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The effects of fermenting pineapple peels (*Ananas comosus* L. Merr) with Loog-pang at various ensiled times on the nutrient content and pH

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ABSTRACT: The research herein aimed to compare the effects of supplementing with Loog-pang from different fermentation methods of pineapple peel (*Ananas comosus* L. Merr) on nutrient contents and pH levels. We investigated both fresh and dry pineapple peel with 0% or 3% Loog-pang. The study was conducted in a 2×2×4 factorial arrangement in a completely randomized design (CRD). Factor A identified the type of pineapple peel; fresh or dried (for 48 hours). Factor B was the level of Loog-pang used in the fermentation process, at either 0% or 3%. Factor C determined the length of the fermentation process at 0, 3, 6, or 9 days. The treatments were replicated two times each, and the parameters were evaluated: nutrient contents [dry matter (DM); ash, crude fiber (CF); crude protein (CP); ether extract (EE)]; and pH. The results showed no significant differences in the interaction of fermented pineapple peel with Loog-pang at different fermentation times in crude protein, crude fiber, ether extract, or pH ($P>0.01$); but showed significant differences in dry matter content and ash ($P<0.01$). We may, therefore, conclude that nine days of fermentation with fresh pineapple peel and 3% Loog-pang will provide the highest crude protein content, while reducing the crude fiber value at a suitable pH. In addition to the type of pineapple peel, both the Loog-pang supplement and incubation time further impacted changes in nutrient levels during the fermentation process.

Keywords: fermentation; pineapple peel; Loog-pang; crude fiber; crude protein

Introduction

The pineapple (*Ananas comosus*) is one of the world's most important fruits as the most popular edible member of the *Bromeliaceae* family, and is also consumed as juice, concentrate, and jam. Based on data from the Central Bureau of Statistics of Indonesia, the fruit production of pineapple in 2021 was 2,886,417.00 tons. This achievement produces pineapple peel reaching 27% of the total fruit production (Nurhayati et al., 2014). The estimated production of pineapple peel products in Indonesia (2021) totals 779,332.59 tons of pineapple production. Furthermore, pineapple peel has relatively good nutrition, with 88.95% dry matter (DM), 8.78% crude protein (CP), 27.09% crude fiber (CF), 1.15% ether extract (EE), and 3.82% ash (Nurhayati, 2013). Vitamin C and the protein-hydrolyzing enzyme bromelain are abundant in pineapple peel. Bromelain enzymes hydrolyze protein to peptides and improve the digestion of proteins in the digestive tract of poultry (Manosroi et al., 2014; Swan and Nagendran, 2014). Up to 40% of bromelain is absorbed in the digestive tract along with its structure, making it resistant to stomach acid (Seifert et al., 1979). As blood plasma cholesterol, triglyceride, and chylomicron levels decrease in rats after they consume pineapple juice, bromelain also lowers cholesterol levels (Daher et al., 2005). Bromelin's ability

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to reduce cholesterol has not been fully understood; however, the enzyme may improve blood circulation by removing cholesterol plaque from blood vessel walls.

Farmers profit from utilizing pineapple peel as broiler feed, as it is cheap and straightforward to obtain. However, the use of pineapple peel as poultry feed must be limited, due to its low crude protein content and high crude fiber (Rafiu et al., 2022; Ibrahim et al., 2020). The high crude fiber content causes complex nutrients to be poorly digested by poultry. To overcome this constraint, it is significant to explore other treatments to be applied to the peels, such as crushing, to improve their ingestion without degrading their feedstuff value.

The use of microorganisms through fermentation has been well-documented to improve the nutritional value of pineapple peels (Omwango et al., 2013; Ibrahim et al., 2015; Adrizal et al., 2017; Bulkaini, 2021), thereby offering the potential to make dramatic contributions to sustainable livestock production. Mandey et al., 2017; determined that fermented pineapple waste contained increased crude protein content and decreased crude fiber content after a 3% supplement application of "Ragi tape" Indonesia as a pineapple peel fermentation starter; containing *Candida parapsilosis, C. melinii, C. lactosa. C. solani, Hansenula subpelliculosa, Rhizopus oligosporus, Aspergillus f1avus, A. oryzae,* and *Hansenula malanga*.

In Thailand, Loog-pang is a traditional fermentation starter, which is a mixed culture of mold, yeast, and bacteria for traditional alcoholic products derived from raw rice. Loog-pang's type inocula are available in the markets of most Asian countries. They are generally produced by household or village manufacturers using closely guarded recipes. The size and shape of the rice cake, as well as the particular spices added, are the trademarks of the manufacturer. As a commercial product, Loog-pang contains *Amylomyces, Aspergillus, Rhizopus, Mucor, Monascus,* and *Penicillium genus* (Limtong et al., 2005), which can be used to convert pineapple peel into animal feed. *Aspergillus niger* has also been successfully used in various fermentation studies, however, Phromnoi and Laorodphan, 2021; demonstrated that pineapple peels fermented with Loog-pang had higher crude protein than those of other fermentation processes via *in vitro* gas production. To date, there have been no studies on fermented pineapple peel employing Loog-pang in broiler diets. In recognition of this potential, examination of the most efficient fermentation time is needed, since that remains an important factor affecting the feed's nutrient content. We have, therefore, chosen to investigate the use of Loog-pang with different fermentation times and types of pineapple peel on the nutrient contents and pH.

Material and methods

Pineapple peels, Loog-pang, and experimental design

Both fresh pineapple peel and dried pineapple peel were used in this research. Fresh pineapple peels (Pattavia pineapple) were collected from the fruit store at Complex 2, Khon Kaen University, Khon Kaen, Thailand. The fermentation starter, Loog-pang, was purchased at a Thai market in the Mueang district of Khon Kaen, Thailand.

Pineapple peels were cut with a chopper machine (Hammer mill Retsch SK1, GmbH, Haan, Germany) and cleaned. After that, pineapple peels were divided into two containers: one for dried samples and the other for fresh samples. The mixing process is carried out after the dried pineapple peels have been separated and dried at 32°C for 48 hours., as opposed to the fresh sample, which is mixed with 3% of Loog-pang directly. The samples will be

anaerobically incubated in plastic containers when the mixing process is complete and opened on the treatment days.

The experiment was conducted in a 2×2×4 factorial arrangement in a completely random design (CRD). Factor A selected the pineapple peel type, which was either fresh or dried; Factor B designated the level of Loog-pang used in the fermentation process, at either a 0% supplement or with a 3% supplement; and the length of the fermentation process; either 0, 3, 6, or 9 days comprised Factor C. The treatment was packed into a plastic rectangle container, sealed with brown plastic tape to ensure an anaerobic condition, and ensiled at room temperature. Silos of all ensiled pineapple peel samples were exposed after 0, 3, 6, and 9 days and were sampled to determine the chemical composition and pH value.

Nutrient content and pH

In the proximate analysis, samples were dried at 60 °C for 48 h in a forced-air drying oven until constant moisture was reached. The samples were ground by forcing a grinder machine through a 1 mm steel screen (Hammer mill Retsch SK1, GmbH, Haan, Germany) prior to chemical analysis.

Fermented pineapple peel was determined by dry matter (DM), ash, and ether extract (EE), crude fiber (CF) as stipulated in AOAC, 2000 and crude protein (CP) was analyzed by determining CP percentage using a Leco FP-828 P combustion analyzer (Leco FP828 Nitrogen analyzer, LECO Corporation, MI, USA).

$$CP = N \times 6.25$$

(N = nitrogen content; 6.25 = conversion factor)

In the pH test (AOAC, 2005), samples were weighed up to 5 g, 10 mL of distilled water was added, the mixture was homogenized for one minute, and the samples were then placed in a refrigerator overnight. The pH levels were determined using a pH meter, which was calibrated using buffer solutions of pH 4 and pH7 before analysis.

Sample analyses

Data was determined quantitatively through proximate analysis in a 2×2×4 factorial in CRD method. An analysis of variance within the SAS program adhered to the following statistical model:

$$Yijk = \mu + Ai + Bj + ABij + Ck + ACik + BCjk + ABCijk + \boldsymbol{\varepsilon}ijk$$

Where Yijk = observation, μ = overall mean, Ai = the type of pineapple peel effect (i = fresh pineapple peel and dried pineapple peel), Bj = the level of Loog-pang (j = no supplement, and 3% supplement), ABij = the type of pineapple effect × the level of Loog-pang, Ck = the length of the fermentation process (k = 0, 3, 6, or 9 days), ACik = the type of pineapple effect × the length of the fermentation process, BCjk = the level of Loog-pang × the length of the fermentation process, ABCijk = the type of pineapple effect × the level of Loog-pang × the length of the fermentation process, and **ε**ijk = error.

All data were presented as the mean and standard values of two replicates and subjected to one-way analysis of variance (ANOVA) and post-ANOVA. Significance was accepted at P≤0.01. The nutrient content of the samples was determined quantitatively by proximate analysis and a pH meter.

Results and discussion

DM, CP, CF, ash, EE, and pH were performed, as shown in Table 1. The interactions were observed between the type of pineapple peel, amounts Loog-pang, and fermentation times; which determined nutrient composition ($p<0.01$). Interaction between the type of pineapple peel × the level of Loog-pang × the length of the fermentation process did not affect CP, CF, EE, or pH ($p>0.01$), but DM content and ash were affected ($p<0.01$).

Dry matter

We deduced that the combination of fresh pineapple peel, 3% Loog-pang supplement, and six days of incubation most positively impacted DM content ($p<0.01$). On the other hand, the lowest DM values were found on Day 0 of fermentation when fresh and dried pineapple peel were combined with 3% Loog-pang. The results of this study indicate that the fermentation of dried pineapple peel with 3% of Loog-pang culture and a shorter fermentation time can reduce the DM values. In contrast, Hajar et al., 2012; Kurniawan, 2016; Widyastuti and Hidayat, 2018; previously found that fermentation could reduce organic matter (OM) and produce a decrease in DM, which is used by microbes as a source of energy. They further stated that the longer the fermentation process, the greater increase in water production and the decrease in DM content. The supplementation of Loog-pang in this study the decreased DM values of 88.47% and 87.77% by 0% and 3% of Loog-pang, respectively. In line with a previous study by Jude Ojei (2010), microbes used in the fermentation process will continue to grow throughout the process of fermentation. The increased biomass in fermented products will increase water vapor production, increase the moisture content, and decrease the dry matter

Crude Protein

There were no significant differences in crude protein contents within the interaction of fermented pineapple peel with Loog-pang at different fermentation times ($P>0.01$). Our results, presented in Table 1, show that the highest CP values (12.34%) were found in dried pineapple peel ensiled with 3% Loog-pang at nine days of incubation. Conversely, the lowest CP values (6.05%) were found in fresh pineapple peel at 0 day incubation without the benefit of a Loog-pang treatment. The results of this study indicate that the fermentation time 0, 3, 6, and 9 days increased by 7.71%, 8.96%, 10.22%, and 10.83% of CP values, respectively. Earlier findings by Omwango et al., 2013; reported a significant increase in crude protein content in pineapple waste, fermented at 48, <96, and <72 hours with an *Aspergillus niger* supplement ($P<0.05$). The data, however, indicated increasing CP content concomitant with fermentation time and type of pineapple peel. The use of Loog-pang; which may be attributed to the ability of *Rhizopus oligosporous* and *Aspergillus niger* bacteria, which is found in Loog-pang. In studies by Correia et al. (2004), *Rhizopus oligosporous* was used to produce enhanced levels of free phenolics from pineapple residue in combination with soy flour as a potential nitrogen source. Additionally, the inclusion of *Aspergillus niger* has been reported to have high specific activity for cellulases and hemicellulases (Howard et al., 2003). The previous

study by Nurhayati et al. (2014) found increased quality and CP content (10.74%) in pineapple peel fermentation using 3% of yoghurt (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) in 24 hours of incubation.

Crude Fiber

According to proximate analysis, the CF content was highest when dried pineapple peel and 0% Loog-pang were added to the fermentation process (26.24%). However, the combination of fresh pineapple peel with 0% Loog-pang lowered the resulting CF content in the fermented pineapple ($p<0.01$). The best result was found in fresh pineapple peel, at three days of incubation, with no Loog-pang supplement (13.62%). Previous research found higher CF contents in fresh pineapple peel with 0.4% and 0% Loog-pang at 23.38% and 29.22%, respectively; as well as no supplement of Loog-pang at 0, 7, and 14 days of incubation (Phromnoi and Laorodphan, 2021). Mandey, 2017; also found higher CF contents (17.42%) in pineapple peel fermentation with 3% Ragi tape at three days of incubation. The combination of Loog-pang and the fresh type of pineapple peel influence the CF content decrease. Despite the degradation of CF by the fermenting fungi, this is attributed to the ability of fungi to further hydrolyze the simple sugars for use as a carbon source to synthesize fungal biomass rich in protein (Bonilla Loaiza et al., 2022; Oboh, 2006; Skory et al., 1997; Sugiharto, 2017). Additionally, the ability of fungi to degrade and transform cellulose and hemicellulose has often been reported (Lyayi and Aderolu, 2004; Lyayi, 2004; Yoon et al., 2008; Rasmussen et al., 2010; Okamoto et al., 2011; Lee et al., 2015). Earlier findings by Adrizal et al., 2017; showed lower CF values in the fermentation of a fresh type of pineapple peel with a local microorganism named Bamboo sprout at 17.16%, 17.77% and 16.38% by 1, 2 and 3 weeks of incubation, respectively.

Table 1 The effects of fermented Loog-pang on the chemical contents and pH values of pineapple peel at different fermentation intervals

Treatment			Nutrient contents					
Type	Loog-pang (%)	Length	DM (%)	CP (%)	CF (%)	Ash (%)	EE (%)	pH
Fresh	0	0 day	87.55^{h}	6.05	18.34	5.53^{d}	0.63	3.51
Fresh	0	3 days	88.36^{g}	8.14	13.62	5.56^{d}	1.15	3.98
Fresh	0	6 days	88.63^{f}	9.40	15.13	6.48^{c}	1.52	4.66
Fresh	0	9 days	88.97^{e}	9.78	14.44	6.34^{c}	1.44	4.85
Fresh	3	0 day	84.48^{k}	6.83	19.19	4.32^{e}	0.8	3.99
Fresh	3	3 days	89.91^{c}	8.18	16.19	6.77^{b-c}	1.78	4.06
Fresh	3	6 days	91.54^{a}	8.26	18.86	7.49^{a}	1.46	4.99
Fresh	3	9 days	90.96^{b}	9.42	15.95	7.44^{a}	1.42	4.10
Dried	0	0 day	86.96^{i}	8.85	26.24	7.40^{a}	1.24	7.01
Dried	0	3 days	88.62^{g}	9.39	18.77	7.53^{a}	1.27	7.06
Dried	0	6 days	89.07^{e}	11.68	24.34	7.71^{a}	1.91	6.27
Dried	0	9 days	89.64^{d}	11.78	26.04	7.57^{a}	1.88	7.04
Dried	3	0 day	84.61^{k}	9.10	23.69	7.30^{a-b}	0.96	6.10
Dried	3	3 days	85.49^{j}	10.12	21.38	7.32^{a-b}	1.29	6.00
Dried	3	6 days	88.33^{g}	11.52	20.08	7.43^{a}	1.45	5.69
Dried	3	9 days	86.85^{i}	12.34	21.62	7.79^{a}	1.83	5.02
SEM			0.08	0.35	0.08	0.35	1.07	0.19
RMSE			0.11	0.49	0.11	0.49	1.52	0.27
Root Rep			1.41	1.41	1.41	1.41	1.41	1.41
Type		Fresh	88.80	8.26	16.47	6.24	1.28	4.27
		Dried	87.45	10.60	22.77	7.51	1.48	6.28
Loog-pang (%)		0	88.47	9.39	19.61	6.77	1.38	5.55
		3	87.77	9.47	19.62	6.98	1.37	5.00
Length (day)		0	85.90	7.71	21.87	6.14	0.91	5.15
		3	88.09	8.96	17.49	6.80	1.37	5.28
		6	89.39	10.22	19.60	7.28	1.59	5.41
		9	89.10	10.83	19.51	7.29	1.64	5.25
p-value Interaction								
Type x LP			<0.01	0.16	<0.01	<0.01	<0.01	0.23
Type x L			<0.01	0.13	0.05	<0.01	0.02	<0.05
LP X L			<0.01	0.12	0.30	<0.01	0.05	0.03
TypexLPXL			<0.01	0.40	0.06	<0.01	0.51	0.09

Dry matter (DM), crude protein (CP), crude fiber (CF), ether extract (EE); Standard error of the mean (SEM); Root mean square error (RMSE); Loog-pang (LP); L (Length); a, b, c, d, e, f, g, h, i, j, k: different superscripts in the same column and factor indicate differ significantly (P<0.05) and highly significant (P<0.01).

Ash

Interactions were observed between the type of pineapple peel, Loog-pang application, and fermentation times for ash content (P<0.01, **Table 1**). The lowest ash values were found in fresh pineapple peel ensiled with Loog-pang on day 0 of fermentation. The experiment demonstrated that combining dried pineapple peels with

different fermentation times using 3% Loog-pang significantly (P<0.01) enriched the ash value contents of the pineapple peel. This increase in ash content is expected due to reduced organic matter (OM) in the fermentation process. Data from the present study align with previous studies in which the fermentation time could increase ash content (Oboh, 2006; Ezekiel et al., 2010; Omwango et al., 2013; Ahmed et al.,2017; Ogodo et al., 2017). During fermentation, Loog-pang, a yeast fermentation that contained *Aspergillus, Rhizopus,* and *Mucor,* degraded organic matter as food to build their cells and obtain the energy needed for mycelium proliferation and growth (Ali et al., 2015). The less organic matter is degraded, the smaller the proportional decrease in ash content. On the other hand, the more organic matter is impaired, the more the ash content will increase proportionally (Mulyono et al., 2019; Wardono et al., 2022; Yan et al., 2022).

Ether Extract

There were no significant differences in the EE contents through the interactions between the type of pineapple peel supplemented with 0% and 3% Loog-pang at different fermentation times. However, it showed that the combination of pineapple peel types with 0% and 3% Loog-pang impacted EE contents (P<0.01, **Table 1**). In contrast with the findings of Lasekan and Shittu, 2019; the type of pineapple peel used in fermentation has not influenced the fat content. Pineapple peel fermentation combined without the Loog-pang supplement resulted in the lowest EE content compared to the other groups; whereas the highest EE values were found in the fermentation of dried pineapple peel with no Loog-pang supplement (1.91%). A decrease in EE content might be due to the supplementation of Loog-pang, which contained fungi of the genus *Aspergillus, Rhizopus,* and *Mucor* are reported to be lipase-producing organisms that used it as energy for proliferation and degraded EE content (Mahadik et al., 2002; Mhetras et al., 2009; Phromnoi and Laorodphan, 2021).

pH

The type of pineapple peel × the level of Loog-pang × the length of the fermentation process had no effect on pH (p>0.01), as expressed in **Table 1**. However, the combination of the type of pineapple peel and fermentation time affected the pH levels; in which dried pineapple peel combined with any length of fermentation increased the pH contents (p<0.05). The lowest pH values were found in fresh pineapple peel ensiled with 0% Loog-pang at day 0 of fermentation (3.51). The previous study by Suksathit et al. (2011) showed that fresh pineapple waste silage with no yeast produced a low pH value (3.06). This is attributed to the ability of dried pineapple peel supplemented with Loog- pang to effectively increase pH levels.

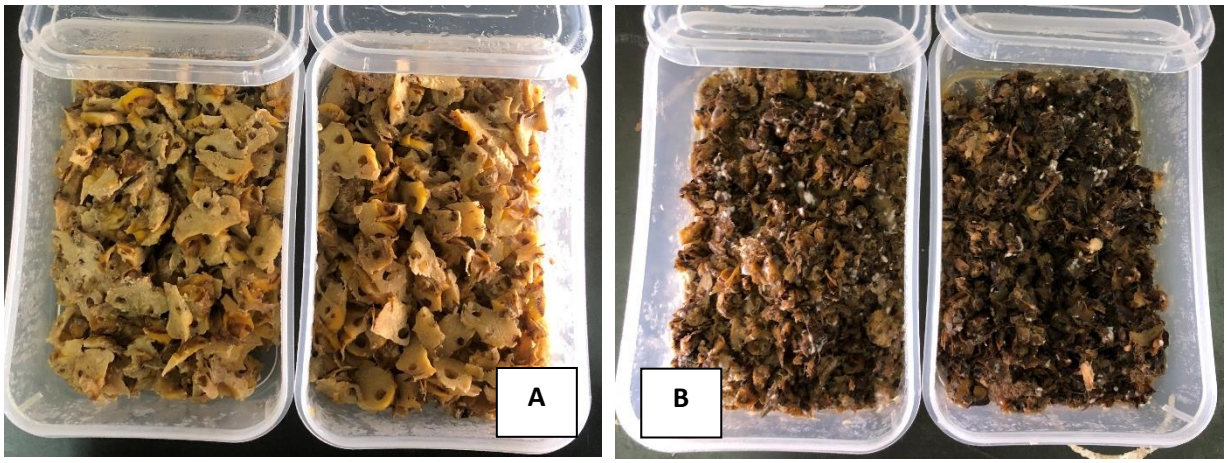


Figure 1 Comparison of the pineapple peel types colour after fermentation with 3% of Loog-pang at 9 days A) Fresh type and B) Dried type.

Additionally, the dried pineapple peel treatment used in this study was seen to have a dark brown color, and a slightly acidic, fruity smell; due to the presence of lactic acid (Limin et al*.,* 2018). The decrease in pH within the fermentation process is likely due to the influence of lactic acid produced by lactic acid bacteria. pH is a critical factor that influences the growth of microorganisms and the formation of products within the fermentation process, as each microorganism in a living environment has an optimal pH range (Horikoshi, 1999; Baker-Austin and Dopson, 2007; Jin and Kirk, 2018; Preetha and Rita, 2020)

Conclusion

The crude fiber contents of fresh pineapple peel without Loog-pang fermentation were lower than those of unfermented pineapple peel. Our study's findings showed that nine days of fermentation with fresh pineapple peel and 3% Loog-pang remarkably increased crude protein contents while significantly reducing the crude fiber value. In addition to the type of pineapple peel, the Loog-pang supplement and incubation time impacted the nutrient levels within the fermentation process. We do recognize, however, that the physical quality of the pineapple peels after the fermentation process; such as color, smell, and texture, is of interest for further investigation.

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