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EDITORIAL OFFICE
THE PROMINENCE OFFICE TOWER
JL. JALUR SUTERA BARAT NO. 15
ALAM SUTERA, TANGERANG 15143
TELP/FAX: +62-21 2977 9596 / +62-21 2977 9598
EMAIL : jffn@sgu.ac.id
WEBSITE : journal.sgu.ac.id/jffn

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EDITORIAL TEAM

Journal of Functional Food and Nutraceutical Secretariat Office
Research Center for Food and Health
Academic Research and Community Service
Swiss German University
The Prominence Office Tower
Jl. Jalur Sutera Barat No. 15
Alam Sutera, Tangerang 15143

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Jalan Jalur Sutera Barat Kav 15
Alam Sutera – Tangerang
Indonesia

Principal Contact
Maria D.P.T. Gunawan Puteri
Managing Editor
Swiss German University
jffn@sgu.ac.id

Support Contact
Maria Lamury
Administration Team
Swiss German University
jffn@sgu.ac.id

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ABOUT THE JOURNAL

Journal of Functional Food and Nutraceutical (JFFN) is an official journal of **Perhimpunan Penggiat Pangan Fungsional dan Nutrasetikal Indonesia or Indonesian Society for Functional Food and Nutraceutical (P3FNI-ISFFN)** that has been established in collaboration with **Research Center for Food and Health Swiss German University (RC F&H SGU).** JFFN publishes review and research result on frontier research, development, and application in the scope of functional food and nutraceuticals**.** The journal is expected to bring together all stakeholders in relation to the food ingredients and nutraceuticals.

Scope of the journal Include:

- Interdisciplinary approach of food technology, food nutrition, and health
- Plant bioactive; dietary fiber, probiotics; functional lipids; bioactive peptides; vitamins, minerals and botanicals and other dietary supplements.
- Nutritional and technological aspects related to the development of functional foods and nutraceuticals.
- Food digestion, bioavailability, mechanism, efficacy, and safety of food ingredients and nutraceuticals.
- Food product development with health benefit
- Characterization of healthy foods and functional constituents
- Preparation of natural and synthetic ingredients for use in foods and supplement
- effects of processing (including packaging and storage) on functionality and improvement of product quality; verification, quality control and traceability of natural and synthetic functional food ingredients and nutraceuticals.
- The regulatory aspects of functional foods and related issues e.g. labelling, substantiation of health claims are also of interest together with those dealing with the value creation on the food chains based on the nutritional/healthy aspects.

JFFN publishes **2 times in a year,** August and February. JFFN adopting **Open Journal System** for fast manuscript management process. All authors are requested to register in advance and submit the manuscript online to support the fast managing and review process and to be able to track the real-time status of the manuscript.

All accepted manuscripts receive individual digital object identifier (DOI) and indexed by Google Scholar. The online PDF version of the journal is open access from **https://journal.sgu.ac.id/jffn**

Subscription of the hard copy can be requested by email to **jffn@sgu.ac.id**

PREFACE

Welcome to the inaugural issue of *Journal of Functional Foods and Nutraceuticals (JFFN).* It is my great privilege and pleasure to present the inaugural volume of this new peer-reviewed journal, a joint publishing journal of Perhimpunan Penggiat Pangan FungsionaI dan Nutraceutical Indonesia (P3FNI) or Indonesian Society of Functional Foods and Nutraceuticals (ISFFN) and Research Center of Food and Health, Swiss German University (SGU). JFFN is a frontier publication devoted to strengthen the development of functional foods, from theoretical aspects to application-dependent studies and the validation of emerging technologies, which naturally complement each other, as well as any grass root issues for practitioners. JFFN aims to provide a highly readable and valuable contribution literature to emerging interest in functional foods science and technology in Indonesia Society. The journal is also dedicated to encourage early bird authors to experience publishing in an international journal by providing a friendly tutorial.

This first issue comprises five manuscripts, connected by a unifying theme: "Functional Food and Nutraceutical for Community Health". The presented articles can be categorized into the following groups:

- Basic research evaluating the functional activities
- Social studies on consumer trends on functional food

It is our hope that the articles of this first issue will become a valuable resource for the readers of JFFN, and will stimulate further research into the vibrant world of functional foods.

As the chairman of P3FNI, I would like to use this inauguration occasion to thank many people who supported the idea to create a new journal JFFN and provided the opportunity for the journal to be born, in particular Dr. Maria S. Gunawan-Putri. I also deeply appreciate the hearty support of SGU as we strive to make JFFN the most authoritative journal on the field of functional foods. Furthermore, as the editor in chief, I would like to extend my sincere thanks to all members of the editorial and the advisory boards, whose service, dedication, and commitment have made the creation of this journal possible. I would also like to acknowledge the highly appreciative effort to all of manuscript reviewers for providing valuable comments and suggestions to the authors. As we are working together, we aim to continue to strive for quality and excellence in published articles. It is without doubt that the success of our journal depends highly on the author contribution of articles. Through seamless collaboration with all of our authors, we aim to continue to strive for quality and excellence in publishing articles.

It is our hope that JFFN could deliver valuable and interesting information to the nationwide and worldwide community of food science and stimulate further exciting research in the diverse area of functional foods and nutraceuticals. I am certain that this first issue will be followed by many others, providing high quality reports on the most advanced developments in food science field. More information about JFFN guidelines for the preparation and submission of papers can be found at JFFN website: https://journal.sgu.ac.id/jffn/index.php/jffn/index .

Finally, as a newly established journal I do realize that there are still a lot of aspects that have to be improved. Therefore, we are sincerely waiting for your mutual suggestions and criticism.

July 2019,

C. Hanny Wijaya
Editor in Chief of Journal of Functional Food & Nutraceutical

Journal of Functional Food and Nutraceutical

STABILIZATION OF RED MELINJO PEEL (*GNETUM GNEMON* L.) ETHYL ACETATE EXTRACT AS ANTIBACTERIAL AGENT

Adolf J.N. Parhusip[1]
Nuri A. Anugrahati[1]
Wenny S.L Sinaga[1]
Johan Honga[1]
Veliana Angel[1]

[1] Food Technology Study Program of Pelita Harapan University

ABSTRACT

Melinjo (*Gnetum gnemon* L.) is a typical Indonesian plant that has many benefits such as antimicrobial agent. The aim of this study was to determine the antimicrobial activity of red melinjo peel extract. In this study, extraction was conducted by maceration using ethyl acetate as solvent for 24 hours at room temperature. 4-16% red melinjo peel extract (w/v) could inhibit the growth of *Staphylococcus aureus* ATCC 6538, *Listeria monocytogenes* ATCC 7644 and *Salmonella* Typhi ATCC 14028. However, 4-16% red melinjo peel extract could not inhibit the growth of *Candida albicans* ATCC 10231. In stability test, the selected extract had a stable inhibition at pH 4-7, heat treatment 65 - 95 °C for 30 minutes, salt 1%-5%, and sugar 10%-50%. The selected extract produced the biggest inhibition diameter at low pH (pH 4) and produced the smallest inhibition diameter at neutral pH (pH 7). Heat treatment 65 °C for 30 minutes produced the biggest inhibition diameter among tested bacteria and decreased with increasing heating temperature. Addition of 1-5% NaCl and 10-50% sucrose worked synergistically with the selected extract in inhibit the growth of the tested bacteria.Abstract in English.

Keywords: *Melinjo, extraction, antibacterial, stabilization, cell damage.*

ABSTRAK

Melinjo (*Gnetum gnemon* L.) merupakan tanaman khas Indonesia yang mempunyai banyak manfaat, salah satunya adalah sebagai senyawa antimikroba. Tujuan penelitian ini adalah mengetahui aktivitas antimikroba dari ekstrak kulit melinjo merah. Ekstraksi dilakukan dengan metode maserasi menggunakan pelarut etil asetat yang berlangsung selama 24 jam pada suhu ruang. Ekstrak kulit melinjo merah konsentrasi 4% (w/v) hingga 16% (w/v) mampu menghambat pertumbuhan *Staphylococcus aureus* ATCC 6538, *Listeria monocytogenes* ATCC 7644, dan *Salmonella* Typhi ATCC 14028. Ekstrak kulit melinjo merah 4-16% (w/v) tidak mampu menghambat pertumbuhan *Candida albicans* ATCC 10231. Ekstrak terpilih memiliki kemampuan penghambatan yang stabil pada pH 4-7, suhu 65 - 95 °C 30 menit, konsentrasi garam 1%-5%, dan konsentrasi gula 10%-50%. Ekstrak terpilih menghasilkan diameter penghambatan terbesar pada pH rendah (pH 4), sedangkan pH netral (pH 7) menghasilkan diameter penghambatan terkecil. Pemanasan pada suhu 65 °C selama 30 menit menghasilkan diameter penghambatan terbesar pada bakteri uji dan diameter penghambatan semakin menurun seiring dengan meningkatnya suhu pemanasan. Penambahan NaCl 1-5% dan sukrosa 10-50% pada ekstrak bekerja sinergis dengan kemampuan ekstrak dalam menghambat pertumbuhan bakteri uji.

Kata kunci: *Melinjo, ekstraksi, antibakteri, stabilitas, kerusakan sel.*

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Corresponding author:
Johan Honga
Tangerang, Indonesia, 15811
Email: johanhonga@gmail.com

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INTRODUCTION

Melinjo (*Gnetum gnemon* L.) is a typical Indonesian plant which is rich in flora diversity. Melinjo belongs to *Gnetaceae* family that is originated from Indo-Malaya and Melanesia and widely cultivated from Southeast Asia to Fiji (Manner and Elevitch, 2006). In Indonesia, melinjo is commonly processed into *emping* (chips from melinjo seeds) and vegetables in various soup (Kato *et al*., 2009).

According to BPS or Indonesian Central Bureau of Statistics (2016), melinjo production increased about 7.78% from 197,648 tons in 2014 becoming 213,025 tons in 2015. This increasing of melinjo production consequently will increase the melinjo peel waste as well. Unfortunately, the utilization is still limited. In fact, melinjo peel has many benefits, one of them is as an antimicrobial agent. Parhusip and Sitanggang (2011) explains that melinjo peel had antibacterial activity against *Bacillus cereus*, *Staphylococcus aureus*, *Enterobacter aerogenes* and *Pseudomonas aeruginosa*.

The use of red melinjo peel in this study was based on the study conducted by Cornelia *et al*. (2009) that it had the highest phenolic compounds (0.386 mg GAE/g samples) compared to yellow and green melinjo peels. The phenolic compounds can inhibit the growth of Gram positive bacteria (Septiadi *et al*., 2013). In this study, the melinjo peel extract will be used to test its antimicrobial activity toward *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella* Typhi, and *Candida albicans* which represented Gram positive bacteria, Gram negative bacteria, and yeast. The four types of microbes are pathogenic microbes that often contaminate food products and cause disease if consumed (Parhusip and Sitanggang, 2011)

The melinjo peel extract was obtained through extraction and maceration using ethyl acetate as a solvent. Parhusip and Sitanggang (2011) reported that ethyl acetate extract of red melinjo peel produced a better inhibition diameter compared to ethanol extract. In addition, ethyl acetate has low toxicity, and is able to attract polar and nonpolar compounds, easily evaporated, inexpensive, and easily obtained (Putri, 2013).

In this study, ethyl acetate extract of red melinjo peel will be tested to inhibit *S. aureus*, *Listeria monocytogenes*, *Salmonella* Typhi, and *Candida albicans*, then the optimum concentration of ethyl acetate will be determined to inhibit the growth of the tested micorbes. Stability test will be tested using the selected extract which is carried out at pH of 4-5, temperature of 65-95 °C for 30 min, and addition of 1-5% NaCl solution, and 10-50% sucrose. Stability test is required to see its inhibition in certain pH, heat temperature, salt, and sugar concentration as an initial step to be applied on food products. Based on the previous study, the 1-5% salt concentration was chosen in this study because in general the addition of 1-2% salt gives a salty taste in food products. Salt with a concentration of 5-15% used to preserve food or food products (Yusmita, 2018). Meanwhile, the sugar concentration was chosen from 10% to 50%, because in general the addition of sugar concentration up to 40% gives a sweet taste to food products. If the concentration is more than 40%, the sugar will act as a preservative in food products (Utomo *et al*., 2015).

METHODOLOGY

Materials and Tools

The materials used in this study were red melinjo peel (*Gnetum gnemon* L.) that was obtained from Bogor-West Java (evenly perfect red/merah sempurna dan merata), ethyl acetate, distilled water, Gram positive bacteria cultures (*S. aureus* ATCC 6538 and *L. monocytogenes* ATCC 7644), Gram negative bacteria culture (*S.* Typhi ATCC 14028), and yeast culture (*C. albicans* ATCC 10231), Nutrient Agar (NA), Nutrient Broth (NB), Potato Dextrose Agar (PDA), Potato Dextrose Broth (PDB), tartaric acid solution, K2PO4, HCl, NaOH, NaCl, Tween 80, sucrose, and aluminum foil.

Material Preparation and Red Melinjo Peel Extraction (Parhusip and Sitanggang, 2011)

Melinjo seed was sorted, washed, and drained, then, the peel was separated from its seed. Melinjo peel was dried in a cabinet dryer until its moisture content was around 10%. The dried peel was grinded using a blender and sifted to obtain red melinjo peel powder.

The dry powder of melinjo peel was mixed with ethyl acetate as an extraction solvent with a ratio of (1:4 w/v). The mixture was stirred using a shaker at 150 rpm for 24 h at room temperature, then filtered using a Whatman filter paper No. 1 and vacuum pump. The filtrate was concentrated using a rotary evaporator at temperature of 55 °C and blown with Nitrogen to obtain a crude extract. The crude extract was diluted into 4-16% concentration by weighting 0.4, 0.8, 1.2, and 1.6 mg in 10 mL ethyl acetate.

Antimicrobial Activity Test of Melinjo Extract with Well Diffusion (Parhusip and Sitanggang, 2011)

A 40 µL culture of each test microbe was distributed into the media on a petri dish and contacted with the extract, so the concentration of each test microbe is about 10^4-10^5CFU/40 Ml. Let the media solidify for 30 minutes and make 5 holes with 6 mm diameter each. A 0.5 mL of each concentration of red melinjo peel extract was aseptically inserted into wells 1-4 (4%, 8%, 12%, and 16% (w/v) respectively). Well 5 was a control which ethyl acetate solution was used as a control. Then, the dish was incubated for 24 h at 37 °C and observed their inhibition diameter on the next day by measured to the nearest mm. The most effectiveness extract concentration obtained in this test will be further used for stability test, it is done by statistical analysis.

Stability Test of the Selected Extract at pH, Temperature, Salt and Sugar Concentration (Romson *et al.*, 2011, Winarti *et al.*, 2008, and Adriansyah *et al.*, 2003)

The selected extract was put into a test tube containing a different pH solution on each tube. The four pH levels were pH 4, 5, 6 and 7. The temperatures for stability were 65, 75, 85, and 95 °C for 30 minutes.

The sugar stability test was done by inserting the selected extract into a tube containing 10%, 20%, 30%, 40% and 50% sucrose, respectively. The stability test for salt was done by inserting the selected extract into 6 tubes containing NaCl solution with different concentrations (1%, 2%,

3%, 4% and 5%). The inhibition diameter was observed using well diffusion method with the selected extract (no sucrose or NaCl added) as a control.

RESULTS AND DISCUSSION

Antimicrobial Activity of Red Melinjo Peel

The inhibition diameter observed on the NA media indicated the antimicrobe activity of the melinjo peel extract. Ethyl acetate extract of red melinjo peel showed the ability to inhibit the growth of test bacteria from concentrations of 4% to 16%. However, the extract was not able to inhibit the growth of *C. albican*s at such Concentrations (Table 1 and Fig. 1).

The inability melinjo peel extract to inhibit *C. albicans* caused by the presence of chlamydospore in yeast, an asexual spore at the end of hyphae which forms a thick wall thus cannot penetrate by the antimicrobial compounds (Jawetz *et al.*, 2005). Besides, yeast is a eukaryotic organism which has more stable membrane than prokaryotic organisms due to sterol component in its cytoplasm. This caused the red melinjo extract hardly interfere the yeast cell permeability (Madigan et al., 2009). The 16% extract concentration showed the largest diameter inhibition compared to other concentrations seen in three microbes

Table 1. Results of various concentration level of red melinjo extract affected the inhibition diameter produced on the test microbes

Microbes	Extract concentration (%)	Inhibition diameter (mm)
S. aureus	0 (Control)	0 ± 0
	4	10.08 ± 0.60^{cd}
	8	11.59 ± 0.47
	12	14.23 ± 0.93^{fg}
	16	16.65 ± 0.31
L. monocytogenes	0 (Control)	0 ± 0
	4	9.25 ± 0.82
	8	10.54 ± 0.60
	12	13.94 ± 0.90^{fg}
	16	14.88 ± 1.07
S. Typhi	0 (Control)	0 ± 0
	4	7.74 ± 0.93
	8	9.74 ± 0.89
	12	11.06 ± 0.98^{de}
	16	13.45 ± 1.18
C. albicans	0 (Control)	0 ± 0
	4	0 ± 0
	8	0 ± 0
	12	0 ± 0
	16	0 ± 0

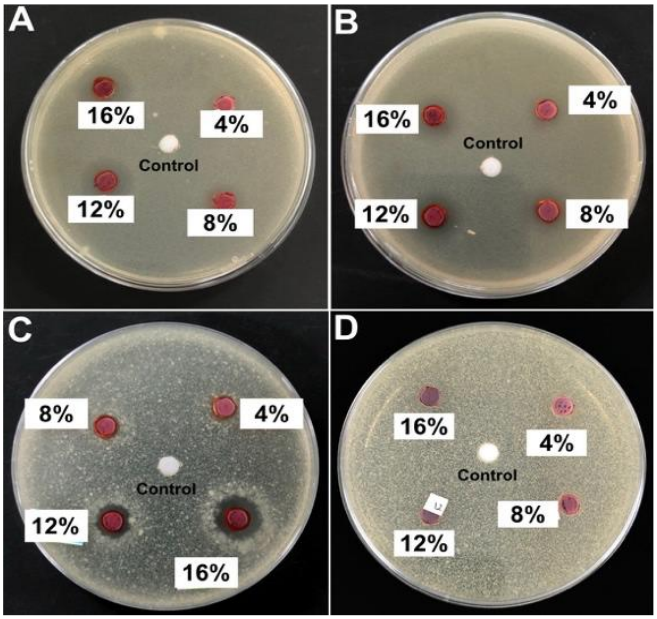

Figure 1. Results of inhibition diameter produced on *S. aureus* (A), *S.* Typhi (B), *L. monocytogenes* (D), and *C. albicans* (D) at 4-16% red melinjo peel extract

The inability melinjo peel extract to inhibit *C. albicans* caused by the presence of chlamydospore in yeast which is difficult to penetrate with antimicrobial compounds. Chlamydospore is an asexual spore at the end of hyphae which forms a thick wall so that it is difficult to penetrate antimicrobial compounds (Jawetz *et al*., 2005). Besides that, yeast is eukaryotic organism which has more stable membrane than prokaryotic organisms due it has a sterol component in its cytoplasm. This caused the red melinjo ethyl acetate extract at concentrations of 4%, 8%, 12%, and 16% couldn't interfere yeast cell permeability (Madigan et al., 2009).

The 16% extract produced the largest diameter inhibition compared to other concentrations. *S. aureus* and *L. monocytogenes* had a larger inhibitory diameter than *S.* Typhi. This result was match with theory that Gram negative bacteria have more complex cell walls composed of lipopolysaccharide, proteins and have peptidoglycan (Madigan *et al*., 2009). From the results of visual observations and statistical analysis showed that each concentration could be used as the chosen concentration. The selection of selected extract will be based on extract efficiency and suitability with the increase in inhibition diameter produced. The selection of extracts concentrations referred to Widyasanti *et al*. (2016) theory, that the inhibition diameter of 20 mm or more classified as very strong, the inhibition diameter is 10-20 mm classified as strong category, the inhibition diameter is 5-10 mm classified as medium category, and the inhibition diameter is 5 mm or less classified as weak category. Based on the theory, red melinjo peel ethyl acetate extract was classified as strong category. Table 1 showed that at 12% concentration, all test bacteria produced inhibition diameters greater than 10 mm. Therefore, the extract selected in this study was 12% concentration extract.

Extract Stability Test on pH

Testing the extract at the pH aims to see the stability of the selected extract in various acidic to neutral conditions (pH 4-7). The selected extract itself has a pH of 4.93 before it was tested into various pH solutions. Most foods are produced at that pH range, thus was chosen in this study (FDA, 2008).

Fig. 2 shows that the average inhibition diameter for each pH level was greater than 10 mm, thus can be classified as a strong inhibitory category (Widyasanti *et al*., 2016). This means that red melinjo ethyl acetate extract had a good stability and strong inhibition capacity, even though the pH varied from 4 (acid) to 7 (neutral). pH 4 gave the highest value, while other pH levels were not significant. According to Silhavy *et al*. (2010), bacteria would be more difficult to grow at acidic condition. This stability test reflects that red melinjo extract is potential to be applied for food products that have pH ranging from 4 to 7, such as meat, fish, milk, corn, spinach, asparagus, beets, and yellow walnut (FDA, 2008).

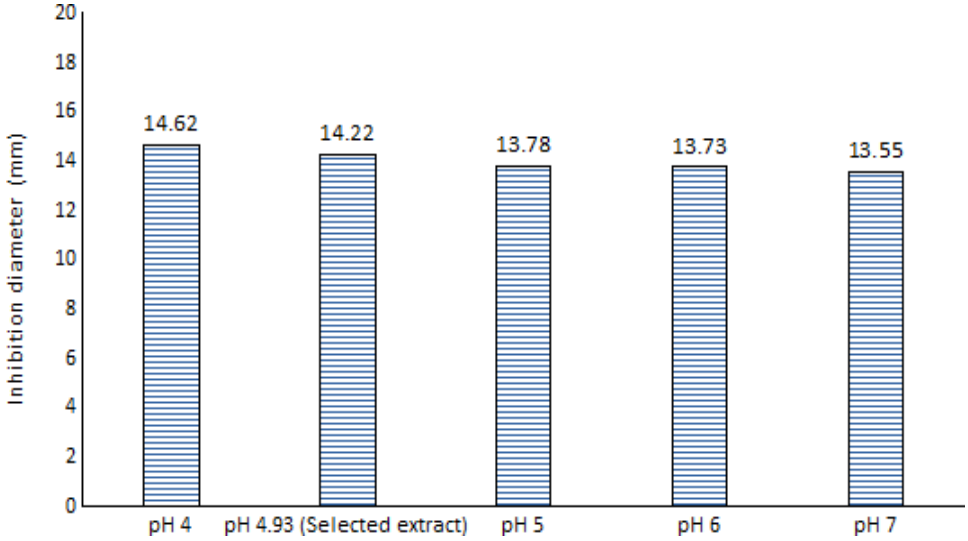

Figure 2. Test results of pH level affect the average of inhibition diameter on pH stability test

There was no interaction between the type of test bacteria and the pH level on the inhibitory diameter., However, significant effect was seen on the inhibitory response of the test bacteria (Fig. 3). *S. aureus* had the highest inhibition diameter (14.81 mm), followed by *L. monocytogenes* and S. Typhi (both were not significant) with inhibition diameter of 13.57 mm (Fig. 4). These results are in accordance with the study of of Silhavy *et al*. (2010) that the growth of Gram-positive bacteria was easier to be inhibited than Gram negative due to differences in the composition of membrane cell.

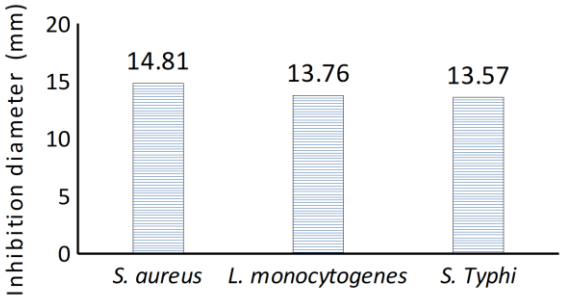

Figure 3. Test result of bacteria type affect the average of inhibition diameter on pH stability test

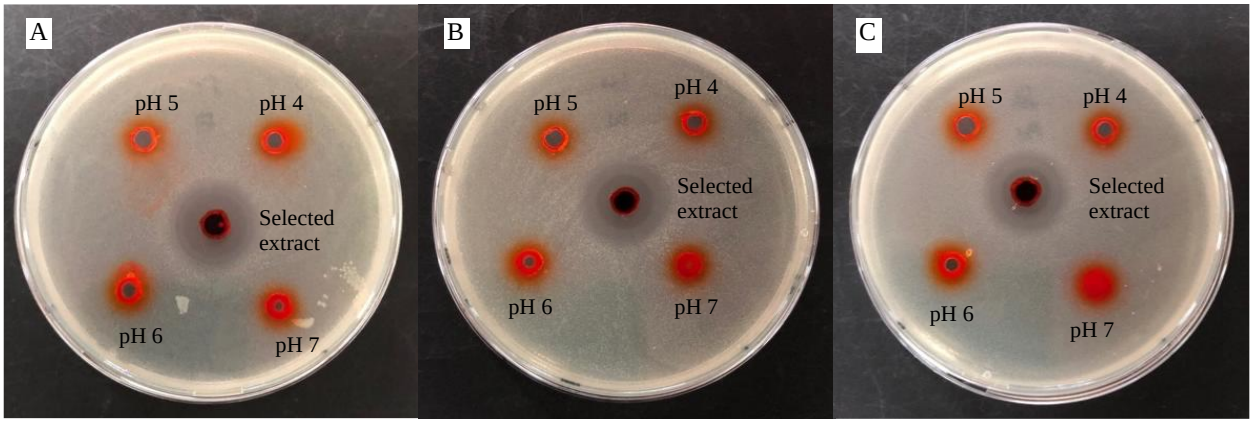

Figure 4. Inhibition diameter of *S*. Typhi (A), *L. monocytogenes* (B), and *S. aureus* (C) at 4-7 pH

Extract Stability Test on Temperature

Heating temperature of the selected extract was chosen between 65 and 95 °C to see extract ability for heat treatment, especially for pasteurization and blanching. Table 2 and Fig. 5 showed that heating temperature at 65 °C for 30 minutes produced the largest inhibition diameter compared to other temperature.

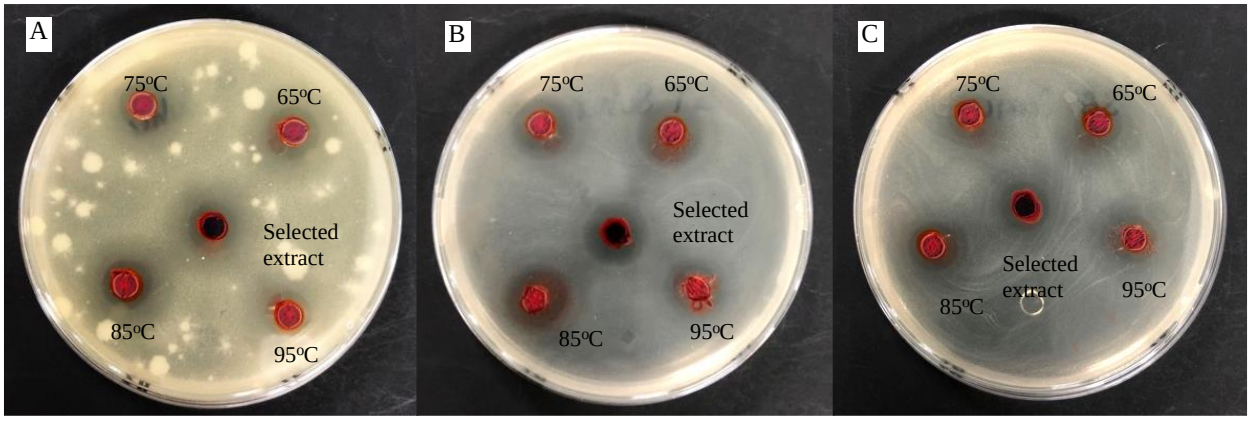

Figure 5. Inhibition diameter of *L. monocytogenes* (A), *S.* Typhi (B), and *S. aureus* (C) at 65-95ºC heating temperature

Table 2 Impact of heating temperature to the average of inhibition diameter on heating temperature stability test

Bacteria	Heating temperature (°C)	Diameter inhibition (mm)
S. aureus	Selected extract (without heating)	14.23 ± 0.93^{efg}
	65	15.60 ± 1.01^{g}
	75	14.34 ± 1.05^{efg}
	85	13.89 ± 0.93^{def}
	95	13.51 ± 0.60^{de}
L. monocytogenes	Selected extract (without heating)	13.94 ± 0.90^{ef}
	65	14.99 ± 0.58^{fg}
	75	13.20 ± 1.0^{de}
	85	12.53 ± 1.07^{cd}
	95	11.75 ± 0.46^{bc}
S. Typhi	Selected extract (without heating)	11.06 ± 0.98^{b}
	65	15.10 ± 0.59^{fg}
	75	13.31 ± 0.98^{de}
	85	10.44 ± 1.01^{ab}
	95	9.69 ± 0.61^{a}

Increasing the diameter caused by the breakdown of bioactive components to be antibacterial agent during heating. The antibacterial activity from heated bioactive component is usually higher than the initial bioactive component in inhibiting bacteria (Kyung *et al*., 1997). From the statistical

analysis showed that there is an interaction between the type of bacteria and the diameter of inhibition produced. *S.* Typhi produced the smallest diameter average (resistance) to the heating temperature and *S. aureus* produced the largest (vulnerable) average diameter of the heating temperature. This results are in accordance with the study of Silhavy *et al*. (2010) that Gram positive bacteria are easier to inhibit growth than Gram negative

Elevated heating above 65°C showed a decrease in the inhibition diameter of each test bacteria. The heating treatment at 95 °C produced the smallest inhibition diameter compared to the control or other heating temperatures. This was caused by bioactive component damage when exposed to high temperatures (Ewald *et al.*, 1999). From the stability test of extracts to heating temperature, red melinjo ethyl acetate extract had to be applied for pasteurized food products, such as milk and fruit juice drinks, as well as leaf products, such as fruit and vegetables on blanching process (Singh and Lovedeep, 2009).

Extract Stability Test on Salt

The addition of 1-5% salt concentration was chosen in this study because in general the use of 1-2% salt gives an acceptable salty taste in food products. Meanwhile, salt with a concentration of 5-15% is used to preserve food or food products (Yusmita, 2018). Table 3 and Fig. 6 showed the greater salt concentration added to the selected extract, the greater inhibition diameter produced in each test bacteria. There is an interaction between salt concentration and the response of each bacteria. Statistical analysis showed that the inhibition diameters produced by the control differed significantly from the inhibition diameter at 1-3% salt concentration. Meanwhile there was no significant difference in inhibition diameter produced at concentrations of 1-3%, but differed significantly from the concentration of 4%. The diameter of inhibition produced at a concentration of 4% was significantly different from the concentration of 5% ($p \leq 0.05$). These results reaffirm that the ethyl acetate extract of red melinjo peel had a good stability and works synergistically with salt concentration.

Table 3. Test results of salt concentration affect the average of inhibition diameter on salt stability test

Bacteria	Salt concentration (%)	Inhibition diameter (mm)
S. aureus	Selected extract	12.93 ± 0.66^{c}
	1	15.89 ± 0.19^{e}
	2	16.19 ± 0.23^{e}
	3	16.19 ± 0.21^{e}
	4	18.25 ± 0.53^{fg}
	5	18.78 ± 0.26^{gh}
L. monocytogenes	Selected extract	10.93 ± 1.21^{b}
	1	16.16 ± 0.75^{e}
	2	16.49 ± 0.19^{e}
	3	16.64 ± 0.79^{e}
	4	18.14 ± 0.87^{fg}
	5	19.44 ± 0.53^{gh}
S. Typhi	Selected extract	9.73 ± 0.44^{a}
	1	14.41 ± 0.65^{d}
	2	14.53 ± 0.61^{d}
	3	14.58 ± 0.29^{d}
	4	17.66 ± 0.29^{f}
	5	17.96 ± 0.53^{fg}

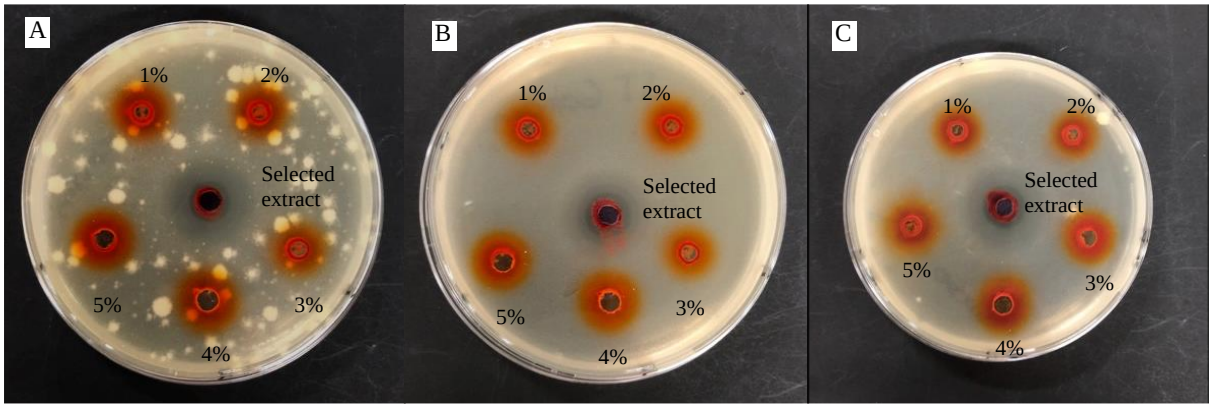

Figure 6. Inhibition diameter of *L. monocytogenes* (A), *S.* Typhi (B), and *S. aureus* (C) at 1-5% NaCl solution

Salt works synergistically with the extract in inhibiting the growth of test bacteria as salt has a high osmotic pressure that causes plasmolysis in bacterial cells (Ahillah *et al*., 2017). In addition, NaCl is hydroscopic thus it can bind water molecules, resulting in lower Aw (Madigan *et al*., 2009).

Based on this stability test, red melinjo extract had a potential to be applied for food products that contain 1-5% salt, such as biscuits, extruded foods, cakes, noodles, porridge, chocolate drinks, canned tuna, and selected seafoods (NHRI, 2018).

Extract Stability Test on Sugar Addition

The sugar concentration was chosen from 10% to 50% in this study, because the addition of sugar concentration up to 40% to food products generally gives an acceptable sweet taste. If the concentration is more than 40%, the sugar will act as a preservative in food products (Utomo et al., 2015).

Based on the stability test (Table 4 and Fig. 7), the greater the concentration of sugar added to the extract, the greater diameter of inhibition produced in each test bacteria. The presence of sugar works synergistically with the extract in inhibiting the growth of test bacteria as it can reduce the water content of bacterial cells, thus limits the microbial living activities. This result in disruption of cell metabolism which leads to cell death. When sugar with high concentrations added to food, it can block the microbial growth and decrease the water activity (a_w) (Buckle et al., 2009).

There is an interaction between the sucrose concentration and inhibition diameter of test bacteria (Fig. 10). *S. aureus* produced the largest (sensitive) average inhibition diameter when added with sugar concentration, while *S.* Typhi produced the smallest inhibition diameter (resistant). Statistical analysis showed that both controls and 10-50% concentrations did not differ significantly.

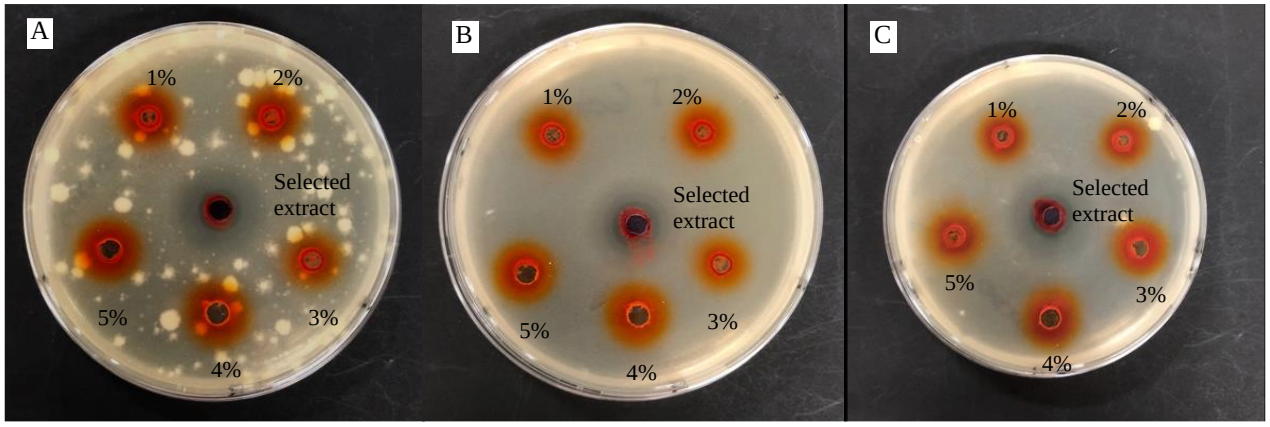

Figure 7. Inhibition diameter of *S.* Typhi (A), *L. monocytogenes* (B), and *S. aureus* (C) at 1-5% sucrose solution

Table 4. Test results of sugar concentration affect the average of inhibition diameter on sugar stability test

Bacteria	Sugar concentration (%)	Inhibition diameter (mm)
S. aureus	Selected extract	13.64 ± 0.38^{abcd}
	10	13.29 ± 0.88^{abc}
	20	13.79 ± 0.40^{bcde}
	30	15.09 ± 0.96^{ghi}
	40	15.33 ± 0.61^{hi}
	50	15.91 ± 0.63^{i}
L. monocytogenes	Selected extract	13.84 ± 0.98^{bcde}
	10	14.57 ± 0.32^{defgh}
	20	14.61 ± 0.30^{defgh}
	30	14.20 ± 0.53^{cdefg}
	40	14.74 ± 0.60^{efgh}
	50	14.93 ± 0.24^{fgh}
S. Typhi	Selected extract	12.76 ± 0.71^{a}
	10	13.06 ± 0.65^{ab}
	20	13.11 ± 0.29^{ab}
	30	13.99 ± 0.97^{bcdef}
	40	14.59 ± 0.42^{defgh}
	50	15.33 ± 0.38^{hi}

This confirmed that the red melinjo peel ethyl acetate extract had a good stable at 10-50% sugar concentration and synergistic with extract. Silhavy *et al*. (2010) revealed that Gram positive bacteria are more sensitive to the antimicrobial agent than Gram negative because the differences in their cell membrane structures. From this stability test, red melinjo ethyl acetate extract had a potential to be applied as an antimicrobial agent to food products that have 10- 50% sugar content, such as carbonated drinks, fruit juices (Walker, 2014), formula milk, yogurt, wafers, brownies, crackers, donuts, and some baby foods (Walker and Goran, 2015).

CONCLUSION

A 12% red melinjo peel ethyl acetate extract was able to inhibit the growth of *S. aureus*, *L. monocytogenes*, and *S.* Typhi with categorized strong inhibitory strength (average inhibition diameter was 13.08 mm). The selected extract had a good stability at 4-7 pH, 65-95 ^{o}C for 30 minutes heating temperature, 1-5% NaCl concentration, and 10-50% sucrose concentration. So, red melinjo peel ethyl acetate extract had a potential to be applied to food products widely. Further research is needed regarding quantitative phytochemical testing to see the percentage of bioactive components contained in the extracts. In addition, an *in vivo* toxicity test is needed to be carried out as an initial step for the extract application as an antibacterial agent in food products.

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STUDY ON FUNCTIONAL INGREDIENTS AND CLAIMS OF READY TO DRINK (RTD) FRUIT JUICE IN MODERN RETAIL

Hendry Noer Fadlillah [1,2]
Tina Nurkhoeriyati[1]
Lira Felanesa [2]
Andika Wahyu Utomo[3]

[1]Department of Food Technology, Faculty of Life Sciences, International University Liaison Indonesia
[2]PT Industry Navigator Indonesia (INAVIGA Indonesia)
[3]Departemen Ilmu dan Teknologi Pangan, Fakultas Teknologi Pertanian, Institut Pertanian Bogor

ABSTRACT

Fruit juice is well known as a healthy food. The purpose of fruit juices consumption is not only for refreshment, but also for gaining health benefits. The aim of this research is to identify health-related claim in juice and the functional ingredient used to meet the regulation on claim requirement. The research is conducted by collecting RTD (ready to drink) juice in modern retail. All the information on the label is recorded, identified, and classified based on regulation document, then processed and analyzed statistically. The result shows that 70% of product samples provide claim on the label. Claim on vitamin C is the most used claim for RTD fruit juice. As many as 52% of products with claim, has vitamin C – related claim. After vitamin C, the next popular claims are claims regarding dietary fiber (11%) and vitamin A (10%). Among all the products with claims, 63% of them provide more than one nutrient claims. They combine two or more nutrients content as the claim (e.g. vitamin C and vitamin A, fiber and vitamin C, etc.). There were 20% of RTD fruit juice enriched or fortified by functional ingredients, mostly in vitamin premix. The juice industry can still provide claims, without fortification or enrichment, if they are able to maintain their nutritional content to meet regulatory requirements regarding claims.

Keywords: *Claim; nutrient; regulation; RTD juice; vitamin.*

ABSTRAK

Sari buah (jus) merupakan produk yang dikenal menyehatkan. Konsumen mengonsumsi sari buah tidak hanya untuk mendapatkan kesegaran, tetapi juga untuk memperoleh manfaat kesehatan. Riset ini bertujuan untuk mengidentifikasi jenis klaim terkait kesehatan pada produk sari buah dan ingridien fungsional yang digunakan untuk memenuhi persyaratan regulasi dalam pemberian klaim. Penelitian dilakukan dengan mengumpulkan sampel produk sari buah siap minum di ritel modern. Semua informasi dalam label dicatat, diidentifikasi, dan diklasifikasi berdasarkan regulasi. Kemudian diolah dan dianalisis secara statistik. Hasilnya menunjukkan 70% sampel memberikan klaim dalam label kemasannya. Klaim terkait vitamin C adalah yang paling sering ditemukan. Sebanyak 52% dari produk berklaim mengandung klaim terkait vitamin C. Setelah vitamin C, klaim lain yang populer adalah terkait serat pangan (11%) dan vitamin A (10%). Menariknya, 63% produk berklaim memberikan klaim terkait lebih dari satu jenis zat gizi. Produk tersebut mengombinasikan dua atau lebih kandungan gizi sebagai klaim, seperti vitamin C dan vitamin A, serat pangan dan vitamin C, serta lainnya. Terdapat 20% produk sari buah siap minum yang difortifikasi atau diperkaya oleh ingridien fungsional dari luar. Industri sari buah tetap dapat memberikan klaim, tanpa fortifikasi atau pengayaan, jika mereka mampu menjaga kandungan gizinya untuk memenuhi persyaratan regulasi tentang klaim.

Kata kunci: *Klaim, regulasi; sari buah siap minum; vitamin; zat gizi..*

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Corresponding author:
Hendry Noer Fadlillah
Tangerang, Indonesian, 15345
Email: hendry_nf@yahoo.com

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INTRODUCTION

Indonesia as a tropical country, has a bright prospect for fruit industries. Many variants of fruit are produced and potentially to be developed to the many kinds of high economic value products, including juice beverages (Kemenperin, 2009). Fruits are good sources for several active components and phenolic compounds to support health and reduce the risk of chronic diseases. Fruit juice consumption is associated with several health benefits, including to lower cardiovascular disease risk and obesity (Clemens *et al*., 2015) and to improve intestinal microbiota (Henning *et al.*, 2017).

Juice market is growing very well in the global market. Juice consumption keeps growing, with the main market in China, France, Germany, the United Kingdom, and the United States. Other countries, including Indonesia, are also expected to have a large annual growth in juice market in the future (Anonym, 2019). Anonym (2018) stated that fruit juice is one of the best segments in beverage industry. This product is consumed by all of consumer levels of any background, education, and job (Rahmawati, 2013). In Indonesia, juice beverage can be easily found. The products can be obtained at both traditional and modern market. Market of fruit juice in Indonesia has an enormous potential. It will develop continuously since the increasing of health awareness. Ready to drink (RTD) juice becomes more popular, because of their convenience. The increasing of human activity is the main factor of its popularity. Currently, RTD juice product is served in many types of packaging and variant (Rahmawati, 2013). Fadlillah *et al*. (2019) reported that in market, fruit juice products can be found in some types of packaging such as PET (polyethylene terephthalate), carton, and PP (polypropylene). Not only in packaging type, RTD juice products are available with many flavors. Regarding flavor, orange juice is the one which dominates the market, followed by guava, mango, and other fruits.

There are two major ingredients in juice formulation. Firstly, are fruit and water, and secondly is sweetener. In its development, the fruit juice ingredients become more varied for some reasons, include the big interest from the consumers for health promoting juice (Buech, J., 2018) and the interest of the producers to enhance the shelf life of the products. Moreover, the consumers request for the better taste and healthier products also contribute to the development of the fruit juice products. Those factors encourage more variants of ingredients to be used, such as preservative, flavoring, vitamin, mineral, fiber, etc. (Taylor, 2016). A good understanding of the juice product in the market is very useful for industries, consumers, researchers, and regulation institution. Thus, this research is aimed to provide better knowledge regarding the fruit juice products. The research is conducted to analyze the type of RTD fruit juice in the retail modern; identify the claim of the products; analyze the strategy of the fruit juice industries to meet the claim requirement; and identify the addition of functional ingredient in the formulation.

MATERIALS AND METHOD

Materials for this research are ready to drink (RTD) fruit juices that are collected from modern retail in Bogor. Then, all the RTD fruit juices found in supermarket and minimarket are bought to be analyzed. Regulation documents such as Regulation of the Minister of health for food additive and some technical regulation of Indonesia National Agency of Drug and Food Control/ *Badan Pengawas Obat dan Makanan* (NADFC/ BPOM) for food category, claim, food additive, and others were also used to identify label information.

All the information on the label of products were recorded, identified, classified, and categorized based on the regulation from Minister of Health and NADFC. Samples were collected based on juice definition on food category (BPOM, 2016a). Claim information was classified based on regulation of NADFC; Food additive was identified by using regulation of Minister of health for food additive. Then, all the information analyzed statistically by using Microsoft Excel to understand the correlation of each other.

RESULTS AND DISCUSSION

Fruit juice is defined as a liquid processed from the edible part of fruit that is washed, crushed, purified (if needed), with or without pasteurization and packaged to be consumed directly. The basic characteristics of fruit juice are the ethanol level no more than 0,5%, except for certain fruits; it is allowed to be added by juice concentrate from the same fruit; and is also allowed to be added by sugar, to a maximum concentration of 50 g/kg (BPOM, 2016a). Fruit juice category could be identified from the label. BPOM (2018) requires food industries to provide the name of the product on the label, together with the net weight, name and address of the producer or importer, date and production code, and also the expiration date. The total collected sample from modern retail in Bogor is 82 products. Based on database on NADFC website, there are many of registered fruit juice products, both domestic and import products (BPOM, 2019), but not all the products are available in the market. Some of them are no more exist. Amongst all samples, 70% of fruit juice products provide nutrition or health claim on the label (Figure 1).

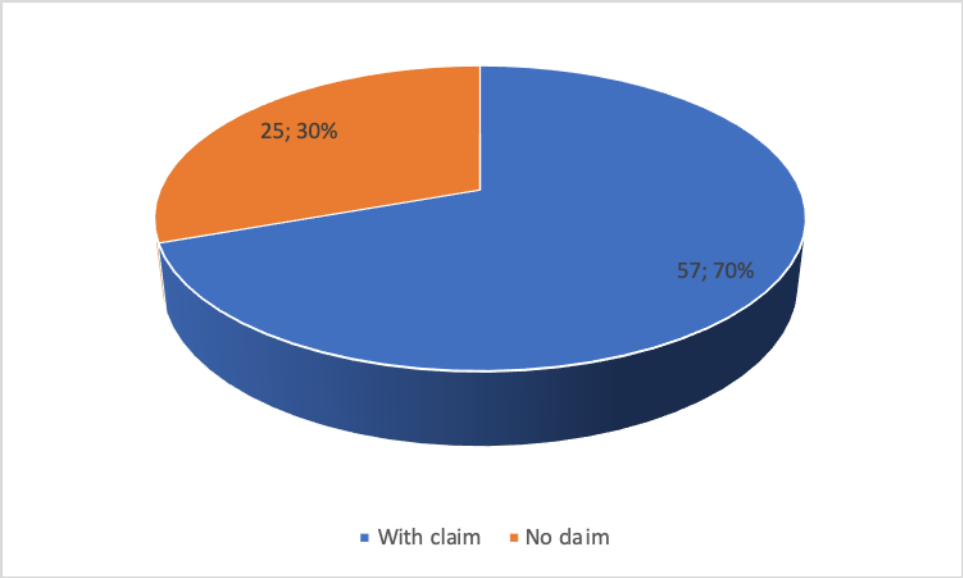

Figure 1. Percentage of fruit juice products with or without the claim

Claim is defined as any description that state and suggest both directly or indirectly, regarding certain characteristics of a food about the origin, nutrient content, nature, production, processing, composition or others quality factors (BPOM, 2016b). Nutrition and health claims on food are useful for consumers to select products according to their needs. Fadlillah *et al.* (2015) reported that claim information is the most considered to be in the label by consumers with age of 15 – 24 years old. Health claims may also contribute to the improvement of the industrial competitiveness. The type of claim significantly influences on the product credibility and purchasing intention (Hoefkens and Verbeke, 2012). Moreover, healthy choice label increases positive impact to the desire of buying (Yang, 2014).

Most of the fruit juice products in this study claim a single nutrition or health function. However, there are some of claimed products provide more than one of nutrient content. Around one third of claimed products offer two or more excellences in nutrition (Figure 2). This phenomenon can be understood, because fruit as main juice raw material is rich in various vitamins, minerals, and fiber. Fruits contain energy and nutrients in a great number (Slavin and Lloyd, 2012). Nevertheless, during processing, the nutrient could be reduced or lost, so that not all of the nutrients can be claimed at the end products.

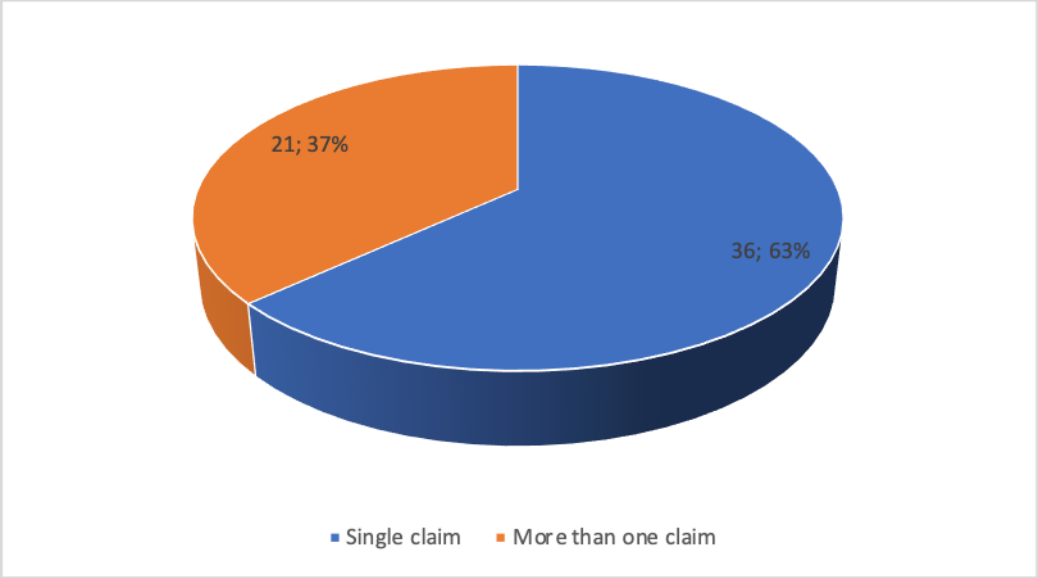

Figure 2. Number of claims in fruit juice products

Vitamin-related content is the most used as claims. There are 43% of claimed products regarding vitamin C-related, followed by fiber-related content (11%), vitamin A (10%), and others (completely, see in Figure 3). Fruit juice contributes to the nutrient intake (Bellisle *et al*., 2018). The nutrient content of fruit juice varies. It depends on the raw material used. Fadlillah *et al*. (2019) stated that the most used raw materials for fruit juice products in Indonesia are orange and guava. Orange juice is also the most consumed fruit juice around the world (Chanson-Rolle *et al*., 2016).

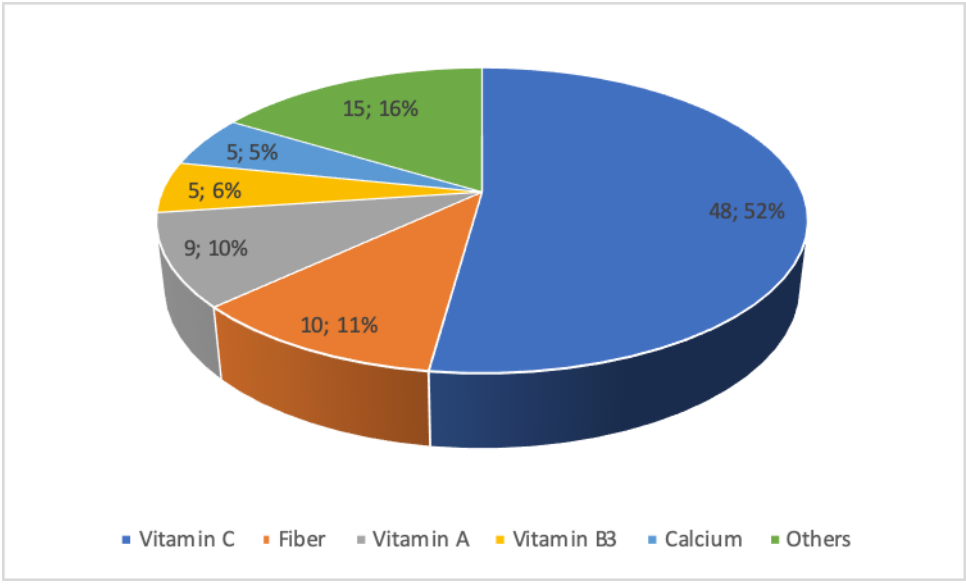

Figure 3. Nutrition-related claims in fruit juice products

Naturally, vitamin C or ascorbic acid is found in fruits. Orange or citrus is rich in vitamin C (Slavin and Lloyd, 2012). Chanson-Rolle *et al* (2016) also stated that, orange juice consumption will contribute to several micronutrients' intakes, including vitamin C. Guava is also high in vitamin C (Ali *et al*., 2014). Guava is an affordable source of vitamin C for society (Sinha, 2014). Based on the fact, orange and guava become the most used raw material in fruit juice, so it is logical, if vitamin C is the micronutrient that is the most often found as claim in fruit juices. Commercial fruit juices are

often enriched with vitamin. The enrichment is conducted, because of the benefit popularity of this nutrition (Baba et al., 2016). Rodriguez-Bernaldo *et al*. (2009) reported that fruit juice is a good vehicle for vitamin C. It is relative stable during storage.

Fiber-related claim is also popular in fruit juice, after vitamin C. Naturally, fruit contribute to the fiber intake, and it is very useful to support health including to lower cardiovascular diseases risk and obesity (Slain and Lloyd, 2012). Food industry have to maintain the fiber content of the fruit juice during the processing. Clemens *et al.* (2015) stated that fiber level in juice product decreased. Some of the industries modify their process to minimize the loss of fiber during juice processing. The other method is by enriching the juice with the additional fiber (Thongsombat *et al*., 2007).

Claim statement must comply with the regulation requirement. Figure 4. shows that 80% of the products are made without the addition of functional ingredients from outside. It means that producers maintain nutrient content in the raw material to meet the regulation requirement. Nutrient degradation is affected by condition of processing, storage and cooking. It is highly variable depend on the commodity (Rickman *et al*., 2007). Barrett and Lloyd (2011) stated that nowadays, there are some advanced technologies that provide opportunity for juice producers to retain the nutrient optimally. Those advanced processing includes high-pressure processing and some electric method, such as microwave, pulsed electric fields, ohmic processing, and others. For example, to retain vitamin A and vitamin C in the fruit juices, without causing any risk in food safety, producers can use high pressure processing (HPP). Vitamin A and C relatively are unaffected by HPP.

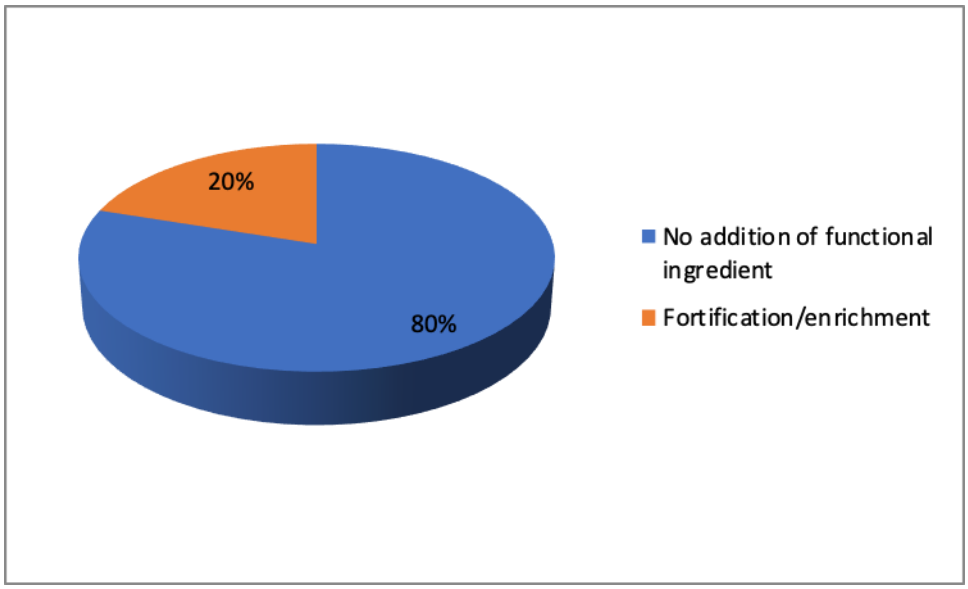

Figure 4. Percentage of fruit juice products with fortification or enrichment

The other method to improve the nutritional quality is by conducting fortification or enrichment. There are 20% of products in this study that are fortified or enriched. Fortification is defined as the practice to improve or increase the content of micronutrient, such as vitamin and mineral (including trace elements) in food. The main purpose of fortification is to improve the nutritional quality status of society (WHO, 2018). Enrichment is similar with fortification, but this term is more to improve the nutrient content of food that is lost during processing (PP, 2004). Juice also can be used as vehicle to deliver a certain nutrient. There are 45% fortified juices in the United States. They added in the nutrient into the products that include calcium, vitamin A, D, E, and others (Hyde *et al*., 2012).

Figure 5 shows the comparison between fortified/enriched products and unfortified/enriched

products. The comparison states that not all fortified products provide claim. Vice versa, to provide claim, producer does not always have to fortify or enrich their products.

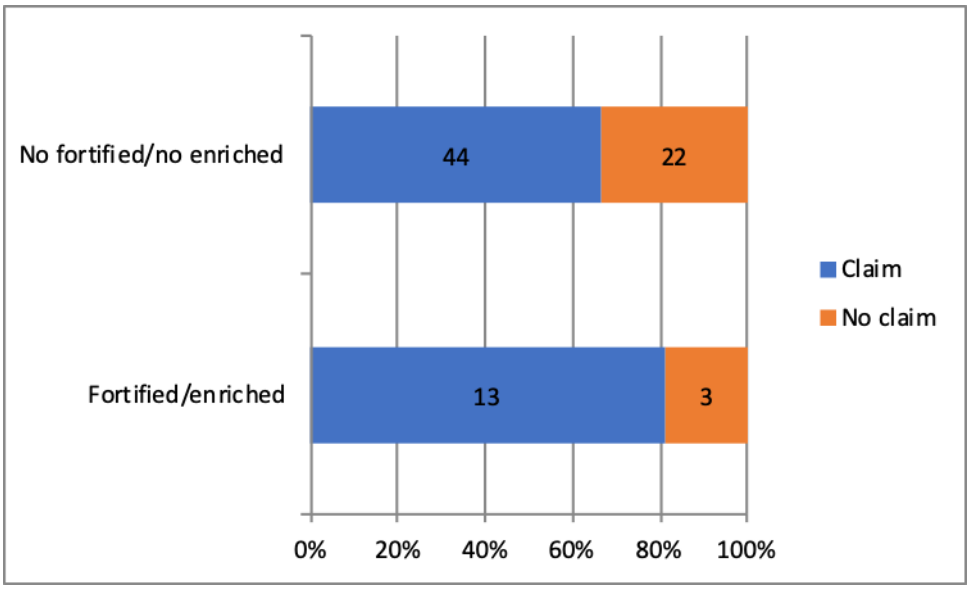

Figure 5. Comparison product with or without claim between fortified/enriched and no fortified/no enriched

From the fortified/enriched fruit juice, the vitamin content is mostly in the form of vitamin premix (35% of the fortified/enriched fruit juice) and mineral (23%). Guinot *et al* (2012) stated that vitamin and mineral premix is the most significant solution to reduce the cost for large-scale food fortification programs. Addition of micronutrient in premixes will be more convenient, accurate and economical than trying to add the individual nutrients separately. Besides premix, there are also single addition of vitamin B3 and vitamin B12 in samples (Figure 6).

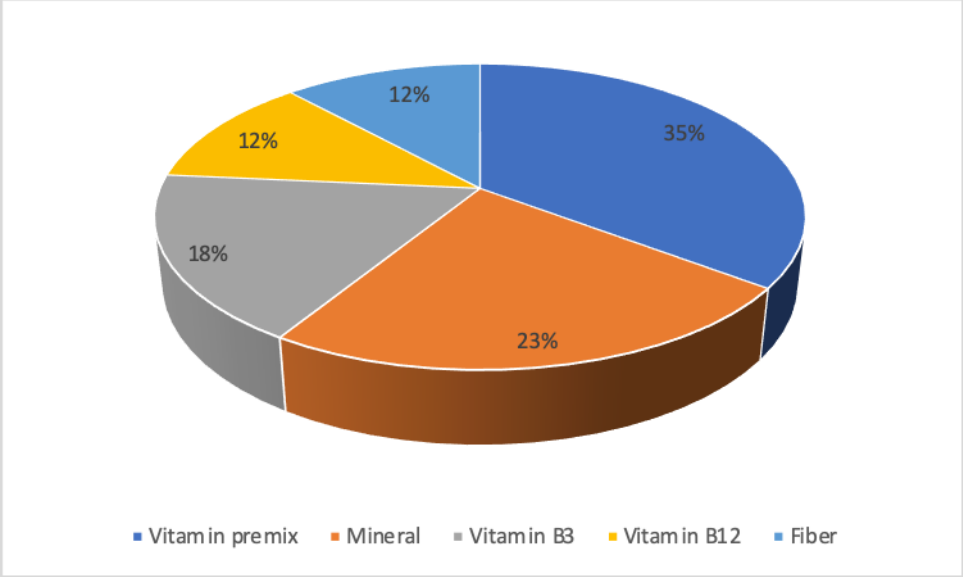

Figure 6. Type of functional ingredients added to the fruit juice products

BPOM (2016b) divided claim into some categories, they are nutrition claim, healthy claim, and other claims. Definition of nutrition claim is that all the description that state, show, or imply that food has a certain nutrition character, including content of energy, protein, fat, carbohydrate, vitamin, and mineral. There are several types of nutrition claim, include claim of nutrient content, and claim of nutrient comparison.

Healthy claim is that all the description that state, recommend, or imply that there is correlation between the food or its ingredient and the health. There are three types of health claim, they are claim of nutrition function, claim of other function, and claim of lowering risk of a disease (BPOM, 2016b).

Among the samples with claims which are studied, the most claims provided is nutrition claim. There are 68% of claimed products have claims of "source of" or "contain" certain nutrient and 29% of claimed products have claims of "high" or "rich" in certain nutrient claim. There is also health claim for the nutrient function (see Figure 7). To be clearer, Table 1 shows the claim statement on the label. Some of the products provide more than one claim. For example, there are products that claims are not only high in vitamin C content, but also high in fiber content. Other products claim to contain other nutrients, such as vitamin B3, vitamin B6, vitamin E, mineral Ca, and mineral Zn.

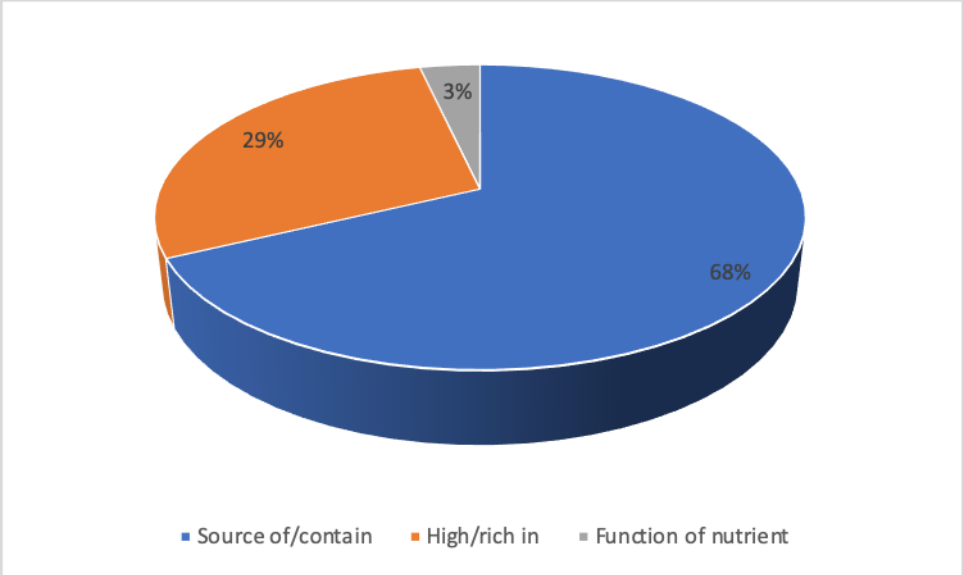

Figure 7. Type of claims in fruit juice products

Not all products can provide claim. There is regulation to be fulfilled before the claim is approved. Nutrition claim with the statement of "source of" contained micronutrient, the product must comply to 15% of ALG (*Acuan Label Gizi* or nutritional label reference) for the respective micronutrient content per 100 g for solid product, or 7,5% of ALG per 100 ml for liquid product. To the fruit juice products to claim "high" or "rich", the product must contain twice as many "source of" the respective micronutrient (BPOM, 2016b).

For dietary fiber, producers can claim their products as "source of" dietary fiber, if they can proof that the content of the fiber in the product is no less than 3 g per 100 g (in solid product) or 1,5 g per 100 kkal (in liquid product). The value increases if they claim as "high" fiber or "rich" in fiber. For this claim, the content of fiber must be no less than 6 g per 100 g (in solid product) or 3 g per 100 kkal (in liquid product) (BPOM, 2016b).

Health claim that provide function of nutrient on the label is approved if the content of the nutrient meets the minimal requirement of "source of"

claim. There are two types of nutrient function claims in fruit juices studied. Firstly, is about the role of calcium in bone formation and teeth maintenance. Secondly, is the claim about function of dietary fiber to maintain digestion health

Table 1. Some of claim statements on the label

Type of claims	Category of claim	Claim on the label
Source of nutrient	Nutrition claim	*Sumber vitamin C*, source of vitamin C
		Mengandung vitamin C, contain vitamin C
		Sumber nutrien (B3, B6, E, Ca, Zn), Source of nutrient (B3, B6, E, Ca, Zn)
		Mengandung vitamin A, vitamin B12, vitamin C; Contain vitamin A, vitamin B12, vitamin C
		Mengandung vitamin C, B3, dan B6; Contain vitamin C, B3, and B6
High/rich in nutrient	Nutrition claim	*Tinggi vitamin C*, High vitamin C
		Tinggi serat, High fiber
		High calcium, high vitamin C, high vitamin D; *Tinggi kalsium, tinggi vitamin C, dan tinggi vitamin D*
		Tinggi serat, dan tinggi vitamin C; High fiber and high vitamin C
		Memenuhi 100% AKG vitamin C; Meet 100% of RDA
		Kaya vitamin A dan C, tinggi serat: Rich in vitamin A and C, high fiber
Function of nutrient	Healthy claim	*Berisi kalsium yang telah berperan dalam pembentukan dan pemeliharaan tulang dan kepadatan gigi*; Contain calcium with role for bone formation and teeth maintenance
		Dengan serat inulin yang membantu menjaga fungsi pencernaan; With inulin fiber which maintain the function of digestion

CONCLUSION

Most of the RTD fruit juices in the modern retail provide claim on the label. Nutrition claim is the most often found, including "source" or "contain" certain nutrient claim and "high" or "rich" claims. The others are health claims, that describe the function of certain nutrient. Most of claimed fruit juices do not add in any functional ingredients from the outside, and only 20% of claimed products are fortified.

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ANTI-INFLAMMATORY ACTIVITY OF PECTIC ENZYME-TREATED PECTIN ON LIPOPOLYSACCHARIDE-INDUCED RAW 264.7 CELLS

Cian-Song Huang[1]
Qiao-Lin Li[1]
Diana Lo[2]
Mei-Li Wu[1]
Yuh-Tai Wang[3]
Ming-Chang Wu[1]

[1]Department of Food Science, National Pingtung University of Science and Technology, No. 1, Hsueh Fu Road, Nei-Pu Hsiang, 91201 Pingtung, Taiwan
[2]Food Technology Department, Faculty of Engineering, Bina Nusantara University, Jakarta, Indonesia, 11480
[3]Life Science Center, Hsing Wu University, No. 101, Sec.1, Fen-Liao Road, Lin Kou, Taipei 244, Taiwan

ABSTRACT

Even inflammation is a body defense response, but excessive inflammation causes chronic inflammatory conditions. The purpose of this study was to investigate the ability and pathway of the pectic enzyme-treated (PET) pectin to inhibit the inflammation of macrophage RAW 264.7 induced by lipopolysaccharide. The PET-pectin produced by commercial pectinase enzyme hydrolysis for 24, 48 and 72 h. Results showed that PET-pectin produced from 48 h reaction time had the highest antioxidative activity, thus these parameters were used to produce PET-pectin used in this study. PET-pectin showed no cell cytotoxicity to normal macrophage RAW 264.7 and reduce the nitrite secretion from LPS-induced RAW 264.7 by 20%. Finally, the expression of cytokines, including NO synthase (iNOS), nitric oxide (NO), cyclooxygenase-2 (COX-2), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and tumor necrosis factor (TNF-α) were analyzed by western blot. In the western blot method, it was found that iNOS, COX-2, NF-κB, TNF-α and other proteins that activated NO production had a downtrend. It was found that PET-pectin possess promising activity to mitigate the inflammatory response. Further study on experimental animals is needed to conclude its activity against inflammation.

Keywords: *Pectic enzyme-treated pectin; inflammation; lipopolysaccharide; RAW 264.7.*

ABSTRAK

Meski inflamasi merupakan respon pertahanan tubuh, inflamasi yang berlebihan dapat menyebabkan kondisi inflamasi kronis. Tujuan dari penelitian ini adalah mengamati kemampuan dan mekanisme dari pektin yang telah direaksikan dengan enzim pektinase dalam mencegah inflamasi yang disebabkan oleh lipopolisakarida pada RAW 264.7. Sebelum digunakan, pektin direaksikan dengan enzim pektinase komersial selama 24, 48, dan 72 jam untuk menghasilkan *PET-pectin*. Hasil menunjukkan bahwa pektin yang direaksikan selama 48 jam menghasilkan aktivitas antioksidan tertinggi, sehingga pektin ini yang digunakan dalam penelitian selanjutnya. *PET-pectin* tidak menyebabkan sitotoksisitas pada sel RAW 264.7 dan dapat mereduksi sekresi nitrit sebesar 20% pada RAW 264.7 yang telah diinduksi lipopolisakarida. Ekspresi dari sitokin, termasuk iNOS, NO, COX-2, NF-κB, TNF-α, dianalisis menggunakan *western blot*. Hasil *western blot* menunjukkan bahwa ekspresi iNOS, COX-2, NF-κB, dan TNF-α menurun dengan adanya *PET-pectin*. Berdasarkan penelitian ini, *PET-pectin*mempunyai kemampuan yang menjanjikan untuk mencegah respon inflamasi. Penelitian menggunakan hewan dibutuhkan untuk menyimpulkan aktivitas *PET-pectin* dalam mencegah inflamasi.

Kata kunci: *Pektin yang direaksikan dengan enzim pectinase; inflamasi; lipopolisakarida; RAW 264.7.*

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Corresponding author:
Ming-Chang Wu
Pingtung, Taiwan, 91201
Email: mcwu@mail.npust.edu

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INTRODUCTION

Inflammation is a body defense response to destructive stimulus by initiating healing process regulated by immune system; however, excessive inflammation causes chronic inflammatory conditions including arthritis, asthma, multiple sclerosis and atherosclerosis (Wang *et al*., 2016). Lipopolysaccharide (LPS) is commonly used as compound to induce inflammation in RAW 264.7 mouse macrophages cell due to its ability to trigger the secretion of pro-inflammatory cytokines (Agarwal *et al*., 1995).

Pectin is a common gelling agent used for jam and jelly making extracted from citrus peel or apple pomace. Pectin is composed of hetero-polysaccharides rich in galacturonic acid primarily found in the cell walls of terrestrial plants. Beside galacturonic acid, pectin also consists of rhamnose, arabinose, galactose and other 13 different monosaccharides (Naqash *et al*., 2017). Pectin which act as dietary fiber also has been reported to possess anti-diabetic activity (Liu *et al*., 2016).

Several methods used to hydrolyze pectin into pectic-oligosacharides which has lower molecular weight are acid, enzyme and hydrothermal hydrolyses. Pectin can be hydrolyzed by pectic enzymes into pectic enzyme-treated (PET) pectin without high temperature and extreme pH. Basically, pectic enzyme consists of three enzymes, which is deesterifying enzymes (pectinesterases), depolymerizing enzymes (hydrolases and lyases) and protopectinases (Alkorta *et al*., 1997). Previous researches have shown a promising result of PET-pectin as antioxidant and emulsifying agent (Huang *et al.*, 2011), anti-cancer (Huang *et al*., 2012; Huang *et al.*, 2018), anti-bacterial (Wu *et al*., 2014) and prebiotics (Ho *et al*., 2017). Huang *et al*. (2011) reported that hydrolyzed pectin showed higher radical scavenging activity and reducing power than untreated pectin. However, research of PET-pectin on inflammation has not been studied. Therefore, the aims of this study were to investigate the ability and study the pathway of the PET-pectin in inhibiting the inflammation of macrophage RAW 264.7 induced by LPS.

MATERIALS AND METHOD

Materials

Citrus pectin with 99% purity and 60% DE (degree of esterification) was purchased from Nacalai Tesque (Kyoto, Japan). Commercial pectic enzyme, Peclyve CP produced from *Aspergillus niger* was purchase from Lallemand, Australia. RAW 264.7 murine macrophage cell (BCRC 60001) was purchased from Bioresource Collection and Research Center (Hsinchu, Taiwan). Other materials used in this research is analytical grade.

Enzyme-Hydrolyzed Pectin Preparation

Citrus pectin was firstly added into water to reach 1% (w/v) concentration of pectin. Afterwards, 5% (v/v) pectic enzyme was added and reacted at 45°C for three different durations, which were 24, 48, and 72 h. Resulted products were freeze dried and analyzed for ABTS radical scavenging activity at different concentrations based on method described by Re et al. (1999). Chosen parameter to produce PET-pectin was used for further analysis.

RAW 264.7 Cell Culture and Viability Determination

RAW 264.7 murine macrophage cell was cultured in DMEM (Dulbecco's Modified Eagle Medium) containing 10% heat-inactivated fetal bovine serum and 1% penicillin-streptomycin, and then incubated at 37°C in a 5% CO_2 incubator (NU-4500, NuAire, MN, USA). The cells were grown as monolayer and subcultures were performed with 1.5 ml of 1× trypsin in phosphate buffered saline (PBS). The morphology of RAW 264.7 cells was observed using an inverted microscope (CK30-F100, Olympus, Tokyo, Japan). Firstly, RAW 264.7 cells were seeded in a 96 well-plate at concentration of 1×10^4 cells/well and incubated at 37°C overnight to let the cell adhere to the well-plate. Afterwards, cells were treated with various concentrations of PET-pectin (0, 400 and 800 µg/ml) and lipopolysaccharide or LPS (1 µg/ml) for 24 h at 37°C. MTT (3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay was used to assess the cell viability. Cells were added with 10 µL of MTT solution (5 mg/ml) and 100 µL

medium. The reaction between cell and MTT was conducted at 37°C for 4 h. The crystal formed by viable cell was then dissolved in 100 µL of DMSO for 10 min (Wang et al., 2016). The absorbance were measured at 570 nm by microplate spectrophotometer (Bio-Tek, VT, USA).

Nitrite Determination

Nitrite released was determined by Griess reagent as described by Gunawardena et al. (2013). Firstly, cells were cultured in a 96 well plate with $1x10^4$ cells/wall. After letting it to adhere overnight, LPS (1 µg/ml) together with different concentration (0, 400, and 800 µg/ml) of PET-pectin were added into cells and incubated for 24 h. Supernatant after incubation (80 µl) was taken to new 96 well plate and added with 80 µl of Griess reagent (1% sulfonamide and 0.1% naphthylethylenediamine in 5% HCl) for 10 min. The absorbance determined at 540 nm using microplate spectrophotometer (Bio-Tek, VT, USA).

Western Blot Analysis

Western blot analysis were used to determine cytokines production on LPS induced RAW 264.7 cell according to the method carried out by Kim et al. (2016). Cytokine observed were STAT-3, NF-κB, COX-2, iNOS, and TNF-α, inflammatory related cytokines. After treated with PET-pectin at the concentration (0, 400 and 800 µg/ml) on LPS induced RAW 264.7 cell, control (untreated cells) and different PET-pectin treatment on LPS induced cell were collected and washed with phosphate-buffered saline (PBS). The proteins were then extracted using Halt^{TM} protease inhibitor cocktail (100x): 0.5M EDTA solution (100x): RIPA (radioimmunoprecipitation) lysis and extraction buffer with ratio 1:1:100 at 4°C for 10 min. Equivalent amounts of proteins were separated by 10% SDS-PAGE, and then electro-transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Schwalbach, Germany). After blocking with 5% skim milk, the membranes were incubated with primary antibodies (β-actin and detected cytokines) at 4 °C overnight, followed by incubation with horseradish peroxidase (HRP)-conjugated secondary antibodies for an hour. Finally, signals were detected by an enhanced chemiluminescence system (G:Box, Syngene, MD, USA).

RESULTS AND DISCUSSION

The antioxidant activity observed as ABTS radical scavenging activity of produced pectic-enzyme treated pectin (PET-pectin) was observed in different pectin concentration and reaction time in 1% pectinase enzyme solution. The results of the antioxidant activity were shown in Table 1.

Table 1. Radical scavenging activity of pectic-enzyme treated pectin (PET-pectin) produced from different treatment time and concentrations

Reaction time (hour)	PET-pectin concentration in reaction (mg/ml)		
	25	50	100
24	36.92±0.19^{g}	52.50±0.50^{e}	76.69±0.34^{b}
48	37.25±0.91^{g}	59.30±0.48^{c}	82.89±0.33^{a}
72	38.62±1.08^{f}	58.09±0.25^{d}	77.13±0.33^{b}

$^{a-g}$Data expressed as mean ± standard deviation that do not share a letter are significantly different observed by one-way analysis of variance with Duncan Multiple Range Test post-hoc.

Results showed that there was a higher increase in antioxidant activity found in the PET-pectin made with higher pectin concentration, while lower changes found in PET-pectin made from different reaction time. From the findings, 100 mg/ml pectin concentration and 48-hours-reaction time was chosen as the optimum condition to produce PET-pectin as it showed significantly higher antioxidant activity.

The higher the concentration of pectin represented higher substrate provided in the reaction to be degraded by pectinase enzyme. Pectin is a complex polysaccharide consisting galacturonic acid (sugar

acid) as main component. Degrading pectin into shorter component may increase reducing sugar end, which in turn increased antioxidant activity. The reducing end of sugar derivative can reduce other components by oxidizing its carbonyl end into carboxyl group (Wade, 2013). This finding is supported by the research done by Alrahmany and Tsopmo (2012) showing that carbohydrases (viscozyme, celluclast, and amyloglucocidase) treatment on oat bran increased its radical scavenging activity.

The anti-inflammatory activity of PET-pectin was observed on RAW 264.7 macrophage cells. Figure 1a showed the cell viability of RAW 264.7 cells treated with different concentration of enzyme-hydrolyzed pectin. It showed that enzyme-hydrolyzed pectin caused no inhibition on the RAW 264.7 cell growth, which also refers that there is no toxicity caused by enzyme-hydrolyzed pectin on RAW 264.7 cells. Even at the PET-pectin concentration of 200 µg/ml, there was significant increase of cell viability, which showed that at low concentration, PET-pectin can be used as growth substrate of RAW 264.7 cells.

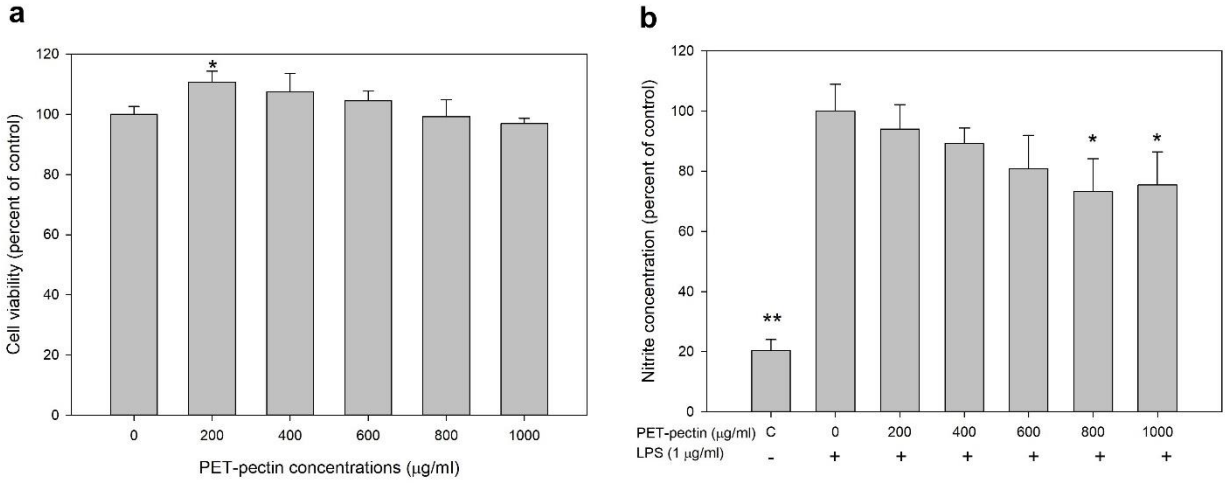

Figure 1. The cell viability (a) and nitric oxide production of lipopolysaccharide (LPS) induced injury (b) on RAW 264.7 cell after treated with different concentrations of pectic-enzyme treated pectin (PET-pectin). *Significantly different at 95% confidence and **significantly different at 99% confidence to 0 µg/ml PET-pectin determined by independent t-test.

Nitrite oxide (NO), a short-lived free radical, is cytokines activated molecule produced by macrophage cells as inflammatory response whose production is associated with iNOS via activated NF-κB signaling pathways (Tripathi et al., 2007). Figure 1b showed the nitric oxide production of lipopolysaccharide (LPS) injured RAW 264.7 cells. Result showed that LPS caused an excessive increase in nitric oxide produced by RAW 264.7 cells. However, PET-pectin added into LPS injured RAW 264.7 cells decreased of the produced nitric oxide.

The morphology of the RAW 264.7 can be seen in Figure 2. It showed the condition of RAW 264.7 before (Figure 2a) and after (Figure 2b) stimulated with LPS without PET-pectin. After stimulated with LPS, the morphology of RAW 264.7 cells changed from round form into irregular form with pseudopodia formation. This irregular form and pseudopodia formation were found lesser on the cell co-treated with PET-pectin (Figure 2c and d), indicating that PET-pectin can prevent the damage cause by LPS on RAW 264.7 cells as observed from the morphology of the cells.

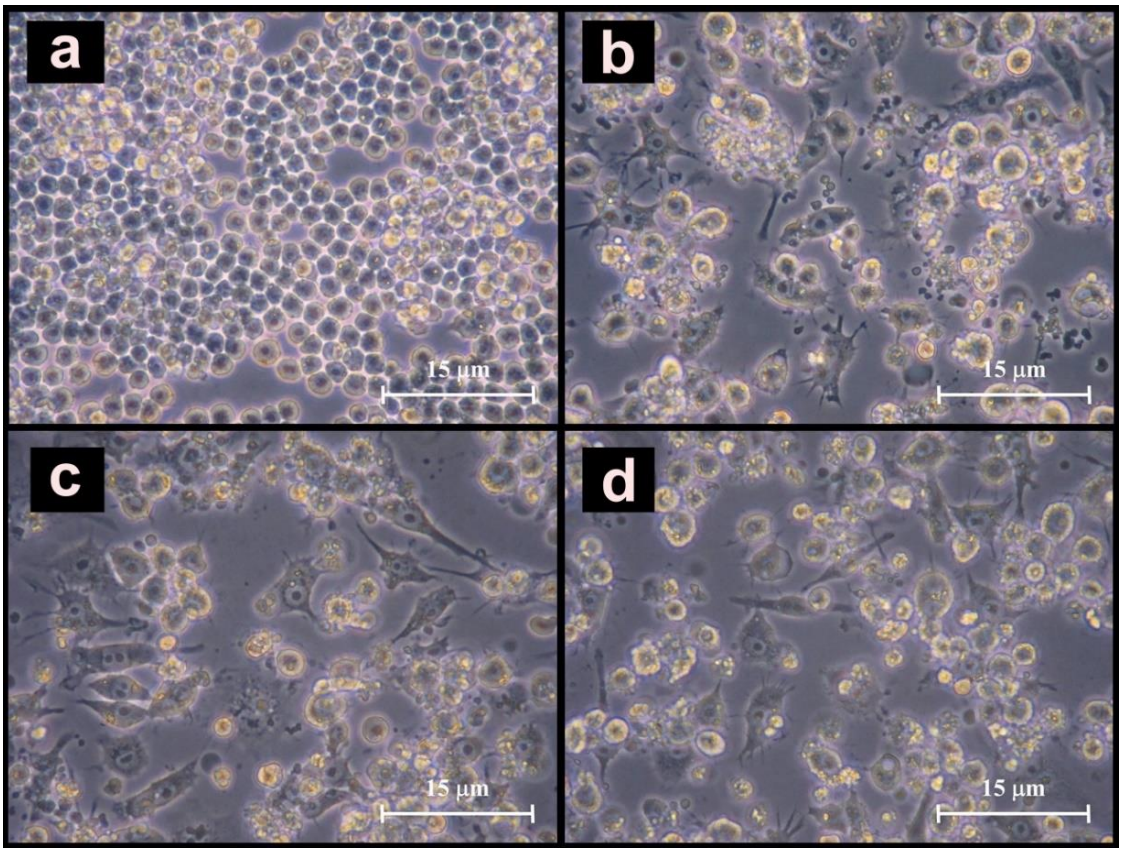

Figure 2. RAW 264.7 Cell morphology at different PET-pectin concentrations. (a) Control (b) 1 µg/ml lipopolysaccharide (LPS) without PET-pectin (c) 1 µg/ml LPS with 400 µg/ml PET-pectin (d) 1 µg/ml LPS with 800 µg/ml PET-pectin.

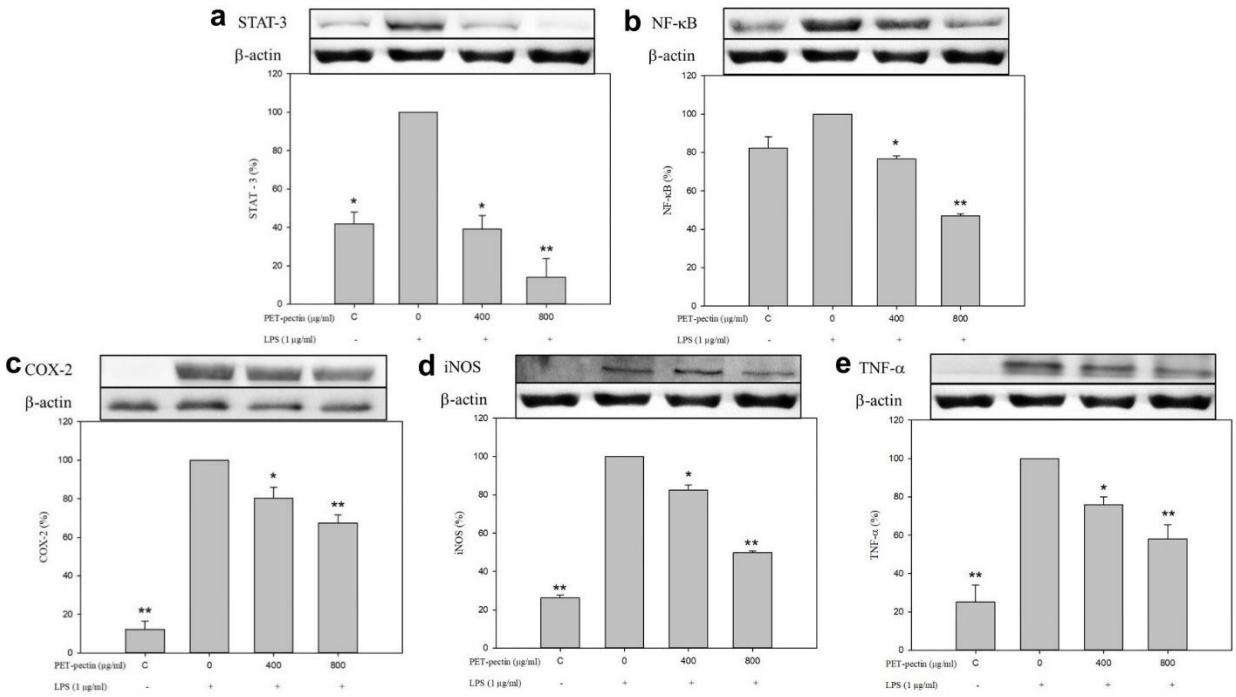

Figure 3. Protein expression of inflammation related response (a) STAT-3, (b) NF-κB, (c) COX-2, (d) iNOS, and (e) TNF-α observed by western blot methods. *Significantly different at 95% confidence and **significantly different at 99% confidence to 1 µg/ml lipopolysaccharide (LPS) stimulated without PET-pectin determined by independent t-test.

To observe the mechanism of PET-pectin on anti-inflammatory activity, western blot was conducted to observe different protein expression involved in inflammation reaction. The protein observed were STAT-3, Nf-κB, COX-2, iNOS and TNF-α as shown in Figure 3a-e.

All the protein expression increased after stimulated with 1 µg/ml LPS. For the cell co-treated with LPS and PET-pectin, the protein expression was significantly lower compared to the cell without PET-pectin treatment in a dose-dependent manner.

The mechanism of action observed in this study was shown in Figure 4. LPS acted as stimuli to trigger inflammation caused the increase in STAT-3 and NF-κB protein expression. STAT-3 and NF-κB, two key proinflammatory pathways control essential tumor-promoting functions in various malignancies, including cell survival, cell proliferation, and suppression of an immune response (Bollrath and Greten, 2009). Afterwards, the expression TNF-α, COX-2, and iNOS increased. Inducible isoform of NOS or iNOS then caused an increase in NO expression. PET-pectin can cause reduction in inflammation as shown in the reduction of STAT-3, NF-κB, iNOS, COX-2, and TNF-α expressions.

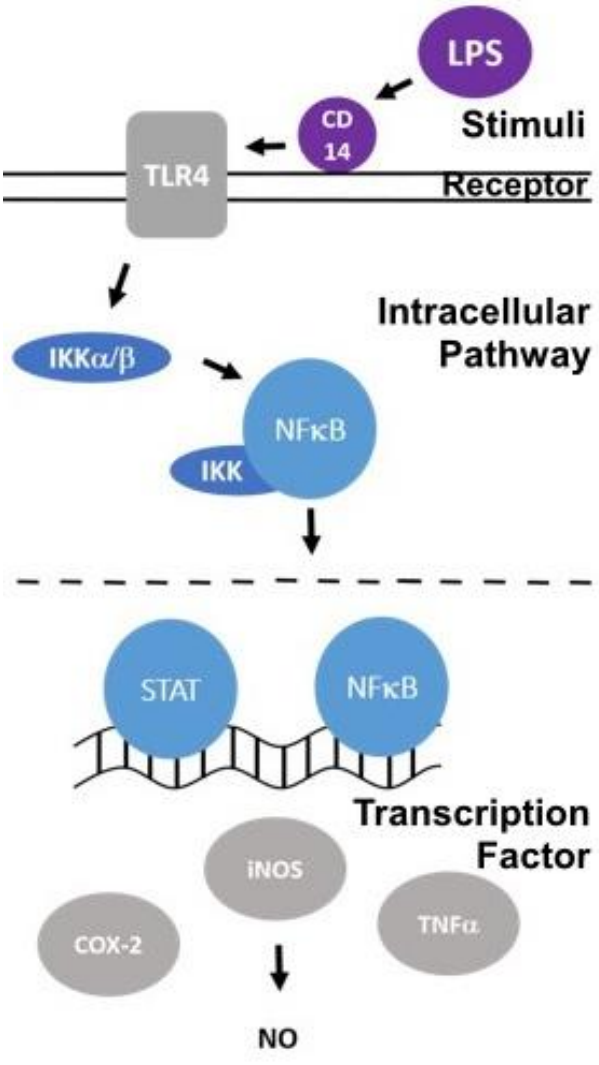

Figure 4. The pathway of mechanism of action

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PERCEPTION OF THE MILLENIAL GENERATION TOWARD FUNCTIONAL FOOD IN INDONESIA

Isma Amaliah[1]
Wahyudi David[1]
Ardiansyah[1]

[1]Department of Food Science and Technology, Universitas Bakrie

ABSTRACT

Currently, the functional food trend is growing and developing in Indonesia. Consumers' perceptions are one important factor to picture consumer knowledge and attitude in the future. The aim of this study is to determine the perception of the millennial generation toward functional food in Indonesia. An online cross-sectional survey was carried out among 1982 respondents (aged between 18–38 years) and distributed through the social media platforms during two weeks in April 2018. The questionnaire measured demographic characteristics, awareness, knowledge, the priority to purchase, and future buying motivation for functional food. The result showed that 55% of the respondents claimed that they were aware of functional food. However, the knowledge of respondents regarding health component was still insufficient. The most important reasons for purchasing functional food were health benefits, availability, affordability, tasty, easy to consume, and clear label information. Most of the millennial generation was interested in purchasing functional food in the future. In conclusion, this study provided information regarding the millennial generation's perception toward functional food in Indonesia and how it might contribute to increase the development of functional food in Indonesia.

Keywords: *Cross-sectional surve; functional food; millennial generation's; perception.*

ABSTRAK

Saat ini, tren makanan fungsional tumbuh dan berkembang di Indonesia. Persepsi konsumen adalah salah satu faktor penting untuk menggambarkan pengetahuan dan sikap konsumen di masa depan Tujuan dari penelitian ini adalah untuk mengetahui persepsi generasi milenial terhadap pangan fungsional di Indonesia. Survei *cross sectional* online dilakukan dengan jumlah 1982 orang responden (usia 18-38 tahun) dan didistribusikan melalui media sosial selama dua minggu pada bulan April 2018. Pada kuesioner ini mengukur karakteristik demografi, kesadaran dan pengetahuan, prioritas untuk membeli, dan motivasi membeli pangan fungsional di masa yang akan datang. Sebanyak 55% responden menyatakan bahwa mereka telah mengetahui pangan fungsional (memiliki kesadaran terhadap pangan fungsional). Pengetahuan responden tentang komponen kesehatan pada pangan fungsional masih kurang. Alasan paling penting untuk membeli pangan fungsional menurut generasi milenial adalah manfaat kesehatan, ketersediaan, harga yang terjangkau, enak, mudah dikonsumsi, dan informasi pada label. Sebagian besar generasi milenial tertarik untuk membeli pangan fungsional di masa yang akan datang. Studi ini memberikan informasi tentang bagaimana persepsi generasi milenial terhadap pangan fungsional di Indonesia dan diharapkan dapat berkontribusi untuk meningkatkan pengembangan pangan fungsional di Indonesia.

Kata kunci: *Cross sectional surve; pangan fungsional; generasi milenial; persepsi.*

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Corresponding author:
Ardiansyah
Jakarta, Indonesia, 12940
Email: ardiansyah@bakrie.ac.id

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INTRODUCTION

Food is one of the main human needs used to eliminate hunger and obtain nutritional value for the body. At present, changes in the lifestyle of people and the desire for a healthier life have transformed the philosophy of food, where food is not just limited to eliminating hunger and obtaining nutritional value, but has a function to prevent diseases and improve human health. This food is commonly referred to as functional food. According to Martirosyan & Singh (2015), functional food is food either naturally or certain processes that have bioactive components in quantities clear and safe for consumption, and have been scientifically proven to be beneficial for health in terms of prevention of diseases. Recently, the Indonesian Society for Functional Food and Nutraceutical conducted a focus group discussion with other stake holders, such as the goverment (Indonesian Institute of Science-LIPI, National Standardization Agency of Indonesia-BSN, and Indonesia National Agency of Drug and Food Control-BPOM), industries, and researchers of functional food in Yogyakarta, Indonesia; the forum defined the term functional food in Indonesia. Functional food is food (fresh/processed) that contains components, useful for improving certain physiological functions and/or reduce the risk of diseases, proven by scientific studies; it must have a beneficial function as normally consumed as part of the daily diet (P3FNI, 2019).

The current health costs incurred by Indonesia are increasing; therefore, one of the solutions is using food for medicinal effect. Functional food trends have begun to grow and develop along the increasing research, publications and consumer awareness about the relationship between food, nutrition and health. Data from the WHO (2017) shows that in 2015, the health costs incurred by Indonesia were USD 1737.21 per capita; this cost was greater than that of the previous year 2014 amounting to USD 1205.20 per capita.

At present, Indonesia is beginning to try to develop functional foods known to have functional effects on body health by increasing various research, publications, and consumer awareness (Rahardjo, 2018). The increasing development of functional food in Indonesia will indirectly impact the health costs incurred. The progress of the functional food industry can be attributed to numerous factors, such as innovations in food, science and technology, increasing aging population, the regulatory environment to allow health claims on foods, and increased awareness of consumers regarding food products that claim health function (Arvanitoyannis et al., 2005).

Japan is well-known as a pioneer and successful country in developing and marketing various functional food products. This is due to the situation in which Japanese consumers began to realize the relationship between food and health and the importance of maintaining health, which motivated them to live healthier by consuming certain foods that had health functions. Kotilainen et al. (2006) explained that the increasing demand for functional food is in line with the increasing attention and awareness among consumers to consume healthy products that can improve their quality of life. In Indonesia, even though the development of functional food has been getting much attention, knowledge about consumers' perceptions in choosing and receiving functional food products is still relatively rare. Granqvist & Ritvala (2016) state that some consumers do not know how to classify products appropriately; hence, they do not understand the function of a product more specifically. This is one of the problems that can cause delays in the development and marketing of functional food in Indonesia.

Some studies indicate that the millennial generation comprising individuals born in the early 1980’s to 2000 can be one of the important target consumers because of the large population in the near future (Meier & Crocker, 2010). Badan Pusat Statistik (2016) states that one fourth of Indonesia's population belongs to the millennial generation. Recently, the population of Indonesia was around 257.89 million, while Indonesia's millennial generation was around 62.06 million (24.07%) of the total population in Indonesia (Susenas, 2016). In addition to the sizeable population, the millennial generation also represents consumers in the future who can greatly influence the

development of the food industry (Kljusurić & Čačić, 2014).

The young generation is known as the millennial generation. This generation tends to have characteristics, such as motivation, hope and high curiosity about anything. Based on their characteristics, it is interesting to understand how their behavior in choosing products can assist in developing innovation and marketing strategies for functional food products. To date, there has been no investigation into the perception of information related to functional food consumers in Indonesia. The millineals are a consumer segment, poised to benefit from the incorporation of functional foods in their diets due to health concerns in recent years.

MATERIALS AND METHOD

Sample and procedure

The cross-sectional survey was conducted among 1982 respondents 18 to 38 years old from almost all regions in Indonesia (Meier & Crocker, 2010). The survey was conducted by using Google forms, propagated in all regions in Indonesia during two weeks in April 2018. A link to the questionnaire list was distributed through the social media channels, such as Line, Instagram, WhatsApp, Facebook, and Twitter in the current study. The social media is an effective way to attract the millennial generation. They easily completed the survey and gave feedback voluntarily.

Questionnaire

The questionnaire started with a brief introduction. The respondents were informed about the purpose of the survey. At the beginning of the questionnaire, to make sure that the term "functional food" is well understood, the definition of functional food was given to the respondent. The questionnaire contained the definition so that each of the respondents was able to get freely acquainted with it.

Awareness of functional food was asked by inquiring if the respondents had ever heard or known about functional food before receiving the questionnaire ("Yes" or "No"). The knowledge about functional food was asked by inquiring if respondents know about food components that have health effects like probiotic, catechins as antioxidants, peptide, dietary fiber, resistant starch, and isoflavone ("Yes" or "No"). Their priorities to purchase functional foods and future buying motivation of functional food were also inquired. The respondents were also asked about gender, age, income, and education.

Statistical analysis

Quantitative data was analyzed through the calculation of summary statistics including frequencies and percentages. Data were analyzed using descriptive statistics and cross tabs using the program *SPSS* v.22. Chi-square which was a non-parametric test was used for testing the relationships between categorical variables at 95% significance level.

RESULTS AND DISCUSSION

Characteristics of respondents

The respondents (n = 1982) consisted of 304 males (15.3%) and 1678 females (84.7%). The characteristics of respondents are shown in Table 1. Majority of the respondents were females, between 20 to 23 years old. In terms of income distribution, this study found that 52.5% of the respondents earned no income. Regarding education level, most of the respondents (51.5%) had graduated from senior high school and 45.9% of the respondents had completed their bachelor/diploma levels.

Functional Food Awareness

Awareness is the most critical factor that can affect functional food consumption (Vella et al., 2014). After reading the definition, most of the respondents (55.5%) stated that they had heard about functional food before the survey (aware respondents) and 44.5% stated that they had not heard about functional foods (unaware respondents). It can be seen that most of the millennial generation knows about functional food. Millennial generation tends to be more aware of

functional food; this study is confirmed by the research conducted by Kljusurić & Čačić (2014).

Table 1. Characteristics of Respondents

Characteristics	Category	N	%
Gender	Male	304	15.3
	Female	1678	84.7
	Total	**1982**	**100**
Age	18	99	5.0
	19	135	6.8
	20	208	10.5
	21	372	18.8
	22	321	16.2
	23	231	11.7
	24	147	7.4
	25	139	7.0
	26	90	4.5
	27	88	4.4
	28	42	2.1
	29	29	1.5
	30	24	1.2
	31	16	0.8
	32	14	0.7
	33	9	0.5
	34	6	0.3
	35	3	0.2
	36	5	0.3
	37	2	0.1
	38	2	0.1
	Total	**1982**	**100**
Income	0	1041	52.5
	< 1.500.000	236	11.9
	1.500.000 - 2.500.000	247	12.5
	2.500.001 - 3.500.000	148	7.5
	> 3.500.000	310	15.6
	Total	**1982**	**100**
Education	Junior high school	6	0.3
	Senior high school	1020	51.5
	Bachelor/Diploma	909	45.9
	Master/Doctor	47	2.4
	Total	**1982**	**100**

Another study conducted in Spain showed that youth aged between 18 to 34 years (millennial generation) knew more about functional food compared to non-millennial generation aged between 35–76 years (Carrillo et al., 2013). This data also corresponded with the survey of The Centre for Strategic and International Studies (CSIS) (2017); they mentioned that the millenial generation aged between 17–29 years realized that health is the key factor to be happy. Further, to be happy they have to maintain a healthy lifestyle and intake healthy food.

The share of aware and unaware respondents did not differ between gender, age, and education (Table 2). Meanwhile, the income level had a significant effect on aware and unaware respondents (Chi-square *p<0.05)*. It might have occurred as based on the opinion of Kotilainen et al. (2006). Functional food products are relatively expensive compared to ordinary food products, especially in developing countries, as the market sector of these products is still relatively smaller.

Table 2. Functional Food and Demographic Characteristics Awareness

Characteristic		Awareness about Functional Food*				Sig.
		Aware Respondent		Unaware Respondent		
		n	%	n	%	
Gender	Man	159	14.44	145	16.48	0.240**
	Woman	942	85.56	736	83.52	
Income (Rp)	0	613	55.68	428	48.58	0.033♣
	> 1.500.000	126	11.44	110	12.49	
	1.500.000-2.500.000	128	11.63	119	13.51	
	2.500.001-3.500.000	73	6.63	75	8.51	
	> 3.500.000	161	14.62	149	16.91	
Education Level	Junior High School	2	0.18	4	0.45	0.423♦
	Senior High School	576	52.31	444	50.40	
	Bachelor/Diploma	494	44.87	415	47.11	
	Master/Doctor	29	2.64	18	2.04	

*Eligible respondent for Awareness Respondent (n=1101) and Unaware Respondent (n=881).
**Chi-square 1.38 df: 1; ♣Chi-square 10.49 df: 4; ♦Chi-square 2.80 df: 3

Additionally, there are no specific regulations governing and supervising functional food labeled products in Indonesia, based on Perka BPOM No. 13 (2016) about Claims on Labels and Processed Food Ads. Functional food has been defined in Perka BPOM RI No. HK.03.1.23.11.11.09909. In 2011, it was redefined and was better known as food with claims. The requirement to fulfill claimed food is also still relatively difficult and requires considerable costs, especially research costs in terms of proving the effectiveness of the expected function in food products. Therefore, food products that have health effects and functions to prevent certain diseases are still rare and relatively expensive in the market.

Food Components on Functional Foods Knowledge

After awareness, it is also necessary to know how respondents understand the components of food products that have health effects as functional foods. The second factor that can influence functional food consumption, besides awareness, is knowledge (Vela et al., 2014). Respondents' knowledge regarding the components of food that have health effects are shown in Figure 1.

The knowledge about food components was insufficient; most of the respondents did not know that the components of food have health effects or could reduce the risk of diseases in the body. Six food components were presented in the questionnaire. However, most of the respondents claimed that they only knew about probiotics and dietary fiber for digestive health compared to other components, like catechins, peptides, resistant starch, and isoflavones (Figure 1). Respondents tended to know more about the components of probiotics and dietary fiber because in the markets, food containing probiotic and food fiber were available around them.

Priority Attribute to Purchase Functional Foods

The respondents were free to choose more than one of their priorities in purchasing functional food. The priority in purchasing functional food based on the preference of respondents is shown in Figure 2. The availability and displaying of products can potentially increase priority to purchase the product (Soon and Wallace, 2018).

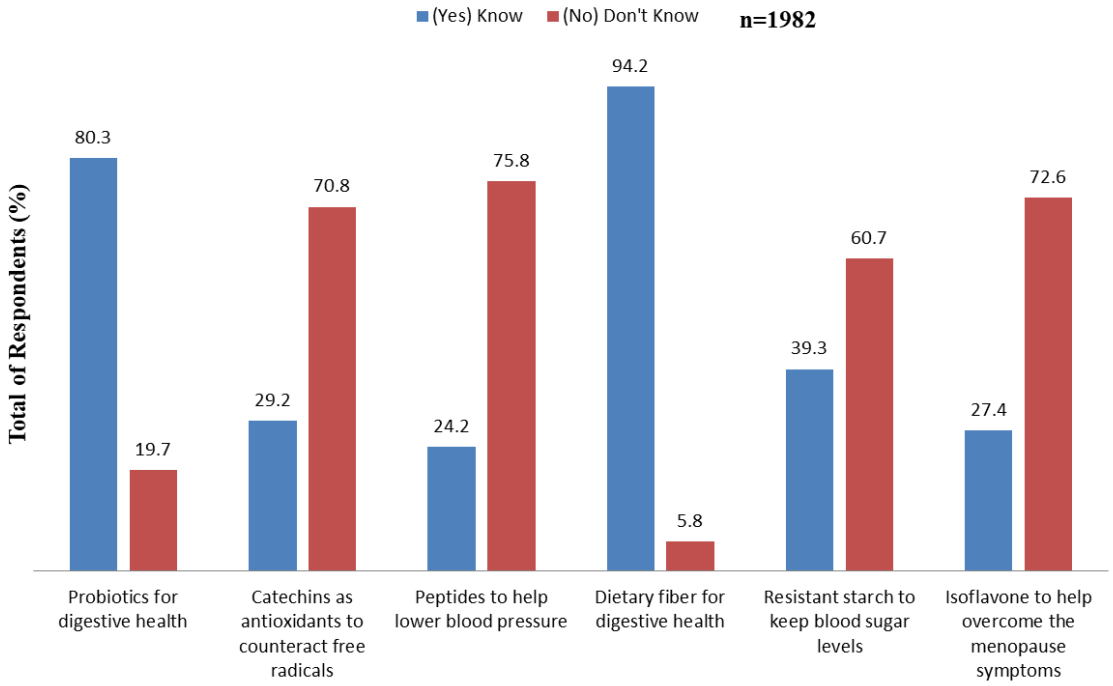

Figure 1. Knowledge about Food Component that Have Health Effect

The respondents expressed the important factors in terms of motivating to buy functional food in the future. The respondents were free to choose more than one of the priority factors to express their opinion. This information is very important to understand what consumers need in an effort to improve functional food development. Based on Figure 2, the most chosen attributes as the priority of respondents from the total respondents (1982) in purchasing functional foods are health benefits, availability, affordability, tasty, easy to consume and label information. Health benefits were the most important factor for respondents (1751 respondents comprising 88.3% of the total respondents), followed by 1422 (71.7%) respondents who considered the availability of products as important, then 1383 (69.7%) respondents rated affordable prices, and 1376 (69.4%) respondents chose good taste in the decision to buy functional food. Nutrition and health information has the potential to influence acceptance of functional food products by communicating the health benefits of such products. Several other factors, also a priority in purchasing functional food, are shown in Figure 2.

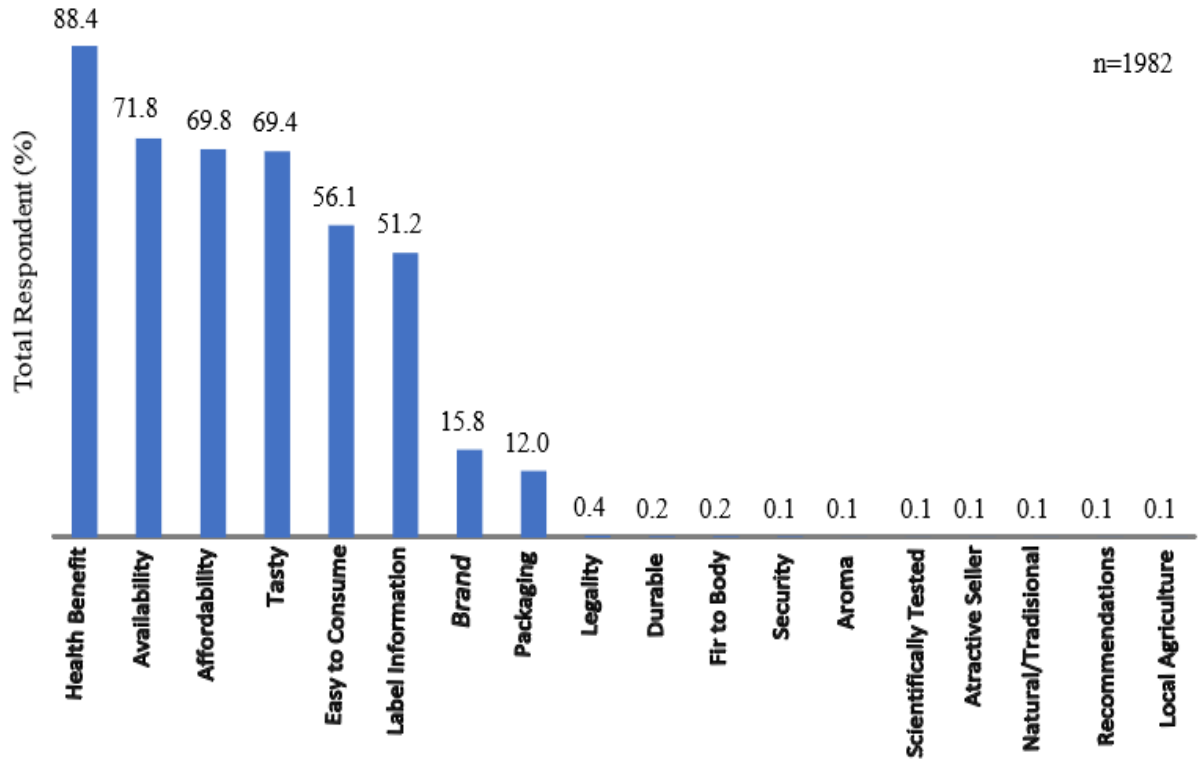

Figure 2. Priority to Purchase of Functional Food

These studies generate information about potential consumer wants and needs. Thus, strategic plans to target consumers effectively can be formulated for functional food development. Functional food has huge potential to be developed, but it is important to understand the target market that would bring together what consumers want in terms of product development by producers. This is in line with the research of Van der Zanden et al. (2015); the functional food market still has not reached the target in marketing because it still fails to meet consumer expectations. Most functional food industries still cannot find effective strategies to meet what consumers expect. This corresponds to what Del Giudice & Pascucci (2010) stated that in the marketing strategy the functional food market segment is still difficult to identify specifically. Functional food products still need to be promoted and clear information needs to be given to consumers to make them more recognizable among consumers.

Future Buying Motivation

The motivation of respondents to buy functional food in the future is shown by looking at the decision of respondents when buying functional food related to disease symptoms. Respondents were asked about their future buying motivation related to affordability and suffering symptoms of a disease; this information has been summarized in Table 3. Food choices stated in different sources, such as direct messages through articles, advertisements, images and editorials can influence future buying motivation (Hamadeh and Maruis, 2008).

Most of the respondents stated that they would be willing to buy functional foods if they had more money or if they had symptoms of a disease in the future. Price is one of the factors that influence respondents' consumption in choosing and consuming functional food. Expensive prices of functional food products can be an obstacle for respondents to not consume them. From the total respondents (n = 1982), 97.7% or 1937 wanted to consume functional food if they had more money, while only 2.3% or 45 did not want to eat functional food even though they had more money. This illustrates that most of the millennial generation is interested in consuming functional food if they had more money in the future. There is a large pretension of millennial generation respondents to consume functional food, if they

had more money in the future. The respondents want to pay higher price to get the health benefits.

Table 3. Future Buying Motivations

Statements	Category	n	%
If respondents have more money, would they like to buy functional foods	Yes	1937	97.7
	No	45	2.3
If respondents have symptoms a disease, would they like to buy functional food	Yes	1827	92.2
	No	155	7.8

One of the most important factors related to consuming functional food are health factors. The awareness of healthy living in the millennial generation is quite high. Respondents' decision regarding whether they want to consume functional food as an alternative prevention, when they have symptoms of a disease are shown in Table 3. Among the total respondents (n = 1982), 92.2% or 1827 stated that they wanted to consume functional food as an option of risk reduction of a disease; only 7.8% or 155 decided to not consume functional food for risk reduction of disease. According to Jain et al. (2014), in India, awareness about health and changes in lifestyle affect consumers to choose functional food products in India. This also illustrates that most of the millennial generation in Indonesia is interested in consuming functional food as an alternative measure for risk reduction if they had symptoms of a disease; awareness about health is one of the important things and can influence respondents in their decision to choose functional food.

To increase the literacy of the millennial generation about functional food, regulating food labels may contribute positively and motivate them to not just decide to buy based on the price, but also based on health properties. Furthermore, functional food must have quality and effects in accordance with the price and added value they can provide to consumers (Lyly et al., 2007).

Study's limitations and strengths

The current study, which explored the information sources for functional foods, and the awareness and perceptions of health claims of functional foods on the millenial generation among Indonesia, is not without limitations. All data collected was self-reported and therefore there may be discrepancies between the reported information and participants' actual understanding and perceptions related to functional foods. There are still contrary opinions regarding the term functional food, since there is no specific regulation on functional food in Indonesia. These could also affect the perception of the respondents. Furthermore, another limiting factor is that the participants were predominantly students who earned no income and all their income was subsidized by their parents.

Despite these limitations, the current study has strength in that it utilized a researcher-administered questionnaire and examined the completeness of the data. Since there is no study related to the perception on functional food in Indonesia, this is the first study on the perception on functional food in a specific age called the millenial generation. In addition, the study questionnaire consisted of a variety of open and close-ended questions, which enabled the collection of a wide breadth of data.

CONCLUSION

Almost 55% or 1101 millennial generation respondents in Indonesia claimed that they had heard about functional food or had been aware of functional food. Respondents' knowledge regarding food components that have health effects is still insufficient. This knowledge is important to be able to improve functional food marketing. Consumer literacy related to knowledge of food components has a positive impact on increasing market and products development. Health benefits, availability, affordability, tasty, easy to consume and label information need to be considered in

functional food products. Consumer expectations of the millennial generation can enhance the development of the functional food market.

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GARLIC PEEL EXTRACT PHYTOCHEMICAL EVALUATION AND EXTRACTION OPTIMIZATION

Della Rahmawati[1],
Dylan Andika[1],
Stacia Andani Fortunata[1]

[1] Department of Food Technology, Faculty of Life Sciences and Technology, Swiss German University, The Prominence Tower, Alam Sutera, Jalan Jalur Sutera Barat No 15, Tangerang 15143, Indonesia

ABSTRACT

Garlic plant has been known to have various beneficial properties beside as a condiment. However, the garlic peel is still considered as waste and the research of its functional properties are still very limited. The purpose of this study was to explore the potential phytochemical activities of garlic peel extract which might be utilized as natural food additive or even functional ingredient in the future. The experiment was divided to two phases. The first phase identifies and compares the phytochemical content and activities between aqueous and ethanolic extract. The presence of saponins was detected along with the absence of alkaloids on both aqueous and ethanolic extract. The aqueous extract possessed lower flavonoid content (7.593±0.299 mg/l quercetin equivalent), but higher phenolic content (64.688±1.865 mg/l GAE) and stronger antioxidant activity (17.042±0.380%) compared to the ethanolic peel extract. Aqueous extract showed antimicrobial activity against *S. cerevisiae*, while the ethanolic extract did not. Moreover, both types of extract also did not show any α-glucosidase inhibition activity. In the second phase, optimization attempts for extraction method were done and it was found that the highest amount of antioxidant activity along with flavonoid and phenolic content could be obtained in treatment of the raw material-solvent ratio to 20 gr/1000ml.

Keywords: *garlic peel extract, phytochemical activities, antioxidant, antimicrobial, extraction method.*

ABSTRAK

Bawang putih merupakan bumbu yang telah dikenal dengan berbagai keunggulannya. Meskipun demikian, kulit bawang putih sering dianggap limbah dan riset mengenai sifat fungsionalnya sangat terbatas. Pada penelitian ini potensi dari kandungan dan aktivitas fitokimia ekstrak kulit bawang putih akan dieksplorasi untuk penggunaannya di masa depan sebagai bahan tambahan pangan alami atau bahan baku pangan fungsional. Eksperimen yang dilakukan dibagi menjadi dua tahap. Pada tahap pertama dilakukan perbandingan antara kandungan dan aktivitas fitokimia dari eksrak air dan ekstrak etanol. Hasil penelitian menunjukkan keberadaan saponin pada kedua ekstrak, sedangkan keberadaan alkaloid tidak terdeteksi pada keduanya. Ekstrak air memiliki kandungan flavonoid yang lebih rendah (7.593±0.299 mg/l quercetin equivalent), namun memiliki kandungan fenolik yang lebih tinggi (64.688±1.865 mg/l GAE) dan kandungan antioksidan yang lebih kuat (17.042±0.380%) dibandingkan ekstrak etanol. Ekstrak air menunjukkan aktivitas antimikroba terhadap *S. cerevisiae*, yang tidak dimilik ekstrak air. Kedua ekstrak tidak menunjukkan aktivitas inhEXibisi terhadap enzim α-glukosidase. Pada tahap kedua, ekstraksi optimisasi dilakukan dan ditemukan bahwa aktivitas antioxidant terkuat yang didukung dengan kandungan flavonoid dan fenolik tertinggi diperoleh pada perlakuan rasio ekstraksi bahan baku terhadap solvent 20g/1000ml.

Kata kunci: *kulit bawang putih, aktivitas fitokimia, antioksidan, antimikroba, ekstraksi.*

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Corresponding author:
Stacia Andani Fortunata
Tangerang, Indonesia, 15143
Email:
staciafortunata@gmail.com

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INTRODUCTION

Garlic plant has been known since the ancient times to have many benefits. Through various studies, different parts of the plants have been shown to possess many properties which are valuable for functional ingredients for food and promoting health-beneficial effects (Durairaj, S., Srinivasan, S., & Lakshmanaperumalsamy, P. 2009; Amagase, H. et al. 2001). However, behind the well-known usefulness of garlic plant, there is one part of it which is still widely considered as waste. The garlic peel/skin is usually discarded directly without any further use. Moreover, in 2015 Indonesia had been reported to produce at least 23,991 tons of garlic (BPS. 2015). Therefore, the amount of garlic peel waste produced was also very significant.

There have been several efforts to study the potential of garlic peel even though they are still very limited. Garlic peel has been proven to contain six phenyl propanoids which act as strong antioxidants (Ichikawa M, et al. 2003). Another research has also reported the antioxidant and antimicrobial activities of garlic peel extract (Ifesan et al. 2014). Moreover, it is also able to reduce blood sugar level of laboratory mice which have been induced by alloxan (Wijayanti, R. et al. 2015).

Based on the evidence provided by the studies above, there might be a possibility to utilize garlic peel extract as a natural food additive or even as a functional food in the future. However, natural extracts can only give their maximum benefits if they are obtained by proper extraction methods. Since the research on the potential of garlic peel is still very limited, the optimum extraction method for garlic peel is yet unclear. Therefore, in the hope that it may provide basic knowledge for the future applications of garlic peel extract, this study was conducted to evaluate the phytochemical activities of garlic peel extracts obtained by using various extraction methods (aqueous and ethanolic extraction by maceration) and try to improve the quality of the extract by optimizing the extraction method (re-maceration and ratio change). The extraction method of garlic peel can be optimized once the phytochemical activities have been observed, and the optimization technique can be guided to produce better extract. The optimization was aimed to improve its phytochemical activities and yield.

MATERIALS AND METHOD

The study was divided into two phases. The first phase was intended to identify the phytochemical properties of garlic peel extract obtained by using water and ethanol 96% as extraction solvents using maceration process. The extraction was done according to modified method by Ifesan et al. 2014.

Garlic peel extraction

Twenty gram of ground garlic peel was macerated using 500 ml of solvent for 24 hours. The extracts obtained using both solvents were then filtered and concentrated to an equivalent concentration of 200mg/ml and analyzed for phytochemical activities.

Qualitative analysis

The analyses consisted of qualitative alkaloid content (Wagner's test), qualitative saponin content (foam test), total phenolic content (Folin-Ciocalteau assay), total flavonoid content (aluminium chloride assay), antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Saccharomyces cerevisiae* and *Aspergillus niger* (well diffusion method), antioxidant activity (DPPH scavenging assay) and alpha-glucosidase inhibition activity. Based on the result obtained from the first phase, the extraction method which produces extract with better activities was chosen for optimization attempts in the second phase.

Optimization of extraction process

In the second phase, the optimization for extraction method was done through two different approaches. Extraction method with better activities in the first phase was modified using re-maceration and raw material-solvent ratio change. Re-maceration technique was divided into two variations. The solvent was changed and collected every 8 hours and every 12 hours during 24 hours extraction using the same ratio of 20 gram/500ml. The raw material-solvent ratio change was divided into 3 different concentrations (20gram/1000ml, 20 gram/1500ml, 20 gram/2000ml). The extracts obtained from each treatment were then concentrated and the phytochemical activities of the extracts were analyzed and compared to the result obtained in the first phase. Only activities regarded significant or potential in the first phase will be analyzed in phase two.

RESULTS AND DISCUSSION

The phytochemical activity analyses conducted in first phase indicated that both type of extracts were distinctive from each other. This phytochemical analysis will check whether the garlic peel has same bioactive compound with garlic bulb or not. A health property of garlic depends upon the bioactive compounds such as organo-sulfur and non-sulfur compounds (Kim et al., 1997; Rahman, 2007; Sendl et al., 1992; Tepe et al., 2005). Those compounds are vitamins, fatty acids, glycolipids, phospholipids, essential amino acids, phenolics, and flavonoids (Fenwick and Hanley, 1985; Tsiaganis et al., 2006).

Qualitative analyses showed in Table 1. showed that alkaloid was not present in both ethanolic and aqueous garlic peel extract. This may indicates that both types of extract possess a very low quantity of alkaloids that it could not be detected visually, or they do not contain alkaloids at all. While saponin were detected in both of them. Saponins from garlic have been found to have pharmacologic activities such as antifungal, antibacterial, anti-inflammatory and hypocholesteremic influences (Lacaille-Dubois and Wagner 1996). However, antimicrobial activity was only existed in aqueous extract against *S. cerevisiae*.

Tabel 1. Comparative Qualitative analysis data of the phytochemicals of the garlic

Secondary metabolites	Ethanol Extract Garlic Peel	Water Extract Garlic Peel	Methanol Extract Garlic Bulb*
Alkaloids	-	-	+
Saponins	+	+	+
AGI	-	-	NA
Antimicrobial activity (*S. cerevisiae*)	-	+	NA
Flavonoid	+	+	+
Antioxidant activity	+	+	NA
Phenolics	+	+	+

+ indicates presence, - indicates absences

*Divya BJ, Suman B, Venkataswamy M, Thyagaraju K. (2017)

Antimicrobial activity of aqueous garlic extract against *Saccharomyces cerevisiae* has already been previously studied (Durairaj et al., 2009) and according to the research by Kivanc & Kunduhoglu (1997), *Saccharomyces cerevisiae* exhibit the highest sensitivity against various plant juices including the garlic juice, compared to other type of yeasts and bacteria. This may possibly have a connection to the antimicrobial activity of aqueous garlic peel extract against *Saccharomyces cerevisiae.*

There were also no alpha-glucosidase activity inhibitions detected in both extract (Table 1). Negative results were obtained possibly due to the absence of inhibitor and the presence of sugar in the extract, therefore the absorbance of sample solution will be considerably high. Since sugar is far more soluble in water than in ethanol, the negative percentage in the aqueous extract will be greater as well, because more of the sugar will be broken down by the enzyme.

The flavonoid in both extracts were detected. The ethanolic extract of garlic has higher flavonoid content than aqueous extract, based on quantitative analysis of flavonoid content. The flavonoid content (dilution factor/DF=1) of aqueous and ethanolic extract were respectively 7.593±0.299 mg/l quercetin equivalent and 14.019±0.539 mg/l quercetin equivalent. These results show that flavonoids in garlic peel are tend to be more soluble in less polar solvent such as ethanol. This might happen since various kinds of phenolic compounds, including flavonoids have different solubility in various solvents (Ganora, 2009).

The total phenolic content of aqueous extract higher than ethanolic extract. The total phenolic content (DF=5) of aqueous and ethanolic extract were respectively 64.688±1.865 mg/l gallic acid

equivalent (GAE) and 47.043±0.750 mg/l GAE. Based on the result, it can be concluded that the phenolic compounds contained in garlic peel can be more optimally extracted using water as solvent.

The antioxidant activity (DF=5) of aqueous and ethanolic extract were respectively 17.042±0.380% and 13.706±0.668%. This antioxidant activity may be heavily related to the phenolic compounds of garlic peel, especially phenyl propanoids which have been already identified and studied previously by Ichikawa et al (2003). Based on the experimental results, the antioxidant activity seems to be directly proportional to the total phenolic compound. As the total phenolic compound of aqueous extract was higher, the antioxidant activity was also higher as expected. Aqueous extraction was chosen for the second phase. Since the antioxidant properties of aqueous extract was regarded significant, only antioxidant activity, total flavonoid content and total phenolic content were analyzed and compared in second phase. Moreover, extraction method using water is cheaper, more flexible for processing and it will not have any problem with halal certification if it is applied for food or pharmaceutical industry in the future.

In the second phase optimization was performed. The optimization process of extraction procedure is important due to changes in conditions such as extraction technique, ratio of solvent and sample determine the extraction yield of individual chemical constituents extractable (Hinneburg and Neubert, 2005). In other hand, different temperature, extraction time were also affected the yields.

Maceration was chosen as method of extraction. Maceration is one of suitable widely used technique for extractions of plant material. Therefore, in this study the optimization process was used re-maceration technique. Purpose of re-maceration technique is to maximizing yields of the compounds of interest, while minimizing the extraction of unwanted compounds with two time maceration (Sibul F.S, et al. 2016)

The result of total flavonoid content, total phenolic content and antioxidant activity of aqueous extract after optimization attempts are shown in Tables 2,3,4,5,6,7.

Table 2 Total flavonoid content after ratio change

Optimization Method	Total Flavonoid Content (mg/l quercetin equivalent), DF=1
Ratio 20 gr/1000ml	17.617±0.486
Ratio 20 gr/1500ml	8.528±0.256
Ratio 20 gr/2000ml	9.393±0.162

Table 3 Total flavonoid content after re-maceration

Optimization Method	Total Flavonoid Content (mg/l quercetin equivalent), DF=1
Re-maceration 8 hours	6.121±0.377
Re-maceration 12 hours	6.331±0.299

Table 4 Total phenolic content after ratio change

Optimization Method	Total Phenolic Content (mg/l GAE), DF=5
Ratio 20 gr/1000ml	80.817±1.609
Ratio 20 gr/1500ml	59.207±0.529
Ratio 20 gr/2000ml	56.923±0.999

Table 5 Total phenolic content after re-maceration

Optimization Method	Total Phenolic Content (mg/l GAE), DF=5
Re-maceration 8 hours	71.394±0.506
Re-maceration 12 hours	69.879±0.418

Table 6 Antioxidant activity after ratio change

Optimization Method	Antioxidant Activity (%), DF=5
Ratio 20 gr/1000ml	25.856±0.408
Ratio 20 gr/1500ml	21.881±0.305
Ratio 20 gr/2000ml	23.168±0.186

Table 7 Antioxidant activity after re-maceration

Optimization Method	Antioxidant Activity (%), DF=5
Re-maceration 8 hours	23.834±0.326
Re-maceration 12 hours	21.904±0.683

Based on the experimental results, optimization process showed significant difference in total flavonoid content compared to the control. Changing the ratio between raw material and solvent gave significant improvement towards flavonoid content, while doing re-maceration decreased the flavonoid extracted.

The re-maceration process should increase the yield of substances extracted theoretically. However, both extract with 8 hours and 12 hours of re-maceration similarly exhibit lower flavonoid content. This phenomenon may be explained by the fact that in phase one, the flavonoids in garlic peel tend to be less polar (higher flavonoid value with ethanolic extraction).

The flavonoids, as one of the phenolic compounds, have aglycones forms which are less polar than glicosidic flavonoids. These less polar aglycones flavonoids may bound with saponin which was also present in aqueous extract. Saponin is a natural surfactant which may help dissolving less polar substances into polar solvent. Since the basic principal of re-maceration is changing the solvent over time and the fact that saponin is hydrophilic, the saponin may have been exhaustedly extracted in earlier extraction solvent (Gonzalez-valdez et al.,2013).

As the result, in the later extraction solvent, flavonoids were not extracted optimally and the flavonoid content was lower in total. This did not happen to the extraction method which used the same solvent during 24 hours extraction (without re-maceration).

The optimization process of aqueous garlic peel extraction also showed significant difference in total phenolic content compared to the control. The re-maceration process significantly increased the amount phenolic content as expected. However, the change in raw material-solvent ratio indicated lower values.

The irregularly high phenolic content of 20 g/ 1000 ml extract may also be related to the mishandling issue. However, it is unclear why the 20 gr / 1500 ml extract and 20 gr/2000 ml extract exhibited lower values compared to the control extract. Moreover, the phenolic content also seemed to decrease as the amount of solvent gets higher. This may come from the evaporation process, which involves longer time to evaporate higher volume sample. The smaller ratio of raw-material-solvent requires higher volume to achieve the same concentration of 200 mg raw material/ ml solvent and consequently will lengthen the evaporation time. This longer time will expose more of the extract to the heat and degrade more of the phenolic compounds.

In general, all of the optimization process increased the antioxidant activity of aqueous garlic peel extract despite of several reduction in phenolic and flavonoid content mentioned in Table 6 and 7. This may indicate that other compounds which act as antioxidant were also extracted in higher amount besides flavonoid and phenolic compounds.

The considerably high antioxidant activity of 20 gr/ 1000 ml extract is believed to be related to mishandling issue mentioned before. The antioxidant activities of re-macerated extracts were significantly higher than control extract and directly proportional to the total phenolic compound mentioned in Table 7. The antioxidant activities of re-macerated extract were also generally higher than the extract with ratio-changing.

Based on the results above, optimization technique (changing ratio of solvent) in general were able to improve the phytochemical activities of aqueous garlic peel extract compared to the results previously obtained in the first phase with the highest total flavonoid content, total phenolic content and antioxidant activity can be achieved by using 1000 ml solvent for 20 mg sample. However, re-maceration technique was not able to improve activities.

CONCLUSION

Aqueous garlic peel extract had better phytochemical activity in terms of total phenolic content, antioxidant activity and antimicrobial activity compared to the ethanolic extract. Nevertheless, there were no alpha-glucosidase inhibition activity detected in both extract. Total flavonoid content, total phenolic content and antioxidant activity of garlic peel extract could be optimized by changing amount of solvent to 1000 ml for 20 mg sample. However, optimization by using re-maceration technique was not able to improve activities.

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Journal of Functional Food and Nutraceutical

Activity Report (Laporan Kegiatan)

Mini Simposium dan AKSI P3FNI

Serta

Focus Group Discussion tentang Definisi dan Regulasi Pangan Fungsional

Dengan Tema

"Penguatan dan Diseminasi Pangan Fungsional untuk Kesehatan Masyarakat"

Diselenggarakan oleh

Perhimpunan Penggiat Pangan Fungsional dan Nutrasetikal Indonesia

Bekerjasama dengan

Fakultas Teknologi Pertanian, Universitas Gadjah Mada

Yogyakarta, 17 Januari 2019

1. Latar Belakang

Penyakit Tidak Menular (PTM) di Indonesia semakin meningkat pertumbuhannya. Laporan dari The World Bank menunjukkan bahwa pada tahun 1995 angka prevalensi Penyakit Menular (PM) lebih tinggi dibandingkan PTM, namun pada tahun 2001 dan selanjutnya, angka prevalensi PTM jauh melampaui jumlah prevalensi PM. Sedangkan berdasarkan data Riskesdas (2013), telah terjadi peningkatan prevalensi beberapa penyakit tidak menular seperti diabetes melitus, kanker, stroke, obesitas, dll. WHO memperkirakan PTM menyebabkan 56 % dari semua kematian dan 44 % dari beban penyakit dalam negara-negara di wilayah Asia Tenggara. Tingginya kasus PTM sangat berdampak pada kualitas kesehatan masyarakat, menurunnya produktivitas, meningkatnya biaya perawatan kesehatan, sehingga mengakibatkan turunnya pertumbuhan ekonomi, menurunnya daya saing dan sebagainya.

Indonesia kaya akan sumber alam dengan kandungan komponen bioaktif yang sangat potensial untuk dikembangkan. Dengan jumlah penduduk yang besar, meningkatnya kesejahteraan dan semakin meningkatnya kesadaran masyarakat untuk hidup sehat, maka dapat diprediksi bahwa permintaan makanan fungsional akan meningkat di masa yang akan datang. Hal ini memberi harapan bahwa pengembangan makanan fungsional di Indonesia sangat prospektif. Pangan fungsional juga mempunyai peluang dalam perdagangan ekspor, antara lain ke negara Jepang, Eropa dan Amerika.

Untuk menangkap peluang ini sangat diperlukan kerjasama antar pemangku kepentingan (peneliti, penggiat/asosiasi, pemerintah, industri, dan konsumen). Dukungan dari berbagai pemangku kepentingan diharapkan akan semakin meingkatkan awareness konsumen tentang pangan fungsional. Kerjasama simultan dan terus-menerus menjadi salah satu kunci untuk pengembangan pangan fungsional di Indonesia.

Mengingat pentingnya peran pangan fungsional dan nutrasetikal dalam mengatasi berbagai penyakit dan melihat kecenderungan saat ini telah terjadi peningkatan konsumsi pangan fungsional dan ketertarikan pemangku kepentingan di bidang ini, maka perlu adanya tindakan nyata dalam bentuk diseminasi dan aksi. Untuk itu **Perhimpunan Penggiat Pangan Fungsional dan Nutraseutikal Indonesia (P3FNI)** akan melakukan kegiatan Mini Simposium dan AKSI, dilanjutkan FGD dengan tema “Penguatan dan Diseminasi Pangan Fungsional untuk Kesehatan Masyarakat”

2. Tujuan dan Luaran Kegiatan

Kegiatan FGD dan AKSI bertujuan untuk

1) Mendefinisikan pangan fungsional dan nutrasetikal di Indonesia
2) Sosialisasi pangan fungsional untuk kesehatan masyarakat
3) Mengenalkan peran P3FNI kepada penggiat pangan fungsional dan masyarakat, khususnya kepada pemangku kepentingan (pemerintah, akademisi, dan pengusaha/industri)
4) Meningkatkan kesadaran pemangku kepentingan potensi pangan fungsional yang ada di Indonesia,
5) Menjalin komunikasi dan potensi kerjasama di antara pemangku kepentingan yang bergerak dalam bidang pangan fungsional dan nutrasetikal

Luaran dari kegiatan ini adalah:

1) Definisi pangan fungsional dan nutrasetikal
2) Bertambahnyapengetahuandankesadaranmengenaipentingnyapanganfungsional di Indonesia
3) Terjalinnya kerjasama antar pemangku kepentingan dalam bidang pangan fungsional dan nutrasetikal (pemerintah, akademisi, penggiat, dan pengusaha)

3. Pelaksanaan Kegiatan

Mini simposium dan FGD dilaksanakan pada hari Kamis tanggal 17 Januari 2019 bertempat di Kampus Fakultas Teknologi Pertanian Universitas Gadjah Mada Yogyakarta. Jumlah peserta yang hadir pada seminar kurang lebih sebanyak 180 peserta yang terdiri dari akademisi, peneliti, pemerintah, UMKM Pangan, dan pemerhati pangan fungsional. Acara dibuka oleh Ketua P3FNI yaitu Prof. Dr. C. Hanny Wijaya. Agenda mini simposium dibagi menjadi 3 sesi dengan narasumber yang ahli di bidangnya.

Sesi Pertama terdiri dari:

1. Prof. Dr. C. Hanny Wijaya yang menyampaikan materi **"Pangan Fungsional dan Nutrasetikal: sejarah dan perkembangan terkini"**
2. Prof. Dr. Laksono Trisnantoro yang menyampaikan materi **"Sosial ekonomi dan potensi pangan fungsional di Indonesia"**
3. Prof. Dr. Ir. Eni Harmayani, M.Sc. yang menyampaikan materi **"Potensi Pangan Nusantara sebagai Pangan Fungsional"**

Sesi kedua terdiri dari:

1. Dr. Agus Nurudin, Managing Director of PT The Nielsen Company, Indonesia yang menyampaikan materi **Potensi pasar dan konsumen pangan fungsional di Indonesia**
2. Ibu Yunawati Gandasasmita dari Kalbe Nutritionals yang menyampaikan materi tentang **"Industri pangan fungsional di Indonesia"**
3. Dr. Ardiansyah yang menyampaikan materi tentang **"Bagaimana membedakan klaim ilmiah dan HOAX?"**
4. Dr. A. Muzi Marpaung yang menyampaikan materi tentang **"Functional Food Made Easy (Membumikan Pangan Fungsional dalam Kehidupan Sehari- hari)"**

Pada sesi ke-3 dilanjutkan dengan deklarasi penguatan pangan fungsional di Indonesia. Deklarasi dibacakan oleh Sekjen P3FNI, Dr. Ardiansyah, kemudian dilanjutkan dengan penandatanganan deklarasi oleh Ketua P3FNI dan Dekan FTP UGM. Pada sesi ke-3 ini juga disampaikan sosialisasi keanggotaan P3FNI dan jurnal resmi P3FNI yaitu Journal of Functional Food and Nutraceuticals. Acara mini simposium ditutup oleh Prof. Dr. Ir. Eni Harmayani, MSc. selaku dekan FTP UGM.

Agenda ke-2 setelah mini simposium adalah FGD tentang definisi dan regulasi pangan fungsional dengan menghadirkan narasumber yang sama pada acara mini simposium ditambah narasumber dari BPOM, BSN, dan LIPI. Hadir sebagai narasumber dari BPOM yaitu Ibu Yusra Egayanti selaku Kasubdit Standardisasi Pangan Olahan Tertentu, sedangkan dari BSN diwakili oleh Ibu Ellia Kristiningrum dan LIPI oleh Dr. Agus Haryono selaku Kepala Pusat Penelitian Kimia Fungsional LIPI. Diskusi dihadiri oleh kurang lebih 55 peserta dari akademisi, peneliti, dan industri. Acara FGD

dipimpin oleh moderator yaitu Dr. Anton Apriantono. Hasil dari FGD yaitu definisi pangan fungsional sebagai berikut :

"Pangan fungsional adalah pangan (segar / olahan) yang mengandung komponen yang bermanfaat untuk meningkatkan fungsi fisiologis tertentu, dan / atau mengurangi risiko sakit yang dibuktikan berdasarkan kajian ilmiah, harus menunjukkan manfaatnya dengan jumlah yang biasa dikonsumsi sebagai bagian dari pola makan sehari-hari"

Selanjutnya definisi pangan fungsional ini akan disosialisasikan sebagai definisi pangan fungsional menurut P3FNI pada acara P3FNI di tahun 2019 yang akan ditentukan waktunya kemudian.

4. Jumlah Peserta

- ✓ Mini simposium : 164 orang (simposium 136 peserta, 17 pembicara dan moderator, serta 11 panitia mahasiswa).
- ✓ FGD : 56 orang

5. Foto-Foto Kegiatan

Sesi I

Sesi II

Peserta Mini Simposium

Peserta FGD

Panitia

Code of Ethics

For Authors

Main Concern:

- Originality and plagiarism
- Authorship of the paper
- Data access and retention
- One journal submission
- Conflict of interest
- Timeliness

Plagiarism

When an author deliberately uses another's work without acknowledgment, credit, or permission. Plagiarism has many different forms, from literal copying to paraphrasing someone else's work or your work and can include:

- Data
- Words and Phrases
- Ideas and Concepts

Authorship

Authorship should be limited to those who have made a significant contribution to the conception, design, execution, or interpretation of the reported study. You must obtain their agreement beforehand.

Data Access and Retention

Authors may be asked to provide the raw data in connection with a paper for editorial review, and should be prepared to provide public access to such data. Authors are responsible for their data and the analysis.

One Journal Submission

Submitting your manuscript to one journal only at a time. Avoid to submit the same manuscript to various journals.

Conflict of Interest

All submissions must include disclosure of all relationships that could be viewed as presenting a potential conflict of interest.

Timeliness

Probably, there will be several revisions in order to meet our journal standard. Be prompt to deal with it. Contact the editorial team if you require more time.

How to Avoid Plagiarism

1. *Use your own ideas*

Write your own work with your own idea.

2. *Cite the sources*

Always acknowledge the sources. That is why you need a **good citation** and **reference system**.

3. *Rewrite someone ideas in your own words*

Effective **paraphrasing** can help you prevent plagiarism! **Remember to CITE!**

4. *Use notes*

Record all details about the source and **distinguish** carefully between any idea from your reading and your own ideas.

Journal of Functional Food and Nutraceutical

User Account Registration Guideline

REGISTRATION

To make a submission to Journal of Functional Food and Nutraceuticals, you need to **register** a user account and log in. After log in, click the **submission** tab and **make a new submission**, then you will be directed to your Dashboard.

SUBMISSION

1. Click **New Submission** on the left side of the screen.

In the **Section** area, choose which **type of papers** you want to submit → Read and agree the **Submission Requirement** by clicking all the checkboxes → In addition, you can write an email to the editor by filling the **Comments for Editor** or leave it blank → Click **Save and Continue**

2. You will be directed to the **Upload Submission File** section.

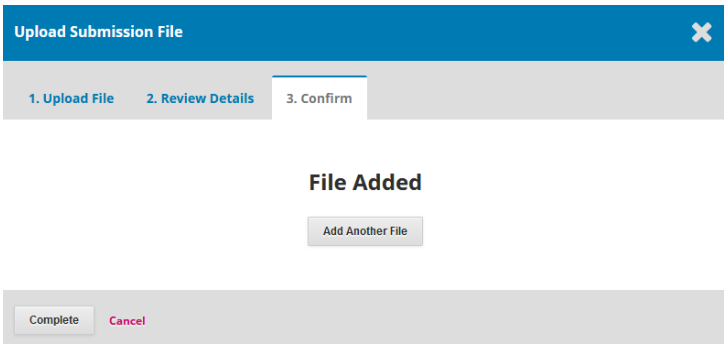
A. Upload your file in the **Upload Submission File** section.

Select **Article Text** in the **Article Component** → **Upload** your **file**. The type of file that accepted is in **.doc** or **.docx** → Click **Continue** to go to the next process

B. You need to **reconfirm** that you have uploaded the correct file in the **Review Details**.

You can edit the file name by clicking the **Edit** button → Click **Continue** to go to the next step

C. On the **Confirm** section, you can add another file.



You may be able to **add another file** for your submission, otherwise leave it blank → Click **Complete** button

Note:
1. You will be able to see your uploaded file in the **Submission Files** list.
2. You will be able to upload another file by clicking the **Upload File** button on the right side of the **Upload Submission** section.
3. You will be able to modify your file by clicking button next to your uploaded file.
4. To continue, click the **Save and Continue** button.

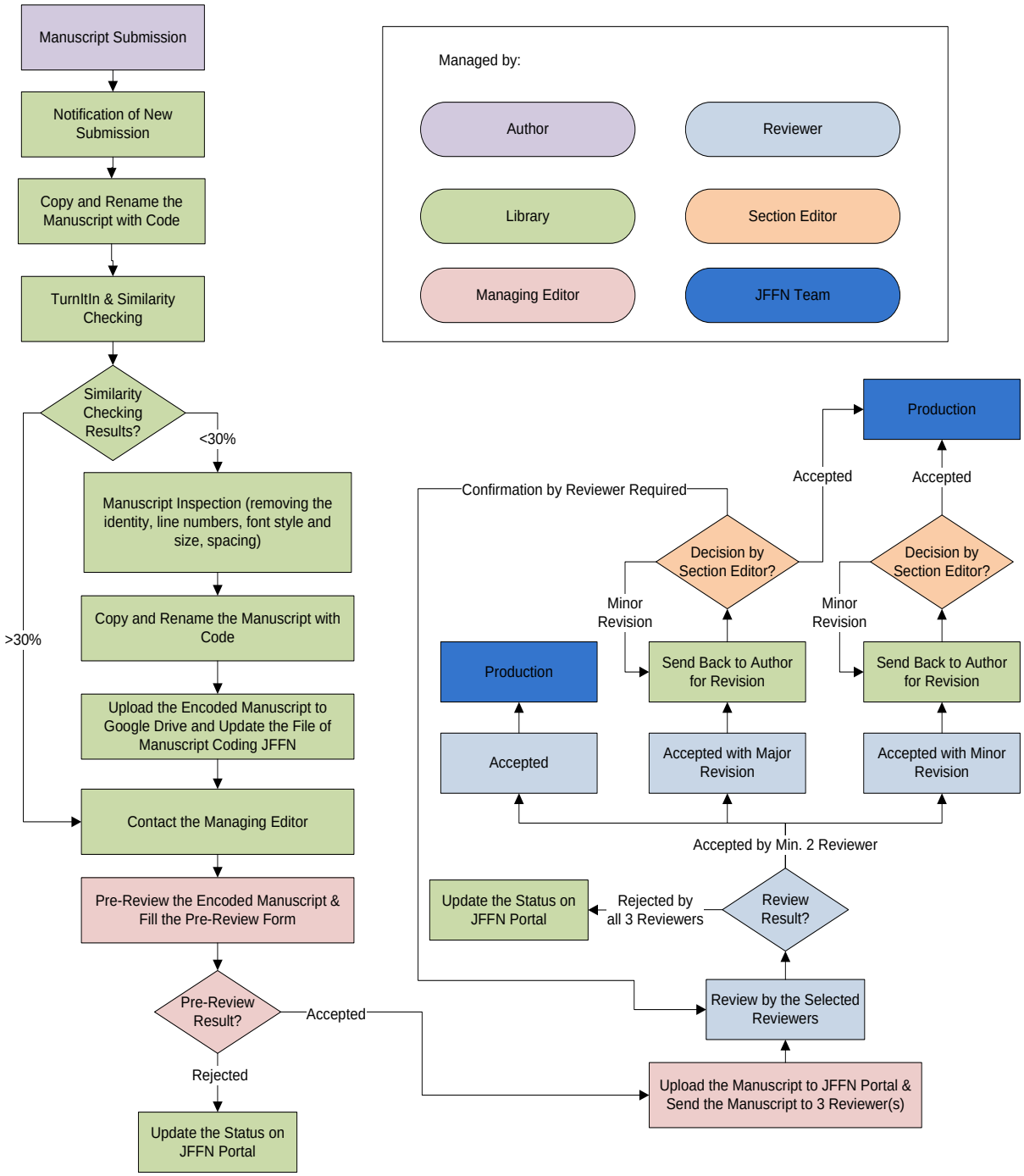
3. You have to enter the **Metadata** of your article.

Fill the **Title** with capital letters → Fill the **Abstract** of your article → You can add more **contributors** if you wrote the article with another author(s) → Fill the **keywords** by pressing enter button at the end of each → Click **Save and Continue**

4. For the last step, you will be asked to **Confirm** your submission.

After you check all the submitted document and data, click the **finish submission** button. You will receive an **email confirmation** that your article has been sent for editorial review. In your Dashboard, you can see that your article is on the **submission** process.

Flow of Manuscript Acceptance Process in JFFN



Guideline for Authors

NOTE: Please read the instructions carefully and strictly follow them to ensure smooth submission process. Papers that do not adhere to the guidelines will be rejected, hence full cooperation from the authors is highly appreciated.

- **Type of Papers**

There are three types of paper which are accepted by Journal of Functional Food and Nutraceutical: Research Papers, Review Articles, and Short Communication and Notes. Please note that the papers have not been and will not be published elsewhere, the Chief Editor reserves the right to change the paper into Short Note. The Author(s) shall retain all copyright rights held by the Author in the Manuscript.

a. **Research Papers**
   Original full-length research papers that have not been published previously, except in a preliminary form, and should not excess 7,500 words from introduction to conclusion (not including references). Research paper should not contain more than 40 references.

b. **Review Articles**
   By invitation only.

c. **Short Communications and Notes**
   Short communications of up to 3000 words from introduction to conclusion (optional), not including references, describing work that may be of a preliminary nature but merits publication. These papers should not contain more than 40 references.

- **Manuscript Preparation**

Manuscripts are accepted either in English or Indonesian language. For content written by Indonesian author/s, the author should provide title and abstract in both Indonesian and English.
Author is asked to provide manuscripts as electronic files and should be prepare using common word processor software (e.g. Microsoft-Word®) in following formats: DOC, DOCX, or RTF.
For *Research Papers* and *Short Communications and Notes* please refer to the **Template** to prepare the manuscript accordingly. Please note that the paper should be uploaded in a **SINGLE** file where title page information, manuscript, figure(s) and/or table (s) are included.

- **Originality**

The manuscript that submitted must be an original work. Authors should refer to the Code of Ethics to ensure its originality. Ensure the manuscript has not been previously published, nor is it before another journal for consideration (including published in different language).

It is recommended to check the manuscript for any possible plagiarism by using any program such as TurnitIn or any other software before submitting the manuscripts. Authors are responsible for the integrity of the work as a whole (including method, analysis, calculation, or other details), from inception to published article.

- **Publication Fee**

Submission Fee
Journal of Functional Food and Nutraceutical **will be charged IDR 500.000 per article** at the time of manuscript submission. Submission fee exemption can be applied with term and condition.

Membership Fee
For P3FNI and Swiss German University members will receive 50% discount for the submission fee.

- **Submission Preparation Checklist**

As part of the submission process, authors are required to check off their submission's compliance with all of the following items, and submissions may be returned to authors that do not adhere to these guidelines.

- The originality of the manuscript and ensure the manuscript has not been published previously – Please see "Originality"
- Manuscript text is prepared in accordance with the author guidelines and given template (for *Research Papers* and *Short Communications and Notes*).
- Ensure that some of the provisions are checked and present – **Please see file Check List:** http://bit.ly/checklistjffn.

- **Article Submission**

Online submission via *Open Journal System* (https://journal.sgu.ac.id/jffn). Please refer to the User Account Registration Guideline for submission process. After the submission, author who submit the manuscript will get a confirmation email and able to track their submission status by logging in to the system. The submission tracking includes a status of manuscript review and editorial process. If authors have any problems with the online submission, please contact JFFN admin at the following email: jffn@sgu.ac.id

Reviewer Guideline

Interested to become a reviewer?

The JFFN Editorial Team will send the manuscripts to the relevant reviewers according to the expertise of respective reviewers. If you are interested in becoming a reviewer of JFFN, please fill out the reviewer application form: http://bit.ly/revregform along with a brief summary of your expertise and your CV. Send all the documents to jffn@sgu.ac.id. The reviewers who pass the selection will obtain many benefits. All review process will be processed through JFFN online system.

1. **Confirmation (Accept or Decline)**

   The reviewers will receive an email invitation that will be sent by the JFFN system. Use the links to accept or reject the invitation. If you decide to accept the invitation as the reviewer, you will be responsible to input the review result/s to the JFFN journal editor as the requirement whether the manuscript is appropriate to be published in JFFN.

2. **Submitting the review**

   Reviews must be entered in the JFFN submission system. Drop us an email if you encounter trouble accessing the manuscript or entering your comments to jffn@sgu.ac.id.

3. **Timing**

   The deadline for completing the manuscript review process is 14 days. If you are unable to complete or need additional time for the review process, please notify us immediately so that we can keep the authors informed and assign alternative solution if necessary.

Confidentiality

All reviewers are required to maintain the confidentiality of the manuscript and never share information to the other parties without the editor's consent. The involvement of third parties in the review process, must be declared during the review process. Correspondence as part of the review process is also to be treated confidentially by all parties.

All reviewers are strongly required to keep the confidentiality of process reviews, maintain material confidentiality of manuscripts, and will not take advantage during the review process.

Anonymity

Reviewers are anonymous by default. Reviewers' identities are not revealed to authors or to other reviewers unless reviewers specifically request to be identified by signing their names at the end of their comments.

All reviewer's identity will be kept confidential. The reviewer's identity will not be revealed to anyone unless reviewers specifically request to notify the identity by writing the name on the review form or comments.

Writing the Review

Here are the generic questions to the reviewer. Please evaluate the submission based on the general scientific journal guideline. Please download the review form from: http://bit.ly/revformjffn.

The form covers:

1. Is the manuscript technically sound and do the data support the conclusion?
2. Has the statistical analysis been performed appropriately and rigorously?
3. Is the manuscript presented in an intelligible fashion and written in standard English/Indonesian?
4. Review comments to the author? Please state the positive suggestion that might support the authors to improve the manuscript.
5. If you would like your identity to be revealed to the authors, please include your name here (optional) *Your name will not be published in the manuscript.

Revisions

When an author revises a manuscript, the Academic Editor will often ask the original reviewer(s) to evaluate the authors' revision. We expect the reviewers to be available to provide these additional comments. You will be requested to suggest the acceptance of the manuscript.

In the revision process, the editorial team frequently will ask reviewers to evaluate the author's revision. The editorial team expects that all reviewers will be available to conduct evaluation and provide valuable suggestions to improve the manuscript quality. In the end, the reviewer will be asked to decide the appropriateness of the manuscript according to several categories:

- Accept without revision
- Accept with minor revision
- Accept with major revision
- Decline

Thank you to our reviewers

Peer-review is an important step to maintain the high quality of a journal. Reviewers provide scientific critiques based on their expertise that assist editors to make acceptance decision professionally. Therefore, the Editors would like to acknowledge our reviewers listed below who have contributed their valuable time for maintaining the quality of Journal of Functional Food and Nutraceutical.

List of reviewers JFFN volume 1 no 1 August 2019:

Anastasia Fitria Devi	Pusat Penelitian Kimia Lembaga Ilmu Pengetahuan Indonesia (P2Kimia LIPI)
Antonius Herry Cahyana	Universitas Indonesia
Della Rahmawati	Swiss German University
Diah Indriani Widiputri	Swiss German University
Eduan Effendi	Sekolah Tinggi Ilmu Kesehatan 'Aisyiyah Palembang
Erliana Ginting	Balai Penelitian Tanaman Aneka Kacang dan Umbi (Balitkabi), Kementerian Pertanian
Irvan S Kartawiria	Swiss German University
Kholis A. Audah	Swiss German University
Lily Arsanti Lestari	Universitas Gadjah Mada
Melanie Cornelia	Universitas Pelita Harapan
Mutiara Pratiwi	Swiss German University
Nina Artanti	Pusat Penelitian Kimia Lembaga Ilmu Pengetahuan Indonesia (P2Kimia LIPI)
Riyadh Rizky Adam	Swiss German University
Slamet Widodo	Universitas Negeri Makasar
Sylvia Yusri	Swiss German University
Tabligh Permana	Swiss German University
Wilbur Donald R. Pokatong	Universitas Pelita Harapan
Zita Sarungallo	Universitas Papua

Registrasi anggota P3FNI

Perhimpunan Penggiat Pangan Fungsional dan Nutrasetikal Indonesia (P3FNI) juga mengembangkan kontribusinya di kancah internasional bersama dengan International Society for Nutraceutical and Functional Food (ISNFF). Secara internasional terdapat klaster ISNFF seperti di Korea dan China. Untuk kepentingan percaturan internasional P3FNI menggunakan nama Indonesian Society for Functional Food and Nutraceutical (ISFFN).

Keterlibatan P3FNI atau ISFFN ini memberi manfaat anggotanya untuk memberikan kontribusi ilmu pengetahuan dan teknologi serta mengikuti pemutakhiran pangan fungsional dan nutrasetikal. Bersosialisasi dan berkesempatan dalam pertemuan ilmiah bersama penggiat pangan fungsional dan nutrasetikal dari berbagai negara untuk terus-menerus mengarah pada optimalisasi penggunaan pangan untuk kesejahteraan manusia secara bijaksana dan menjaga kelestariannya.

Siapa Yang Perlu Menjadi Anggota?

1. Akademisi dan peneliti yang terus-menerus mengembangkan dan memajukan ide alternatif dan kreatif untuk menuju kemajuan dan kesejahteraan manusia dengan menggunakan data basis ilmiah.
2. Praktisi kesehatan maupun industri yang menerapkan pangan fungsional dan nutrasetikal.
3. Mahasiswa sebagai penerus masa depan untuk melestarikan praktik-praktik pengadaan dan penggunaan pangan fungsional dan nutrasetikal secara bijaksana dan berkelanjutan.
4. Memberi advokasi dalam kasus-kasus pangan dan kesehatan.
5. Individu yang memiliki perhatian dalam pangan fungsional dan nutrasetikal.

Fasilitas Anggota P3FNI

1. Link Internasional untuk kegiatan atau program riset dan pembelajaran pangan fungsional dan nutrasetikal meliputi direktori laboratorium dan professor, lembaga kesehatan, LSM/NGO, skim hibah, dan internship/magang di industri.
2. Biaya partisipasi dalam pertemuan ilmiah yang diselenggarakan P3FNI.
3. Informasi tentang pangan fungsional dan nutrasetikal Indonesia dan isu internasional terbaru (international current issues).

Iuran Keanggotaan P3FNI

Iuran dari anggota digunakan untuk mendanai kegiatan yang diselenggarakan P3FNI untuk peningkatan keahlian anggota melalui kegiatan ilmiah. Pembayaran menurun progressif 25% jika pembayaran iuran keanggotaan untuk jangka pembayaran 2 tahun sekaligus.

Akademisi, Peneliti non-komersial	Rp 400.000
Praktisi industri, kesehatan, komersial	Rp 500.000
Mahasiswa S2 dan S3	Rp 200.000
Mahasiswa S1 (Perlu Rekomendasi)	Bebas Biaya

Pendaftaran on line anggota P3FNI dapat dilakukan dengan masuk melalui web dengan alamat: **sia.p3fni.org**

Pembayaran dapat dilakukan melalui **setor**, **transfer**, **pembayaran langsung/cash**.
Pembayaran setor dan transfer ditujukan ke no rekening berikut :
Bank BNI
Cabang HR MUHAMMAD
No. rekening 0390796832
a.n. Indah Epriliati