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EVALUATION OF TAPUY LEES AS A FUNCTIONAL INGREDIENT IN THE SNACK FOOD POLVORON

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ABSTRACT

Rice wine lees is a major by-product generated in the Philippine rice wine or tapuy production. Flour was developed using tapuy lees and used as a functional ingredient in polvoron. Preparation of flour involved washing of the pressed lees, filtration, sterilization, drying and grinding to a fine powder. The water activity of the sample was <0.400 and microbial loads were within acceptable limits, with 2.30×10^4 cfu/g total plate count and 2.50 cfu/g mold count. The flour had high crude protein ($45.03 \pm 0.14\%$) and dietary fiber ($13.10 \pm 0.08\%$) levels. When used in polvoron, up to 50% (w/w) of lees can be substituted to all-purpose flour with minimal effect on quality. Using 33% lees, significant improvement in crude protein content was noted, without adversely affecting the taste and overall sensory quality of the product. Results demonstrated the feasibility of producing a functional food ingredient using a major by-product of tapuy production.

PRACTICAL APPLICATIONS

Lees is a nutritious, but underutilized, by-product of rice wine manufacture. In tapuy production, a large volume of lees (about 27% of the weight of raw rice) is generated and is merely discarded. The use of lees in the development of value-added food products will be a step toward making nutrient-dense foods available to consumers, while addressing waste management problem from rice wine production. Results of this study would provide manufacturers of rice wine a valuable resource to make their process more efficient and productive. This will also serve as a basis to further explore the nutritional quality of rice wine lees and its potential applications in other food products.

INTRODUCTION

Processing of various crops for food or other uses generates by-products and wastes. Most of these by-products are usually processed into animal feeds to address ecological problems associated with food processing (Sugiura *et al.* 2009). However, some of these by-products have been found to still contain high levels of certain food components, particularly protein and fiber, which may be used as functional food ingredients. Functional foods are those having beneficial health effects beyond basic nutrition, and include conventional foods, fortified and enriched foods, and dietary supplements (IFT 2005). For instance, brewer's spent grains (BSG), or the by-product of beer production, improved the total dietary fiber (DF) and protein levels in breadsticks (Ktenioudaki *et al.* 2012). Distiller's dried grains and defatted maize germ (DMG), the by-products of corn bioethanol and maize milling industries, respectively, were found to be effective flour extenders as they increase the protein and fiber contents of baked goods without compromising eating quality (SDSU 1993; Nasir *et al.* 2010). DMG was shown to improve the crude protein, crude fiber and ash contents of the wheat-DMG flour blends. The use of DMG at 5% level produced acceptable cookies (Nasir *et al.* 2010). Tate *et al.* (1990), on the contrary, found that the processed cake meal from peanut expeller can be blended with wheat flour at 10% (w/w) level to prepare acceptable cookies with enhanced protein and mineral contents.

Rice wine or tapuy is a traditional alcoholic beverage in Northern Philippines derived from the fermentation of polished rice. In the tapuy production process, a large volume of residue, called lees, is generated after the fermentate, or the wine, is collected. Lees is currently discarded as its properties and potential applications have not yet been fully investigated. Initial analysis of the chemical composition of dried lees in our laboratory revealed that this underutilized by-product contains high levels of crude protein and crude fiber (Corpuz 2009), which can be explored as a functional ingredient in value-added food products.

There has been considerable worldwide interest in healthful and nutritious foods (Sloan 2010; Lempert 2012), mainly driven by population's changing lifestyles. The active lifestyles, particularly in urban communities, necessitate the availability of healthful and nutritious convenience foods and snacks. The incidence of lifestyle-related diseases is generally increasing globally (Sassi and Hurst 2008; DOH 2011; Pappachan 2011) and the number of older people is continually growing (WHO 2013). Consequently, the demand for foods that meet specific medical and health needs is high (Sloan 2010). Meanwhile, access to low-cost nutritious foods remains to be a major concern in developing countries. These situations reflect the scenario in the Philippines (Stanton, Emms & Sia 2011).

Polvoron is a Philippine snack food made of toasted flour, powdered milk, sugar and butter. This crumbly treat is popular among children and adults alike, either as snack or dessert and is therefore a good vehicle for supplementation with functional ingredients. Nutritional enhancement of polvoron was successfully done using other food processing by-products, such as fish protein concentrate from milkfish (Orejana *et al*. 1984) and peanut fines (San Juan *et al*. 2006). Preparation of polvoron does not require special equipment or methods, i.e., all ingredients are simply mixed, and thus, polvoron is a good model system for testing supplementation. In this study, we evaluated the potential of tapuy lees flour (TLF) as a high-protein, high-fiber functional ingredient in polvoron. The physicochemical and microbial properties of lees flour were determined and the incorporation of TLF in polvoron was assessed in terms of microbial, sensory and physicochemical properties of the products.

MATERIALS AND METHODS

Materials

Lees from the glutinous local rice variety NSIC Rc15 was obtained from the Rice Wine Manufacturing Plant of the Philippine Rice Research Institute (PhilRice), Maligaya, Science City of Muñoz, Nueva Ecija, Philippines. Ingredients for polvoron were procured from a local supermarket. All chemicals used in the physicochemical evaluation were of analytical grade. Agar media were purchased from HiMedia Laboratories Ltd. (Mumbai, India).

Flour Preparation from Tapuy Lees

Lees from tapuy production was collected, repeatedly washed with distilled water, autoclaved at 103 kPa for 15 min and then air-cooled. The lees was spread at about 1.27-cm thick on a sterilized aluminum tray and then dried in an air-convection oven at 40C for 48 h. The dried lees was first pulverized using mortar and pestle and then ground with Cyclotec 1093 Sample Mill (Tecator, Höganäs, Sweden), packed in polyethylene (PE) plastic bag and stored at 4–5C. Samples were analyzed within 1 week after processing.

Characterization of TLF

Physicochemical Analysis. Rice wine lees flour was passed though a 250-μm sieve. Color was measured as CIE *L*, *a* and *b* color values using a Minolta CR-110 choma meter (CIE standard observer with illuminant source C, 0° viewing angle; Minolta Camera Co., Ltd., Osaka, Japan), wherein *L* pertains to the measure of the lightness of the color with 100 as white and 0 as black, *a* for redness (+) or greenness (−) and *b* for yellowness (+) or blueness (−).

The moisture content (MC), crude protein, crude fat, ash and total carbohydrates of the lees flour were analyzed based on the AOAC standard methods. DF was determined by enzymatic-gravimetric method (AOAC 2005). Water activity (a_w) was measured using a Durotherm a_w-Wert-Messer (Lufft, Germany) water activity meter at 23 ± 1C.

Microbial Load Determination. Ten grams of the sample was mixed with 90 mL of 0.1% peptone solution. Successive dilutions were made by transferring 1 mL of the suspension medium to 9 mL of 0.1% peptone solution. The components were plated in duplicate using dilutions from 10^{-1} to 10^{-2} on potato dextrose agar specific for molds and malt yeast extract agar for yeast; and 10^{-2} to 10^{-5} on plate count agar for total plate count (TPC) determinations. Plated samples were incubated at 37C and were counted after 48 h.

Development of Lees-Supplemented Polvoron

Polvoron Formulation. Polvoron samples were prepared using 226 g of all-purpose flour, 116 g of lard, 98 g of sugar, 144 g of buttermilk and 114 g of butter. All-purpose flour was first toasted in a pan over moderate heat until it turned light brown and then allowed to cool and set aside. Lard and butter were melted in a sauce pan over moderate heat. In a bowl, all ingredients were combined and mixed thoroughly. Mixing was done until the melted shortening mixture was evenly dispersed. Round molder was used to shape about 13-g portions of the mixture to produce a round-shaped polvoron (diameter 3.2 cm, thickness 1.3 cm). Lees was substituted to toasted all-purpose flour at different levels (0%, as unsubstituted control, to 100% w/w). The polvoron samples were individually packed in transparent PE bags.

Sensory Evaluation. Laboratory sensory evaluation was carried out using a 15-cm unstructured scale scorecard with 10 experienced PhilRice staff as panelists. Sensory evaluation was conducted at around 3:00 p.m. in individual booths with controlled white light. Each panelist was given four coded samples and water for rinsing in between sample tasting. One piece per sample was presented as packed, but additional pieces were provided upon request of the panelists. The attributes evaluated were pleasant/buttery aroma, color (white to dark brown), off-odor (rancid, alcoholic and burnt), smoothness/mouthfeel, buttery and sweet taste, and off-taste (rancid and alcoholic). For the positive attributes, a mean score of at least 7.5 cm, the halfway point of the scale, was considered acceptable. For off-flavors, ratings that significantly differed with that of the control were considered unacceptable.

Determination of Microbial Load and Physicochemical Properties. Twenty-five grams of the sample was mixed with 225 mL of 0.1% peptone solution and processed in a blender (Osterizer cycle blend 10, model 4172-074) at speed 9 for 30 s. Successive dilutions were made by transferring 1 mL of the suspension medium to 9 mL of 0.1% peptone solution. TPC and mold counts were determined using the standard plate count techniques as described earlier.

Nutritional Analysis. Nutritional composition was determined by measuring the MC, crude protein, crude ash, crude fat, ash, total carbohydrates and DF content of control sample and the treatment with the highest acceptability and low microbial load. The best formulation based on sensory and microbial evaluation was selected only for the nutritional analysis because fortification or nutritional supplementation of food products is often limited by these characteristics.

Statistical Analysis

All analyses were conducted in duplicate. *t*-Test, analysis of variance and subsequent comparison of means using Tukey's HSD (honestly significant difference) were determined using SAS statistical software v. 9.1 (SAS Institute, Cary, NC) at $P < 0.05$.

TABLE 1. PHYSICOCHEMICAL PROPERTIES AND MICROBIAL LOAD OF TAPUY LEES FLOUR (TLF)

Parameter	Values
Color†	
L	77.60 ± 0.00
a	−0.45 ± 0.07
b	9.90 ± 0.14
Water activity (a_w)	<0.400
Total plate count (×10^4 cfu/g)	2.30
Molds (cfu/g)	2.50
Yeast (cfu/g)	ND

† Means of two replications ± SD.
ND, not detected.

RESULTS AND DISCUSSION

TLF Preparation and Characterization

Physical and Microbial Qualities. The color values of TLF are shown in Table 1. The lees flour developed has a brownish color, as indicated by its *L* and *b* (yellow) values (Table 1). Browning of lees was expected from the various thermal treatments carried out in the preparation of TLF.

A shelf-stable flour was prepared from lees, as indicated by its a_w of <0.400 (Table 1) and MC of 7.23 ± 0.02% (Table 2). Dried foods with good storage stability usually have MC values ranging from 5 to 15%, while foods with a_w below 0.6 are less susceptible to microbial spoilage (deMan 1999). MC and a_w are important factors affecting food quality as the progress of chemical and microbiological spoilage reactions in food items depends on them.

The TPC of the lees flour was 2.30×10^4 cfu/g and mold count, 2.50 cfu/g (Table 1). These values were within acceptable limits ($<10 \times 10^4$ cfu/g for TPC and bacterial count and <1000 cfu/g for mold count), based on the standards of the World Food Program of the United Nations (UN 2009). The low a_w value may explain the absence of yeast and low levels of other microorganisms in the lees flour sample. For molds and yeasts, growth starts at a_w between 0.7 and 0.8, whereas for bacteria, the critical a_w value is 0.8 (deMan 1999).

TABLE 2. CHEMICAL COMPOSITION OF FLOUR FROM TAPUY LEES

Parameter (% w/w, dry basis)	Values†
Moisture	7.23 ± 0.02
Ash	0.46 ± 0.09
Crude fat	4.59 ± 0.03
Crude protein	45.03 ± 0.14
Total carbohydrates	42.69 ± 0.05
Dietary fiber	13.10 ± 0.08

† Means of two replications ± SD.

FIG. 1. POLVORON WITH DIFFERENT SUBSTITUTION LEVELS (W/W FLOUR) OF TAPUY LEES FLOUR
T_0 = control, no lees; T_1 = 25% lees; T_2 = 33%; and T_3 = 50%.

Chemical Composition. TLF contained high amounts of crude protein and DF at 45.03 ± 0.14% and 13.10 ± 0.08%, respectively (Table 2). Raw NSIC Rc15 rice contains 7.1% crude protein (NSIC 2012). Enhancement of protein content can be due to concentration effect after starch in rice was converted to sugars and then alcohol in the rice wine fermentation process. Additional protein source could be the yeast used in the fermentation (Manabe *et al.* 2004). The increase in fiber level, on the contrary, could be due to retrograded amylose (Sanchez 2008), which was left unfermented when the readily available starch was converted into alcohol. Retrograded amylose is a type of resistant starch, or starch that can withstand actions of amylolytic enzymes. Resistant starch is a component of DF (Englyst *et al.* 1987). In supplying DF in foods, TLF was therefore comparable to some whole grains. Cooked long-grain brown rice contains around 3.5% DF (USDA 2012), whole grain cornmeal, 7.3%; whole oats, 10.3% (Slavin 2007); and whole grain wheat flour, 14.6% (USDA 2012). The high amount of DF is considered responsible for the many health benefits of whole grains, including lowering of low-density lipoprotein and total cholesterol levels, prevention of cardiovascular diseases and improvement in digestive health. Results of this study demonstrated that TLF can be used as an ingredient to augment the protein and DF content of foods and provide the beneficial effects of the said food components to consumers.

Utilization of TLF in Polvoron

Sensory Attributes. A preliminary test was conducted with different substitution levels of TLF: 0, 33, 67 and 100% (w/w) flour. Ten panelists evaluated the samples and noted that overall quality of the samples decreased significantly ($P < 0.05$) with increasing level of lees substitution (data not shown). Hence, substitution with lower amount of lees (0, 25, 33 and 50% [w/w] flour) was utilized in subsequent trials. Figure 1 shows the different polvoron samples prepared using these treatments.

Sensory assessment revealed that there were no significant differences in odor, off-odor (rancid, alcohol, burnt) and sweetness ratings among treatments (Table 3). Lees has a characteristic alcoholic and yeasty aroma. Processing of the lees into flour either significantly reduced these off-odor components or other food ingredients (butter, toasted flour) in polvoron effectively masked the undesirable odors even up to 50% flour replacement. However, rating for smooth texture decreased significantly with increasing TLF substitution, with perceptible roughness observed in samples with at least 33% lees. Texture is considered as the limiting sensory attribute of polvoron (San Juan *et al.* 2006) and this could be affected by MC. Moisture in flours affects particle aggregation when shortening is added to polvoron and results in a more crumbly product with less creamy mouthfeel. Color is another important characteristic of polvoron as it usually determines a food product's consumer appeal (Liu *et al.* 2011). The brown color of polvoron intensified with corresponding increase in the percentage of TLF, with apparent increase even at the lowest level of replacement. This can be ascribed to the evident brown

TABLE 3. SENSORY SCORES OF TAPUY LEES-SUPPLEMENTED POLVORON

	Lees substitution (% w/w flour)			
Sensory properties	0	25	33	50
Odor/Aroma (pleasant/buttery)†	7.98^{a}	8.07^{a}	7.84^{a}	7.65^{a}
Off-odor‡				
Rancid	0.04^{a}	0.23^{a}	0.27^{a}	0.34^{a}
Alcohol	0.23^{a}	0.22^{a}	0.47^{a}	0.54^{a}
Burnt	0.45^{a}	0.34^{a}	0.37^{a}	0.32^{a}
Color§	8.41^{c}	10.70^{b}	11.24^{ab}	12.66^{b}
Smoothness¶	10.43^{a}	8.93^{ab}	8.12^{bc}	6.87^{c}
Flavor/Taste†				
Buttery	9.61^{a}	8.67^{ab}	8.95^{ab}	7.44^{b}
Sweetness	8.75^{a}	7.60^{a}	8.15^{a}	6.99^{a}
Off-flavor/Off-taste††	0.23^{b}	0.31^{ab}	0.33^{ab}	1.10^{a}
Overall quality‡‡	11.49^{a}	10.24^{ab}	9.45^{ab}	8.79^{b}

Mean values in the same row with the same letter are not significantly different at $P < 0.05$ ($n = 10$).
† 0 = none; 15 = very intense.
‡ 0 = none; 15 = very perceptible.
§ 0 = white; 15 = brown.
¶ 0 = rough; 15 = very smooth.
†† 0 = none; 15 = very distinct.
‡‡ 0 = dislike extremely; 15 = like extremely.

color of the lees powder. DMG, which has a visible shade of yellow, likewise affected the color of cookies even at 5% incorporation (Nasir *et al.* 2010). In terms of taste, all products were comparably sweet, but the sample with 50% lees had a reduced buttery taste and perceptible off-flavor. Off-flavors and off-odors negatively impact product taste, which is a prime attribute affecting consumer acceptability (Drewnowski 1997).

The overall acceptability of each sample was estimated using the same laboratory panel. "Overall acceptability" was used as a criterion to select which samples to use in the subsequent nutritional evaluation and reflected "overall quality." The panelists rated their degree of liking for each product using a 15-cm hedonic line scale (0 = dislike extremely, 15 = like extremely). As indicated in Table 3, the overall acceptability of the product with 50% lees was significantly lower than those of the control and other treatments, although this should be verified using a greater number of subjects through affective sensory tests (Meilgaard *et al.* 2007). Overall acceptability ratings, particularly that of the sample with 50% lees, could be attributed to the texture, color, buttery taste and off-taste scores, as described earlier.

Among polvoron samples with TLF, the sample with 33% lees was determined as the most acceptable treatment, with off-flavor, as well as overall acceptability, rating comparable with those of the unsubstituted control (Table 3). Around the same level of polvoron supplementation (22–36%) was reported acceptable by San Juan *et al.* (2006) using peanut fines as supplementing ingredient. However, polvoron with 50% TLF did not differ significantly from the other treatments in terms of color, buttery flavor and overall acceptability, although it received higher off-flavor rating and lower overall acceptability score than the control (Table 3). Polvoron with 50% TLF was still considered acceptable because its overall acceptability mean rating (8.79) was higher than 7.5, the rating set as the minimum acceptable level.

Physicochemical and Microbial Quality. Physicochemical properties and microbial load of the 33% lees-substituted polvoron, the treatment with sensory characteristics comparable to those of the control sample, were determined. The polvoron with TLF and the control did not exhibit significant differences in terms of their a_w values and microbial load (Table 4), but their physicochemical characteristics differed significantly at $P < 0.05$ (Fig. 2). The MC of polvoron with 33% TLF was significantly higher at 3.76 ± 0.01% than that of the control (3.02 ± 0.03%). This could explain the lower smoothness scores of the products with lees, as discussed earlier.

No significant improvement in DF level was observed for the sample substituted with 33% powdered lees. A significant enhancement in DF level might be observed in the sample with 50% lees, but chemical analysis for the said sample was not conducted because of its inferior sensory quality. To ensure commercial success of high-fiber foods, sensory quality must be the prime consideration in developing the said products (Berry 2013). Addressing this is a great challenge for food formulators because of the adverse effect of incorporation of high-fiber ingredients on the appearance, texture and taste of foods (Ktenioudaki *et al.* 2012). Improvement in the sensory quality of polvoron with elevated levels of lees is necessary for consumers to take advantage of the nutritional benefits of the said product. This may be done by reducing off-flavors though the use of masking agents or developing efficient methods of identifying and reducing the levels of the components in the lees that give off undesirable flavors. Also, fiber enrichment using higher levels of lees could be explored using other food products as delivery systems.

Replacement of all-purpose flour with TLF predictably resulted in decreased carbohydrate content, but the most notable effect was the improvement in crude protein level (Fig. 2). Polvoron with 33% TLF had comparably higher crude protein content than the control. The lees-supplemented sample had a crude protein level of 11.71 ± 0.18%, whereas the control had 7.97 ± 0.30%. Other

TABLE 4. WATER ACTIVITY AND MICROBIAL COUNTS OF TAPUY LEES-SUPPLEMENTED POLVORON (n = 2)

Parameter	Control	With 33% lees
Water activity	<0.400	<0.400
Total plate count (×10^4 cfu/g)	1.00	1.00
Mold (cfu/g)	15	10

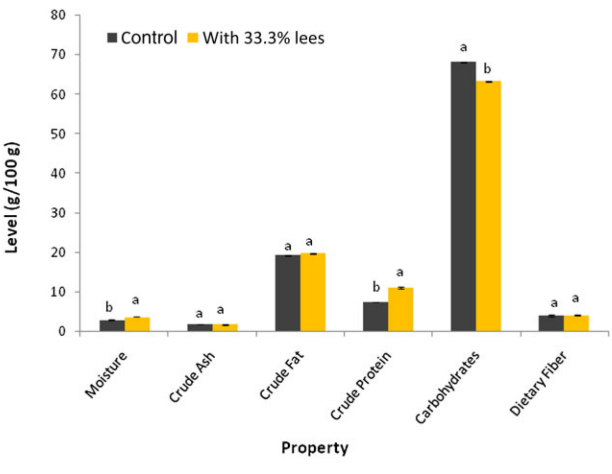

FIG. 2. CHEMICAL PROPERTIES OF TAPUY LEES-SUPPLEMENTED POLVORON (n = 2; MEANS WITH THE SAME LETTER IN EACH PARAMETER ARE NOT SIGNIFICANTLY DIFFERENT AT $P < 0.05$)

by-products shown to have protein enhancing effects in food products are peanut cake meal, which increased the protein level in cookies starting at 10% level (Tate *et al.* 1990); DMG in cookies starting at 5% level (Nasir *et al.* 2010); and BSG in breadsticks starting at 25% (Ktenioudaki *et al.* 2012). The results show that TLF can be used as an ingredient to improve the protein content of polvoron without affecting the overall quality of the food product. The flour developed from tapuy lees, as shown in this study, could be used as a high-protein ingredient to augment the nutritional value of a traditional Filipino dessert, which meets the current needs of the food industry to deliver nutrient-enriched food products to consumers.

CONCLUSIONS

Good quality flour with low microbial load can be prepared from lees, a major by-product in rice wine processing. Lees flour had high crude protein content and its DF level was comparable with those of some whole grains. Lees flour can be substituted to all-purpose flour by up to 50% level in polvoron, but optimum substitution level was 33%. Replacement of all-purpose flour with 33% TLF can increase the crude protein content of polvoron by 3.74% without affecting the overall quality of the product. The results established the feasibility of preparing a flour product from tapuy lees and its suitability as a high protein, high fiber polvoron ingredient.

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