

# Utilization of Defatted Coconut Kernel Residue as a Raw Material For Preparation of Fiber-Enriched Biscuits

A.P.D.RUPANJALI<sup>1</sup>, J.M.M.A. JAYASUNDERA<sup>2</sup>, M.N.D. FERNANDOPULLE<sup>1</sup> and J.M.N. MARIKKAR<sup>2</sup>

<sup>1</sup>Department of Plantation Management, Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka, Makandura, Gonawila (NWP).

<sup>2</sup>Coconut Processing Research Division, Coconut Research Institute, Lunuwila, Sri Lanka

## ABSTRACT

The whitish defatted kernel residue left in the virgin coconut oil extraction process has potential application in high protein, fiber enriched food products. In this study, it was attempted to investigate its use as a raw material for biscuit preparation. For this, wheat flour was fortified with ground defatted kernel residue (defatted coconut flour) in varying proportions (w/w, 0, 10, 20, 30, 40, 50, 60, and 70%) to prepare a series of blends for biscuit preparation according to a standard recipe.

A sensory evaluation by a 36-member semi-trained panel was conducted using a 7-point hedonic scale in order to determine the critical limit of wheat flour fortification with defatted coconut flour for good quality biscuits. Results of the sensory evaluation indicated that wheat flour could be substituted up to a level of 40 % with defatted coconut flour as source of dietary fiber without affecting the overall quality. Proximate compositional analysis were conducted to find moisture (%), ash (%), protein (%), fat (%) , crude fiber (%) and soluble sugar (%). The fiber content of biscuits was about 10 times higher than that of control biscuit sample. Palmitic and oleic acid were found to be the predominant fatty acids in biscuit. In vitro protein digestibility of biscuits was a maximum at 20% substitution of defatted coconut flour and it decreased with 30% and 40% due to increased fiber content in the biscuit samples. Biscuits showed a good keeping quality over a period of three months.

**KEY WORDS:** Defatted coconut flour, Fiber- enriched biscuits, In- vitro protein digestibility, Proximate composition, Sensory evaluation, Storability

## INTRODUCTION

Coconut (*Cocos nucifera* L.) has been part of the peoples' diet and livelihoods in the tropical countries of Asia, and Pacific for over a period of thousand years. It has got a wide range of uses to mankind. The food value of coconut is enormous. The whitish kernel is the most valuable component of coconut. It is a wholesome food containing moisture, fat, protein, carbohydrates, minerals and vitamins. Therefore, it is used as a raw material for a range of food industries. Nearly fifty per cent of the coconut kernel is moisture. When dehydrated, the dried kernel is found to contain 68-70 % oil (Salunkhe *et al*, 1992). Therefore, coconut is considered as a food crop as well as an oil crop.

Extraction of oil from coconut is an important industry in many countries. For this, both aqueous and dry extraction processing methods are employed. In the aqueous extraction process, coconut milk is taken out of the kernel, which is subsequently converted into coconut oil. The dry process involves the conversion of coconut meat into copra. Subsequently, copra is cut into pieces in order to be fed into oil expellers for extraction of oil. After the oil extraction, an oil cake is left behind as a by-product. Since the commercial oil expellers are operated under high temperature conditions, the oil cake so produced is burnt and subjected to various chemical changes. As a result its quality is poor and it is not suitable for human consumption. However, the recently introduced technology for virgin coconut oil extraction has many advantages over the traditional method of coconut oil production. In this process, a

special type of micro expeller is used and the raw material is desiccated coconut which is dried under mild temperature conditions. Since the oil expeller operates at mild temperature conditions, the oil cake so produced is not severely burnt or subjected to any thermal degradation. Under these conditions, the defatted kernel residue (DKR) looks whitish in color and its carbohydrates and proteins are preserved for human consumption (Beansch *et al*, 2004a).

According to the past reports, the DKR contained carbohydrates as predominating component (Beansch *et al*, 2004b). Of these carbohydrates, one portion is soluble sugars and the rest is mostly fibrous materials. The fibrous component of the carbohydrates is of major interest in many studies due to its beneficial health effects. Dietary fiber is now defined as the plant material, which is not degraded by the endogenous secretions of the human dietary track. The major beneficial effects of dietary fiber are its bulking capacity, and the water-holding capacity which plays an important role in the prevention of constipation (de Man, 1990).

DKR when ground forms defatted coconut flour (DCF) which can be a substitute to wheat flour (WF) in many food preparations. It is a good source of protein, high in dietary fiber and low in digestible carbohydrates. It contains no gluten so it is ideal for humans with celiac disease. As a source of dietary fiber, coconut flour provides a number of health benefits in relation to coronary artery diseases cancer, diabetes and mineral absorption in the human body. Researchers have indicated that coconut flour may reduce the concentration of cholesterol in

blood (Aroncon, 1999). The study done by Trinidad *et al* (2003) has revealed that coconut flour has an ability to lower the glycemic index of foods, when it is incorporated into foods such as bakery food items. A series of studies have been conducted to utilize DCF.

With the current nutritional trend, people are more concerned about their health and the nutritional status of their diet. Consumers need nutritious and ready- to -eat processed foods with good keeping quality and a satisfying taste (Mbofung *et al*, 2002). Bakery products like biscuits are identified as food items which satisfy these criteria (Chavan and Kadam, 1999). People of all ages, ethnicity and cultures use biscuits as snack products throughout the world.

Due to the nutritional values and various health benefits of the DCF, it can be ideally used for the preparation of fiber-enriched biscuits. Therefore, this study was undertaken to develop fiber-enriched biscuits by incorporating DCF and to determine the proximate composition, in-vitro protein digestibility (IVPD) and the storage stability of the biscuits.

## MATERIALS AND METHODS

### 1. Biscuit Baking

A standard dough formulation, shown in Table 1, was used for biscuit baking. Commercially milled WF was obtained from Prima Ceylon Co Ltd. A sample of DCF was obtained from the virgin coconut oil production facility of the Coconut Research Institute. WF and DCF were weighed separately and mixed in different proportions. A total of eight mixtures (treatments) were prepared, T<sub>0</sub>- 0:100, T<sub>1</sub>- 10:90, T<sub>2</sub>- 20:80, T<sub>3</sub>- 30:70, T<sub>4</sub>- 40:60, T<sub>5</sub>- 50:50, T<sub>6</sub>- 60:40, T<sub>7</sub>- 70:30, (w/w) and identified by the mass ratio of DCF to WF.

The biscuits were prepared by first creaming the margarine, sugar and egg to a smooth whitish soft paste and then mixing in DCF, WF and baking powder with required amount of water. The creaming and mixing were carried out using a mixer. The resulting dough was kneaded gently and rolled out to a thickness of 0.5 cm.

After cutting it into shape of biscuits using a biscuit cutter, they were baked at 180°C for 20 minutes. After taking out of the oven the biscuits were allowed to cool before packing in low-density polyethylene (LDPE) bags. Sample bags were stored at room temperature until sensory evaluation, compositional analysis and shelf-life studies were conducted.

### 2. Sensory Evaluation

Samples were ranked according to the preference of the thirty-six member semi-trained panelists. Panelists were asked to rank the eight coded samples according to the intensity of overall quality (appearance, texture, taste, smell & overall acceptability) of biscuits. A seven point Hedonic scale (1: dislike very much; 2: dislike; 3: dislike slightly; 4: neither like nor dislike; 5: like slightly; 6: like; 7: like very much) was used to evaluate degree of liking for each sensory attribute.

Results of sensory evaluation were analyzed using non-parametric Friedman rank sum test in the Minitab software package version 14.00.

### 3. Proximate Compositional Analysis of Biscuit Samples

Biscuit samples were ground into powder using mortar and pestle. Moisture content was determined using standard oven (Gallenkamp, SANYO Gallenkamp PLC, U.K.) method by drying at 105 °C for 4 hrs and then to constant weight (SLS 251: 1991); Test for fat content was carried out by Soxhlet extraction using petroleum ether (40-60 °C) as solvent (Pearson, 1973); Mineral content determination was done by dry ashing method (Pearson, 1973); Crude protein content by micro Kjeldahl method (AOAC, 1999a); Crude fiber content by Weende method (AOAC, 1999b) and total sugars by phenol sulfuric acid method (Bemiller and Low, 1998).

Analysis of variance was carried out to find out the significant differences among the biscuit samples using Statistical Analysed System (SAS) soft ware package (Anon, 1998a).

**Table 1 - Recipes of Biscuit Formulation with Different Treatments:**

Treatment	Wheat flour (g)	Coconut flour (g)	Margarine (g)	Sugar (g)	Baking Powder (g)	Egg (g)	Water (ml)
T <sub>0</sub>	500	0	250	200	20	80	0
T <sub>1</sub>	450	50	250	200	20	80	0
T <sub>2</sub>	400	100	250	200	20	80	0
T <sub>3</sub>	350	150	250	200	20	80	0
T <sub>4</sub>	300	200	250	200	20	80	100
T <sub>5</sub>	250	250	250	200	20	80	200
T <sub>6</sub>	200	300	250	200	20	80	250
T <sub>7</sub>	150	350	250	200	20	80	300

#### 4. Fatty Acid Composition

The oils extracted from the samples using the Soxhlet method were derivatised to form their fatty acid methyl esters (FAME) and analysed by Gas Liquid Chromatography (PORIM Test Methods, 1995a).

#### 5. *In-Vitro* Protein Digestibility

IVPD was determined using the method of Savoie and Gauthier (1986) modified as follows: A precise weight (0.500g) of finely ground flour sample was suspended in 17ml of 0.1N HCl and incubated for 5 minutes in a shaking water bath at 37°C. The pH of the mixture was then adjusted to 1.9 and the total volume made up to 20ml. The lot was then transferred into a dialysis tubing (Molecular weight cut-off of approximately 1200–Medicell International Ltd London) to which was added 2.5ml of freshly prepared pepsin (2500-32000 units/g obtained from Sigma Chemical Co., St. Louis, Mo.) enzyme solution (7mg/ml). The dialysis tube was then introduced into a beaker (placed in a shaking water bath set at 37°C) containing 200ml of 0.1N HCl (175 ml 0.1N HCl and 25ml distilled water). 100µl of dialysate samples were withdrawn at 0, 30, 60, 90, 120 and 150 minutes and analyzed for protein content by the Lowry *et al.* (1951) method. The digestibility of each sample was calculated.

#### 6. Shelf Life Studies

Analyses for shelf life studies were done once in two weeks for a period of three months. Moisture content was determined using standard oven method (SLS 251: 1991). Oils of biscuit samples were extracted by Soxhlet extraction procedure using petroleum ether (40-60 °C) as solvent (Pearson, 1973). Peroxide Value (PV) of oil was determined by dissolving oil sample (5g) in acetic acid-chloroform solution and titrated with 0.01N sodium thiosulphate in the presence of saturated potassium iodide and starch indicator (PORIM Test Methods, 1995b). Free fatty acid content of oil samples (5g) was determined by dissolving the sample in 95% ethyl alcohol followed by titration with sodium hydroxide (0.1N) using phenolphthalein indicator (AOCS, 1987). Enumeration of aerobic colony count was done by incubating micro-organisms in nutrient agar (NA) medium at 37°C for 48 hours and the yeast and mould count was done by incubating in potato dextrose agar (PDA) medium with 0.01% Chloramphenicol held at room temperature (SLS 516:1991). Analysis of variance was carried out to find out significant differences among different time intervals using Statistical Analysed System (SAS) soft ware package (Anon, 1998b).

### RESULTS AND DISCUSSION

#### 1. Biscuit Formulation and Dough Consistency

In biscuit formulation, the main ingredient is usually WF. According to previous reports, DCF is rich in both soluble and non-soluble carbohydrates

(Beansch *et al.*, 2004c). Therefore, supplementation of WF with DCF could not only increase fiber content, but also it could help reduce the addition of sugar and fat in biscuit formulations.

However, it is of interest to look into the effect of incorporation of DCF on the water requirement to obtain the desired dough quality. According to the formulations in Table 1, the control and the blends up to 30% DCF level were able to give dough with good consistency with the availability of moisture from ingredients in recipe. When substitution increased beyond 30%, the handling of the dough was difficult as it became non-cohesive. Therefore, from 40% DCF substitution and above, the water had to be added. The increase in the water requirement may be due to the increasing amount of non-soluble carbohydrates in the fortified blends (Leelavathi and Rao, 1993).

**Table 2 - Comparison of Dough Weight and Biscuit Weight<sup>1</sup>:**

Treatment	Dough Weight (g)	Biscuit Yield (g)
T <sub>0</sub>	988.2	849.5
T <sub>1</sub>	1008.1	873.1
T <sub>2</sub>	1040.6	881.0
T <sub>3</sub>	1095.5	884.3
T <sub>4</sub>	1155.3	887.3

<sup>1</sup>Each value in the table represents the mean of duplicate analyses

According to the data presented in the Table 2, the increasing DCF substitution caused to increase in dough weight and biscuit yield. Although supplementation of WF with DCF is mainly for the purpose of dietary fiber enrichment, it is necessary to pay attention to the effects on the sensory attributes.

#### 2. Sensory Evaluation

Sensory attributes such as appearance, smell or aroma, taste and texture are good indicators of consumer acceptance of food. Statistical analysis of the sensory evaluation data are presented in Table 3. The Friedman test for the sensory attributes showed that there was no significant difference between the control and the different levels of DCF incorporated samples with respect to appearance and smell. This confirms that the different levels of DCF substitution for WF do not have any direct impact on the appearance and smell of biscuits. However, attributes such as taste, texture, and overall acceptability seems to be very sensitive to the changing composition of the flour component. With regard to taste, texture, and overall acceptability there was no significant difference between the control and the DCF substituted biscuit samples up to the level of 40% substitution. Therefore, it can be confirmed that a 40% incorporation of DCF with WF is possible with regard to biscuit formulations.

**Table 3 - Results of Friedman Test Along with Sum of Ranks of Sensory Attributes of Different Treatments<sup>1</sup>:**

Treatment	Appearance	Texture	Taste	Smell	Overall acceptability
T <sub>0</sub>	164.0 <sup>a</sup>	197.0 <sup>a</sup>	196.0 <sup>a</sup>	154.5 <sup>a</sup>	178.5 <sup>a</sup>
T <sub>1</sub>	170.0 <sup>a</sup>	200.5 <sup>a</sup>	212.0 <sup>a</sup>	169.5 <sup>a</sup>	184.5 <sup>a</sup>
T <sub>2</sub>	160.5 <sup>a</sup>	195.0 <sup>a</sup>	204.0 <sup>a</sup>	195.5 <sup>a</sup>	173.5 <sup>a</sup>
T <sub>3</sub>	185.0 <sup>a</sup>	195.0 <sup>a</sup>	189.0 <sup>a</sup>	174.0 <sup>a</sup>	180.5 <sup>a</sup>
T <sub>4</sub>	192.0 <sup>a</sup>	170.5 <sup>a,b</sup>	176.0 <sup>a,b</sup>	175.0 <sup>a</sup>	158.0 <sup>a,b</sup>
T <sub>5</sub>	136.0 <sup>a</sup>	128.0 <sup>b,c</sup>	111.5 <sup>b</sup>	136.0 <sup>a</sup>	110.0 <sup>b</sup>
T <sub>6</sub>	132.5 <sup>a</sup>	99.5 <sup>b,c</sup>	99.0 <sup>b</sup>	138.0 <sup>a</sup>	100.5 <sup>b</sup>
T <sub>7</sub>	156.0 <sup>a</sup>	110.5 <sup>c</sup>	108.0 <sup>b</sup>	153.5 <sup>a</sup>	102.5 <sup>b</sup>
Significance	ns	***	***	ns	***

<sup>1</sup>Means in the same column bearing different letters are significantly different from each other. Abbreviations: ns, not significant, \*\*\*, p<0.001, N=3

Most of the panelists indicated that, had the present recipe contained little more salt and sugar, the biscuits would have been much tastier and more acceptable. Therefore this suggestion could be included for further improvement of the DCF based biscuit formulation recipe. Based on the sensory evaluation study, it was decided to limit the DCF substitution up to 40% and compositional analyses and storage studies were carried out only for biscuit samples made of flour containing 0, 10, 20, 30 and 40%DCF supplementation.

**3. Proximate Composition of Biscuit Samples**

Proximate composition of biscuit samples prepared with different treatments was compared with the control as shown in Table 4. When compared to the control sample, significant increases were noticed in moisture and ash contents of biscuit samples prepared with WF/DCF blends.

The increase in moisture content may be due to the fact that increasing level of DCF in the formulation would have increased the water holding capacity of biscuit samples. However, these values were within the ranges found in market available samples as indicated in previous reports (Awasthi and Yadav, 2000). Similarly, an appreciable increase in ash content was a positive development.

When compared to the control samples significant increases were also noticed with regard

to fat and protein contents. According to previous reports, DCF is a low-fat but high protein material having about 20 % protein and 6.5% fat on a dry basis (Beansch *et al*, 2004c). Since these values are higher than those reported for commercially milled WF (10.33% and 0.98%) (Anon, 2005), it definitely helps to raise the fat and protein contents of biscuit samples produced from WF/ DCF blends.

Although there was difference in values of soluble sugar contents between the control and DCF-fortified biscuit samples, the difference was statistically non-significant. However, with regard to carbohydrate content a significant decrease was noticed between the control and DCF fortified samples. Energy values show no significant difference between treatments. However, significant increases were noticed between the control and DCF-fortified samples with respect to crude fiber contents.

**4. Fatty Acid Composition**

Margarine and shortening have an important role in bakery products. Choice of oil or fat may vary depending on the type of application and properties required. In this study, margarine was the source of fat and according to the FAME analysis data presented in Table 5, Palmitic and oleic acids were detected as the predominating fatty acids. It looks as the margarine has been formulated with palm oil.

With the supplementation of DCF, the fatty acid

**Table 4-Proximate Composition of biscuit samples out of different treatments (g/ 100 g dry matter basis)<sup>1</sup>:**

Treatment	Moisture	Ash	Protein	Fat	Crude Fiber	Soluble Sugar	Other Carbohydrates (by difference)	Energy (kilojoules /kg)
T <sub>0</sub>	1.72 <sup>a</sup>	1.49 <sup>a</sup>	5.93 <sup>a</sup>	20.82 <sup>a</sup>	0.60 <sup>a</sup>	24.66 <sup>a</sup>	44.77 <sup>a</sup>	2043.61 <sup>a</sup>
T <sub>1</sub>	2.06 <sup>b</sup>	1.67 <sup>b</sup>	8.31 <sup>b</sup>	22.46 <sup>b</sup>	2.50 <sup>b</sup>	27.86 <sup>a</sup>	35.15 <sup>b</sup>	2037.52 <sup>a</sup>
T <sub>2</sub>	2.34 <sup>c</sup>	1.99 <sup>c</sup>	8.99 <sup>b</sup>	22.93 <sup>b</sup>	3.65 <sup>c</sup>	28.35 <sup>a</sup>	31.74 <sup>b</sup>	2018.18 <sup>a</sup>
T <sub>3</sub>	2.58 <sup>c</sup>	2.19 <sup>d</sup>	10.80 <sup>c</sup>	23.70 <sup>c</sup>	5.30 <sup>d</sup>	30.84 <sup>a</sup>	24.60 <sup>b,c</sup>	1599.42 <sup>a</sup>
T <sub>4</sub>	3.02 <sup>d</sup>	2.44 <sup>c</sup>	12.36 <sup>c</sup>	24.90 <sup>d</sup>	6.10 <sup>e</sup>	32.29 <sup>a</sup>	18.88 <sup>c</sup>	1599.76 <sup>a</sup>
P Value	0.0004	<.0001	0.0013	<.0001	<.0001	0.4061	0.0215	0.5448

<sup>1</sup>Means in the same column bearing different letters are significantly different from each other. P value (p≤ 0.05) significantly different

Table 5 - Distribution of Fatty Acids (Area %) of Biscuits out of Different Treatments<sup>1</sup>:

Treatment	Fatty acid							Unknown
	Caprylic (C8:0)	Capric (C10:0)	Lauric (C12:0)	Myristic (C14:0)	Palmitic (C16:0)	Oleic (C18:1)	Linoleic (C18:2)	
T <sub>0</sub>	0.05	0.00	0.35	1.02	55.06	37.66	5.72	0.13
T <sub>1</sub>	0.14	0.16	0.96	1.28	54.63	37.20	5.48	0.14
T <sub>2</sub>	0.22	0.17	1.76	1.61	54.20	36.50	5.44	0.10
T <sub>3</sub>	0.31	0.25	2.69	1.96	53.24	36.05	5.39	0.10
T <sub>4</sub>	0.50	0.38	3.63	2.36	52.19	35.45	5.37	0.10

<sup>1</sup>Each value in the table represents the mean of duplicate analyses.

profile of all the treatments seems to have been affected. This could be attributed to the fact that the increasing level of DCF proportionately increased the coconut oil content of the finished product. The proportion of short and medium chain fatty acids increased from 0.4% in the control to 4.5% in the 40% DCF substitute biscuits sample.

### 5. In Vitro Protein Digestibility

IVPD is an important factor in assessing the nutritional status of a food product. Initially an increase of IVPD was observed with time and it reached an optimum at 60 minutes of digestion and decreased afterwards. IVPD has a close relationship with dietary fiber. The study done by Bilgicli *et al* (2005) had revealed that addition of fibers to biscuit formulations tends to decrease the IVPD of the products. However in this study protein digestibility increased up to 20% substitution of DCF. Protein digestibility decreased beyond 20% of DCF substitution.

### 6. Storability of Biscuit Samples

With time, keeping quality of any food item may be affected and thereby its quality characteristics such as taste, smell, colour, and texture can deteriorate. Initial moisture content, storage temperature and packaging material are the key factors which determine the keeping quality of food materials. In order to monitor the keeping quality of biscuit samples, moisture content, free fatty acid content, and peroxide values are useful parameters. Particularly, moisture absorption is a critical factor which might affect the longer storage stability of biscuits. According to the data presented in Table 6, moisture content of the control and DCF-fortified samples increased slightly. However, during the entire storage period, none of these samples exceeded the 6% limit prescribed by the standardization agencies (SLS 251:1991; BIS 1974).

The changes in free fatty acid content and peroxide values of the biscuit samples during the storage are also shown in Table 6.

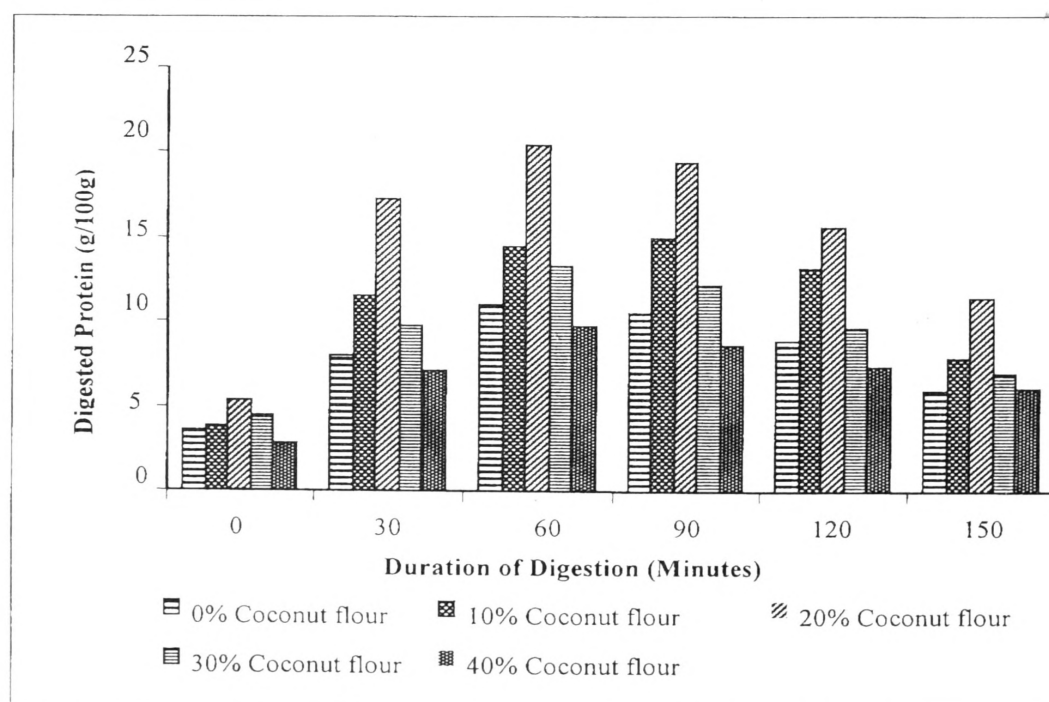


Figure 1- Effect of Incorporation of Different Level of Coconut Flour on the In Vitro Protein Digestibility of Biscuit

**Table 6 - Variation of Moisture Content, Free Fatty Acid Content, and Peroxide Value of Biscuit Samples with Time<sup>1</sup>:**

Treatment	Time (weeks)						
	0	2	4	6	8	10	12
<b>Moisture (%)</b>							
T <sub>0</sub>	1.72 <sup>a</sup>	2.95 <sup>b</sup>	3.18 <sup>c</sup>	3.48 <sup>d</sup>	3.89 <sup>e</sup>	4.61 <sup>f</sup>	5.50 <sup>g</sup>
T <sub>1</sub>	2.06 <sup>a</sup>	2.72 <sup>b</sup>	2.97 <sup>b</sup>	3.65 <sup>c</sup>	4.16 <sup>d</sup>	4.44 <sup>d</sup>	5.43 <sup>e</sup>
T <sub>2</sub>	2.34 <sup>a</sup>	2.58 <sup>b</sup>	2.86 <sup>c</sup>	3.28 <sup>d</sup>	3.71 <sup>e</sup>	3.81 <sup>e</sup>	4.14 <sup>f</sup>
T <sub>3</sub>	2.58 <sup>a</sup>	2.87 <sup>b</sup>	3.21 <sup>c</sup>	3.86 <sup>d</sup>	4.16 <sup>e</sup>	4.88 <sup>f</sup>	5.10 <sup>f</sup>
T <sub>4</sub>	3.02 <sup>a</sup>	3.13 <sup>a</sup>	3.32 <sup>a</sup>	3.87 <sup>b</sup>	4.21 <sup>c</sup>	5.23 <sup>d</sup>	5.64 <sup>e</sup>
<b>Free fatty acids (% palmitic)</b>							
T <sub>0</sub>	0.24 <sup>a</sup>	0.28 <sup>a</sup>	0.42 <sup>b</sup>	0.54 <sup>c</sup>	0.58 <sup>d</sup>	0.64 <sup>e</sup>	0.78 <sup>f</sup>
T <sub>1</sub>	0.26 <sup>a</sup>	0.30 <sup>b</sup>	0.43 <sup>c</sup>	0.58 <sup>d</sup>	0.61 <sup>e</sup>	0.66 <sup>f</sup>	0.79 <sup>g</sup>
T <sub>2</sub>	0.33 <sup>a</sup>	0.34 <sup>a</sup>	0.44 <sup>b</sup>	0.59 <sup>c</sup>	0.62 <sup>c</sup>	0.69 <sup>d</sup>	0.81 <sup>e</sup>
T <sub>3</sub>	0.34 <sup>a</sup>	0.42 <sup>b</sup>	0.50 <sup>c</sup>	0.63 <sup>d</sup>	0.66 <sup>d</sup>	0.75 <sup>e</sup>	0.84 <sup>f</sup>
T <sub>4</sub>	0.34 <sup>a</sup>	0.48 <sup>b</sup>	0.52 <sup>b</sup>	0.64 <sup>c</sup>	0.70 <sup>d</sup>	0.78 <sup>e</sup>	0.86 <sup>f</sup>
<b>Peroxide value (meq/kg)</b>							
T <sub>0</sub>	1.49 <sup>a</sup>	3.00 <sup>b</sup>	4.26 <sup>c</sup>	6.88 <sup>d</sup>	8.56 <sup>e</sup>	9.88 <sup>f</sup>	12.51 <sup>g</sup>
T <sub>1</sub>	1.99 <sup>a</sup>	4.18 <sup>b</sup>	4.30 <sup>b</sup>	7.21 <sup>c</sup>	9.37 <sup>d</sup>	10.90 <sup>e</sup>	11.96 <sup>f</sup>
T <sub>2</sub>	2.99 <sup>a</sup>	4.31 <sup>b</sup>	4.42 <sup>b</sup>	7.76 <sup>c</sup>	9.49 <sup>d</sup>	10.50 <sup>e</sup>	11.46 <sup>f</sup>
T <sub>3</sub>	3.99 <sup>a</sup>	4.68 <sup>b</sup>	5.08 <sup>c</sup>	8.18 <sup>d</sup>	9.78 <sup>e</sup>	11.25 <sup>f</sup>	12.58 <sup>g</sup>
T <sub>4</sub>	4.42 <sup>a</sup>	4.99 <sup>b</sup>	5.31 <sup>c</sup>	8.50 <sup>d</sup>	10.62 <sup>e</sup>	11.02 <sup>f</sup>	12.92 <sup>g</sup>

<sup>1</sup>Each value in the table represents the mean of duplicate analyses. Means in the same column bearing different letters are significantly different from each other

The increases in FFA content of all the biscuit samples were very minute. Similarly, only a slight variation was seen in peroxide values of samples. However, these samples did not show any off flavor or taste during the storage period.

Micro flora usually affects most of the food items and therefore, it is very necessary to monitor

their growth during storage. The data presented in Table 7 shows slight variation of the aerobic plate count and yeast and mould count of biscuit samples during the initial period of three months. However, the variation is within the tolerable limits. The pattern of variation is similar between in the control and the DCF-fortified samples.

**Table 7 - Changes in Aerobic Plate Count and Yeast and Mould Count of Biscuit Samples with Time<sup>1</sup>:**

Treatment	Time (Weeks)						
	0	2	4	6	8	10	12
<b>Aerobic plate count (CFU/g)</b>							
T <sub>0</sub>	4.51×10 <sup>2a</sup>	5.35×10 <sup>3b</sup>	1.05×10 <sup>4c</sup>	1.75×10 <sup>4d</sup>	2.55×10 <sup>4f</sup>	3.10×10 <sup>4f</sup>	3.75×10 <sup>4g</sup>
T <sub>1</sub>	4.30×10 <sup>2a</sup>	5.15×10 <sup>3b</sup>	9.75×10 <sup>3c</sup>	1.60×10 <sup>4d</sup>	2.10×10 <sup>4e</sup>	2.60×10 <sup>4f</sup>	3.25×10 <sup>4g</sup>
T <sub>2</sub>	3.85×10 <sup>2a</sup>	5.15×10 <sup>2b</sup>	9.40×10 <sup>3c</sup>	1.50×10 <sup>4d</sup>	2.20×10 <sup>4e</sup>	2.60×10 <sup>4f</sup>	3.45×10 <sup>4g</sup>
T <sub>3</sub>	3.95×10 <sup>2a</sup>	4.90×10 <sup>3b</sup>	9.45×10 <sup>3c</sup>	1.40×10 <sup>4d</sup>	1.85×10 <sup>4e</sup>	2.40×10 <sup>4f</sup>	3.55×10 <sup>4g</sup>
T <sub>4</sub>	3.90×10 <sup>2a</sup>	4.75×10 <sup>3b</sup>	9.10×10 <sup>3c</sup>	1.25×10 <sup>4d</sup>	1.80×10 <sup>4e</sup>	2.10×10 <sup>4f</sup>	3.05×10 <sup>4g</sup>
<b>Yeast and mould count (CFU/g)</b>							
T <sub>0</sub>	7.85×10 <sup>1a</sup>	1.45×10 <sup>2a,b</sup>	3.05×10 <sup>2b,c</sup>	4.10×10 <sup>2c,d</sup>	5.25×10 <sup>2d</sup>	7.00×10 <sup>2e</sup>	9.55×10 <sup>2f</sup>
T <sub>1</sub>	1.15×10 <sup>2a</sup>	1.85×10 <sup>2a,b</sup>	3.55×10 <sup>2b,c</sup>	4.90×10 <sup>2c</sup>	6.95×10 <sup>2d</sup>	9.20×10 <sup>2e</sup>	1.15×10 <sup>3f</sup>
T <sub>2</sub>	1.45×10 <sup>2a</sup>	2.05×10 <sup>2a</sup>	4.60×10 <sup>2b</sup>	5.30×10 <sup>2b</sup>	7.45×10 <sup>2c</sup>	1.35×10 <sup>3d</sup>	1.45×10 <sup>3d</sup>
T <sub>3</sub>	1.60×10 <sup>2a</sup>	2.25×10 <sup>2a</sup>	3.80×10 <sup>2b</sup>	5.40×10 <sup>2c</sup>	9.45×10 <sup>2d</sup>	1.75×10 <sup>3e</sup>	2.05×10 <sup>3f</sup>
T <sub>4</sub>	1.90×10 <sup>2a</sup>	2.30×10 <sup>2a</sup>	4.25×10 <sup>2b</sup>	5.40×10 <sup>2b</sup>	1.05×10 <sup>3c</sup>	2.05×10 <sup>3d</sup>	2.45×10 <sup>3e</sup>

<sup>1</sup>Each value in the table represents the mean of duplicate analyses. Means in the same column bearing different letters are significantly different from each other. Abbreviation: CFU, Colony Forming Units

## CONCLUSION

From the results of this investigation it is concluded that DCF can be used in making fiber enriched biscuits up to a level of 40% without affecting the sensory quality adversely and the stability of biscuits for three months under ambient conditions. Although the protein digestibility on biscuits decreased beyond 20% DCF substitution, the dietary fibers in DCF plays an important role in providing health benefits and encourages the industry to produce value added products. Therefore further investigations are needed to increase level of substitution of DCF, to improve sensory quality and to extend storability on biscuits.

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