

Original Article

FORMULATION AND STORAGE STABILITY OF COCONUT FLOUR AND DIETARY FIBRE ISOLATE

MANIKANDAN ARUMUGAM^{*1}, MEERA RAMAN¹, KANNAN EAGAPPAN²

¹Department of Food and Nutrition, RVS college of Arts and Science, Coimbatore, Tamil Nadu, India, ²Department of Clinical Nutrition, PSG college of Arts and Science, Coimbatore, Tamil Nadu, India.
Email: manikandan.nutrition@gmail.com

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ABSTRACT

Objective: The general objective of the study is to formulate high percentage dietary fibre 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 fibre isolate (c) to analyse the storage stability of coconut flour and dietary fibre isolate.

Methods: The coconut fibre isolate was prepared by hydrolysis with CaOH₂ as per the established protocol.

Results: The dietary fibre content of dietary fibre isolation was 72.25% and further it was found to be 42% and 48% in coconut flakes and coconut flour respectively. With respect CaOH₂ to hydrolysis, 0.3M and 0.4M concentrations were found to be very ideal in suppressing the dominant coconut taste. With water holding, retention and swelling capacities, isolate was found to be the best (8.27, 7.42, 21.33 ml water/g samples). According to BIS (Bureau of Indian Standard), the microbial load and peroxide value were within safe limits in isolate (up to 10 months).

Conclusion: Results from the above study can be a basis in the development of dietary fibre isolate as a functional food.

Keywords: Coconut flakes, Coconut flour, Dietary fiber isolate, CaOH₂ hydrolysis, Functional food.

INTRODUCTION

Coconut palms are raised in more than 80 nations of the globe, with a total yield of 61 million tones per year. India is one of the largest coconut producing countries in the universe with a share of 15.90% in area and 21.54% in yield. Although the distribution of coconut is mostly limited to coastal states, its products are in demand throughout the state for various functions. The field of coconut farming in India has been registering a consistent increase over the last 25 years. During the last two decades, there has been a significant expansion of the area under coconut in the eastern and also in north-eastern regions of the country. Kerala, Karnataka, Tamil Nadu and Andhra Pradesh states account for 89% of the total production of coconut in the country [2]. India desiccated coconut is commercially known as "Coconut Powder". Desiccated coconut contains saturated and unsaturated fatty acids and they are highly digestible. The protein present in it has all the amino acids essential for human growth. Sugars are present as sucrose, glucose, and galactose [3]. The natural volatile flavor components of fresh coconut meat and/or oil are mainly lactones [4].

During the wet processing of coconut, fresh coconuts, after shelling and paring, are disintegrated and expressed in a screw press to extract coconut milk, which is either used for the preparation of virgin coconut oil or converted to spray dried coconut milk powder. The defatted coconut residue obtained after milk extraction and drying can be utilized as dietary fibre [5] [6]. Modern nutrition and food science professionals view dietary fibre as essential nutritional components [7]. The interest in foods rich in dietary fibre increased in recent decades and the importance of this food constituent has led to the development of a large market for fibre-rich products and ingredients [8].

A high dietary fibre intake has been related to several physiological and metabolic effects [9]. In the digestive tract, dietary fibre exerts a buffering effect that links excess of acid in the stomach, increases the faecal bulk and stimulates the intestinal evacuation, besides, it provides a favourable environment for the growth of beneficial intestinal flora. It has been reported that these specific properties of dietary fibre play an important role in the prevention and treatment of obesity, atherosclerosis, coronary heart diseases, colorectal cancer and diabetes [10]. Though the above mentioned features of

dietary fibre are attributed to health benefits yet there are less commercially available fibres are existing. Similarly the fibres are available as functional food stuff only and not much as exclusive fibre isolates. As the present study has documented the presence of dietary fibre to a level of 72%, it really made us to incline to study the storage ability and thereby popularize the same for the needy to reap the health benefits out of it. Our earlier publications have also shown that this coconut fibre isolate has significant antidiabetic and lipid lowering property in animal models [11].

The general objective of the study is to formulate highest percentage dietary fibre 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 fibre isolate (c) to analyse the storage stability of coconut flour and dietary fibre isolate.

MATERIALS AND METHODS

Formulation of coconut flour and dietary fibre isolate

Coconut meal was processed by selecting 30 days matured, full fat coconut. The coconut meal was de shelled using a coconut de sheller. Uniformly the coconuts were purchased locally. The coconuts were de shelled 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 a fine thread like structures. The shredded thread structures of coconut meal were treated under a temperature of 55 – 60 ° C in a fluid bed dryer for 30 – 35 minutes. The dehydrated full fat coconut meals were then subjected to cold press at 10- 15 ° C temperature without applying any heat. 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. Usually coconut flakes possess a strong dominant natural coconut aroma which indeed even after any processing, generally it is retained. Hence, it is imperative that such dominant flavor needs to be removed. Only then the resultant end product such as coconut flour or dietary fiber isolate may be suitable for using it as base or main ingredient in any other formulations. Similar to this concept, Usman

et al.,(2003)[1] has documented that thevitia perviana seed, though it is rich in protein and oil but still it is poisonous due to the presence of cardiac glycosides that are bitter. Their preliminary studies here revealed that solvent extraction is not very effective, as the seed cake still retained some of its bitter taste. Hence, they tried hydrolysis process with NaOH, Ca(OH)₂ and HCL and succeeded in removing the strong bitter taste. In this regard, in our study also an effort was taken to mask the strong coconut flavor by solvent extraction however it did not yield a good result, hence similar to the above authors protocol, hydrolysis method was applied which in fact brought up a favourable outcome and thus the strong dominant coconut flavor was removed. It is for this reason, we set out to use preferably the hydrolysis method. Added advantage of using this technology lies in the production of DFI too, because by solvent extraction the fiber isolate can be extracted whereas, hydrolysis process yields this unique high fiber isolate other than removing the stubborn strong coconut aroma.

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). The extracting solvent of food grade hexane (non polar solvent) and acetone (a polar solvent) in the ratio of 60:40 were used. The boiling point of the solvent mixture was 60 – 80 ° C. The samples were withdrawn after 6 hours, and kept in a hot air oven at 55 to 65°C for 3 hours to remove hexane and acetone mixture, and the flakes were ground by multi milling for 1 hour without heat treatment. And thus the coconut flour was obtained and weighed.

Solvent extraction by hydrolysis

Acid 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 HCl solution (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 a water bath and maintained at 75 ° C for an hour, which has been established as an ideal time for hydrolysis in preliminary investigations. Products were then washed with water (Demineralised water), dried in fluid bed dryer for further process.

Alkalinity and Calcium hydroxide 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 NaOH solution (Equivalent to 0/002 mole 0.004 mole, 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.

They were placed in a water bath and maintained at 75 ° C for an hour, which has been established as an ideal time for hydrolysis in preliminary investigations [1]. The Products were then washed with water (Demineralised water), dried in fluid bed dryer for further process. Out of these, only CaOH₂ hydrolysis yielded a good quality dietary fibre isolation without the dominance of coconut aroma.

Storage stability

Coconut flour and dietary fibre isolate were transferred to aluminium foil pack individually and placed in plastic containers intact with the lid. The containers were closed and stored at room temperature in the dark. Periodically (every month) for 10 months a suitable volume of coconut flour and dietary fibre isolate were withdrawn from each container and subjected to determination of total plate count and peroxide value by standard protocol [12,13].

Determination of hydration properties

The hydration properties of the coconut residue were determined as per the following procedure reported by [14,15].

Water holding capacity

Water holding capacity, defined by the quantity of water that is bound to the fibre without the application of some external force (except for gravity and atmospheric pressure), was determined by accurately weighing dry sample (1 g) into a graduated test tube, and adding around 30 ml of water, and it was allowed to hydrate for 18 h

at ambient temperature. The supernatant was removed by passing through a sintered glass crucible (G4) under vacuum. The hydrated residue weight was recorded and it was dried at 105°C for 2 h to obtain the residue dry weight.

$$\text{Water holding capacity (g/g)} = \frac{\text{Residue hydrated weight} - \text{Residue dry weight}}{\text{Residue dry weight}}$$

Water retention capacity

Water retention capacity, defined as the quantity of water that remains bound to the hydrated fiber following the application of an external force (pressure of centrifugation) was determined by accurately weighing dry sample (1 g) into a graduated centrifuge tube, adding 30 ml of water and it was hydrated for 18 h, centrifuged (3000g, 20 min) and the supernatant solution was removed by passing through a sintered glass crucible (G4) under applied vacuum. The hydrated residue weight was recorded and then the sample was dried at 105°C for 2 h to obtain its dry weight.

$$\text{Water retention capacity } \left(\frac{\text{g}}{\text{g}} \right) = \frac{\text{Residue hydrated weight} - \text{Residue dry weight (After centrifugation)}}{\text{Residue weight}}$$

Swelling capacity

Swelling capacity is defined as the ratio of the volume occupied when the sample is immersed in excess of water after equilibration to the actual weight. Accurately weighed dry sample (0.2 g) was placed in a graduated test tube, around 10 ml of water was added and it was hydrated for 18 h, and the final volume attained by the sample was measured.

$$\text{Swelling capacity (ml/g)} = \frac{\text{Volume occupied by sample}}{\text{Original sample weight}}$$

Proximate composition

The moisture, crude fibre, protein, fat, calcium, iron and dietary fibre were analysed out of coconut flour, coconut flakes and dietary fibre isolate by standard methods suggested AOAC, 2005 [13]. However, carbohydrate and energy were analysed according to the method of Gopalan et al. 2001 [16], but available lysine of Pellet and Young, 1980 [17] respectively.

Physico chemical properties

Total ash and acid insoluble ash percentage by weight was analysed by the method suggested [13]. The absence of hexane residue was confirmed by standard procedure using gas chromatography. However, peroxide value of all the three products was determined by AOAC,2005 [13] protocols.

Assessment of microbial quality

Assessment of Aflatoxin (B₁, B₂, G₁ and G₂) was performed in accordance with the procedure of APHA [American Public Health Association] [12]. Whereas total plate count, counting and detection of Salmonella was determined by same [12] methods.

Statistical analysis

All experiments and analytical measurements were run in triplicate except blood tests. Means of each parameter was analysed by analysis of variance (ANOVA) and students' independence't' test. Adhoc tests were also performed for ANOVA. Differences between treatments at the 5% (P≤0. 05) and 1% (P≤0. 01) levels were considered significant.

Table 1: proximate composition of coconut flakes, coconut flour and dietary fibre isolate, whereas, values with 'c' were significantly different at the 1 % level (p≤0. 01). Means ± SD, each value in the table is the mean of three replications

S. No.	Testing parameters	Coconut flakes	Coconut flour	Dietary fiber isolate
1	Moisture (%) by wt	3.71±0.11 ^a	5.4±0.99 ^b	5.1±1.37 ^b
2	Crude fibre (%) by weight.	11.50±1.25 ^a	6.54±1.14 ^b	11.0±1.11 ^a
3	Protein, (%) by wt. (Nx6.25)	23.24±0.22 ^a	23.15±0.01 ^a	14.0±0.02 ^b
4	Fat (%) by wt.	49.34±0.46 ^a	3.2±0.24 ^b	4.0±1.12 ^c
5	Carbohydrate, by difference (%) By wt.	20.60±0.02 ^a	48.89±0.01 ^b	61.2±0.02 ^c
6	Calcium mg/100g	19±0.01	20±0.05 ^a	336±1.10 ^b
7	Iron mg/100g	1.4±0.01	3 ±0.56 ^a	6.86±0.67 ^b
8	Available lysine, g/100g protein	0.1±0.01	0.4±0.2 ^a	6.7±0.2 ^b
12	Dietary fiber, (%) by wt.	42±0.6 ^a	48±0.56 ^b	72.15±0.70 ^c
13	Energy (kcal)	619.62±0.14 ^a	369.70±1.1 ^b	340±2.18 ^c

*a, b Values with different letters in the same row are significantly different (p≤0. 05).

RESULTS AND DISCUSSION

The results of the proximate composition of coconut flakes, coconut flour and dietary fibre isolate, stored under ambient condition are presented in table 1. The dietary fibre for dietary fibre isolate (DFI) was 72.25%, whereas; Coconut Flakes (CF) and Coconut Flour (CFLR) had 42% and 48% respectively. The protein content of flakes and flour was almost similar (23.24% and 23.15%) whereas; the isolate was having only 14%. However, the fat content was high (49.34%) in flakes, but was only 3-4% in flour and isolation. Correspondingly energy value was also two folds more in flakes than the other two, which may be attributed to the high fat content of flakes. The dietary fibre was analysed in all the three samples of coconut flakes, coconut flour and isolation. However, only in coconut dietary fibre isolate, all the three types of dietary fibre namely, Soluble Dietary Fibre (SDF), Insoluble Dietary fibre (IDF) and total dietary fibre were analysed. As it is given in the table now. 1, the

total dietary fibre content of all three varieties of coconut products significantly variance with each other. The isolate had a high total dietary fibre content of 72.15± 0.70 whereas the coconut flakes and flour had a value of 42 ± 0.6 and 48± 0.56 respectively. With regard to dietary fibre [19] had found coconut residue fibre made from coconut gratings to have soluble Dietary fibre of 3.41 ± 0.2, insoluble dietary fibre of 33.97 ± 0.67 and total dietary fibre of 33.38 ± 0.91. Whereas, Lee et al., 1992 [20] documented that of total dietary fibre of 37.51 ± 0.72, insoluble dietary fibre of 35.08± 0.60 and that of soluble dietary fibre of 2.43 ± 0.12 from the coconut residue fibre. But in our study, the coconut dietary fibre isolate was found to have a significantly higher level of total dietary fibre of 72.15 ± 0. 70, insoluble dietary fibre of 68.15 ± 0.20 and soluble dietary fibre of 3.65 ± 0.70. The total dietary fibre, insoluble dietary fibre and soluble dietary fibre of the isolate was substantially higher than 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) [22].

Table 2: Assessment of microbial quality of coconut flakes, coconut flour and dietary fibre isolate

S. No.	Testing parameters	Coconut flakes	Coconut flour	dietary fibre isolate
1	Aflatoxin B1	Below detection Limit 1.0	Below detection Limit 1.0	Below detection Limit 1.0
2	Aflatoxin B2	Below detection Limit 1.0	Below detection Limit 1.0	Below detection Limit 1.0
3	Aflatoxin G1	Below detection Limit 1.0	Below detection Limit 1.0	Below detection Limit 1.0
4	Aflatoxin G2	Below detection Limit 1.0	Below detection Limit 1.0	Below detection Limit 1.0
5	Total Plate Count / g	4500	5200	6100
6	Coliform Count / g	<10	<10	<10
7	Salmonella in 25 gm	Absent	Absent	Absent

In the present study, as an alternative source of solvent hexane, a combination of hexane and acetone at the ratio 60:40 was used. This novel attempt of using the above mixture of the solvent was found to yield a higher quantity of oil and the total processing time taken to yield Dietary fibre isolate was only 6 hrs. Whereas, generally the solvents like hexane alone may consume about 8 - 12 hours. Various authors [23, 25] have reported varying extraction yields with different solvents or seeds.

In the present work, the acetone was chosen as a co-solvent for hexane because not only as polar solvent but it significantly reduces the burden of removing co-extracted water due to azeotrope distillation. Secondly, unlike alcohols, acetone does not form an azeotrope with water, and the heat of vaporization for acetone is about equal to that of n- hexane [26] Hence, the energy cost for the solvent recovery, part of an acetone or an acetone / hexane process, would be comparable the above facts the hexane / acetone mixture in the ratio of 60:40 was employed as the successful extraction solvent.

The results of a study performed by [26] revealed that mixtures of acetone and n-hexane as an extraction solvent helped to produce cottonseed oil without the concomitant development of odorous meals. Though [27] in his study identified that solvent hexane brought up at the highest yield yet he warns in terms of safety, particularly if such oil is not purified. 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 coconut taste. This was similar to a study by Usman et al [1], 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. This implies that at particular concentrations of hydrolyzing solution the characteristic taste principles get diminished or get completely lost.

The fat content of dietary fibre plays an important role in determining hydration properties such as water holding capacity (WHC), water retention capacity (WRC) and swelling capacities (SWC). As coconut flakes in the present work were found to have nearly 50 % fat (Table no 1), it was not considered in assessing hydration properties and table 4 shows water holding capacity, water retention capacity and swelling capacity of coconut flour (7.17, 6.72, 14.35 g water/g samples respectively), and it quietly increased in dietary fibre isolate (8.27, 7.42, 21.33 g water /g sample). The swelling capacity is dependent on the characteristics of individual components and the physical structure (porosity, crystallinity) of the fibre matrix [28, 29].

Table 3: Hydration properties of coconut flour and dietary fibre isolate

S. No.	Parameters	Coconut flour	Dietary fibre isolate
1.	Water holding capacity (WHC) (g water/g sample)	7.17±0.74 ^a	8.27±0.04 ^b
2.	Water retention capacity (WRC) (g water/g sample)	6.72±0.13 ^a	7.42±0.65 ^b
3.	Swelling capacities (SWC) (g water/g sample)	14.35±0.71 ^a	21.33±0.09 ^b

^{ab} Values with different letters in the same column is significantly different ($p \leq 0.05$), Means \pm SD, each value in the table is the mean of three replications.

The swelling capacity of coconut dietary fibre isolate was significantly higher (21.33 ml/g) in comparison with that of coconut flour (14.75 ml/g). However, Ragavendra *et al* [30] observed that SWC of sugar beet and citrus fibre was of the same order. Though the particle size of the fibre matrix was not determined in the present study. Yet, as smaller the particle size tend to have higher packing density, further particle composition and structure will contribute to the overall distribution of water [31] Thus, it may be understood that the isolate in the present study could also possess

high porosity and crystallinity that could have rendered it high appreciable swelling capacity. Hydration properties and the water retention capacity of coconut fibre were higher as compared to the other samples (sugar beet, apple, pea, wheat and carrot). Similarly, water holding and swelling capacities of coconut fibre were higher than that of the soluble dietary fibres [15] [30]. This shows that coconut flour and dietary fibre isolate has a great swelling capacity, which is most desirable for the physical functioning of dietary fibre.

Table 4: Changes in peroxide value (m \pm q O₂ / kg fat) of coconut flour and coconut dietary fibre Isolate at room temperature over 10 months of period

Test food	Month interval / Peroxide value (Meq 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

From the table no 4, it was clearly evident that the peroxide value of coconut dietary fibre 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 fibre isolate was only aluminium foil. In this line, a study conducted by [32] demonstrated that the virgin coconut meal incorporated Sooji Halwa mix withstood peroxidation much in metallised polyester packing than the one packed and stored in polypropylene.

Table 5: 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 Fibre 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

Table no 5 shows the total plate count of the experimental substances. Total plate count of coconut flour increased from 4200 to 57100 per g and dietary fibre 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 fibre isolate.

Stipulation of BIS without treating with any preservatives. Conversely, in accordance with BIS (Bureau of Indian Standard) recommendations, only a total plate count of coconut flour has crossed beyond 50000 max per g at the 8th month but till 10th month dietary fibre isolate could withstand the stipulation of BIS. According to "Microbial Food safety-Indian Regulation" [33] solvent extracted soya flour should contain a total bacterial count of less than 50,000/g, Cali-form bacteria should be less than 10/ g, and salmonella bacteria should be absent in 25 grams of the sample for consumption. Similar to these guidelines, our product isolate satisfied these conditions.

CONCLUSION

In conclusion: (a) Dietary fibre isolate is a rich source of dietary fibre, 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 fibre (72.5%) than any other cereals (b) dietary fibre isolate stored up to 10 months ambient conditions, did not produce any rancid odour and the microbial load was also within the safe limits.

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CONFLICT OF INTEREST

None.

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