per 100 g. These findings are in agreement with studies conducted by Gobinath *et al.* (2014) reported that crude protein content of cookies prepared from composite flour of wheat, Barnyard millet and mushroom flour ranged from 8.93 to 10.31 per cent. Tiwari *et al.* (2011) reported that protein content of cookies added with pigeon pea *(Cajanus cajan* L) had crude protein content ranging from 6.20 - 8.00 g per 100 g

Table 6: proximate analysis of nutrients

Nutrients	T_0	T_1
Moisture (g)	2.80	2.73
Carbohydrate (g)	66.54	65.59
Protein (g)	13.65	**13.15**
Fat (g)	20.12	20.57
Crude Fibre (g)	0.46	**1.92**
Ash (g)	1.00	0.84
Calcium (mg)	11.62	**31.42**
Iron (mg)	1.83	1.74

Hypoglycaemic effect of multi millet cookies in albino rats

A large number of animal studies and human nutrition intervention trials proved that millet foods are known to be hypoglycaemic because of high fibre content. Hypoglycemic effect of the multi millet cookies by conducting experiments on wistar albino rats and the results are presented in table 7.The initial weight of the various groups of rat ranged from 190 ± 7.4 to 212 ± 8.6g. After 28 days of study there was a statistically significant increase in body weight of the normal group fed with refined wheat cookies and multi millet cookies (G3 to G6) with values ranging from 222 ± 9.6 to 232 ± 9.9g when compared with G1. Similarly trend was observed in the diabetic group the rats The initial blood glucose level of the normal rats was 77.50 ± 3.20 (G1) while that of the diabetic group was 140.62 ± 3.64mg per 100 ml (G2). At the end of the feeding trial the blood glucose level was decreased in G1 and increased in G2. The initial blood glucose level of perse control group ranged between 90.15± 4.05 (G6) to 91.90 ± 3.75 (G3), whereas the diabetic group ranged from 154.22±3.55 (G10) to 172.09 ± 4.30 (G7) mg per 100ml. Among the perse control group (G3-G6) the blood glucose level was observed to be decreased in the rats fed with multi millet cookies when compared to rats fed with refined wheat cookies. The overall result indicated that the reduction in the blood glucose levels was high in the rats fed with multi millet cookies than the rats fed with control cookies.

Jothilakshmi *et al.*, 2012 also reported a statistically significant reduction in the blood glucose level at the

Table 7: Effect of multi millet cookies on body weight and blood glucose in normal and treated animals.

Group	Body weight (g)		Blood glucose (mg / 100ml)	
	Initial	Final	Initial	Final
G1	205 ± 8.8	220 ± 9.1	77.50 ± 3.20	73.54 ± 4.25
G2	195 ± 7.6	150 ± 5.2**(a)	140.62 ± 3.64	220.25 ± 9.19**(a)
G3	207 ± 8.4	222 ± 9.6	91.90 ± 3.75	99.05 ± 4.15
G4	190 ± 7.4	228 ± 9.4	90.35 ± 2.95	80.25 ± 3.85
G5	204 ± 8.2	230 ± 9.8	91.42 ± 3.93	86.15 ± 3.68
G6	212 ± 8.6	232 ± 9.9	90.15 ± 4.05	83.15 ± 4.26
G7	220 ± 8.8	226 ± 8.4	172.09 ± 4.30	205.25 ± 6.60**(b)
G8	215 ± 8.0	230 ± 8.9	158.12 ± 4.65	115.16 ± 6.95**(b)
G9	222 ± 8.9	235 ± 9.1	163.15 ± 4.70	129.45 ± 5.92**(b)
G10	218 ± 8.5	232 ± 8.8	154.22 ± 3.55	121.20 ± 6.88**(b)

G1*- Normal;* ***G2****- Diabetic Control;* ***G3- G6*** *(Perse control) (Normal Rat +Multi millet cookies));* ***G7-G10****- Treatment control (Diabetic Rat +Multi millet cookies).*

Values are expressed as mean ± SEM.

*** (a) Values are significantly different from normal control G1 at P<0.001.*

*** (b) Values are significantly different from Diabetic control G2 at P<0.01.*

end of the four weeks of feeding trial of millet incorporated multigrain mixes to normal and diabetic rats. Dietary fibre is nutritionally important non nutrient roughage. Millets are good sources of soluble fibre. During digestion, soluble fibre dissolves in water, and traps nutrients inside its gummy gel and slows down considerably while moving through the digestive tract. Inside the gel, nutrients are shielded from digestive enzymes and less likely to reach the wall of the intestines. Dietary sugars like carbohydrates and starch are among the nutrients trapped inside this gel. Consequently, sugar is absorbed into the bloodstream more slowly, blunting the sharp hike in blood glucose typically experienced by diabetic patients after a meal. Lesser hike in blood glucose leads to greater sensitivity of the action of insulin. Avoiding high peaks and low valleys in blood glucose places less stress on the pancreas and is important not only to diabetics but also to those who want to prevent the onset of type 2 diabetics.

It may be inferred from this study and results that acceptable and good quality cookies could be produced from multi millet composite flour. The development of composite flours for bakery industry will create a new avenue to utilize tropical raw materials. The resulting cookies would be expected to have better nutritional quality at a reduced cost. Hence utilization of small millets is important in the light of changing climate conditions, growing population and rising rates on non communicable diseases.

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