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Modification of Tannia (*Xanthoxoma sagittifolium*) Starch using Lactic Acid Bacteria and Its Application for Cookies Products

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This work was performed to evaluate the physico-chemical properties of modified tannia flour (MTF) with lactic acid bacteria. Physico-chemical properties of MTF by analyzing the degree of acidity, swelling power and solubility, functional group analysis by FTIR and XRD patterns, then determine proximate composition of MTF cookies. Modified tannia flour is indicated by intensity decreased of the FTIR and XRD patterns during fermentation. Proximate composition of MTF and cookies with moisture, ash, fat, protein and glucose content products approximately, respectively 7.63 ± 0.05 %, 2.94 ± 0.02 %, 0.01 ± 0.21 %, 0.86 ± 0.09 %, 43.00 ± 0.14 % and 8.76 ± 0.22 %, 2.67 ± 1.18 %, 48.07 ± 0.23 %, 16.52 ± 0.03 %, 9.08 ± 1.77 %. Physico-chemical properties of MTF with lactic acid bacteria had pH of MTF decreased but swelling power and solubility increased during fermentation and pasting characteristics were modified, so MTF can be applied for making cookies product, where the addition of anchovy meat as a source of protein can increase the protein content of cookies product.

Keywords: Tannia, Modified starch, Lactic acid bacteria, Cookies.

INTRODUCTION

Tannia (*Xanthosoma sagittifolium*) is one of the local food that can be used as an alternative carbohydrates. Jointly with taro (*Colocasia esculenta*), tannia represents the third most important root crop after yam and cassava [1]. Both tannia and taro is classified as cocoyam. Cocoyam corm has a variation in chemical composition namely 63-85 % moisture, 4800-5210 kJ/kg energy values, 0.10-0.11 % fat contents, 0.42-1.01 % sugar contents, 13-29 % starches, 0.60-1.18 % dietary fibers, 1.4-3.0 % proteins and 0.60-1.3 % ash [1-3]. Tannia flour contains small granules, therefore, substitution of wheat flour using tannia flour can increase the utilization of tuber tannia, which the functional characteristics and physico-chemical can be used as food [4-11]. Taro flour processing can increase the chemical composition, *e.g.*, decreased levels of calcium oxalate, protein and fat but increases the levels of ash and dietary fiber [3].

Several studies have been reported about characteristic of cocoyam flour. FTIR analysis of cocoyam flour confirmed the presence of rich carbohydrates in the samples [5,8,10], moreover, its flour has a pattern like its starch [6-10,12,13]. The pasting properties of cocoyam starch are reported by several workers [4,8-11,13]. Applications of starch in food system are primarily governed by swelling power, solubility and pH [4,8,9,12].

Tannia flour can be modified by several methods. Modified starches are used to improve viscosity, shelf stability, particulate integrity, processing parameters, textures, appearance and emulsification [14]. Natural fermentation, enzyme fermentation, oxidized, acetylated and acid thinned modification treatments were used [12,14-17]. Fermentation techniques can increase chemical composition of taro flour [15-17], which is useful tool that can be used to improve the nutritional quality and safety profile of tannia flour as a raw material for food products development. Successful performance of modified cocoyam flours using natural fermentation, glucoamylase fermentation, oxidized, acetylated and acid thinned as food ingredients depend upon the functional characteristics. The functional characteristics of starch including swelling power, solubility, pH and pasting properties have been studied [12,15,17]. Lactic acid bacteria (LAB) are found to be useful in flavouring foods, in inhibiting spoilage bacteria and pathogens, in intestinal health and other health benefits related to blood cholesterol levels, immune competence and antibiotics production [16]. Wahyuni *et al*. [18] made a succesful bread using modified wikau maombo flour by LAB to substitution wheat flour.

Ojinnaka *et al*. [14] reported that cocoyam starch modification by acid and enzyme glucoamylase can be used for cookies production. Sensory and qualities cookies showed cookies prepared at 5 % level of substitution were most aceptable. Pricilia *et al*. [19] produced a sweet bread that choose from panelist preferred the colour, aroma and flavour with a modified tannia flour composition of 70 % lactic acid bacteria and 30 % wheat. Based on organoleptic test, it is found that the higher content of tannia flour in the cookies, the lower protein content [20]. Similiarly, Abera *et al*. [3] reported that to increase the protein value of tannia cookies, it is necessary to substitute anchovy meat as a protein source, given enough anchovy available and often consumed by people without causing allergic effects. Making cookies with anchovy substitution is expected to increase the protein content of tannia cookies that could potentially be a product that has a functional value.

In this study, tannia flour was modified using lactic acid bacteria (LAB). The physico-chemical parameters of modified tannia flour (MTF) were evaluated by proximate analysis, FTIR, XRD, pasting characteristics, swelling power, solubility and pH.

EXPERIMENTAL

Chemicals used in this work were of analytical grade. Materials used in the making process of flour is tannia root were obtained from Kendari city and starter lactic acid bacteria (LAB) UP.1.3 isolate was obtained from collection of Food Science and Technology Laboratory, Agricultural Faculty Halu Oleo University, Kendari, Indonesia. Materials used for the manufacturing of cookies were modified tannia flour (100 g), anchovy meat, chicken eggs (160 g), butter (75 g), Hilo teen milk (20 g), sodium bicarbonate (0.75 g) and sugar flour (75 g) obtained from the local supermarket of Kendari city. Media *Lactobacillus* deMan Rogose Sharp (MRS) was purchased from Merck, Germany.

Preparation of modified tannia flour (MTF): Preparation of modified tannia flour were determined by previous method [21]. Tannia were peeled, washed, chipped and then soaked in 5 % salt water for 3 h. Then, tannia was washed with fresh water for 2 days to remove the formed mucus during immersion, thinly sliced with a thickness of ± 0.3 cm and then dried using an oven at 60 ºC for 48 h. Once dried, crushed and sieved using 80 mesh sieve until be tannia flour. The process continued with the addition of lactic acid bacteria (± 200 colony for 300 g of roots) with methods of bribery and fermented for 3 days in incubator (35 ºC) by means of dry fermentation. Furthermore, the process of downsizing, drying oven and flouring to be modified tannia flour.

Standard AOAC methods [22] were adopted for estimating moisture, ash, protein, fat and carbohidrat contents.

FTIR: Infrared analysis of modified tannia flour (MTF) was analyzed using infrared spectroscopy (Shimadzu).

X-ray diffraction: Modified tannia flour (MTF) were characterized using XDR JEOL JDX-3530 X-ray diffractometer in order to determine the crystal phase and crystallinity. Sample (1 g) was placed in a cell diffraction (sample holder) and then irradiated with CuKα radiation at wavelength λ = 1.541 Å, voltage at 40 kV and current at 30 mA with a range of angles 2θ = 5-50º.

Pasting characteristics: Pasting profiles of tannia starch dispersions were obtained using a Rapid Visco Analyser (RVA, Newport Scientific, Warriewood, Australia). A 3.0 g of 12 g/100 g wet basis of modified tannia flour (MTF) was dispersed in 25 mL of distilled water. Samples were heated from 50 to 95 ºC with rotation speed was 960 rpm for the first 10 s and changed to 160 rpm until the end. Pasting temperature (PT), peak viscosity (PV), hold viscosity (HV), breakdown viscosity (BV) and setback viscosity (SV) were recorded from the graph.

Swelling power and solubility: Swelling power and solubility were analyzed using the method performed by Tattiyakul *et al*. [4] modified by the suspension sample is heated in a water bath at 90 ºC for 30 min. The suspension was centrifugated at 5000 g for 10 min. The supernatant was decanted and dried at 110 ºC until a constant weight was reached. Solubility was expressed as the percentage of dried solid weight based on the dry sample weight. Swelling power was represented as the sedimented fraction, which was weighed and its mass related to that of dry starch to give the swelling power (g/g).

pH: Modified tannia flour (20 g) was prepared with 100 mL water. pH of the samples was determined using a pH meter Jenway 3505 (Camlab, UK). The pH was adjusted with buffer solution (pH 4 and 7) and the measurement were performed in triplicates.

Preparations of anchovy meat: The preparation process of anchovy meat includes removing bones and head up the entire contents of its stomach, then washed to remove dirt, anchovy meat back washed with lime juice and rinsed with clean water and then pulverized in blender, then drained and pressed with a cloth.

Cookies test: Preparation of cookies were done by weighing each ingredient and then eggs, butter, sugar and salt whipped until soft. After that added the flour mixture (baking soda and anchovy meat) little by little. Once evenly mixed, the dough is shaped and molded crushed, then grilled in oven at 130 ºC for 20 min. Chemical constituents of cookies were analyzed by standard AOAC [22] for estimating moisture, ash, protein and fat contents while carbohydrate content was analyzed using Nelson-Smogyi method [23].

Statistical analysis: Analysis were done in triplicate and standard deviations were calculated. Analysis of variance was performed to calculate significant differences in treatment means and LSD ($P < 0.05$) was used to separate means.

RESULTS AND DISCUSSION

Chemical composition of tannia flour: Carbohydrates, proteins, minerals and vitamins are some of the basic constituents, which determines its nutritional value. Table-1 shows the effect of fermentation of tannia flour using lactic acid bacteria on the chemical composition. The moisture content of modified tannia flour was found to be 7.63 ± 0.05 %, which is higher than cocoyam flour (natural fermentation) [16] and lower than native tannia flour [12]. Low content of MTF, thereby giving the flours a better shelf life than native tannia flour as the moisture causes the microorganisms metabolism. Moisture contents in modified tannia (oxidized, acetylated, acid-thinned) is less when compared with native tannia flour [1].

TABLE-1
CHEMICAL COMPOSITION OF TANNIA FLOUR

Analysis (%)	MTF (LAB fermentation)	Cocoyam flour (natural fermentation)[a]	Native tannia flour[b]
Moisture	7.63 ± 0.05	5.15 ± 0.50	8.39 ± 0.12
Ash	2.94 ± 0.02	1.91 ± 0.12	2.47 ± 0.18
Fat	0.01 ± 0.21	2.61 ± 0.01	0.09 ± 0.01
Crude fiber	12.10 ± 0.45	0.19 ± 0.25	0.89 ± 0.29
Protein	0.86 ± 0.09	18.75 ± 0.01	0.16 ± 0.06
Glucose	43.00 ± 0.14	–	–
Carbohydrate	–	71.57 ± 0.01	–
Amylose	–	–	22.7 ± 0.14

The number after the sign (±) is the standard deviation value
(-) Not reported
[a]Reported by Igbabul *et al*. [16]
[b]Reported by Lawal [12]

In this study, MTF has a higher ash content 2.94 ± 0.02 % than cocoyam flour and native tannia. Lawal [12] reported that ash content reduced while tannia flour was modified by oxidized, acetylated, acid-thinned. Kaur *et al*. [25] reported that taro flour has ash content 1.2 ± 0.12 %, which was similiar as reported by Igbabul *et al*. [16].

Fat content of MTF is found to be 0.01 ± 0.21 %. Its value is lower than native tannia [1,12,24,25] where fat content ranged between 0.09-0.97 %. Low value of MTF was caused by LAB which produces lipase enzymes that degrade fat even lipase activity tend rather low [26-28]. However, different result was reported by Igbabul *et al*. [16], where fat content was increased during natural fermentation (using deionized water).

Fat and water can bind as a role of crude fiber. The crude fiber content of MTF (12.10 ± 0.45 %) was higher than native tannia. It was caused due to the degradation of cellulose by cellulase enzyme of LAB [29,30]. Igbabul *et al*. [16] reported that cocoyam flour fermented by deionized water also decreased during fermentation. Protein content plays important role in water absorption capacity of flour in food systems. Protein in MTF (0.86 ± 0.09 %) was higher than native tannia (0.16 ± 0.06 %). It was caused by LAB which secreted some extracellular enzymes (proteins) such as amylase, linamarase and cellulase [31].

The glucose (sugar) contents of MTF (43.00 ± 0.14 %) was high as compared to other parameters, thus signified that MTF is a rich source of energy for human nutrition. Fermented tannia flour by LAB can increased glucose content [21].

FTIR analysis: FTIR spectra of MTF in fermentation 1 day, 2 day and 3 day are shown in Fig. 1. Modified tannia flour showed an absorption at wavenumbers 574, 930, 1048, 1080, 1154, 1365, 1471, 1610, 2920 and 3421 cm^{-1}, which indicated that absorption of carbohydrates. In this study, FTIR spectra are similiar with the six varieties of taro flour of Cameroon [5] and four cultivars of taro in India [8]. A peak at 3421 cm^{-1} showed O-H bond stretching, while peak at 2920 cm^{-1} indicated a streching vibration of aliphatic group $-CH_2$ reinforced by its uptake C-H bending vibration at 1471 and 1365 cm^{-1}. A peak appearance at 1610 cm^{-1} is attributed due to a COO^- stretching vibration and a bond streching of C-C and C-O is observed at 1154 cm^{-1} while bending bond C-O-H is observed at 1080 cm^{-1} [31]. The peaks at 1047 and 1022 cm^{-1} are due to the presence of crystalline and amorphous starch while at 930 and 574 cm^{-1} showed the skeleton vibration and ring D-glucopyranosyl [8]. The result obtained in this work was consistent with literature on some tuber starches [5,8,10].

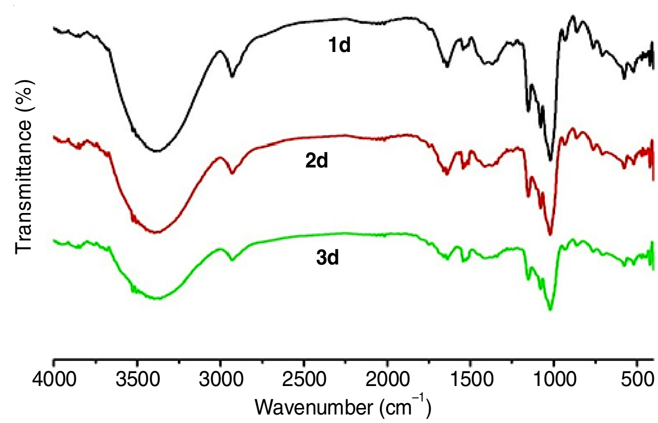

Fig. 1. FTIR spectra of MTF using starter LAB during fermentation

Longer fermentation time caused decrease in peak intensity (Fig. 1) because LAB is capable of producing enzymes which degrade starch functional group [18]. Moreover, fermentation time increased the activity of amylolytic to hydrolyze starch which lowers the amount of bonds in the sample. It is clear that starch of tannia flour had been modified.

XRD analysis: The objective of XRD analysis in MTF to known the XRD pattern that characterize crystalline starch granules. Fig. 2 indicate that the starch belongs to type A. It can be seen from peak position of a strong intensity at 15° and 23° (2θ), a double peak at 17-18° (2θ) and low peak intensity at 20° (2θ) [10]. Similar observations are also reported by Lawal [12] on native, oxidised, acetylated, acid-thinned tannia flour. The results is also consistent with literature on some other tuber starches [7,8,10,13].

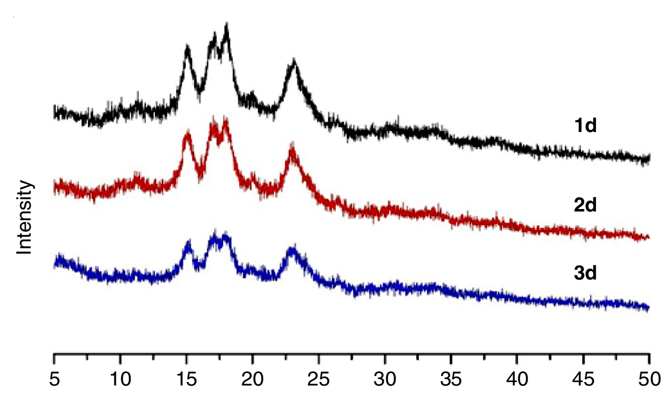

Fig. 2. XRD pattern of MTF using starter LAB during fermentation

Pattern A of amylopectin showed that crystalline starch are due to sequential packing of double helices where amylopectin is the main contributor to crystalline order within the granule [12]. Moreover, peak intensity decreases during fermentation. Fermentation time increased the activity of amylolytic to hydrolyze amylopectin starch which lowers the levels in the samples. Thus, it is confirmed that starch of tannia flour had been modified.

Pasting parameters: Pasting temperature is the temperature at which the viscosity of starch pastes begin to rise. Table-2 showed that pasting temperature of modified tannia flour was lowest than native taro flour and cocoyam flour. It suggests that resultant MTF from this process require a lower energy and will cook for a shorter period than other as due to the weak granular [21]. Based on previous reports [15,21], a decrease in pasting temperature of MTF was caused due to long fermentation period.

TABLE-2
PASTING PROPERTIES OF MODIFIED TANNIA FLOUR (MTF)

Pasting properties	MTF (LAB fermentation)	Cocoyam flour (natural fermentation)[a]	Native taro flour[b]
Pasting temp. (°C)	76	63.30 ± 0.5	87.20 ± 0.21
Peak viscosity (cP)	2674.5	124.46 ± 6	3379 ± 22.2
Hold viscosity (cP)	1651	117.50 ± 0.2	1553 ± 21.3
Final viscosity (cP)	2406.5	157.58 ± 0.8	2871 ± 16.4
Break down (cP)	1023.5	66.96 ± 5.3	622 ± 31.1
Seatback (cP)	775.5	42.08 ± 1.1	996 ± 29.2

The number after the sign (±) is the standard deviation value
[a]Reported by Oke and Bolarinwa [15]
[b]Reported by Sit *et al*. [8]

Other parameters were obtained from pasting characteristics *i.e.* peak viscosity (PV), hold viscosity (HV), final viscosity (FV), breakdown viscosity (BV) and setback viscosity (SV).

Peak viscosity is known as a rapid increase in viscosity when an adequate number of starch granules become swollen which is related with swelling capacity of the starch granules during heating [11]. Table-2 showed that MTF had a lower peak viscosity than native taro but MTF had a higher peak viscosity than cocoyam flour. It indicated that flour with a lower peak viscosity had a lower thickening power than flour with a higher peak viscosity. The reason is attributed due to that during fermentation occurs leaching starch, especially starch granules amorphous namely amylose.

Hold viscosity of MTF (1651 cP) was increased when compared with native taro (1553 cP). Hold viscosity increased with increase in fermentation period. The same reason is attributed as in case of peak viscocity. The highest hold viscosity of MTF indicated that MTF showed lowest resistance against heating and shear stress applied than native taro. Final viscosity defines the viscous gel forming capability of the material after cooking and determining quality of the end product [11]. Fermentation caused decreased final viscosity, which may be due to the leaching of starch during fermentation, especially when the starch granules were amorphous [15,21].

Breakdown viscosity showed the stability of swollen granules at constant temperature and mechanical agitation [11]. Fermentation can increased breakdown viscosity (Table-2), where breakdown viscosity of MTV and native taro was found 1023.5 cP and 622 cP, respectively. Higher breakdown viscosity value shows that lower resistance of the samples to shear and temperature which causes quality degradation in food products [11].

Setback viscosity provides information about retrogradation and syneresis of starch. The setback viscosity of MTF was found to be approximately 775.5 cP and has a lower value than native taro (996 cP). Thus, the pasting properties showed that native taro flour was better than MTF but it was better than wikau maombo flour [21].

Swelling power and solubility analysis: Swelling power defines that amount of water can be absorbed by 1 g of starch granules, if it is in excessive amount of water at high temperatures. Swelling power increases with the length of fermentation, however, this increase is not significant as the longer fermentation process results more broken starch. Modified tannia flour had a swelling power of 5.27 ± 0.14 g/g; 5.28 ± 0.49 g/g and 5.43 ± 0.19 g/g in fermentation 1 day, 2 days and 3 days. From Table-3, it can be seen that solubility increased during the fermentation process from 5.43 to 6.63 %. In this study, increasing the solubility of MTF during fermentation was caused due to the amorphous nature of starch [6].

TABLE-3
PHYSICO-CHEMICAL PROPERTIES OF THE MODIFIED TANNIA FLOUR BY LACTIC ACID BACTERIA

Type of starter	pH	Swelling power (g/g)	Solubility (%)
LAB 1 day	5.45 ± 0.13	5.27 ± 0.14	5.43 ± 0.18
LAB 2 day	5.42 ± 0.33	5.28 ± 0.49	5.97 ± 0.38
LAB 3 day	5.35 ± 0.32	5.43 ± 0.19	6.63 ± 0.26

The number after the sign (±) is the standard deviation value

Swelling power and solubility of MTF did not differ significantly. According to Oke and Bolarinwa [15], swelling power and solubility profiles indicate that MTF has a strong intragranular relationship, which required large energy for relaxation. This is similar to that performed by Tattiyakul *et al*. [4] where swelling power and solubility of taro flour at 80 ºC was 16 g/g and 14 g/100 g.

pH value: Measurements of pH values aim to determine the level of acidity or alkalinity of MTF. Swelling power and solubility increased progressively as the pH increased. Table-3 showed that a slight decrease in the pH value of MTF observed until the 3rd day of fermentation. The pH values observed in storage period were influenced by the activity of microorganisms during the processing of MTF. Accumulation of acid produced through metabolism LAB can lower the pH of medium.

The result obtained in this work is consistent with literature on some tuber starches. These include wikau maombo (cassava fermentation using sea water) [18], native, oxidized, acetylated and acid-thinned tannia starch [12].

Analysis of protein raw materials of flour and anchovy meat: Unfermented tannia flour contains lower protein content while the fermentation process provides significant changes in the protein content of flour [16,17,32]. Increased levels of protein affected by increasing the microbial activity during fermentation caused by the hydrolysis of protein molecules into amino acids and small peptides [16]. Table-4 showed that MTF increases the protein content upto 1.49 %. However, this result is very low when compared with cocoyam flour protein [16] amounted to 15.61 to 18.75 % with a fermentation time of 1-2 days, while Obadina *et al*. [17] reported the protein content 6.25 % during 2 weeks fermentation.

TABLE-4
PROTEIN CONTENT OF RAW MATERIALS

Raw materials	Protein content (%)
Anchovy meat	13.32 ± 0.08
Modified tannia flour	1.49 ± 0.03

The number after the sign (±) is the standard deviation value

Around 6-8 % protein content of flour are needed to make a cake and crispy products such as biscuits and chips, whereas bakery products, donuts, noodles and pasta requires a protein content of about 11-13 % [33]. Therefore, to increase the protein content of MTF required a combination anchovy meat containing 13.32 % so that it can support protein needed for cookies.

Cookies product: In this study, modified tannia flour were fermented for 3 days for making cookies having composition MTF 70 %, wheat 20 % and anchovy meat 10 %. Fig. 3 showed that cookies product made from MTF had pourus structural more hollow, which makes cookies good crumb properties (Fig. 3b) as compared to those cookies which consisted only wheat flour (Fig. 3a). It may be attributed due to the high swelling properties of MTF. In addition, crumb properties of cookies had relationship with starch content. High starch can function in maintaining the compactness and stability cookies.

Proximate composition of MTF cookies: Table-5 showed that proximate composition of MTF cookies is higher than cookies using wheat flour. Similiar values were reported by Ojinnaka *et al*. [14] for native tannia cookies and its modified using HCl treatment. Moisture content of MTF cookies was 8.76 %. It was not significant different with MTF cookies using HCl. Elisabeth [32] also reported that moisture content of product chip was higher than MTF cookies.

MTF cookies had higher protein contents than tannia cookies using HCl treatment due to its proteolytic activity so that tannia flour increased protein contents. Increased protein contents of cookies from the result of adding all the ingredients into one product excellence which benchmarks the nutritional value of food product is protein content. Fat contents of MTF cookies was also found higher than tannia cookies using HCl treatment. Alozie and Chinma [34] reported that cake using fermented cocoyam flour has a fat content 18.33 %. Fat added to the dough as more brittle cookies can be generated. Ash content of MTF was also higher than tannia cookies using HCl treatment, which may be due to the fact that fermentation increased ash content of cookies.

Modified starch is fermented using LAB which LAB has ability to use the mineral as a material to support its growth. It is reported that bacteria growth to produce compounds such as inorganic substrates require the consumption of minerals and vitamins [35]. During fermentation affects the amount of bacteria growing. Many LAB growth will support the fermentation process to produce flour tannia modifications so the resulting modified tannia flour contains many minerals and also increase the ash content of cookies.

Conclusion

Tannia flour was modified by using lactic acid bacteria (LAB). Pasting properties showed that modified tannia flour

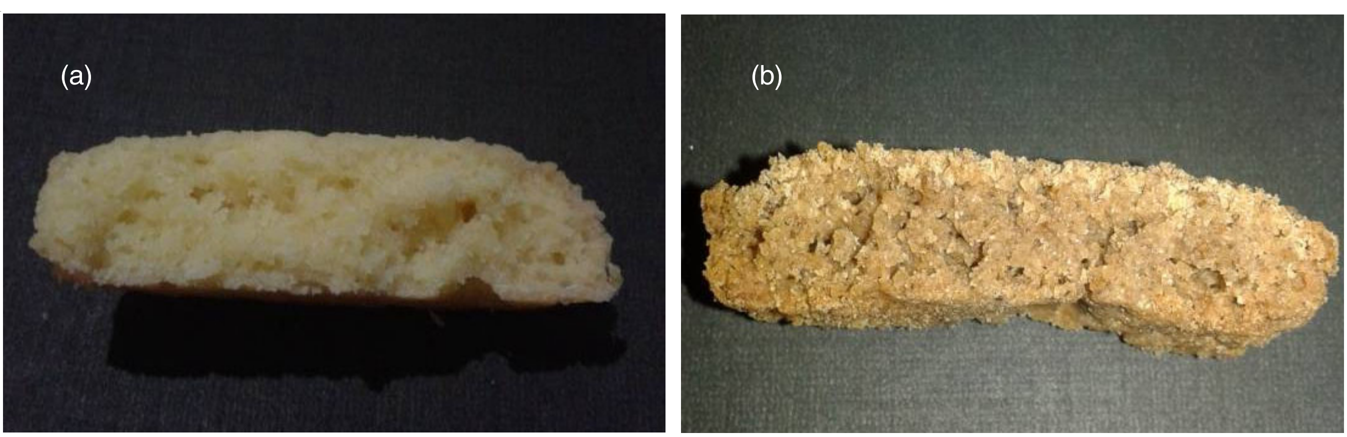

Fig. 3. (a) Cookies controls (wheat 90 %: anchovy meat 10 %), (b) Cookies MTF (modified tannia flour 70 %: wheat 20 %:anchovy meat 10 %)

TABLE-5
PROXIMATE COMPOSITION OF COOKIES

Analysis (%)	MTF cookies (LAB fermentation)	Control cookies*	Native tannia cookies [Ref. 14]	Tannia cookies (HCl modified) [Ref. 14]
Moisture	8.76 ± 0.22	5.99 ± 0.57	8.73	9.14
Protein	16.52 ± 0.03	12.40 ± 0.36	4.38	5.58
Fat	48.07 ± 0.23	44.06 ± 0.02	2.31	2.33
Ash	2.67 ± 1.18	1.37 ± 0.48	1.05	1.05
Glucose	9.08 ± 1.77	3.63 ± 0.53	–	–
Carbohydrate	–	–	82.30	81.38

*Values after ± is the standard deviation value; MTF cookies: MTF 70 %: anchovy meat 10 %: Wheat flour 20 %; Control: tannia flour 0 %.

can be applicated and use of the flour in various food products where physico-chemical characteristics *i.e.* ash, crude fiber, protein contents, swelling power and solubility were increased while moisture, fat and pH values were decreased. Structure pourus of modified tannia flour (MTF) cookies product was more hollow pourus so that its had a good crumbs with increased moisture, protein, fat, ash and glucose contents.

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CONFLICT OF INTEREST

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

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