

Philippine langkauas (*Alpinia pyramidata* Blume) rhizomes as potential functional food ingredient: characterization, safety assessment, and product development

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Abstract

Langkauas (*Alpinia pyramidata* Blume), an indigenous plant in the Philippines, belongs to the neglected and underutilized plant species that shows potential functional properties. This study characterized langkauas as a potential functional food ingredient that is safe for human consumption. Nutrient composition and dietary fiber were determined by adopting the AOAC INTERNATIONAL Official Method. Fiber fermentability was determined *in vitro*. Phytonutrients and their antioxidant activities were determined using UV-VIS spectrophotometer. Safety assessment was conducted by evaluating the heavy metal contents and microbial load of the langkauas rhizome. Different percentages of langkauas extracts (LE) were infused in the production of lemonade, which was later subjected to sensory evaluation for general acceptability using a 9-point hedonic scale. Nutrient composition of langkauas rhizome per 100 g sample was as follows: 81.76±0.01 g moisture, 14.37±0.01 g carbohydrates, 1.45±0.00 g protein, 1.68±0.01 g ash and 0.74±0.04 g total fat. Freeze-dried langkauas rhizome is a high source of dietary fiber (5.49±0.24 g) and contains a significant amount of insoluble fiber (2.86±0.01 g) and soluble fiber (2.63±0.25 g). It also produced short-chain fatty acids after *in vitro* colonic fermentation with significant amounts of propionate (2.89±0.39 mg/g) while acetate and butyrate were not detected. Phytonutrient contents showed that the sample has a total polyphenol of 618.96±8.02 mg/100 g, and total anthocyanidin of 140.92±4.93 mg/100 g. Total flavonoids were not detected. DPPH radical scavenging activities of the langkauas rhizome are 27.77±0.78% inhibition, and its FRAP assay is 522.62±6.65 mg Trolox/100 g. The microbiological load and heavy metal content of langkauas rhizome were within the acceptable level, implying that the rhizome is safe for consumption. General acceptability showed that lemonades with 10% and 15% LE have no significant difference from the control. The most preferred lemonade was with 15% LE and the least preferred was with 20%. Thus, langkauas rhizomes may be considered a potential functional food ingredient which is safe to use in the development of food products.

1. Introduction

Food can be considered as functional if it satisfactorily demonstrates its beneficial effect on one or more target functions in the body beyond regular nutritional contribution, in a way which is applicable to either the state of welfare and health or risk reduction (Roberfroid, 2002). The scientific basis for the development of health, nutrition and food processing of functional foods has been established enormously (Mermelstein, 2002; van Kleef *et al.*, 2005) and was found to ensure overall good health and/or manage specific conditions in a convenient way as compared to

conventional foods (Sloan, 2000; van Kleef *et al.*, 2005). It is an interesting opportunity for the food industry when food offers an additional health value that persuades people to engage in healthier food selection (van Kleef *et al.*, 2005). The extent to which the availability of bioactive components such as phytonutrients, antioxidants, and dietary fibers, as well as their safe use, is a great challenge for business and food sectors (Pap *et al.*, 2004; Gunders, 2012) to further understand and employ neglected functional foods in more diverse applications.

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Several countries, such as India, Nepal, Malaysia, and the Philippines, have reported good uses of the neglected and underutilized plant species (Padulosi *et al.*, 2013). In the Philippines, important policies and projects relating to Neglected and Underutilized Species (NUS) have been implemented over the last decade. However, agricultural statistics released by the Philippine Bureau of Agricultural Statistics (BAS) have listed ginger as a minorly utilized. According to Shakirin *et al.* (2010), the lack of promotion, minimal planting area, and having an economical potential which has not been fully explored are classified as under-exploited or underutilized plants.

At present, one of the fundamental concerns of both consumers and food industries is food safety (Oonmettaree *et al.*, 2006) and quality retention (Tambunan *et al.*, 2001). In addition, phytochemical constituents together with antioxidants have raised interest among scientists, food manufacturers, producers, and consumers for their role in the maintenance of human health (Milner, 1999). In tropical countries, various kinds of gingers are being cultivated and used not only for traditional medicines but also as spices. The genus *Alpinia* from the family of Zingiberaceae consists of about 230 species, which are probably distributed and originated somewhere in tropical Asia, but are now being cultivated in several parts of the world (Raina *et al.*, 2002). Langkauas is commonly planted in open areas in backyards. It is a perennial herb with a pseudostem which can grow up to 2.2 m with thick rhizomes. Oblong-elliptic leaves measuring 45 cm × 12 cm are arranged alternately in two rows with short petioles; a thin light green marking all around is ciliated to the margin; the stipule has brownish green markings with patches of vicious hairs all around. Inflorescences in terminal panicles, a 10 cm long corolla tube is divided into 3 petals with 1.9 cm × 1.6 cm each; with 2 deep lips and up to 2 cm lip; white with red streaks. Orange-red round ripe fruit grows up to 1.4 cm (Mohtar *et al.*, 2024).

Langkauas bears underground stems termed as rhizomes (Figure 1), which hold a strong aromatic smell with conspicuous nodes and internodes (Jirawan, 2005; Subramanian and Nishan, 2015). The rhizomes are cut into various pieces, usually cylindrical in shape. It is often marked with narrow and whitish short-colored interval body when cut while fresh, which gives rise to raised rings (Subramanian and Nishan, 2015). It is externally characterized with a dark reddish-brown color, the inner part has a dark center enclosed by a wider and paler bed on the outer rim, which also darkens when dried. The langkauas rhizomes possess a strong aromatic odor and pungent or spicy taste (Subramanian and Nishan, 2015). Although sufficiently characterized in its physical form, there are still wide gaps in the studies

concerning the nutrition, phytonutrients, antioxidants and dietary fiber content of langkauas in the Philippines. It is essential to look into the presence of the said bioactive components from resources that are locally available, which can be readily available sources of functional constituents. Moreover, information regarding the safe use of langkauas is insufficient. Further research exploring the functional properties of langkauas should be carried out to uncover its great potential to contribute to the fields of nutrition and health.



Figure 1. Langkauas (*Alpinia pyramidata* Blume) rhizomes.

The present undertaking is comprised of three parts, as reflected in Figure 2 (conceptual framework): the characterization, safety assessment, and product development. Langkauas rhizomes have a great potential as a functional food ingredient due to their phenolic compounds, such as phenolic acids (Mayachiew and Devahastin, 2008), that may provide beneficial effects beyond nutrition in maintaining human homeostasis. The characterization was done to determine the composition and phytonutrients that would be utilized and reflected in the product development. Safety assessment was also included to address food safety concerns.

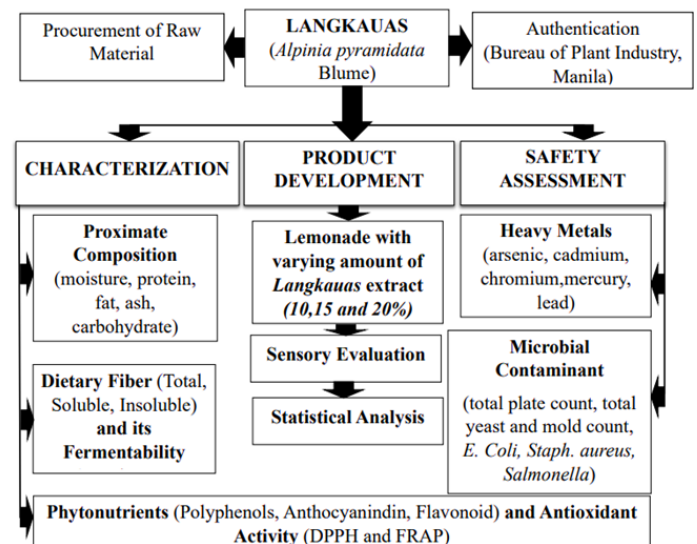


Figure 2. Conceptual framework

2. Materials and methods

2.1 Sample collection and preparation

The langkauas plant was subjected to authentication

with the Bureau of Plant Industry, San Andres Street, Malate, Manila. Approximately 2 kg of rhizome samples were obtained from Tabaco City, Albay (13°21'N123°44'E), Bicol Region, Philippines, where langkaus plants are abundant. Freshly harvested mature rhizomes (10-12 months after planting), free from physical and microbial damage, were used in the study. The rhizomes were sorted and washed with distilled water. The peels were removed using a stainless steel knife. The rhizomes were cut into thin slices, washed, and placed in sterile plastic airtight containers and stored at -40°C prior to further use.

The thinly sliced rhizomes were lyophilized using ScanVac Coolsafe freeze dryer (Lyngø, Denmark). Homogenization of the sample was done using a 1095 Foss Knifetec mill (Hoganas, Sweden). The homogenized samples were immediately placed into a sterile airtight plastic container and stored at chilling temperature (<4°C) before further analysis.

2.2 Proximate analysis

2.2.1 Protein determination

The Kjeldahl method aligned with AOAC Official Method 985.29 (AOAC INTERNATIONAL, 2019) was used in the analysis of protein. An amount of 1 g of the sample was used and digested. Approximately 15 mL of concentrated sulphuric acid (H₂SO₄), three Hengar granules, and two (2) pieces of Kjeltabs (3.5 g K₂SO₄ + 0.4 g CuSO₄•5H₂O) were added to the flask. The sample was digested at 420°C for 1 h using Foss digestion block Kjeltach 2200 (Hilleroed, Denmark). The digest was cooled, added with 30 mL distilled water and 15 mL 60% NaOH and distilled for 15 min using the distilling apparatus Kjeltac 8100 (Hilleroed, Denmark).

The condensate was recovered in with 5 mL of 2% boric acid and 2 drops of mixed indicator and back-titrated with 0.01 N HCl until the endpoint of light grayish pink color appeared.

2.2.2 Moisture determination

Moisture content analysis was conducted using AOAC Official Method 925.10, wherein 2 g of sample was placed in a pre-weighed aluminum dish and was heated in 105°C for 1 h and cooled in a desiccator before weighing. Sample was reheated until constant weight was achieved.

2.2.3 Fat determination

AOAC Official Method 922.06 by acid hydrolysis method was utilized for the analysis of fat. About 1 g of sample was placed in dry crucible and was heated in oven at 110°C for 8 hrs. The samples in dried crucible

underwent acid hydrolysis for 30 min at 70-80°C using 8.3 N HCl. The fat was extracted using FOSS Soxtec 2045 (Hilleroed, Denmark) and petroleum ether as solvent. The fat content was expressed as g/100 g.

2.2.4 Ash determination

Ash analysis was performed using gravimetric method aligned with AOAC Official Method 923.03 wherein crucibles were gradually heated in muffle furnace until 550°C and were cooled in a desiccator before weighing. This was repeated until constant weight was attained. Then 2 g of sample was placed in the pre-weighed crucible, heated again in the muffle furnace for 18 h at 550°C and was cooled in a desiccator before weighing. The final weight was determined and was reported as g/100 g ash content.

2.2.5 Carbohydrate determination

Total carbohydrates was calculated by difference, adopting the method of Millena and Sagum (2018) using the following formula:

$$\text{Total Carbohydrate} = 100 - (\text{moisture} + \text{ash} + \text{protein} + \text{fat} + \text{Total Dietary Fiber})$$

2.2.6 Determination of insoluble, soluble, and total dietary fiber

The determination of the total dietary fiber was based on AOAC Official Method 985.29. To determine the total insoluble dietary fiber, a crucible containing celite was weighed. The celite bed was previously soaked in water and distributed with the use of distilled water. Suction filtration was applied to the crucible to draw celite into the fritted glass as an even mat. The enzyme-digested mixture was placed in the crucible and filtered into the suction flask. The residue was washed twice with 10 mL portion of water, 10 mL ethanol, and 10 mL acetone. The crucible containing the residue was dried for 5 h at 105°C using an air oven, cooled in a desiccator and then weighed. The weight of the celite and crucible was subtracted from the weight of the crucible with residue to determine the residue weight. The residue, celite, and fiber mat was scraped and analyzed for protein; the other duplicate was analyzed for ash through incineration at 525°C. The insoluble dietary fiber was computed using the following formula:

$$\% \text{ Insoluble Dietary Fiber (IDF)} = \frac{\text{Wt. of Residue} - \text{Protein} - \text{Ash} - \text{Blank}}{\text{Wt. of Test Portion}} \times 100$$

To quantify the soluble dietary fiber, the combined filtrate and water washing from the previous experiment for the IDF were adjusted to 100 g with deionized water. The mixture was placed in a beaker, and 400 mL of 95% ethanol was added. The solution was preheated at 60°C. Precipitate was formed at room temperature after 60 min. Crucible containing celite was weighed, wherein the

celite bed was moistened and redistributed using 78% ethyl alcohol. Celite was drawn into the fritted glass as an even mat by applying suction to the crucible. The precipitate mixture was filtered with three 20 mL portions of ethyl alcohol, two 10 mL portions of 95% ethyl alcohol and two 10 mL portions of acetone. The crucible containing the residue was dried for 5 h at 105°C in an air oven, cooled in a desiccator, and then weighed. The weight of the celite and crucible was subtracted from the weight of the crucible with residue to determine the residue weight. The residue, celite, and fiber mat were scraped and analyzed for protein; the other duplicate was analyzed for ash through incineration at 525°C. The formula used to quantify soluble dietary fiber is as follows:

$$\% \text{ Soluble Dietary Fiber (SDF)} = \frac{\text{Wt. of Residue} - \text{Protein} - \text{Ash} - \text{Blank}}{\text{Wt. of Test Portion}} \times 100$$

The total dietary fiber (TDF) was computed by combining the soluble and insoluble dietary fiber using the following formula:

$$\% \text{ TDF} = \% \text{ IDF} + \% \text{ SDF}$$

2.3 *In vitro* fermentation

In vitro fermentation procedure was performed using a published method of McBurney and Thompson (1989) and Mallillin *et al.* (2008). A 0.5 g of fiber isolate was placed in a 100-mL serum bottle. Fermentation media (40 mL of a mixture of 2 L deionized water, 1 L 0.5 M sodium bicarbonate buffer solution, 1 L macromineral solution, 5 mL 0.1% resazurin) and reducing solution (2 mL of a mixture of 1.25 g cysteine-HCl + 50 pellets of potassium hydroxide in 100 mL deionized water and 1.25 g sodium sulphide in 100 mL deionized water) was added to the serum bottle. The bottle was flushed with carbon dioxide until the mixture became colorless, then sealed with rubber stoppers and an aluminum crimp seal. The solution was stored at 4°C overnight. The bottle was placed in a water bath for 1-2 h at 37°C. A 10 mL faecal inoculum (1:15 dilution of fresh faeces from a healthy human volunteer eating an unspecified diet with no intake of antibiotics for a year) was added to the bottle. The mixture was incubated for 24 h at 37°C without mixing. The fermented digest was filtered and analyzed using HPLC (LC10 Shimadzu, Shimadzu, Japan) to measure short-chain fatty acid (SCFA) against a volatile acid standard mixture of acetate, propionate and butyrate (Supelco, USA).

2.4 Phytonutrient content and antioxidant activity

2.4.1 Extraction of bioactive compounds

The bioactive compounds of langkauas powder were extracted following the method used in the studies of Shakirin *et al.* (2010) and Omena *et al.* (2012). Approximately 1 g of freeze-dried langkauas powder

was weighed and placed in Erlenmeyer flask with a cover. Approximately 25 mL of acidified 70% ethanol was added to the sample and shaken for 1 h in a shaking water bath at room temperature. After 1 h, 25 mL of 70% acetone was added again to the solution and was continued in a water bath shaker for another 1 h. The mixture was then transferred and centrifuged at 2500 rpm for 15 min. The extract was then placed in different glass vial containers for further analysis.

2.4.2 Total polyphenol content determination

The method of Chew *et al.* (2011) was used to determine the total polyphenol content of the sample. In a 5 mL test tube, an aliquot (2 mL) of appropriately diluted extract was mixed with 1.5 mL of 1 N Folin-Ciocalteu reagent and the mixture was allowed to stand at 22°C for 5 min. Then, 1.5 of saturated sodium carbonate solution (60 g/L) was added to the mixture. The end volume of the mixture was made up to 10 mL with distilled water and mixed thoroughly. Also, the absorbance quality of the reaction mixture was measured at 725 nm against a blank after 90 min at 22°C. Sample extracts were analyzed in triplicate. Gallic acid (25-150 µg/mL) was used to construct a standard curve for quantification and the results were expressed as mg gallic acid equivalent (GAE)/g DW of sample.

2.4.3 Total flavonoid content determination

Total flavonoids content (TFC) of the sample extract was measured following the aluminum chloride colorimetric assay as described by Chew *et al.* (2011). An aliquot (1 mL) of appropriately diluted extract was mixed with 4 mL double double-distilled water and 0.2 mL of 5% sodium nitrite. After 5 min, 0.3 mL of 10% aluminum chloride was added to the mixture. After 6 min, 2 mL of 1 M sodium hydroxide was also added, then the volume was diluted with double distilled water. Absorbance of the reaction mixture was measured at 510 nm against a blank. Catechin (50-300 mg/L) was used to construct a calibration curve for quantification, and the results were expressed as mg catechin equivalent/g DW of sample.

2.4.4 Total anthocyanidin content determination

The total anthocyanidin content (TAC) of langkauas rhizomes was determined using the method of Zheng *et al.* (2013). Briefly, an aliquot (1 mL) of plant extract was diluted with 2.5 mL of 1% (w/v) vanillin in ethanol and 2.5 mL of 9.0 N HCl in ethanol. The solution was mixed and incubated for 20 min at 30°C. Absorbance of the reaction mixture was measured at 500 nm against a blank. All sample extracts were analyzed in triplicate. Catechin (50-300 mg/L) was used to construct a calibration curve for quantification, and the results were

expressed as mg catechin equivalent/g DW of sample.

2.4.5 1,1-Diphenyl-2-picrylhydrazyl radical scavenging assay

Scavenging activity of DPPH radical of langkaus rhizomes extract was determined as per Shakirin *et al.* (2010) with some modifications. An aliquot of 0.5 mL of the sample extract was mixed with 2 mL of DPPH solution. The mixture was shaken vigorously and left to stand in a dark room for 10 min at room temperature. The absorbance was measured at 515 nm against a blank, using a UV-VIS spectrophotometer. All sample extracts were analyzed in triplicate. Percent inhibition was determined using the formula:

$$\% \text{ inhibition} = \frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{control}}} \times 100$$

2.4.6 Ferric reducing ability plasma assay

FRAP assay was conducted in accordance with the method of Omena *et al.* (2012). The method was based on the reduction of ferric-tripyridyl triazine complex to its ferrous, dark blue color in the absence and presence of antioxidants. Briefly, the FRAP reagent was prepared by mixing 5 mL of 10 mM TPTZ 2,4,6-Tris(2-pyridyl)-s-triazine solution in 40 mmol/L HCl, and 5 mL of 20 mmol/L FeCl₃ and 50 mL of 0.30 mol/L acetate buffer (pH=3.6). The reagents were heated to 37°C for 10 min. A 0.1 mL of sample aliquot was mixed with 3 mL of FRAP reagents and was incubated at 37°C for 10 min. The absorbance of the reaction mixture was measured at 593 nm using UV-VIS spectrophotometer against a calibration curve prepared with Trolox (90-455 µM). Afterwards, the following formula was used:

$$\text{FRAP} \left(\frac{\text{mg}}{100 \text{ g}} \right) = \frac{\text{Concentration} \times 0.05 \text{ L}}{\text{sample weight}} \times 100$$

2.5 Safety assessment

2.5.1 Heavy metal content

The levels of heavy metals and total microbial load were quantified for the safety assessment study. The Atomic Absorption Spectrophotometry (AAS)-Cold Vapor Technique was used for the determination of Mercury, while the Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES) was used to determine Arsenic, Cadmium, Chromium and Lead from the langkaus rhizome samples.

2.5.2 Microbiological analysis

Aerobic Plate Count, Yeast and Mold Count, and determination of *Salmonella*, *Staphylococcus aureus*, as well as *Escherichia coli* count were performed as part of the microbiological analyses in accordance with the methods from the Bacteriological Analytical Manual (BAM, 2001) and AOAC INTERNATIONAL (2014).

The fresh langkaus rhizomes were analyzed for microbiological quality based on the following parameters and methodologies: (a) Total Plate Count by FDA BAM Ch.3; (b) Yeast and Mold Count by FDA BAM Ch. 18; (c) *Staphylococcus aureus* by FDA BAM Ch. 12; (d) *Salmonella* by AOAC Official Method 2014.017; and (e) *Escherichia coli* and Coliform Count by FDA BAM Ch. 4. The tests were performed in triplicate.

Prior to analysis, 50 g of sample was weighed for consistency. A 450 mL of 0.1% peptone water was added to each weighed sample for Yeast and Molds Count, and a 450 mL of Butterfield's Phosphate-Buffered Dilution (BPB) was added for Aerobic Plate Count. The samples were subjected to homogenization in a stomacher (Stomacher 400 Circulator, Seward Company, USA) for 2 min. The entire analysis was performed in Biosafety Cabinet Class II (Nuaire Biosafety Cabinet NU-543-600S).

The Aerobic Plate Count was determined using the Pour Plate Method. A diluted sample using a decimal dilution series (10⁻¹, 10⁻², 10⁻³) based on the estimated microbial density was prepared. A 1 mL of each dilution was dispensed into labeled petri dishes in triplicate. Cooled plate count agar (45±1°C) was added to each plate within 15 min of making the dilution, in quantities of 12-15 mL. Control plates were also prepared with dilution water. The petri dishes were incubated at 35°C for 48±2°C. All counts were rounded off to two significant figures.

The yeasts and molds were determined using the Spread Plate Method. A 10 mL of the previous dilution was transferred to 90 mL of diluent using sterile pipette. A 0.1 mL of each dilution was aseptically pipetted onto pre-poured DRBC agar plates that had been solidified, and the inoculum was spread using a sterile spreader. Each dilution was plated in triplicate to ensure accuracy and reliability of the results. The plates were incubated at 25°C for 5 to 7 days in a low-temperature incubator (ThermoStable 1R-250, DAIHAN Scientific Co., Ltd., Korea). Only the plates with 10 to 150 colonies were counted using a colony counter (BOECO Colony Counter CC-1, Boeckel Co (GmbH Co.) KG, Germany).

To determine *S. aureus* count, approximately 450 mL of phosphate-buffered dilution water was added to the stomacher and homogenized for 2 min. Serial dilutions were prepared, mixing each by shaking 25 times over a 30 cm arc for 7 s. For each dilution, 1 mL was aseptically distributed across three Baird-Parker agar plates (0.4 mL, 0.3 mL, and 0.3 mL) and spread using a sterile bent glass rod. Plates were kept upright for 10 min

to allow absorption, then incubated upright at 35°C for 1 hour, followed by inversion and incubation for 48 h at 35°C. Plates with 20–200 colonies were selected; suspected *S. aureus* colonies were transferred to BHI broth, emulsified, and incubated at 35°C for 18–24 hours. To 0.5 mL of BHI culture, 0.5 mL of reconstituted coagulase plasma with EDTA was added, mixed, and incubated at 35–37°C for up to 6 h. Clot formation, assessed by tube inversion, indicated a positive result. Colony counts were performed using a colony counter and reported as CFU/g.

Escherichia coli enumeration was performed using the Most Probable Number (MPN) method via the Multiple-Tube Fermentation Technique. Serial dilutions from aerobic plate counts were used to inoculate triplicate Lauryl Sulfate Broth (LSB) tubes (1 mL each) across at least three consecutive dilutions. Tubes were incubated at 35±0.5°C for 48±3 h and scored positive based on turbidity and gas production. Presumptive positive tubes were confirmed by transferring a loopful of suspension into Brilliant Green Lactose Bile (BGLB) broth with inverted Durham tubes, incubated under identical conditions, with gas production indicating confirmed positives.

From confirmed tubes, a loopful was inoculated into EC broth and incubated at 44.5±0.5°C for 24±2 h; negative tubes were re-incubated and re-examined at 48±2 h. Gas-positive EC tubes indicated the presence of fecal coliforms.

To confirm *E. coli*, broth from gas-positive EC tubes was streaked onto L-EMB agar and incubated at 35±0.5°C for 18–24 h. Colonies with dark centers and flat morphology, with or without metallic sheen, were selected (up to five per plate), subcultured onto PCA slants, and incubated under the same conditions. Isolates were Gram-stained and subjected to IMViC biochemical tests: indole production (Kovacs' reagent, positive indicated by red layer), Voges-Proskauer (eosin-pink color after α -naphthol and KOH treatment), Methyl Red (red color after methyl red reagent addition), and citrate utilization (turbidity in Koser's citrate broth). Gas production was also assessed in LST broth. Isolates identified as Gram-negative, non-spore-forming rods that fermented lactose with gas production within 48 h and exhibited IMViC patterns of ++-- (biotype 1) or +--- (biotype 2) were confirmed as *E. coli*. The MPN of *E. coli* was calculated based on positive EC tubes across serial dilutions.

Salmonella spp. were analyzed using 3M™ Petrifilm™ *Salmonella* Express (SALX) System in accordance with AOAC 2014.017.

2.6 Product development

2.6.1 Preparation of langkauas extract

Freshly harvested langkauas rhizomes were used for the extraction of juice. The extraction process was patterned from the study of Sasi Kumar *et al.* (2013) with minor modifications. Each rhizome was thoroughly washed, brushed, and cleaned. The peels were removed using a knife and grated into a pulp. The juice was extracted by filtering through muslin cloth and was stored in a refrigerated temperature separately.

2.6.2 Lemonade production with langkauas extract

The ingredients to produce lemonade, including pure lemon juice, sucrose, citric acid, ascorbic acid, lemon flavor, and distilled water, were purchased at the local supermarkets. The preparation of lemonades was patterned from the study of Tamer *et al.* (2017) with minor modifications. The basic formulation of lemonade consists of 17 g of lemon juice concentrate, 120 g of sucrose, 2.1 g citric acid, 0.5 g ascorbic acid, 0.4 mL natural lemon flavor, and 860 g distilled water. The amount of water was substituted with 10%, 15% and 20% of langkauas extract. For the lemonade production, sucrose, citric acid, ascorbic acid, natural lemon flavor, and distilled water were added to pure lemon juice as determined in the formulation (Table 1). The langkauas extract was heated at 98°C for 5 min and was then cooled down to room temperature. After cooling, the extract was transferred to sterile glass bottles, capped, and stored at 4°C before sensory evaluation.

Table 1. Formulation of lemonade with langkauas extract.

Ingredients	Control	Formulation 1	Formulation 2	Formulation 3
Langkauas extract (g)	-	86	129	172
Pure lemon juice (g)	17	17	17	17
Sucrose (g)	120	120	120	120
Citric acid (g)	2.1	2.1	2.1	2.1
Ascorbic acid (g)	0.5	0.5	0.5	0.5
Natural lemon flavor (mL)	0.4	0.4	0.4	0.4
Distilled water (g)	860	774	731	688

2.6.3 Sensory evaluation

Approval from the Ethics Review Committee of the University of Santo Tomas Graduate School (ERC-USTGS) was sought before the conduct of the product development. Formulated lemonade with varying amounts of langkauas extract was subjected to sensory evaluation. The consumer acceptability of all formulated lemonade was determined using 50 untrained randomly

selected panelists (Mihafu *et al.*, 2020) from the municipality of Tabaco City, Albay, ages 18-56 years old. The sensory attributes evaluated were color, appearance, aroma, mouthfeel, flavor, and general acceptability.

Each panelist received a participant's information letter, consent form, and sensory evaluation form with four 3-coded samples. Each sample (100 mL) was served in a clear plastic cup with a bottle of water. The panelist was tasked to evaluate the samples for each of the sensory attributes according to their preference using the 9-point hedonic scale (1-dislike extremely to 9-like extremely).

2.7 Data analysis

Results were expressed as mean values and standard error of the means (SEM). Proximate analyses, total dietary fiber, soluble and insoluble dietary fiber analysis, and fermentability tests were performed in triplicate. Heavy metals, microbial analysis, phytonutrient content and antioxidant activity were also analyzed in triplicate. ANOVA was used to determine any significant differences among mean values ($p < 0.05$), followed by Duncan's multiple range test to determine which mean value was significantly different, using SPSS Statistics software (Version 20).

3. Results and discussion

3.1 Proximate composition of langkauas rhizomes

The proximate composition analysis was conducted to provide the nutritional information for langkauas rhizome, which includes moisture, carbohydrate, protein, fat, and ash. As shown in Table 2, results revealed that the langkauas rhizome has a high moisture content of 81.76 ± 0.01 g/100 g which may indicate high perishability of the sample, and was comparable to the moisture content of other common spices of the family Zingiberaceae such as ginger (*Zingiber officinale*) with 81.3%; and turmeric (*Curcuma longa*) with 91.5 ± 0.65 g/100 g (Chan *et al.*, 2011; Hirun *et al.*, 2012; Jayashree *et al.*, 2014).

Table 2. Proximate composition of langkauas rhizome

Nutrient	Mean \pm SEM, g/100 g
Ash	1.68 ± 0.01
Total Fat	0.74 ± 0.04
Protein	1.45 ± 0.00
Moisture	81.76 ± 0.01
Carbohydrates	14.37 ± 0.01

Carbohydrates, proteins and fats form the major parts of the diet (Indrayan *et al.*, 2009). According to the Food and Drug Administration-Philippines (FDA, 2015),

a food product that contains 3 g of fat/100 g sample is considered to be low-fat, and a food with 0.5 g of fat/100 g of sample is considered to be fat-free. Values reported by Indrayan *et al.* (2009) and Yaneff (2017) showed that the total fat of langkauas rhizome was 1.14% and 1.0 g/100 g, respectively, which were higher than the obtained value of 0.74 ± 0.04 g/100 g in this study. According to Okpala and Gibson-Umeh (2013), low-fat food is less prone to lipid-related forms of deterioration. Langkauas rhizome has a protein content of 1.45 ± 0.00 g/100 g. The value obtained was much lower than the study of Indrayan *et al.* (2009), which is 4.44%, but slightly higher than the reported value of 1.0 g/100 by Yaneff (2017). Protein and its amino acid contents are important in cell structure, enzyme and hormone regulation, repair and growth of tissue in the body, and proper functioning of antibodies in fighting infections (Muruet *et al.*, 2007).

Carbohydrates are one of the major components found in the langkauas rhizome. Based on the present study, the carbohydrate content obtained was 14.37 ± 0.01 g/100 g, which was lower than the reported value of 15g/100 g by Yaneff (2017). The ash content of langkauas rhizome in this study (1.68 ± 0.01 g/100 g) was lower compared to the findings of Indrayan *et al.* (2009) with 3.04g/100 g. However, Indrayan *et al.* (2009) and Muhammad *et al.* (2015) reported that the ash content of a plant is relatively smaller compared to other proximate compositions but has nutritionally important mineral contents.

The varying results of the proximate composition of langkauas rhizome from the other studies can be attributed to the soil and geographical location of the plant source, as well as variety (Odeunmi *et al.*, 2010; Millena and Sagum, 2018).

3.1.1 Dietary fiber content of langkauas rhizome

Table 3 presents the results of the dietary fiber analysis, wherein data show that the langkauas rhizome contains a total dietary fiber of 5.49 ± 0.24 g/100 g sample, with 2.86 ± 0.01 g/100 g of insoluble dietary fiber (IDF) and 2.63 ± 0.25 g/100 g of soluble dietary fiber (SDF). Based on the United States Food and Drug Administration (USFDA, 2002) and the study of Anderson *et al.* (2010), a food product that contains 2.5-4.75 g/100 g of fiber in the food sample is considered to be a good source of dietary fiber; and a content of at least 5 g/100 g per serving is considered to be a high source of dietary fiber. The values obtained from this study revealed that langkauas rhizome can be considered a high source of dietary fiber. SDF and IDF are important for both their dietary and functional properties. Results of this study showed a 1:1 ratio of soluble to insoluble

dietary fiber, which is not common in other food sources. Food ingredients with an SDF/IDF ratio close to 1:2 may indicate balanced dietary and functional properties (Figuerola *et al.*, 2005).

Table 3. Dietary fiber content of langkauas rhizome.

Dietary Fibers	Mean±SEM, g/100 g
Total	5.49±0.24
Insoluble	2.86±0.01
Soluble	2.63±0.25

3.2 *In vitro* fermentability of langkauas rhizome

The dietary fiber content of langkauas rhizome sample was isolated and fermented using human fecal inoculum. The short-chain fatty acids produced from *in vitro* fermentation of fiber isolates were analyzed, and the results are revealed in Table 4. According to Wong *et al.* (2006), dietary carbohydrates, specifically dietary fiber and resistant starches, are substrates for fermentation that produce SCFAs, primarily acetate, propionate, and butyrate, as end products. Acetate enters the peripheral circulation to be metabolized by peripheral tissues; propionate is largely taken up by the liver, and butyrate is the major energy source for colonocytes. As shown in the table, the study obtained an amount of 2.89±0.39 mg/g propionate; however, acetate and butyrate were not detected. Propionate has a significant role in the prevention of risks of hypercholesterolemia and cardiovascular disease since they are known to affect lipid and cholesterol metabolism (Jonathan *et al.*, 2012) and may also have systemic effects, specifically a hypolipidemic action (Wong *et al.*, 2006).

Table 4. Short-chain fatty acid content of langkauas rhizome.

Short-chain fatty acid	Mean±SEM, g/100 g
Acetate	N.D.*
Propionate	2.89±0.39
Butyrate	N.D.*

*Not Detected

3.3 Phytonutrient content of langkauas rhizome

According to Chudiwal *et al.* (2010), terpinen-4-ol is one of the most active compounds present in langkauas plant and also contains some important phytoconstituents, such as alkaloids, glycosides, saponins, terpenoids, phenolics, phytosterols, and carbohydrates. As shown in Table 5, the study obtained notable amounts of total polyphenols and total anthocyanidins, with 618.96±8.02 mg/100 g and 140.92±4.92 mg/100 g, respectively. Total flavonoids, however, were not detected. One of the important classes of natural antioxidants found in plants that scavenge free radicals is polyphenols. Polyphenols are able to neutralize free radicals, break down peroxides and

scavenge singlet and triplet oxygen (Chew *et al.*, 2009). The polyphenols that have been isolated from *Alpinia* species include quercetin, kaempferol, isorhamnetin, myricetin, flavone C-glycoside, and proanthocyanidins, which act as hydrogen donors, reducing agents and singlet oxygen quenchers (Wong *et al.*, 2009). According to Chew *et al.* (2011), the amount of TPC could be categorized into 4 classes: high (>5000 mg GAE/100 g), medium high (3000-5000 mg GAE/100 g), medium low (1000-3000 mg GAE/100 g) and low (<1000 mg GAE/100 g). The langkauas rhizome in this study showed a low level of TPC of 618.96±8.02 mg/100 g, similar to the study of Tangkanakul *et al.* (2009), which also showed relatively low antioxidant activity of 216.63±3.33 mg/100 g.

Table 5. Phytonutrient content of langkauas rhizome.

Phytonutrient content	Mean±SEM
Total Polyphenols (mg gallic/100g)	618.96±8.02
Total Anthocyanidins (mg catechin/100g)	140.92±4.93
Total Flavonoids	N.D.*

*Not Detected

The langkauas rhizome used was yellowish in color, which somehow explains the low anthocyanidin content of 140.92±4.93 mg/100 g. Anthocyanins are the glucosides of anthocyanidins, which are responsible for blue, red, violet and purple colors. Anthocyanidins include delphinidin, cyanidin, pelargonidin, malvidin, petunidin and peonidin (Martinez *et al.*, 2017), which may be responsible for some biological activities, including lowering or preventing the risk of cardiovascular disease, cancer, diabetes and arthritis (Miguel, 2011).

The data gathered reveals that the langkauas rhizomes can be a promising source of bioactive compounds and could enhance the quality of food when used as an ingredient.

3.4 Antioxidant activity of langkauas rhizome

The antioxidant activity of langkauas rhizome was measured through the DPPH free radical scavenging assay and ferric reducing antioxidant power (FRAP). DPPH radical scavenging activity was used to determine the antioxidant capacity by the measurement of the decrease in DPPH radical absorption after exposure to radical scavengers, while FRAP was used to measure the ability of the sample to reduce metals (Stanojevic *et al.*, 2009). As shown in Table 6, the antioxidant activities of langkauas rhizome were 522.62±6.65 mg Trolox/100g in FRAP assay, and 27.77±0.78% inhibition in DPPH assay. Antioxidants observed in langkauas rhizome can act as radical scavengers that may protect the cells against a number of diseases, which include

neurodegenerative disorders and cancer (Wong *et al.*, 2009). The value obtained for the DPPH assay in this study was lower than the values obtained by Mahae and Chaiseri (2009), possibly due to the differences in preparing the raw material, as well as the use of different concentrations of solvents during sample extraction.

Table 6. Antioxidant activity of langkauas rhizome.

Antioxidant activity	Mean±SEM
DPPH (% inhibition/0.2g)	27.77±0.78
FRAP (mg Trolox/100g)	522.62±6.65

In this study, the reduction of the violet of DPPH to a pale yellow was due to the abstraction of a hydrogen atom from the antioxidant compound (Shakirin *et al.*, 2010). The main reason for high antioxidant activity towards DPPH radicals is due to the high phenolic content of langkauas rhizome (Jayaprakasha and Patil, 2007), and more DPPH reduction happens when there are more antioxidants in the extract (Molyneux, 2003). Primarily, the FRAP assay treats the antioxidants in the sample as a reductant in a redox-linked colorimetric reaction (Guo *et al.*, 2003). As shown in Table 6, langkauas rhizome exhibited good ferric reducing antioxidant power (FRAP) of 522.62±6.65 mg/100 g. This may imply that the phytonutrients present in langkauas rhizome can inhibit free radical scavenging activity and reduce oxidation.

3.5 Heavy metals evaluation of langkauas rhizome

According to the Codex Alimentarius (2017), there is no established standard yet for langkauas rhizomes and their food products. Values obtained were patterned from the established standards for root and tuber commodities. Based on Table 7, results for the analysis of arsenic, cadmium, chromium, and mercury showed that there is no trace of the heavy metal elements detected from the langkauas rhizome. The amount of Lead showed that the langkauas rhizome contained 0.022 µg/g, but within the established permissible limit of 10 µg/g (Gupta *et al.*, 2010). The presence of small amounts of lead may have been absorbed from air, soil and water runoff. According to Bandaranayake (2006), the potential intake of toxic metals, specifically lead, can be estimated based on the level of its presence in the product and the estimated or recommended dosage of the product. The possible exposure can be put into a toxicological perspective called the Provisional Tolerable Weekly Intake values (PTWI) for toxic metals, which have been established by the Food and Agriculture Organization of the World Health Organization (FAO-WHO). According to Vracko *et al.* (2007), exposure to lead should not exceed the PTWI of 25 µg/kg (25000 µg/g) bodyweight/week. Hence, the results obtained indicate that langkauas

rhizome can be processed and utilized into a food product and considered to be safe for consumption.

Table 7. Heavy metal content of langkauas rhizomes.

Heavy metals	Mean±SEM, µg/g
Arsenic (As)	ND
Lead (Pb)	0.022±0.00
Cadmium (Cd)	ND
Chromium (Cr)	ND
Mercury (Hg)	ND

ND: Not detected.

3.6 Microbiological evaluation of langkauas rhizome

The results of microbiological evaluation of langkauas rhizomes are presented in Table 8. Total plate count was 5.4×10^6 CFU/g and *S. aureus* was 2.1×10^3 CFU/g, which means that the viable organisms present in langkauas were within the acceptable limit of 10^6 cfu/g and 10^4 CFU/g, respectively. *Salmonella* was absent in the sample. The detection limit based on the volume of sample analyzed was negative for *E. coli*. Since there is no established standard yet for langkauas rhizome, values were patterned from the established standards of the Food and Drug Administration (FDA, 2022) for spices.

Table 8. Microbiological assessment of langkauas rhizome.

Parameters	CFU/g	FDA standards	
		m	M
Total plate count	5.4×10^6	10^5	10^6
Yeast and molds	$>6.5 \times 10^6$	10^3	10^4
<i>Staphylococcus aureus</i>	2.1×10^3	10^2	10^4
<i>Escherichia coli</i>	Negative	Negative	Negative
<i>Salmonella</i>	Absent	Absent	Absent

m: acceptable level of microorganism determined by a specified method; the values are generally based on levels that are achievable under GMP, M: level which, when exceeded in one or more samples, would cause the lot to be rejected as this indicates a potential health hazard or imminent spoilage.

Since the obtained value of yeast and molds exceeded the standard limits, various physical cleaning treatments have been performed to lessen the presence of yeast and mold present in the sample. Physical cleaning treatments included: (a) thorough washing, (b) brushing and washing, and (c) peeling and washing. Analysis of yeast and molds was repeated to meet the requirements for safe use of langkauas. As shown in Table 9, thorough washing of langkauas rhizome has a yeast and mold count of 3.6×10^4 CFU/g; brushing and washing treatment yielded 3.0×10^4 CFU/g; and peeling and washing treatment resulted in 1.4×10^4 CFU/g. Based on the values obtained, the application of the different physical cleaning treatments of langkauas rhizomes resulted in the

lessening of yeast and mold count and met the standard limits established by the FDA upon re-testing.

Table 9. Yeast and mold count of langkauas rhizome utilizing different cleaning treatments.

Treatments	CFU/g
(a) - thorough washing	3.6×10^4
(b) - brushing and washing	3.0×10^4
(c) - peeling and washing	1.4×10^4

ND: Not detected.

All findings from the different parameters tested fell within the standards established by the FDA. Hence, the langkauas rhizomes are deemed acceptable and safe for consumption. However, growing conditions, harvesting, processing method, storage conditions, and post-harvest treatment should be carefully observed to prevent microbial contamination.

3.7 Sensory evaluation of lemonades with langkauas extracts

The sensory assessment is the primary aspect for designing new foods because changes in color, aroma or texture of the original matrices can substantially affect the acceptability of the consumer (Sanchez-Bel *et al.*, 2015). According to Li *et al.* (2012), the commonly used test in conducting sensory evaluation is a 9-point hedonic scale because the outcome has been reported to be closest to a consumer's preference. Moreover, it still remains the most reliable sensorial test because it enables the overall sensory attributes. The 9-point structure scale evaluation was done, with 9 as the best and 1 being the worst quality.

Lemonade with langkauas extract (LE) was developed to utilize the raw material. Different formulations were developed with varying amounts of langkauas extract (0%, 10%, 15% and 20%) labeled with a 3-digit code number as shown in Figure 3. Color, appearance, aroma, mouthfeel, flavor, and general acceptability were the sensory attributes evaluated.

The mean scores of the sensory evaluation using 50

untrained panelists and 9-point hedonic scale are summarized in Table 10. In terms of appearance, sensory evaluation revealed that lemonade with 10% and 15% LE concentration has no significant effect on the control lemonade, but incorporation of 20% LE concentration would lead to significant changes in the appearance. A similar observation was noted with the color attribute. The 20% LE inclusion in the lemonade would lead to a change in color in terms of transparency.



Figure 3. Lemonade with varying amounts (0%,10%,15% and 20%) of langkauas extract (from left to right).

A different observation was noted with aroma attributes. Statistically, there is a significant difference observed between control, lemonade with 10%, 15% and 20% LE. An amount of 10% and 15% LE gave a langkauas-like aroma from the control. However, inclusion of 20% LE concentration increases the distinct langkauas-like aroma, leading to the lowest rating in terms of aroma. As for the mouthfeel, lemonade without LE and lemonade with 10% and 15% LE were not significantly different, except for the lemonade with 20%. It was observed that an increasing amount of substitution of LE affects the mouthfeel attribute of the lemonade. With regard to the flavor attribute, significant differences were observed between the control and lemonade with 10, 15 and 20% LE. This may imply that the astringent taste of LE was perceived by the panelists.

The general acceptability reflects the overall acceptability of the panelists on the product, considering the sensory attributes. The appearance, color, aroma, mouthfeel and taste were all considered in evaluating the general acceptability of the product developed. As shown in the table, lemonade without LE was highly acceptable

Table 10. Sensory evaluation of lemonades with langkauas extracts.

Sensory attributes	Lemonade w/o LE (Control)	Lemonade w/ 10% LE (Sample 1)	Lemonade w/ 15% LE (Sample 2)	Lemonade w/ 20% LE (Sample 3)
Appearance	8.38±0.12 ^a	8.26±0.11 ^{ab}	8.24±0.12 ^{ab}	7.92±0.17 ^b
Color	8.30±0.13 ^a	8.30±0.10 ^a	8.22±0.12 ^a	7.78±0.18 ^b
Aroma	8.72±0.06 ^a	8.06±0.14 ^b	8.08±0.13 ^b	7.54±0.20 ^c
Mouthfeel	8.66±0.08 ^a	8.52±0.12 ^a	8.46±0.09 ^a	8.04±0.15 ^b
Flavor	8.82±0.05 ^a	8.40±0.12 ^b	8.50±0.12 ^b	8.20±0.12 ^b
General Acceptability	8.72±0.06 ^a	8.44±0.10 ^{ab}	8.54±0.09 ^{ab}	8.26±0.13 ^b

Values are presented as mean±SD. Values with different superscripts in the same row are statistically significantly different ($p < 0.05$). LE: langkauas extract.

for the panelists, while the lemonade with 20% LE was the least acceptable. However, no significant difference was observed between control and lemonades with 10% and 15% LE concentration. Furthermore, among all the samples incorporated with LE, the lemonade with 15% LE was the most acceptable formulation and obtained 8.50, which is closest to the rating of the control with 8.72. Although a significant difference was observed in different sensory attributes and overall acceptability, this implies that the lemonades produced with 10%, 15% and 20% LE concentration were still acceptable to the panelist with a degree of likeness that falls “like very much” in the 9-point hedonic scale.

4. Conclusion

The langkauas rhizome is a good source of carbohydrates, dietary fiber, phytonutrients, and has a significant reducing property against free radical formation. The most preferred lemonade was the one infused with 15% langkauas extract. Hence, langkauas rhizomes may be considered as a potential functional food/ingredient and safe in the development of food products that would greatly benefit human health. It is recommended to conduct efficacy studies to further validate the functional properties of langkauas rhizome when used as an ingredient in newly developed food products. A shelf-life study of the product is also recommended to determine how long this product may be consumed. Incorporation of langkauas rhizomes in other food categories may also be done.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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