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Journal of Functional Food and Nutraceutical

SCREENING OF IMMUNOSTIMULATORY ACTIVITY FROM INDONESIAN KAMPUNG (*GALLUS DOMESTICUS*) EGG WHITE WATER EXTRACT: *IN VITRO* STUDY

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ABSTRACT

The most popular Indonesian native chicken, known as kampung chicken, is maintained under free-range conditions therefore it is prone to high environmental stress. Indonesian native chicken and its product are always regarded as having health benefits compared to commercial chicken by Indonesian society. But there is still limited report regarding Indonesian native chicken and its product. This study focused on screening immunostimulatory activity from Indonesian native chicken egg white using *in vitro* approaches as a functional food. Indonesian native chicken egg white (NEW) was extracted using distilled water supplemented to human-human hybridoma HB4C5 cells to examine the IgM production stimulating activity using ELISA. The gene expression was also examined using qRT-PCR. The ability of NEW on stimulating immunoglobulin production by mouse splenocytes was analyzed. Commercial egg white water extract (CEW) was used as a comparison. The data were analyzed using One-way ANOVA and continued by using post-hoc analysis using Tukey's multiple comparison test. The results showed that NEW and CEW modulated IgM production by the HB4C5 cells 8.72-fold and 6.75-fold, respectively, compared to control. NEW stimulated immunoglobulin (Ig) production by the mouse splenocytes higher than CEW. To conclude, NEW provides an immunostimulating activity that can potentially act as a health-promoting food.

Keywords: gallus domesticus, HB4C5 cells; immunoglobulin production; immunostimulatory activity; Indonesia native chicken egg white

ABSTRAK

Ayam asli Indonesia yang paling banyak diternakkan, dikenal dengan nama ayam kampung, dipelihara dengan keadaan diumbar sehingga rentan terhadap tekanan lingkungan yang tinggi. Ayam Kampung dan produknya oleh masyarakat Indoneisa selalu dianggap memiliki manfaat kesehatan dibandingkan dengan ayam komersil. Namun penelitian terkait ayam kampung dan produknya masih terbatas. Penelitian ini difokuskan pada skrining aktivitas imunostimulasi dari putih telur ayam kampung dengan menggunakan pendekatan *in vitro* sebagai potensi bahan pangan fungsional. Pertama, putih telur kampung diekstraksi terlebih dahulu menggunakan akuades. Kemudian ekstrak akuades telur kampung (NEW) ditambahkan ke sel HB4C5 untuk dilakukan pemeriksaan aktivitas stimulasi IgM menggunakan ELISA. Selain itu, ekspresi gen menggunakan qRT-PCR juga diperiksa. Tidak hanya itu, kemampuan NEW dalam menstimulasi produksi imunoglobulin pada splenosit tikus juga dianalisis. Ekstrak akuades putih telur komersial (CEW) digunakan sebagai perbandingan. Data dianalisis menggunakan ANOVA satu arah dan dilanjutkan analisis posthoc menggunakan uji perbandingan berganda Tukey. Hasil penelitian menunjukkan bahwa NEW dan CEW menstimulasi produksi IgM oleh sel HB4C5 masing-masing 8,72 kali lipat dan 6,75 kali lipat dibandingkan dengan kontrol. Tidak hanya itu, NEW menstimulasi produksi immunoglobulin (Ig) oleh splenosit tikus lebih tinggi dari CEW. Sebagai kesimpulan, NEW menyediakan aktivitas imunostimulan yang berpotensibertindak sebagai makanan yang dapat meningkatkan kesehatan.

Kata kunci*:* aktivitas immunostimulant; gallus domesticus; produksi immunoglobulin; putih telur ayam kampong; sel HB4C5

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INTRODUCTION

Eggs are widely known as a nutritious food source containing essential nutrients such as amino acids, unsaturated fatty acids, vitamins, minerals, and folate. Egg protein has always been regarded as an excellent source of bioactive peptides distributed in the egg white and egg yolk amounting to 50% and 40%, respectively (Kovacs-Nolan et al., 2005; Mine, 2007). Besides their nutritional properties, some bioactivities such as antibacterial, anti-inflammatory, antihypertensive activities were also observed and contributed to the health-promoting functions (Lee et al., 2018; Tagashira et al., 2018).

Recently, there is a high demand for natural products, especially food-source, as health-promoting agents. This is due to the increasing evidence of immunological disease (Nieman and Wentz, 2019). Immunostimulatory agents are essential to regulate immune response by enhancing the optimal defensive capacity of human immunity (Veldhoen and Brucklacher-Waldert, 2012). Thus, fresh and innovative idea approaches are needed to develop.

Researchers found that certain daily nutritional interventions are advantageous compared to chemical compounds as immunostimulants in terms of side effects and price (Veldhoen and Brucklacher-Waldert, 2012). Some bioactive protein fractions obtained from food products were reported to have immunostimulatory effects. Besides, animal protein is considered to be more nutritious and effective in improving innate and adaptive immunity, resulting in the enhancement of the human immune system from infection and disease (Chalamaiah et al., 2017).

Previous studies added that egg white water-soluble protein fractions could modulate the immune system through the innate and adaptive immune system. Based on *in vitro* study, ovotransferrin, ovomucin, and lysozyme were found to modulate the immune system through the innate immune mechanism (Tanizaki et al., 1997; Lee et al., 2018; Tagashira et al., 2018). Meanwhile, lysozyme was found to be a strong candidate as an immunomodulator due to its ability on stimulating adaptive immunity through immunoglobulin M (IgM) production by HB4C5 cells (Sugahara et al., 2000).

There are few factors that influence the composition and concentration of egg white, such as the wide variety of chicken breeds, physiological status, and the age of the laying hens (Robert, 2004; González Ariza et al., 2021). A previous study reported that the traditional breed had a different egg white concentration. This might be due to immunological adaptation, reflecting their original need to fight pathogens under environmental stress of free-rearing of traditional chicken (Bílková et al., 2018). Moreover, Javůrková et al. (2019) found out that there was a close correlation between eggshell pigmentation in traditional egg and the egg concentration, especially lysozyme and ovotransferrin. They explained that tinted eggshell on the traditional egg was more likely to have a higher lysozyme component compared to other traditional eggs. Another research on characterizing the protein component in traditional egg also reported lysozyme and other egg white components vary widely in traditional egg (Bílková et al., 2018).

Kampung chicken (*Gallus domesticus*) is well known as an Indonesian native chicken. Indonesian native chicken is maintained under free-range conditions; therefore, they are prone to environmental stress (Muladno, 2008). In addition to that, the differences in strain and feed of native chickens and commercial layer chickens cause differences in the eggs' exterior and interior quality (Robert, 2004). So far, the study conducted by Wulandari et al. (2015) has reported that the lysozyme isolated from Indonesian native chicken egg worked as an antibacterial agent towards *Staphylococcus aureus* and *Escherichia coli*. Ragland and Criss (2017) found that the ability of lysozyme to work as antibacterial was correlated with its ability to modulate immune system because components released from bacteria in a lysozyme-dependent manner can alter innate immune function. However, the reports on the effect of the Indonesian native chicken egg white on immunological aspects are not available.

Considering all the previous reports, it is reasonable to evaluate the immunostimulatory

activity of Indonesian native chicken egg white using *in vitro* approaches. Thus, this finding could be beneficial for the search for the new and effective natural immunostimulatory agent as well as potential functional food agent.

MATERIALS AND METHOD

Materials

A total of 90 eggs from commercially available Indonesian native chicken egg (*n*=45) and commercial chicken egg (*n*=45) was purchased from Yogyakarta, Indonesia. The eggs were kept in the refrigerator no more than three days before further analysis. ERDF medium was purchased from Kyokuto Pharmaceutical (Tokyo, Japan). Fetal bovine serum (FBS), insulin, transferrin, ethanolamine, and sodium selenite were obtained from Sigma-Aldrich (St. Louis, MO, USA). Goat anti-human IgM antibody, horseradish peroxidase (HRP)-conjugated anti-human IgM antibody, goat anti-mouse IgM, goat anti-mouse IgG, rabbit anti-mouse IgA, HRP-goat anti-mouse IgM, HRP-goat anti-mouse IgG, and HRP-goat anti-mouse IgA were obtained from Invitrogen (Carlsbad, CA, USA). Oxalic acid, ABTS (2.2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), trypan blue solution, and Sepasol were obtained from Nacalai Tesque (Kyoto, Japan). MMLV reverse transcriptase was from Promega (Madison, WI, USA), Thunderbird SYBR qPCR Mix was obtained from Toyobo (Osaka, Japan).

Characterization of albumen from Indonesian native egg and commercial egg

The shell cleanliness of prepared egg was checked through direct observation. First, the eggshells were cracked open. The presence of meat spot and bloodspot inside the egg was checked through direct observation. The albumen and yolk were separated. The height of the thick albumen was measured within a tripod micrometer at three different points. The height of albumen was measured to describe the quality of the egg. The egg white was then pooled, homogenized, and further lyophilized. The moisture and protein content of albumen from each egg was measured using the method from Association of Official Analytical Chemist (AOAC) method (AOAC, 2005) with three replications.

Water extraction of egg white samples

Lyophilized Indonesian native egg and commercial egg white was dissolved in distilled water and stirred for 24 h at 10°C. After that, they were centrifuged at 25,000 × g at 4°C for 20 minutes to remove insoluble substances. Then, supernatant was adjusted to pH 7.4 and sterilized using 0.22 µm filter. The sterilized samples were kept in the refrigerator and used as egg white water extracts for further experiments.

HB4C5 cell culture and assay condition

This experiment utilized human hybridoma cell line HB4C5 cells. This cell line was used to assess the IgM production stimulation activity of egg white samples. HB4C5 cells were cultured in ERDF medium supplemented with 10% FBS at 37°C in a humidified atmosphere containing 5% CO_2.

The ability of NEW and CEW on stimulating IgM production was examined by measuring the concentration of IgM secreted by HB4C5 cells into the culture medium.—HB4C5 cells were sub-cultured in ERDF medium supplemented with 10 µg/mL of insulin, 20 µg/mL of transferrin, 20 µM ethanolamine, and 25 nM sodium selenite (ITES-ERDF medium). HB4C5 cells were inoculated in 96-well culture plate at cell density of 3×10^5 cells/well in ITES-ERDF medium supplemented with various concentrations of NEW or CEW (0.04 mg/mL, 0.16 mg/mL, 0.625 mg/mL, 2.5 mg/mL, and 10 mg/mL). Distilled water was added to ITES-ERF medium instead of sample as control. After six (6) hours incubation period, the supernatant was collected, and the concentration of IgM secreted into the culture medium was measured by the enzyme-linked immunosorbent assay (ELISA).

First, 100 µL of the primary antibody using goat anti-human IgM antibody diluted 1000 times with 50 mM carbonate buffer as coating solution was applied to the 96-well plate and incubated at 4°C overnight. Phosphate-buffered saline with Tween

20 (PBS-T) was used three times as a washing solution in between the reaction. After that, each well was blocked with PBS containing 5% skim milk for two hours at 37°C. Next, each well was treated with 50 µL of culture supernatant and distilled water as a control for one hour at 37°C. Next, each well was treated with washing solution. After that, 100 µL of secondary antibody consisting of HRP-conjugated anti-human IgM antibody diluted 2,000 times with PBS containing 5% skim milk was added to each well and incubated for one hour at 37°C. Then, 0.6 mg/mL of ABTS dissolved in 50 mM citrate buffer (pH 4.0) containing 0.03% H_2O_2 was applied to the well at 100 µL/well. After that, 1.5% oxalic acid was added to the well to stop the reaction. The absorbance was measured at 415 nm with 655 nm as the reference wavelength (Sugahara et al., 2000).

Determination of cell viability

Trypan blue dye exclusion test was conducted to evaluate the cytotoxicity of samples to HB4C5 cells. HB4C5 cells were inoculated at concentration 3×10^5 cells/well with various concentration of NEW and CEW (0.04 mg/mL, 0.16 mg/mL, 0.625 mg/mL, 2.5 mg/mL, and 10 mg/mL) into a 24-well culture plate. In addition to that, distilled water was added as a control instead of sample. After inoculation, HB4C5 cells were cultured for 6 h in CO_2 incubator at 37°C and 5% CO_2. After that, the cell suspension was collected from each well into microtubes and centrifuged 3,000 rpm at 4°C for 10 min. The supernatant was removed, and the cell pellet was resuspended with 10 µL medium. Next, 10 µL trypan blue solution was added to each cell suspension in dark room. After 5 min, 10 µL of cell suspension was put in the haemocytometer. The number of living cells and dead cells were counted. Cell viability (%) was calculated by dividing the number of viable cells with total number of cells times 100.

Determination of gene expression using reverse transcription-polymerase chain reaction (qRT-PCR)

The effect on IgM gene expression was measured using quantitative real-time RT-PCR (qRT-PCR). The method for determining gene expression was conducted under previous experiment from Nishi et al. (2011) with few modifications. HB4C5 cells were cultured in ITES-ERDF medium at 5×10^5 cells/mL containing egg white water extract at 37°C for 6 h. The harvested cells were washed twice with PBS and stored at −80 °C. Sepasol RNA I Super G Total RNA and chloroform (Nacalai Tesque, Kyoto, Japan) were used for isolating RNA according to the manufacturer's instructions, and the isolated RNA was spectrophotometrically quantified using a Nanodrop (Biospec-nano, Shimadzu, Kyoto, Japan). The first strand cDNAs were synthesized from total RNAs using an oligo-dT primer, MMLV reverse transcriptase, and dNTP. The qRT-PCR mixture consisted of 2.5 µg of a cDNA sample, Thunderbird SYBR qPCR Mix, 10 µM forward primer, and 10 µM reverse primer were spined for 5 minutes before applying to StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The relative gene expression was calculated by the comparative CT method using StepOne Software v2.1 (Applied Biosystems). Data are shown as the number-fold differences in IgM expression normalized to the Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), housekeeping gene, as an endogenous reference. The PCR primer sequences used in this experiment for amplification were as follows: human GAPDH 5'-GCACCGTCAAGGCTGAGAAC-3' (sense) and 5'-TGGTGAAGACGCCAGTGGA-3' (antisense); and human IgM 5'-CTCCCAAAGTGAGCGTCTTC-3' (sense) and 5'-CAGCCAGGACACCTGAATCT-3' (antisense) (Nishi et al., 2011).

Determination of Ig-production stimulating activity of egg white water extract on mouse splenocytes *in vitro*

Animal experiment was carried out under an approved protocol by Ehime University Animal Care and Use Committee and was performed under applicable guidelines and regulations. Three 6-week-old female BALB/c mice were bought from Japan CLEA (Tokyo, Japan). The mice were housed with a pelleted basal diet and water *ad libitum* in the animal room under controlled environment: 12 h light/dark cycle at a temperature of 24 ± 1°C.

The mouse splenocytes were prepared under a previous experiment by Kumalasari et al. (2012). The mice were sacrificed and the spleens were taken out from mice. The spleen was minced through 40 μm pore size mesh into the culture dish. The splenocytes suspension was centrifuged at 190 × g at 4°C for 5 min and hemolyzed 2 times using hemolysis buffer. After that, the splenocytes were washed with PBS and centrifuged at 190 × g for 5 min. Following the centrifugation, the cells were suspended in 5% FBS-RPMI 1640 medium and inoculated into each well of a 96-well culture plate at 1×10^6 cells/well. After that, the NEW or CEW was the added into the 96 well-plate. As a comparison, distilled water was also added as a control. The mixture of mouse splenocytes and the samples were incubated in a CO_2 incubator at 37°C for 48 h. The availability of cells was checked using WST-8 test. Thereafter, the IgA, IgG, and IgM secreted into culture medium were determined by ELISA as previously described by Nishi et al. (2011).

First, the 100 μL coating solution was added into a 96-well plate for 2 h at 37°C. The coating solution consisted of goat anti-mouse IgA, goat anti-mouse IgG, or rabbit anti-mouse IgM diluted 1,000-folds with 50 mM carbonate buffer. After that, the 96-well plate was washed using PBS-T. Following the washing step, each well was blocked with 250 μL of 5% skim milk-PBS solution for 2 h at 37°C to prevent non-specific binding which suppresses accurate measurement. After washing, each well was treated with 50 μL of culture supernatant for 1 h at 37°C. Distilled water was added to blank well. After incubation, the plate was washed and treated with 100 μL secondary antibody consisting of HRP-goat anti-mouse IgA, HRP-goat anti-mouse IgG, or HRP-goat anti-mouse IgM diluted 1,000 times with 5% skim milk-PBS for 1 h at 37°C. After washing the wells, 0.6 mg/mL of ABTS dissolved in 50 mM citrate buffer (pH 4.0) containing 0.03% H_2O_2 was added to the wells at 100 μL/well. The absorbance was measured using a microplate reader at 415 nm with 655 nm as the reference wavelength (to eliminate non-specific absorbance) after the addition of 1.5% oxalic acid to terminate the coloring reaction at 100 μL. The assays were triplicated.

Statistical analysis

All the data are expressed as a mean ± standard deviation (SD). The data of egg quality was analyzed using t-test. Meanwhile, all the data of immunostimulatory activity were analyzed using one-way variance (ANOVA) using SPSS (ver. 22). Tukey HSD was used as a post-hoc analysis. Values of $p<0.05$ were regarded as statistically significant.

RESULTS AND DISCUSSION

One of the utmost strategies on promoting and maintaining a human health is through human diet containing natural bioactive compound especially the one that has low toxicity to normal cells (Faria et al., 2013; Teodoro, 2019). The finding of the more effective natural compounds is a very important goal. Therefore, this research was succeeded to identify the immunostimulatory potential agent from native chicken egg white through stimulating IgM production by HB4C5 cells and antibody production by mouse splenocytes.

Characterization of Indonesian native chicken egg white

First, the characterization of the egg was conducted to understand the difference between native egg and commercial egg (Table 1). The interior quality of the egg was described by looking at the height of the egg white. While the chemical characteristic of egg white was described by examining the moisture and protein content. This study described that native egg has a lower egg white height than commercial egg ($p<0.05$). In addition, native egg has 86.91% moisture content, which was lower than ($p<0.05$) that of commercial egg (87.72%). In addition, the protein content of native egg showed a higher percentage ($p<0.05$) than that of the commercial egg white, which was 10.89% and 10.26%, respectively.

A good quality egg has higher thick albumen describing the freshness of the egg. As the temperature and storage time increase, the height of albumen will decrease periodically (Samli et al., 2005). Not only that, but the height of thick

albumen is an important indicator of calculating Haugh Unit (HU) as well. Haugh Unit (HU) describes the internal quality of egg based on the height of its albumen and egg weight. The height of thick albumen is directly correlated with HU. It is generally accepted that a good quality egg has higher HU (Robert, 2004). However, previous research reported that the egg's weight could be a biased factor in calculating HU and might influence the height of thick albumen(Silversides and Villeneuve, 1994; Shi et al., 2009). It is safe to say that the lower height of thick albumen in native egg was caused by the lower egg weight in native egg. The albumen height of native egg in this experiment still in the range of thick albumen height of native egg according to the previous experiment, which was 3.3-7.22 mm (Wulandari et al., 2015).

Protein content in raw native chicken egg white was slightly higher than that in commercial chicken egg white (Table 1). This result was supported by previous result (Wei et al., 2019). Previous study has reported that the water-soluble protein content in egg white was known to have immunostimulatory activity, such as ovalbumin, ovomucoid, ovotransferin, and lysozyme (Huang et al., 2012; Rupa et al., 2015; Sugahara et al., 2000). We assume that NEW contained mostly water-soluble fractions, such as ovotransferrin, ovomucin, lysozyme, unknown peptides, and amino acids. It is reasonable to say that water-soluble fraction that has immunostimulating activity in NEW is higher than in CEW.

Table 1. Comparative physical and chemical properties of fresh indonesian native chicken egg and commercial chicken egg using proximate analysis (mean ± SD, *n*=3)

Parameters	Native egg	Commercial egg	*p* value
Egg white height (mm)	4.87±1.16	8.11±1.18	<0.05
Moisture (%)	86.91±0.01	87.72±0.01	<0.05
Protein content (%)	10.89 ±0.07	10.26 ±0.01	<0.05

The effect of Indonesian native chicken egg white on IgM production and cell viability of HB4C5 cells

In order to demonstrate the immunostimulatory activity of Indonesian native chicken egg white, it was extracted using distilled water, thereafter activity on IgM-producing stimulation was evaluated using HB4C5 cells. The cells used in this experiment was the human-human hybridomas producing a monoclonal IgM. HB4C5 cells are commonly used in the screening of antibody production stimulation activity in foodstuffs.

Previous studies screened the IgM production stimulating activity from various foodstuffs using human hybridoma HB4C5 cells (Sugahara et al., 2005; Daifuku et al., 2012; Nishi et al., 2011). In current investigation, as shown in Figure 1, egg white water extract stimulated the production of IgM by HB4C5 cells in a dose-dependent manner. This result suggested that both egg white samples from Indonesian native chicken egg and commercial egg significantly enhance IgM production by HB4C5 cells. However, NEW more effectively activated IgM production compared to CEW, where NEW significantly enhanced 8.72-fold, while CEW enhanced 6.75-fold compared to the control at 20 mg/mL sample concentration.

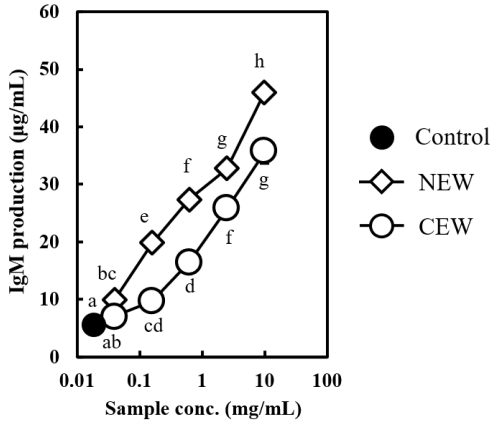

Figure 1. The effect of egg white water extract on IgM production by HB4C5 cells. means denoted by a different letter indicate significant difference between treatments (Tukey's HSD, $p<0.05 n=3$)

Interestingly, at the lowest concentration, CEW did not show any IgM production-stimulating activity (p>0.05) on HB4C5 cells. In the same concentration, NEW stimulated IgM production slightly higher than control (p<0.05). According to the relative cell viability result (Figure 2), both samples did not show cell toxicity at any concentration.

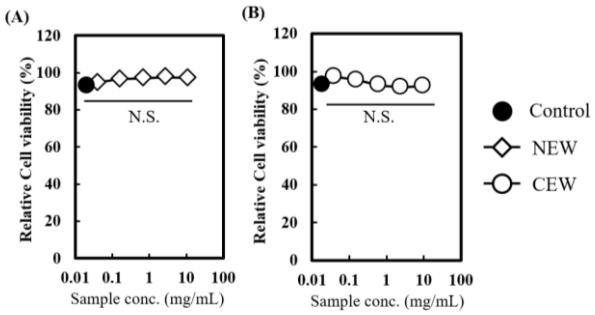

Figure 2. Cell viability of HB4C5 cells treated with egg white water extracts and control. (A) relative cells viability of HB4C5 cells treated with NEW, (B) relative cell viability of HB4C5 cells treated with CEW (Tukey's HSD, p<0.05, n=3)

The effect of NEW on IgM gene expression in HB4C5 cells

To further analyze the IgM production enhancing ability of NEW, the gene expression level of IgM in HB4C5 cells was investigated using quantitative RT-PCR. HB4C5 cells were harvested after cultivation with 150 µg/mL for 6 h. As shown in Figure 3, NEW upregulated IgM gene expression, meanwhile CEW did not sgnificantly different with control. This result showed that NEW more effectively upregulates IgM gene expression level in HB4C5 cells than CEW at the same sample concentration as NEW (p<0.05).

The immunostimulatory activity of Indonesian native chicken egg white has not been explored before. This study added new information regarding the potential immunostimulatory agent from native egg. This study revealed that NEW activates IgM production by HB4C5 cells through elevation of mRNA level. The potential active substance is water-soluble protein from egg white. This is supported by previous experiment conducted by Sugahara et al. (2000) who examine the mode of action of water-soluble fraction, lysozyme, from egg white. The study showed that lysozyme performed immunostimulatory activity through enhancing IgM production by HB4C5 cells through accelerating the translation process. Further experiments are needed to examine the active substance and the mode of action of Indonesian native chicken egg white water extract.

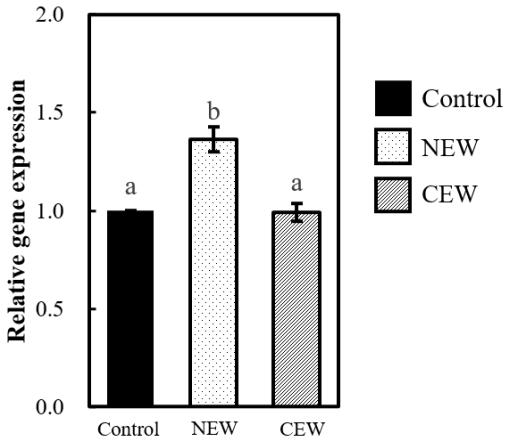

Figure 3. Effect of egg white water extract on IgM gene expression (mean ± SD). means denoted by a different letter indicate significant difference between treatments (Tukey's HSD, p<0.05, n=3)

The effect of NEW on mouse splenocytes *in vitro*

The immunostimulatory activity of NEW and CEW was evaluated by using mouse splenocytes *in vitro*. As indicated in Figure 4, NEW and CEW accelerated the production of IgA and IgM dose-dependently.

NEW and CEW facilitated IgA production 4.4-fold and 1.98-fold, respectively, at the highest concentration compared to control (p<0.05). Unlike NEW, CEW did not significantly enhanced IgA production at the lowest concentration but significantly increased at the highest concentration. IgG concentration was not significantly increased by both NEW and CEW, thus there was no significant difference between both samples (p>0.05). IgM production was enhanced by both

NEW and CEW (p<0.05). NEW effectively enhanced 3.2-fold and CEW enhanced 2.6-fold at the highest concentration compared to control.

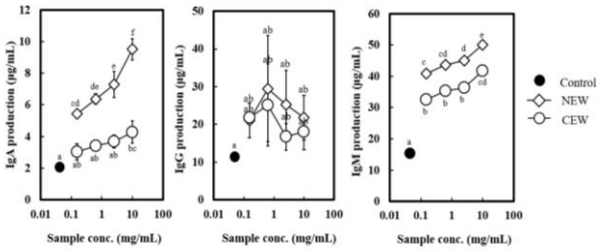

Figure 4. The effect of egg white water extract on mouse splenocytes. means denoted by a different letter indicate significant difference between treatments (Tukey's HSD, p<0.05, n=3)

This study demonstrated that both NEW and CEW were able to stimulate IgA and IgM production by mouse splenocytes. Furthermore, the production of IgG from both samples were not significantly different (p>0.05). In addition, NEW has an ability to enhance IgA and IgM production higher than CEW. In other words, NEW has immunostimulatory activity through stimulating Ig production more effectively compared to CEW by mouse splenocytes. This result is supported by Song et al. (2014) who reported that egg white water extract promoted host defense mechanism through producing immunoglobulin production.

Ig production provides total humoral immunity (short-term and long-term protection) of the body to fight against all sorts of pathogens, cancer cells, and toxic substance (Janeway et al., 2001). The result of this study was supported by the previous findings which pointed out that production of IgA, IgG, and IgM plays an important role to provide humoral immunity in human body (Schroeder and Cavacini, 2010). This finding was considered novel since there was no previous research evaluating the ability of egg white extract on Ig production using mouse splenocytes *in vitro* and this is the first study that evaluated immune stimulating effect of native egg white.

Further detailed *in vitro* studies are needed to determine the exact substance in NEW that modulates IgM production. Besides, *in vivo* humoral and cellular immunological investigations are required to determine its capability on immunostimulating potential and molecular mechanisms of actions.

CONCLUSION

NEW stimulates the immune system through enhancing IgM production by the HB4C5 cells without any cytotoxicity. NEW was also found to more effectively upregulate IgM gene expression level in HB4C5 cells than CEW. This study also demonstrated that NEW has an ability to modulate Ig production by mouse splenocytes *in vitro*. NEW enhanced not only IgA and IgM production but also IgG production only at the highest concentration. the immunostimulating activity of NEW can be a promising source of an immunostimulator agent. Further investigations of active substances and *in vivo* studies are needed to understand the mechanism of immunostimulatory activity.

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Journal of Functional Food and Nutraceutical

THE IMPACT OF PERCEIVED VALUE OF JAMU TOWARDS THE MILLENNIAL PURCHASE DECISION: THE CASE STUDY OF GENERATION Z

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ABSTRACT

The aim of this research is to identify the impact of perceived value of Jamu towards the millennial purchase decision in the case study of generation Z. The indicators use for perceived value are awareness, perception, and family environment. As for purchase decision, the indicator used are consumer behavior and purchase decision. The data were collected from 125 who have previously consumed Jamu. Simple linear regressions and descriptive data analysis were used to examine the data collected. The findings indicate that perceived value of Jamu significantly impacts the millennial generation and with descriptive data analysis each indicator were examined. Among all indictors of perceived value, perceptions have the highest impact towards the millennial purchase decision.

Keywords: awareness; consumer behaviour; family environment; perceived value of jamu; purchase decision

ABSTRAK

Tujuan dari penelitian ini adalah untuk mengetahui pengaruh persepsi nilai jamu terhadap keputusan pembelian milenial pada studi kasus generasi Z. Indikator yang digunakan untuk nilai yang dipersepsikan adalah kesadaran, persepsi, dan lingkungan keluarga. Sedangkan untuk keputusan pembelian, indikator yang digunakan adalah perilaku konsumen dan keputusan pembelian. Data dikumpulkan dari 125 orang yang sebelumnya telah mengkonsumsi Jamu. Regresi linier sederhana dan analisis data deskriptif digunakan untuk memeriksa data yang dikumpulkan. Hasil penelitian menunjukkan bahwa nilai persepsi jamu berpengaruh signifikan terhadap generasi milenial dan dengan analisis data deskriptif setiap indikator diuji. Di antara semua indikator perceived value, persepsi memiliki pengaruh paling tinggi terhadap keputusan pembelian milenial.

Kata kunci*:* kesadaran; keputusan pembelian; lingkungan keluarga; perilaku konsumen; persepsi nilai jamu

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INTRODUCTION

Indonesia is Southeast Asia's largest country, with approximately 5,100 km from east to west and 1,800 km from north to south. The country itself is estimated to have 271,056,000 people in population. In addition, the nation has more than 300 various ethnic groups and more than double the number of different languages (Mc Divitt et al., 2020).

Jamu is traditional Indonesian herbal medicine used in Indonesia for many centuries to treat diseases and to maintain good health (Amalia & Aprianingsih, 2017). Today, while preserving its traditional value, *Jamu* itself is struggling to survive in the modern lifestyle. 49.53% of Indonesians consume *Jamu* to help them maintain their health and to cure diseases (Andriati. R.M. Teguh Wahjudi, 2016). Previously *Jamu* was known as *Jamu gendong* which is a traditional herbal medicine in the form of bottled drinks that are sold in a basket which carried by the seller (KBBI, 2016).

Furthermore, *Jamu* also has a lot of amazing benefits, *Jamu* uses dozens of vegetables, plants, and spices which are almost all antive to Indonesia (Bali, 2019). Here are the 10 most common herbs and spices used in traditional Indonesian for healing:

1. Turmeric
   It is naturally an anti-inflammatory, highly antioxidant, helps to prevent cancer, heart disease and Alzheimer's disease.
2. Ginger
   It helps with digestion, encourages weight loss, relives morning sickness and diarrhea, relieves cold and flu, controls blood sugar levels, and reduces cholesterol.
3. Galangal
   It is part of the ginger family, used as in anti-microbial, anti-fungal, anti-parasite, analgesic, wound disinfectant, regulator of blood pressure and heart tonic, helps with arthritis and rheumatism.
4. Kaemferia Galangal
   It helps in relieving cold and cough, headaches and migraine, pneumonia, treat rheumatic disorders, cure asthma, and helps get rid of acne.
5. Curcuma
   Curcuma's essential oil is believed to enhance the role of the kidney and liver, anti-inflammatory, antioxidant, cancer prevention, anemia care.
6. Cinnamon
   It is filled with antioxidants, has anti-inflammatory properties, decreases cholesterol rates, heart disease and blood pressure, lowers blood sugar levels and has strong anti-diabetic impact.
7. Nutmeg
   It promotes good sleep, decreases skin inflammation and acne, helps with Alzheimer's disease and dementia, detoxifies the liver, and relieves stone in the kidneys.
8. Tamarind
   It reduces blood sugar levels, reduces hair loss, rich in vitamin and minerals such as cardiovascular essential potassium, anti-inflammatory, ideal for reducing joint pain and connective tissue.
9. Cardamom
   It potentially decreases colorectal cancer by improving antioxidant activity in the body, blood circulation. Anti-diabetic effect, the essential oil believed to have anti0depressant effects, protecting against bad breath, a nausea and vomiting remedy.
10. Betel Leaves
    These leaves are common for enhancing digestion, reducing stomach pain, treating breathing problems, preventing body odor and nose bleeding, relieving cough, and treating headaches.

Though, the advantages of *Jamu* can clearly be seen as something that is beneficial and healthy for the body, there few disadvantages too look out for. Singh (2018) states that, herbal medicinal products take too long to function and the whole process is terribly slow. They contain various ingredients that occasionally cause allergic reactions. Herbal medicinal products are not good for serious cases like heart attacks and broken bones. Such medications are also unsuccessful in unexpected injuries and illness. He further discusses that; Herbal remedies and medication may have harmful

side effects for some conditions which often take a long time to reveal. Herbal medicines also interfere with prescription medicines. Herbs harvested in the wild are endangered. Furthermore, faulty identification of the appropriate herb may even result in poisoning. Herbal medicines are also not well regulated and hence they do not bear any guarantee of safety.

Today, the selling of *Jamu* is modernized into capsules, pills, and sachets. Afdhal & Welsch (1988) states that *Jamu* has become increasingly identified with the rapidly growing range of powders, creams, tablets, capsules, and cosmetic packages that are being produced in both small-scale cottages industries and increasingly sophisticated factories. With the modernization of *Jamu*, it is no longer necessary to wait for *Jamu Gendong*, as the access to *Jamu* is easier.

A study by Boparai et al (2017) found a lack of knowledge and understanding of herbal medicines and herbal-drug interaction amongst students, most participant lies in the millennial age group and most students that participated on the study, did not use herbal medicine for personal use. Therefore, a research would be conducted to analyze the perceived value of *Jamu* towards the millennial lifestyle.

Raines (2002) states that, millennials are people who were born between the year 1980 and 2000. In addition, (Fry, 2016) states that millennials have outgrown Baby Boomers as the largest living generation. Furthermore, the millennial population is estimated to peak at 81.1 million in the year 2036. According to Goldmansachs (2020), millennial is the first generation of digital natives and their technological affinity helps shape the way they shop. They are used to collecting price comparisons, product information and peer reviews instantaneously. Furthermore, they are committed to fitness, to the right exercise and eating time and money. Their active lifestyle affects all trends, from food and drink to fashion.

Moreover, Costin (2019) states that millennials are currently the biggest spender in the society. 60% of millennials spend over $4 on one single coffee, 70% of millennials are willing to spend more to eat in trendy restaurants, 69% of millennials purchase clothes for reason apart from basic needs, and over 50% of millennials spend money on taxis and Ubers, compared with Gen X only 29% and Boomers 15% do the same.

Family environment also influences lifestyle, Shaw (2014) discuss that, healthy relationships with parents, characterized by low tension, high support rates and open communication, are particularly important for adolescents as they undergo adolescents physical and emotional changes. For example, regular parent-adolescents contact, and positive parent recognition are associated with lower drug use, including lower teenage drinking and smoking. Similarly, adolescents who report having a positive relationship with at least one parent are more likely to have good physical and mental health. Adolescent family conflicts are to be anticipated and can even serve a significant development purpose. However, adolescents who experiences high rates of tension with their parents and/or low levels of support are more likely to be involved in risk behaviors, such as early alcohol use or drinking and smoking and are more likely to have depressive symptoms to contend with. Thus, it is shown that family environment participates in influencing the lifestyle.

MATERIALS AND METHOD

Perceived value of jamu

The independent variable of the research is perceived value of *Jamu*. According to Chen & Chen (2009), Perceived value can be defined as the general assessment by the customer to the usefulness of a product based on expectations of what is received and what is offered. Furthermore, Wang & Wang (2010) mentioned that, perceived value can be described as the difference between the evaluation by the potential customer of all the benefits and all the costs of the offer and the perceived alternatives. He further discusses that value denotes customers who feel that their options are better than any other option and will choose what they think is best for them. Perceived value may be the sense of trade-off between benefits and costs. Amalia & Aprianingsih (2017) states that *Jamu* is traditional Indonesian herbal medicine

used in Indonesia for many centuries to treat diseases and to maintain good health. According to Shinoda (2013) translated to English, *Jamu* is a traditional herbal medicine made from natural cultural heritage which has been passed down for generations for health.

The indicators used for perceived value of *Jamu* are awareness, perception, and family environment.

Awareness

Cambridge Dictionary states that awareness can be define as the information that something occurs, or perception of a situation or topic based on knowledge or experience at this time. In addition, according to Gafoor (2012), awareness in general, is being competent, educated, informed alert.

Awareness is also having the ability to experience or be conscious of things, objects, or sensory patterns. Moreover, Reinhardt, Mletzko, Sloep, & Drachsler (2011) mentioned that awareness is an understanding of other people's activities which creates a background for your own activity. In addition, He further states that there are six different forms of awareness:

1. Activity awareness
   Activity awareness is an action that deals with an object's history, present and future.
2. Cultural awareness
   Cultural awareness is the knowledge and perception of an individual about foreign cultures, their values, beliefs, and perceptions. This awareness is especially important when interacting with people from other cultures.
3. Social awareness
   Social awareness is the social knowledge that explains the things people are conscious of. It provides detail about others' attentiveness, movements and facial expressions that mirror a person's emotional stat, as well as hints about a person's interest in a subject.
4. Workplace awareness
   Workplace awareness refers to comprehension of co-worker's workplace layout and task characteristics and is intricately linked to other types and aspects of awareness.
5. **Location awareness**
   Location awareness can refer to understanding an object's physical location. This could be correlated with one's own location.

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9. Workplace awareness
   Workplace awareness refers to comprehension of co-worker's workplace layout and task characteristics and is intricately linked to other types and aspects of awareness.
10. Location awareness
    Location awareness can refer to understanding an object's physical location. This could be correlated with one's own location.
11. Knowledge awareness
    Knowledge awareness refers to a person's ability to assess the information of a given entity by another. In addition, information awareness may also refer to information about the competences and skills of someone else, as well as his method of operation.

Perception

According to Al-Jeraisy (2008), Perception can be defined as, the impression, selection and perception of the stimuli the user is receiving from the outside world. In addition, the process of organizing and interpreting information and sales offers obtained through five senses.

According to Torri (2012), interviewed consumer expressed the consumption of *Jamu* products with the sense of reassurance and safety. Most interviewees have thought that *Jamu* works slowly and gentle compared to conventional medicines and are less effective. Several consumers have indicated that their protection is proven by the long history of human use of *Jamu*:

"*Jamu* has been used for many generations and it has been tested on many people in the past… the *Jamu* producers know what is the good way to mix the herbs and the other ingredients in the *Jamu* has they have learnt from their mothers or grand-mothers, so it is something already experimented and proofed…."

"Some people say that it is important to make some laboratory trials for *Jamu* and traditional medicine to see if it is toxic…I know that some plants are toxic but in the case of *Jamu*, the Javanese people have been using the herbs of the *Jamu* for such a long time and it has been proved that it is good for health…"

Younger people aged between 25-35 were much less likely to find herbal medicines as an option than any other age group.

There are also risks involve in consuming *Jamu*, consumers did not compare their own use of herbal medicines to what they had learned about the risks:

"My mother-in-law always used to say but I don't know if it's true, that if you combine different plants and roots and if you mix different types of *Jamu* it can make you ill"

When asked about the relative risks and benefits of *Jamu* and other herbal medicines, those aged between 25-35 years or 60+ were more likely to give a 'don't know' answer.

Family environment

According to Zastrow & Kirst-Ashman (2010), The family environment is involving circumstances and conditions of social climate within families. Because each family is composed of different people in a diverse community, each family climate is special. The environment can differ from one way to another. One clear distinction, for example, lies in socio-economic status.

Kalavana, Lazarou, & Christodoulou (2011) mentioned that, family cohesion was related to healthy eating and physical activity strongly and positively, while family tension was positively linked to unhealthy eating, smoking, alcohol intake and inadequate sleep. In addition, Kalavana et al., (2011) also states that personal parameters and interpersonal parameters are important factor that influence healthy lifestyle habits for adolescents.

However, the previous research by Torri (2012) focuses more on the perception and risk of *Jamu*, while this research focuses on perceived value of *Jamu*. Therefore, this study proposes the following hypothesis:

H1o: The perceived value of *Jamu* is not impacting the purchase decision.

$H1_1$: The perceived value of *Jamu* is impacting the purchase decision.

Purchase decision

The dependent variable of this research is purchase decision. Rita & M (2018) mentioned that purchase decision is the method of choosing two or more alternative options resulting in the decision to purchase or not to purchase. There must be alternate options when consumers decide. The decision-making process for purchases needs specific details to be checked or obtained. She further explains, purchase decision is the mechanism whereby consumers determine which products to buy. Consumers would buy the most desired brand, but can be affected by two factors, namely the behavior of other people and unpredictable circumstances, which are between purchasing intentions and purchasing decision. The intention to buy will change if the situation faced

by customers hampers or forces them to cancel the buy or turn to other alternatives.

According to Al-Jeraisy (2008), the purchase decision process consists of three phases:

- First: Pre-purchase stage

1. Finding a question about the consumption and feeling the need to fix it.
2. To scan for and gather relevant data.
3. Evaluating alternative approaches.
4. Choosing the right alternatives.

- Second: Purchase stage

5. The purchase id finalized.

- Third: Post-purchase stage

6. Use and measure of the purchased brand before and after use.
7. Product disposal.

Millennial

According to Moreno, Lafuente, Carreon, & Moreno (2017) Generation Millennials were born between 1977 and 2000. They are children of the baby boomers and they now reach almost 83 million or more, eclipsing Generation X members and being even larger segment than the baby boomers. The indicators used for purchase decision are consumer behavior and purchase motivation.

Consumer behavior

According to Al-Jeraisy (2008), consumer behavior can be defined as the practices the customer participates in, in addition similar to decision-making processes, in the search for a good or a service to fulfill his need or desire and in assessing, receiving, using and disposing of it.

Keys to understanding consumer behavior

1. Consumer behavior is based on motives and incentives.
   Motives are internal factors that motivates the customer to act, while incentives are external factors that reflect benefits that the customer expects from buying the product.
2. Consumer behavior includes several activities.
   Consumer behavior consists of a collection of behavior which ultimately lead to a customer making the purchase decision.
3. Consumer behavior goes through successive steps.
   Three stages of consumer behavior in decision:
   - Pre-purchase decision
   - Purchase decision
   - Post-purchase decision
4. Consumer behavior varies according to time and structure.
   Time refers to when a transaction happens and the period of purchase.
   Structure refers to the number of stages or phases of purchase and the collection of activities performed at each point.
5. Consumer behavior includes different roles.
   Consumer behavior can be described by several roles starting with discovering a purchase idea, going through the purchasing decision, and ending up using the product.
6. Consumer behavior is influence by external factors.
   External factors that influence consumer behavior:
   - Culture
   - Reference groups
   - Social Class
   - Family
   - Marketing methods
   - Situational Factors

Purchase motivation

According to Herawati, Prajanti, & Kardoyo (2019) motivation can be defined as the individual person's drive and compel him or her to do so, and this motivation is created by the pressure arising from the unmet needs.

Phat (2014) mentioned that a motive can be interpreted as a drive or desire of a person who is seeking satisfaction for. He further discusses, that purchase motivation can be defines as those factors or forces which offer an impulse to purchase or cause action or decide the choice of goods or services purchased.

Type of Study

This study will apply descriptive studies. According to Cooper & Schindler (2014) descriptive studies is an attempt to identify or define a subject, often by creating an account of a group of concerns, individuals or events, by gathering information and tabulating frequencies on or communicating with research variables; the study reveals who, what, how, where, or how much; the study involves a univariate query or hypothesis in which research asks or states something about the subject.

Population and sample

Because the population will only consist of millennial (Generation Z), the sampling method is non-probability method, which means not all people may participate in the survey. Using Rao Purba' formula, it is determined that the minimum sample for this research is 100.

Type of data and collection method

The primary data will be collected from questionnaires as its quantitative collection method, then interviews and observations as its qualitative collection method. While secondary data will be collected from previous studies and journals.

Data analysis technique

Descriptive data analysis will be used using the questionnaires as its data. This technique will identify which statements is agreed the most by the respondents, and which is agreed the least. The result is valuable to create useful recommendation for future research.

After that, validity and reliability will be tested using SPSS. This is to ensure the questions are suitable for the researched variables, and that the questionnaire is dependable to be used for this research and other future research with similar variables. The tests are effective to detect any mistake such as typo or difficult lingo that the public may not understand. For the questions to be considered valid, the Pearson correlation must surpass the minimum R value, which for this research will be 0.361 for pre-test, and 0.175 for post-test. Meanwhile, for reliability must surpass the minimum 0.70 to be considered a good questionnaire.

Descriptive data analysis will be conducted on this research, According to Cooper & Schindler (2014) descriptive data analysis is an attempt to identify or defined a subject, often by creating an account of a group of concerns, individuals or events, by collecting and tabulating data on or communicating with research factors; the study shows who, what, when, where or how; the study involves a univariate query or hypothesis in which research ask or state something about the subject.

Table 1. Interval likert scale

Interval likert	
4.20 – 5.00	Strongly Affected
3.40 – 4.19	Affected
2.60 – 3.39	Neutral
1.80 – 2.59	Not Affected
1.00 – 1.79	Strongly Not Affected

Next will be normality test, Ainiyah, Deliar, & Virtriana (2016) mentioned that, to verify the normal distribution of the collected data, P-Plot, Histogram, and Kolmogorov-Smirnov test are applied. P-plot and Histogram test-if the fata followed a diagonal line or the histogram is bell-shaped while with Kolmogorov-Smirnov test – if Asymp. Sig. (2-tailed) is > 0.05 (95% confidence level).

Next is heteroscedasticity test, Cooper & Schindler, (2014) states that, analysis of regression will produce an acceptable result if the data are free of heteroscedasticity, or, to be said, homoscedasticity. The Glejser approach would be used to evaluate the heteroscedasticity, therefore the importance of Sig. To be free of heteroscedasticity, must be above alpha 0.05.

Next is simple linear regression, Cooper & Schindler (2014) states that, simple linear regression can be defined as a statistical technique used to construct a self- weighting estimation

formula that forecast values for a dependent variable from the values of independent variables; monitors confounding variables to better evaluate the input of other variables; test and describes a casual theory. Then the last test will be hypothesis test, which includes both f-test and t-test.

RESULTS AND DISCUSSION

Profile of respondent

Out of 125 respondents, 62.4% of them are males, and 48.8% of them are between the age of 19-21.

Descriptive data analysis

The following statements are the ones with the highest mean score by the respondents:

a. It is safe to consume *Jamu* because it is natural. (Perception)
b. *Jamu* is cheaper for daily usage and should be educated to the community. (Awareness)
c. Family Involvement with *Jamu* makes me more aware about *Jamu*. (Family Environment)
d. *Jamu* as an alternative medicine. (Purchase Decision)

The following statements are the ones with the least mean score by the respondents:

a. Herbal medicine works faster than conventional medicine. (Perception)
b. Consuming *Jamu* soon. (Awareness)
c. Family influences decision making process. (Family Environment)
d. Consuming *Jamu* as daily supplement. (Purchase Decision)

Validity and reliability tests

After collecting the responses from the questionnaires (pre-test), the result is tested using the SPSS software, the results of the questions all pass the validity test; all items surpass the Pearson correlation with the minimum R value of 0.361 and for the reliability test, all items surpass the minimum Cronbach's Alpha value of 0.70. As for the post-test, the result surpasses the Pearson correlation minimum R value of 0.175, and for the reliability test, all items suppress the minimum Cronbach's Alpha value of 0.70.

Normality test

Table 2. Normality test

One-Sample Kolmogorov-Smirnov test		
		Unstandardized Residual
N		125
Normal Parameters[a.b]	Mean	.0000000
	Std. Deviation	3.09438632
Most Extreme Differences	Absolute	.056
	Positive	.035
	Negative	-.056
Test Statistic		.056
Asymp. Sig. (2-tailed)		.200[c.d]
a. Test distribution is Normal.		
b. Calculated from data.		
c. Liliefors Significance Correction.		
d. This is a lower bound of true significance.		

As shown from the table above, the Asymp. Sig. (2-tailed) is 0.200, which indicates that the data is distributed normally since it is higher than 0.05. To reinforce if the data is normally distributed, another method is used, Histogram graphic and Normal Probability Plot (P-Plot Test).

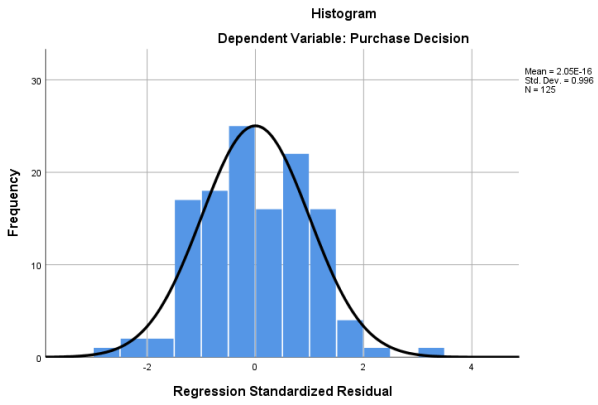

Figure 1. Histogram normality test

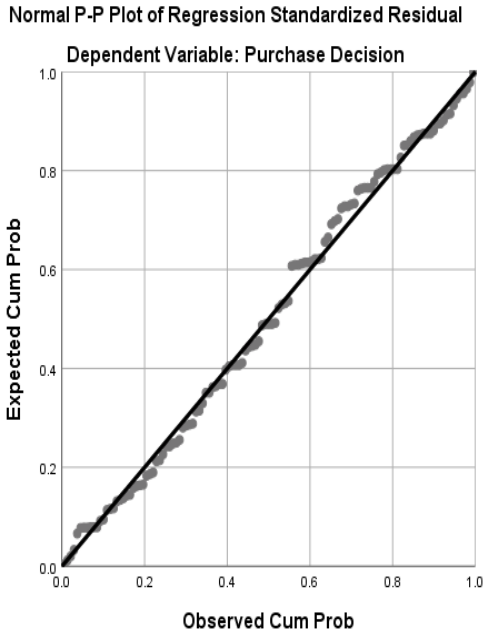

Figure 2. p-plot normality test

From the figures 1 and 2 shown above, the data can be seen that it followed the diagonal line for P-Plot Test and a bell-shaped line for the Histogram Graphic. Thus, the data can be considered as normally distributed.

Heteroscedasticity

Table 3. Heteroscedasticity test

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	.044	.0944		.046	.963
	Perceived Value	.050	.019	.234	2.674	.009

b. Dependent Variable: RES2

From the table 3 shown above, the output data with significance value of 0.009 lower than the 0.05 value, which indicates that it did not passed the heteroscedasticity test.

Hypothesis test (F-test and T-test)

From the table 4, the result of F-Test is 94.287 with significance level of 0.000. The F-table used in this study is 3.92 with significance level of 0.05. Since the result of the test is higher than the F-table value, therefore, $H1_1$ can be accepted.

Table 4. F-test

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	910.160	1	910.160	94.287	.000[b]
	Residual	1187.328	123	9.653		
	Total	2097.488	124			

c. Dependent Variable: Purchase Decision
d. Predictors (Constant), Perceived Value

Table 5. T-test

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-5.282	1.706		-3.096	.002
	Perceived Value	.327	.034	.659	9.710	.000

a. Dependent Variable: Purchase Decision

From the table 5 shown that the result of T-Test is 9.710 with significance level of 0.000. The T-table used in this study is 1.97944 with significance level of 0.05. Since the result of the test is higher than the T-table value, therefore, $H1_1$ can be accepted.

CONCLUSION

In short, *Jamu* is traditional Indonesian herbal medicine that has been used for many centuries to treat diseases and to maintain good health. Today, *Jamu* is struggling to survive in the modern lifestyle, 49.53% of Indonesian consume *Jamu* to help maintain their good health. For *Jamu* to survive in the modern lifestyle they must modernize into capsules, pills, and sachets. With today's one of the largest generation population, millennial generation Z, they are currently the biggest spender in the society. The objective of this study is to analyse the impact between perceived

value of *Jamu* and the millennial purchase decision. For Indonesia to preserve it culture, *Jamu*, the current generation should be knowledgeable about the perceived value of *Jamu*.

In analyzing the impact of perceived value of *Jamu* towards the millennial purchase decision, questionnaires were distributed using Google Forms online to 125 respondents. Based on data, Simple Linear Regression, it can be concluded that perceived value of *Jamu* affects the millennial purchase decision by 42.9%. In addition, descriptive data analysis was conducted to further analyze the variables. The result of the descriptive data analysis shows that most millennials have previously consume *Jamu* and are willing to consume it again. However, the availability of *Jamu* is scarce. Moreover, the millennials are well knowledgeable about *Jamu* and agree that the surface knowledge of *Jamu* should be educated in schools for the younger generations.

Therefore, perceived value of *Jamu* significantly affects the millennial purchase decision by 42.9%. It shows that the main hypothesis of this study is considered accepted.

Managerial implication

Based on the result of this study, not all millennial generation Z but most millennial generation Z are knowledgeable about *Jamu*. Most have consumed *Jamu* and are satisfied with the benefits of *Jamu*. Based on the questionnaire result, most respondent agree with the information of *Jamu* being educated in the community. Hence, *Jamu* company and businesses to host events for younger generation to further educate about *Jamu*. In addition, *Jamu* can also be implemented as a topic in the educational field.

According to the questionnaire result, over 40% does not intend to consume *Jamu* in the next few days, the availability of *Jamu* is scarce, though there are other factors that could affect why the respondent would not want to consume *Jamu* in the next few days. Hence, a collaboration should be conducted between local government and start up business to develop product of *Jamu* for modern, hygienic and socialize to the millennial.

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NON-SOY LEGUMES AS ALTERNATIVE RAW INGREDIENT FOR *TEMPE* PRODUCTION IN INDONESIA WITH ADDITIONAL HEALTH BENEFITS: A REVIEW

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ABSTRACT

The availability of legumes in Indonesia is abundant. Many of them show great potential as an alternative ingredient to suppress the deficiency of nutrient intake. However, the utilization needs to be improved. The aim of this review is to evaluate the potential of selected non-soy legumes which are jack bean, mung bean, red kidney bean, and cowpea based on some consideration such as productivity and their potential to be used as raw ingredient for *tempe* production related to the nutrient content and functional properties. Despite of the high production of non-soy legumes, the utilization is still considerably low. Several researches stated that non-soy legume shows a great nutrient profile and good functionalities after being processed into *tempe*. Nutrient content of jack bean, mung bean, red kidney bean, and cowpea were improved due to the removal of antinutrients by the processes involved in *tempe* production. It shows a similarity and comparability to nutrient content of soybean *tempe* and even shows better functionality.

Keywords: alternative; health benefit; legumes; tempe

ABSTRAK

Ketersediaan kacang-kacangan (legumes) di Indonesia sangat melimpah. Banyak diantaranya menunjukkan potensi sebagai bahan alternatif dalam pemenuhan kebutuah gizi, namun demikian pemanfaatannya masih belum optimal. Kajian ini bertujuan untuk mengevaluasi potensi kacang-kacangan (legumes) non-kedelai, yaitu kacang koro, kacang hijau, kacang merah, dan kacang tunggak berdasarkan produktivitas dan potensinya sebagai bahan baku pembuatan tempe, dalam kaitannya dengan kandungan nutrisi dan sifat fungsional untuk kesehatan. Meskipun produksi kacang-kacangan non-kedelai ini cukup tinggi, pemanfaatannya terbilang masih sangat rendah. Beberapa penelitian menunjukkan bahwa kacang-kacangan non-kedelai ini menunjukkan profil gizi dan sifat fungsional yang baik setelah diolah menjadi tempe. Kandungan gizi keempat kacang non-kedelai ini menjagi lebih baik karena hilangnya zat anti gizi selama proses produksi tempe. Tempe yang terbuat dari keempat kacang non-kedelai ini memiliki kandungan gizi setingkat dengan tempe kedelai dan menunjukkan manfaat untuk kesehatan.

Kata kunci: bahan baku alternatif; manfaat kesehatan; kacang-kacangan leguminosa; tempe

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INTRODUCTION

Tempe is one of Indonesian fermented food that was usually made from soybean (*Glycine max (L.) Merr.*). The process of making *tempe* is much influenced by the activity of certain microorganisms, such as *Rhizopus oligosporus, R. oryzae, R. stolonifer, R. arrhizus, R. formosaensis, Mucor* spp, yeast, lactic acid bacteria, and different gram-negative bacteria. However, the main fungus of *tempe* production is *R. oligosporus* (Sharma and Sarbhoy, 1984).

According to Badan Pusat Statistik (2019), people in Indonesia consumed an average of 0.146 kg of *tempe* per capita per week in 2018. This amount of consumption is increasing compared to previous years while on the other hand the productivity of soybean of each province in Indonesia decreased by 4.62% from 2017 to 2018 (Statistik, 2019). Additionally, Indonesia imported as much as 2.52 million tons of soybean from America in the same year (Statistik, 2019). The national dependency of imported soybean influenced *tempe* producers to suffer due to the fluctuated currency. Therefore, other source of raw material is needed to reduce this problem.

Soybean has become the only legume which is being utilized massively as ingredient for *tempe* compared to other legumes. The utilization of soybean is mainly for *tempe* production and its derivatives. While on the other hand other legumes also show great potential to be utilized as *tempe* ingredients.

Tempe from other raw materials has been known by Indonesian since mid-1970s and it is reported in Shurtleff & Aoyagi (2007). Production of non-soy legumes as *tempe* ingredient is expected to increase demand of non-soy legumes. Based on previous study, it was shown the potency of non-soy legumes as *tempe* ingredient as *tempe* production contribute to the improvement of nutritional content and functionality. Therefore, non-soy legumes in Indonesia have a promising potential to be utilized (Belinda, A, 2015).

There are many types of legumes that can easily be cultivated in Indonesia. The cultivation of non-soy legumes is relatively easy, and they have commonly been used in Indonesia. In some research, it has been shown that their physical properties as well as the nutrient profile show a great potential to be utilized as raw ingredient for *tempe* production. (Pupitojati, Indrati, Cahyanto, & Marsono, 2019; Dewi, 2014; Grace, 2017; Radiaty & Sumarto, 2016). Non-soy legumes such as mung bean, cowpea, jack bean, and red kidney bean have shown their potential in some aspects.

Cowpea is potential for its sustainability and nutrient content. According to (Utomo & Antarlina, 1998) the cultivation of cowpea could take place in a marginal area which may not have such a good drainage and nutrients-rich soil. In some regions in Asia and Africa, cowpea is utilized to prevent stunting and improve protein intake. Red kidney bean is famously known for their role in improving nutrients intake (Astawan, 2009). Utilization of plant-based food has been reported to be able in maximizing protein intake and decreasing health issues (Shehzad, et al., 2015). Several market researches showed that the demand and awareness of foods with certain functionality and health benefit are increased. Mung bean and jack bean utilization may also be promising in the functional food industry. Mung bean contains folate which may help to prevent the defect of neural tube (Czeizel, Dudás, Vereczkey, & Bánhidy, 2013). While jack bean, an underutilized legume was found to contain l-canavanine which has potential to inhibit cancer cells in pancreas (Swaffar, Ang, Desai, & Rosenthal, 1994).

Regardless of the potential of non-soy legumes, the current consumption is still considerably low. Utilization of non-soy legumes as a raw ingredient of *tempe* could be a promising approach in improving the consumption. This study will discuss Indonesian popular non-soy legumes, namely mungbean, red kidney bean, cowpea, and jack bean, their potentials for its utilization as raw ingredient in *tempe* production with additional benefits. Scope of the discussion include of physical, chemical, nutritional content, and health benefit of the legumes in comparison to soy and as ingredient in *tempe* processing. This study will also discuss the different in production process of each legume to become tempe.

Variation of *tempe* in Indonesia

As the demand of soybean increases, the prevalence of other local legumes is hindered, while the production of soybean in each city in Indonesia is still considered to be not adequate (Statistik, 2019). The import of soybean also costs a lot more, therefore many researches have been conducted related to the innovation of making *tempe* with different non-soy legumes *as* raw ingredients.

Traditionally and due to scientific curiosity, there have been a lot of variations of *tempe* made of various raw ingredients. In Indonesia, many types of legumes can be found in each island. And there has been a lot of involvement of many legumes to be used as raw ingredient to produce tempe (Table 1). Some of the variations that have been consumed in Indonesia are *tempe semangit*, *tempe kacang merah*, *tempe kacang hijau* (Jateng), *tempe bongkrek*, *tempe enjes tempe kedelai* (Malang), *Oncom merah* and *hitam* (West Java) (Suprapti, 1996).

Table 1. Raw materials from Indonesia used for *tempe* fermentation

Raw Material	Name of *Tempe*	Origin
Soybean *(Glycine max)*	*T. Kedelai*	Malang, East Java
Mung bean *(Vigna radiata)*	*T. Kacang hijau*	Yogyakarta, Central Java
Lamtoro *(Leucaena)*	*T. Lamtoro*	Wonosari, Central Java
Pigeon Pea *(Cajarus cajan)*	*T. Kacang Iris*	Lombok, East Bali
Lablab *(Lablab purpureus)*	*T. Koro Wedus*	ND
Jack bean *(Canavalla ensiformis)*	*T. Koro Pedang*	ND
Cowpea *(Vigna unguiculate)*	*ND*	ND
Kidney bean *(Phaseolus vulgaris)*	*ND*	ND
Winged bean *(Psophocarpus tetragonolobus)*	*T. Kecipir*	Papua New Guinea
Coconut pressed cake	*T. Bongkrek*	Banyumas, East Java
Velvet bean *(Mucuna pruriens)*	*T. Koro Benguk*	Cental & East Java

ND: Not Defined
Source: (Shurtleff & Aoyagi, 2007)

Existence of the *tempe* variats based on the raw ingredient showed potential for *tempe* development using non-soy legumes. Cowpea is suitable as a raw ingredient for *tempe* production (Haliza, Purwani, & Thahir, 2007). Cowpea seeds in Indonesia are mostly utilized as an additional vegetable cooked with jack fruit stew and vegetables with coconut soup or mixed with other ingredients for porridge, *bakpia*, and rice cake. Through fermentation process, cowpea is gaining some attributes that give it the potential to be utilized as an ingredient for *tempe* (Haliza,

Purwani, & Thahir, 2007). According to Wardiah, Samingan, & Putri (2016), cowpea *tempe* contains p-coumaric acid and ferulic acid which is expected to be the most powerful antioxidant. Ferulic acid contained in cowpea *tempe* can suppress blood pressure and glucose level in the blood. Red kidney bean is one of various commodity of local legumes that is well known in Indonesia. Fermentation is able to increase the nutrient and digestibility profile of red kidney beans (Maryam, 2016). In other hand, mung bean also found to have greater soluble protein. Another bean such as jack bean, fermentation resulted a bioactive compound with high ACE inhibitory activity after fermentation by *Rhizophus sp*. molds (Pupitojati, Indrati, Cahyanto, & Marsono, 2019).

Characteristic and potency of non-soy legumes as ingredient for *tempe*

Classification and physical characteristic

Based on Table 2, all legumes are having the same division and family when compared to soybean. Therefore, it can be said that cowpea, mung bean, red kidney, and jack bean have the possibility to be raw ingredients for *tempe* although each of those non-soy legumes have different physical characteristic.

Table 2. Classification of legume

Legumes	Classification	
	Genus	**Species**
Soybean	*Glycine*	*Glycine max L.*
Cowpea	*Vigna*	*Vigna unguiculata*
Mung bean	*Vigna*	*Vigna radiate (L.) R.*
Red kidney	*Phaseolus L.*	*Phaseolus vulgaris L.*
Jack bean	*Canavalia*	*Canavalia ensiformis*

Each legume has difference in size, color, peels, and weight. Size of seeds influences the fermentation process. Hence, the quality of *tempe* will also be determined by the seeds size. As it can be seen in Table 3 and Figure. 1, cowpea has a similar weight to soybean. The weight of 50 cowpea seeds is 7.0 grams compared to that of soybean which is 8.0 grams. The similarity of the weights seemed to be the advantage of cowpea to be used for *tempe* ingredient based on size. This is also supported by Haliza, Purwani, & Thahir, (2007) that the seed size of cowpea can be used as potential *tempe* ingredient.

Figure 1. Peeled legumes. A. Jack bean, B. Red kidney bean, C. Cowpea, D. Soybean, E. Mungbean.

The physical changes of each non-soy legumes were also observed. The observation was done by applying some processes involved in *tempe* production such as soaking, dehulling, and boiling since the observation aimed to know the ability of each non-soy legumes to absorb water during *tempe* processing and further, influence the ability of each of these non-soy legumes to be dehulled that might also be different due to the rigidity difference. Due to the rigid texture of red kidney bean, cowpea, and jack bean, longer soaking was required in order to be processed further based on *tempe* production process. Therefore they were soaked for 24 hours instead of 12 hours. It appears that all legumes experienced increases in weight after 12 or 24 h of soaking. Shown in Table 3.

The time variation of heat treatment of the legumes, resulted in different antinutritional content of each legume (Popova & Mihaylova, 2019). When introduced to heat treatment the antinutrient content in mung bean and red kidney bean are faster to be removed, therefore it requires only 15 minutes to be softened and able to be processed further. The ability to be softened is an

important factor for *tempe* making, because it influences the effectivity of fermentation by the molds. The softer the legumes, the more effective the fermentation. This proves that mung bean and red kidney bean are more potential in term of texture. Another factor that influences heat treatment time of each non-soy legumes is carbohydrate content. It is known that mung bean and red kidney has the highest carbohydrate content. Higher carbohydrate content will result in faster heat treatment process. When introduced to heat treatment, the raw tissue of seeds will be broken, and this might allow the starch to be ruptured and dispersed causing leaching of amylose.

Table 3. Physical characteristics of each legume based on *tempe* processing. **A.** Weight of seeds in different soaking treatment. **B.** Weight of seeds in different heat treatment

A

Type of Seeds	Initial	Weight of seeds (g)			
		12 h		24 h	
		Weight of 50 seeds (g)	Expansion percentage (%)	Weight (g)	Expansion percentage (%)
Soybean	8.0	19.0	137.5	-	-
Mung bean	3.0	8.0	166.7	-	-
Red kidney bean	22.0	43.0	95.5	45.0	104.5
Cowpea	7.0	14.0	100.0	16.0	128.6
Jack bean	74.0	112.0	51.4	117.0	58.1

B

Type of Seeds	Initial	Weight after heat treatment			
		15 min		30 min	
		Weight of 50 seeds (g)	Expansion percentage (%)	Weight (g)	Expansion percentage (%)
Soybean	8.0	16.0	100.0	15.0	87.5
Mung bean	3.0	11.0	266.7	-	-
Red kidney bean	22.0	37.0	68.2	-	-
Cowpea	7.0	13.0	85.7	13.0	85.7
Jack bean	74.0	121.0	63.5	118.0	59.5

Nutrient content

The utilization of non-soy legumes is still considerate as low. While they are also showing great potential from nutrient profile. Referring to Table 4. It was shown that the protein content of each non-soy legumes is lower than compared to soybean. However, when processed into *tempe*, could improve protein content of each legume (Radiaty & Sumarto, 2016).

Table 4. Nutrient content of each legume per 100 g

Nutrients	Legumes				
	Soybean	**Cowpea**	**Mung bean**	**Red kidney**	**Jack bean**
Water	8.54 g	11.05 g	9.05 g	11.75 g	15.5 g
Protein	36.49 g	23.85 g	23.86 g	22.53 g	27.6 g
Fat	19.94 g	2.07 g	1.15 g	1.06 g	3.9 g
Carbohydrates	30.16 g	59.64 g	62.62 g	61.29 g	56.9 g
Total soluble sugar	ND	ND	ND	ND	4.20 g *
Fibre	9.3 g	10.7 g	16.3 g	15.2 g	8.0 g
Calcium	277 mg	85 mg	132 mg	83 mg	247 mg *
Phosphorus	585 mg	438 mg	367 mg	406 mg	338 mg *
Iron	15.7 mg	9.95 mg	6.74 mg	6.69 mg	7 mg
Potassium	1797 mg	1375 mg	1246 mg	1359 mg	141 mg

Source: (Gebhardt & Thomas, 2002) (Rodrigues & Torne, 1992)*
ND: Not defined

Mung bean

Proximate analysis data was collected from several researches of *tempe* made from non-soy legumes (Table 5). When compared to the Standard Nasional Indonesia (SNI) of *tempe* by BSN, (2015) protein content of mung bean *tempe* is lower than the required amount. This result is similar to a research by Angelina, et al., (2016) who reported that the protein in mung bean *tempe* was only 11.73 % at the highest and met the required *tempe* standard by SNI 3144:2009 for *tempe*. This difference may be influenced by the different in cultivars.

Despite the lower protein content of mung bean, the protein digestibility, however, was reported to be better than soybean *tempe*. This is supported by the findings that the protein digestibility and soluble amino acid in mung bean *tempe* was found to be better than in soy *tempe* and remain the same after the production was upscaled industrially (Belinda, 2015; Angelina, 2016; Grace, 2017)

Cowpea

The initial carbohydrates content in cowpea was shown to be 59.64 g/100 g in Table 5. However, after fermentation the carbohydrate content was decreased to 18.97 % (Table 5). This might be due to enzymatic degradation of carbohydrate by lactic acid and molds fermentation. This is supported by (Madodé, et al., 2013) that galacto-oligosaccharides (GOS) was reduced effectively after fermentation followed by soaking and boiling.

Table 5. Proximate analysis of mung bean, cowpea, jack bean, red kidney *tempe*

Tempe	Protein Content (%wb)	Fat (%wb)	Carbohydrate (%wb)	Moisture (%wb)	Ash content (%wb)
Soybean	17.93	7.82	49.38	65.65	1.28
Mung bean	14.96	0.2	20.27	64.32	0.25
Cowpea	16.04	0.67	18.97	63.47	0.83
Jackbean	28.29	0.68	22.83	67.02	0.18
Red kidney	23.75	0.11	34.19	41.71	0.24
SNI (Soybean *Tempe*)	Min. 16	-	-	Max. 65	Max 1.5

*wb: wet basis; *)remark of result that is not in wet basis and with unknown units.
Source: (Iswandari, 2006; Dewi, 2014; Widaningrum, Sukasih, & P, 2015; Wicaksono, 2014)

The protein content in cowpea after *tempe* fermentation was shown in Table 6. The protein content of cowpea *tempe* was 16.04 % and it was lower when compared to *tempe* standard (BSN, 2015). The SNI for *tempe* is ideally 16 (%wb) meaning that the protein content of cowpea *tempe* by Dewi, (2014) has already met the standard. Fermentation time also gives an impact to the protein content. It was increased gradually the longer the fermentation. The release of amino group from protein and the release of the nitrogen from vitamin B12 was predicted to be the reason (Dewi, 2014).

Jack bean

The proximate analysis of whole jack bean *tempe* can be seen in Table 6. The protein content was 28.29% and was lower than the raw form which was 34% (Widaningrum, Sukasih, & P, 2015). The decrease in protein content is due to the hydrolysis of protein into a simpler amino acid (Belinda, 2015). This is similar to the findings during fermentation of jack bean for which it was reported that there was a significant increase in soluble protein that might be caused by the activity of proteolytic enzyme of Rhizopus sp. (Widaningrum, Sukasih, & P, 2015). The nutrient content from combination of 25% jack bean mixed with 75% of jack bean was known to be good and meet the SNI 3144:2009 for *tempe* except for fat content. The fat was lower than the standard because of the 25% combination of jack bean. (Kusumawardhani, 2015).

Regardless of the excellent nutrient content, the consumption of jack bean *tempe* is still low due to its low sensory acceptance (Kusumawardhani, 2015). This could be improved by turning jack bean *tempe* into flour. The shelf-life may be extended while at the same time the application might be broadened.

Red kidney bean

Red kidney bean was reported to be a good ingredient for *tempe* (Srapinkornburee, Tassanaudom, & Nipornram, 2009). The whole *tempe* processing was reported to increase the content of protein, soluble protein, and amino acid. In contrast, the ash content, carbohydrate, oligosaccharide, fat, and antitrypsin was decreased (Karisma, 2014). Modification of the seeds surface give a difference in nutrient profile of the resulted *tempe*. The reduction of size was found to give influence on the chemical characteristic of red kidney *tempe* such as soluble protein. A size of 10 mesh grits was reported to improve the protein content and lower the fat content (Wicaksono, 2014).

Functional Properties

In Table 6 the functional properties of the different non-soy legumes are being displayed as well as with which method they have been determined. The result will be discussed in the following paragraphs.

In general, every legume has functional properties. Many researches have shown their roles in providing bioactive compound. However, their bioavailability is depending on the antinutritional components that might give a disruption on the functional properties of legumes.

Mung bean

Phenolic compound contained in mung bean seeds is considered to be high with the amount of 0.62 to 1.08 g/100 g of dry matter (Anwar, Latif, Przybylski, Sultana, & M, 2007). Vitexin and isovitexin (Figure 2) are known to be the most common flavonoids found in the seeds coat as they give a 96.2% contribution to the total of antioxidant activity contained in mung bean seeds (Cao, et al., 2011). However, due to dehulling process, in *tempe* fermentation, it is expected that isovitexin and vitexin amount was also reduced in significant amount.

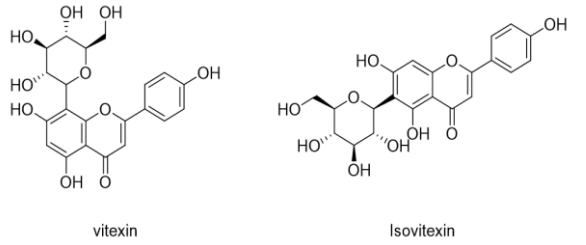

Figure 2. Chemical structure of vitexin (left) and isovitexin (right) in mung bean

According to Belinda (2015), *tempe* fermentation increased total phenolic compound of mung bean from 238.24 mg GAE/100g dry base on its original seed into 404.89 mg GAE/100 g dry base. And upscaled *tempe* production seemed to benefit the antioxidant activity, as Grace (2017) showed that DPPH radical scavenging activity of 20kg-batch produced *tempe* (86.91%) was higher compared to laboratory scale (5 kg) and small scale (500 grams). Looking up to the changes, mold fermentation seemed to recover the antioxidant of mung bean. This is supported by the findings from Limón, et al., (2015) who found that solid state fermentation of beans gave an improvement in antioxidant activity. Antioxidant activity of mung bean *tempe* was expected to be influenced by some compounds such as beta-carotene, phenolics, and protein (Belinda, 2015). However, further analysis showed that there is no correlation of beta-carotene to the antioxidant activity of mung bean *tempe* while phenolic content and protein have correlation to it. A finding by Angelina (2016); Grace (2017) even found that beta-carotene was not detected while at the same time they found a higher antioxidant activity in mung bean *tempe*.

Cowpea

In cowpea, polyphenols are found to be the major bioactive compound which is mostly contained in the husk of the seeds and ranging from 63.14 ± 4.45 mg gallic acid equivalents (GAE) /100 g to 692.0 3 ± 9.58 mg GAE/100 g (Awika & Duodu, 2017; Sombié, et al., 2018). And it was found to be significantly contributing to the antioxidant activity. Exposure to heat may affect the availability of this compound. Dehulling and cooking process with boiling water as involved in *tempe* production was found to decrease the total phenolic compound significantly as well as flavonoids in cowpea such as daidzein, myricetin, genistein, and quercetin. Despite the decrease of the total phenolic compound, the health-promoting effect still remained (Barros, Rocha, Glória, Araújo, & Moreira-Araújo, 2017).

Antioxidant activity as well as total phenolic content of cowpea were found to be 59.67% and 1.58% respectively (Table 6). Referring to Dewi, (2010) there is an increase in both compound as fermentation time increased. During the first 30 h of mold fermentation, total phenolic content of cowpea was reported to be the lowest compared to soybean. However, at 42 h of fermentation, total phenolic content of cowpea was increased and higher compared to soybean. Similar finding by Martins, Petropoulos, & Ferreira, (2016), found

that natural lactic acid fermentation in cowpea was reported to give an increase for its ability to inhibit lipid oxidation as well as an increase in total phenolic compound and vitamin E. Another compound contributing to antioxidant activity such as ferulic acid, p-coumaric, tyrosol, and vanillic was also increased significantly due to fermentation process. Another similar finding by Yadav, et al., (2018) reported that a 16 h of natural lactic acid fermentation process applied to cowpea seeds enhance the antioxidant activity of cowpea cultivars. The total phenolic content was reported to increase at 16- 24 hours while total flavonoid content of cowpea was significantly reduced after 6 h of soaking. This is due to the fact that the flavonoids in cowpea seeds are mainly contained in the seed coat and are water soluble.

Table 6. Functional properties of bioactive compound and nutrients of each non-soy *tempe*

Non-soy tempe	Functional Properties	Amount	Method	Notes
Mung bean	Antioxidant activity	86.091% [1]	DPPH radical scavenging activity	Upscaled production
		28.19 mg dry[2] base/ml		IC50
		15.284 mg dry[3] base/ml		
	total phenolic	250.70 mg GAE/g[1] dry base		Upscaled production
		404.89 mg[3] GAE/100g dry base	Folin-Ciocalteu	
		397.92 mg[3] GAE/100g dry base		
	Beta- carotene	15.31 mg/100g [2]dry base	HPLC	
	Amino acid	104.20 mg protein/ 100 g dry base [2]	Lowry	
Cowpea	Antioxidant	59.67% [4]	DPPH	
	total phenolic	1.58% [4]	Folin Ciocalteu	
Jack bean	Antioxidant	3.59% [5]	DPPH	Plastic
		2.53% [5]		Banana leaves
	Resistant Starch	10.27% [6]	AOAC	Fermentation with *L. casei* & *Bifidobacterium*
Red Kidney	Amino acid	37.19 g/100 g [7]	HPLC	
	Soluble protein	51.92 g/ 100 g [7]	Bradford	
	Isoflavone	104.08 mg/ 100 g [8]	AOAC	

Source: (Grace, 2017)[1] (Belinda, 2015)[2] (Angelina, 2016)[3] (Dewi, 2014)[4] (Ardinati, et al., 2018)[5] (Nur'afiah, 2014)[6] (Karisma, 2014)[7] (Maryam, 2016)[8]

Jack bean

Consumption of jack bean has been associated to the health promoting effect. Functional properties such as ferric reducing power, radical scavenging activity of superoxide and DPPH inhibiting power of jack bean showed a higher amount compared to other legumes (Swaffar, Ang, Desai, & Rosenthal, 1994). Raw seeds of jack bean have been known to have a total free phenolic content of 12.98 g catechin equivalent/ 100 g and showed a positive correlation towards the antioxidant activity (Vadivel, Cheong, & Biesalski, 2012). Jack bean shows successful inhibition activity against alpha amylase and alpha glucosidase at 77.56% and 75.45% inhibition in vitro respectively (Vadivel, Cheong, & Biesalski, 2012). Cooking, soaking, and

the combination of both, however, results in a significant reduction of these compounds. While on the other hand, scavenging activity against superoxide and DPPH free radical was increased after such processing (Vadivel, Cheong, & Biesalski, 2012).

Fermentation by lactic acid bacteria was also reported to give an impact on jack bean. It was found that fermentation in jack bean was able to produce glucose and resistant starch (Nur'afiah, 2014). One beneficial aspect of resistant starch is to reduce some diseases related to digestion systems like colon cancer since it can act as prebiotic which is able activate microflora in the gut (Nur'afiah, 2014).

Modification of packaging for jack bean *tempe* influenced the antioxidant activity. The antioxidant activity for jack bean *tempe* wrapped in plastic packaging was higher significantly and lipid was lower compared to the one which used banana leaves (Ardinati, et al., 2018). This is supported by the finding from Kurniawan, Setiani, & Dwiloka, (2019) that explained that plastic packaging could produce *tempe* with higher antioxidant activity compared to banana leaves and other kind of leaves since plastics has ability to minimize oxygen exchange around the environment. Another functionality of jack bean *tempe* was investigated. Additionally, jack bean may provide a bioactive peptide with high ACE inhibitory activity when fully fermented into *tempe* (Pupitojati, Indrati, Cahyanto, & Marsono, 2019).

Red kidney

The consumption of red kidney beans, any other legumes and plant-based food has been associated with combating diseases like cancer, obesity, and diabetes. It was reported that phenolic compounds contained in red kidney beans provide anticancer properties (Duranti, 2006; Scalbert, Manach, Morand, Remesy, & Jimenez, 2005). These phytochemicals could be improved by fermentation. A study by Magana, et al., (2019) reported that phenolic content and antioxidant activity may be improved with solid state bioconversion. Similar findings were reported by Limón, et al., (2015), solid fermentation of red kidney beans by *Bacillus subtilis* causes a high phenolic content of 31- 36 mg/g and a high antioxidant activity of 508- 541 µg trolox equivalents/g. On the other hand, liquid fermentation by *Lactobacillus plantarum* was observed to cause high amounts of gamma-aminobutyric acid and angiotensin converting enzyme which are potentially able to be antihypertensive.

Fermentation process involved in *tempe* production gives an impact to red kidney (Limón et al., 2015). As shown in Table 7, red kidney *tempe* contains isoflavones such as genistein, daidzein, glycytein, and factor-2 (Maryam, 2016). Fermentation by molds was found to improve the total isoflavones in red kidney. This is because fermentation facilitate the release of aglycones of isoflavones from its sugar, resulting in higher amount of isoflavones.

Antinutrient

Antinutrients are compounds which have detrimental effect on the consumption of the soybeans. Generally, antinutrients naturally present in any type of legumes. Some of them are beneficial and some may act as an antinutrient. Phytic acid, tannins, antitrypsin, derivatives of carbohydrates and lectin are examples of antinutrients that may not give contribution to the functionality of legumes (Dixit, Jix, Sharma, & Tiwari, 2011). It may interfere the availability of nutrients and minerals by showing behaviors such as inhibition of enzymes activity as well as formation of complex with nutrients resulting in the unavailability of nutrients and minerals.

According to Ogun, Markakis, & Suwanto (1989), cowpea seeds contain about 16.5- 32.0 TIU/mg antitrypsin. This factor is relatively heat resistant which makes it difficult to be removed (Utomo & Antarlina, 1998). Cowpea seeds also contain oligosaccharide and they are mostly raffinose, stachyose, and galactose. These could disturb the digestion system and lead to flatulence (Utomo & Antarlina, 1998). Mung bean contains only a few amounts of sulphur containing amino acid and that makes mung bean only contain a small amount of digestible protein (Kataria, Chauchan, & Punia,

1989). Red kidney bean contains 1.82% of dry weight phytic acid and tannins which is mainly contained in the hulls (Astawan, 2009). Jack bean contains some different antinutrients that is interesting to be examined. Cyanogen, canavanine and concanavalin A are major antinutrients that can be seen as a highlight in jack bean. Concanavalin A can be naturally found in jack bean and represents 20% of total protein in seeds (Dalkin & Bowles, 1983) and its presence of canavanine may inhibit the growth of chickens. As shown in Table 7-9, several processes are able to significantly reduce some antinutrients contained in each legume.

In soybean roasting gives a better and more effective performance in removing antinutrients than compared to cooking. Tannin was found to be the highest among the other antinutrients and roasting seemed to be significantly reduced tannin better than cooking. Further process, which is fermentation, the data shows that fermentation give the best reduction of all antinutrient factors compare to both cooking and roasting. This is due to the presence of α-galactosidase enzyme produced by lactic acid bacteria which can hydrolyze the antinutrient factors (Adeyomo & Onilude, 2013).

Table 7 Antinutrients of milled soybean through some processing

Type of treatment	Antinutrients (mg/ g)			
	Antitrypsin	Tannin	Protease inhibitor	Phytic acid
Raw	1.20 ± 0.12	1.93 ± 0.19	1.20 ± 0.02	1.16 ± 0.05
Cooked	0.05 ± 0.05	1.12 ± 0.02	0.05 ± 0.05	0.28 ± 0.02
Roasted	0.02 ± 0.25	0.49 ± 0.12	0.03 ± 0.03	0.25 ± 0.03
Fermentation (Isolated LAB)	0.010 ± 0.02	0.120±0.05	0.020 ± 0.03	0.047 ± 0.03

Some processing like fermentation, soaking, dehulling, and boiling are possible to reduce the phytic acid concentration and are effective in removing tannins (Astawan, 2009). The effect of processing through some antinutrients in cowpea is shown in Table 9.

Phytic acid in cowpea seeds was found to not be affected by any kind of treatments while for red kidney bean as it can be seen on Table 10 the phytic acid content was reduced to 19% after being soaked for 12 h. This is also similar to phytic acid contained in mung bean which was reduced to 30% after being soaked for 18 h. Additionally, soaking treatment with CaCl2 for 72 hours was investigated to be efficient to remove hydrogen cyanide content in jack bean (Kusumawardhani, 2015). For other antinutrients in cowpea such as antitrypsin, the concentration was found to be massively decreased by cooking process and steaming (Ogun, Markakis, & Suwanto, 1989) (Emire & Rakshit, 2007). For oligosaccharides, both stachyose and raffinose were significantly decreased by almost all treatments except for cold soaking. Tannins were not detected after being dehulled and steamed. This is because most tannins in cowpea are contained in the hulls and are sensitive to heat (Utomo & Antarlina, 1998).

Result showed that soaking and cooking process of red kidney beans does not sufficiently remove the amount of the shown antinutrients, but only lowers it by between on average 26% for phytic acid and on average 52% for α-galactoside. Sprouting is removing a bigger amount of antinutrients with 74-88% which shows that these biological processes are being more effective than just soaking and cooking it. In addition to that germination for 48 h followed by autoclaving is successfully removing phytic acid and tannin. However, the processes were not 100% effective to reduce α-galactosides (Shimelis & Rakshit, 2007). On the other hand, similar treatment such as germination could reduce the content of hydrogen cyanide in jack bean (Akpapunam & Sefa-Dedeh, 1997). Raw form of red kidney beans is also known to contain hemagglutinin which is toxic and able to agglutinate red blood cells. These antinutrients could be removed with the help of heat treatments to the beans at a temperature of 100^0C for a few minutes (Astawan, 2009). Similar with some antinutrients contained in jack bean that urease contained in jack bean was also reported to be easily removed by introduction to heat processing to the seeds.

Table 8. Antinutrient of cowpea through some processing

Treatments	Antinutrients				
	Phytic Acid (% dry wt)	**Tannins (% dry wt)**	**Antitrypsin (TIU/mg)**	**Stachyose (% dry wt)**	**Raffinose (%dry wt)**
Raw	1.2 ± 0.2	0.10 ± 0.05	27.6 ± 8.2	3.1 ± 0.4	1.4 ± 0.5
Dehulled	1.2 ± 0.2	ND	26.6 ± 7.8	2.5 ± 0.3	1.1 ± 0.5
Cold-soaked	1.1 ± 0.2	0.07 ± 0.02	25.7± 7.2	2.7 ± 0.2	1.2 ± 0.5
Hot-soaked	1.1 ± 0.2	0.08 ± 0.03	10.7 ± 3.3	2.6 ± 0.3	1.1 ± 0.5
Cooked	1.0 ± 0.2	0.06 ± 0.02	2.5 ± 0.8	2.0 ± 0.1	1.0 ± 0.5
Steamed	1.0 ± 0.1	ND	2.4 ± 0.6	1.0 ± 0.1	0.9 ± 0.5

ND: Not Detected
Source: (Ogun, Markakis, & Suwanto, 1989)

Table 9. Antinutrients reduction of different cultivars of red kidney bean when processed into tempe

Cultivars	Treatment	Antinutrients reduction (%)		
		Phytic acid	**Tannin**	**α-galactoside**
Type 1	Soaking, 12 h	17	23	45
	Sprouting, 48 h	79	75	88
	Cooking	28	35	53
	Germination 48 h + autoclaving	100	100	93
Type 2	Soaking, 12 h	18	25	41
	Sprouting, 48 h	87	74	80
	Cooking	25	34	49
	Germination 48 h + autoclaving	100	100	91
Type 3	Soaking, 12 h	19	24	45
	Sprouting, 48 h	87	80	88
	Cooking	26	27	53
	Germination 48 h + autoclaving	100	100	93

Source: (Emire & Rakshit, 2007)

CONCLUSION

The use of soybean as raw ingredient for *tempe* production other than non-soy legumes is inevitable. On the other hand, locally produced non-soy legumes in Indonesia such as mung bean, cowpea, jack bean, and red kidney bean have shown greater potential. Utilization of these non-

soy legumes as raw ingredient for *tempe* production could be the answer to improve the value of them and thus, the dependency of soybean as ingredient for *tempe* production could be minimalized.

There have been many researches related to the utilization of non-soy legumes as raw ingredient for *tempe*. Further processing into *tempe* showed that nutrient content of each non-soy legumes was improved and compared to soybean *tempe* even shows greater functionalities.

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CANDLENUT MILK AND CREAM APPLICATION FOR NON-DAIRY ICE CREAM WITH HIGHER POLYUNSATURATED FATTY ACID

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ABSTRACT

Lower ability to digest lactose limits the consumption of dairy products for children and teenagers. Candlenut is a known source of polyunsaturated fatty acid that can be processed into candlenut milk and cream as an alternative ingredient in the production of non-dairy food products, such as candlenut ice cream. However, the application of candlenut to the food products requires some optimizations. This research aimed to explore the optimized formula and overcome the limitations in nutritional requirement, sensory acceptance, and saponin content. Compared to dairy products, the utilization of candlenut will result in undesirable texture due to its unsaturated fatty acid content. The addition of stabilizer was able to improve the texture and was comparable to the dairy ice cream. The formulation was optimized by Design Expert software, resulted in selected formula using 49.7% candlenut cream, 29.8% of candlenut milk, 19.9% of sugar, and 0.55% stabilizer which had score 7.07±1.3 ("like moderately") of the overall acceptance by hedonic test. The iodine value of the candlenut ice cream was higher (21.56 ± 0.60) compared to dairy ice cream (4.89 ± 0.13). The selected formula of candlenut ice cream also passed the Indonesian National Standard (SNI) in fat, sugar, protein content, total soluble solid and total plate count spoilage aspects. The saponin content (49.34 ± 0.23) was also in the range of daily intake limit.

Keywords: candlenut; ice cream; unsaturated fatty acid

ABSTRAK

Laktosa intoleran membatasi asupan produk laktosa pada anak-anak dan remaja. Kemiri diketahui sebagai sumber asam lemak tak jenuh ganda yang berpotensi untuk diolah menjadi susu dan krim kemiri sebagai bahan baku alternatif untuk memproduksi produk pangan bebas laktosa seperti es krim kemiri. Namun, pengolahan kemiri menjadi produk pangan memerlukan penyesuaian. Penelitian ini bertujuan untuk mengeksplorasi formula optimal dan mengatasi keterbatasan akan kebutuhan nutrisi, penerimaan sensorik, dan kandungan saponin. Jika dibandingkan dengan produk berbahan dasar laktosa, pemanfaatan kemiri akan menghasilkan tekstur yang tidak diinginkan karena kandungan asam lemak tak jenuhnya. Penambahan stabilizer dapat membantu memperbaiki tekstur yang sebanding dengan es krim laktosa. Formulasi dioptimasi dengan software Design Expert, menghasilkan formula terpilih dari 49,7% krim kemiri, 29,8% susu kemiri, 19,9% gula, dan 0,55% stabilizer dengan skor penerimaan keseluruhan 7,07±1,3 "Suka" pada tes kesukaan. Nilai iodin es krim kemiri lebih tinggi (21,56±0,60) dibandingkan dengan es krim laktosa (4,89±0,13). Formula es krim kemiri yang terpilih sesuai dengan Standar Nasional Indonesia (SNI) dalam aspek lemak, gula, kadar protein, total padatan terlarut, dan angka lempeng total. Kandungan saponin (49,34±0,23) juga berada dalam batasan asupan harian.

Kata kunci: asam lemak tak jenuh; es krim; kemiri

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INTRODUCTION

Around 70% of children and teenagers around the world were shown to suffer from lactose indigestion or intolerance due to the depleting amount of lactase enzymes. The decrease of lactase activity is directly proportional to age, as the decrease of lactase activity starts at an early age of 2-3 years old (Sitepu et al., 2020; Hegar and Widodo, 2015). This population usually suffers adverse effects upon dairy product consumption and calls for alternative ingredients to enjoy food products that are commonly made from milk.

Candlenut is known as a spice with high fat content and has a potential to be processed into a plant-based milk and cream. Candlenut has also been studied for its high proportion of polyunsaturated fatty acid (PUFA), namely linolenic acid, linoleic acid, and oleic acid, which are known to have many beneficial effects in health. The risk of cardiovascular diseases could be decreased by the substitution of dietary saturated fats with polyunsaturated fat. The greater the reduction in saturated fat will reduce greater risk of cardiovascular diseases (Hooper et al., 2020). In addition to its fat content and profile, high availability of candlenut in Indonesia may also support its utilisation as non-dairy ingredients for animal milk and cream replacement in Indonesia. Predominantly grown in East Nusa Tenggara, South Sulawesi, Aceh and North Sumatera, candlenut production reaches 100.6 tons from 214.1 thousand hectare of candlenut plantation area in 2014 (Badan Pusat Statistik, 2020).

Despite the health benefits and potential production, candlenut oil is also shown to have weak stability in high temperature processing and give off flavor (rancid) to the end product. The phytochemical content also gives a distinct musty and bitter taste that may become challenging in its application to food with subtle flavor (Rasid et al., 2019). The low temperature processing in ice cream production and storage provide conditions to avoid the rancid flavor formation. Wide range of flavoring applications are also very common in ice cream and might help to overcome the unpleasant characteristics brought by candlenut phytochemical content. The application of additives such as stabilizer may also be applied for texture improvement of the resulting ice cream. While low temperature processing and additive application may support candlenut as a promising alternative ingredient in the production of non-dairy ice cream, it is important to also study the resulting ice cream quality especially for its compliance to the commercial standard such as, Indonesian National Standard (SNI) of dairy ice cream.

MATERIALS AND METHOD

Materials and equipment

The candlenut kernels were purchased from a local market in South Tangerang. For candlenut ice cream production, food grade mineral water, sugar, salt, CMC, and xanthan gum were used. Reagents used for analysis were potassium iodide (KI), hydrochloric acid (HCl), sodium hydroxide (NaOH), potassium dichromate ($K_2Cr_2O_7$), chloroform, sodium thiosulfate ($Na_2S_2O_3$), Bromocresol Green Methyl Red indicator soluble starch, Wijs solution, plate count agar and saline solution (NaCl 0.85%). All reagents were analytical grade and obtained from Merck (Germany).

Equipment used in candlenut processing include food processor (Mitzui, Korea), cheesecloth, water bath with hotplate (Benstead Thermolyne CIMAREC, USA) rotary evaporator (IKA, China) and ice cream maker (Cuisinart, USA), viscometer (Brookfield, USA), refractometer (ATAGO, Japan) Biosafety cabinet (ESCO, USA), hotplate (CIMAREC, USA), autoclave (HIRAYAMA, Japan), incubator (Memmert 100-800, Germany) and micropipette (Eppendorf, Germany).

Candlenut milk and cream production

Washed candlenut kernels were soaked in room temperature water (25ºC) for 1 hour, and drained afterward. The candlenut kernels were blended into the food processor with ration 125 g per 1000 ml mineral water for 3 minutes until homogenized to produce candlenut puree. The candlenut puree was filtered with cheesecloth to remove the solid particle, yielding the liquid part of the candlenut milk. To improve the stability of the candlenut milk, 6 grams of soy lecithin was added per liter

and blended for 2 minutes. All the process was done at room temperature. The resulting candlenut milk was further pasteurized in a hot water bath at 60 ºC for 30 minutes. The pasteurized candlenut milk was evaporated at 60 ºC, 150 rpm using vacuum evaporator to reach fat content above 35% (Australian New Zealand Food Standards Code, 2016), to produce candlenut heavy cream.

Candlenut ice cream formulation

Two ice cream controls were made according to ice cream machine guidelines (CuisinArt Recipe Booklet) for simple ice cream of 250 grams of heavy cream, 150 grams of whole milk, 100 grams of granulated sugar, and a pinch of salt. The first control was using cow milk and cream while the second control was using candlenut milk and cream. Design expert is used for the formulation of candlenut ice cream using candlenut milk and cream and also stabilizer (CMC and xanthan gum). The ice cream mixtures were mixed randomly using spatula for 3 minutes and pre-cool at 2-5 °C for 120 min prior to cold mixing in the ice cream machine for 60 minute and then stored at -20°C for 24 hour. Formula variation was designed using lower and upper limits of four ingredients shown in Table 1 and randomization using Design Expert software (v 9.0). Each formula was observed in response to viscosity of the pre-cooled mixture, the meltdown rate of the ice cream, and cost of ingredients. Formula is selected based on its proximity to the first control. And the impact of stabilizer addition is observed in comparison to the second control. The minimum level and maximum level of sugar quantity were 95.8 grams and 100.0 grams. The xanthan gum was considered 2.0 grams as the minimum level and 4.0 grams as the maximum level.

Table 1. Lower and upper limit of ice cream ingredients

Ingredients	Minimum level (gr)	Maximum level (gr)
Candlenut Cream	248.5	252.2
Candlenut Milk	148.5	150.0
Sugar (SNI 01-3713-1995)	95.8	100.0
Xanthan Gum (Naresh and Shailaja, 2006)	2.0	4.0

Physical properties analysis

Viscosity analysis

For the ice cream mixture viscosity analysis, 200 ml of samples were transferred into a beaker glass. The spindle of the viscometer was adjusted inside the beaker until the sample surface reached the designated level which was marked in the spindle rod. The viscometer was turned on and the speed adjusted to 50 rpm. Viscosity of the sample was displayed on the screen of the viscometer.

Overrun analysis (Marshall et al. 2003)

Candlenut ice cream mixture was measured in the beaker glass before inserting it into the ice cream maker. After the candlenut ice cream was frozen in ice cream maker for 30 minutes, the ice cream was measured again in beaker glass before it was stored in the chest freezer. The overrun of candlenut ice cream was calculated with the following formula:

$$\%\ overrun = \frac{volume\ of\ ice\ cream - volume\ of\ mixture\ used}{volume\ of\ mixture\ used}\ x\ 100\%$$

Meltdown rate analysis (Alamprese et al., 2002)

To analyze the meltdown rate of candlenut ice cream, 50 grams of candlenut ice cream was weighed using digital balance. Candlenut ice cream sample that was stored in the chest freezer with the temperature of -20°C was thawed until it reached -15°C on the surface. The thawed samples were put in the sieve and let it melt at room temperature (25°C). The melted candlenut ice cream was held

in the beaker glass and weighed every 5 minutes using a digital balance for about 60 minutes (1 hour). The meltdown rate was calculated:

$$\%\ meltdown = \frac{weight\ of\ melted\ sample}{weight\ of\ sample}\ x\ 100\%$$

Total soluble solid analysis (AOAC, 2005)

From meltdown rate analysis, the melted ice cream was collected and used to analyze the total soluble solid. The total soluble solid analysis was using a pocket refractometer at room temperature (25ºC). Refractometer was calibrated using pure water and cleaned before usage. Two drops of sample were applied in the glass of the refractometer and the "start" button was pressed. The result of total soluble solid analysis was displayed on the screen of the refractometer (in ºBrix).

Chemical properties analysis

Moisture content analysis (SNI 01-2891-1992)

One gram of the sample was weighed and dried in the oven at 105°C for 8 hours. The weight was measured periodically and the analysis stopped when constant weight was achieved. The moisture content was then calculated using this formula:

$$\%\ moisture\ content = \frac{weight\ of\ sample\ (fresh - dried)}{weight\ of\ fresh\ sample}\ x\ 100\%$$

Total ash content analysis (sni 01-2891-1992)

Porcelain crucible was dried in the oven at 105°C for 2 hours. After 2 hours of drying, the dried porcelain crucible was transferred into a desiccator for 30 minutes. The sample as much as 1 g was weighed and added into the dried porcelain crucible. The crucible was then put into a 600°C furnace. The sample was heated with a furnace for 12 hours or until the white ashes were produced and no smoke was emitted. The crucible then moved into a desiccator for another 30 minutes. The ash was weighed and calculated with the following formula:

$$\%\ ash\ content = \frac{weight\ of\ ash}{weight\ of\ sample}\ x\ 100\%$$

Chemical properties analysis

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$$\%\ ash\ content = \frac{weight\ of\ ash}{weight\ of\ sample}\ x\ 100\%$$

Protein content analysis (SNI 01-2891-1992)

In the protein content analysis, 0.25 grams of sample was weighed and mixed in a 100 ml Kjeldahl flask. 0.25 grams of selenium and 3 ml of concentrated H_2SO_4 was added to the flask. The prepared sample in the flask was boiled for 60 minutes until it became clear. Twenty ml NaOH 40% and 50 ml of aquadest were added to the flask once the sample was cooled down and distilled. The distilled sample was added with 10 ml of H_3BO_3 2% in Erlenmeyer flask and mixed with 2 drops of Bromocresol Green Methyl Red indicator. The distillation was stopped when the sample condensed to 10 ml and indicated with green bluish color. The product was titrated with 0.1 N HCl until the color of the sample became pink. A blank sample was also made the same way as the procedure above. The result was calculated using the formula:

$$\%\ N = \frac{(S - B)x\ N\ HCl\ x\ 14}{w\ x\ 1000}\ x\ 100\%$$

Where:

S = Volume for sample (ml)

B = Volume for blank (ml)

N = HCl Normality

14 = Relative mass of nitrogen atom

w = Sample weight (g)

Crude fat content analysis (SNI 01-2891-1992)

In the analysis of crude fat content, 2 grams of sample were weighed and spread over on top cotton and filter paper. The sample was rolled into a thimble shape and drubbed in an oven at 80°C for about an hour. The dried sample was put in a Soxhlet apparatus that has been connected with fat flasks containing boiling stones that already dried with known weight. The sample then extracted over 6 hours using 150 ml hexane. The hexane was distilled and the fat extract was dried in the oven at 100°C for an hour. The extract was cooled down and weighed. The fat content was calculated by using the following formula:

$$\%\ fat\ content = \frac{weight\ of\ extracted\ fat - weight\ of\ empty\ flask}{weight\ of\ sample}\ x\ 100\%$$

Carbohydrate analysis (SNI 01-2891-1992)

Carbohydrates in the sample were calculated by the weight differences of sample and other constituents. The constituents of the sample were water, ash, protein, fat and fibre. Carbohydrate content was calculated with the following formula:

$$\%\ carbohydrate = 100\% - (\%water + \%ash + \%protein + \%fat + \%fibre)$$

Iodine value (Council of Europe, 2004)

The Iodine value of an ice cream was measured by titration. Two grams of ice cream sample was weighed in the Erlenmeyer flask. Chloroform as much as 15 ml was added into the flask and shaken vigorously. The flask was added with 10 ml of Wijs solution and mixed until homogeneous. The mixture was stored in dark condition for 30 minutes. After 30 minutes, the mixture was added with 10 ml of 10% Potassium Iodide solution and shaken vigorously. The flask then was added with 100 ml Aquadest and homogenized. The sample were titrated with 0.1 N Sodium Thiosulphate until the yellow color was almost faded. After that, 5 ml of 1% starch were added to the mixture and shaken vigorously. The sample were titrated again until the blue color disappeared. The iodine value was calculated by using the following formula:

$$Iodine\ Value = \frac{(Black\ Titration\ Volume - Test\ Titration\ Volume)\ x\ 1.269}{Weight\ of\ sample}$$

Unsaturated fatty acid (Omega-3, Omega-6 and Omega-9) analysis (Pontoh, 2016)

The unsaturated fatty acid content (omega-3, omega-6 and omega-9) of formulated candlenut ice cream was analyzed using a gas chromatography using 30 m x 0.25 mm column (ID 0.25 µm), oven temperature gradient of 170 - 225 °C (1 °C/min), injection temperature 250 °C. The omega-3, -6, and -9 fatty acids quantitative determination were detected using FID with a helium gas carrier.

Quantitative analysis of saponin compound (SNI 01-3555-1998)

Two grams of sample were weighed and transferred into an Erlenmeyer flask. The sample was added with 25 ml KOH alcohol 0.5 M. The mixture was heated for 1 hour. After that, the sample was added with 1 ml phenolphthalein 0.5 M and mixed vigorously. The sample was titrated with HCl 0.5 N until the solution became colorless. The Blank sample was prepared without sample addition and titrated as the procedure above. The saponin content were calculated using the following formula:

$$Saponin\ Content = \frac{56.1\ x\ HCl\ normality\ x\ (black\ titration\ volume - test\ titration\ volume)}{weight\ of\ sample}$$

Microbial analysis

Total plate count analysis was conducted by using Plate Count Agar (PCA) as a media. The saline solution was made by mixing 4.25 gram of sodium chloride (NaCl) with 500 ml of distilled water. For media preparation, 18 grams of PCA powder was weighed and mixed with 800 ml distilled water in

hot temperature until boiled. Both of the saline solution and media were sterilized in autoclave at 121°C for 30 minutes.

For the sample preparation, 1 ml of candlenut ice cream sample was taken and diluted in 9 ml saline solution (1:10). Series of dilutions were made into ratio 1:100, 1:1000, 1:10000, and 1:100000 from the 1:10 dilution sample. One ml of each sample from different dilutions was transferred into petri dishes and added by 20 ml of PCA agar. The samples were incubated at 37°C for 48 hours inside an incubator.

Sensory analysis

The hedonic 9-point scale was used for affective testing with texture, taste, bitterness, and overall acceptance as the sensory attributes that should be evaluated. The samples were 3 different candlenut ice cream formulas which were optimized by Design Expert (v 9.0). Thirty untrained panelists were asked to score the products using numeric scores ranging from 1 until 9 (1 represented "dislike very much" and 9 represented "like extremely"). The samples were coded with 3-digit random code. The sample was prepared by weighing 20 grams of sample from each formula and served in a coded ice cream cup. The results were analyzed statistically using Friedman's Test, and continued with Wilcoxon's Test if there was a significant difference.

RESULTS AND DISCUSSION

The candlenut milk that underwent fat content analysis resulted in ±1000 grams of candlenut milk containing ± 78.75 grams of fat or 7.875% fat. Based on Australian New Zealand Food Standards Code (2016), the fat content of heavy cream should be 35%. To produce a candlenut cream which contained 35% fat, the candlenut milk was evaporated by a vacuum rotary evaporator until it reached the desirable fat content. Candlenut milk and cream only are not able to build ice cream texture as shown by significantly lower viscosity of candlenut ice cream without stabilizer (1,393.33±71.47 cP) in comparison to dairy ice cream (4,358.33±151.18 cP). The resulting ice cream also differs in the creaminess and crystal formation. The application of stabilizer is deemed necessary in the texture improvement of the resulting candlenut ice cream. (Goff & Hartel, 2013). As shown in Table 2, the application of CMC and xanthan gum were able to increase the pre-cooled mixture to a similar level of dairy ice cream. Nevertheless both stabilizers gave different impacts to the ice cream meltdown rate. CMC application increases meltdown rate up to 89.69 % and very hard texture of the candlenut ice cream. Xanthan gum increases meltdown rate only up to 57.40% in candlenut ice cream which is relatively similar to dairy ice cream of 49.59%. Therefore xanthan gum was chosen as the stabilizer of candlenut ice cream and used in the following ice cream formulation.

Table 2. Viscosity and meltdown rate comparison between stabilizer addition and dairy ice cream

Ice Cream Sample	Stabilizer		Viscosity (cP)	Meltdown Rate (%)
	Type	Quantity (%)		
Candlenut Ice Cream	n/a	n/a	1,393±71.40	n/a
Candlenut Ice Cream	CMC	0.06	4,345±35.36	89.69±1.50 %
Candlenut Ice Cream	Xanthan Gum	0.50	4,300±14.14	49.59±0.77 %
Dairy Ice Cream	n/a	n/a	4,358±151.18	57.40±0.31 %

Ice cream formulation using design expert

Based on the minimum and maximum limit of each ingredient from Table 1, twenty formulas were generated and the viscosity of the ice cream mixture was evaluated in response to the viscosity of pre-cooled mixtures, the meltdown rate of ice cream, and the calculated cost of ingredients (Table

3). The matrix of formula to responses value resulted in the equation as shown in Table 4 that was used for further optimization to target 4,358 cP viscosity and 57.4% meltdown rate. The optimization offered five alternative formulas which had the same responses (viscosity and meltdown rate) as the targeted ice cream (control positive ice cream), as shown in Table 5. Three optimized formulas (formula 1 - 3) with highest desirability (>0.85) were chosen and analyzed further for its overall acceptance and perceived sensory attributes (Table 6). The panelists were not able to distinguish bitterness attributes between formula 1 and formula 3, as well as color between formula 1 and 2. However, other sensory attributes differences seemed to be significantly different. The candlenut ice cream formula 1, 2 and 3 were accepted over the range 5-7 points (neither like or dislike-like moderately), with formula 2 receiving the highest score for overall and taste acceptance.

Table 3. Alternative formula provided by design expert and its responses

Formula #	Ingredients (%)				Responses		
	Candlenut Cream	Candlenut Milk	Sugar	Stabilizer	Viscosity (cP)	Meltdown Rate (%)	Economical Value (IDR)
1	49.70	29.90	20.00	0.40	2830	61.34	11808.36
2	50.04	30.00	19.16	0.80	5780	54.28	12198.83
3	50.04	30.00	19.16	0.80	5800	54.43	12198.83
4	49.70	29.85	19.65	0.80	5820	52.02	12163.59
5	50.44	30.00	19.16	0.40	2720	60.77	11890.63
6	49.90	29.70	20.00	0.40	2790	61.17	11836.19
7	50.17	29.70	19.73	0.40	2800	61.33	11867.10
8	50.07	30.00	19.53	0.40	2640	59.86	11848.27
9	50.34	29.70	19.16	0.80	5900	55.49	12240.57
10	50.34	29.70	19.16	0.80	5780	53.92	12240.57
11	49.70	30.00	19.70	0.60	4780	56.21	11982.90
12	50.02	29.70	19.48	0.80	5810	51.88	12203.93
13	50.44	29.70	19.46	0.40	2600	60.07	11898.02
14	49.70	29.70	20.00	0.60	4800	56.17	11990.29
15	49.70	29.85	19.65	0.80	5820	52.17	12163.59
16	49.90	29.80	19.79	0.50	3130	57.03	11918.39
17	49.70	30.00	19.70	0.60	4760	56.33	11982.90
18	50.24	30.00	19.16	0.60	4720	56.86	12044.73
19	50.44	30.00	19.16	0.40	2760	60.11	11890.63
20	50.16	29.84	19.37	0.63	4980	53.84	12068.94

Physicochemical and microbial analysis of selected formula

The selected formula 2 of candlenut ice cream (Figure 1) was analyzed for its physicochemical characters and microbial count to observe its compatibility with national standard (Table 7). The addition of stabilizer will increase the total soluble solid in ice cream as the stabilizer has the ability to bind water with another compound, such as sucrose (Saati and Sundari, 2009). Therefore, the addition of xanthan gum as stabilizer increased the total soluble solid of ice cream.

Figure 1. Selected formula of candlenut ice cream

The overrun measurement of the ice cream was done by calculating the percentage difference of volume of ice cream before and after the freezing process. In the freezing process, there was a mixing process, the air whipped towards the ice cream mixture. By the air movement into the ice cream mixture, the volume of the mixture was expanded from 0 into maximum 100%. In some areas of overrun, it provided a pleasing texture, structure and body to the ice cream. Based on the texture and quality of the ice cream, ice cream with lower quality (economic ice cream) has higher overrun, while high quality ice cream (premium ice cream) will have lower overrun (Hartolo, 2011). The overrun of the control positive ice cream was between 37.53% and 38.05%, and the overrun of the selected formula of candlenut ice cream.

Table 4. Equation of design expert responses

	Viscosity	Meltdown Rate	Economical Value
Cream	+15,921.29135	+110.71732	+35.000
Milk	+70,082.40957	+451.51694	+8.075
Sugar	+19,935.7397	+144.99509	+13.000
Stabilizer	-1.73656×10^5	+523.26934	+190.000
Cream+ Milk	-298.45259	-1.95963	
Cream+ Sugar	-43.05408	-0.37484	
Cream+ Stabilizer	+360.01920	-1.03134	
Milk+ Sugar	-281.43017	-1.79811	
Milk+ Stabilizer	+113.01462	-2.32099	
Sugar+ Stabilizer	+374.96192	-1.47446	

Table 5. Optimized formula provided by design expert

Optimized Formula #	Ingredients (%)				Responses			Desirability
	Candlenut Cream	Candlenut Milk	Sugar	Stabilizer	Viscosity (cP)	Meltdown Rate (%)	Economical Value (Rp)	
1	49.700	29.914	19.836	0.552	4250.00	56.6101	11942.1	0.883948582
2	49.700	29.808	19.942	0.550	4250.00	56.6126	11944.2	0.881706645
3	49.854	29.992	19.602	0.552	4250.00	56.6099	11957.8	0.868173204
4	50.318	29.700	19.428	0.554	4234.25	56.6124	12020.2	0.796302702
5	50.438	29.840	19.160	0.562	4239.73	56.6099	12037.1	0.776290035

Ice cream quality can be determined as bad when the meltdown rate of it was high. Higher meltdown rate means the ice cream can melt easily. The ice cream quality can be determined as undesirable

also when the meltdown rate of it was too low, because the texture of the ice cream will be too tough to consume (Hendriani, 2005). The meltdown rate of the selected formula of candlenut ice cream was 57.38±0.52% which was comparable with the control positive ice cream 57.40±0.31%.

Based on the proximate analysis results from Table 7, protein and fat content met the requirement from the National Standard Indonesia (SNI) value. For the total ash, moisture, and carbohydrate, there are no minimum requirements of SNI in the ice cream.

Table 6. Hedonic test result of optimized formula of candlenut ice cream

Sensory Attribute	Formula 1	Formula 2	Formula 3
Texture	6.43±1.30 [a]	7.20±1.19 [b]	6.60±1.10 [c]
Taste	6.40±0.97 [d]	6.60±1.00 [e]	5.93±1.28 [f]
Bitterness	6.50±1.07 [g]	7.00±1.20 [h]	5.83±1.32 [g]
Color	6.70±0.95 [i]	7.10±0.99 [i]	6.43±1.38 [j]
Overall Acceptance	5.63±0.96 [k]	7.07±1.31 [l]	6.70±1.24 [m]

*the hedonic test used 9 point scale for each sensory attribute; 1=dislike extremely, 2=dislike very much, 3=dislike moderately, 4= dislike slightly, 5=neither like or dislike, 6=like slightly, 7=like moderately, 8=like very much, 9=like extremely

Table 7. Physicochemical and microbial analysis of candlenut ice cream

Character	Selected Formula of Candlenut Ice Cream	Dairy Ice Cream	SNI 01-3713-1995
Total Soluble Solid (°Brix)	22.63±0.30	21.45±0.18	Minimum 3.4
Overrun (%)	29.54±0,19	37.79±0.26	n/a
Meltdown Rate (%)	57.38±0.52	57.40±0.31	n/a
Viscosity (cP)	4,273.33±20.21	4,358.33±151.18	n/a
Protein Content (%)	3.48±0.02	n/a	Minimum 2.7
Fat (%)	11.66±0.03	n/a	Minimum 5
Total Ash (%)	0.65±0.02	n/a	n/a
Moisture (%)	63.23±0.04	n/a	n/a
Carbohydrate (%)	21.07±0.12	n/a	n/a
Iodine Value (cg/g)	21.56±0.60	4.89±0.13	n/a
Saponin (mg/g)	49.34±0.23	n/a	Limit 10-200
Total Plate Count (CFU/ml)	4.90×10^4	n/a	Maximum 2×10^5

The iodine value is a number which represents the amount of iodine in grams consumed by 100 grams of a chemical substance. Normally iodine value is used to determine the degree of unsaturation in fatty acid. In the iodine value analysis, the iodine will react with a double bond chain of unsaturated fatty acid. The higher the iodine value is, the greater the number of double bonds. Therefore, the high iodine values result in more unsaturated fatty acids. (Daun et al., 2011). The iodine value of

candlenut ice cream was much higher than the dairy ice cream, which indicated the number of unsaturated fatty acids were also high.

Table 8. Categorization of frozen dessert based on overrun measurement (Marshall et al., 2003)

Product	Overrun (%)
Super premium ice cream	20-40
Premium ice cream	60-75
Ice cream, packaged	75-95
Ice cream, bulk	90-100
Sherbet	30-40
Ice	25-30
Soft ice cream	30-50
Milk shake	10-15

The results of saponin analysis (Table 7) showed the saponin content in the candlenut ice cream was around 49.34 mg/100g of saponin, and in one serving of ice cream (60 g) would contain 29.6 mg of saponin. According to Hosettmann and Marston (2005), the average limit of saponin daily intake for Asian people is 214 mg per person daily. And referring to Arnelia (2002), the recommended daily intake of saponin in food samples was 10-200 mg/100g daily. Thus, the saponin content in the candlenut ice cream was considered safe to be consumed.

The maximum number of total plate count from the SNI is 2.00 x 10^5 CFU/ml. From the results on Table 7, the total plate count of the candlenut ice cream was 4.90 x 10^4 ±0.02 CFU/ml which is below the maximum standard of SNI.

The selected formula of candlenut ice cream was analyzed for the unsaturated fatty acid content, namely omega-3, omega-6, and omega-9. The unsaturated fatty acid content was compared to the unsaturated fatty acid content in the dairy ice cream. The results showed in Table 9**.** All of the omega-3, omega-6, and omega-9, often called triple omega fatty acids, content in the candlenut ice cream showed higher amounts compared to the dairy ice cream. A study done by Elbossaty (2018) found some health benefits of the triple omega fatty acids to the body. The omega-3 is able to reduce cardiovascular disease by keeping blood vessels healthy and reducing the cholesterol and triglycerides level in the body. Rheumatoid arthritis and cancer prevention also can be triggered by omega-3. The omega-6 helps to reduce the development of diabetes type 2 and a good treatment to allergy and sclerosis. The omega-9 is able to reduce bad cholesterol (LDL) and increase good cholesterol (HDL) in blood vessels, increase immune system, protect nerves, and prevent heart disease. The recommended daily intake of the triple omega fatty acids was < 3gram/daily for omega-3, 12 – 17 gram/daily for the omega-6, and no adequate intake recommendation for the non-essential fatty acids like omega-9 (Robertson, 2020). The omega fatty acids content for one cup serving of ice cream (60 g) were 1,707.66 mg for omega-3, 2,743.29 mg for omega-6, and 1,815.93 mg for omega-9. The candlenut ice cream was able to fulfill the recommended daily intake for the omega-3, however for fulfilling the omega-6, additional intake from other food resources might be needed.

Table 9. Unsaturated fatty acid composition in the candlenut and dairy ice cream

Unsaturated Fatty Acid Composition	Selected Formula of Candlenut Ice Cream (mg/100g)	Dairy Ice Cream (mg/100g) (Lukmanto, 2012)
Omega – 3	2,846.10±7.14	13.03
Omega – 6	4,572.15±0.35	21.07
Omega – 9	3,026.55±6.72	1510.91

CONCLUSION

The application of candlenut kernel in ice cream as an alternative ingredient for non-dairy products can be promising. Xanthan gum as a stabilizer can improve the ice cream texture. The candlenut ice cream was also considered as safe to be consumed as the amount of saponin was still in the range of daily intake limit. The amount of polyunsaturated fatty acid in the candlenut ice cream was higher compared to the dairy ice cream. The selected formula of candlenut ice cream (49.85% candlenut cream, 30.00% candlenut milk, 19.60% sugar and 0.55% stabilizer) also had an overall acceptance

score of 7.07±1.3 ("like moderately"). The effect of long-term storage by shelf life analysis to the food products can be explored furthermore. Addition of flavours and vitamin fortification are also recommended for further development.

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OVEN DRYING AND WATER EXTRACTION OF *CURCUMA XANTHORRHIZA* FOR HYGIENE IMPROVEMENT IN THE PRODUCTION OF *JAMU CEKOK*, A TRADITIONAL APPETITE STIMULANT HERBAL MEDICINE

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ABSTRACT

Toddlers (<5 years old) suffering from malnutrition have been a concern in Indonesia, and 53% of them are known to relate with appetite problems. *Jamu cekok* is a traditional appetite stimulant commonly applied by hand-squeezing the water out of herbal mixture directly into the throat of the children. Main ingredient of *jamu cekok* is the rhizome of *Curcuma xanthorrhiza* Roxb (*Temulawak* or Javanese turmeric) with curcumin and xanthorrhizol for its active compounds. Despite empirical evidence of the function and promising market potential, the common practice for the material preparation including sun-drying and hand squeezing has a higher risk for microbial contamination. For hygiene improvement, oven drying and water extraction can be applied to prepare *C. xanthorrhiza* water extract for material of *Jamu cekok* production. In this research, several drying temperatures (30 and 50 °C) as well as extraction temperatures (50, 75, and 100 °C) and solvents to mass ratio (10:5, 10:2, 10:1) were observed in the production of *Temulawak* water extract. Estimation of curcumin content and qualitative determination of xanthorrhizol to evaluate the effectiveness of water extraction, while microbial growth is observed to evaluate the improvement of hygiene conditions. Solvent to mass ratio 10:2 was shown to have the highest curcumin content. Higher extraction temperatures are shown to give higher curcumin content but less xanthorrhizol content. The oven drying and water extraction is shown to successfully improve the sanitation condition shown with significantly lower microbial growth.

Keywords: curcumin; jamu cekok; microbial growth; temulawak; xanthorrhizol

ABSTRAK

Balita usia di bawah 5 tahun dengan kondisi malnutrisi yang disebabkan oleh rendahnya nafsu makan merupakan permasalahan yang banyak ditemukan Indonesia. Jamu cekok umumnya menjadi alternatif untuk meningkatkan nafsu makan pada anak. Jamu cekok adalah obat tradisional Indonesia yang cara memberikanya dengan meremas campuran herbal langsung kedalam mulut bayi. Bahan utama jamu cekok adalah rimpang *Curcuma xanthorrhiza* Roxb (Temulawak) dengan bahan aktif kurkumin dan xantorizol. Walaupun fungsi secara empiris dan potensi pasar sudah cukup menjanjikan, tetapi proses pengeringan dan cara pemberian memiliki potensi kontaminasi yang cukup besar. Perubahan proses pengeringan menggunakan oven dan ekstraksi menggunakan air dapat diaplikasikan untuk meningkatkan sanitasi dari jamu cekok. Pada penelitian ini, dilakukan ekstraksi temulawak dengan pelarut air pada beberapa kondisi suhu pengeringan (30°C dan 50°C), suhu ekstraksi (50°C, 75°C, dan 100°C), serta rasio berat dari pelarut dan bahan baku (10:5, 10:2, 10:1). Estimasi kandungan kurkumin dan xantorizol secara kualitatif digunakan untuk menentukan efektivitas dari proses ekstraksi menggunakan air, sedangkan peningkatan sanitasi dapat dilihat dari jumlah pertumbuhan bakteri. Kandungan kurkumin paling tinggi dihasilkan dari rasio pelarut terhadap bahan baku 10:2. Suhu ekstraksi paling tinggi menghasilkan kandungan kurkumin yang paling tinggi, tetapi sebaliknya memiliki kandungan xantorizol yang paling rendah. Perubahan proses pengeringanan dan metode ekstraksi menunjukan peningkatan sanitasi yang terlihat dari penurunan jumlah bakteri yang sangat signifikan.

Kata kunci: jamu cekok; kurkumin; pertumbuhan bakteri; temulawak; xantorizol

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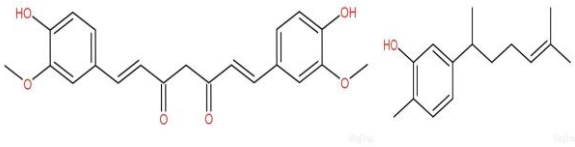
INTRODUCTION

Malnutrition remains to be one of the most common public problems occurring in Indonesia. The prevalence of toddlers (<5 years old) suffering from malnutrition due to appetite problems was up to 53% (Indonesia's Ministry of Health, 2012). To accommodate the appetite problems in children, *Jamu cekok* a traditional herbal mixture is commonly applied for treating appetite problems in children. *Cekok* means a method of giving the *herbal* mixture into the throat of the children by force (Figure 1a). In most applications, *Curcuma xanthorrhiza* Roxb (temulawak or Javanese ginger, Figure 1 (b)) is used as the main ingredient of *Jamu Cekok* as it is known to be effective for increasing appetite in children.

Figure 1. (a) Method of *jamu cekok* (b) Rhizome of *temulawak*

The active compounds in *C. xanthorrhiza*, such as curcumin, xanthorrhizol and essential oils contribute as the main factors of its benefit (Sidik et al., 1995). Curcumin (Figure 1a) is a polyphenol compound that is derived from a dietary spice, such as *C. longa* and *C. xanthorrhiza*, and has been studied for wide application including appetite stimulation (Agarwal *et al*, 2003). *Xanthorrhizol* (Figure 1b) is an active compound that majorly exists and has become a distinctive feature for *C. xanthorrhiza* to differentiate the plant from other Curcuma species (Halim *et.al*, 2012). Study by Choi *et.al* (2005), showed a wide health benefit potency of xanthorrhizol including those related to appetite stimulation. Besides, curcumin content from *temulawak* can increase bile secretion and elevate the activity of enzymatic activity in the digestive system, which results in increasing appetite levels *(Platel and Srinivasan, 2000)*.

While *jamu cekok* has empirical evidence for its efficacy and the demand is there, the commercial product is currently not available in the market. Besides, the preparation of *jamu cekok* is commonly done under an unhygienic method. Thus, there is the opportunity to develop a standard of *temulawak* utilization to produce hygienic *jamu cekok*. Water extraction could become a preferable alternative method for *jamu cekok* hygienic production if proven for their efficacy and hygienic improvement.



(A) (B)

Figure 2. (A) Chemical Structure of Curcumin (B) Chemical Structure of Xanthorrhizol

MATERIALS AND METHOD

Material

The materials that were used in this research are fresh temulawak (*C. xanthorrhiza*), which were obtained from Tangerang Traditional Market, distilled water, curcumin and xanthorrhizol standards from Nacalai Tesque Inc (Put city name, Japan), Plate Count Agar (Merck, city name, Germany), and HPLC-grade ethanol 99.5%.

Method

Temulawak extract preparation

The fresh temulawak was obtained from Tangerang traditional market. It was washed and cut into 2-cm-thick pieces. It was dried within two variations of oven drying temperature (30°C and 50°C) and milled. The fresh *temulawak* was separated into 2 batches and dried at 30°C and 50°C for 72 hours, separately. The dried temulawak were extracted using water at various temperatures (50°C, 75°C, 100°C) and various solvents to mass ratio (10:5, 10:2, 10:1). Optimum conditions for drying and extraction ratio were defined in correlation with the estimation for curcumin content.

Content of curcumin, to ensure the efficacy, and

the number of microbial growths in the extract, to ensure its hygienic standard of production. The evaluations are conducted triplo and the results were statistically evaluated for significance.

Determination of curcumin content (Rohman, 2012; Pawar et al., 2014)

The curcumin content was estimated by using UV-Vis spectrophotometry analysis at the wavelength of 420 – 430 nm, in which curcuminoids show the intensive absorption intensity in some organic solvents (Rohman, 2012). The crude extract is evaluated for its curcumin content estimation based on the standard curve made using curcuminoid standard (Pawar et al., 2014).

Xanthorrhizol quantitative determination (Choi et al., 2017)

The crude extract was chromatographed on a C18 reversed-phase column using ethanol containing 0.1% phosphoric acid. Xanthorrhizol standard with known concentration is used to calculate the amount of xanthorrhizol content in the sample. Sample only, sample with xanthorrhizol standard, and xanthorrhizol standard only were injected into the HPLC system and calculated using an embedded software program to calculate the amount of xanthorrhizol content in the sample.

Total microbial growth (AOAC, 1990)

Total plate count method was used to analyze the hygiene quality of the sample. Each extract was taken using separate sterile pipettes, and diluted to 10^{-2}, 10^{-3}, and 10^{-4}. From each dilution 1 ml of extract sample was pipetted into a petri dish, poured with 12-15 ml plate count agar (cooled to 45 ± 1°C), and mixed immediately by alternate rotation and back-and-forth motion of plates on flat level surface. After the agar solidified, the petri dishes were placed inversely and incubated for 48 h at 35°C. Microbial growth was counted and was calculated for its microbial concentration within serial dilution.

Statistical analysis

The result from the research was analysed statistically by using ANOVA and a post hoc test using Tukey HSD. Differences at $p < 0.05$ are considered statistically significant with 95% confidence level (Kopjar *et al.*, 2009).

RESULTS AND DISCUSSION

Selection of drying temperature and extraction ratio

Sun-drying remains the common practice in preparing dried temulawak prior to consumption or further processing in Indonesia. In this study temperatures of 30°C and 50°C are selected as the minimum and maximum temperatures in Indonesian climates and oven drying is selected as a method due to its simplicity and high processing unit availability. Following the oven-drying, the dried *C. xanthorriza* was extracted with several variations of temperature and ratio solvent to mass.

Table 1. Curcumin Content on Dried Temulawak with Different Temperature of Drying and Water Extraction Process

Drying Temperature	Extraction Temperature	Solvent to Mass Rasio	Curcumin (mg)
30°C	50°C	10:5	21.038 ± 0.52[a]
		10:2	19.308 ± 0.57[a]
		10:1	22.467 ± 0.40[a]
	75°C	10:5	27.996 ± 0.24[b]
		10:2	47.415 ± 1.83[c]
		10:1	70.584 ±1.60[d]
	100°C	10:5	66.972 ± 0.29[e]
		10:2	112.721 ± 0.33[f]
		10:1	87.589 ± 0.67[g]
50°C	50°C	10:5	38.60 ± 0.20[a]
		10:2	47.01 ± 0.40[b]
		10:1	21.13 ± 0.80[c]
	75°C	10:5	52.05 ± 0.60[d]
		10:2	66.89 ± 0.15[e]
		10:1	43.94± 0.50[b]
	100°C	10:5	189.27 ± 0.60[f]
		10:2	417.75 ± 2.33[g]
		10:1	351.04 ± 2.66[h]

The content of curcumin from each sample were shown in Table 1. The evaluation showed that extracts resulting from dried temulawak which was dried at 50 °C, has higher estimated content of curcumin in comparison to those dried at 30 °C.

The result was in agreement with prior study of Halder *et. al* (2010), showing that higher drying temperatures cause the difference in moisture ability to diffuse in the cells of biological material and may increase yield of the following extraction up to three times higher compared to those that were dried at low temperatures. It is interesting to note that higher estimated content of curcumin was shown in the extract resulting from ratio extraction of 10:2 solvent to mass (Table 1). Higher solvent ratio will increase extraction yield as it facilitates conditions that favors good mass transfer from the solid matters to their solvent (Sayyar et al., 2009). However, in this study, it is shown that the optimum extraction ratio was not the highest ratio of solvent to mass. This is probably due to the limit of solvent capacity. According to the evaluation of estimated content of curcumin, 50 °C is selected as the drying temperature and extraction at ratio solvent to mass 10:2 are selected as extraction conditions.

Impact of extraction temperature to curcumin and xanthorrhizol content and microbial growth

*C. xanthorrhiza e*xtract resulting from extraction at higher temperatures have higher estimated content of curcumin in comparison to those coming from lower temperatures extractions (Table 1). In contrast, the highest content of xanthorrhizol was shown in extract resulting from extraction at lower temperature (Table 2). Xanthorrhizol is known to have less stability in temperatures above 50 °C and was not able to maintain its stability at higher temperatures (Yulianti, 2010).

Table 2. Xanthorrhizol Content from Various Extraction Temperature

Temperature	Xanthorrhizol Content (mg/L)
50°C	1966.81
75°C	406.81
100°C	306.06

Note: Used best ratio (1:20) and optimum drying temperature(50°C)

C. xanthorrhiza is known to have anti-microbial activity from xanthorrhizol content, however heat treatment is also known to inhibit microbial growth. Evaluation of total microbial count in *C. xanthorrhiza e*xtract from extraction at higher temperatures has lower microbial count (Table 3). This has shown that high temperature treatments have more impact than xanthorrhizol content in the inhibition of microbial growth from *C. xanthorrhiza e*xtract. Nevertheless all extract tested has met the hygiene standard of traditional herbal medicine production of $<10^4$ CFU/ml (Kepmenkes, 1994). Study by Shohlichah (2012) showed that *Jamu* with traditional preparation can have as high as 4.8 x 10^6 CFU/ml. Therefore, oven drying and water extraction of *C. xanthorrhiza* is shown to successfully improve the hygiene level in the preparation of *Jamu cekok* ingredients.

Table 3. Microbial Growth in The Sample

Temperature (°C)	Number of Bacteria (CFU/ml)
50	25.7 x 10^3
75	10.8 x 10^3
100	4.3 x 10^3

CONCLUSION

In the effort of hygiene improvement for the preparation of *jamu cekok*, oven-drying and water extraction were applied in replacement of sun drying and hand-squeezing. In concern to the processing efficiency and resulted extract efficacy, oven drying at 50°C followed by extraction in ratio solve is shown to yield better curcumin content in the resulting extract. Extraction temperature showed a contrast effect in the curcumin and xanthorrhizol content. Highest curcumin content is shown in extract resulting from highest extraction temperature while highest xanthorrhizol content is shown in extract resulting from lowest extraction temperature. Nevertheless, all extracts met the microbial growth requirements in hygiene standard of traditional herbal medicine production and improvement in the preparation of *jamu cekok* ingredients.

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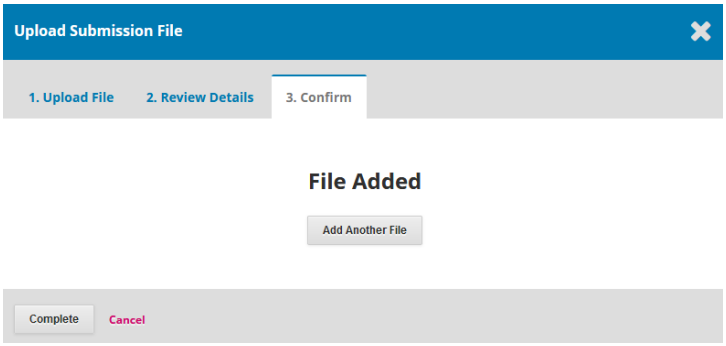

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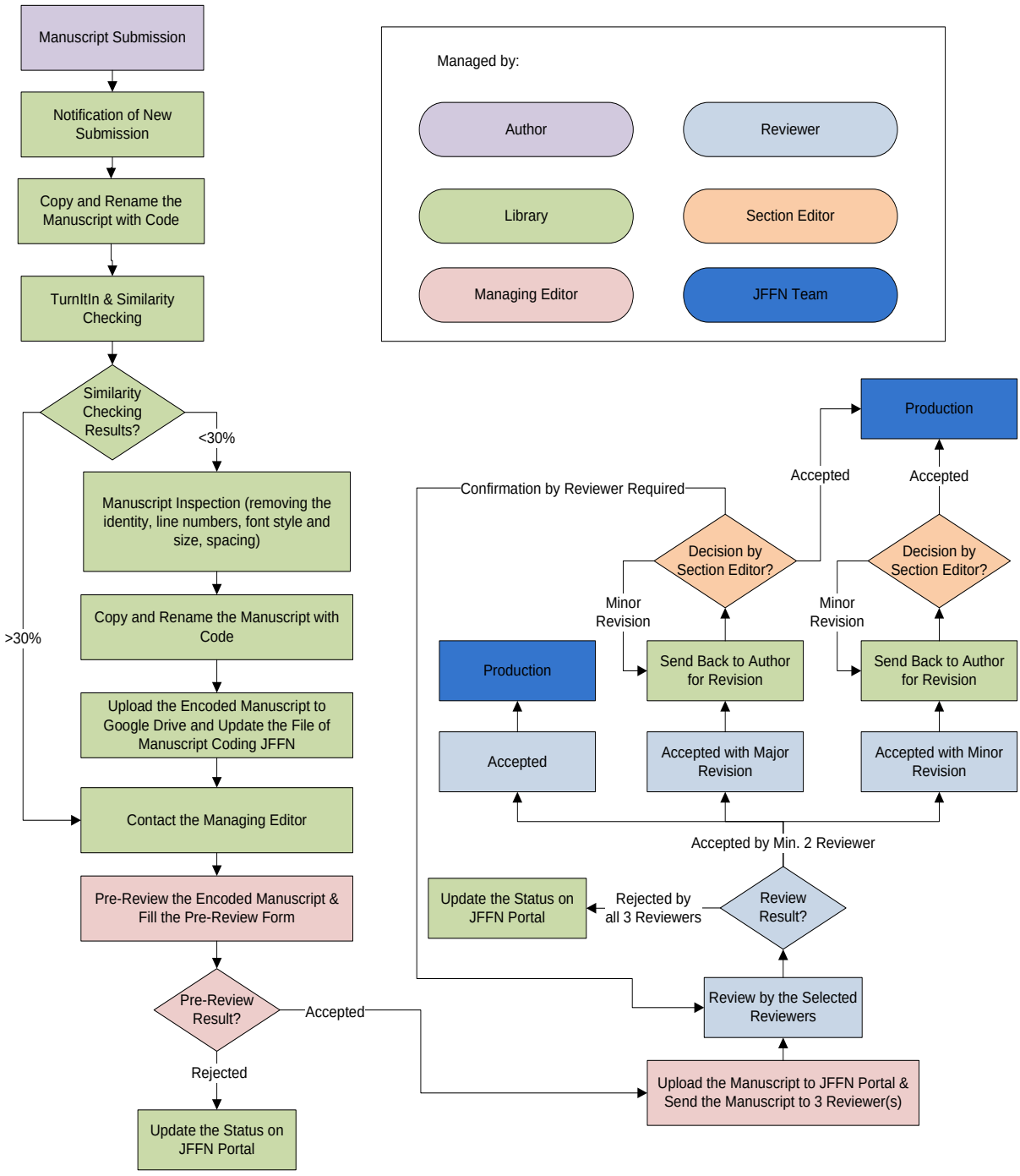

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