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Nutrition, chemical and physical evaluation of flour of local Madura corn and powder of underutilized *Holothuria* species

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Abstract

The purpose of this study was to evaluate the nutrition, chemical, and physical properties of flour of the local Madura corn and sea cucumber of underutilized *Holothuri*a species, which may be valuable for future use in formulating new products from the two materials. Tambin corn was collected from farmers in the Bangkalan regency in Madura, Indonesia. Untreated (non-nixtamalized) and nixtamalized corn was dried, ground, sifted in 60 mesh, and then analysed for proximate analysis, mineral content, gelatinization profile, FT-IR spectrum and colorimetry. Three abundant and underutilised sea cucumber species, identified as *Holothuria atra, H. leucospilata*, and *H. impatiens,* were collected from Bawean islands, cooked in sea water until rubbery, dried, and then analyzed for proximate analysis and mineral content. Results showed that the protein content of non-nixtamalized and nixtamalized corn was 6.40% and 6.59%, respectively; while those of *H. atra, H. leucospilata*, and *H. impatiens* were 66.64%, 79.23%, and 60.12%, respectively. Ash content of non-nixtamalized corn, nixtamalized corn, *H. atra, H. leucospilata*, and *H. impatiens,* were 2.30%, 1.82%, 26.29%, 27.78% and 39.18%, respectively. The fat content of non-nixtamalized corn, nixtamalized corn, *H. atra, H. leucospilata*, and *H. impatiens,* were 7.69%, 6.02%, 1.44%, 1.48% and 1.35%, respectively; while calcium content was 32.227, 163.546, 337.471, 314.910 and 307.670 mg/100 g, respectively; iron content were 23.367, 20.207, 16.796, 15.136 and 15.611 mg/100 g, respectively; zinc content was 13.095, 12.076, 10.015, 7.142 and 8.073 mg/100 g, respectively. The gelation profile indicated that peak viscosities were 1368 cP and 281 cP, peak temperatures were 75°C and 53°C, set back viscosities were 1443 cP and 94 cP, for non-nixtamalized and nixtamalized corn respectively. The addition of sea cucumber powder at 5% and 10% reduced all gelatinization parameters. Fourier transform infrared (FTIR) spectrum indicated that the main bands of nixtamalized corn were less intense than those shown by raw corn, with an absorption band at 3009 cm^{-1} corresponding to the C-H group and at 1646 cm^{-1} corresponding to the N-H bending vibration of primary amines. Colorimetry analysis on corn results showed a reduction of L and an increase of a and b values by nixtamalization.

1. Introduction

Corn is among the three staple foods consumed by 80% of regions worldwide (Food and Agriculture Organization of the United Nations (FAO), 2022), and provides more than 30% of calories to the world population (FAOSTAT, 2010). Corn posted higher annual production (752.04 MT) (Shiferaw *et al*., 2011) than rice and wheat (Zhou, 2009), and the number was increasing and was projected to double by the year 2050 (Shiferaw *et al*., 2011). Global demand was also increasing higher than rice and wheat (Shiferaw *et al*., 2011). Therefore, corn is an important crop to support food security (CIMMYT, 2024), for some other reasons (Shiferaw *et al*., 2011). First, there is high corn consumption in developing countries which are more prone to hunger. Around half of world corn production (392 MT) was from developing countries (Shiferaw *et al*., 2011). Secondly, corn is drought tolerant, and grows

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in a wider range of temperature, humidity, and soil fertility. Third, corn is a traditional crop that has been recognized by ancient people (Wacher, 2003), cultivated almost all over the world, thus providing high diversity to survive climate change (Langner *et al*., 2019). Fourth, corn is ready to be consumed, where no need for hull removal which can be a laborious process (Langner *et al*., 2019).

The use of a local variety of corn is encouraged to have better adaptability to local environmental stress (Langner *et al*. 2019). Tambin is one of the local varieties of corn in Madura Island (East Java, Indonesia) an area with average rainfall ranging from 38 mm (August) to 275 mm (January), and an average temperature of 19-30°C (worldweather online, 2024). The protein content of Madura corn is considerably low when compared to other corn in common (around 10%) (Shiferaw *et al*., 2011). Annual production of Tambin corn (3.482 MT/ha) was also relatively higher than other Madura corn varieties (2.978 to 3.847 MT/ha) (Amzeri, 2009). Nevertheless, corn production in Madura was lower than world average production (3.64 MT/ha) (Shiferaw *et al*., 2011).

Although corn production is essential for food security, corn is lacking in essential amino acids tryptophan and lysine, minerals, especially zinc, iron, calcium, and vitamin B (Bathla *et al*., 2019; Waller *et al*. 2020), which leads to health-related problems such as pellagra and anemia in population consuming corn as a staple food (Waller *et al*., 2020). Improvement of nutrition content had been carried out to result in corn with relatively high concentrations of lysine and tryptophan (Gallego-Castillo *et al*., 2021). Some efforts have been made to increase lysine and tryptophan content in corn by fertilization (Ramirez-Silva *et al*., 2020; Szostek *et al*., 2021). Florine concentration in fertilizer increased total amino acids in corn, but reduced lysine content (Szostek *et al*., 2021). A similar result was shown when adding mycorrhizae and *Azospirillum* biofertilizer to synthetic fertilizer to corn (Ramirez-Silva *et al*., 2020), where lysine and tryptophan were reduced, although grain production was increased. Fortification of maize with high lysine and tryptophan legume seemed to improve the amino acid profile of corn. Locus bean added to fermented corn increased considerable lysine and tryptophan content (Makanjoula and Moses, 2017). Mineral and vitamin fortification had been applied to corn flour, especially iron, zinc and folic acid (Waller *et al*., 2020), which needed encouragement and education for people to use the fortified product (Waller *et al*., 2020). Moreover, fortification has also been applied to corn products such as tortillas, using soybean (Obatolu *et al.,* 2007), or mixing of soybean and sesame seed (Serna-Saldivar *et al.,* 1988), protein concentrate of lima bean (Lecuona-Villanueva *et al.,* 2012), chickpea hydrolysate (Acevedo-Martinez and de Meija, 2021), lupin flour (Hernandez-Chavez *et al.,* 2019), and squid muscle flour (Heredia-Sandoval *et al.,* 2021), mix of vitamin B1, B2, niacin, folic acid, and minerals of iron and zinc (Stylianopoulos *et al.,* 2002). The effectiveness of fortification was determined by several factors including types of fortifying agents (Stylianopoulos *et al.,* 2002; Lecuona-Villanueva *et al.,* 2012) or carrier food (Serna-Saldivar *et al.,* 1988, Vazquez-Rodriguez *et al.,* 2013). Sensory acceptability and textural properties are important to be considered in every fortified food. The addition of squid muscle flour gave tasteful properties (Heredia-Sandoval *et al.,* 2021), and better sensory acceptance than the unfortified product (Lecuona-Villanueva *et al*., 2012). Textural properties of fortified products seemed to be prone to alteration (Hernandez-Chavez *et al.,* 2019; Heredia-Sandoval *et al.,* 2021), although some reports mentioned no adverse effect of fortification on product acceptance (Lecuona-Villanueva *et al.,* 2012).

It is apparent that one of the possibilities to improve the nutrition of corn is fortification. Sea cucumber is one of the prospective materials for fortification, especially when it is available in the area. There are around 400 species of sea cucumber live in Indonesia (Setyastuti *et al.,* 2019), and more than 60 species of them are export commodities (Purcell *et al*., 2012). Therefore, more species of sea cucumber have low prices or even no significant economic value. Around 47% of the world's commercial sea cucumber species grow in Asia (Choo, 2008). The protein content of sea cucumber widely ranged from around 3.5 (Barzkar *et al*., 2017) to 70% (Azad *et al*., 2017). While some species showed a considerable amount of lysine (around 1%) (Wen *et al*., 2010; Ardiansyah *et al*., 2020) and tryptophan (around 0.2%) (Wen *et al*., 2010; Ardiansyah *et al*., 2020), some other species did not contain tryptophan (Sicuro *et al*., 2012; Widianingsih *et al*., 2016). Calcium and iron were reported to be present in sea cucumber (Barzkar *et al*., 2017; Rasyid, 2017; Ardiansyah *et al*., 2020), with calcium may be up to around 4000 mg/100 g (Barzkar *et al*., 2017), and iron was up to 521 mg/100 g (Ardiansyah *et al*., 2020). Vitamin B was absent in a number of previous reports (Rasyid, 2017; Ardiansyah *et al*., 2020; Rasyid *et al*., 2020), but vitamin E was present in some species (Ardiansyah *et al*., 2020). Sea cucumber is low in fat (around 0.38%) (Widianingsih *et al*., 2016), but contains some polyunsaturated acids especially oleic acid (Widianingsih *et al*., 2016; Ardiansyah *et al*., 2020) and linoleic acid (Ardiansyah *et al*., 2020), with the total amount of polyunsaturated fatty acids was 0.35%. Previous works reported the absence of heavy metals in the body of sea cucumber (Rasyid, 2017; Ardiansyah *et al*., 2020), indicating that their consumption is safe. Sea cucumbers are reported to show several health benefits including immune-enhancing, anti-proliferative, hypocholesterolemic, and wound healing activities (Pangestuti and Arifin, 2018).

A preliminary study was carried out to determine characteristics of nixtamalized local variety of corn (called Tambin) and underutilized species of sea cucumber, the data which may be valuable for formulating corn-based high-nutrient new products.

2. Materials and methods

2.1 Collection of corn

Dried grain of Tambin corn was bought from Mr. Moh. Dahri, a farmer in the production area of Tragah district, Bangkalan regency, in East Java province, Indonesia. The corn was then kept in a tight container and stored in the laboratory of the University of Trunojoyo Madura, Indonesia, for further treatment.

2.2 Collection of sea cucumber

Three species of sea cucumber were collected by a local trader, Mr. Pandi, from the coastal area of Bawean island, East Java province. These three cucumbers are considered to be of very low economic value, abundance, easy for collection as they live in shallow water near coastal areas, and are not utilized by local people. Fresh sea cucumbers were washed with clean seawater, then sun-dried for three or four days until dry as indicated by no visible water coming out when the sea cucumber was bent around 90°. The dried sea cucumber was packed in a water-proof container, and shipped to the University of Trunojoyo Madura. The sea cucumber was then transferred to an air-tight container, and kept at room temperature, until further treatment.

2.3 Nixtamalization

Corn was first washed and then put into boiling lime water (5% of corn weight) for 20 mins, or until the testa was easily removed by fingers. The heating was stopped, and the mixture was let stand for 12 hrs. After that, the testa of the corn kernel was removed by hand, and the kernel was washed thoroughly until the lime was completely removed. Corn kernel was dried in a drying oven at 50°C for 8 hrs until fully dry as indicated by light sound when grains were shaken, and then kept in an airtight plastic container until further use.

2.4 The preparation of corn flour

Dried nixtamalized corn kernel was ground using an electric grinder, and then sifted using 60-mesh sieve. The flour was then kept in an airtight plastic container for further use.

2.5 The making of sea cucumber powder

Dried sea cucumbers were cut longitudinally to enable removing internal organs and sand and stones inside the body. The sea cucumbers were then fried in hot clean white sand until the body of the sea cucumbers expanded and became completely porous. The fried sea cucumbers were then ground using an electric grinder and sifted with 60-mesh sieve. The sea cucumber powder was then kept in an airtight container until further analysis.

2.6 Proximate analysis

Proximate analysis was performed on both non-nixtamalized and nixtamalized corn flour, and sea cucumber flour, following standard Association of the Official Analytical Collaboration (AOAC) International (2000) methods: moisture content (925.09), protein content (920.87), lipids content (968.21), and ash content (923.03). All measurements were performed at least in duplicate. Carbohydrate content in corn was determined by difference.

2.7 Mineral analysis

Minerals Ca, Fe, and Zn in corn flour and sea cucumber flour were determined using an atomic absorption spectrometer (NOVA 400, Analytik Jena AG, Jena, Germany), with an air/acetylene flame and hollow-cathode lamps, following a previous method (Ooi *et al.,* 2012). Wavelength, slits, and lamp current used for the determination of each element were 213.9 nm, 0.5 nm, 4.0 mA, for zinc; 422.7 nm, 1.2 nm, 4.0 mA, for calcium; and 248.3 nm, 0.2 nm, 6.0 A, for iron; respectively. All measurements were performed in duplicate. Carbohydrate content in corn was determined by difference.

2.8 Gelatinization profile analysis

Pasting properties analyses of flour of nixtamalized or non-nixtamalized corn, sea cucumber powder, or a mixture of the two, were performed using a rapid visco analyzer (Newport, Sydney, Australia), following a previous method (Zhou and Hou, 2012). A sample of each flour of 3.5 g (moisture content of 14%) was transferred into a canister, and 25 mL of deionized water was added. The slurry was manually mixed to break lumps. A 20-minute heating and cooling cycle was set. At the beginning of the cycle, the slurry was stirred at 960 rpm for 10 s, and then at 160 rpm until the end of the heating cycle. The heating cycle consisted of heating at 60°C for 4 mins, then heating to 95°C, holding at 95°C

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for 4 mins, and finally cooling to 50°C. Data on peak viscosity, trough viscosity, and final viscosity were collected in the Rapid Visco Unit. Analysis of pasting properties was also carried out for a mixture of nixtamalized corn and sea cucumber powder at 5 and 10% concentrations. The level of sea cucumber powder added to corn flour was determined by estimating the mixture to contain around 2.5 to 5% of adult women's protein daily requirement (46 g/day), in one serving of corn-based product (233.8 g, Fritolaysmartlabel, 2024), without adversely affecting flavour of the mixture. The sea cucumber used in the mixture had the highest protein content. All determinations for non-nixtamalized and nixtamalized corn were carried out in duplicate. RVA for mixtures of corn and sea cucumber powder was only performed once due to a shortage of sea cucumber powder.

2.9 Fourier transform infrared analysis

To identify functional groups of starch and sea cucumber flour, Fourier Transform Infrared (FTIR) (Varian GTA 120, Dresden) method was performed (Abdullah *et al.,* 2003).

2.10 Colorimetry

To determine color parameters of corn starch, a color reader (Konica Minolta color reader CR-20, Singapore) was utilized to read L, a, and b values.

2.11 Statistical analysis

Data were analyzed for One-way Anova using a statistical package IBM SPSS Statistics 23 to see if there is any difference among means. When there were differences among means, a posthoc test was carried out using the Tukey method at P=0.05. Data were presented as means.

3. Results and discussion

3.1 Proximate analysis

The result of the proximate analysis of corn flour is presented in Table 1. Nixtamalization did not alter significantly the protein content of corn, although a slight increase was noticed (Sefa-Dedeh, 2004; Kadir *et al.,* 2019), as also shown in our work (Table 1). However, previous work reported an increase in protein content due to prolonged immersion time during nixtamalization (Musita *et al.,* 2018). In general, the protein content in corn was around 10% (Wu and Messing, 2014) or slightly lower than 10% (Vazquez-Rodriguez *et al*., 2013; Moreno *et al*., 2015; Zhang *et al.,* 2016; Kadir *et al.,* 2019), thus Tambin corn had lower than average level of protein. The protein content of nixtamalized corn varied from 4% (Morales and Zepeda, 2017) to 9% (Moreno *et al*., 2015; Sanchez-Madrigal *et al.,* 2015), apparently depending on the variety of corn and growing environment (Wu and Messing 2014; Zhang *et al.,* 2016). The protein concentration of Tambin corn was still in the range.

Ash content in Tambin corn (1.8 to 2.3%) (Table 1) was similar to an earlier report (Rojas-Molina *et al.,* 2020), but slightly higher than reported in several previous works (Sanchez-Madrigal *et al.,* 2015; Zhang *et al.,* 2016; Musita *et al.,* 2018; Kadir *et al.,* 2019). Variety and growth environment (Zhang *et al.,* 2016) seem to determine the ash content of corn. Earlier reports on the effect of nixtamalization on ash content seemed inconclusive, since nixtamalization could increase slightly ash content (Kadir *et al.,* 2019), or reduce it (Musita *et al.,* 2018). In this study, nixtamalization did not alter ash concentration in corn (Musita *et al.,* 2018).

The fat content of Tambin corn was above 5% (Table 1), thus categorized as high-oil corn (Sanjeev *et al.,* 2014). Previous studies from Indonesia (Musita *et al.,* 2018; Kadir *et al.,* 2019) reported that the fat content of corn is lower than 5%. Nixtamalized corn showed fat content ranging from 2.26 to 4.2% (Moreno *et al*., 2015; Rojas-Molina *et al.,* 2020). Nixtamalization reduced fat content (Musita *et al.,* 2018; Kadir *et al.,* 2019), due to its melting nature during heat treatment (Musita *et al.,* 2018; Kadir *et al.,* 2019). In this study, nixtamalization did not alter fat content significantly.

The moisture content of non-nixtamalized and nixtamalized was 11.01 and 11.91%, respectively (Table 1). In previous reports, the water content of nixtamalized corn flour ranged from 8.5% (Rojas-Molina *et al*., 2020) to 14.2% (Johnson *et al*., 2010). Therefore, moisture content in nixtamalized Tambin corn was within the range.

The three species of sea cucumber collected from the coastal area of Bawean island were later identified as *Holothuria atra*, *H. impatiens* and *H. leucospilata*. The three species were not excessively utilized by locals, low

Table 1. Proximate analysis of Tambin corn or sea cucumber flour.

Types of flour	Water content* (%)	Protein content*(%)	Ash content* (%)	Fat content * (%)	Carbohydrate (%)
Non-nixtamalized Tambin corn	11.01	7.056	2.302	7.694^a	71.998^b
Nixtamalized Tambin corn	11.909	6.592	1.819	6.061^b	73.773^a

Values with different superscripts within the same column are statistically significantly different (P<0.05).

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price, and were known as 'black sea cucumbers' (Setyastuti, 2015).

Protein content in sea cucumber varied greatly due to the high diversity of species. In this study, the three species showed significant differences in protein content, with *H. leucospilata* indicating the highest protein concentration (79.02%), while *H. impatiens* exhibited the lowest concentration of protein (60.99%) (Table 2). The three species used in this experiment showed higher protein content than the same species from East Nusa Tenggara, Indonesia, where the protein content of *H. atra*, *H. impatients*, and *H. leucospilata* were 42.32, 39.94 and 39.87%, respectively (Oedjoe, 2017). The protein content of *H. leucospilata* in this study was twice higher than that reported earlier (Oedjoe, 2017). Other previous studies on *Holothuria* species reported lower protein content, less than 30% (Salarzadeh *et al.,* 2012; Li *et al.,* 2018; Ardiansyah *et al.,* 2020). On the contrary, some works mentioned protein content higher than 40% (Ibrahim *et al.,* 2015; Azad *et al.,* 2017; Sroyraya *et al.,* 2017; Andriamanamisata and Telesphore 2019). Protein in sea cucumbers was categorized as extracellular proteins, muscle proteins, and proteases, with extracellular proteins dominating (Wang *et al.,* 2020). Extracellular proteins in sea cucumbers are important food components to determine the textural properties of food (Wang *et al.,* 2020). Protein in sea cucumber consists of high concentrations of essential and non-essential amino acids (Ardiansyah *et al.,* 2020). Therefore, sea cucumbers are a rich source of protein to support human health.

Ash content among the three species studied was significantly different, with *H. leucospilata* exhibiting the highest ash concentration (39.18%), and *H. atra* showing the lowest ash content (26.29%) (Table 2). Ash content in sea cucumber varied greatly, some works mentioned less than 5% (Morales and Zepeda 2017; Rasyid *et al.,* 2020), while others reported 30% or more (Sicuro *et al.,* 2012; Ibrahim *et al.,* 2015; Oedjoe, 2017). This discrepancy was likely due to the incorporation of sea cucumber protective skin which contains chalk grains (Oedjoe, 2017). *Holothuroidea* species are famous for microscopic spicules in their skin as their skeletal structure (manoa.hawaii.edu) which might be around 14% of total weight (Oedjoe *et al.,* 2017). Among the three species identified in this study, *H. leucospilata* showed abundant thorns on its body surface. This high ash concentration did not make carbohydrate by difference method to be carried out, since the sum of protein, fat, and ash, had exceeded 100%.

3.2 Mineral content analysis

Calcium content in non-nixtamalized corn was reported elsewhere ranged from 2 (Szczepaniak *et al.,* 2016) to over 20 mg/100 g (Moreno *et al.,* 2015), depending on variety, season, and mineral concentration in soil (Szczepaniak *et al.,* 2016). Therefore, the calcium content in Tambin corn (5.25%) (Table 3) could be categorized as low. Nixtamalization allowed the diffusion of calcium ions into the internal tissue of corn during hot steeping, resulting in a considerable increase in calcium concentration to reach around 100 – 300 mg/100 g (Moreno *et al.,* 2015; Morales and Zepeda, 2017). Such a phenomenon was also shown in our work, where nixtamalized corn had a significantly higher concentration of calcium (34.1%) (Table 3).

On the other hand, iron content in Tambin corn (Table 3) was slightly higher than in earlier reports (Moreno *et al.,* 2015; Bathla *et al.,* 2019) which varied from 2.4 (Bathla *et al.,* 2019) to 4 mg/100 g (Moreno *et al*., 2015). Nixtamalization significantly reduced iron content in Tambin corn (Table 3). The effect of nixtamalization on iron content was different from

Table 3. Mineral contents of corn and sea cucumber flour.

Types of flour	Ca (mg/100 g)	Fe (mg/100 g)	Zn (mg/100 g)
Non-nixtamalized Tambin corn	5.25^{d}	4.89^{a}	2.73^{a}
Nixtamalized Tambin corn	34.10^{c}	4.21^{ab}	2.54^{ab}
H. atra	67.01^{a}	3.38^{ab}	$2b^{c}$
H. impatiens	65.99^{ab}	3.20^{b}	1.51^{c}
H. leucospilata	62.26^{b}	3.22^{b}	1.62^{c}

Values with different superscripts within the same column are statistically significantly different (P<0.05).

Table 2. Proximate analysis of sea cucumber flour.

Types of powder	Water content* (%)	Protein content*(%)	Ash content* (%)	Fat content * (%)	Carbohydrate (%)
H. atra	13.661	67.690^{c}	26.293^{c}	1.439	Not determined**
H. impatiens	11.651	60.998^{b}	27.779^{b}	1.475	Not determined**
H. leucospilata	12.636	79.021^{a}	39.181^{a}	1.345	Not determined**

Values with different superscripts within the same column are statistically significantly different (P<0.05).
**Carbohydrate by difference in sea cucumber could not be performed since ash content was too high to be calculated by difference method, due to high calcium as microscopic spicules in their skeletal structure, leading to the sum of all non-carbohydrate components exceeding 100%.

previous reports. While iron content reduced during nixtamalization, due to leaching in cooking (Morales and Zepeda, 2017), iron concentration also increased as a result of different nixtamalization agents (Moreno *et al.,* 2015).

Zinc is very limited in corn, around 1.5 to 30 mg/kg (Liu *et al.,* 2020). It could reach 2.15 mg/100 g (Moreno *et al.,* 2015). Tambin corn was, thus, relatively high in zinc (Table 3). Zinc concentration in corn was correlated to its concentration in soil so high zinc in fertilizer improved zinc content in corn (Liu *et al.,* 2020). Nixtamalization reduced zinc concentration in corn, except when certain types of nixtamalization agents were applied (Moreno *et al.,* 2015). In this study, nixtamalization significantly lower zinc levels in corn, which is likely due to leaching during steeping (Moreno *et al.,* 2015).

Sea cucumber can be a rich source of minerals, with calcium usually in higher concentration than others. Calcium concentration varied greatly among species and their living environment, ranging from around 100 mg/100 g (Barzkar *et al.,* 2017) to around 4000 mg/100 g (Ardiansyah *et al.,* 2020). Calcium in the three species in our work (62.26 to 67.01%) (Table 3) may be categorized as low, with the calcium content of *H. leucospilata* did not significantly differ from that of *H. impatiens*, which was higher than that of *H. atra* (Table 3).

Previous publications on iron content in sea cucumber reported that it ranged from around 4 mg/100 g (Ardiansyah *et al.,* 2020) to 26 mg/100 g (Li *et al.,* 2018). Thus, the three species of sea cucumbers used in this study (3.20 to 3.38%) showed relatively low concentrations of iron (Table 3).

Zinc concentration in sea cucumber may range from 0.04 mg/100 g (Barzkar *et al.,* 2017) to 5 mg/100 g (Li *et al.,* 2018). Three Holothuria species in this study seemed to contain zinc (1.51 to 2%) at moderate level (Table 3).

3.3 Gelatinization profile analysis

The gelatinization profile of non-nixtamalized and nixtamalized Tambin corn was significantly different, where all parameters, except peak time, of nixtamalized corn, were considerably lower than those of non-nixtamalized corn (Table 4). Peak viscosity of nixtamalized Tambin corn (49 cP) seemed very low compared to other reports which mentioned 1850 to 2900 cP (Johnson *et al*., 2013), 203.7 to 285.6 cP (Musita *et al*., 2018), 500 to 1000 cP (Moreno *et al*., 2015). Nixtamalization rendered corn starch resistant to breakdown, and thus maintained constant viscosity (Gomez *et al*., 1992), as also shown in this study (Table 4). During nixtamalization, crosslinking between Ca++ ions and starch lowers maximum viscosity due to inhibition of starch swelling (Gomez *et al.,* 1992; Musita *et al.,* 2018). The phenomenon was shown by higher peak time; lower peak viscosity, trough, breakdown, final, and setback viscosity (Tabel 4). Nixtamalization also increased gel stability as shown by a smaller difference between peak and final viscosity (Gomez *et al.,* 1992; Musita *et al.,* 2018), and reduced retrogradation tendency (Gomez *et al.,* 1992; Musita *et al.,* 2018). Nixtamalized flour had a high molecular weight of amylopectin which may resulted from the recrystallization of starch during steeping (Gomez *et al.,* 1992), which in turn determined its heat resistance and gel stability (Gomez *et al.,* 1992).

The addition of 5% sea *H. leucospilata* powder, however, seemed to increase the gelling susceptibility of nixtamalized corn flour (Table 4). On the contrary, when 10% sea cucumber powder was mixed with nixtamalized corn flour, gelatinization seemed to be almost completely hindered, as shown by very low viscosity and long gelatinization time (Table 4). The increase in viscosity by the addition of 5% sea cucumber powder seemed to relate to nixtamalization-induced aggregation of protein molecules to increase gelation strength, which then interacted with starch to further exaggerate the effect (Yang *et al.,* 2021). On the contrary, gel weakening by the addition of 10% sea cucumber powder could be due to several factors: starch had better gelling properties than protein, protein resided in between swelling starch granules to function as inactive filler, and protein changes gel's microstructure to affect textural properties

Table 4. Gelatinization profile of Tambin corn flour and its mixture with sea cucumber (*Holothuria leucospilata*) powder.

Type of corn flour	Peak viscosity (cP)	Trough viscosity (cP)	Breakdown (cP)	Final Viscosity (cP)	Setback viscosity (cP)	Peak time (min)
Non-nixtamalized Tambin corn	2470[a]	1677[a]	793[a]	5135.5[a]	3458.5[a]	4.94[a]
Nixtamalized Tambin corn	49[b]	42.5[b]	6.5[b]	69.5[b]	27[b]	6.9[b]
Nixtamalized corn and 5% sea cucumber powder	1846	211	1635	209	200	4.20
Nixtamalized corn and 10% sea	23	21	2	37	16	6.33

Note: Statistics were only performed for non-nixtamalized and nixtamalized corn, due to shortage of sea cucumber stock. Values with different superscripts within the same column are statistically significantly different (P<0.05).

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(Bravo-Nunesz *et al.,* 2019). High protein corn also showed lower parameters of gelatinization, than those of common corn (Abiose and Ikunjenlola, 2014).

3.4 FTIR analysis

The result of FTIR analysis for non-nixtamalized and nixtamalized Tambin corn (Figure 1) was similar to earlier reports (Arambula-Villa *et al.,* 2018), where nixtamalization lowered intensity without altering major absorption bands (Arambula-Villa *et al.,* 2018). Lower absorbance was interpreted as the presence of cross-linking between starch components (Mondragon *et al.,* 2004). Similarly, broadband absorption was shown at around 3000-3600 cm^{-1} (Arambula-villa *et al.,* 2018; Abdullah *et al.,* 2018), which related to the O-H group, and stretching of water structure (Arambula-Villa *et al.,* 2018). Another absorption was shown at 2900-3000 cm^{-1}, which corresponded to the C-H group (Arambula-villa *et al.,* 2018; Abdullah *et al*., 2018), indicating tension of CH4 groups (Arambula-Villa *et al.,* 2018). The absorption at around 1560-1600 cm^{-1} correlated to N-H bending and C-N stretching vibration (Arambula-Villa *et al.,* 2018). At around 1000 cm^{-1}, absorption bands were shown and related to C-O stretching vibration (Arambula -Villa *et al.,* 2018; Abdullah *et al.,* 2018). Lastly, absorption at around 700-900 cm^{-1} indicated a C-O-C group in the skeleton of amylose and amylopectin (Arambula-Villa *et al.,* 2018). The absorption pattern in corn starch was similar to that of cassava and potato starch (Abdullah *et al.,* 2018).

3.5 Colorimetry analyses

Colorimetry analysis showed that nixtamalization significantly reduced L and a values, but increased b values (Table 5).

The color of corn was correlated to anthocyanin concentration in corn (Moreno *et al.,* 2017), which resided in aleuron in the pericarp (Moreno *et al.,* 2017). Alkalization during nixtamalization solubilizes the pericarp, to alter color of corn (Moreno *et al.,* 2017). Luminosity, hue, and chroma were reduced due to nixtamalization and made color of corn yellow (Moreno *et al.,* 2017). A similar result was shown in our work, where the yellowish index increased by nixtamalization (Table 4). L and a value of nixtamalized corn in our work were about similar to those reported earlier (Sanchez-Madrigal *et al.,* 2015; de Oliveira *et al.,* 2019; Rojas-Molina *et al.,* 2020). However, the b value of Tambin corn was above earlier reports (Sanchez-Madrigal *et al.,* 2015; Rojas-Molina *et al.,* 2020).

Table 5. Color analysis of corn flour.

Type of flour	L	a	b
Non-nixtamalized tambin corn	83.430^a	1.677^b	25.003^b
Nixtamalized Tambin corn	79.113^b	3.207^a	27.107^a

Values with different superscripts within the same column are statistically significantly different (P<0.05).

4. Conclusion

Holothuria leucospilata showed around 80% protein which was the highest among the three species examined, and all species were relatively low in calcium and iron, but moderate levels of zinc. Tambin corn contained a relatively low concentration of calcium and iron, but a moderate level of zinc. Gelatinization of nixtamalized corn was hindered by the addition of 5% sea cucumber powder, and with the addition of 10% sea cucumber powder, gelatinisation was almost completely obstructed. Nixtamalization induced crosslinking among starch

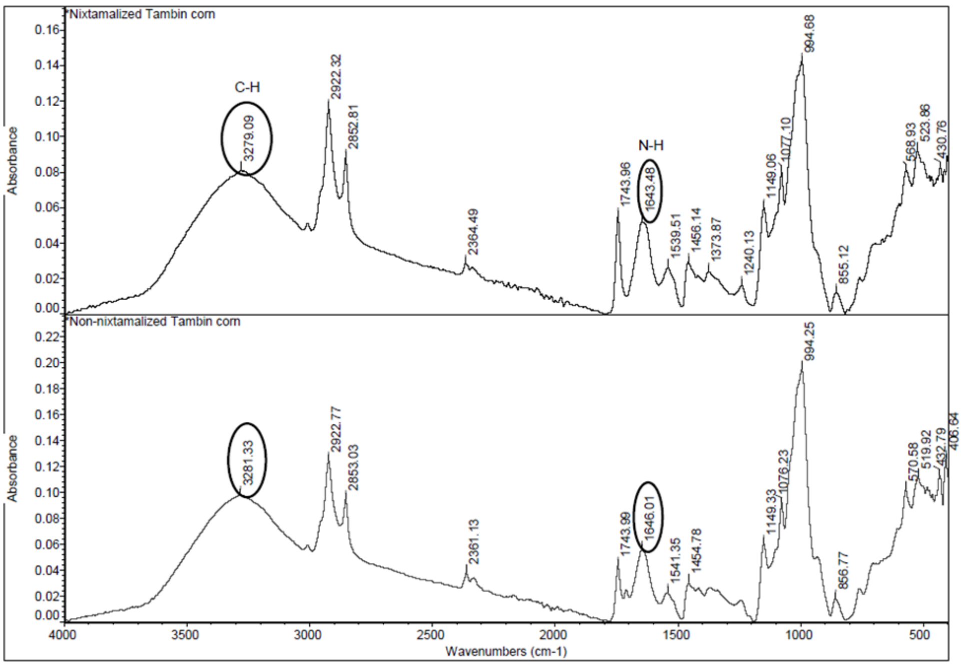

Figure 1. Infrared spectrograms (FTIR) of non-nixtamalized and nixtamalized Tambin corn.

components reduced L and a values, but increased the b value. The results of the study may serve as fundamental information for development of new product made from corn and *H. leucospilata*.

Conflict of interest

The authors declare no conflict of interest in conducting this study.

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