Research Article

Dietary fiber isolate from coconut flakes – A functional food

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ABSTRACT

The general objective of the study is to formulate high percentage dietary fiber isolate from coconut flakes, as a functional food, and the specific objectives are as follows: (a) to formulate coconut flour from coconut flakes (b) to determine the proximate composition and microbial analysis of coconut flakes, coconut flour and dietary fiber isolate (c) to analyze the storage stability of coconut flour and dietary fiber isolate (d) to determine the anti diabetic activity and cholesterol lowering effect of dietary fiber isolate. The coconut fiber isolate was prepared by hydrolysis with $CaOH_2$ as suggested by Usman et al. The dietary fiber content of dietary fiber isolate was 72.25% and further it was found to be 42% and 48% in coconut flakes and coconut flour respectively. The protein content of flakes and flour was almost similar (23.24% and 23.15%) while; the isolate was having only 14%. However, the fat content was high (49.34%) in flakes but was only 3-4% in flour and isolate. Correspondingly energy value was also two folds more in flakes than the other two. With respect $CaOH_2$ hydrolysis, 0.3M and 0.4M concentrations were found to be very ideal in suppressing the dominant coconut taste. According to BIS (Bureau of Indian Standard), the microbial load and peroxide value were within safe limits in isolate (up to 10 months). When, 500 mg/day of dietary fiber isolate was administered to experimental group, appreciably showed a decrease of 32%, 42% and 51% ($p < 0.05$) of the blood glucose at the end of 1st 2nd and 3rd week respectively. Serum total cholesterol of the same group was significantly lower after consumption of 300mg and 500 mg of dietary fiber isolate for forty two days ($P<0.05$); The results indicated 500 mg/day of dietary fiber isolate reduced blood glucose and lipids viz total cholesterol, low density lipoprotein and triglycerides. Fruitful outcome from the above study can be a basis in the development of dietary fiber isolate as a functional food.

Keywords: Coconut flakes, Coconut flour, $CaOH_2$ hydrolysis, Dietary fiber isolate, Functional food.

INTRODUCTION

Coconut processing sector in India is currently confined to copra production, oil extraction, desiccated coconut, manufacture of coir and its products. Out of the total coconut production, 5% is utilized as tender nuts and the rest as mature nuts, which find different uses such as household and religious purposes (56%), milling copra (35.6%), edible ball copra (6.5%) and desiccated coconut (1.6%). One third of the annual production is used by processing industry for the manufacture of coconut oil, while rest is processed into desiccated coconut and other products. Even though India is the third largest coconut growing country in the world, its contribution to the export market is insignificant.[1-8]

The most widespread consumed dietary fiber products are those derived from cereals. However, over the past decade high dietary fiber materials from fruits (citrus, apple, and others) have been steadily introduced in the world markets. Fruit dietary fiber concentrates have better nutritional quality than those found in cereals, because of their significant contents of associated bioactive compounds (flavonoids, carotenoids, etc.) and more balanced composition (fibre content, SDF/IDF ratio, hydration capacities, lower metabolic energy value, and phytic acid content).[8-11]

One by-product of the coconut milk industry is the coconut residue taken after extraction of the coconut milk. The coconut residue is made into coconut flour and believed to contain dietary fiber. Dietary fiber has been shown to have important health implications in the prevention for risk of chronic diseases such as cancer, cardiovascular diseases and diabetes mellitus.[24] Only one report, by Trinidad et al. (2006)[26] is available in the literature regarding the utilization of spent coconut fiber as dietary fiber, in which only the physic- chemical and nutritional properties of coconut fiber were emphasized.

The general objective of the study is to formulate high percentage dietary fiber isolate from coconut flakes, as a functional food, and the specific objectives are as follows: (a) to formulate coconut flour from coconut flakes (b) to determine the proximate composition and microbial analysis of coconut flakes, coconut flour and dietary fiber isolate (c) to analyze the storage stability of coconut flour and dietary fiber isolate (d) to determine the anti diabetic activity and cholesterol lowering effect of dietary fiber isolate.

MATERIALS AND METHODS

Formulation of Coconut flour and Dietary fiber isolate

Coconut was processed by selecting 30 days matured full fat coconut meal. The coconut meal was deshelled using a coconut desheller. Uniformly the coconuts were purchased locally. The coconuts were deshelled to remove the kernel from the shell. Coconuts were scrapped to remove the outer brown layer. This was then cut to remove the water followed by scraping to form fine thread like structures. The shredded thread structures of coconut meal were treated under a temperature of 55 – 60 ^{0} C in a fluid bed dryer for 30 – 35 minutes. The time taken for drying 1 ton of scraped coconut meal was between 30 and 35 minutes. The dehydrated full fat coconut meals were then subjected to hydraulic press (cold press) at room temperature. Two products were obtained namely: Virgin coconut oil and coconut flakes. The dehydrated coconut flakes were formed as a byproduct of virgin coconut oil. The coconut flakes were treated using solvent extraction with and without hydrolysis.

Solvent extraction without hydrolysis

Coconut flakes of 5 kg were extracted with 18 liters of extracting solvent for 8 hours using mini solvent extraction unit (6kg capacity of samples). Food grade hexane (non polar solvent) and acetone (polar solvent) in the ratio of 60:40 were used. The boiling point of solvent mixture was 60 – 80 ^{0}C. The samples were withdrawn after 6 hours, and kept in a hot air oven at 55 to 65^{0}c for 3 hours to remove hexane and acetone mixture, and the flakes were ground by CAD milling for 1 hour without heat treatment. Then the coconut flour was weighed.

Solvent extraction by hydrolysis

Acid hydrolysis of Coconut flakes

10 gm of the coconut flakes was weighed separately into five separate boiling tubes. Each was moistened with 20 ml of 0.1 M to 0.5 M HCl solutions (Equivalent to 0.002 moles, 0.004 moles, 0.006 moles, 0.008 moles and 0.01 moles of the prepared solutions). They were placed in water bath and maintained at 75 ^{0}C for an hour, which has been established as an ideal time for hydrolysis in preliminary investigations.[13] The Products were then washed with water (Demineralised water), dried with fluid bed dryer for further process.

Alkaline and Calcium hydroxide hydrolysis of coconut flakes

Similarly 10 gm of the coconut flakes was weighed separately into five separate boiling tubes. Each was moistened with 20 ml of 0.1 M to 0.5 M NaOH solutions (Equivalent to 0.002 moles, 0.004 moles, 0.006 moles, 0.008 moles and 0.01 moles of the prepared solutions). The remaining five were moistened with 20 ml of 0.1 M to 0.5 M calcium hydroxide solutions. The products were then washed with water (Demineralised water), dried with fluid bed dryer for further process. Out of these, only $CaOH_2$ hydrolysis yielded a good quality dietary fiber isolate without the dominance of coconut aroma.

Proximate Composition

The moisture, crude fiber, protein, fat, calcium, iron and dietary fiber were analyzed out of coconut flour and dietary fiber isolate by standard method suggested.[4] However, carbohydrate and energy were analyzed according to the method of Gopalan (1996)[19], but available lysine of Pellet et al., (1980)[20] respectively.

Storage stability

Coconut flour and dietary fiber isolate were transferred to aluminum foil pack and sealed with plastic containers. The containers were closed and stored at room temperature in dark. Periodically (every month) for 10 months a suitable volume of coconut flour and dietary fiber isolate were withdrawn from each container and subjected to determination of total plate count and peroxide value by standard protocol.[4,5]

Anti-diabetic effect

Animal experiment was carried out as per guidelines of Institutional Animal Ethical Committee and approval (Bearing number: 1012/c/06/CPCSEA). 32 male Wister albino rats reared at animal house of RVS College of Pharmaceutical Science, Coimbatore, Tamil Nadu, INDIA. Animals with body weight of 150 – 200 g were selected, based on uniform food intake and weight gain was maintained with standard laboratory conditions (12 h light/dark cycles). The animals were divided into 4 groups of 8 rats in each, the first group being non-diabetic control. Animals of groups II, III and IV were rendered diabetic by a single intraperitoneal (i.p.) injection of 60 mg/kg of Streptozotocin (STZ) freshly prepared in 0.1 M of citrate buffer (pH 4.5). Second, the diabetic control group was administered ½ unit of Insulin per day for avoiding morbidity and mortality as it was positive control group. Third and fourth groups were forced fed with 300 mg and 500 mg of Dietary fiber isolate per day respectively. The experimental duration was for three weeks. Food intake and body weight gain were monitored weekly. The blood glucose levels were monitored 5 days once, by obtaining blood samples from tail vein; with standard kit. At the end, animals were sacrificed, after overnight fasting, by cervical dislocation.

Cholesterol activity

The rats were divided into four groups, each of six animals were maintained under standard laboratory conditions (12 h light/dark cycles). The first group (gp I) was kept as the control (receiving Vehicle). The second group (gp II) third and fourth groups (gp III and gp IV) were fed with 1% cholesterol powder (1g/kg/day) of hypercholesterolemic gp by oral administration for 10 days as prescribed by Reeves et al (1993).[23] Then, the hypercholesterolemic condition was confirmed by using respective diagnostic kits on the 11th day of experiment using blood drawn by retro orbital plexus. After the

confirmation of hypercholesterolemic conditions of rats, the day on which rats were treated with dietary fiber isolate powder at a low dose of 300 mg/day (gp III) and high dose of 500 mg/day (gp IV) body weight was considered as 1st day of experiment. Blood samples from the retro orbital plexus were drawn after an overnight fasting (more than 12 hr) before treatment (0 week), then 10 days of treatment. Total cholesterol (TC), triglycerides (TGs) and high density lipoprotein (HDL)-cholesterol were measured using kits (Bio-Diagnostic). Low density lipoprotein (LDL)-cholesterol was calculated by Friedwalds formula.

Statistical Analysis

All experiments and analytical measurements were run in triplicate except blood tests. Means of each parameter were analyzed by analysis of variance (ANOVA) and students independence't' test. Adhoc were also performed for ANOVA. Different between treatments at the 5% ($P \leq 0.05$) and 1% ($P \leq 0.01$) levels were considered significant.

RESULTS AND DISCUISSION

Table 1: Proximate composition of Coconut flakes, Coconut flour and Dietary fiber isolate

Testing Parameters	Coconut Flakes	Coconut Flour	Dietary Fiber Isolate	Test Methods
Moisture (%) by wt	3.71±0.11[a]	5.4±0.99[b]	5.1±1.37[b]	AOAC 18th Edn.2005,925.10
Crude fiber (%) by wt.	11.50±1.25[a]	6.54±1.14[b]	11.0±1.11[a]	AOAC 18th Edn.2005,962.09
Protein , (%) by wt. (Nx6.25)	23.24±0.22[a]	23.15±0.01[a]	14.0±0.02[b]	AOAC 18th Edn.2005,984.13
Fat (%) by wt.	49.34±0.46[a]	3.2±0.24[b]	4.0±1.12[a]	AOAC 18th Edn.2005,920.85
Carbohydrate by difference (%) By wt.	20.60±0.02[a]	48.89±0.01[b]	61.2±0.02[a]	Nutritive value of Indian Foods, Gopalan C. etal; NIN,ICMR,1996
Calcium mg/100g	-	20±0.05[a]	336±1.10[b]	AOAC 18th Edn.2005,985.35
Iron mg/100g	-	3 ±0.56[a]	6.86±0.67[b]	AOAC 18th Edn.2005,985.35
Available lysine, g/100g protein	-	0.4±0.2[a]	6.7±0.2[b]	Carpenter, K.J and booth, H.V.H. In nutritional evaluation of protein foods 1960 [9]. Ed., Pellett, P.L and young V.R. The United Nations University, (P.95-97;1980)
Dietary fiber, (%) by wt.	42±0.6[a]	48±0.56[b]	72.15±0.70[a]	AOAC 18th Edn.2005,991.43
Energy (kcal)	619.62±0.14[a]	369.70±1.1[b]	340±2.18[a]	Nutritive value of Indian Foods, Gopalan C. etal; NIN,ICMR,1996

a,b Values with different letters in the same row are significantly different ($p \leq 0.05$). Means ± SD, each value in the table is the mean of three replications.

Table 2: Changes in Peroxide value (mεq O_2 / kg fat) of coconut flour and coconut dietary fiber isolate at room temperature over 10 months of period.

Test food	Month interval / Peroxide value (mεq O_2 / kg fat)									
	1	2	3	4	5	6	7	8	9	10
Coconut flour	Nil	Nil	Nil	Nil	1.25	1.80	2.65	3.16	3.85	3.95
Dietary fiber isolate	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	1.45	2.85
Specification	Max 3	Max 3	Max 3	Max 3	Max 3	Max 3	Max 3	Max 3	Max 3	Max 3

Table 3: Changes in Microbial load of coconut flour and coconut dietary fiber isolate at room temperature over 10 months of period.

Test food	Month interval / Microbial Load colonies / g									
	1	2	3	4	5	6	7	8	9	10
Coconut flour	6150	8200	11150	13200	16700	22800	29100	36700	51300	57000
Dietary fiber isolate	5750	6600	8500	11200	15400	19600	25400	30900	34700	38900
Specification	Max 50000	Max 50000	Max 50000	Max 50000	Max 50000	Max 50000	Max 50000	Max 50000	Max 50000	Max 50000

The results of the proximate composition of coconut flour and dietary fiber isolate, stored under ambient condition are presented in Table 1. The dietary fiber for dietary fiber isolate (DFI) was 72.25% whereas; Coconut Flour (CFLR) had 42% and 48% respectively. Whole cereals tend to have both soluble and insoluble dietary fiber. However, depending on the variety of grain the proportions of their two dietary fibers do vary. In a study conducted by Sudha et al (2007) [25] found that Soluble Dietary fiber (SDF) content of wheat bran and rice bran were 5.01% and 4.33% respectively, Whereas it was 8.9 and 10.8 % in oat bran and barley bran respectively. The protein content of flour was 23.15%, whereas, the isolate was having only 14%. However, the fat content was only 3-4% in flour and isolate.

The dietary fiber was analyzed in all the two samples of coconut flour and isolate. However, only in coconut dietary fiber isolate, all the three types of dietary fiber namely, Soluble Dietary Fiber (SDF), Insoluble Dietary fiber (IDF) and total dietary fiber were analyzed. As it is given in the table 1, the total dietary fiber content of all three varieties of coconut products significantly varied with each other. The isolate had a highest total dietary fiber content of 72.15± 0.70 whereas the coconut flour had a value of 48± 0.56 respectively. With regard to dietary fiber, Asp et al (1983) [3] had found coconut residue fiber made from coconut gratings to have soluble Dietary fiber of 3.41 ± 0.2, insoluble dietary fiber of 33.97 ± 0.67 and total dietary fiber of 33.38 ± 0.91. whereas, Lee et al (1992) [10] documented that of total dietary fiber of 37.51 ± 0.72, insoluble dietary fiber of 35.08± 0.60 and that of soluble dietary fiber of 2.43 ± 0.12 from coconut residue fiber. But in our study, the coconut dietary fiber isolate was found to have a significantly high level of total dietary fiber of 72.15 ±0.70, insoluble dietary fiber of 68.15 ± 0.20 and soluble dietary fiber of 3.65 ± 0.70. The total dietary fiber, insoluble dietary fiber and soluble dietary fiber of isolate was substantially higher than of wheat bran (TDF 44.5, SDF 2.9 and IDF 41.6 %) [21] and oat bran (TDF 23.8, SDF 3.6 and IDF 20.2).[17]

Residues obtained from sodium hydroxide and hydrochloric acid hydrolysis, irrespective of the strength of the solution used was found to have lost its coconut taste. This establishes complete removal of all available coconut taste principles. However, in comparison with that of calcium hydroxide hydrolysis, it was found that only the samples hydrolyzed with 0.3 M and 0.4 M Ca $(OH)_2$ solution lost their coconut taste. This was similar to a study by Usman et al 2003 [13], in which with that of calcium hydroxide hydrolysis, it was found that only the samples of *Thevitia peruviana* seed cake hydrolyzed with 0.4M and 0.5M Ca $(OH)_2$ solution lost their bitter taste.

From the Table no 2, it was clearly evident that peroxide value of coconut dietary fiber isolate was within safe limits up to 10 months but whereas coconut flour could not keep up their peroxide value within the BIS (Bureau Of Indian Standard) prescribed level of 3 per 100g maximum, which was soaring ahead of 3 per 100g maximum at 8th month. However, with regard to packaging material used to store both flour and dietary fiber isolate was only aluminum foil. In this line, a study conducted by Khan et al (2012) [15] demonstrated that the virgin coconut meal incorporated sooji Halwa mix withstood peroxidation much in metalized polyester packing than the one packed and stored in polypropylene.

Table no 3 shows the total plate count of the experimental substances. Total plate count of coconut flour increased from 4200 to 57100 per g and dietary fiber increased from 5750 to 38900 per g. over a period of 10 months. However, our study indicates that the microbial load was within the limit up to 8 months for coconut flour and 10 months for dietary fiber isolate without treating with any preservatives. Conversely, in accordance with BIS (Bureau of Indian Standard) recommendations, only total plate count of coconut flour has crossed beyond 50000 max per g at the 8th month but till 10th month dietary fiber isolate could withstand the stipulation of BIS. Hence the coconut flour with aluminum foil packing met the above mentioned specification up to 8 months and dietary fiber isolate up to 10 months and it was ascertained as suitable for consumption. According to "Microbial food safety- Indian Regulation"[27] solvent extracted soya flour should contain total bacterial count of less than 50,000/g, coli-form bacteria should be less than 10/ g, and salmonella bacteria should be absent in 25 gram of the sample for consumption. Similar to these guidelines, our product isolate satisfied these conditions.

Table 4: Effect of different concentration level of dietary fiber isolate for anti diabetic activity

Groups	Food Intake mg/kg/day	Weight gain/loss (g/21 days)	Blood glucose mg/dl			
			5th day	10th day	15th day	21th day
Control Non diabetic	11.3 ± 1.2^{a}	24.2 ± 1.6	110.10±2.14	112.52±0.94	119.64±1.95	108.41±1.04
Control diabetic (Insulin)	20 ± 1.5^{b}	-27 ± 3.8	288.16±4.16	269.50±3.14 (6%)	247.80±4.39 (14%)	226.80±3.02 (21%)
Dietary fiber isolate (300 mg / day)	20 ± 1.16^{b}	-20 ± 4.3	262.66±7.78	210.83±7.06 (19%)	170.50±7.54 (35%)	153.50±7.33 (41%)
Dietary fiber isolate (500 mg /day)	20 ± 1.3^{b}	-13.5 ± 0.6	283.30±4.53	192.16±11.6 (32%)	163.16±10.3 (42%)	138.50±10.2 (51%)

Values are mean of ± SD of 8 rats; Values bearing different superscripts in the same row are significantly different (p <0.05)

Table 5: Cholesterol lowering effect

Test food	Total cholesterol		LDL		HDL		Triglycerides	
	Day 1	Day 42	Day 1	Day 42	Day 1	Day 42	Day 1	Day 42
Control	299±7.84	298.5±3.96	154±3.75	155.4±4.96	50.2±5.41	48.98±3.12	233±14.14	233.2±18.01
1% Cholesterol powder	275±7.11	270±3.96*	144.3±6.9	142±0.11*	47.4±4.20	45.4±7.53	277±16.02	255±15.05*
Dietary fiber isolate (300mg/day)	287±20.03	268±15.52*	152.7±7.89	144.7±9.10*	53.4±4.54	50.28±4.95	328±24	274±23.14*
Dietary fiber isolate (500 mg/day)	290±10.11	257±7.52*	160.8±4.74	140±3.10*	41.9±3.19	40.87±8.45	242±19.10	202±17.58*

*Significantly different at P<0.05.

Effect of Dietary fiber isolate on Blood Glucose level

Increased food intake with body weight loss was observed in all the diabetic groups, lowest weight loss was observed in 500 mg/kg/day concentration (Table 4) of dietary fiber isolate (-13.5±0.6). Gradual decrease in blood glucose was observed in all the 3 groups of animals. Insulin (control diabetic) was able to decrease the blood glucose level from 288.16 mg to 226.500 mg during 3 weeks (6%, 14% and 21% at the end of 1st, 2nd and 3rd week respectively). Significant decrease in Insulin fed was observed at the end of 2nd week and 3rd week respectively. In dietary fiber isolate 300 mg/day group significant decrease was observed from 1st and 2nd week till 3rd week (The percentage decrease was 19%, 35% and 41% at the end of 1st, 2nd and 3rd week respectively). Dietary fibers isolate 500 mg/day group decreased the blood glucose of 32% 42% and 51% ($p < 0.05$) at the end of 1st 2nd and 3rd week respectively. Similarly the blood glucose and cholesterol levels of treated groups of rats showed a significant reduction after 7 weeks of treatment with *plantago psyllium.*[10]

Table 5 shows the serum total, LDL and HDL cholesterol, and triglycerides of rats before and after feeding of the dietary fiber isolate. Serum total cholesterol were significantly lower after consumption of 300mg dietary fiber isolate and 500 mg dietary fiber isolate for 42 days (P<0.05; Table 5). Similar results were also observed for LDL cholesterol. For both the concentration of dietary fiber isolate there was a significant reduction in serum triglycerides (P<0.05; Table 5). However, there was no significant increase or decrease in HDL cholesterol that was observed in the study. In corroboration to the present study, a reduction in glucose and cholesterol levels was observed in diabetic patients fed with coconut dietary fiber from coconut flakes without significant adverse effects.[26] In another animal study, the blood glucose and cholesterol levels of treated groups of rats showed significant reduction after 7 weeks of treatment with *plantago psyllium.*[10] The findings of Schneeman and Richter (1993).[25] Supported the phenomenon of cholesterol reduction with diets containing psyllium husk fiber; they reported decrease in serum cholesterol of rats by providing normal diet with psyllium husk. They concluded that psyllium husk can alter lipid metabolism. Some other studies are also in corroboration with recent findings of decreasing plasma lipid profile especially cholesterol with psyllium husk.[9,12, 29] The research of Fang (2000)[6] exposed that in rats after administration of psyllium fiber caused significant reduction in total cholesterol. In another study, Agarwal et al., (2007)[1] documented decrease in serum cholesterol level of normal subjects consuming psyllium husk as a source of dietary fiber. The same kind of reduction in cholesterol level was also observed in the present study after feeding with dietary fiber isolate. Such dramatic reduction in glucose and lipids in the present research could be attributed to coconut dietary fiber isolate. Though the basic source is coconut, yet it has not impaired the lipid parameters in vivo. Thus, the results make us to get interested to further explore the mechanism of attenuation of lipo-glycemic entities.

CONCLUSION

In conclusion : (a) Dietary fiber isolate is a rich source of dietary fiber, when it was treated with calcium hydroxide hydrolysis and it was found that coconut flakes lost their coconut taste and produced highest percentage of dietary fiber (72.5%) than any other cereals (b) dietary fiber isolate stored up to 10 months ambient conditions, did not produce any rancid odour and the microbial load was also within the safe limits (c) dietary fiber isolate administration substantially brought down the blood glucose level and reduced the lipid parameters of the rats. Hence, dietary fiber isolate prepared from coconut flakes rendered as a safe, odourless therapeutic functional food. As dietary fiber isolate potentially ameliorates glucose and lipid levels, this may be used as a functional food for human beings. Further, a systematic human study may be carried out using dietary fiber isolate in order to explore its impact in humans.

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