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ORIGINAL RESEARCH PAPER

PHYSICOCHEMICAL AND MICROBIAL PROPERTIES OF GLUTEN-FREE SAPAL FLOUR

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Abstract: Gluten constitutes protein, essential for obtaining the elasticity and mouldable structure of food products. However, gluten intolerant (GIP) victims are recommended to abstain from gluten intake, indicating that they must focus solely on gluten-free diets. As a result, this affects their level of dietary fiber consumption that is vital for intestinal health. Hence, this study was initiated to explore Left-Over Sapal (LOS) potential usage to produce a gluten-free alternative flour high in dietary fiber. Series of analyses were conducted on the LOS, encompassing physical analysis, peroxide value, and microbial analysis. All analyses were compared with the control sample (wheat flour). The results from physical analysis showcased 20 % better water activity as compared to the control sample. Milling yield value of 51.43 % and color analysis results of 65.94 ± 0.04 (L*), 9.67 ± 0.02 (a*), and 12.25 ± 0.01 (b*) were obtained. The peroxide values manifested were all below 18.9 meq·kg^{-1} during the ten weeks, satisfying the standard. The microbial analysis exhibited a total plate count of; 0-day (5.04 ± 0.02), week 2 (4.85 ± 0.06), week 4 (5.85 ± 0.04), week 6 (4.00 ± 0.05) and week 10 (3.62 ± 0.11) cfu·g^{-1}. Finally, the yeast and mould results showcased during the ten weeks were coherent with the standard (≤ 5 cfu·g^{-1}).

Keywords: *coconut, physical analysis, peroxide value, total plate count, yeast & mould count*

INTRODUCTION

In a nutritional context, gluten refers to the type of proteins in cereal grains, such as wheat, barley, and rye [1]. The first person to establish gluten from wheat was Jacopo Beccari in 1745 [2]. Gluten is used as a food additive in many products, acting as a thickening agent in processed meats, diluted seafood, and sauces. Gluten plays an essential role in food manufacturing. It provides water absorption, flexibility, viscosity, and adhesion properties for food products such as dough; hence it can be suggested that gluten improves quality in baked foods [2]. It is also able to extend the shelf-life of baked foods [1]. Thus, it can be concluded that gluten is one of the most critical elements necessary for improving the quality of food products.

However, recent studies showed that gluten is abundantly found in wheat flour and negatively impacts individuals with gluten sensitivity. The resultant effect leads to gluten-related diseases (GRD) such as Celiac Disease (CD), gluten ataxia, dermatitis herpetiformis, wheat allergy, and non-gluten celiac sensitivity. Gluten in wheat is associated chiefly with causing CD [1], an immune-based enteropathy due to ingestion of gluten that leads to inadequate nutrient absorption because of the small intestine malfunction [3]. CD cannot be cured, and the only remedy is to avoid gluten consumption. Hence, a continuous gluten-free diet is the only inevitable treatment for people with CD [4]. This disease needs to be monitored as stats showed that about 1 % of the global population are CD sufferers, with Malaysia having 1.5 % of its population identified as CD victims [5].

Gluten ataxia (GA) is an immunologically driven illness triggered by gluten intake in sensitive individuals [6]. A previous study suggested that GA is responsible for 40 % of patients with sporadic idiopathic ataxia [7]. This disease is generally considered curable [8]. Dermatitis herpetiformis (DH) is a skin disease that causes itchy skin that manifests on elbows, knees, and hips [9]. Wheat Allergy (WA) is another disease found in people intolerant to gluten. Wheat allergens are found in various glutens, globulins, and albumins. However, patients usually overcome WA by the age of 6 [10]. Wheat-dependent exercise-induced anaphylaxis is formed when wheat is taken up before extreme physical activity and mostly shows in teenagers [11]. Non-celiac gluten sensitivity (NCGS) is developed when gluten is ingested in the absence of CD and WA [12]. The symptoms show shortly after gluten intake and then disappears within hours or a few days of abstinence from gluten and show up again if gluten is reintroduced [13]. Thus, it can be suggested that the usage of wheat flour in foods needs to be restricted for gluten-related diseased patients due to its gluten content.

In pursuit of a possible substitute, one of the alternatives of gluten-free dietary fiber is coconut [14]. Dietary fiber is an edible component of plants or plant-like carbohydrates resistant to digestion in the small intestine and partially or wholly fermented in the large intestine [15]. According to their definition, even though dietary fibers are carbohydrates based on their chemical structure, they have not been included in this macronutrient group. They are also indicated separately on the nutritional food labels [15]. Dietary fiber in foods assists in lowering bowel passage duration, inhibiting constipation, lowering the risk associated with colorectal cancer, lowering blood cholesterol level, creating short-chain fatty acids and increasing the beneficial intestinal microflora [16]. This benefit is also an essential biochemical process in the body for gluten-intolerant people. Thus, it reveals the crucial role of fiber in remedying the impact of gluten-related diseases for sufferers of gluten sensitivity.

There are two main categories of dietary fiber: soluble and insoluble. The soluble fiber ferments faster in the small intestine and is more easily accessible for hydrolytic enzymes; hence, it is absorbed into the body system, while insoluble fiber is excreted as faeces, increasing the stool mass [15]. Surprisingly, coconut, abundantly found in Malaysia, is incredibly rich in dietary fiber (38.5 g·100 g^{-1}) [16].
Coconut (*Cocos nucifera*) is one of Malaysia's most-produced local commodities, which sets the country among the top 10 producers in the world [17]. In Malaysia, coconut is the second-largest local commodity (517 K tones) produced after rice (2901 K tonnes), followed by cassava (44 K tones) and potatoes (41 K tones) as reported by FAO (2018) [17]. The coconut-based industry is ranked fourth place in Malaysia after palm oil, rubber, and rice. Coconut has contributed 3 % to Malaysia's gross domestic product (GDP) for five years consecutively [18]. Today, coconut products in factories are coconut water, coconut milk, and oil. However, residues of coconut milk (i.e., sapal) are disposed of as waste or used for livestock feeding [19]. Coconut milk residue is a diet rich in fiber [14]. Therefore, this study aims to produce low-cost dietary fiber, and gluten-free flour obtained from coconut residue to serve as a gluten-free alternative for GRD patients.

MATERIALS AND METHODS

Materials

The left-over Sapal (LOS) utilized was obtained from a local market in Shah Alam, Selangor. The recognized supplier was contacted a day before the LOS collection. About 5 kg of LOS was collected early hours of the day (around 6.30 am), after which the coconut milk was extracted immediately. The clean portion of the LOS having the least impurity was collected and weighed before being transported to the laboratory in an airtight icebox (2 - 4 °C). Wheat flour (i.e., control flour sample), sugar, bicarbonate, rice flour, evaporated milk, xanthan gum, egg, corn cream, and peanut were purchased from the commercial market in Shah Alam.
The chemicals used in this study encompasses 30 mL 3:2 acetic acid-chloroform, 0.5 mL potassium iodide, 30 mL water, 0.1 M sodium thiosulfate, 0.5 mL 1 % starch, 2.5 g yeast extract, 30 g agar, 5 g tryptone, phosphate dilution buffer, alcohol, 11g glucose, 5 g bacteriological peptone, 1 g potassium phosphate, 0.5 g magnesium sulphate heptahydrate, 0.5 mL 5 % (w/v) rose Bengal solution, 1 mL 0.2 % (w/v in ethanol) dicloran solution, 0.1 g chloramphenicol, 2 L sterilized distilled water, 5 mL 0.1 % peptone water; which were all supplied by Sigma Integrated Chemical Shah Alam, Selangor.

Preparation of the Sapal Flour (SF)

The LOS was divided into 70 g and placed into dehydrators (Excalibur 3926TBX, USA). The LOS was evenly distributed using a height interval of 1cm between the dehydrator trays. The LOS was dried at 40 °C for 3 hours. After the drying process, LOS was removed and subjected to the grinding process by using a food grinder (PANA-MX-800, Panasonic, Japan) for 1 minute. Finally, the dried LOS was sieved

using a test sieve with a 0.18 - 0.20 mm particle size. The resultant LOS flour was then stored in an airtight container at 4 °C in preparation for the anticipated analysis.

Physicochemical Analysis of the Sapal Flour (SF)

The physicochemical analysis conducted on SF included various analyses involving water activity, color, milling yields, and peroxide value analysis. Details of these analyses are highlighted in the following sections.

Water Activity

About 5 g from each Sapal Flour (SF) were placed in different Petri dishes, and their water activity was measured using water activity Rotronic HygroLab, HP23-AW-A Set, USA at 25 ± 0.2 °C. Three specimens were taken from the SF and each of these specimens was subjected to three separate water activity analyses [20].

Color Analysis

In the color analysis, a Hunter colorimeter Konica Minolta Chroma Meter, CR-410, USA machine based on the CIE (Commission Internationale de l'Eclairage) scale was used in triplicate to measure the color of LOS flour sample, using L *, a *, b * based on CIE scale. L* value measured the lightness (0)/white (100), a* value measured green (-) /red (+), and b* value measured blue (-) /yellow (+). In the analysis, three samples were taken from the SF sample. Each of these samples had three different color analyses performed on them. Also, color analysis was conducted on a sample of wheat flour, which acted as the control sample to compare.

Milling Yields Analysis

The yield analysis was designed to find the yield percentage of the SF after the drying process. The milling yield result of wheat flour was obtained from [21]. The yield percentage was primarily hinged on two main variables that encapsulate the SF's weight before drying and the weight of the SF after drying expressed mathematically by the formula below [22].

$$\text{Yield } \% = \frac{Weight\ of\ sample\ before\ drying - Weight\ of\ sample\ after\ drying}{Weight\ of\ sample\ before\ drying} \quad (1)$$

Peroxide Value

The following steps estimated the peroxide value of the SF. First, 5 g of the SF was placed into a 250 mL Erlenmeyer flask with a glass stopper. Next, 30 mL of acetic acid-chloroform, having a 3:2 solution, was added to it and stirred until it dissolved. After that, a 0.5 mL saturated potassium iodide solution was added, with the mixture allowed to stand and then shaken for 1 min, before adding 30 mL of water. Slow titration was done with 0.1 M sodium thiosulfate and vigorously shaken until the yellow color manifested, disappeared. Then, a 0.5 mL starch solution was added. The titration process continued with vigorous shaking until all the iodine was released from the chloroform layer and the blue color showcased vanished. The peroxide value was then calculated using the formula by Method Cd 8 - 53 [23].

$$Peroxide\ Value = S \times M \times \frac{1000}{g}\ Test\ Portion \quad (2)$$

Peroxide Value = milliequivelent peroxide·kg oil of fat
S = mL sodium thiosulfate
M = molarity sodium thiosulfate solution

Microbial Analysis of Sapal Flour (SF)

The microbial analysis conducted on SF included various analyses involving total plate count and yeast & mould count. Details of these analyses are highlighted in the following sections.

Total Plate Count

The Total Plate Count (TPC) method is a technique used to estimate the number of microorganisms in food and is measured in the colony-forming unit (CFU) g [24]. A colony-forming unit is the scientific means of counting and reporting the population of live bacteria or yeast and mould in a product [25]. A high total plate count indicates a high presence of microorganisms, consisting of spoilage types [26]. Since flour is generally a product with low water activity, it is considered a microbiologically safe product. While it is true that pathogenic bacteria cannot satisfactorily flourish in arid conditions, pathogens that contaminate flour can survive for a long time [27].

According to the FDA, the estimation of microorganisms such as E. coli in SF was done using the Total Plate Count (TPC) method [28]. Immediately after the SF was obtained from LOS (day 0), it was kept at a storage temperature of 27 °C ± 2 °C, 75 % RH ± 5 % RM in a polyethylene airtight container. Thereafter, the initial step was to prepare agar media plates inside the laminar flow cabinet, with 1 L of media prepared by mixing 2.5 g yeast extract, 1 g glucose, 15 g agar, and 5 g of tryptone, and then the addition of sterilized distilled water to bring up the volume to 1 L, with the *p*H value adjusted to 7 using a *p*H meter (PHOENIX/ EC-45 *p*H/ Germany). The agar media was autoclaved at 121 °C for 15 min, then poured with its hot temperature onto Petri dishes and allowed to solidify. The same amount of agar media was poured on all plates to ensure the same height presented to the spiral stylus tip to maintain contact angle. After this process, the serial dilution process was done using phosphate dilution buffer to produce flour sample dilutions of 10^{-1} to 10^{-4}.

The agar plate was placed on the platform, the stylus tip was placed on the surface of the agar, and the motor was started. The plater deposited 49.2 µL of the diluted sample in an Archimedean spiral pattern on the surface of the agar plates. The dispensing system was manually sanitized between samples using 70 % alcohol and distilled water to minimize cross-contamination. After inoculation, the next diluted sample was introduced for the same procedure. Each dilution was inoculated three times. The plates were covered, labelled, inverted, and then incubated at 35 ±1 °C for 48 hours. The colonies that formed were counted with the help of Colony Viewer (BIOMIC/ V3/ USA) and the numbers were converted to cfu·g^{-1} of flour.

Yeast & Mould Count

The yeast and mould count are indicators of the poor quality of food. The assessment of yeast and mould growth in SF was done using the spread-plate method according to FDA [28]. Agar media known as Dichloran Rose Bengal Chloramphenicol (DRBC) was prepared by mixing 10 g glucose, 5 g bacteriological peptone, 1 g potassium phosphate, 0.5 g magnesium sulphate heptahydrate, 0.5 mL 5 % (w/v) rose Bengal solution, 1 mL

0.2 % (w/v in ethanol) dichloran solution, 0.1 g chloramphenicol, 15 g agar, and added sterilized distilled water to boost the volume to 1 L, with the *p*H value adjusted to 5.6 using a *p*H meter. Chloramphenicol is an antibiotic that inhibits bacterial growth in the media. The DRBC media was autoclaved at 121 °C for 15 min and poured while still hot onto Petri dishes and allowed to solidify in the dark.
The serial dilution process was done using 0.1 % peptone water to produce flour sample dilutions of 10^{-1} to 10^{-6}. About 0.1 ml of each dilution was aseptically pipetted onto the solidified DRBC agar media, and the inoculum was spread with a sterile glass rod. Each dilution was inoculated thrice. The plates were incubated in the dark at 25 °C. The incubation period was 5 days, and after that, the plates that contained 10 - 150 colonies were counted, and the numbers were converted to cfu·g^{-1} of flour.

Statistical Analysis
All the experimental analyses in this study were constantly being benchmarked to the control sample. All the analyses were made in triplicates (n = 3). Computations involving mean values and standard deviations were determined for all the conducted analyses. Additionally, Excel software© (2016 version) was utilized for implementing the one-way analysis variance (ANOVA), which birthed the significant difference in means values that were gauged from a salient point of "$p < 0.05$".

RESULT AND DISCUSSION

This section highlights and discusses the results obtained from the various analyses conducted.

Physical Properties of Sapal Flour (SF)

The physicochemical properties of SF in comparison to the wheat flour are shown in Table 1 below:

Table 1. *Physical properties of SF*

Parameters	SF	Control (Wheat Flour)
(L*)	65.94 ± 0.04^{a}	95.70 ± 0.61^{b}
(a*)	9.67 ± 0.02^{b}	4.30 ± 0.05^{a}
(b*)	12.25 ± 0.01^{b}	5.84 ± 0.44^{a}
Water Activity (a_w)	0.47 ± 0.01^{a}	0.59 ± 0.01^{b}
Milling Yields (%)	51.43 ± 0.54^{a}	64.7 ± 0.42^{b}

Notes: (L*) = lightness, (a*) =redness and (b*) = yellowness of hunter color analysis
The 'a-b' indicates a significant difference ($p < 0.05$) between the samples
The value of the milling yield of control flour was obtained from [24]

Water Activity
The degree of water activity in food products plays a significant role in determining the estimated onset and severity of mould spoilage. According to [29], food products that have relatively high water content have the propensity to deteriorate rapidly due to biological and chemical alterations. Table 1 shows the water activity of SF as compared

to the conventional wheat flour as the control sample. The SF had a water activity value of 0.47 ± 0.01 and proves to be lower than that of the wheat flour (control sample), which had a water activity value of 0.59 ± 0.00. This result portrays about 20 % significant difference ($p < 0.05$) lower between the SF and wheat flour.
However, a study done by [30] posits that the resultant declination in the water activity of the samples (i.e. black carrot fiber) may be due to the presence of fiber in coconut, which is present in the SF sample and has a high water absorption capacity. Therefore, it instigates a minimal proportion of the water made available [31]. However, both samples had a water activity value that was below the threshold ($a_w < 0.60$) at which microorganisms require to proliferate and induce spoilage [32]. A study by [33], suggested the specification for flour would be $a_w = 0.62$ to 0.68. Also, a study implemented by [34], also had some of its water activity results below the threshold ($a_w < 0.6$) which involved samples such as quinoa flour having $a_w = 0.512 \pm 0.00$ and rice flour having $a_w = 0.635 \pm 0.01$.

Color Analysis

The color appearance is one of the characteristics that influence consumers' preferences. Table 1 shows the color result of SF as compared to the control sample. The color analysis result showed that the lightness (L*) value of SF was 65.94 ± 0.04 while the control sample recorded 95.70 ± 0.61, with the SF manifesting a 31 % significantly lower L* value than the control sample. The significantly lower ($p < 0.05$) L* value may have been contributed by the darker color shown in the SF. This color change is because LOS had a brownish color due to the thin brown layer between coconut shell and coconut meat (testa). A study on coconut haustorium flour done by [35] mentioned that the L* value of unpeeled coconut haustorium flour was lower than the peeled coconut haustorium flour due to the yellowish color of the skin.
Moreover, according to [36], the chemicals used to speed up the ageing process in bleached flour causes it to have a whiter color, finer grain, and softer texture. Hence, this suggests that unbleached flour showcases a denser grain and harder texture. The bleaching process involved in the production of conventional wheat flour makes the control sample relatively lighter than SF. Hence, the reason for the lower L* value of the SF.
Regarding redness (a*), SF and wheat flour recorded a value of 9.67 ± 0.02 and 4.30 ± 0.05, respectively. In terms of a*, SF was 56 % significantly ($p < 0.05$) higher than the control sample. Finally, the outcome of yellowness (b*) for SF and wheat flour was 12.25 ± 0.01, and 5.84 ± 0.44, respectively. The SF was significantly ($p < 0.05$) higher (52 %) than the control sample. However, based on studies on coconut haustorium flours done by [35] and on pigmented rice flour done by [37], when lightness (L*) decreases, redness (a*), and yellowness (b*) values increase. Nonetheless, this resultant outcome could result from the high a* and b* values of SF.

Milling Yields

Regarding the productivity analysis, Table 1 shows that the result obtained for the milling yield of SF was 51.43 ± 0.54 %. This result suggests a 20.51 % declination in the SF's yield value compared to the wheat flour (control). Whereas, a study done by [21] on samples involving wheat, rye, hulled barley, triticale, and hulled oat gave milling yield values of 64.7 ± 0.42, 59.1 ± 0.28, 63.0 ± 0.47, 58.9 ± 0.35, and 35.1 ±

0.14, respectively. The study posited by [38] proposes that three different factors influence the yielding value of various food products. First, the quality of the raw materials, encompassing its nutritional composition, size, and physical form; second, the quality of the utilized equipment, involving its condition; third, the parameters involved in the processing, encircling the time and temperature used for drying the flour, its grinding time, and milling time. The lower value in milling of SF than wheat flour can be attributed to the coconut milk extraction that had removed more than half of the main ingredients, which are moisture and fat. Also, after the extraction process, the drying process of the flour in the dehydrator further led to its moisture content being reduced to 7.1 ± 0.37 g, and its fat content to 20.6 ± 0.42 g [39]. This finding suggests that water is not the sole underlying factor that influences the milling yield and this was concurred by [21], who posited that the low milling yield observed in oat is due to its high-fat content (i.e., oat 5.2 ± 0.07 g and wheat 2.1 ± 0.03 g). Hence, besides moisture, fat is another factor that affects the milling yield of a sample, as the milling yield is indirectly proportional to fat content.

Peroxide Value

The peroxide values of SF varied for 10 weeks, with a 4.30 ± 0.01 meq·kg^{-1} in day 0, 11.34 ± 0.13 meq·kg^{-1} in the 2nd week, 19.13 ± 0.04 meq·kg^{-1} in the 4th week, 27.14 ± 0.01 meq·kg^{-1} in the 6th week, and 18.90 ± 0.07 meq·kg^{-1} in the 10th week as shown by Figure 1.

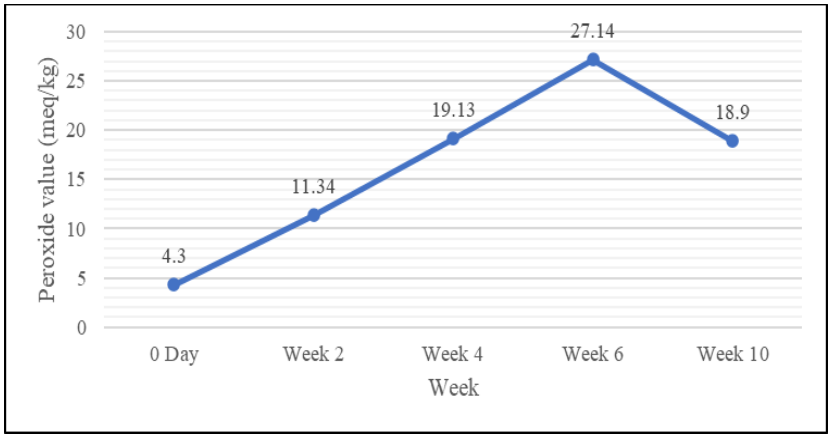

Figure 1. *The peroxide value result of the* SF *for 10 weeks*

All the results obtained were above the standard limit of 3 meq·kg^{-1} [40]. Nevertheless, a study by [41] mentioned that when the peroxide value of a product is 35.5 meq·kg^{-1}, it is still edible as long the product is kept at a temperature of 70 °C. However, when it exceeds 35.5 meq·kg^{-1}, it is not comestible anymore despite being stored at that temperature. According to the findings made by [41], all the peroxide values obtained from the analysis of the various samples had rancidness values below 35.5 meq·kg^{-1} which were then incubated at a temperature of 70 °C and were found to be still eatable. This indicates that a mild heat treatment at 70 °C given to SF products is sufficient to inactivate the peroxidase and lipoxygenase enzymes. This finding is backed up by a study on the peroxidase and lipoxygenase enzymes in Brussels sprouts and cabbage done by [42], showing a similar examination observation, and proving a decline of the

peroxide value as a decrease in the peroxidase and lipoxygenase enzyme activities. However, the current study exhibited a relatively higher peroxide value due to the enormous amount of fat content (i.e., lauric short-chain fatty acid) (20.6 g·100 DW) in SF [41, 42]. Peroxide value is expressed by the amount of peroxide oxygen per 1 Kg of fat or oil in the sample; higher fat or oil content results in high peroxide value [43].
In contrast, a study on the storage stability of coconut flour at room temperature during 10 months done by [40], that had a minimal oil content in the flour, resulted in peroxide values of 2.65 meq·kg^{-1}, and 3.95 meq·kg^{-1} in the seventh month and tenth month, respectively. These relatively low peroxide values resulted from low-fat content (i.e., 3.2 grams of oil). Hence, the peroxide values in the study by Arumugam et al. were below the standard (3 meq·kg^{-1}) during 7 months period. Accordingly, the SF used in this study manifested about 84.47 % fat content higher than the flour used by [40]. Therefore, it is justified to assume that the peroxide values recorded in this study result from the fat content in the SF. Also, according to [44], unsaturated free fatty acids react with oxygen to form peroxides and lead to a series of chain reactions causing an odor of volatile substances. High temperature and proximity to light and oxygen speed up these reactions. Hence, the resultant peroxide value may be because SF is a coconut milk by-product usually considered a waste material. Therefore, making the oxidation process inevitable during the extraction of milk.

Microbial Analysis

Total Plate Count

Figure 2 shows the result of the total plate count during the 10-week experiment. In the analysis of TPC, SF was above the standard in the first 4 weeks, and then it decreased below the standard. From day 0 to week 2, a slight decrease of 4 % was recorded. Shortly after, in week 4, an increase of 17 % was recorded. The comparable count for day 0, and week 4 might be attributed to the fact that bacteria are in the lag phase before rapid multiplication followed by a decline [45]. According to the Bureau of Indian Standard (BIS), the recommended limit of the total plate count should be a maximum of 4.7 cfu·g in flour [40]. However, until week 4, SF was not in conformance to this standard. This may be associated with several factors, including possible contamination at the time of purchase, improper packaging, and the relatively low drying temperature (40 °C).

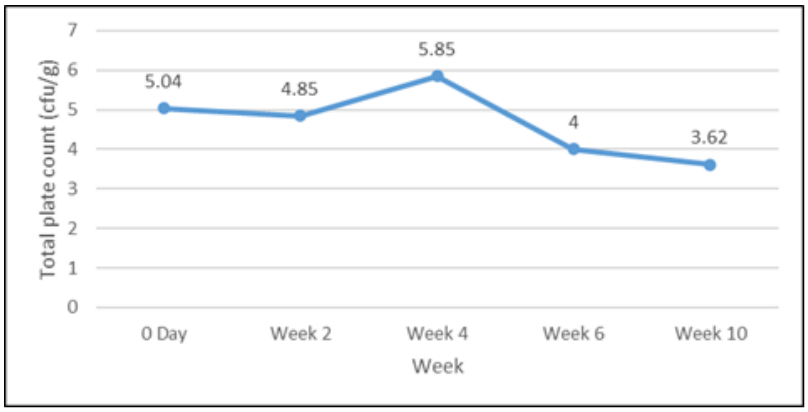

Figure 2. *The total plate count of the* SF *for 10 Weeks*

Nonetheless, a study by [46] recommended a 70 °C drying temperature, which would be adequate to kill the present pathogenic organisms in the food material. It is supported by a study on the determination of the microbiological quality of packed and unpacked bread, carried out by [47], which showed that unpacked flour sold in local markets has a high TPC of 9.08 cfu·g^{-1}. Total heterotrophic bacteria in packed flour were found to be 7.55 cfu·g^{-1}. There was a difference of 1.55 cfu·g^{-1} in the TPC values of packed flour, manufactured and packaged in a factory, and unpacked flour, processed and sold in local markets. These findings suggest that the interaction of the flour with its environment affects the flours' microbial status, as the locally processed flour could have allowed more growth of the microorganisms. This growth may be due to lower manufacturing and processing standards (hygiene, appropriate temperate) than factory flour. In addition, the products sold by the local market vendors contain high microorganisms because their products are kept exposed to the surroundings due to the negligence of good hygiene by the local market vendors relative to supermarket vendors [47]. These findings explain the high total plate count rating in this study, as the material (LOS) used was purchased from a local market supplier; hence the LOS might have been contaminated before analysis.

Yeast & Mould Count

The yeast and mould count estimate the number of colony-forming units present per gram of a product, which is measured in the CFU·g^{-1}. Figure 3 shows the result of yeast & mould count analysis of SF during day 0, week 2, week 4, week 6, and week 10 at a temperature of 30 ± 2 °C. According to the World Food Program, the yeast & mould content limit is 5 cfu·g^{-1} [47]. The SF sample was below the standard value during storage for 10 weeks, and therefore, it complied with the limit. From day 0 to week 2, there was a sharp increase of 33 % in yeast and mould count, which might be attributed to the 'log/exponential phase of the bacteria cycle known for rapid multiplication. Then, from week 2 to week 4 it slightly decreased by 6 %. Afterward, in week 4 to week 6, it further slightly decreased by 3 %. Lastly, from week 6 to week 10, it slightly decreased by 2 %. The systematic decline from the second to the tenth week is due to the last stage of the bacterial life cycle, which is the 'decline phase'. Another factor affecting the yeast and mould count in SF is its low water activity (a_w) and moisture content. Since the SF had an a_w value of 0.47 ± 0.01 and a moisture value of 7.1 g·100 g DW, it is considered to be within the safety standards (a_w < 0.6, and moisture < 14 %) in which microorganisms cannot grow [39].

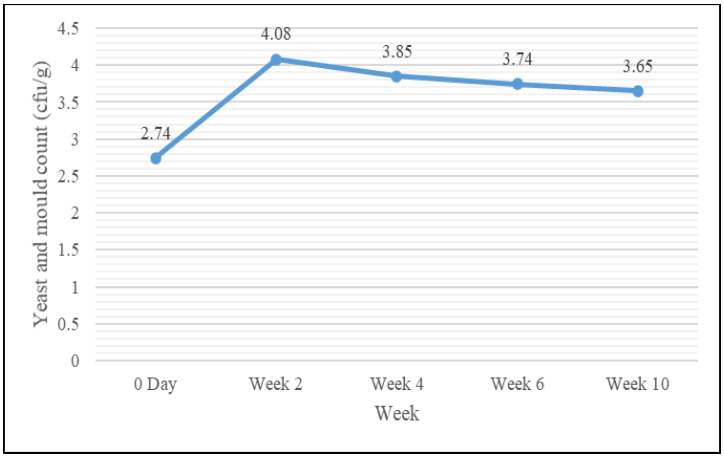

Figure 3. *The yeast and mould count of the* SF *for 10 Weeks*

A study on the nutritional and microbiological quality of germinated soy flour by [48] reported quite similar results, saying that the yeast and mould count was found to be within acceptable levels throughout the six months of storage at ambient temperature. Another study [47] determined the microbiological quality of packed and unpacked bread obtained yeast and mould count values below the recommended limit for all samples; unpacked flour in the local market; and packed flour in the supermarket). However, the unpacked flour sample had a value of 6 cfu·g^{-1}.

CONCLUSION

The value of water activity in food products plays an essential role in determining the estimated onset and severity of mould deterioration. The result of water activity of SF had a significantly ($p < 0.05$) lower value than wheat flour. Both treated and control samples were below the safety level ($a_w < 0.6$). The resultant color analysis recorded a lightness (L*) value for the SF that was about 31 % significantly ($p < 0.05$) darker than the wheat flour. The reason for this was that the LOS had its inherent brownish color from the tester layer of the coconut. Furthermore, the acceptable limit for PV is 35.5 meq·$kg^{-1,}$ and the SF was still good after ten weeks of storage.

The high TPC indicates the increased presence of microorganisms, which usually leads to the spoilage of food products. The resultant total plate count in LOS flour was higher than the acceptable limit except in week 10. The contamination probably instigated this during the time of purchase from the local market.

The result of SF on yeast & mould count was below the acceptable limit at the end of week 10, which proved that the SF can still be comestible during this storage duration. Finally, the results obtained from the physical analyses and microbial analyses suggest that SF manifested a high potential of being an excellent gluten-free flour alternative with a long shelf life that could benefit gluten intolerant individuals, pharmaceutical, and other food industries.

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