EFFECT OF COCONUT TESTA FLOUR ON COOKIE CHARACTERISTICS

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ABSTRACT

A study was conducted to assess the effect of coconut testa flour (CTF) on quality characteristics of cookies by varying the proportion of CTF in wheat flour from 10 to 60% (w/w). Cookies samples prepared according to a standard recipe were evaluated by proximate composition, hardness, amylose content, and shelf-life stability. A 30 members sensory panel was employed to determine the critical limit of CTF fortification for acceptable quality cookies. Results showed that CTF substitution up to 30% was possible without affecting the overall acceptability of cookies. Keeping quality of the cookies remains within the acceptable range throughout three-month storage period.

Keywords: coconut testa flour, cookies, functional properties, storability, wheat flour

1. INTRODUCTION

Proper use of agriculture byproducts is an integral part of the sustainable agriculture development. The trend in the world today is to convert byproducts into useful products through the manipulation of microorganisms or recycle them as much as possible. Coconut testa, for instance, is a byproduct generated on a daily basis by coconut processing industries such as desiccated coconut, virgin coconut oil and milk powder. Previous studies showed that coconut testa constitutes approximately 18% (w/w, wet basis) of the total weight of the whole coconut endosperm (MARIKKAR and MADURAPPERUMA, 2012). By using the statistics provided by PERERA *et al* (2014), it can be estimated that around 30,000 kg of coconut testa could be generated out of 100,000 nuts of Sri Lankan tall variety. As the removed testa is either wasted or under-utilized by the processing factories without any value addition, it is timely to undertake research studies to use them in food applications. This might have various direct and indirect environmental and economic impacts reducing the disposal costs and increasing the added value of the final products, giving emphasis to explore their health benefits. Recently, ADEKOLA *et al* (2017) suggested that testa of coconut can be used to produce a flour to supplement wheat flour to prepare foods such as cookies with low-glycemic index.

According to KLUNKLIN and SAVAGE (2018), cookies are hardly regarded as healthy snack because of their high levels of rapidly digestible carbohydrate, high fat content, low levels of fiber and only modest amounts of protein. Nevertheless, the nutritional value of these product is considered low due to the nutritional composition of refined wheat flour composition. In this background, in recent years, a large variety of vegetable flour products derived from legumes, oilseed, tubers, corn, rice, etc., have been evaluated in cookies formulation in the attempt to produce nutritionally fiber and/or protein enriched products (ZUCCO *et al*., 2011; CHUNG *et al*., 2014; PACIULLI *et al*., 2018; DIAZ *et al*., 2019).

As of date, supplementation of wheat flour with defatted coconut testa flour in cookie formulation has been scantily investigated. Hence, in this study, the effect of partial substitution of wheat flour by coconut testa flour on the proximate composition, storage stability and consumer acceptance of cookies has been investigated.

2. MATERIALS AND METHODS

2.1. Materials

Wheat flour, coconut testa flour, salted butter, icing sugar, fresh milk, potable water and soybean oil, baking powder, distilled water were used for cookie dough preparation. Samples of commercial wheat flour were obtained from Serendib Flour Mills (Pvt) Ltd and samples of partially-defatted coconut testa flour (CTF) of Sri Lankan commercial variety were supplied as a generous gift by the coconut research institute, Lunuwila, Sri Lanka. Other ingredients namely, table salt, fresh milk, soybean oil, and baking powder were obtained from Puttlam Salt Ltd, Ambewela Milk Ltd, Lanka Soy (Pvt) Ltd and Cargills Food City (Pvt) Ltd, respectively.

2.2. Chemicals

Agar culture media, potato dextrose agar were obtained from Himedia (Pennsylvania, USA); petroleum ether, concentrated sulfuric acid, potato starch, sodium hydroxide , hydrochloric acid, boric acid were purchased from Merck (Darmstadt, Germany); dimethyl sulfoxide, 3,5-dinitrosalicylic acid, sodium potassium tatrate, bromocresol, methyl red, ethanol, methanol, iodine reagent, deionized water, sodium chloride, monosodium phosphate and disodium phosphate were obtained from Fluka (Buchs, Switzerland).

2.3. Functional properties of flours

2.3.1 Water and oil absorption capacity

The water and oil absorption capacities were determined by the method of SOSULSKI *et al.* (1976). Flour samples (one gram, in triplicate) were mixed with 10 ml distilled water and 10 ml of soybean oil separately in 2 centrifuge tubes. Samples were kept at room temperature for 30 min and centrifuged with Eppendorf 8510R centrifuge (Eppendorf, Germany) at 3000 rpm for 30min. The volume of free water and free oil were read directly from the centrifuge tube. The water absorption and oil absorption were examined as percent water-bound per gram of flour and percent oil-bound per gram of flour, respectively.

2.3.2 Bulk density

Bulk density of flour sample was examined using the method described by JONES *et al.* (2000) with slight modifications. Flour samples (ten grams, in triplicate) were measured in a 50 mL measuring cylinder. The cylinder was tapped on a wooden plank until no visible decrease in volume was noticed and volume of flour sample was recorded. The bulk density was calculated using the following formula:
Bulk Density (g/cc) = Weight of sample / Volume of sample

2.3.3 Swelling capacity

The method of OKAKA and POTTER (1977) with some modifications was used for determining the swelling capacity. Flour samples (one gram, in triplicate) were filled up to 10 ml mark in a 100 ml graduated cylinder was added with water to adjust total volume to 50 ml. The top of the cylinder was tightly covered and mixed by inverting the cylinder. The suspension was inverted again after 2 min and allowed to stand for further 30 min. The volume occupied by the sample was taken after 30 min.

2.3.4 Emulsion activity

The emulsion activity and stability were followed using the method of YASUMATSU *et al.* (1972). Emulsion was prepared in calibrated centrifuged tube by adding flour samples (one gram, in triplicate) into 10 ml distilled water and 10ml of soybean oil. The emulsion was centrifuged at 3000 rpm for 5 min. The emulsion activity was estimated using following equation:

$$\text{Emulsion activity } (\%) = (\text{Height of emulsion layer} / \text{Total height of mixture}) \times 100$$

2.3.5 Least gelation concentration

Least gelation concentration was evaluated using the method of COFFMAN and GARCIA (1977) with slight modification. Flour samples [2 to 30% (w/v), in triplicate] were mixed with 5 ml distilled water and heated at 90°C for 1 h in a hot-water bath. After allowing it to cool under tap water, it was kept at 4 °C for 2 hrs. The least gelation concentration was determined at the concentration when the sample from inverted tube did not slip.

2.4. Composite flour preparation

Composite flour samples were prepared according to the following mixing ratio: $T_{0,}$ 100% WF; $T_{1,}$ WF supplemented with 10% CTF; $T_{2,}$ WF supplemented with 20% CTF; $T_{3,}$ WF supplemented with 30% CTF; $T_{4,}$ WF supplemented with 40% CTF; $T_{5,}$ WF supplemented with 50% CTF; $T_{6,}$ WF supplemented with 60% CTF.

2.5. Cookies preparation

Formulation of cookies was carried out according to a method described in AACC with slight modification (SCIARINI *et al.*, 2013). Cookies were baked in three batches, each with 60 cookies. Initially, salted butter (30g) was creamed with icing sugar (40 g), and baking powder (2 g) for 3 min at low speed in a mixer (the bowl will be scraped every minute). Water was added thereafter in varying proportions to make the required consistency of dough. Mixing was continued using Kenwood mixer (Model KM200, Korea) for 2 min at a high speed. Finally, 100g of flour was added and mixed for 2 min at low speed while scraping the bowl at every 30 sec. The dough was allowed to rest for 10 min before rolling-on and cutting (62 mm diameter, 5.5 mm thick). The cut dough pieces were then baked for 25 min in an oven (BIOBASE forced air drying oven, China) pre-heated at 150 °C. The cooled cookies were then left at 25 °C for 30 min and packed in sealed plastic bags until further analysis.

2.6. Sensory evaluation

This is a ranking test, which is aimed to rank cookie samples according to the preference of a thirty-member semi-trained panelists. Panelists were asked to rank seven coded samples according to the intensity of sensory attributes namely color, smell, taste, texture and 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.

2.7. Proximate compositional analysis

For sampling under each treatment category, 7 cookies were selected randomly from each of the three batches. Moisture, crude fat, crude protein and ash contents of coconut testa flour were determined according to methods described in AOAC (2005) manual. The carbohydrate content of the flour was calculated by difference [100- (crude protein + crude fat + ash + moisture+ crude fiber)].

2.8. Determination of amylose content

Amylose content was determined according to the iodimetric method described by JULIANO *et al.* (1968) with slight modification. Iodine reagent was prepared dissolving 1.0 g of iodine and 10 g potassium iodide in water and the volume was made up to 500 ml. A series of standard amylose solutions was prepared dissolving appropriate amounts of amylose in 10 ml of 1.0 N solution of sodium hydroxide and made up to100 ml with water. Powdered cookie sample of 100 mg each from T_0, T_1, T_2, and T_3 was weighed accurately, and dissolved in 1.0 mL 95% ethanol and 10 mL of 1.0 N sodium hydroxide and heated for 10 min in boiling water bath. The solution was made up to 100 ml in a volumetric flask. An aliquot (2.5 ml) of this solution was then added with 20 ml distilled water and three drops of phenolphthalein. Subsequently, 0.1 N hydrochloric was added dropwise until the pink color just disappear. An aliquot (1 ml) of iodine reagent was added into this and the volume was made up to 50 ml. The resulting color was left for 20 min to fully develop before the absorbance reading was monitored at 620 nm with a Perkin-Elmer Lambda 3B double beam UV/Visible spectrophotometer. Iodine solution of same concentration as above but without amylose was used as reference cell. Concentration of amylose in individual cookie sample was calculated using the standard curve.

2.9. Hardness test

For sampling under each treatment category, three cookies were selected randomly from each of the three batches. Hardness of all samples was carried out using Brookfield CT3 texture analyzer using the following test criteria was applied in this test. Test Type: TPA; Target: 4.0mm; Hold Time: 0s; Trigger Load: 15.0g; Test Speed: 0.50mm/s; Return Speed: 0.5mm/s. The value for hardness was derived from the automatic calculation from texture profile analysis curve.

2.10. Shelf life studies

For storability test, biscuit samples containing 5 cookies were packed under hygienic condition in clean packages and sealed immediately to protect product quality. Low-density polyethylene (LDPE) bags were used for packaging. During shelf life study, sample bags were kept at room temperature (27°C and RH 60%) until all analyses were performed. The shelf life of cookies was evaluated based on moisture content and the microbial count (total plate count and yeast and mould count) at once in every two weeks' time continuously for three months.

2.11. Microbiological analysis

Enumeration of aerobic colony count was done by incubating microorganisms in nutrient agar (NA) medium under 37°C for 48 hours; 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).

2.12. Statistical analysis

Samples were selected randomly from three batches. All measurements were carried out in triplicate data (n=3). All the results were presented in the form of mean ± standard deviation (SD). Data were statistically analyzed by one-way analysis of variance (ANOVA) with using Tukey's Test of MINITAB (version 15) statistical package at 0.05 probability level. Results of sensory evaluation results were analyzed using non-parametric Friedman rank sum test using the Minitab software package version 14.00 (SAS 1998).

3. RESULTS AND DISCUSSION

3.1. Functional properties of flours

The functional properties of cereal and non-cereal flours are known to influence the quality attributes of finished bakery products. A comparative analysis of the physicochemical and functional properties of wheat and coconut testa flours would therefore be beneficial. According to the data presented in Table 1, both of these displayed significant ($p<0.05$) differences in all of the properties studied. The swelling capacity, which is related to the irregular positioning of polymer in granules and small size of starch granules (WONG and LELIEVRE, 1982) was higher for CTF (30±0.01) than WF (20±0.01). SURESH and SAMSHER (2013) stated that varietal differences, particle size differences, and methods of processing are other factors that may influence the swelling capacity of flour. In an earlier report, CHANDRA *et al.* (2015) noticed that the swelling capacity of composite flours increased with increasing level of incorporation of rice, green gram and potato flour into wheat flour. From the results of this study, it can be predicted that a composite flour made by mixing WF with CTF would gain a higher swelling capacity with reference to the control.

According to Table 1, the bulk density of CTF (0.66±0.01) was slightly higher than that of WF (0.49±0.01). The bulk density (g/cm^3) of flour is defined as the density measured without the influence of any compression (CHANDRA *et al.*, 2015). According to LAM *et al.* (2008), bulk density of flour samples depends on particle size, density of individual particle, moisture as well as surface characteristics, which are generally influenced by the method of preparation. Undoubtedly, the preparation methods of CTF and WF were not similar as one which was cereal based while the other was non-cereal-based. According to SURESH and SAMSHER (2013), the high bulk density of flour suggests its suitability for multiple uses in food preparations. Water absorption capacity of WF and CTF were 65±0.0% and 75±0.0%, respectively. BERTON *et al.* (2002) stated that water absorption capacity is associated with protein and starch contents of flour. However, the particle size reduction and high content of fiber would increase the water absorption capacity of flour (SURESH and SAMSHER, 2013; CHANDRASHEKER and DESIKACHAR, 1984). Our preliminary investigation too confirmed that CTF was a rich source of protein and crude fiber, which were relatively higher than those of wheat flour (Table 1).

As the capacity of protein to enhance the formation and stabilization of emulsions is important for many applications in food products, information on emulsion activity of flour is beneficial (CAUVAIN and YOUNG, 2006). In the present study, emulsion activity of CTF was lower (25±0.00) than WF (50±0.00). According to previous researchers, the emulsion activity of flour would increase with higher amounts of soluble proteins (GABRA and KAUR, 2014; YASUMATSU *et al.*, 1972) and reduce with fiber content (YASUMATSU *et al.*, 1972). The higher crude fiber content as noticed before in CTF could be a probable reason for lower emulsion activity displayed by CTF. When considering least gelation concentration of the two flour samples, WF showed lower amount (8%, w/v) while CTF displayed a higher value (18%, w/v). This means that WF would form gel quickly at lower concentration than CTF. In an earlier report, AKINTAYO *et al*. (1999) stated that lowest gelation concentration increased with the gelation of protein.

Table 1. Comparative functional and nutritional properties of coconut testa flour (CTF) and wheat flour (WF).

Functional/Nutritional properties	Flour	
	CTF	WF
Swelling capacity (ml)	30^{b}±0.01	20^{a}±0.01
Water absorption capacity (%)	75^{b}±0.00	65^{a}±0.00
Oil absorption capacity (%)	52.7^{a}±2.52	58.50^{b}±1.32
Emulsion activity (%)	25^{a}±0.00	50^{b}±0.00
Least gelation concentration(%,w/v)	18^{b}±0.00	8^{a}±0.00
Bulk density(g/cc)	0.66^{b}±0.01	0.49^{a}±0.01
Moisture (%)	2.27±0.42*	14.0±0.03†
Ash (%)	4.50±0.14*	1.82±0.29 †
Protein (%)	23.82±0.99*	11.68±0.11 †
Fat (%)	10.17±1.84*	1.91±0.09 †
Total carbohydrate (by difference)	59.24*	70.59 †

Each data in the table represents mean of triplicate analysis. Means in the same raw bearing different superscripts are significantly different (p<0.05) from each other. *Marasinghe *et al.* (2019) and †Majzoobi *et al.* (2011).

The oil absorption capacity is an indicator of the suitability of the flour in facilitating enhancement of flavor and mouth feel in food preparations. The oil absorption capacity was higher for WF (58.50±1.32) than CTF (52.7±2.52). According to CHANDRA *et al.* (2015), the presence of high fat content in flours might affect adversely their oil absorption capacity. In this study, CTF having a lower oil absorption capacity was reasonable because our earlier investigation showed that the fat content of CTF was relatively higher (10.17 %) than those of WF (Table 1) (MAIZOOBI *et al*., 2011). SURESH and SAMSHER (2013) commented that amino acid composition, protein conformation, surface polarity and hydrophobicity are other factors that may exert influence on the oil-binding capacity of flour.

3.2. Sensory attribute evaluation

Flour is the main ingredient in any biscuit formulations. There have been a number of attempts to improve the nutritional qualities of biscuits by partially substituting WF with non-wheat flour ingredients (KLUNKLIN and SAVAGE, 2018). Replacing WF with other types of flour would most likely affect the nutritional values as well as the organoleptic characteristics of biscuits (HOODA and JOOD, 2005). In order to determine the critical limit of fortification of CTF in biscuit formulation, a proper method of sensory evaluation and statistics are necessary. According to Table 2, the preference of the panelists for color attribute sharply dropped after substitution of 10% CTF due to unappealing off-white grainy appearance; but the score values for T_2 and above were tended to increase due to brown color development caused by higher amounts of CTF. Apart from Maillard reaction, the mild brown color of CTF could also be a contributory factor for brown color development in biscuits. This has caused the score values of colour to go up with further substitution. There was, however, no significant difference between the control and the different levels of CTF substitution with respect to smell characteristics (Table 2). Other attributes such as taste, texture, and overall acceptability were more sensitive to the changing content of CTF in the formulation. With regard to these three sensory attributes, there was no significant difference between the control and the CTF substituted biscuit samples up to the level of 30% substitution (Table 2). According to the panelists, a slight bitter taste and undesirable mouth-feel were noticed in biscuit samples exceeding 30% CTF level. Therefore, CTF substitution up to 30% would be maximum permissible limit for good quality cookies. Hence, the proximate compositional analyses and storage studies were performed only for biscuit samples made out of composite flour containing 0, 10, 20 and 30% CTF supplementation.

Table 2. Results of Friedman test along with sum of ranks of sensory attributes of different treatments.

Treatments	Color	Smell	Taste	Texture	Overall acceptability
T_0	158.0^{e}	128.0^{a}	152.0^{c}	161.0^{c}	166.5^{c}
T_1	86.5^{a}	126.5^{a}	153.5^{c}	156.5^{c}	151.0^{c}
T_2	103.5^{b}	107.5^{a}	136.5^{c}	150.0^{c}	148.0^{c}
T_3	110.0^{b}	112.0^{a}	141.5^{c}	139.5^{c}	132.5^{bc}
T_4	123.0^{c}	116.5^{a}	101.0^{b}	105.5^{b}	111.5^{b}
T_5	126.5^{c}	116.5^{a}	78.5^{a}	67.5^{a}	77.0^{a}
T_6	132.0^{d}	133.0^{a}	77.0^{a}	60.0^{a}	53.5^{a}
CL	***	ns	***	***	***

Means in the same column bearing different superscripts are significantly different from each other. Abbreviations: CL, confidence level; ns, not significant; ***p<0.0001. T_0, biscuit formulation by wheat flour; T_1, biscuit formulation by wheat flour mixed with 10% coconut testa flour; T_2, biscuit formulation by wheat flour mixed with 20% coconut testa flour; T_3, biscuit formulation by wheat mixed with 30% coconut testa flour; T_4, biscuit formulation by wheat flour mixed with 40% coconut testa flour; T_5, biscuit formulation by wheat flour mixed with 50% coconut testa flour; T_4, biscuit formulation by wheat flour mixed with 60% coconut testa flour.

3.3. Proximate composition of biscuits

Proximate compositions of cookies samples displaying good overall acceptability were compared with those of control sample as shown in Table 3. Generally, cookies available in the market are generally prepared from wheat flour, which lacks dietary fibre contents and good quality protein due to deficiency in lysine. Our preliminary investigation confirmed that composite flour preparation using CTF would be beneficial because CTF was found to be rich in protein (Table 1) as well as crude fiber (Table 3). In a previous study, APPAIAH *et al.* (2014) also indicated that testa of copra was a rich source of protein and crude fiber; it contained about 9.3% of protein and 11.6 % of crude fiber. When compared with the control, cookies prepared with composite flour showed significant increase ($p<0.001$) on the moisture, protein and crude fiber contents, but with regard to ash and fat contents of cookies, there was no significant ($p>0.05$) difference among all substitution levels (Table 3). This indicated that substitution of WF with CTF did not have any direct impact on both ash and fat contents of the finished products. As previously noticed in Table 1, water absorption capacity of CTF was higher than WF due to high fiber content of CTF. This necessitated an extra amount of water to be added to maintain better consistency of the dough at higher level of substitution of CTF. With regard to total carbohydrate content of cookies, there was a significant ($p<0.001$) decline along with the rising substitution level of CTF.

Table 3. Proximate composition of cookie samples made out of different treatments (g/ 100 g dry matter basis).

Treatment	Moisture	Ash	Protein	Fat	Crude Fiber	Other Carbohydrates (by difference)
T_0	0.79^a±0.04	1.11^a±0.15	5.59^a±0.50	14.28^a±0.41	7.90^a±0.14	70.32^d± 0.12
T_1	0.95^b±0.04	1.17^a±0.23	7.43^b±0.15	14.59^a±0.23	8.95^b±0.07	66.91^c±0.58
T_2	1.24^c±0.02	1.33^a±0.00	9.63^c±0.18	14.99^a±0.47	11.25^c±0.5	61.56^b±0.75
T_3	1.48^d±0.02	1.50^a±0.23	11.79^d±0.60	15.54^a±0.16	15.85^d±0.1	53.84^a±0.33
CL	***	ns	***	ns	***	***

Each data in the table represents mean of triplicate analysis. Means in the same column bearing different letters are significantly different from each other. Abbreviations: ns, not significant; ***($p<0.0001$). For other abbreviations, see Table 2.

3.4. Amylose content and hardness

The data presented in Table 4 compares the amylose content of CTF substituted cookie samples with that of the control sample. Amylose being a linear polymer contribute important characteristics to starch present in cereals, and hence it can affect the physicochemical properties of the finished products (MAJZOOBI *et al.*, 2011). The control showed the highest amylose content (27.4 %), which fell within the range of amylose content of wheat flour of different cultivars (20 to 30 %) reported in other studies (MAJZOOBI *et al.*, 2011). The amylose contents of T_1, T_2, and T_3 were tended to decline with the decrease of wheat flour component in the composite. In terms of texture, cookies made out of 100% wheat flour was seemed to be the hardest (14.86 N) and the hardness of T_1, T_2, and T_3 were found to decline, with the cookie sample T_3 which represented CTF substituted cookies at 30% level displaying the lowest value of 8.27 N. Generally, the hardness of cookie is strongly influenced by the development of gluten network whereby gluten promotes the network development by attracting water molecules from the mixture. BARAK *et al.* (2013) previously pointed out that WF having a higher gluten-content would influence the hardness of cookies. The composite flour made out of CTF substitution would contain lesser amount of gluten, which leads to the decline in the harness of the cookies.

Table 4. Variation of amylose content and harness of cookies[1].

Treatment	Amylose content (%)	Hardness (N)
T_0	$27.40^c \pm 0.79$	$14.86^d \pm 0.01$
T_1	$22.77^b \pm 0.50$	$13.42^c \pm 0.02$
T_2	$16.50^a \pm 0.75$	$11.63^b \pm 0.01$
T_3	$15.10^a \pm 0.26$	$8.27^a \pm 0.01$

[1]Each data in the table represents mean of triplicate analysis. Means in the same column bearing different superscripts are significantly different ($p<0.05$) from each other. For abbreviations, see Table 2.

3.5. Shelf-life stability

Moisture and microbiological stability are important attributes to test the shelf-life stability of products. When considering keeping quality of food materials, the initial moisture content, storage temperature, microbiological quality and packaging materials are important determining factors. In this study, keeping quality of cookies samples are tested in terms of moisture content and microbial growth along the storage period of three months. Generally, moisture absorption would not only influence microbiological stability but also textural properties such as hardness. CAUVAIN and YOUNG (2006) stated that moisture content of freshly produced cookies should not exceed the limit of 5% while other standards specified that the moisture content of cookies should be less than 6% during storage period (SLS 251:1991; BIS 1974). According to data presented in Table 5, moisture content of samples significantly increased with storage period but none of them exceeded the upper limit of moisture content throughout the three months of storage period.

Table 5. Changes in the moisture content of cookie samples with time.

Treatment	Time (in weeks)						
	W0	W2	W4	W6	W8	W10	W12
T_0	$0.79^a \pm 0.04$	$0.88^a \pm 0.04$	$1.04^b \pm 0.06$	$1.07^b \pm 0.05$	$1.88^c \pm 0.05$	$2.17^d \pm 0.04$	$2.98^e \pm 0.09$
T_1	$0.94^a \pm 0.04$	$1.22^b \pm 0.03$	$1.35^c \pm 0.04$	$1.49^c \pm 0.01$	$2.07^d \pm 0.1$	$2.59^e \pm 0.04$	$3.20^f \pm 0.03$
T_2	$1.25^a \pm 0.03$	$1.40^b \pm 0.02$	$1.57^b \pm 0.01$	$1.87^c \pm 0.05$	$2.43^d \pm 0.08$	$3.11^e \pm 0.09$	$3.41^f \pm 0.07$
T_3	$1.47^a \pm 0.03$	$1.65^b \pm 0.02$	$1.60^b \pm 0.03$	$2.22^c \pm 0.16$	$2.74^d \pm 0.06$	$3.34^e \pm 0.08$	$3.97^f \pm 0.05$

Each value in the table represents the mean of triplicate analyses. Means in the same row bearing different superscripts are significantly ($p<0.05$) different from each other. Abbreviations: W0, week0; W2, week2; W4, week4; W6, week6; W8, week8; W10, week10; W12, week12. For abbreviations, see Table 2.

The observed gain in moisture content might be due to high water permeability of LDPE as well as the high-humidity conditions of the storage. Previously, SIRIPATRAWAN (2009) stated that there was a moisture migration between the atmosphere and the packaged low-moisture food product such as rice crackers through packaging material during storage until the food reaches equilibrium with the environment. He noticed that the moisture barrier performance of packaging materials was dependent on permeability coefficient; rice crackers packaged in polypropylene pouches had higher shelf-life values than those in polyethylene pouches because polypropylene had lower permeability coefficient.
In this study, the changes in total plate count and yeast and mold count through the three-month storage period were compared with initial amount as shown in Table 6. According to WHO standard (WHO, 1994), total plate count and yeast and mould count of biscuit samples should not exceed 2.0×10^5 cfu g-1 and 1.0×10^4 cfu g-1, respectively. Although both microbial counts of all samples were found to increase with storage period, none of them exceeded the upper limit along the three-month storage period. This clearly showed that microbiological quality of cookies remains within the safe limit throughout the three-month storage period.

Table 6. Changes in total plate count, yeast and mold count of cookies samples with time.

Treatment	Time (in weeks)			
	W0	W4	W8	W12
	Total plate count (CFU/g)			
T_0	$(1.30^a\pm0.07)\times10^3$	$(1.42^a\pm0.09)\times10^3$	$(2.08^b\pm0.03)\times10^3$	$(2.53^c\pm0.05)\times10^3$
T_1	$(1.47^a\pm0.04)\times10^3$	$(1.57^a\pm0.05)\times10^3$	$(2.18^b\pm0.03)\times10^3$	$(2.97^c\pm0.06)\times10^3$
T_2	$(1.57^a\pm0.05)\times10^3$	$(1.70^a\pm0.02)\times10^3$	$(2.46^b\pm0.04)\times10^3$	$(4.46^c\pm0.15)\times10^3$
T_3	$(1.67^a\pm0.05)\times10^3$	$(2.05^b\pm0.04)\times10^3$	$(2.97^c\pm0.04)\times10^3$	$(5.53^d\pm0.15)\times10^3$
	Yeast and mould count (CFU/g)			
T_0	$(2.23^a\pm0.2)\times10^2$	$(2.67^{ab}\pm0.15)\times10^2$	$(3.13^b\pm0.12)\times10^2$	$(6.27^c\pm0.25)\times10^2$
T_1	$(2.30^a\pm0.26)\times10^2$	$(3.03^b\pm0.15)\times10^2$	$(3.50^b\pm0.10)\times10^2$	$(7.50^c\pm0.30)\times10^2$
T_2	$(2.70^a\pm0.10)\times10^2$	$(3.40^b\pm0.10)\times10^2$	$(4.30^c\pm0.41)\times10^2$	$(8.30^d\pm0.25)\times10^2$
T_3	$(3.03^a\pm0.15)\times10^2$	$(4.03^b\pm0.20)\times10^2$	$(5.23^c\pm0.20)\times10^2$	$(9.10^d\pm0.10)\times10^2$

Each value in the table represents the mean of triplicate analyses. [1]Means in the same row bearing different superscripts are significantly (p<0.05) different from each other. Abbreviations: W0, week0; W4, week4; W8, week8; W12, week12; For other abbreviations see Table 2.

4. CONCLUSIONS

This study attempted to evaluate the suitability of CTF as a partial substitute for WF in cookies formulation. Sensory evaluation showed that CTF substitution up to 30% was possible without affecting the overall acceptability of cookies. According to proximate analysis, cookies partially substituted with CTF could have enhanced levels of protein and crude fiber contents. The effect of substation on hardness of cookies was positively correlated with amylose content of composite flour; this could also be due to lowering of gluten network formation, which usually brings hardness to cookies. Shelf-life studies showed that moisture content and microbiological quality parameters of cookies would remain within the safe limit throughout the three-month storage period. The findings of this study could eventually help enhance the economic value of coconut testa generated as byproduct by the coconut processing industries in Sri Lanka.

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