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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

THE CHARACTERISTICS OF SYNBIOTIC YOGHURT ICE CREAM MADE FROM ICE CREAM MIX AND PURPLE YAM YOGHURT (*DIOSCOREA ALATA*)

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ABSTRACT

Synbiotic yoghurt ice cream is a kind of ice cream made from ice cream mix (milk, skim milk and sugar) and purple yam yoghurt as the main ingredient. The inulin, prebiotic compound of purple yam, inulin, can trigger the growth of beneficial bacteria so it would beneficial for the health. The addition of stabilizer, xanthan gum, aims to produce yoghurt ice cream with a soft texture. The aim of this study to determine the effect of proportion of ice cream mix:yoghurt and concentration of xanthan gum on yoghurt ice cream characteristics. This study used a completely randomized design-factorial pattern with two factors and three replications. The factors are proportion of ice cream mix:yoghurt (70:30; 60:40; 50:50) and concentration of xanthan gum (0.1%; 0.2%; 0.3.%). The observed data were analyzed using ANOVA, followed by DMRT test at 5% level if there were significant differences. The study revealed that the best treatment, based on physicochemical properties of ice cream, is the proportion of ice cream mix:yoghurt (60:40) and xanthan gum concentration (0.2%) with total LAB of 8.55 log CFU/ml; overrun 27.69%; melting time 13.51 min/10 gr; total dissolved solids 31.00 ^{O}Brix; viscosity 517.00 mPas.

Keywords: purple yam; synbiotic; xanthan gum; yoghurt ice cream

ABSTRAK

Es Krim yoghurt sinbiotik adalah jenis es krim yang dibuat dari *ice cream mix* dan yoghurt uwi ungu sebagai bahan utamanya. Komponen prebiotik di dalam uwi ungu, yaitu inulin, dapat meningkatkan pertumbuhan bakteri baik sehingga bermanfaat untuk kesehatan manusia. Penambahan bahan penstabil, xanthan gum, bertujuan untuk menghasilkan es krim yoghurt dengan tekstur yang lembut. Penelitian ini bertujuan untuk mengetahui pengaruh *ice cream mix*:yoghurt dan konsentrasi xanthan gum terhadap karakteristik es krim yoghurt. Penelitian ini menggunakan Rancangan Acak Lengkap Pola Faktorial dengan dua faktor dan 3 ulangan. Faktor-faktor yang digunakan adalah proporsi *ice cream mix*:yoghurt (70:30; 60:40; 50:50) dan konsentrasi xanthan gum (0,1%; 0,2%; 0,3.%). Data yang diperoleh dianalisa menggunakan analisis ragam dan diikuti dengan Uji Duncan's (pada taraf 5%) jika terdapat perbedaan yang nyata. Hasil penelitian menunjukkan perlakuan terbaik (berdasarkan sifat fisiko kimia es krim), adalah proporsi *ice cream mix*:yoghurt (60:40) dan konsentrasi xanthan gum (0.2%) yang mempunyai total BAL 8,55 log CFU/ml; pH 5,33; total asam tertitrasi 0,46%; *overrun* 27,67%; kecepatan meleleh 13,51 menit/10 gr; total padatan terlarut 31,00 ^{O}Brix; dan viscositas 517,00 mPas.

Kata kunci: es krim yoghurt; uwi ungu; sinbiotik; xanthan gum

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INTRODUCTION

Synbiotic yoghurt ice cream is a kind of ice cream made from ice cream mix and synbiotic yoghurt as the main ingredient. Synbiotic yoghurt is made by combining probiotic bacteria and prebiotic agents (Puspitasari et al., 2015). Making yoghurt ice cream is done by mixing yoghurt and ice cream mix (ice cream dough) then frozen (Goff and Hartel, 2013). In this study, synbiotic yoghurt was made by mixing milk and purple yam filtrate as a source of prebiotics and lactic acid bacteria as a source of probiotics (Rosida, et al., 2019).

Winarti (2010) stated that purple yam (*Dioscorea alata*) contains prebiotic agent in the form of inulin. In the large intestine, inulin undergoes fermentation due to the activity of the microflora contained in the large intestine so that it has positive implications for body health (Widowati, 2005). Based on the research of Maryati et al. (2016), *L. acidophilus* FNCC0051 was able to utilize inulin better than other lactic acid bacteria. Thus, in this study, the probiotic bacteria *L. bulgaricus* was used, besides *S. thermophilus* and *L. acidophilus*.

The characteristics of yoghurt ice cream can be influenced by the proportion or addition of yoghurt to the ice cream mix. According to Soukolis and Tzia (2008) the mixed yoghurt ranges from 5-70% of the volume of the ice cream mix. The addition of yoghurt to the ice cream mix resulted in the texture of the yoghurt ice cream being rough, so it was necessary to add a stabilizer to overcome this problem. The stabilizer serves to increase the thickness of the dough, prolong the melting rate, improve the texture and reduce the formation of large ice crystals, with the presence of a stabilizer making the dessert smoother and softer (Goff and Hartel, 2013).

Research by Soukolis and Tzia (2008) revealed that the use of xanthan gum 0.2% in yoghurt ice cream was the best treatment compared to guar gum and Carboxymethyl Cellulose (CMC). Based on this, this research study the production of synbiotic yoghurt ice cream made by the proportion of ice cream mix:yoghurt and the concentration of xanthan gum.

MATERIALS AND METHOD

The materials used for the research were purple yam obtained from farmers in Nganjuk, ice cream mix (consists of milk, skim milk, sucrose), xanthan gum, yoghurt starter mix (consists of *L. bulgaricus, S. thermophilus* and *L. acidophilus)* obtained from the Faculty of Science and Technology-Airlangga University.

The equipments used for the research are analitycal balance, blender, refrigerator, mixer, ice cream maker, pan, stirrer, measuring glass, knife, filter cloth, thermometer, laminar air flow, incubator.

Making purple yam synbiotic yoghurt

The purple yam filtrate was made by crushing the purple yam (purple yam: water = 1:4 (w/v)) using blender. The juice was filtered using filter cloth to obtain the purple yam filtrate. Heating the filtrate at a temperature of 80^{o}C for 15 minutes while stirring continuously. Milk was pasteurized at 80^{o}C for 15 minutes and mixed with purple yam filtrate (50:50) (v/v), 10% skim milk (w/v) and 5% sugar (w/v). The medium was then cooled down to 40^{o}C. Yoghurt starter (*S. thermophilus: L. bulgaricus: L. acidophilus* =1:1:1) was inoculated (5%) in the medium (v/v), and then incubated at 37°C for 18 hours. (Rosida et al., 2019)

Making synbiotic yoghurt ice cream

Making ice cream mix is done by heating milk, 10% skim milk (w/v), 18% (w/v) sugar to a temperature of 45°C, then added xanthan gum according to treatment (0.1%; 0.2 % ; 0.3%) (w/v). Then pasteurization (temperature 85^{o}C for 10 minutes), homogenization, and aging (temperature 4^{o}C for 12 hours). Then mixed ice cream mix and purple yam synbiotic yoghurt according to treatment (70:30 ; 60:40 ; 50:50) and processed using an ice cream maker for 30 minutes, and frozen of the ice cream (temperature -10^{o}C for 24 hours) (Venkateshaiah et al., 1997). The ice cream yoghurt was directly analyzed for Total LAB by Total Plate Count Method (Fardiaz, 1992), pH (SNI, 2009) , overrun

(Zahro and Fithri, 2015), melting time (Zahro and Fithri, 2015), total soluble solid (SNI, 2009), and viscosity (Zahro and Fithri, 2015).

RESULTS AND DISCUSSION

Analysis of purple yam synbiotic yoghurt

Table 1. Average of chemical composition of purple yam synbiotic yoghurt

Parameter	Analysis Results
Total lactic acid bacteria (logCFU/ml)	9.31±0.137
pH	4.53±0.05
Total acid (%)	1.15±0.041
Total soluble solid (^{O}Brix)	13.83±0.764
Inulin content (%)	0.54±0.010
Dietary fiber content (%)	1.51±0.437

Table 1. Showed that total lactic acid bacteria in accordance with the requirements of SNI yoghurt which requires a minimum of 10^7 CFU/ml starter or 7 logCFU/ml. Gustaw et al., (2011) stated that the activity of lactic acid bacteria will increase when added with prebiotics. The prebiotics found in water yam are able to stimulate the growth of probiotic bacteria found in yoghurt, it will increase the number of probiotic bacteria (Andriyani et al., 2013).

Characteristics of purple yam synbiotic yoghurt ice cream

Table 2. Showed that the average total LAB of yoghurt ice cream was not significantly different, which ranged from 8.54-8.63 logCFU/ml. This was because in the process of making ice cream there was no further fermentation, so that the treatment with different proportions produced almost the same total LAB. This was in accordance with the research of Pangga et al. (2018) and Baay et al. (2018), which stated that the addition of yoghurt in yoghurt ice cream production resulted in LAB populations that were not significantly different.

Table 2. The average value of the total lactic acid bacteria and ph of yoghurt ice cream from the treatment of the proportion of ice cream mix: yoghurt and xanthan gum concentration

Treatment		Total LAB (log CFU/ml)*	pH*
Ice cream mix : yoghurt	Xanthan gum (%)		
70:30	0.1	8.55 ±0.073	5.47 ±0.115
	0.2	8.55 ±0.069	5.53 ±0.115
	0.3	8.54 ±0.068	5.57 ±0.058
60:40	0.1	8.63 ±0.053	5.30 ±0.100
	0.2	8.56 ±0.004	5.33 ±0.058
	0.3	8.54 ±0.058	5.43 ±0.058
50:50	0.1	8.63 ±0.019	5.20 ±0.100
	0.2	8.61 ±0.068	5.23 ±0.115
	0.3	8.59 ±0.011	5.27 ±0.058

Note: *The average value in the same column showed no significant difference at p≤0.05

Table 2. Revealed that the lower the proportion of ice cream mix or the higher the proportion of yoghurt, the lower the pH of yoghurt ice cream. The yoghurt used in this study had a pH of 4.53. This was in accordance with Pangga et al. (2014) The pH of yoghurt ice cream was influenced by the proportion of yoghurt used as the main ingredient in making yoghurt ice cream.

Table 3. Showed that the lower the proportion of ice cream mix or the higher the proportion of yoghurt and the higher ice cream overrun, however the the higher xanthan gum concentration, the lower the yoghurt ice cream overrun. Mahdian et.al. (2012) stated that the lactic acid present in yoghurt lowered the pH of the yoghurt ice cream dough so that it clumps the protein contained in the yoghurt ice cream dough. The clumping increases the viscosity of the yoghurt ice cream dough, so that during the agitation process, the dough cannot expand optimally which results in low product overrun. Purwadi (2019) added that protein solubility decreases when the pH reaches 4.5-5.4 (isoelectric pH) then the protein would coagulate.

The higher the xanthan gum concentration, the lower the overrun and the longer the melting time of ice cream. This is because xanthan gum would bind to water thereby preventing the formation of large ice crystals and slowing down the melting

time. The increase in the concentration of xanthan gum caused the dough becomes thicker so that the binding power to water is stronger so that the yoghurt ice cream did not melt quickly. Goff and Hartel (2013) stated that the stabilizer would increase the viscosity of ice cream so that the resulting ice cream had low overrun and soft texture due to the formation of small ice crystals and slow melting time. Goff and Hartel (2013) stated that good quality of melting time for ice cream ranges from 15- 20 minutes.

Table 3. The average of overrun, melting time, total dissolved solids and viscosity of yoghurt ice cream

Treatment		Overrun (%)	Melting time (min/10 g)	Total soluble solid (°Brix)	Viscosity (mPas)
Ice cream mix : yoghurt	Xanthan gum (%)				
70:30	0.1	27.69 ^{a} ±0.541	13.51 ^{i} ±0.060	31.00 ^{d} ±0.000	490.00 ^{i} ±2.598
	0.2	27.08 ^{a} ±0.541	14.23 ^{h} ±0.053	31.17 ^{e} ±0.289	512.83 ^{h} ±2.566
	0.3	26.81 ^{b} ±0.130	15.54 ^{g} ±0.127	33.00 ^{f} ±0.000	545.50 ^{g} ±1.803
60:40	0.1	25.33 ^{c} ±0.577	15.53 ^{f} ±0.032	26.67 ^{c} ±1.155	553.33 ^{f} ±1.041
	0.2	24.05 ^{c} ±0.820	16.33 ^{e} ±0.010	28.67 ^{d} ±0.577	566.67 ^{e} ±3.329
	0.3	23.34 ^{c} ±0.649	16.51 ^{d} ±0.020	30.17 ^{d} ±0.289	571.17 ^{d} ±0.289
50:50	0.1	22.51 ^{d} ±0.493	17.36 ^{c} ±0.021	23.33 ^{a} ±0.577	575.50 ^{c} ±0.500
	0.2	20.39 ^{e} ±0.907	19.22 ^{b} ±0.012	24.50 ^{a} ±0.500	579.17 ^{b} ±1.143
	0.3	18.88 ^{f} ±0.467	21.43 ^{a} ±0.115	25.00 ^{b} ±0.000	604.50 ^{a} ±4.093

Note: The average value in the same column followed by different notations showed a significant difference at $p \leq 0.05$

Table 3 showed the higher the proportion of ice cream mix or the lower the proportion of yoghurt and the higher the concentration of xanthan gum, the higher the melting time, viscosity and total dissolved solids of yoghurt ice cream. Mahdian et al. (2012) stated that yoghurt added to yoghurt ice cream dough has a low pH causing protein clumping so that the dough becomes thicker and difficult to trap air causing increased viscosity, decreased yoghurt ice cream overrun and ice cream did not melt easily. Goff and Hartel (2013) stated that high acidity in ice cream would increase the viscosity and the melting time of ice cream would be longer. Besides that, the addition of stabilizer would increase the viscosity and total dissolved solids. Agustina et al. (2019) stated the mechanism of xanthan gum as a stabilizer, namely the hydroxyl group on xanthan gum would absorb water thereby increasing viscosity. The study revealed that the best treatment is the proportion of ice cream mix:yoghurt 60:40 and xanthan gum concentration 0.2% because it had the highest overrun and total LAB 8.63 logCFU/ml which had met minimum LAB (7.0 logCFU/ml) in yoghurt standard (SNI 2981-2009)

CONCLUSION

The results showed that the best treatment, based on physicochemical properties of ice cream, was the proportion of ice cream mix:yoghurt treatment 70:30 with a concentration of 0.1% xanthan gum, that produced purple yam synbiotic yoghurt ice cream with total LAB of 8.55 logCFU/ml; overrun 27.69%; melting time 13.51 min/10gr; total dissolved solids 31.00°Brix; viscosity 517.00 mPas.

REFERENCES

Agustina, M., Fahrizal dan Indarti, E. 2019. Penambahan CMC, Gum Xanthan, dan Pektin Sebagai Stabilizer Pada Sirup Air Kelapa. *Jurnal Ilmiah Mahasiswa Pertanian Unsyiah*. 4 (2), pp.266-273.

Arbuckle, W.S. and Marshall, R.T. 2000. *Ice Cream*. 5th ed New York: Chapman And Hall

Azhar, M. 2009. Inulin Sebagai Prebiotik. *SAINSTEK*. XII(1), pp.1-8.

Baay, M.G. Ramirez, M.A.J.R. and Gorne, A.S.C. 2018. Product Optimization of Yoghurt Ice Cream Flavored With Fermented Cacao Butter (*Theobroma cacao* L.). *Journal of Science, Engineering and Technology* 6, pp.137-148.

Budhiarto, S. 2003. Pengaruh Lama Pengukusan dan Sistem Pengeringan (Kabinet dan Vakum) Terhadap Kualitas Tepung Ubi Ungu Jepang. Skripsi. Malang: Universitas Brawijaya.

Chotimah, S.C. 2009. Peranan *Streptococcus thermophilus* dan *Lactobacillus bulgaricus* Dalam Proses Pembuatan Yoghurt. Review. *Jurnal Ilmu Peternakan*. 4(2), pp.47-52.

Ezeocha, V.C. and Ojimelukwe, P.C. 2012. The impact of cooking on the proximate composition and anti-nutritional factors of water yam (*Dioscorea alata*). *Journal of Stored Products and Postharvest Research*. 3(13), pp.172–176.

Fardiaz, S. 1992. *Mikrobiologi Pangan I*. Jakarta: Gramedia Pustaka Utama.

Franck, A. and De Leenher, L. 2005. *Polysaccharides and Polyamides in The Food Industry*. 1st ed. Weinherim: Wiley-Vch.

Goff, H.D. and Hartel, R.W. 2013. *Ice Cream*. 7th ed. New York: Springer.

Gustiani, S., Helmy, Q., Kasipah, C. and Novarini, E. 2017. Produksi dan Karakterisasi Gum Xanthan dari Ampas Tahu Sebagai Pengental Pada Proses Tekstil. *Arena Tekstil.* 32(2), pp.51-58.

Jannah, A. M., Legowo, A. M., Pramono, Y.B., Al-Baari, A.N., dan Abduh, S.B.M. 2014. Total Bakteri Asam Laktat, Ph, Keasaman, Citarasa dan Kesukaan Yoghurt Drink dengan Penambahan Ekstrak Buah Belimbing. *Jurnal Aplikasi Teknologi Pangan*. 3(2), pp.7-11.

Kailasapathy, K. 2002. Microencapsulation of

Technology and Potential *Applications. Curr. Issues Intest. Microbiol*. 3, pp.39-48.

Korengkeng, A.C., Yelnetty, A., Hadju, R. dan Tamasoleng, M. 2020. Kualitas Fisikokimia dan Mikrobia Yoghurt Sinbiotik yang Diberi Pati Termodifikasi Umbi Ungu (*Dioscorea alata*) dengan Level Berbeda. *Zootec*. 40(1), pp.124-133.

Mahdian, E., Tehrani, M.M. and Nobahari, M. 2012. Optimizing Yoghurt-Ice Cream Mix Blend in Soy Based Frozen Yoghurt. *J. Agr. Sci. Tech*. 14, pp.1275-1284.

Maryati, Y., Nuraida, L. dan Hariyadi, R. D. 2016. Kajian Isolat Bakteri Asam Laktat Dalam Menurunkan Kolesterol Secara *in vitro* dengan Keberadaan Oligosakarida. *AGRITECH*, 36(2), pp.196-205.

Mudannayake, D.C., Wimalasiri, K.M.S., Silva, K.F.S.T. and Ajlouni, S. 2015. Selected Sri Lankan Food Plants and Other Herbs as Potential Sources of Inulin-Type Fructans. *J. Natn. Sci. Foundation Srilanka*. 43(1), pp.35-43.

Mulyani, T., Sudaryati dan Susanto, A. 2013. Kajian Peran Susu Skim dan Bakteri Asam Laktat Pada Minuman Sinbiotik Umbi Bengkuang (*Pachyrrhizus erosus*). *Jurnal Teknologi Pangan*. 5(1), pp.76-100.

Mustika, S., Yasni, S. dan Suliantari. 2019. Pembuatan Yoghurt Susu Sapi Segar dengan Penambahan Puree Ubi Jalar Ungu. *Jurnal Pendidikan Teknologi Kejuruan*. 2(3), pp.97-101.

Ochoa, F.G., Santos, V.E., Casas, J.A. and Goamez, E. 2000. Research Review Paper Xanthan Gum: Production, Recovery, and Properties. *Biotechnology Advances*. 18, pp.549-579.

Padaga, M. dan Sawitri, M.E. 2015. *Es Krim Yang Sehat*. Surabaya: Trubus Agrisarana.

Pangga, N. R.. Rossi, E. dan Rahmayuni. 2014. Penggunaan *Whipping Cream* Es Krim

. *JOMFAPERTA*. 1 (1).

Purwadi. 2019. *Ilmu Dan Teknologi Pengolahan Keju*. Malang : UB Press.

Puspitasari, D., Radiati, L.E. dan Thohari, I. 2015. Pengaruh Lama Simpan Es Krim Yoghurt Sinbiotik dengan Penambahan Pati Ubi Cilembu Terhadap Viskositas, Eksopolisakarida, dan Total Padatan. Malang : Universitas Brawijaya.

Ramadhan, K., Atmaka, W. dan Widowati, E. 2015. Kajian Pengaruh Variasi Penambahan Xanthan Gum Terhadap Sifat Fisik dan Kimia Serta Organoleptik Fruit Leather Kulit Buah Naga Daging Super Merah (*Hylocereus costaricensis*). *Jurnal Teknologi Hasil Pertanian*. 8(2), pp.115-122.

Rini, A.S. 2019. Pasar Indonesia Menjanjikan Produsen Es Krim Bermunculan. [diakses tanggal 5 Maret 2020]. https://ekonomi.bisnis.com/read/20190411/9/910680/pasar-indonesia-menjanjikan-produsen-eskrim-bermunculan.

Rosida dan Santi, S.S. 2019. Pengembangan produk yoghurt dan es krim sinbiotik uwi ungu (*Dioscorea alata* var purpurea) sebagai pangan fungsional. *Laporan Penelitian Riset Inovasi dan Penerapan Ipteks*. Surabaya : LPPM UPN Veteran Jawa Timur.

Sawitri, M.E. dan Sari, E.P. 2020. Prospek Frozen Yoghurt Sinbiotik Fortifikasi Dengan Ekstrak Kulit Buah Naga Merah (*Hylocereus polyrhizus*) dan Fruktosa, Mendukung Gaya Hidup Sehat Pasca Pandemi Covid-19. *Prosiding Seminar Teknologi Dan Agribisnis Peternakan VII, Purwokerto*.

SNI. 1995. SNI 01-3713-1995. Es Krim . Jakarta : Badan Standarisasi Nasional.

SNI. 2009. SNI 2981-2009. Yoghurt. Jakarta : Badan Standarisasi Nasional.

Soukolis, C. and Tzia, C. 2008. Impact of the Acidification Process. Hydrocolloids and Protein Fortifiers on the Physical and Sensory Properties of Frozen Yoghurt. International Journal of Dairy Technology, 61 (2):17-177.

Widodo, W. 2002. Bioteknologi Fermentasi Susu. Pusat Pengembangan Bioteknologi.Universitas Muhammadiyah, Malang.

Widowati, S., Sunarti, T.C. dan Zaharani, A. 2005. Ekstraksi. Karakterisasi. dan Kajian Potensi Prebiotik Inulin dari umbi Dahlia *(Dahlia pinnata* L.). *Prosiding Seminar Rutin Puslitbang Tanaman Pangan, Bogor*.

Winarti, S. 2010. Makanan Fungsional. Surabaya: PT. Graha Ilmu.

Venkateshaiah, B.V., Atmarara, K., and Gireesh, T. 1997. Development of a Frozen Yoghurt Dessert of Sensory Acceptability. *Tropical Agricultural Research*. 9, pp.62-68.

Zahro, C., dan Fithri, C.N. 2015. Pengaruh Penambahan Sari Anggur (*Vitis vinifera* L.) Terhadap Karakteristik Fisik, Kimia Dan Organoleptik Es Krim. *Jurnal Pangan dan Agroindustri*. 3(4), pp.1481-1491

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EFFECT OF ETHANOL CONCENTRATION AND EXTRACTION TIME WITH MICROWAVE ASSISTED EXTRACTION ON ANTIOXIDANT ACTIVITY OF TEMULAWAK-EXTRACT *(Curcuma xanthorrhiza.Roxb)*

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ABSTRACT

Temulawak (*Curcuma xanthorrhiza* Roxb) is commonly used as traditional medicine. This study aimed at determining the effect of ethanol concentration and of the duration of microwave-assisted extraction (MAE) on antioxidant activity of *C. xanthorrhiza* ethanol extract. *C. xanthorrhiza* rhizomes were extracted using 70%, 80% and 90% (v/v) ethanol for 5, 7 and 9 minutes. Compared to control (without microwave extraction) and analyzed for curcumin content, total phenolic content (TPC), and 2.2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity (RSA). Increasing the ethanol concentration from 70% to 90% as solvent and increasing the extraction time from 0 to 7 minutes at 240 watt caused an increase in curcumin content, TPC, and DPPH RSA. Curcumin, TPC, and DPPH RSA of *C. xahorrhiza* extract using 90% ethanol solvent for 7 minutes were the largest compared to other treatments, namely 184 mg/g, 319.5 mg/g and 71.08%; respectively.

Keywords: antioxidant; ethanol extract; microwave assisted extraction

ABSTRAK

Temulawak (*Curcuma xanthorrhiza* Roxb) biasa digunakan sebagai obat tradisional. Penelitian ini bertujuan untuk mengetahui pengaruh konsentrasi etanol dan lama ekstraksi berbantu gelombang mikro (MAE) terhadap aktivitas antioksidan ekstrak etanol *C. xanthorrhiza*. Rimpang *C. xanthorrhiza* diekstrak menggunakan etanol 70%, 80% dan 90% selama 5, 7 dan 9 menit dibandingkan kontrol (tanpa ekstraksi gelombang mikro) dan dilakukan analisis kurkumin, total fenol, dan kapasitas penangkapan radikal DPPH. Peningkatan konsentrasi etanol dari 70% sampai 90% sebagai pelarut dan peningkatan lama ekstraksi 0 sampai 7 menit daya 240 watt menyebabkan peningkatan kadar kurkumin, total fenol, dan kapasitas penangkapan radikal DPPH. Kurkumin, total fenol, dan kapasitas penangkapan radikal bebas dari ekstrak *C. xanthorrhiza* menggunakan pelarut etanol 90% selama 7 menit adalah paling besar dibandingkan perlakuan yang lain yaitu masing-masing 184 mg/g, 319,5 mg/g dan 71,08%.

Kata kunci: antioksidan; ekstrak etanol; ekstraksi berbantu gelombang mikro

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INTRODUCTION

Lipid oxidation can occur in food products and in the human body. Oxidation of food causes rancidity and damages to vitamins, pigments and proteins. Lipid oxidation can also lead to the emergence of degenerative diseases, such as coronary heart disease, stroke and diabetes mellitus. Antioxidants can inhibit lipid oxidation.

Temulawak (*Curcuma xanthorrhiza Roxb*) is an Indonesian native plant with important medicinal value (Dharma 1985). *C. xanthorrhiza* as a medicinal plant has properties as hepatoprotection, anti-inflammatory, anticancer, antidiabetic, antimicrobial, preventing cholera, and antihyperlipidemia (Hwang et al., 2006). Traditionally, this plant is used as a health supplement known as "herbal medicine" or to treat particular health disorders including hepatitis, liver complaints, diabetes, rheumatism, anticancer, hypertension, and heart disorders (Salleh et al., 2016).

The efficacy of *C. xanthorrhiza* may be due to its activity as an antioxidant. According to Gordon (1990), antioxidants are substances with the ability to prolong or prevent the lipid oxidation process. *C. xanthorrhiza* and turmeric acetone extract from sliced rhizomes dried using a cabinet dryer for 14 h have a higher inhibitory activity of peroxide and malonaldehyde formations from linoleic acid than ginger (Septiana et al., 2006a). The antioxidant activity of decoction of dried *C. xanthorrhiza* is greater than that of dry turmeric (Samsudin and Panigoro, 2013). *C. xanthorrhiza* extract is potential to be used as antiatherosclerosis because it has inhibitory activity of LDL oxidation and macrophage cholesterol accumulation (Septiana et al., 2006b). The bioactive components of *C. xanthorrhiza*, such as curcuminoids (Menon and Sudheer, 2007) and xanthorrhizol (Jantan et al., 2012) can function as antioxidants. *C. xanthorrhiza* also contains bioactive components in the form of alkaloids, saponins, quinones and triterpenoids (Panigoro et al., 2013).

Various extraction methods have been developed to extract bioactive components from natural sources, including several innovative technologies such as microwave-assisted extraction (MAE), ultrasonic-assisted extraction, enzyme-assisted extraction, and several conventional methods such as maceration and Soxhlet extraction. Compared to the maceration and soxhlet methods, MAE method has some advantages, such as shorter extraction time and lower temperature, which leads to less degradation of thermally labile compounds (Li et al., 2017). In addition, MAE is also compatible with water as an extraction solvent so that the use of organic solvents is reduced or eliminated, and more non-polar organic compounds from plant materials could also be extracted by MAE (Zghaibi et al., 2019). The use of a solvent mixture of ethanol and water is widely used for extraction because of the difference in polarity of the two, so that the polarity can be adjusted based on the proportion of ethanol with water, and the solvent mixture is safe for humans. This study was conducted with the aim of determining the optimum proportion of ethanol with water as a solvent in the extraction of *C. xanthorrhiza* and the optimum duration of microwave-assisted extraction (MAE) on the antioxidant activity of the resulting extract.

MATERIALS AND METHOD

Materials

The main materials used in this study were *C. xanthorrhiza* from farmers in Purbalingga, Central Java, while the chemicals used in this study were methanol, ethanol, standard curcumin, gallic acid, Na_2CO_3, folin ciocalteau, and DPPH or 1,1-diphenyl -2-picrylhydrazyl (Merck, Germany).

The tools used in this study were scales (Ohaus, United States), erlenmeyer, beaker glass, test tubes (Pyrex, Germany), vortex, microwave (Electrolux), blender (Philip, Neetherlands), UV-Vis spectrophotometer (Shimadzu 1240, Japan), and cabinet dryer.

Method

Preparation of *C. xanthorrhiza* ethanol extract

First, the rhizomes of *C. xanthorrhiza* were washed, drained and dried and powderized. The *C.*

xanthorrhiza powder was obtained from fresh *C. xanthorrhiza* rhizomes sliced 4 mm, dried at 57^0C for 14 h using a cabinet dryer, crushed and filtered using a 60 mesh filter. A total of 5 g of *C. xanthorrhiza* powder was dissolved in 75 ml 70%, 80% or 90% ethanol solvent (1:15 g/ml), allowed to stand for 20 min. (Handayani *et al*., 2014), and put in a 240 Watt microwave for 0, 5, 7 and 9 min. Every one minute, the radiation will be paused for one to two minutes, which was carried out to avoid the temperature from increasing to above the boiling point of the solvent (Li et al., 2009). The extract was then cooled down to room temperature, and filterred using a filter cloth, then filterred again using Whatman No.1 Filter Paper to produce the filtrate. Next, the filtrate was concentrated using a rotary evaporator, at a temperature of 60°C.

Analysis of curcumin content (Delfiya et al., 2014)

Curcumin content of ethanolic extract of *C. xanthorrhiza* tested using curcumin as a standard. The standard curve made by making a series of dilutions of curcumin in ethanol with various concentrations (0.2; 0.4; 0.6; 0.8 and 1.0 µg/mL). Analysis of curcumin from various ethanolic extracts of *C. xanthorrhiza* with different MAE durations and standard curcumin carried out by placing each sample in a spectrophotometer cuvette and the absorbance measured at a 425 nm. Absorbance vs curcumin standard concentration curve was made by connecting a line at these points and the correlation equation was made. Curcumin concentrations were calculated using a curcumin standard absorbance vs concentration graph. Tests on aqueous extracts were carried out in the same method as the standard based on the curcumin standard concentration.

Analysis of total phenolic content (Hammerschmidt and Pratt, 1978)

Total phenolic content (TPC) of *C. xanthorrhiza* extract was tested using gallic acid as a standard. The standard curve was made by making a series of dilutions of gallic acid in ethanol. Each gallic acid and 0.1 mL ethanol extract of *C. xanthorrhiza* were added with 2 mL of Na_2CO_3, then mixed and allowed to stand for 2 minutes, added 0.1 mL of folin ciocalteau and mixed using a vortex. The mixture was then kept in the dark for 30 minutes and the absorbance was measured at a wavelength of 750 nm using spectrophotometry.

Free radical scavenging capacity (Sheikh et al., 2009)

The extract solution was prepared by dissolving the sample of ethanol extract of *C. xanthorrhiza* in methanol. Then, 2 ml of ethanol extract was mixed with 2 ml of 0.16 mM DPPH solution in methanol. The mixture shaken for 1 minutes, left for 30 minutes in the dark and the absorbance was measured at 517 nm. The ability to capture DPPH radicals is calculated by the following equation:

Radical scavenging capacity (%)

$$\frac{Abs.Control - Abs.Sample}{Abs.\ Control} \times 100\%$$

Abs: Absorbance

Statistical analysis

DPPH radical scavenging capacity, curcumin content, and total phenolic content analysed using Analysis of variance (ANOVA) at the 5% level. If there was a significant effect on the treatment, the analysis continued with Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

The results in Table 1 show that the increase in ethanol concentrations of 70% to 90% increased TPC of *C. xanthorrhiza* extract. The results of this study are in line with the study conducted by Do et al (2014), stating that the use of ethanol concentrations of 50% to 100% increased total phenolic content of *Limnophita aromatica* extract. The increase in ethanol concentration does not necessarily increase total phenolic content depending on the type of sample. The highest total phenol content of *Vermonia amygdalian* ethanol extract using microwave assisted extraction (MAE) was obtained using 60% ethanol solvent compared to 40% and 80% ethanol solvent (Alara et al,, 2020).

Total phenolic content test used to test compound

content belonging to the phenolic compounds group. Phenolic compounds are characterized by the presence of a phenyl ring containing one or more hydroxyl substitutes. Thus, total phenolic content has a very diverse structure identified as phenolic monomer, dimer, and polymeric. Some phenolic compounds include simple phenolics, benzoquinones, phenolic acids, flavonoids, and condensed tannins (Lattanzio, 2013). Thus, phenolic compounds have different polarities depending on the material.

Table 1. Curcumin content, total phenolic content and DPPH radical scavenging capacity of *C. xanthorrhiza* ethanol extract

Treatment	Curcumin content (mg/g)	Total phenolic content (mg/g)	DPPH scavenging at 4000 ppm
Ethanol concentration			
70% ethanol	100.5^{c}	170.75^{c}	55.67^{b}
80% ethanol	124.5^{b}	232^{b}	58.51^{b}
90% ethanol	153.25^{a}	259.5^{a}	64.96^{a}
Duration of MAE			
0 minutes	106^{c}	202.5^{c}	53.40^{c}
5 minutes	128.25^{b}	229^{b}	62.57^{b}
7 minutes	151^{a}	271.5^{a}	65.47^{a}
9 minutes	119^{b}	182.75^{c}	57.40^{b}

Curcumin belongs to a group of phenolic compounds that are commonly found in *C. xanthorrhiza*. The increase in ethanol solvent concentration of 70% to 90% increased curcumin content of ethanolic extract of *C. xanthorrhiza*. The results of this study are in line with study on the curcumin content of turmeric extract (Paulucci et al., 2013) and multistage extraction of *C. xanthorrhiza* (Anggoro et al., 2015) extracted using 96% ethanol which was higher than that using 70% ethanol.

Curcumin content and total phenolic content of *C. xanthorrhiza* extract were also affected by the duration of Microwave-Assisted Extraction (MAE) from 0 to 9 minutes. The increase in the extraction time from 0 to 7 minutes at 240 watt increased curcumin content and total phenolic content of *C. xanthorrhiza* extract. Extraction using MAE method utilizes microwave radiation to accelerate selective extraction through heating the solvent quickly and efficiently, it generates tremendous pressure on cell wall due to swelling of plant cells and the pressure developed pushes cell wall from inside, stretch the cell wall and ruptures them (Jain et al., 2009) thereby increasing the contact between the sample and the solvent. The increase in the extraction time up to 7 minutes increased the contact time between the sample and the solvent, so that the amount of compounds extracted increased. The increase in the extraction time from 7 to 9 minutes reduced curcumin content and total phenolic content of *C. xanthorrhiza* extract because it was suspected that prolonged extraction cause the degradation of some antioxidants. Similar results were reported in studies on the extraction of total phenolic from blackthorn flowers (Lovric et al., 2017).

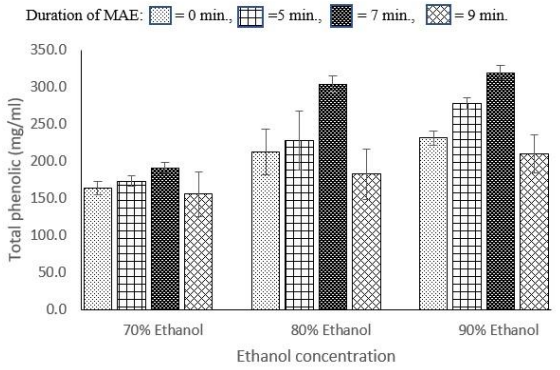

Figure 1. Effect of solvent type and duration of microwave-assisted extraction on total phenolic content of *C. xanthorrhiza* ethanol extract

The results of this study in Figure 1 show that at a concentration of 70%, the addition of extraction time does not play a role in the changes in the total phenolic content when compared to the concentrations of 80% and 90%. The increase in duration of MAE from 0 min. to 7 min. further increased total phenolic content of *C. xanthorrhiza* extract with 90% ethanol compared to 70% ethanol. The results of this study in line with the study conducted by Lee et al. (2011) on the ethanol extract of eleuthero. Higher water concentration causes more non-phenolic

compounds, such as carbohydrates to be extracted. Carbohydrates do not only cause phenolic content extracted to be lower, but also protect phenolic compounds so that at radiation for more than 7 min., the decrease in phenolic content of 70% ethanol extract was lower than that of 80% and 90% ethanol extract. In addition, it was suspected that more phenolic compounds in *C. xanthorrhiza* were non-polar than polar compounds. One type of phenolic compound commonly found in rhizomes of *C. xanthorrhiza* is curcumin.

The increase in total phenolic content and curcumin content through the increase in ethanol concentration of 70% to 90% and the increase in duration of microwave-assisted extraction to 7 minutes increased DPPH free radical scavenging capacity. DPPH radical scavenging capacity can be used to determine the antioxidant activity of *C. xanthorrhiza* extract. The results of this study are in line with the study conducted by Do et al (2014), indicating that the use of 50% to 100% ethanol increased total phenolic content and antioxidant DPPH radical scavenging activity of *Limnophita aromatica* extract.

Curcumin and other phenolic compounds in *C. xanthorrhiza* are compounds with the potential as antioxidants. The correlation analysis between total phenolic content and curcumin content with antioxidant activity aims the determination the relationship between the compounds and antioxidant activity. The relationship between curcumin content and total phenolic content with antioxidant activity as DPPH scavenger has a correlation coefficient (R^2) of 0.92 and 0.80; respectively. The resulting value was positive, therefore the relationship between total phenolic content and curcumin content on antioxidant activity was directly proportional. Based on the correlation coefficient (R^2) value that greater than 0.8; both phenolic and curcumin compounds (as part of phenolic compounds) had a significant correlation or the bioactive compounds had a significant effect on antioxidant activity based on DPPH radical scavenging capacity. The results of this study in line with the study conducted by Septiana et al (2021).

The highest curcumin content, total phenolic content, and free radical scavenging capacity of *C. xanthorrhiza* extract were obtained through MAE using 90% ethanol solvent for 7 minutes these are 184 mg/g, 319.5 mg/g and 71.08%; respectively. DPPH free radical scavenging capacity was carried out at a fairly small concentration of 4000 ppm. DPPH radical scavenging capacity testing at large concentrations generaly higher. Lemongrass antioxidant activity testing at a concentration of 20,000 ppm was 79.98% (Suri et al., 2020).

CONCLUSIONS

The increase in concentration of ethanol solvent from 70% to 90% and the increase in extraction time from 0 to 7 minutes at 240 watt caused an increase in curcumin content, total phenolic content, and DPPH radical scavenging capacity, but an increase in extraction time from 7 to 9 minutes decreased the extract parameters. The highest curcumin content, total phenolic content, and free radical scavenging capacity of *C. xanthorrhiza* extract were using 90% ethanol for 7 minutes compared to other treatments, namely 184 mg/g, 319.5 mg/g and 71.08%; respectively.

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REFERENCES

Alara, O.R., Abdurahman, N.H., and Olalere, O.A. 2020. Ethanolic extraction of flavonoids, phenolics and antioxidants from *Vernonia amygdalina* leaf using two-level factorial design. *Journal of King Saud University – Science*. 3, pp.7-16

Anggoro, D., Rezki, R.S., dan Siswarni MZ. 2015. Ekstraksi multi tahap kurkumin dari temulawak *Curcuma xanthorriza* Roxb.) menggunakan pelarut etanol. *Jurnal Teknik Kimia USU*. 4(2), p.39-45

Delfiya, A., Thangavel, K., Natarajan, N., Kasthuri, R., and Kailappan, R. 2014.

Microencapsulation of turmeric oleoresin by spray drying and *in vitro* release studies of microcapsules. *Journal of Food Process Engineering*. 38**,** pp.37–48.

Dharma, A.P. 1985. *Tanaman Obat Tradisional Indonesia*. Jakarta: PN Balai Pustaka.

Do, Q.D., Artik Angkawijaya, A.E., Tran-Nguyen, P.L, Huynh, L.H., Soetaredjo, F.E., Ismadji, S., and Ju. Y. 2014. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. *Journal of Food and Drug Analysis*. 22, pp.296-302

Gordon, M. H. 1990. The mechanism of antioxidants action *in vitro*. *In:* B.J.F Hudson (Ed), *Food Antioxidant.* London: Elseiver Applied Science.

Hammerschmidt, P.A. and Pratt, D.E. 1978. Phenolic antioxidants of dried soybeans. *Journal of Food Science.* 43(6723), pp.556-559.

Handayani, D., Mun'im, A., & Ranti, A. S. 2014. Optimation of green tea waste extraction using microwave assisted extraction to yield green tea extract. *Traditional Medicine Journal*. 19(1), pp.29–35.

Hwang, J.K., Shim, J.S. and Pyun. 2006. Antibacterial activity of xanthorrizol from *Curcuma xanthorrhiza* against oral pathogens. *Fitoterapia*. 71, pp.321-323.

Jantan I, Saputri FC, Qaisari MN, and Buang F. 2012. Correlation between chemical composition of *Curcuma domestica* and *Curcuma xanthorrhiza* and their antioxidant effect on human low density lipoprotein oxidation. *Evidence Based Complementary and Alternative Medicine*. 2012 (438356), pp.1-10.

Handayani, D., Mun'im, A. and Ranti, A. S. 2014. Optimation of green tea waste extraction using microwave assisted extraction to yield green tea extract. *Traditional Medicine Journal*. 19(1), pp.29–35.

Jain, T., V. Jain, R. Pandey, A. Vyas and S. S. Shukla. 2009. Microwave assisted extraction for phytoconstituents – An overview. *Asian Journal Research Chemistry*. 1(2), pp.19-25.

Lattanzio, V. 2013. Phenolic Compounds Introduction. In.: Ramawat, K.G., Me´rillon, J.M. (Eds). *Natural Products*. Berlin Heidelberg: Springer-Verlag, pp.1543-1580. (Accessed 15 September 2021). Available from: https://www.researchgate.net/publication/281611160_Phenolic_compounds

Li, Y., Li, S., Lin, S., Zhang, J., Zhao, C and Li, H. 2017. Microwave-assisted extraction of natural antioxidants from the exotic Gordonia axillaris fruit: Optimization and identification of phenolic compounds. *Molecules.* 22, pp.1481-1495.

Lovric, V., Predrag, P., Danijela B.K., Marijana, J. and Verica D. 2017. Effect of Microwave-assisted extraction on the phenolic compounds and antioxidant capacity of blackthorn flowers. *Food Technol. Biotechnol*. 55(2), pp.243–250

Menon, V.P. and Sudheer, A.R. 2007. Antioxidant and anti-inflammatory properties of curcumin. *Advances in Experimental Medicine and Biology*. 595, pp.105-25.

Panigoro, R., Samsudin, S.and Dhianawaty. 2013. Comparison of curcumin level in fresh and decoction of dried *Curcuma xanthorrhiza* Roxb. rhizome. *International Journal* of *Research* in *Pharmaceutical Sciences*. 4(2), pp.256-259.

Paulucci, V.P., Couto, R.O., Teixeira, C.C.C., and Freitas, L.A.P. 2013. Optimization of the extraction of curcumin from *Curcuma longa* rhizomes. *Brazilian Journal of Pharmacognosy*. 23(1), pp.94-100

Salleh, N.A.M., Ismail, S. and Halim. M.R.A

2016. Effects of *Curcuma xanthorrhiza* extracts and their constituents on phase ii drug-metabolizing enzymes activity. *Pharmacognosy Research* 8(4), pp.309–315.

Samsudin S. and R. Panigoro. 2013. Comparison of antioxidant activity between decoction of dried *Curcuma longa*. L., and *Curcuma xanthorrhiza* Roxb. rhizomes. *International journal of research in phytochemistry and pharmacology*. 3(1), pp. 27-30.

Septiana, A.T. Mustaufik, Dwiyanti, H., and Ola, M.M. 2006a. Pengaruh spesies Zingiberaceae (jahe, temulawak, kunyit, dan kunyit putih) dan ketebalan irisan sebelum pengeringan terhadap kadar dan aktivitas antioksidan ekstrak aseton yang dihasilkan. *Agritech.* 26(2), pp.49-54.

Septiana, A.T., Dwiyanti, H., Muchtadi, D., and Zakaria, F. 2006b. Penghambatan oksidasi LDL dan akumulasi kolesterol pada makrofag oleh ekstrak temulawak (*Curcuma xanthorriza* Roxb). *Jurnal Teknologi dan Industri Pangan.* XVII(3), pp.221-226.

Septiana, A.T., Erminawati and Winarsi, H. 2021. The effect of basic ingredients form, cold storage and precipitate separation on bioactive components and antioxidant activity of *Curcuma xanthorrhiza* Roxb. *Food Research.* 5(4), pp.203 - 209

Sheikh, T. Z. B., Yong, C. L. and Lian, M. S. 2009. In vitro antioxidant activity of the hexane and methanolic extracts of *Sargassum baccularia* and *Cladophora patentiramea*. *Journal of Applied Sciences*. 13(9), pp.2490-2493.

Suri, A., Erminawati, and Naufalin, R. 2020. Antioxidant activities of lemongrass with solvent multi-step extraction microwave-assisted extraction as natural food. preservative. *Journal of Functional Food and Nutraceutical.* 2(2), pp.117-128

Zghaibi, N., Omar, R., Kamal, S.M.M., Biak, D.R.A. and Harun, R. 2019. Microwave-assisted brine extraction for enhancement of the quantity and quality of lipid production from microalgae *Nannochloropsis sp. Molecules. 24*, pp.3581-35101.

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DRAGON FRUIT PEEL EXTRACT AND ENCAPSULATED CATFISH OIL FORMULATION IN GUMMY CANDY WITH POTENTIAL *IN VITRO* ANTIHYPERGLYCEMIA PROPERTIES

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ABSTRACT

Red dragon (*Hylocereus polyrhizus*) fruit peel as household waste was still underutilized although it was high in bioactive compounds with antioxidant and antidiabetic properties. The oil extract of freshwater fish, such as catfish (*Pangasius* sp.) was high in monounsaturated fatty acids with reported antihyperglycemia properties. The objectives of this study were to encapsulate catfish oil extract, determine the most liked formulation of gummy candy with dragon fruit peel extract and encapsulated fish oil beads content, and analyze the proximate content and *in vitro* α-amylase inhibition activity of the most liked gummy candy formulation. The methods consisted of dragon fruit peel extraction, encapsulation of catfish oil extract using sodium alginate, gummy candy formulation, sensory evaluation, and analysis of color, proximate, and α-amylase inhibition activity. Twenty five percent w/w of the gummy candy ingredients were composed of the extracts and encapsulation beads at 1:1, 1:3, and 3:1 weight ratios. Encapsulation of the fish oil could mask the fishy odor in the gummy candy. All formulation could be molded into gummy candy perfectly and showed chewy texture. The addition of dragon fruit peel extract could increase the yellowness value, while the fish oil beads decreased the lightness value of the candy. The most liked gummy candy formulation was F3 (ratio 3:1). It was composed of 54.47% water, 1.59% db ash, 5.77% db fat, 1.82% db protein, 36.36% db carbohydrates and showed 46.49% α-amylase inhibition activity.

Keywords: alginate; α-amylase; catfish; proximate; sensory

ABSTRAK

Kulit buah naga merah (*Hylocereus polyrhizus*) umumnya hanya menjadi limbah rumah tangga padahal banyak mengandung senyawa bioaktif dengan aktivitas antioksidan dan antidiabetes. Ekstrak minyak ikan air tawar, seperti patin (*Pangasius* sp.), tinggi akan kandungan asam lemak tidak jenuh rantai tunggal yang memiliki efek antihiperglikemia. Penelitian ini bertujuan untuk mengenkapsulasi minyak ikan patin, menentukan formulasi permen jeli dengan kandungan ekstrak kulit buah naga dan enkapsulasi minyak ikan patin, serta menganalisis kandungan proksimat dan aktivitas penghambatan α-amilase *in vitro* terhadap formulasi permen yang paling disukai berdasarkan uji sensori. Tahapan dalam penelitian ini adalah ekstraksi kulit buah naga dan minyak ikan patin, enkapsulasi minyak ikan dengan natrium alginat, formulasi permen jeli, uji sensori, analisis warna, proksimat, dan aktivitas penghambatan enzim α-amilase. Ekstrak kulit buah naga dan enkapsulasi minyak ikan menyusun 25% b/b bahan permen jeli dengan rasio bobot 1:1, 1:3, dan 3:1. Enkapsulasi minyak ikan dapat mencegah bau amis pada permen jeli. Seluruh formulasi permen jeli dapat tercetak dengan sempurna dan bertekstur kenyal. Penambahan ekstrak kulit buah naga dapat meningkatkan nilai kekuningan permen, sedangkan penambahan enkapsulasi minyak ikan dapat menurunkan nilai kecerahan permen jeli. Permen jeli F3 dengan rasio ekstrak kulit buah naga : enkapsulasi minyak ikan 3:1 paling disukai oleh panelis dan memiliki kadar air 54,47%; abu 1,59%bk; lemak 5,77%bk; protein 1,82%bk; karbohidrat 36,36%bk; serta menunjukkan aktivitas inhibisi α-amilase sebesar 46,39%.

Kata kunci: alginat; α-amilase; patin; proksimat; sensori

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INTRODUCTION

Kulit buah naga merah (*Hylocereus polyrhizus*) yang menyusun 30-35% total berat buah naga belum dimanfaatkan secara optimal. Pemanfaatan ekstrak kulit buah naga sebagai bahan pembuatan permen jeli dapat memberikan nilai tambah pada limbah konsumsi buah naga, yaitu sebagai sumber pewarna merah alami, agen pembentuk gel dengan kandungan pektin bermetoksil tinggi (Muhammad et al., 2014), dan sumber serat pangan (Huang et al., 2021). Kulit buah naga merah juga mengandung beragam senyawa bioaktif, seperti flavonoid, fenolik, terpenoid, alkaloid, dan tanin (Panjaitan & Novitasari, 2021) dan ekstraknya menunjukkan aktivitas antioksidan yang lebih tinggi dibandingkan daging buahnya secara *in vitro* (Budilaksono, 2014). Selain menunjukkan aktivitas antioksidan yang tinggi, ekstrak kulit buah naga juga menunjukkan aktivitas antidiabetes yang baik yang terbukti pada penelitian pemberian ektstrak etanol kulit buah naga merah pada tikus penderita diabetes mellitus tipe 2 (DM-2) sebanyak 0,4 mg/g berat badan dapat menurunkan kadar gula darah (Panjaitan & Novitasari, 2021).

Ikan air tawar, seperti ikan patin (*Pangasius* sp.) mengandung asam lemak esensial tak jenuh, seperti asam lemak omega 3 (EPA dan DHA) yang penting bagi kesehatan mata, otak, dan perkembangan sistem imunitas (Sugata et al., 2019). Kandungan asam lemak tak jenuh tunggal (MUFA) dalam minyak ikan patin hibrid memiliki aktivitas hipoglikemik dengan meningkatkan sensitivitas insulin, aktivasi AMPK, translokasi GLUT4, dan menekan ekspresi protein pro-inflamasi sitokin (Keapai et al., 2016). Asam lemak tak jenuh memiliki ikatan rangkap yang mudah teroksidasi sehingga mudah terputus menjadi jenuh dan radikal yang ditandai dengan penurunan mutu minyak dan timbulnya aroma amis. Proses enkapsulasi minyak ikan dapat memberikan perlindungan terhadap asam lemak tak jenuh sehingga mencegah terjadinya oksidasi dan mampu meningkatkan mutu minyak ikan ketika diaplikasikan dalam permen jeli (Idrus et al., 2013).

Permen jeli menjadi produk yang disukai hampir seluruh golongan usia dengan komposisi yang umum didominasi oleh gula sederhana. Inovasi permen jeli dengan kulit buah naga yang diteliti oleh Yuwidasari et al. (2019) juga mengandung 60-80% sukrosa. Hal ini menyebabkan konsumsi permen jeli mudah meningkatkan kadar gula darah sehingga perlu dihindari oleh penderita diabetes mellitus tipe-2 (DM-2). Sementara itu, DM-2 telah menjadi penyebab kematian urutan ke-7 di dunia dan angka penderita DM-2 di Indonesia mencapai 10,3 juta penduduk yang menyebabkan Indonesia menempati peringkat ke-6 negara penderita DM-2 tertinggi pada tahun 2017 (Hestiana, 2017). Permasalahan tersebut membutuhkan penanganan khusus untuk mengurangi peningkatan angka penderita DM-2 melalui formulasi pangan fungsional, seperti permen jeli rendah gula yang mengandung bahan pangan dengan potensi antihiperglikemia.

Penelitian ini bertujuan untuk membuat enkapsulasi minyak ikan patin, menentukan formulasi permen jeli rendah gula dengan kandungan ekstrak kulit buah naga dan enkapsulasi minyak ikan patin, serta menganalisis kualitas sensori, warna, kandungan proksimat, dan aktivitas antihiperglikemia. Uji penghambatan enzim α-amilase digunakan sebagai model uji aktivitas antihiperglikemia secara *in vitro*.

BAHAN DAN METODE

Bahan dan Alat. Bahan utama dalam penelitian ini adalah ikan patin dan buah naga yang dibeli dari pasar dan supermarket di daerah Tangerang. Bahan kimia teknis *food grade* yang digunakan adalah polioksietilen-20-sorbitan monolaurat (Tween 20), sorbitan monooleate (Span 80), natrium alginat, kalsium klorida, dan pektin. Reagen kimia *analytical grade* diperoleh dari distributor Merck, seperti n-heksana, asam sulfat, natrium hidroksida, asam borat, dan asam klorida. Peralatan utama dalam penelitian ini adalah kolorimeter (Biobase NH310), blender (Miyako), oven (Denpoo DEO-18T), kompor (Rinnai), inkubator (Memmert), tanur (Carbolite Gero), perangkat Kjeldahl (Behr), perangkat Soxhlet (Iwaki), dan spektrofotometer UV-Vis (Shimadzu). Penelitian dilakukan secara bauran, yaitu di Lab Pangan Molekuler, Fakultas Teknobiologi, Universitas Katolik Indonesia Atma Jaya dan di rumah.

Ekstraksi Kulit Buah Naga. Pembuatan ekstrak kulit buah naga mengacu pada metode Yuwidasari et al. (2019). Kulit buah naga disortasi, dicuci dengan air mengalir, lalu ditimbang. Kemudian kulit buah naga dihancurkan dengan blender dan ditambahkan air mineral dengan perbandingan bobot kulit buah naga : air = 1 : 2. Hasil blender disaring dengan saringan 100 mesh (0,147 mm) sehingga diperoleh ekstrak kulit buah naga halus.

Ekstraksi Minyak Ikan Patin. Minyak ikan patin diekstraksi dengan metode *dry rendering* agar kualitas asam lemak tak jenuh tetap terjaga dan rendemennya lebih tinggi dibandingkan *wet rendering* (Eka et al., 2016). Metode ekstraksi minyak ikan mengikuti metode Rozi et al. (2019). Ikan patin dibersihkan dan dipotong menjadi 4 bagian. Daging ikan patin dipotong menjadi potongan dadu kecil dengan ukuran ± 2 cm. Potongan kecil ikan patin dihancurkan menggunakan blender dengan kecepatan medium selama 3 menit untuk menghasilkan bubur ikan patin. Bubur ikan patin dipanaskan dalam oven pada suhu 100 ºC selama 30 menit. Minyak hasil pemanasan diperas dengan kain kasa dan dihitung persentase rendemennya dengan rumus pada persamaan 1:

$$\text{Rendemen (\%)} = \frac{\text{Bobot minyak kasar (g)}}{\text{Bobot ikan (g)}} \times 100\% \quad (1)$$

Enkapsulasi Minyak Ikan Patin. Metode enkapsulasi mengacu pada Dewandari et al. (2018). Minyak ikan dicampurkan 5% v/v Tween 20 lalu diblender hingga homogen. Campuran minyak ikan dengan Tween 20 dituang ke larutan Span 80 (5% v/v) dengan perbandingan bobot campuran tersebut dan Span 80 1:1 b/b lalu diblender sehingga menghasilkan emulsi. Emulsi dicampurkan dengan larutan Na-alginat 1% b/v dengan perbandingan volume emulsi : Na-alginat = 1:2 lalu diaduk hingga homogen. Campuran tersebut diteteskan ke larutan kalsium klorida 100 mM dengan pipet tetes. Butiran enkapsulasi yang terbentuk disaring dan dibilas dengan air mineral.

Formulasi Permen Jeli. Bahan utama yang digunakan dalam formulasi permen jeli adalah campuran ekstrak kulit buah naga dan butiran enkapsulasi minyak ikan patin sebanyak 25% dari bobot total seluruh bahan permen jeli. Perlakuan formulasi terletak pada perbedaan rasio kedua bahan utama (Tabel 1). Bahan lainnya dalam formulasi permen jeli adalah pektin 2%; xylitol 30%; asam sitrat 1%; perisa 0,1%, dan air 50% b/v (Yuwidasari et al., 2019). Xylitol dan pektin dicampurkan dan dipanaskan dalam 250 g air selama 10 menit pada suhu 80 ºC menggunakan kompor. Larutan diaduk selama 5 menit agar seluruh bahan tercampur merata. Setelah itu asam sitrat dan perisa ditambahkan sesuai formulasi ketika pemanasan masih berlangsung. Adonan dicetak dan didinginkan pada suhu ruang selama 1 jam lalu disimpan pada suhu ± 5 ºC di dalam kulkas selama 1-2 jam untuk memperkuat tekstur.

Tabel 1. Komposisi minyak ikan dan ekstrak kulit buah naga dalam permen jeli 500 g

Formulasi	F1	F2	F3	F4	F5	F6
Ekstrak kulit buah naga (g)	0	125	31,25	62,5	93,75	0
Butiran minyak ikan (g)	0	0	93,75	62,5	31,25	125
Rasio kulit buah : butiran minyak	-	-	3:1	1:1	1:3	-

Pengujian Sensori dengan Metode Uji Hedonik. Uji hedonik (Eka et al., 2016) menggunakan 30 orang panelis tidak terlatih, yaitu mahasiswa Fakultas Teknobiologi, Universitas Katolik Indonesia Atma Jaya. Setiap panelis diberikan 3 buah permen jeli untuk masing-masing formulasi. Tingkat kesukaan konsumen terhadap 6 formulasi permen jeli diukur berdasarkan atribut warna, rasa, tekstur, aroma, dan keseluruhan permen jeli. Skor penilaian yang digunakan adalah 1 (amat sangat tidak suka) hingga 9 (amat sangat suka). Pengujian sensori dilakukan dengan mengirim sampel permen jeli ke rumah panelis. Pengisian form kesediaan panelis (https://tinyurl.com/Formkesediaanpanelis) dan kuesioner penilaian permen jeli dilakukan menggunakan *Google Form* (https://tinyurl.com/borangujihedonik).

Pengujian Warna dengan Metode Kolorimetri. Warna sampel diuji dengan alat kolorimeter. Sampel dimasukkan ke dalam wadah, kemudian *color reader* ditempelkan pada permukaan sampel

(Wibawanti & Rinawidiastuti, 2018). Nilai yang dihasilkan adalah *lightness* (L*), *redness* (a*), dan *yellowness* (b*). *Lightness* (L*) merupakan tingkat kecerahan warna yang nilainya berkisar antara 0 (hitam) hingga 100 (putih). Nilai *redness* (a*) mengindikasikan kemerahan (+) hingga kehijauan (-) pada sampel. Nilai *yellowness* (b*) menunjukkan warna kekuningan (+) hingga kebiruan (-).

Pengujian Proksimat. Pengujian proksimat dilakukan sebagai uji lanjut terhadap permen jeli formulasi terbaik hasil uji sensori. Pengujian kadar air secara gravimetri menggunakan oven pada suhu 105°C selama 5 jam. Pengujian kadar abu secara gravimetri menggunakan tanur pada suhu 600°C selama 6 jam. Pengujian kadar lemak menggunakan metode Soxhlet, sedangkan pengujian kadar protein menggunakan metode Kjeldahl (AOAC, 2007).

Analisis Aktivitas Penghambatan Enzim α-Amilase secara In Vitro. Pengujian dilakukan menurut metode Puspantari et al. (2020). Larutan sampel sebanyak 125 µL dicampurkan dengan 125 µL larutan α-amilase dari *porcine pancreatic* (1 unit/mL) lalu diinkubasi pada suhu 37 ºC selama 10 menit. Larutan pati kentang 1% b/v sebanyak 125 µL ditambahkan dan diinkubasi kembali. Pereaksi DNS 0,096 M sebanyak 500 µL ditambahkan dan diinkubasi selama 5 menit. Sebanyak 5 mL air demineral juga ditambahkan dan diukur absorbansinya dengan spektrofotometer UV-Vis pada 540 nm. Kontrol A dibuat dengan 125 µL buffer untuk menggantikan sampel. Kontrol B disiapkan tanpa penambahan enzim α-amilase. Blangko disiapkan tanpa penambahan sampel dan enzim. Kontrol positif menggunakan akarbosa 1 mg/mL yang dilarutkan dalam 100 mL HCl 2 N. Persentase penghambatan α-amilase ditentukan dengan perhitungan pada persamaan 2:

$$\% \text{ penghambatan} = \frac{\text{Absorbansi (kontrol A - blangko) - Absorbansi (sampel - kontrol B)}}{\text{Absorbansi (kontrol A - blangko)}} \times 100\% \quad (2)$$

Analisis Data. Percobaan dilakukan sebanyak dua kali pengulangan. Analisis warna dilakukan sebanya tiga kali dan analisis proksimat serta uji aktivitas inhibisi a-amilase dilakukan masing-masing sebanyak dua kali. Analisis statistik dilakukan terhadap uji warna dan uji hedonik dengan IBM SPSS 25 pada taraf kepercayaan 95%. Uji sebaran data menggunakan uji Shapiro Wilk. Data uji warna berdistribusi normal sehingga dilakukan analisis varians (ANOVA) satu arah dan uji lanjut Duncan's Multiple Range Test. Data uji hedonik tidak berdistribusi secara normal dan dilakukan analisis non-parametrik Kruskal Wallis dan uji lanjut Mann Whitney.

HASIL DAN PEMBAHASAN

Ekstrak kulit buah naga. Kulit buah naga yang digunakan dalam penelitian ini menyusun sebesar 36,86% b/b dari bobot total buah naga. Ekstrak kulit buah naga yang dihasilkan adalah sebanyak 73,71% b/b dari total bobot buah naga. Ekstrak yang diperoleh lebih tinggi dibandingkan bobot kulit buah naga yang diekstraksi karena adanya penambahan air dengan komposisi bobot kulit buah naga : air = 1 : 2 b/b. Kulit buah naga dapat terekstraksi dengan baik menggunakan air dan masih menunjukkan warna merah keunguan yang diduga merupakan komponen pigmen larut air, seperti betalain dan antosianin (Sharma et al., 2021). Kulit buah naga merah mengandung betasianin (15 mg/g) dan total fenolik (36 mg asam galat ekivalen/g) yang lebih tinggi dibandingkan daging buahnya serta kandungan antosianin (135 mg sianidin klorida ekivalen/g) yang lebih rendah dibandingkan daging buahnya (Huang et al., 2021). Senyawa betalain merupakan pigmen alami pada kulit buah naga merah yang umum digunakan pada produk pangan. Betalain terdiri atas betasianin yang menghasilkan warna merah keunguan serta betaxanthin yang menghasilkan warna kuning jingga (Sharma et al., 2021). Betalain tergolong alkaloid yang merupakan konjugat imonium dari asam betalamat dengan siklo-dopa dan gugus amino (asam amino, amina, dan turunannya) yang bersifat stabil pada pH 4-6, baik dalam keadaan aerob maupun anaerob (Priatni & Pradita, 2015). Kandungan betasianin pada kulit buah naga merah bermanfaat sebagai antioksidan, menurunkan kadar gula darah, dan mencegah akumulasi lipid serta penyakit kardiovaskular (Huang et al., 2021). Potensi efek antidiabetes dari betasianin berkorelasi kuat dengan interaksi betasianin-pati

dan betasianin-amilase (Huang et al., 2021). Senyawa antosianin tergolong flavonoid yang bersifat polar dan dapat turut berperan sebagai agen antihiperglikemia (Budilaksono, 2014).

Ekstrak Minyak Ikan Patin. Minyak ikan patin dapat terekstraksi melalui metode *dry rendering* pada suhu 100 °C dengan produk beraroma amis, rasa pahit, dan warna jernih. Rendemen minyak ikan kasar yang diperoleh sebesar 9,2% (Tabel 2). Aroma amis pada minyak ikan merupakan hasil oksidasi asam lemak tak jenuh (Idrus et al., 2013). Rasa pahit pada minyak ikan dapat terbentuk selama pemanasan akibat degradasi protein ikan dan oksidasi asam amino metionin dari peptida ikan yang akhirnya menghasilkan senyawa peptida siklik (Stamenkovic et al., 2019). Metode *dry rendering* dipilih karena dapat meningkatkan rendemen dan kualitas minyak ikan dibandingkan dengan *wet rendering.* Hal ini disebabkan *dry rendering* tidak menggunakan air sebagai pelarut, melainkan mengeluarkan air dari proses pemanasan dengan oven sehingga kandungan lemak pada daging ikan patin dapat terekstraksi secara optimal (Eka et al., 2016). Menurut Andinata (2013), minyak ikan patin hasil *dry rendering* juga memiliki angka asam lemak bebas dan peroksida yang rendah serta angka penyabunan dan bilangan iod yang lebih tinggi dibandingkan *wet rendering*. Semakin kecil angka peroksida, kualitas minyak semakin baik, sedangkan bilangan iod mengindikasikan keberadaan asam lemak tak jenuh (Andinata, 2013). Asam lemak tak jenuh berpotensi dalam mekanisme antidiabetes (Zheng et al., 2016). Walaupun demikian, pemilihan suhu pada *dry rendering* dapat mempengaruhi rendemen minyak ikan. Ekstraksi minyak ikan patin siam secara *dry rendering* akan menghasilkan rendemen tertinggi pada suhu 70ºC dan menurun seiring peningkatan suhu (Kamini et al., 2016).

Butiran Enkapsulasi Minyak Ikan Patin. Enkapsulasi pada penelitian ini terbentuk akibat terjadinya interaksi ionik antara gugus karboksil bermuatan negatif pada natrium alginat dengan kation divalen pada kalsium yang membentuk ikatan silang antar molekul (Dewandari et al., 2018). Tween dan Span berperan sebagai emulgator dalam pembentukan butiran enkapsulasi. Keduanya memiliki gugus non-polar yang berikatan dengan gugus non-polar minyak ikan serta gugus polar yang berikatan dengan gugus polar air sehingga terbentuk campuran yang homogen (Syaputri & Patricia, 2019). Butiran enkapsulasi yang diperoleh sebanyak 380 g dari 250 g minyak ikan dan berukuran 2-3 mm. Tahapan pembuatan enkapsulasi hanya dilakukan sekali dalam 1 *batch*. Pembuatan permen jeli dilakukan sebanyak 2 kali pengulangan menggunakan butiran enkapsulasi dari *batch* yang sama. Hal ini bertujuan untuk meminimalkan perbedaan atau variasi bahan yang dapat berkontribusi terhadap karakteristik sensori permen jeli. Berdasarkan penilaian sensori secara subjektif oleh peneliti terhadap hasil enkapsulasi, butiran enkapsulasi memiliki tekstur kenyal dan tidak memiliki bau amis, tetapi terdapat rasa pahit (data tidak ditunjukkan). Hal tersebut juga dikuatkan dengan profil sensori permen jeli F3-F6 yang mengandung butiran enkapsulasi minyak ikan (Tabel 3 dan Tabel 4). Permen jeli F3 merupakan permen yang paling disukai dari atribut aroma dan permen jeli F3 dan F6 memiliki aroma yang lebih disukai dibandingkan kontrol tanpa butiran enkapsulasi (Tabel 4).

Tabel 2. Hasil ekstraksi minyak ikan patin

No.	Tahapan ekstraksi	Bobot
1.	Ikan patin awal	2754 g
2.	Potongan kecil ikan patin	2729 g
3.	Bubur ikan patin sebelum pemanasan	2664 g
4.	Bubur ikan patin setelah pemanasan	2648 g
5.	Minyak kasar ikan patin	245 g

Penelitian yang dilakukan ini tidak menguji karakteristik struktur dan fungsi butiran enkapsulasi yang terbentuk, khususnya terhadap kerusakan oksidatif minyak ikan. Hal ini disebabkan enkapsulasi dengan alginat sudah umum dilakukan dan fungsi proteksi terhadap kerusakan oksidatif minyak ikan sudah dibuktikan pada penelitian Idrus et al. (2013). Enkapsulasi dengan alginat efektif menutupi bau amis dan memberikan perlindungan dalam mencegah oksidasi minyak ikan (Idrus et al., 2013), namun tidak mampu menutupi rasa pahit. Rasa pahit tersebut dapat terbawa dari hasil ekstraksi minyak ikan patin yang sudah dijelaskan sebelumnya.

Enkapsulasi dengan alginat memiliki kemampuan rendah untuk menyamarkan persepsi rasa pahit, seperti halnya pada produk mikroenkapsulasi sampel ekstrak fenolik daun zaitun menggunakan alginat/pektin yang diteliti oleh Flamminii et al. (2020).

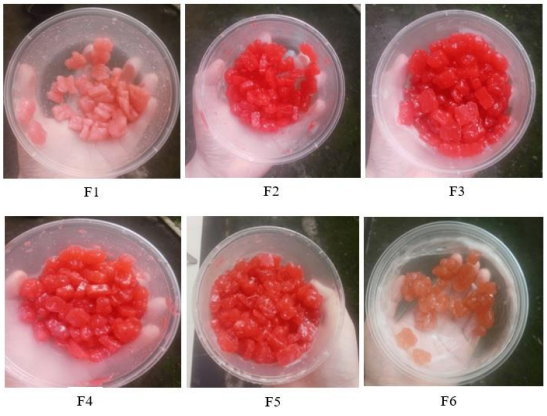
Profil Tekstur, Aroma, dan Warna Permen Jeli. Seluruh formulasi permen jeli dapat tercetak sempurna, bertekstur kenyal, tidak berminyak, dan beraroma tidak amis, tetapi terdapat perbedaan warna antarformulasi yang dapat dilihat pada Tabel 3. Kekenyalan tekstur diperoleh dari penambahan pektin dengan konsentrasi yang sama di setiap perlakuan dan ekstrak kulit buah naga dengan konsentrasi yang bervariasi. Pektin dengan gugus metoksil tinggi berperan sebagai *gelling agent* sehingga menghasilkan tekstur elastis dan kenyal pada permen jeli (Yuwidasari et al., 2019). Aroma yang tidak amis pada permen jeli menunjukkan bahwa hasil enkapsulasi minyak dapat menjaga stabilitas komponen aktif minyak ikan dan memberikan perlindungan terhadap oksidasi selama proses pemanasan dalam pembuatan permen jeli (Dewandari et al., 2018).

Tabel 3. Nilai rata-rata uji *lightness* (L*), *redness* (a*), dan *yellowness* (b*)

Parameter Warna	F1	F2	F3	F4	F5	F6
L	43,73^{a}	38,28^{b}	33,65^{c}	31,85^{cd}	33,42^{cd}	28,03^{d}
a	15,45^{a}	17,89^{a}	20,15^{a}	16,44^{a}	18,1^{a}	16,14^{a}
b	5,95^{c}	13,20^{a}	7,7^{b}	6,43^{bc}	7,08^{bc}	7,56^{bc}

Ket: Huruf yang sama pada baris yang sama menunjukkan perbedaan yang tidak signifikan antarsampel ($p>0.05$). Kode sampel: kontrol tanpa bahan aktif (F1), kontrol tanpa enkapsulasi minyak (F2), rasio formulasi ekstrak kulit buah naga : enkapsulasi minyak 3:1 (F3), 1:1 (F4), 1:3 (F5), dan kontrol tanpa ekstrak kulit buah naga (F6).

Gambar 1 menunjukkan penampakan permen jeli seluruh formulasi. Permen jeli kontrol F1, yaitu tidak ditambahkan kedua bahan utama berupa ekstrak kulit buah naga maupun butiran enkapsulasi minyak ikan, memberikan warna merah bening dan memiliki nilai kecerahan tertinggi. Warna permen F2, F3, F4, F5 menunjukkan kemerahan yang mirip satu sama lain. Warna permen F6 agak kecoklatan dengan adanya penambahan butiran minyak ikan yang terbanyak. Ekstrak kulit buah naga memiliki warna merah terang dan butiran enkapsulasi minyak ikan berwarna putih kekuningan. Penambahan kedua bahan utama akan menurunkan nilai kecerahan permen. Butiran enkapsulasi minyak juga memiliki warna agak gelap sehingga semakin banyak butiran enkapsulasi yang ditambahkan akan semakin menurunkan nilai kecerahan permen jeli. Tingkat kemerahan permen tidak berbeda nyata antarformulasi, namun penambahan ekstrak kulit buah naga dapat meningkatkan intensitas warna kekuningan, khususnya pada kontrol F2 dan sampel F3. Penambahan butiran enkapsulasi minyak akan mengurangi intensitas warna kuning dari ekstrak kulit buah naga pada sampel F3, F4, dan F5 sehingga menyamai kontrol tanpa kedua bahan utama (F1).



Gambar 1. Permen jeli dengan berbagai perlakuan, yaitu (F1) kontrol tanpa ekstrak kulit buah naga dan enkapsulasi minyak ikan, (F2) kontrol tanpa enkapsulasi minyak ikan, sampel dengan rasio ekstrak kulit buah naga dan enkapsulasi minyak ikan (F3) 3:1, (F4) 1:1, (F5) 1:3, dan (F6) kontrol tanpa ekstrak kulit buah naga

Senyawa betasianin pada buah naga stabil pada suhu di bawah 40 °C dan pH 4-6, sedangkan larutan permen jeli memiliki pH sekitar 4 dan dipanaskan pada suhu 80°C. Kondisi pH asam (3-4) dapat mengubah struktur betasianin melalui reaksi dehidrogenasi dan dekarboksilasi sehingga menurunkan intensitas warna merah (Gengatharan et al., 2017). Proses pemanasan pada suhu tinggi (di atas 65 °C) juga dapat mendegradasi senyawa

betasianin melalui proses isomerisasi dan dekarboksilasi yang berangsur-angsur akan menghasilkan warna akhir cokelat muda (Priatni & Pradita, 2015). Selain itu, pemanasan permen dapat menyebabkan terjadinya reaksi Maillard antara komponen gula alami dan asam amino dalam ekstrak kulit buah naga sehingga meningkatkan intensitas warna kuning dan mempengaruhi warna akhir permen (Asra et al., 2019). Wong dan Siow (2015) menyarankan penambahan asam askorbat 0.25%, menjaga pH 4, dan pasteurisasi pada suhu 65 °C selama 30 menit untuk menjaga stabilitas betasianin pada produk jus buah naga merah.

Tabel 4. Skor rerata kesukaan panelis pada keenam formulasi permen jeli

Atribut	F1	F2	F3	F4	F5	F6
Kenampakan	4.57	4.87	7.23*	7.03*	6.8*	6.77*
Aroma	5.27	5.50	7.00*	5.8	5.27	6.53*
Rasa	5.67	5.77	5.50	4.97	4.03	5.13
Tekstur	4.90	5.57	5.23	5.03	3.83	4.33
Keseluruhan	5.50	5.73	5.77	5.50	4.90	5.50

* ($p<0,05$) menunjukkan perbedaan nyata antara sampel dengan kontrol F1 pada setiap baris Kode sampel: kontrol tanpa bahan aktif (F1), kontrol tanpa enkapsulasi minyak (F2), rasio formulasi ekstrak kulit buah naga : enkapsulasi minyak 3:1 (F3), 1:1 (F4), 1:3 (F5), dan kontrol tanpa ekstrak kulit buah naga (F6).

Tingkat Kesukaan Permen Jeli. Skor rerata hasil uji sensori ditampilkan pada Tabel 4. Sampel F3 paling disukai oleh panelis berdasarkan atribut kenampakan (7.23) dan aroma (7.00). Tingkat kesukaan panelis terhadap atribut rasa, tekstur, dan keseluruhan permen tidak menunjukkan perbedaan yang signifikan antara masing-masing sampel dengan kontrol F1. Permen F3 mengandung 18,75% ekstrak kulit buah naga dan 6,25% butiran enkapsulasi minyak ikan (rasio 3:1) yang secara visual menunjukkan warna merah terang (Gambar 1). Hal ini disebabkan oleh pigmen betasianin dan antosianin yang dimiliki oleh ekstrak kulit buah naga (Asra et al., 2019; Rista et al, 2018). Bila dibandingkan dengan F2 yang hanya mengandung ekstrak kulit buah naga, F3 memiliki nilai kecerahan dan intensitas warna kuning yang lebih rendah (Tabel 3). Oleh karena itu, penambahan butiran enkapsulasi minyak ikan dapat menyeimbangkan nilai kecerahan dan intensitas kekuningan permen sehingga kenampakannya menjadi lebih disukai oleh panelis.

Aroma permen jeli F3 juga paling digemari sengan skor 7.00 (suka) dan berbeda nyata dibandingkan kontrol F1, demikian halnya dengan permen F6 yang hanya mengandung enkapsulasi minyak ikan. Hal ini membuktikan bahwa penambahan enkapsulasi minyak ikan dalam permen jeli tidak menghasilkan aroma amis yang mengganggu panelis (Zhang et al., 2016), bahkan dapat meningkatkan skor kesukaan panelis terhadap aroma permen. Walaupun demikian, sampel F4 dan F5 yang juga mengandung enkapsulasi minyak ikan memiliki aroma yang sama-sama disukai seperti kontrol F1. Penambahan ekstrak kulit buah naga dapat meningkatkan kesukaan panelis terhadap aroma. Hal ini ditunjukkan oleh F3 yang mengandung proporsi ekstrak kulit buah naga tertinggi dibandingkan F4 dan F5. Aroma dan rasa khas dari ekstrak kulit buah naga disebabkan oleh kandungan senyawa alami, seperti fenolik, alkaloid, terpenoid, flavonoid, tiamin, niasin, piridoksin, kobalamin, fenolik, karoten, dan fitoalbumin. Senyawa tersebut dapat merangsang indera pengecap dan penciuman sehingga mampu meningkatkan kesukaan panelis terhadap produk formulasi yang mengandung eksrak kulit buah naga merah (Rista et al., 2018).

Komposisi bahan lain dalam formulasi permen jeli sama pada seluruh formulasi sehingga tidak terdapat perbedaan yang nyata pada tingkat kesukaan panelis terhadap rasa, tekstur, dan keseluruhan permen jeli. Rasa asam berasal dari asam sitrat dan manis dari xylitol. Xylitol berperan sebagai pengganti gula dengan tingkat kemanisan 1,0-1,2 kali lebih tinggi dan kandungan kalori yang lebih rendah dibandingkan sukrosa. Indeks Glikemik (IG) yang dimiliki xylitol adalah 7, lebih rendah dibandingkan sukrosa dengan IG 60-70 sehingga permen jeli rendah gula ini aman dikonsumsi oleh penderita DM-2 (Mahyati, 2017).

Kesukaan panelis terhadap tekstur permen jeli tidak terpengaruh secara signifikan dengan variasi penambahan ekstrak kulit buah naga. Kandungan pektin alami dalam ekstrak kasar kulit buah naga cukup kecil untuk dapat mempengaruhi tekstur permen. Keterbatasan dalam penelitian ini adalah

tidak mengukur kadar pektin yang terkandung dalam ekstrak kulit buah naga. Ekstrak kulit buah naga yang mengandung 11% pektin dapat berkontribusi terhadap peningkatan nilai tekstur permen jeli (Yuwidasari et al., 2019). Pektin tergolong polisakarida yang mampu membentuk ikatan hidrogen dan ikatan silang antarmolekul yang memerangkap air sebanyak 8 – 12% air pada kondisi setimbang (Rista et al., 2018). Pektin juga mampu menghambat kristalisasi gula, mengubah cairan menjadi padatan yang elastis, dan memperbaiki bentuk, serta tekstur permen yang dihasilkan (Yuwidasari et al., 2019). Dengan demikian, permen F3 dipilih sebagai formulasi terbaik berdasarkan hasil uji sensori sehingga sampel tersebut diuji terhadap kandungan proksimat dan aktivitas antidiabetes menggunakan model pengahambatan enzim α-amilase secara *in vitro*.

Kandungan nutrisi permen jeli. Tabel 5 menunjukkan hasil pengujian proksimat terhadap permen jeli F3 yang dibandingkan dengan data SNI dan informasi nilai gizi pada tabel nutrisi permen jeli komersial. Permen jeli F3 memiliki kadar abu 1,59% ± 0,21 bk sehingga telah memenuhi SNI 3547.02-2008, yaitu maksimal 3%, namun kadar airnya 54,47% ± 1,19 atau lebih tinggi dibandingkan SNI. Hal ini dapat disebabkan oleh proses pemasakan permen jeli yang dilakukan pada suhu rendah, yaitu 80 ^{o}C, yang dilakukan untuk mempertahankan kandungan senyawa betasianin yang dapat berperan sebagai senyawa antioksidan dan antidiabetes (Sharma et al., 2021). Kadar air yang tinggi dapat menyebabkan permen jeli memiliki umur simpan yang lebih rendah (Asra et al., 2019). Hal ini dapat diatasi pada penelitian selanjutnya dengan cara mengurangi komposisi air dalam formulasi permen jeli dan menentukan suhu pemanasan yang optimal.

Permen jeli F3 juga memiliki kadar lemak sebesar 5,33% ± 0,59 bk, lebih tinggi dibandingkan kadar lemak permen komersial. Hal ini wajar karena permen jeli F3 mendapat penambahan 6.25% butiran enkapsulasi minyak ikan. Kadar lemak yang lebih tinggi berpotensi memberikan manfaat fungsional karena minyak ikan patin dilaporkan mengandung asam lemak tak jenuh sebesar 12,22% b/b PUFA dan 33,94% b/b MUFA (Suseno et al., 2020) yang dapat berkontribusi terhadap aktivitas antihiperglikemia (Zheng et al., 2016). Kadar protein permen jeli F3, yaitu 1,82% ± 1,29, hampir setara dengan permen komersial, yaitu 1 %. Kadar karbohidrat permen jeli F3, yaitu 36,36% ± 1,49, lebih tinggi dibandingkan permen komersial. Hal ini dapat disebabkan oleh kandungan serat pangan dari ekstrak kulit buah naga. Kulit buah naga mengandung serat pangan yang terdiri atas serat pangan terlarut sebesar 14,82% dan serat pangan tidak larut sebesar 56,50% (Puspita et al., 2016). Oleh sebab itu, permen jeli F3 dengan komposisi 6,25% butiran minyak ikan dan 18,75% ekstrak kulit buah naga lebih bernutrisi dibandingkan permen jeli komersial karena memiliki kadar lemak dan serat yang tinggi.

Tabel 5. Kandungan nutrisi permen jeli

Komposisi Proksimat	Permen F3 (Hasil Pengujian)	Permen Jeli Komersial (SNI atau Tabel Nutrisi)
Kadar abu (%bk)	1,59 % ± 0,21	Maks. 3% (SNI 3547.02-2008)
Kadar air (%)	54,47 % ± 1,19	Maks. 20% (SNI 3547.02-2008)
Kadar lemak (%bk)	5,33 % ± 0,59	0% (Marine Gummy)
Kadar protein (%bk)	1,82 % ± 1,29	1% (Marine Gummy)
Kadar karbohidrat (%bk)	36,36 % ± 1,49	3% (Marine Gummy)

Aktivitas penghambatan terhadap enzim α-amilase. Permen jeli F3 memberikan efek antihiperglikemia dengan nilai penghambatan terhadap enzim α-amilase sebesar 46,39% ± 4,43. Nilai penghambatan ini lebih tinggi dibandingkan aktivitas penghambatan *in vitro* oleh kulit buah jenis lain, seperti ekstrak kulit jeruk dengan aktivitas penghambatan sebesar 34,2% (Setyabudi et al., 2015), namun tetap lebih rendah dibandingkan akarbosa yang mencapai 97,29% (Sari, 2015). Akarbosa merupakan senyawa inhibitor α-amilase dan α-glukosida hidrolase yang digunakan sebagai obat penurun kadar gula darah serta sering menyebabkan efek samping, seperti mual, kembung, dan diare. Efek samping tersebut muncul karena banyaknya polisakarida yang tidak tercerna sehingga difermentasi oleh mikrob di usus

besar dan menghasilkan gas yang tinggi. Aktivitas penghambatan α-amilase yang tidak terlalu kuat pada permen jeli F3 menyebabkan permen tersebut berpotensi menjadi pangan fungsional yang dapat mencegah hiperglikemia sebagai gejala awal penyakit DM-2 dengan efek samping yang lebih minimal serta dapat dikonsumsi secara menyenangkan.

Penelitian ini memiliki kelemahan, yaitu tidak menganalisis aktivitas penghambatan α-amilase terhadap kontrol F1 dan antarperlakuan serta hanya membandingkan dengan literatur yang menggunakan metode serupa sehingga tidak dapat diketahui perbandingan hasil tersebut antarperlakuan dan tidak dapat ditentukan rasio mana yang memberikan aktivitas penghambatan α-amilase terbaik. Enzim α-amilase berperan dalam hidrolisis karbohidrat menjadi gula sederhana sehingga penghambatan terhadap enzim tersebut dapat menjadi model penghambatan peningkatan kadar glukosa darah secara *in vitro*. Aktivitas penghambatan diuji dengan spektrofotometer UV-Vis untuk mengukur absorbansi warna kuning yang dihasilkan oleh reaksi asam 2,3-dinitrosalisilat (DNS) dengan gula sederhana. Semakin tinggi kadar pati yang terhidrolisis menjadi gula sederhana, warna kuning yang dihasilkan akan semakin pekat. Sebaliknya, apabila hidrolisis pati menjadi gula sederhana terhambat, absorbansi warna kuning menjadi lebih rendah (Agustinah et al., 2016). Penelitian ini dapat dilanjutkan dengan uji antihiperglikemia secara *in vivo*.

KESIMPULAN

Enkapsulasi minyak ikan patin menggunakan alginat dengan metode gelasi ionik mampu menutupi aroma amis minyak ikan. Enkapsulasi tersebut juga dapat menyatu dengan bahan lain dalam pembuatan permen jeli rendah gula. Seluruh formulasi permen jeli dapat tercetak sempurna, bertekstur kenyal, dan tidak amis. Permen jeli F3 dengan komposisi 18,75% ekstrak kulit buah naga dan 6,25% butiran enkapsulasi minyak ikan paling disukai oleh panelis pada atribut kenampakan (skor 7,23) dan aroma (7,00). Penambahan ekstrak kulit buah naga merah dapat meningkatkan intensitas warna kekuningan, sedangkan penambahan butiran enkapsulasi minyak ikan dapat menurunkan nilai kecerahan permen jeli. Permen jeli F3 tersebut memiliki kadar air 54,47%; abu 1,59%bk; lemak 5,77%bk; protein 1,82%bk; dan karbohidrat 36,36%bk serta menunjukkan aktivitas penghambatan enzim α-amilase sebesar 46,39% sebagai model uji antihiperglikemia secara *in vitro*. Penelitian selanjutnya dapat dilakukan untuk mengonfirmasi aktivitas antihiperglikemia secara *in vitro* dan *in vivo*.

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DAFTAR PUSTAKA

Agustinah, W., Sarkar, D., Woods, F. dan Shetty, K. 2016. Apple and blueberry synergies for designing bioactive ingredients for the management of early stages of type 2 diabetes. *Journal of Food Quality*, 39, pp.370-382.

Andinata, D. 2013. *Profil dan karakteristik minyak ikan patin hasil variasi pakan dan metode ekstraksi*. Skripsi, Universitas Jember.

AOAC. 2007. *Official Methods of Analysis of AOAC International*. 18th Edition. Gaithersburg: AOAC International.

Asra, R., Yetti, R.D., Rusdi, Audina. S. dan Nessa, N. 2019. Studi fisikokimia betasianin dalam kulit buah naga sebagai pewarna merah alami sediaan farmasi. *Jurnal Farmasi Galenika*, 5(2), pp.140-146.

Budilaksono, W. 2014. Uji aktivitas antioksidan fraksi n-heksana kulit buah naga menggunakan metode DPPH. *Jurnal Mahasiswa Farmasi Untan*, 1(1), pp.1-11.

Dewandari, K.T., Sofwan, G. dan Herawan, T. 2018. Preparasi dan karakterisasi beads kalsium alginat yang mengandung nanoemulsi minyak sawit merah dengan metode gelasi

ionik. *Jurnal Penelitian Pascapanen Pertanian*, 15(2), pp. 99-105.

Eka, B., Junianto dan Rochima, E. 2016. Pengaruh metode rendering terhadap karakteristik fisik, kimia, dan organoleptik ekstrak kasar minyak ikan lele *Jurnal Perikanan Kelautan*, 7(1), pp.1-5.

Flamminii, F., Mattia, C.D.D., Sacchetti, G., Neri, L., Mastrocola, D. dan Pittia, P. 2020. Physical and sensory properties of mayonnaise enriched with encapsulated olive leaf phenolic extracts. *Foods*, 9(997), pp. 1-12. doi:10.3390/foods9080997.

Gengatharan, A., Dykes, G.A. dan Choo, W-S. 2017. The effect of pH treatment and refrigerated storage on natural colourant preparations (betacyanins) from red pitahaya and their potential application in yoghurt. *LWT – Food Science and Technology*, 80, pp. 437-445.

Hestiana, D. W. 2017. Faktor-faktor yang berhubungan dengan kepatuhan dalam pengelolaan diet pada pasien rawat jalan diabetes melitus tipe 2 di Kota Semarang. *Journal of Health Education*, 2(2), pp.138-145.

Huang, Y., Brennan, M.A., Kasapsis, S., Richardson, S.J. dan Brennan, C.S. 2021. Maturation process, nutritional profile, bioactivities and utilisation in food products of red pitaya fruits: a review. *Foods*, 10(2862), pp.1-21. https://doi.org/10.3390/foods10112862.

Idrus, S., Affifudin, S. Z. dan Rutumalessy, D. J. 2013. Identifikasi dan mikroenkapsulasi minyak ikan yang mengandung asam lemak omega-3. *Jurnal Riset Industri,* 7(1), pp.53-62.

Kamini, Suptijah, P., Santoso, J. dan Suseno, H. S. 2016. Ekstraksi *dry rendering* dan karakterisasi minyak ikan dari lemak jeroan hasil samping pengolahan salai patin siam. *Jurnal Pengolahan Hasil Perikanan Indonesia*, 19(3):196-2015.

Keapai, W., Apichai, S., Amornlerdpison, D. dan Lailerd, N. 2016. Evaluation of fish oil-rich in MUFAs for anti-diabetic and antiinflammation potential in experimental type 2 diabetic rats. *Korean Journal of Physiology and Pharmacology*, 20(6), pp. 581-593.

Mahyati. 2017. Pemanfaatan xylitol dari limbah tongkol Jagung menggunakan *Candida tropicalis*. In: *Prosiding Seminar Hasil Penelitian, 28 Agustus 2018, Makassar, Indonesia*. Jakarta: SNP2M, pp.71–74. [Accessed 2 April 2021]. Available from: http://jurnal.poliupg.ac.id/index.php/snp2m/article/view/297.

Muhammad, K., Zahari, N.I.M., Gannasin, S.P., Adzahan, N.M. dan Bakar, J. 2014. High methoxyl pectin from dragon fruit (*Hylocereus polyrhizus*) peel. *Food Hydrocolloids*, 42, pp. 289-297.

Panjaitan, R.G.P. dan Novitasari. 2021. Anti-diabetic activity of ther dragon fruit peel (*Hylocereus polyrhizus*) in ethanol extract against diabetic rats. *Pharmacognosy Journal*, 13(5), pp.1079-1085.

Priatni, S. dan Pradita, A. 2015. Stability study of betacyanin extract from red dragon fruit (*Hylocereus polyrhizus*) peels. *Procedia Chemistry*, 16(2015), pp.438-444.

Puspantari, W., Kusnandar, F., Lioe, H. N. dan Laily, N. 2020. Penghambatan fraksi fukoidan rumput laut cokelat terhadap α-amilase dan α-glukosidase. *Jurnal Pengolahan Hasil Perikanan Indonesia,* 23(1), pp.122-136.

Puspita, R., Ardiaria, M. dan Syauqy, A. 2016. Perbedaan efek seduhan kulit dan jus buah naga merah terhadap kadar kolesterol LDL serum tikus *Sprague Dawley* dislipidemia. *Jurnal Kedokteran Diponegoro*, 5(4), pp.1559-1567.

Rista, E., Marianah dan Sulastri, Y. 2018. Sifat kimia dan organoleptik biskuit pada berbagai penambahan ekstrak kulit buah naga merah. *Jurnal Agrotek*, 5(2), pp.127-133.

Sari, R. K. 2015. *Uji efektifitas protein biji melinjo sebagai inhibitor aktivitas α-amilase dan α-glukosidase secara in vitro*. Skripsi, Universitas Jember.

Setyabudi, C., Tanda, S., Santosa, W. I. dan Soetaredjo, F. E. 2015. Studi in vitro ekstrak kulit jeruk purut untuk aplikasi terapi diabetes melitus. *Jurnal Ilmiah Widya Teknik*, 14(1), pp.15-19.

Sharma, M., Usmani, Z., Gupta, V.K. dan Bhat, R. 2021. Valorization of fruits and vegetable wastes and by-products to produce natural pigments. *Critical Reviews in Biotechnology*, 41(4), pp. 535-563.

SNI 3547.2-2008. Kembang Gula Lunak. Departemen Perindustrian dan Perdagangan.

Stamenkovic, A., Ganguly, R., Aliani, M., Ravandi, A. dan Pierce, G.N. 2019. Overcoming the bitter taste of oils enriched in fatty acids to obtain their effects on the heart in health and disease. *Nutrients*, 11(1179), pp. 1-16. doi:10.3390/nu11051179.

Sugata, M., Wiriadi, P.F., Lucy, J. dan Jan, T.T. 2019. Total lipid and omega-3 content in Pangasius catfish (*Pangasius pangasius*) and milkfish (*Chanos chanos*) from Indonesia. *Malaysian Journal of Nutrition*, 25(1), pp. 163-169.

Suseno, S. H., Rizkon, A. K. dan Jacoeb, A. M. 2020. Ekstraksi *dry rendering* dan karakterisasi minyak ikan patin hasil samping industri fillet di Lampung. *Jurnal Pengolahan Hasil Perikanan Indonesia*, 23(1), pp.38-46.

Syaputri, F. N. dan Patricia, V. M. 2019. Pengaruh penambahan emulgator tween dan span terhadap stabilitas krim. *Journal of Science, Technology and Enterpreneurship*, 1(2), pp.140-146.

Wibawanti, J.W.W. dan Rinawidiastuti. 2018. Sifat fisik dan organoleptik yogurt drink susu kambing dengan penambahan ekstrak kulit manggis (*Garcinia mangostana* L.). *Jurnal Ilmu dan Teknologi Hasil Ternak*, 13(1), pp.27-37. DOI: 10.21776/ub.jitek.2018.013.01.3.

Wong, Y-M. dan Siow, L-F. 2015. Effects of heat, pH, antioxidant, agitation and light on betacyanin stability using red-fleshed dragon fruit (*Hylocereus polyrhizus*) juice and concentrate as models. *Journal of Food Science and Technology*, 52(5), pp. 3086-3092.

Yuwidasari, E. A., Yudiono, K. dan Susilowati, S. 2019. Kualitas permen jelly pektin kulit buah naga dan gula pasir. *Jurnal BisTek Pertanian*, 6(1), pp.28-41.

Zhang, Z., Zhang, R., Zou, L. dan McClements, D. J. 2016. Protein encapsulation in alginate hydrogel beads: Effect of pH on microgel stability, protein retention and protein release. *Food Hydrocolloids*, 58(2016), pp.308-315.

Zheng, J., Lin, M., Fang, L. 2016. Effects of n-3 fatty acid supplements on glycemic traits in Chinese type 2 diabetic patients. *Molecular Nutrition & Food Research*, 60(10), pp.2176 - 2184.

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EXPERIMENTAL DESIGN-ASSISTED OPTIMIZATION OF CHROMATOGRAPHIC METHOD FOR THE SIMULTANEOUS QUANTITATION OF PHENOLIC COMPOUNDS IN DRIED FLOWERS EXTRACT

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ABSTRACT

This research aimed to develop and validate a reversed phase-high performance liquid chromatography method to determine phenolic compounds in dried flowers extract simultaneously. The research was divided into two parts: (1) optimization of the separation condition employing a Box Behnken design, and (2) validation test including assessment for the precision, accuracy, and method applicability of a High-Performance Liquid Chromatography (HPLC) coupled with Diode Array Detector (DAD). The studied factors for the optimization of the separation condition were flow rate (0.8−1.2 ml min^{-1}), percentage of the mobile phase at the beginning (0−20% phase B), and end (70−100% phase B) of the gradient program. It was statistically evinced that the chromatographic resolutions (*Rs*>1.0) indicated acceptable separation for protocatechuic acid, p-hydroxybenzoic acid, protocatechuic aldehyde, vanillic acid, *p*-coumaric acid, and ferulic acid. A fast separation method (8.00 min) was achieved by applying the optimum condition of a flow rate of 1 mL min^{-1}, mobile phase composition of 20% acidified methanol at the beginning, and 100% acidified methanol at the end of the gradient program. The validation was then performed for the developed method assuring high precision and accuracy. Additionally, the HPLC-DAD method was successfully applied to determine the phenolic compounds in three dried flower extracts revealing that the method was reliable for routine analyses.

Keywords: bnox-behnken design; edible flowers; method applicability; method validation

ABSTRAK

Penelitian ini bertujuan untuk mengembangkan dan validasi metode kromatografi cair kinerja tinggi untuk mengidentifikasi komponen fenolik pada ekstrak bunga kering secara bersamaan. Penelitian dibagi menjadi 2 bagian: (1) optimasi kondisi pemisahan menggunakan metode desain Box Behnken, dan (2) uji validasi yang meliputi pengujian presisi, akurasi, dan aplikabilitas metode pada Kromatografi Cair Kinerja Tinggi (HPLC) yang dilengkapi dengan detektor *Diode Array* (DAD). Faktor yang dianalisis untuk optimasi kondisi pemisahan adalah laju aliran (0.8-1.2 ml min^{-1}), persentase fase gerak pada awal (0-20% fase B), dan pada akhir (70-100% fase B) program gradien. Dibuktikan secara statistik bahwa resolusi kromatografi (*Rs*>1.0) mengindikasikan pemisahan yang baik untuk asam protokatecuat, asam *p*-hidroksi benzoat, aldehid protokatecuat, asam vanilat, asam *p*-kumarat, dan asam ferulat. Metode separasi yang cepat (8.00 min) didapatkan dengan mengaplikasikan kondisi optimum dari laju aliran yaitu 1 mL min^{-1}, komposisi fase gerak 20% metanol ter-asidifikasi pada tahap awal, dan 100% metanol ter-asidifikasi pada tahap akhir program gradient. Validasi dilakukan terhadap metode yang dikembangkan untuk memastikan tingginya presisi dan akurasi. Metode HPLC-DAD berhasil diaplikasikan untuk mengidentifikasi komponen fenolik dari tiga ekstrak bunga kering dan membuktikan bahwa metode tersebut dapat digunakan untuk analisis rutin.

Kata kunci: aplikabilitas metode; bunga yang daat dimakan; desain box-behnken; validasi metode.

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INTRODUCTION

Edible flowers are a class of harmless flowers that has gained attention in many sectors widely. It is preferred as traditional cuisine, traditional medicine, and primarily as ornaments. Edible flowers could enhance the food flavor, color, and aesthetic value. Consumption of flowers has started in ancient Rome and Greece, and the trend has recently increased [9]. In the recent market, edible flowers are used as salad, soup, and tea from dried ones [24]. Currently, the infusion has become the most common way to utilize edible flowers since it is a simple way to obtain the bioactive substance of the flower. Moreover, edible flowers can be transformed into sauces, jams, jelly, candy, wine, and food preservatives [14]. [13] reported that 97 families, 100 genera, and 180 species of edible flowers are used globally, and their demand is exceptionally high. Some are dianthus, osmanthus, chrysanthemum white, orange lily, calendula, and peach blossom.

The aforementioned edible flowers are well-known because they exhibit an excellent source of bioactive compounds, such as phenolic, flavonoid, anthocyanin, and alkaloid [6] [15]. The phenolic compounds found in the edible flowers include epicatechin, gallic acid, protocatechuic acid, and catechin. The total phenolic compounds were also relatively high [6][17]. The phenolic compounds of edible flowers are attractive to explore due to exhibit antioxidant power, whereas phenolic compounds positively impact several chronic diseases [5][24]. To obtain the advantage of edible flowers, therefore, identifying and quantifying phenolic compounds is necessary. The most frequently used method for determining bioactive compounds is liquid chromatography [5][11].

High-performance liquid chromatography (HPLC) is a separation method for chemical compounds and is the most commonly used method for the analysis of phenolic compounds [2][8][9]. The compound will be separated in the column depending on their polarity. When using the reverse phase column, the less polar compounds will retain in the column for a longer time than the more polar compounds, and hence, the more polar compounds have a shorter retention time. HPLC is a fast method to analyze compounds since it uses high pressure to force the solvents through the column [6].

Retention time is the amount of time a compound spends on the column after being injected. If a sample contains several compounds, each compound will spend a different amount of time on the column according to its chemical composition, i.e., each will have a different retention time. Retention times are usually quoted in units of seconds or minutes [7]. Retention time is used to measure the resolution, which measures how well separated two peaks are from each other. Resolution is defined as the difference in retention time between the peaks divided by the widths of the peaks [5]. Some factors such as gradient program and flow rate could affect the resolution and analysis time of the separation using HPLC [8].

Usually, the interaction among the separation factors may influence the resolution. The chemometric approach can be applied to assist the optimization of the chromatographic method by evaluating the factors concurrently. In evaluating the chromatographic parameters, factorial design is more worthwhile than the single-factor experimental [19]. Moreover, BBD is time efficient since it offers fewer runs for three factors over other factorial designs [7].

After developing an analytical method, validation testing must be performed by checking the linearity, detection and quantification limits, precision, and accuracy [3]. In this study, the ICH Guideline Q2 (R1) and suggestions in ISO 17025 outlined the assessment of the validation parameters. Henceforth, the objective of this study was to evaluate the robustness and other validation parameters of HPLC-DAD for the determination of phenolic compounds in some selected dried edible flowers (dianthus, chrysanthemum, and orange lily).

MATERIALS AND METHOD

Materials

HPLC-grade methanol and acetic acid were

purchased from Merck (Darmstadt, Germany). Standard compounds of the highest available purity were used. Protocatechuic acid (PRO), Protocatechuic aldehyde (PRA), p-hydroxybenzoic acid (*p*-OHB), vanillic acid (VAA), *p*-coumaric acid (*p*-COU), and ferulic acid (FER) were obtained from Sigma Aldrich (St. Louis, MO, USA). Water (aqua pro injection) was obtained from PT Ikapharmindo Putramas (Jakarta, Indonesia). Stock standard solutions of studied compounds were prepared in aqueous methanol 50:50 (v/v) at 1000 mg L^{-1}.

Dried edible flowers

The separation method was developed using a mixture of three commonly used dried flowers for tisane: carnation or dianthus (*Dianthus caryophyllus*), chrysanthemum white (*Dendranthema x grandiflorum*), and orange lily (*Lilium bulbiferum*). The dried edible flowers were purchased from Elif Tea and Tisane (Cirebon, Indonesia). While for the real sample application, the method was utilized to determine the phenolic compounds in each dried flower.

Sample preparation

The dried flowers were firstly ground for 4.5 min with resting for 30 s in every 30 s milling process. The phenolic compounds in 1 g of dried flower samples were extracted using the Ultrasound-Assisted Extraction (UAE) method applying the conditions of 80% ultrasound power at ambient temperature using methanol:water (1:1) as an extraction solvent with a sample-to-solvent ratio of 1:5. The extraction process was performed in three cycles, each for 10 min. The obtained supernatants were separated with a centrifuge of 4000 × g for 10 min. Subsequently, the resulting extracts were collected, and the organic solvent was removed under a vacuum using a rotary evaporator until the remaining extract was 5 mL. The concentrated extract was then filtered with a 45 µm nylon filter prior to the injection to the HPLC-DAD system.

Chromatographic method

The compounds separation was performed by an HPLC Shimadzu prominence with a C18 column Shim-Pac GIST Shimadzu (150 mm, 4.6 mm, 5 µm) supported by a binary pump (LC-20AD) and an auto-sampler (SIL-HTC, Shimadzu, Japan). The diode array detector (DAD SPD M-20A) was set for compound identification using a three-dimensional (3D) scan mode in the wavelength range from 190 to 350 nm. While for compounds quantification, a channel of 260, 280, and 320 nm was selected. This chromatographic system was managed using LabSolutions software. The mobile phases consisted of phase A containing 2% acetic acid, 5% methanol in water, and mobile phase B containing 2% acetic acid, 88% methanol in water. Both phases were filtered using a 45 µm nylon filter then were sonicated in an ultrasound bath for 20 min before use.

Experimental design

A Box Behnken Design (Figure 1) was used to evaluate the effect of three studied independent variables: x_1, flow rate (min); x_2, mobile phase composition at the gradient start (%B initial), and; x_3, mobile phase composition at the end of gradient elution (%B end). Each variable was normalized, resulting in three levels coded as -1, 0, and 1 (Table 1). The composition of the mobile phases in this study was set by earlier studies of phenolic compounds with HPLC [8]. Standard solutions of the six studied phenolic compounds were used to assess the effect of the operating HPLC condition on the separation result. All standard compounds were mixed, achieving a concentration of 200 mg L^{-1} for each compound.

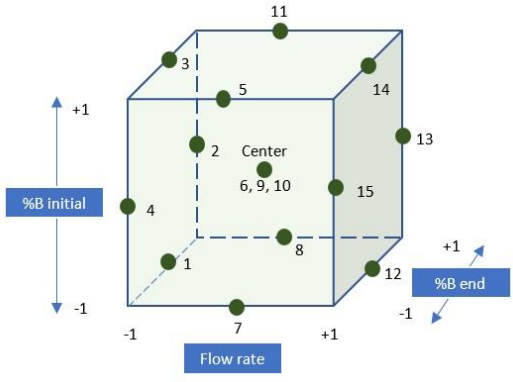

Figure 1. Fifteen run box-behnken design with 3 factors and 3 levels

Table 1. Selected variables and their levels

Factor	Coded			Unit
	-1	0	1	
Flow rate	0.8	1	1.2	mL min^{-1}
Phase $B_{initial}$	0	10	20	%
Phase B_{end}	70	85	100	%

The responses considered for the optimization were analysis time and the resolution (*Rs*) of chromatographic peaks. The analysis time was indicated by the retention time of the last eluted peak in the chromatogram. While the peak resolution (*Rs*) described the separation of two adjacent peaks in terms of their average baseline peak width and was measured using the following equation:

$$Rs = \frac{t2 - t1}{\frac{1}{2}(t2 - t1)} \quad (1)$$

where *Rs* is the peak resolution; t_1 and t_2 are the retention times of the first and second peaks, respectively; w_1 and w_2 are the corresponding widths at the bases of the pair of adjacent peaks.

Method validation

The validation criteria were based on ICH Guideline Q2 (R1) and suggestions in ISO 17025 for the linearity of the calibration curve, limit of detection, the limit of quantification, precision, and accuracy. Standard working solutions were prepared by dissolving the standard stock solution (1000 mg L−1) using aqueous methanol 50:50 (v/v), resulting in concentrations 1, 10, 30, 50, 75, and 100 mg L−1. Regression analysis was performed to measure the coefficient of determination (R^2). The limit of detection (LOD) and limit of quantification (LOQ) were then calculated using the regression result for each compound.

$$LOQ = 3.3\ x\ \frac{Standard\ error\ of\ the\ regression\ curve}{Slope\ of\ the\ regression\ curve} \quad (2)$$

$$LOD = 10\ x\ \frac{Standard\ error\ of\ the\ regression\ curve}{Slope\ of\ the\ regression\ curve} \quad (3)$$

The precision was done by comparing three HPLC-DAD runs intra-day analysis (repeatability, n = 9) and inter-day analysis (intermediate analysis, n = 3×3). Possible outliers were checked using the Q Dixon test. The precisions were indicated by the coefficient of variation (CV) values for each phenolic compound. The value of the CV should not exceed 15 % [1].

$$CV = \frac{Standard\ deviation}{Mean\ value}\ x\ 100\% \quad (4)$$

RESULTS AND DISCUSSION

Data acquisition for the responses

The peaks of phenolic compounds appeared in the order of polarity. As a reverse-phase column of C18 was used, the higher polarity, the faster the compound retained in the column. Therefore, protocatechuic acid (PRO) was first eluted, followed by *p*-OH benzoic acid (*p*-OHB), protocatechuic aldehyde (PRA), vanillic acid (VAA), *p*-coumaric acid (*p*-COU), and ferulic acid (FER). Hereafter, these order numbers indicate the corresponding compounds, as cited in the peak resolution. $R_{s1–2}$ means resolution between PRO and *p*-OHB, and so forth.

The collected extracts were analyzed using HPLC-DAD to identify the existing phenolic compounds in the sample. The compounds were identified based on the comparison of the retention times comparison of the peaks that appeared in the sample chromatogram to the peaks of the studied six standard compounds that were used as references.

Optimization of HPLC-DAD condition

Utilizing a Box-Behnken design (BBD) of three factors and three levels, 15 analyses were performed to assess the effect of HPLC-DAD factors on the separation of the studied phenolic compounds (Table 2). The resolution between each consecutive peak was higher than 1.0, confirming good separations applying different conditions suggested by the BBD. Henceforth, a flow rate change from 0.8 to 1.2 ml min^{-1} as well as altering the mobile phase composition slightly at the beginning (0 to 20% Phase B) and end (70 to 100%

Phase B) of the gradient program did not affect the separation performance of the method.

Table 2. Results obtained in 15 runs performed for peak resolutions analysis time

Run	HPLC factors			Resolutions*					Analysis time
	Flow rate	%B initial	%B end	Rs_{1-2}	Rs_{2-3}	Rs_{3-4}	Rs_{4-5}	Rs_{5-6}	
1	-1	-1	0	6.83	4.90	3.84	7.55	1.34	13.34
2	-1	0	1	6.30	5.23	3.49	7.46	1.28	11.55
3	-1	1	0	6.62	5.73	3.80	9.66	1.76	11.38
4	-1	0	-1	6.74	5.83	4.51	9.59	1.82	13.14
5	0	1	-1	6.35	5.40	3.87	10.20	2.12	10.22
6	0	0	0	6.19	5.41	4.28	9.04	1.76	10.83
7	0	-1	-1	6.93	5.32	4.64	8.85	1.74	12.32
8	0	-1	1	6.25	4.56	3.51	7.13	1.30	10.65
9	0	0	0	6.16	5.44	4.26	9.10	1.78	10.81
10	0	0	0	6.12	5.46	4.30	9.09	1.78	10.81
11	0	1	1	6.56	5.22	3.37	8.51	1.49	9.06
12	1	-1	0	6.36	4.67	4.42	8.37	1.71	10.54
13	1	0	1	5.59	4.93	3.78	8.06	1.54	8.89
14	1	1	0	5.88	5.00	3.52	9.40	1.93	8.57
15	1	0	-1	5.75	4.86	4.23	9.34	1.89	10.39

* Rs1-2, resolution between protocatechuic acid and *p*-OH benzoic acid; Rs_{2-3}, resolution between *p*-OH benzoic acid and protocatechuic aldehyde; Rs_{3-4}, resolution between protocatechuic aldehyde and vanillic acid; Rs_{4-5}, resolution between vanillic acid and *p*-coumaric acid; Rs*5-6*, resolution between *p*-coumaric acid and ferulic acid.

Subsequently, the effects of flow rate and mobile phase composition throughout the gradient program were calculated to optimize the HPLC-DAD factors with the target to minimize the analysis time. The statistical significance for each studied factor was measured (Figure 2). The corresponding bar crossing the vertical line showed the significant factors influencing the analysis time ($p<0.05$). All the main (x_1, x_2, and x_3) and their quadratic effects (x_1x_1, x_2x_2, and x_3x_3), in addition to the interaction of the mobile phase composition at the initial and end of the gradient program (x_2x_3), significantly influenced the analysis time.

The aforementioned significant effects were then used to construct a mathematical model to predict the optimum HPLC-DAD factors. The resulting model for the proposed chromatographic method was as follows:

$$Y = 10.82 - 1.38\,x_1 - 0.95\,x_2 - 0.74\,x_3 + 0.28\,x_1x_1 - 0.14x_2x_2 - 0.11\,x_3x_3 + 0.13x_2x_3$$

where y was the analysis time (min), *xi* were the studied factors (x_1, flow rate; x_2, mobile phase composition at the gradient start (%B initial), and; x_3, mobile phase composition at the end of gradient elution (%B end).

As suggested by the model, a fast separation (8.00 min) was achieved by applying a flow rate of 1 ml min^{-1} with 20% and 100% phase B for the mobile phase composition at the initial and end of the gradient program, respectively.

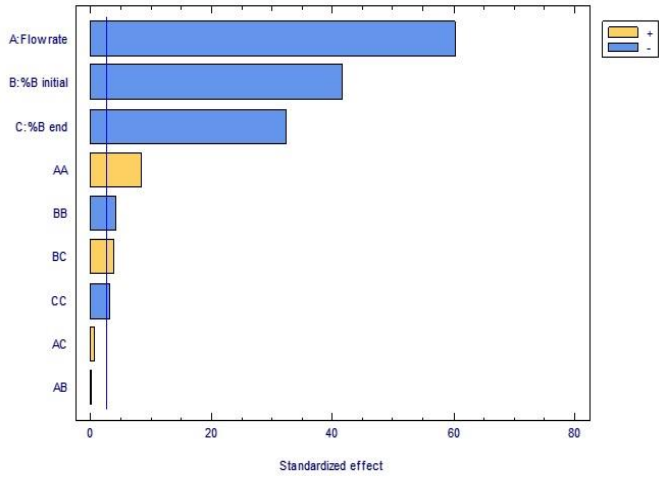

Figure 2 Standardized values of main, interaction, and quadratic effects of HPLC-DAD factors on the analysis time

Validation of optimization

The analytical HPLC-DAD method was validated following the ISO 17025 and ICH Guidelines (R1) (ICH, 2005; ISO, 2005). The linearity of calibration curves for protocatechuic acid, *p*-OH benzoic acid, protocatechuic aldehyde, vanillic acid, *p*-coumaric acid, and ferulic acid was validated with a high coefficient of determination (R^2, higher than 99.69%). The limit of detection (LOD) ranged from 5.07 (*p*-coumaric acid) to 7.60 (ferulic acid) mg L^{-1}, while the limit of quantification (LOQ) ranged from 15.37 (*p*-coumaric acid) to 23.04 (ferulic acid) mg L^{-1}.

Table 3. Concentration of phenolic compounds in dried edible flowers

	Phenolic compounds (µg g^{-1})				
Samples	PRO	PRA	VAA	*p*-COU	FER
Dianthus caryophyllus	169.50±1.64	ND	148.96±0.54	ND	ND
Dendranthema x grandiflorum	ND	4.19± 0.01	ND	ND	ND
Lilium bulbiferum	ND	ND	ND	296.29±2.28	267.96±1.88

Note. PRO, protocatechuic acid; PRA, protocatechuic aldehyde; VAA, vanillic acid; *p*-COU, *p*-coumaric acid; FER, ferulic acid. ND, not detected as the value was lower than the limit of detection.

A Q-Dixon test was performed to evaluate the data for the precisions and showed that there were no outliers in the data set. Two levels of precision of the developed HPLC-DAD method, namely repeatability and intermediate precision, were evaluated. The precision, indicated as CV, of the HPLC-DAD method was ranged from 0.23 (protocatechuic aldehyde) to 0.97% (protocatechuic acid) for repeatability (n=9) and 0.56 (*p*-hydroxybenzoic acid) to 1.50% (ferulic acid) for intermediate precision (n=3×3). Since both CV values for repeatability and intermediate precision were below 1.50%, the developed HPLC-DAD has been proved as a precise separation method.

Real sample application

The compounds were identified based on the comparison of the retention times of the peaks that appeared in the sample chromatogram to the peaks of six standards compounds that were used as references (protocatechuic acid, *p*-OH benzoic acid, protocatechuic aldehyde, vanillic acid, *p*-coumaric acid, and ferulic acid). Five phenolic compounds were detected in the dried flower samples, namely protocatechuic acid, protocatechuic aldehyde, vanillic acid, *p*-coumaric acid, and ferulic acid. These identified compounds were in common to those that have been reported to be presented in edible flowers namely dianthus, chrysanthemum white and orange lily. Chen at al. (2015) reported protocatechuic acid (719.27 µg g^{-1}) and vanillic acid (555.18 µg g^{-1}) were found in *Dianthus caryophyllus*, while in *Dendranthema x*

grandiflorum showed different level of protocatechuic acid content (31.32 µg g^{-1}) in addition to protocatechuic aldehyde. Other report by Sim et al. (2020) revealed the identification of *p*-coumaric acid and ferulic acid in *Lilium lancifolium* with a concentration of 1.14 and 1.46 mg g^{-1} respectively. On the other hand, *p*-OH benzoic acid was not detected in the studied samples as the concentration was lower than the detection limits of the validated HPLC-DAD method.

CONCLUSION

An HPLC-DAD was developed and validated to determine phenolic compounds in selected dried flowers. This method offers the advantage of using a short run time of 8 min to separate six studied phenolic compounds on the C18 column. Sufficient separation of the compounds was achieved (*Rs* > 1.0), applying a mobile phase composition of 20% and 100% at the beginning and end of the gradient program, respectively, at a flow rate of 1 ml min^{-1}. Results from validation of the method proved satisfactory linearity, accuracy, and precision; therefore, we conclude that the method is suitable for routine quantification of individual phenolic compounds in dried edible flowers.

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REFERENCES

Akhtar, I., Javad, S., Ansari, M., Ghaffar, N., & Tariq, A. (2020). Process optimization for microwave assisted extraction of Foeniculum vulgare Mill using response surface methodology. Journal of King Saud University - Science, 32(2), 1451–1458. https://doi.org/10.1016/j.jksus.2019.11.041

Alara, O. R., Abdul Mudalip, S. K., & Olalere, O. A. (2017). Optimization of mangiferin extrated from Phaleria macrocarpa fruits using response surface methodology. Journal of Applied Research on Medicinal and Aromatic Plants, 5, 82–87. https://doi.org/10.1016/j.jarmap.2017.02.002

Alara, Oluwaseun Ruth, Abdurahman, N. H., & Olalere, O. A. (2018). Optimization of microwave-assisted extraction of flavonoids and antioxidants from Vernonia amygdalina leaf using response surface methodology. Food and Bioproducts Processing, 107, 36–48. https://doi.org/10.1016/j.fbp.2017.10.007

Alupului, A., Călinescu, I., & Lavric, V. (2012). Microwave Extraction of active principles from medicinal plants. UPB Scientific Bulletin, Series B: Chemistry and Materials Science, 74(2), 129–142.

Chen, G. L., Chen, S. G., Xie, Y. Q., Chen, F., Zhao, Y. Y., Luo, C. X., & Gao, Y. Q. (2015). Total phenolic, flavonoid and antioxidant activity of 23 edible flowers subjected to in vitro digestion. Journal of Functional Foods, 17, 243–259. https://doi.org/10.1016/j.jff.2015.05.028

Fernandes, L., Casal, S., Pereira, J. A., Saraiva, J. A., & Ramalhosa, E. (2017). Edible flowers: A review of the nutritional, antioxidant, antimicrobial properties and effects on human health. Journal of Food Composition and Analysis, 60(March), 38–50. https://doi.org/10.1016/j.jfca.2017.03.017

Ferreira, S. L. C., Bruns, R. E., Ferreira, H. S., Matos, G. D., David, J. M., Brandão, G. C., da Silva, E. G. P., Portugal, L. A., dos Reis, P. S., Souza, A. S., & dos Santos, W. N. L. (2007). Box-Behnken design: An alternative for the optimization of analytical methods. Analytica Chimica Acta, 597(2), 179–186. https://doi.org/10.1016/j.aca.2007.07.011

Fu, X. Q., Ma, N., Sun, W. P., & Dang, Y. Y. (2018). Microwave and enzyme co-assisted aqueous two-phase extraction of polyphenol and lutein from marigold (Tagetes erecta L.) flower. Industrial Crops and Products, 123(June), 296–302.

https://doi.org/10.1016/j.indcrop.2018.06.087

Guiné, R. P. F., & Florença, S. G. (2020). Edible Flowers, Old Tradition or New Gastronomic Trend: A First Look at Consumption in Portugal versus Costa Rica. Food Research International, 129(April 2019). https://doi.org/10.1016/j.foodres.2019.108868 \

Sim, W., Choi, S., Jung, T., Cho, B., Choi, S., Park, S., & Lee, O. (2020). Antioxidant and anti-inflammatory effects of Lilium lancifolium bulbs extract. https://doi:10.1111/jfbc.13176

Kataoka, H. (2019). Pharmaceutical analysis | sample preparation. In Encyclopedia of Analytical Science (3rd ed., Issue November 2017). Elsevier Inc. https://doi.org/10.1016/B978-0-12-409547-2.14358-6

Li, A. N., Li, S., Li, H. Bin, Xu, D. P., Xu, X. R., & Chen, F. (2014). Total phenolic contents and antioxidant capacities of 51 edible and wild flowers. Journal of Functional Foods, 6(1), 319–330. https://doi.org/10.1016/j.jff.2013.10.022

Llompart, M., Garcia-Jares, C., Celeiro, M., & Dagnac, T. (2019). Extraction | Microwave-Assisted Extraction. In Encyclopedia of Analytical Science (3rd ed., Issue June). Elsevier Inc. https://doi.org/10.1016/B978-0-12-409547-2.14442-7

Lu, B., Li, M., & Yin, R. (2016). Phytochemical Content, Health Benefits, and Toxicology of Common Edible Flowers: A Review (2000–2015). Critical Reviews in Food Science and Nutrition, 56, S130–S148. https://doi.org/10.1080/10408398.2015.1078276

Pinakin, D. J., Kumar, V., Suri, S., Sharma, R., & Kaushal, M. (2020). Nutraceutical potential of tree flowers: A comprehensive review on biochemical profile, health benefits, and utilization. Food Research International, 127(September 2019), 108724. https://doi.org/10.1016/j.foodres.2019.108724

Purohit, S. R., Rana, S. S., Idrishi, R., Sharma, V., & Ghosh, P. (2021). A review on nutritional, bioactive, toxicological properties and preservation of edible flowers. Future Foods, 4(July), 100078. https://doi.org/10.1016/j.fufo.2021.100078

Rombaut, N., Tixier, A.-S., Bily, A., & Chemat, F. (2012). Perspective: Jatropha cultivation in southern India: Assessing farmers' experiences. Biofuels, Bioproducts and Biorefining, 6(3), 246–256. https://doi.org/10.1002/bbb

Rop, O., Mlcek, J., Jurikova, T., Neugebauerova, J., & Vabkova, J. (2012). Edible flowers - A new promising source of mineral elements in human nutrition. Molecules, 17(6), 6672–6683. https://doi.org/10.3390/molecules17066672

Segura-Carretero, A., Puertas-Mejía, M. A., Cortacero-Ramírez, S., Beltrán, R., Alonso-Villaverde, C., Joven, J., Dinelli, G., & Fernández-Gutiérrez, A. (2008). Selective extraction, separation, and identification of anthocyanins from Hibiscus sabdariffa L. using solid phase extraction-capillary electrophoresis-mass spectrometry (time-of-flight/ion trap). Electrophoresis, 29(13), 2852–2861. https://doi.org/10.1002/elps.200700819

Setyaningsih, W., Palma, M., & Barroso, C. G. (2012). A new microwave-assisted extraction method for melatonin determination in dried flower grains. Journal of Cereal Science, 56(2), 340–346. https://doi.org/10.1016/j.jcs.2012.02.012

Setyaningsih, W., Saputro, I. E., Carrera, C. A., & Palma, M. (2019). Optimisation of an ultrasound-assisted extraction method for the simultaneous determination of phenolics in dried flower grains. Food Chemistry, 288, 221–227. https://doi.org/10.1016/j.foodchem.2019.02.107

Wang, L., & Weller, C. L. (2006). Recent advances in extraction of nutraceuticals from plants. Trends in Food Science and Technology, 17(6), 300–312. https://doi.org/10.1016/j.tifs.2005.12.004

Xiang, J., Yang, C., Beta, T., Liu, S., & Yang, R. (2019). Phenolic Profile and Antioxidant Activity of the Edible Tree Peony Flower and Underlying Mechanisms of Preventive Effect on H2O2-Induced Oxidative Damage in Caco-2 Cells. Foods, 8. https://doi.org/doi:10.3390/foods8100471

Zhang, Q. W., Lin, L. G., & Ye, W. C. (2018). Techniques for extraction and isolation of natural products: A comprehensive review. Chinese Medicine (United Kingdom), 13(1), 1–26. https://doi.org/10.1186/s13020-018-0177-x

Zheng, J., Meenu, M., & Xu, B. (2019). A systematic investigation on free phenolic acids and flavonoids profiles of commonly consumed edible flowers in China. Journal of Pharmaceutical and Biomedical Analysis. https://doi.org/10.1016/j.jpba.2019.05.007

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A REVIEW OF THE EFFECTIVENESS NATURAL PIGMENT AS ANTIDIABETIC TO DECREASE THE SIGNIFICANT RISK FOR COVID-19 DISEASE

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ABSTRACT

Diabetes mellitus proved to be a significant risk factor for both COVID-19 infection and poor outcomes among these chronic health problems. Plants are a rich source of chemical components that could block carbohydrate digestion enzymes, and they can be utilized as therapeutic or functional foods. Natural pigments that have potential benefits, such as chlorophyll, anthocyanin as a part of flavonoid, and carotenoid. Chlorophylls are the most significant and widespread pigment molecules in nature, and they are required for photosynthesis to occur. Anthocyanins are the most important group of water-soluble pigments in plants, responsible for the red, purple, and blue colors of many fruits, vegetables, cereal grains, and flowers. Carotenoids, natural pigments found in an array of different foodstuffs, are the most abundant pigments present in the human diet. The most frequent method for determining a substance's antidiabetic potential is to assess the substance's hypoglycemic or antihyperglycemic.

Keywords: anthocyanin; antidiabetic; chlorophyll; carotenoid; natural pigment

ABSTRAK

Diabetes mellitus terbukti menjadi faktor risiko yang signifikan untuk infeksi COVID-19 dan dampak buruk di antara masalah kesehatan kronis lainnya. Tumbuhan merupakan sumber yang kaya akan komponen kimiawi yang memiliki kemampuan untuk memblokir enzim pencernaan karbohidrat, sehingga dapat dimanfaatkan sebagai makanan terapeutik ataupun fungsional. Pigmen alami yang memiliki potensi manfaat antara lain klorofil, antosianin sebagai bagian dari flavonoid, dan karotenoid. Klorofil adalah molekul pigmen yang paling signifikan dan tersebar luas di alam, dan pigmen ini diperlukan untuk terjadinya fotosintesis. Antosianin adalah kelompok pigmen larut air yang paling penting pada tumbuhan, yang bertanggung jawab atas warna merah, ungu, dan biru pada banyak buah, sayuran, biji-bijian sereal, dan bunga. Karotenoid adalah pigmen alami yang ditemukan dalam berbagai bahan makanan yang bervariasi, sebagai pigmen paling melimpah yang ada dalam makanan manusia. Metode yang paling sering digunakan untuk menentukan potensi antidiabetes suatu zat adalah dengan menilai hipoglikemik atau antihiperglikemik dalam kandungan tersebut.

Kata kunci: antosianin; antidiabetes; karotenoid; klorofil; pigmen alami

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INTRODUCTION

A novel coronavirus, the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) was discovered in Wuhan (Hui et al, 2020). Although the virus affected people of all ages, it was more common in elderly people, especially those who had underlying chronic health issues. Diabetes mellitus, in particular, proved to be a significant risk factor for both COVID-19 infection and poor outcomes among these chronic health problems. Diabetes is already linked to an increased risk of death from any acute or chronic disease, including infection (Zoppini et al, 2018). At least one comorbidity was found in 25.1% of COVID-19 patients in a Chinese national study of 1,590 hospitalized patients. Hypertension (16.9%) was the most common comorbidity, followed by diabetes (8.2%) (Guan et al., 2020).

Plants are a rich source of chemical components that have the ability to block carbohydrate digestion enzymes, and they can be utilized as therapeutic or functional foods (Sales et al., 2012). Oxidative stress, on the other hand, is one of the most common issues in diabetes patients, and it can lead to serious consequences. As a result, one of the goals for creating next-generation antidiabetic medicines is to locate antidiabetic compounds or extracts with antioxidant properties (Orhan et al., 2020).

In this review, there are several types of research that can be evaluated as new natural sources in the treatment of diabetes mellitus, especially for the natural pigments that have potential benefits, such as chlorophyll, anthocyanin as a part of flavonoid, and carotenoid. Chlorophyll is chosen because this natural pigment is the largest. Anthocyanin is also reviewed as a water-soluble pigment in nature that is very abundant. Chlorophyll and anthocyanin are found in plants, but carotenoids can be found both in plants as well as animals that are very abundant in the human diet. These three pigments have significantly bioactive compounds such as antidiabetic also be functional foods nowadays.

DIABETES MELLITUS

Diabetes mellitus (DM) is a metabolic disease characterized by excessive blood sugar, dyslipidemia (impaired lipoprotein metabolism), and altered protein metabolism as a result of decreased insulin production and/or action. Diabetes mellitus (DM) is a condition marked by persistently high blood sugar (hyperglycemia) caused by a lack of insulin synthesis, secretion, or resistance. This condition is critical since the number of sufferers is growing, it is estimated that there are presently 200 million sufferers worldwide. Furthermore, DM is critical due to the problems it creates. The majority of chronic consequences of DM are caused by vascular diseases, namely tiny blood vessels (microangiopathy) and big blood vessels (aneurysms) (macroangiopathy) (Kariadi, 2001). Type 1 diabetes mellitus (DM-1) is also known as insulin-dependent diabetes mellitus (IDDM), while type 2 diabetes mellitus (DM-2) is also known as noninsulin-dependent diabetes mellitus (NIDDM).

Insulin production is nonexistent or limited in DM-1 severe pancreatic damage, thus insulin must be obtained from outside the body. DM-1 is also known as insulin-dependent diabetes mellitus, and it can strike at any age (children, adolescents). Insulin insufficiency is present in DM-2, although it is not as severe as it is in DM-1. Insulin resistance is associated with insulin insufficiency in DM-2, meaning that the presence of insulin is unable to control blood sugar levels adequately for the body's demands, and so plays a role in raising blood sugar levels. DM-2 generally manifests itself at the age of 30-40 years, and it can even manifest itself at the age of 50 or 60 years. The most frequent method for determining a substance's antidiabetic potential is to assess the substance's hypoglycemic or antihyperglycemic (blood sugar lowering) impact in experimental animals, generally rats with alloxan-induced diabetes. Due to damage to the β-cells of the islets of Langerhans in the pancreas, alloxan induces a significant reduction in insulin excretion, resulting in hyperglycemia (Marpaung, 2020). Several phytochemicals potentially use to change to hypoglycemic such as chlorophyll, anthocyanin, and carotenoid.

NATURAL PIGMENT AS ANTIDIABETIC

Plants have three mechanisms of reducing blood sugar levels. First, it acts as an astringent, which means it may precipitate intestinal mucous membrane proteins and produce a protective barrier around the intestines, preventing glucose absorption and lowering blood glucose levels. Second, it accelerates the release of glucose from the circulation by accelerating blood circulation, which is closely related to the work of the heart, as well as filtration and renal excretion, resulting in increased urine production, increased glucose excretion through the kidneys, and lower blood glucose levels. Third, increased metabolism or incorporation into fat deposits speeds up the release of glucose. The pancreas is involved in this process because it produces insulin (Suryowinoto, 2005). The importance of reducing postprandial hyperglycemia (PPHG) in the treatment of type 2 diabetes has been demonstrated by a favorable connection between human pancreatic alpha-amylase (HPA) activity and the increase in postprandial glucose levels (Watanabe et al., 1997). The capacity of alpha-amylase enzyme inhibitors to prevent dietary starch from being digested and absorbed in the body has led to the label "starch blocker" (Horii et al., 1986).

Besides, antioxidants in the form of vitamins can help patients with DM-1, both chronic and acute, minimize oxidative stress (Lee, 2002). The majority of antioxidants in plasma can be decreased in individuals with DM-2 as a result of diabetic complications such as atherosclerosis and coronary heart disease (Tiwari & Rao, 2002). Insulin resistance can be greatly improved by taking 132 mg of isoflavones from soybean extract every day for 12 weeks. Consumption of high-carotene vegetables and fruits can protect against hyperglycemia, and plasma levels of lutein and β-carotenoids can help healthy volunteers maintain blood glucose levels indirectly. In DM-2 test animals, carotenoids and astaxanthin can lower non-fasting blood glucose levels. Grape seed extracts include a number of flavonoids, notably proanthocyanidins, which can improve insulin sensitivity and decrease free radical production. The flavonoid quercetin was discovered to be effective in preventing the development of diabetic cataracts (Schoenhals, 2005).

CHLOROPHYLL

Chlorophylls are the most significant and widespread pigment molecules in nature, and they are required for photosynthesis to occur. They serve a crucial part in photosynthesis by absorbing, transmitting, and transducing light energy (Scheer, 2006). Chlorophyll is a blue or green pigment that has a maximum absorption range of 660 to 665 nm. (Hosikian et al, 2010). It is required for photosynthesis because it allows charged electrons to flow through to molecules that produce sugars. All-natural chlorophyll derivatives have a centrally attached magnesium atom and are substituted tetrapyrroles (Ferruzzi & Blakeslee, 2007). The abundant availability of chlorophyll in nature, as well as its biological characteristics, have made it a viable candidate for development as a dietary supplement or functional food (Prangdimurti, 2007). Meanwhile, nearly all chlorophyll-based dietary supplements on the market in Indonesia are imported and sell for a relatively high price (Nurdin et al., 2009).

Chemical structure

Chlorophylls may be classified into two groups based on their distribution in photosynthetic organisms: chlorophylls in oxygenic photosynthetic organisms (abbreviated Chls) and chlorophylls (bacteriochlorophyll) in anoxygenic photosynthetic bacteria (abbreviated BChls) (Chew and Bryant 2007). For a long period, it was believed that there are only four chemically distinct chlorophylls in oxygenic photosynthetic organisms, namely Chls a, b, c, and d (Chen et al., 2010). Plants also contain tiny quantities of pheophytin, protochlorophyllide, and other compounds in addition to Chls and BChls. Chlorophylls are cyclic tetrapyrroles with a distinctive isocyclic five-membered ring (porphyrin ring) that are used in photosynthesis for light-harvesting or charge separation. The structure can be seen in Figure 1. Chlorophyll members have varied characteristics depending on their architectures, allowing different photosynthetic organisms to adapt to different conditions. The IUPAC-IUB nomenclature is used to name the rings and carbon atoms on a chlorophyll structure (Moss, 1988).

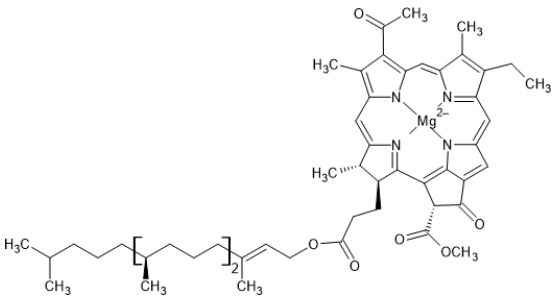

Figure 1. Chlorophyll general structure

Green algae contain chlorophyll b, a green or yellow pigment with a maximum absorption range of 642 to 652 nm. Chlorophyll b is an auxiliary pigment that absorbs light and transfers it to chlorophyll a during photosynthesis (Hosikian et al., 2010). Chlorophyll c is a blue-greenish pigment that has a maximum absorbance range of 447 to 452 nm. Chlorophyll c can also be present in seaweed. Chlorophyll d is found in red algae and marine cyanobacteria, and it absorbs far-red light with a wavelength of 710 nm (Larkum & Kühl, 2005). Porphyrins, chlorins, bacteriochlorins, pheophorbides, bacteriopheophorbides, texaphyrins, porphycenes, and phthalocyanines are some of the types of chlorophyll produced from marine algae and cyanobacteria. These derivatives exhibit chlorin's basic structural skeleton and absorb light significantly in the red band spectrum (Li et al., 2007).

Chlorophyll sources

Chlorophyll is the most significant tetrapyrrolic pigment, found in sea algae and cyanobacteria as part of a chlorophyll-protein complex (Hosikian et al., 2010). Besides, chlorophyll pigment can be found in several types of plants. According to Ashok (2011), a project is underway to demonstrate the effectiveness of wheatgrass in the treatment of diabetes mellitus. *Triticum aestivum* L. is an immunomodulator, antioxidant, astringent, laxative, diuretic, and antibacterial plant that is used in the treatment of acidity, colitis, renal dysfunction, swollen wounds, and vitiated states of the Kapha and Pitta doshas.

Wheatgrass is also thought to have the ability to regulate blood sugar levels. The existence of chlorophyll, which is thought to be a pharmacologically active component in wheatgrass as an antidiabetic drug, was verified by instrumental characterization of wheatgrass (spray-dried powder of juice). Chlorophyll a has a maximum of 661.1, whereas chlorophyll b has a maximum of 642.6. The GC-MS results revealed peaks linked to chemicals that are chlorophyll degradation products. Furthermore, HPLC examination confirmed the existence of chlorophyll a and chlorophyll b.

The pigment content of acetone and ethanol extracts of *G. salicornia*, *T. decurens*, and *H. macroloba* revealed that the green algae *H. macroloba* had the highest content for all types of pigments, with chlorophyll C1+C2 being the pigment with the highest content, with values of 1.85±0.53 and 6.23±0.12 mg/g dry weight for the acetone and ethanol extracts, respectively (Sanger et al., 2018).

Chlorophyll as antidiabetic

In recent years, chlorophyll derivatives have opened a slew of new possibilities for photodynamic treatment (Li et al., 2007). Antibacterial (Alenezi et al., 2017), antioxidant (Lanfer-Marquez et al., 2005), anti-inflammatory (Jelic et al., 2012), and antimutagenic activities (Ferruzzi & Blakeslee, 2007) are all common uses for chlorophyll derivatives in biomedical applications.

According to Shilpa Vs et al. (2019), fresh *E. hirta* leaves were nutritionally analyzed and found to possess adequate quantities of essential components. With an IC_{50} value equivalent to that of the medication metformin, this plant demonstrated considerable inhibition of the enzyme alpha-amylase in a concentration-dependent manner, suggesting that it may yield important antidiabetic chemicals for use in the treatment of diabetes. Antidiabetic efficacy of *Euphorbia hirta* was investigated using an in vitro alpha-amylase inhibition test.

The methanolic extract of *Euphorbia hirta* was found to have concentration-dependent inhibitory

action against the α-amylase enzyme, with an IC_{50} of 0.748 mg/ml in this investigation. The extract's in vitro antidiabetic efficacy was compared to that of the standard medication Metformin, which has an IC_{50} of 0.58 mg/ml. Since *E. hirta* methanolic extract has an IC_{50} value that is equivalent to that of the conventional medication, it inhibits alpha-amylase significantly, and therefore it may have antidiabetic potential. Other Euphorbiaceae plants, such as *Phyllanthus amarus*, *Acalypha indica*, and *Euphorbia thymifolia*, have been found to have considerable antidiabetic potential through their inhibition of the alpha-amylase enzyme.

The antioxidant activity of *G. salicornia* acetone extract was measured at IC_{50} of 1.24±0.1402 mg/mL. *G. salicornia* possesses antioxidant, ion-reducing, chelating, cytotoxic, and antidiabetic properties (Sanger et al. 2013; Saeidnia et al. 2009). According to Hardoko et al. (2015), *G. gigas* includes agarose (0.28%), agar (5.91%), and agaropectin (6.07%), all of which exhibit antidiabetic action by reducing the activity of the α-glucosidase enzyme with IC_{50} values of 0.09±0.004, 0.12±0.005, and 0.15±1.77 mg/mL, respectively.

In alloxan-induced diabetic mice, a dosage of 150 mg/kg of *Clinacanthus nutans* leaf water extract can lower blood glucose levels. This supports its usage as an antidiabetic in the population. When compared to the other fractions, the ethanol precipitate fraction contained the same secondary metabolite compounds as the aqueous extract, namely flavonoids, steroids/triterpenoids, and tannins, which resulted in the greatest reduction in blood glucose levels in mice using the glucose tolerance method (Nurulita et al., 2008).

Adding alfalfa seed to the human diet has been shown to lower triglycerides and LDL, enhance HDL levels, and lower blood glucose levels in previous studies (Asgary et al., 2008; Mehranjani et al., 2008). As a result, alfalfa leaves have long been utilized in South Africa as an effective diabetic therapy (Lust, 1986; Gray & Flatt, 1997). Insulin secretion is stimulated by alfalfa. It also enhances insulin function by lowering blood glucose levels, although its effects on blood lipids have not been well studied (Gray & Flatt, 1997; Winiarska et al., 2007). Fourty experimental rats were randomly divided into four groups, each with ten rats: first, a control group fed normal water and food; second, a diabetic control group; third, an experimental group consisting of diabetic rats given a 250 mg/kg dose of alfalfa aqueous extract; and fourth, a diabetic group consisting of diabetic rats given a 500 mg/kg dose of alfalfa aqueous extract; and fourth, a diabetic group consisting The remaining three diabetic groups were produced by injecting 120 mg/kg alloxan monohydrate intraperitoneally to produce alloxan-induced diabetic rats (Matkovics et al., 1997).

Blood glucose of rats was tested seven days after alloxan monohydrate injection for confirming the diabetic condition in rats, and diabetic rats with blood glucose concentrations more than 200 mg/100 cc were chosen (8 rats out of 10) (Takasu et al., 1991). For equivalence of shock achieved by intraperitoneal injection, the first control group is injected with the physiologic serum. To easily comparing the research respectively, these all experiments compile into Table 1 below.

ANTHOCYANIN

Chemical structure

Anthocyanins are efficient hydrogen donors. Anthocyanins can easily donate protons to highly reactive free radicals, preventing further radical formation, due to their positive charge (Figure 1), the number and arrangement of aromatic hydroxyl groups, the extent of structural conjugation, and the presence of electron-donating and electron-withdrawing substituents in the ring structure (Oliveira et al., 2020).

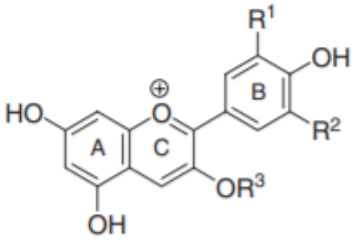

Figure 2. General anthocyanin structure present in fruits (Freitas and mateus, 2006; Kähkönen and heinonen, 2003; Giusti et al., 1999).

Table 1. Effectiveness chlorophyll as antidiabetic in several research

Sources	Extraction	Mechanism	Experiment	Reference
Fresh *Euphorbia hirta* leaves	Methanol	Inhibitory action against the α-amylase enzyme	In vitro	Shilpa Vs et al. (2019)
Gracilaria gigas	Ethanol (agar), Dimethyl sulfoxide (agarose & agaropectin)	Reducing the activity of the α-glucosidase enzyme	In vitro	Hardoko et al. 2015
Clinacanthus nutans leaf	Water	Alloxan-induced diabetic mice	In vivo	Nurulita et al., 2008
Alfalfa seed	Aqueous	Fourty experimental rats were randomly divided into four groups	In vivo	Matkovics et al., 1997; Takasu et al., 1991

Because of their peculiar chemical structure, anthocyanins possess health properties. They are reactive towards reactive oxygen species (ROS), such as superoxide ($O2^{\cdot -}$), singlet oxygen ($^1O_2$), peroxide ($RCOO^{\cdot}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\cdot}$), as well as reactive nitrogen species in a terminator reaction, which breaks the cycle of generation of new radicals, due to their electron deficiency (Magalhaes et al., 2008; Choe and Min, 2006; Kong et al., 2003; Min and Bolf, 2002). The antioxidant properties of phenolic compounds are also linked to chelate metal ions, which participate in the generation of free radicals and so reduce metal-induced peroxidation (Liu, 2010). Anthocyanins exhibit multiple biological effects, but this paper will focus on the antidiabetic effect.

Anthocyanins are glycosilate polyhydroxy or polymethoxy derivatives of 2-phenylbenzopyrilium with molecular weights ranging from 400 to 1200 (medium-size biomolecules) and two benzyl rings (A and B) (Andersen and Jordheim, 2010; He and Giusti, 2010; Castaneda-Ovando et al., 2009; Gould et al., 2009; Ghosh & Konishi, 2007). Anthocyanins are most commonly found as glycosides. The aglycones are rarely found in plants other than as artifacts; the 3-deoxy forms, which can be found in red-skinned bananas, sorghum, and black tea, are the notable exceptions. The sugars most commonly encountered are glucose (the most common), galactose, rhamnose, arabinose, xylose, and glucoronic acid, usually as 3-glycosides or 3.5-diglycosides (Pereira et al., 2009; Freitas and Mateus, 2006); 3-diglycosides and 3-diglycoside-5-monoglycosides are less common. Rutinose, sambubiose, lathyrose, and sophorose are the four main biosides encountered. In terms of glycoside distribution, 3-glycosides occur almost two and a half times as frequently as 3.5-diglycosides, with cyanidin-3-glucoside being the most common anthocyanin (Kong et al., 2003). Anthocyanidins are the de-glycosilated or aglycone forms of anthocyanins.

Anthocyanin sources

Anthocyanins are naturally occurring pigments. Throughout the plant kingdom, it can be found in all plant tissues (Kong et al., 2003). Anthocyanins are phytochemicals that belong to the flavonoid family and are found in nature (Pervaiz et al., 2017). Anthocyanins belong to the phenolics or polyphenolics superfamily of antioxidants (Quideau et al., 2011; Ferretti et al., 2010; Daayf and Lattanzio, 2008). Flavonoids are a class of phytochemicals that make up the most important category of phenolics in foods. They are commonly found in teas, honey, wines, fruits, vegetables, nuts, olive oil, cocoa, and cereals (Raghvendra et al., 2011; Wallace, 2011; Andersen & Markham, 2006).

Anthocyanins are the most important group of water-soluble pigments in plants, responsible for the red, purple, and blue colors of many fruits, vegetables, cereal grains, and flowers. They are odorless and practically flavorless, adding to taste as a somewhat astringent sensation (He & Giusti, 2010; Gould et al., 2009; Motohashi, 2008; Veitch and Grayer, 2008; Andersen & Jordheim, 2006; Escribano-Bailón et al., 2004; Vidal et al., 2004; Williams and Grayer, 2004; Harborne & Williams, 2000; Harborne & Grayer, 1988). Anthocyanins obtained from fruits and vegetative tissues have simpler structures than anthocyanins isolated from flowers (Andersen & Jordheim, 2010).

Anthocyanin as antidiabetic

Diabetes mellitus is a disease with an oxidative stress component. Free radicals react with biomembranes causing oxidative destruction of unsaturated fatty acids to form cytotoxic aldehydes via lipid peroxidation. Furthermore, lipid peroxidation was measured under conditions of thiobarbituric acid reactive substances (TBARS) and lipid hydroperoxides (HPX), which are the end products of lipid peroxidation. Increased lipid peroxidation of membranes and lipoproteins occurs in patients. HPX formed from lipid peroxidation has a direct toxic effect on endothelium cells and degrades to form hydroxyl radicals. This can be seen in beta pancreatic cells (Pari & Latha, 2005).

Free radicals are unstable, reactive, and have the ability to damage biological molecules so that free radicals in excess in the body are very dangerous because they cause damage to cells, nucleic acids, proteins, and fatty tissues. Free radicals are formed in the body as a by-product of metabolic processes or because the body is exposed to free radicals through breathing (Tias, 2010). The normal body contains endogenous antioxidant or anti-free radical mechanisms. Antioxidants are compounds that can inhibit free radical reactions in the body (Wardatun, 2011). Antioxidants can stop the process of cell destruction by donating electrons to free radicals. Antioxidants will neutralize free radicals so they do not have the ability to steal electrons from cells and DNA. The mechanism of action of primary antioxidants is to prevent the formation of new free radical compounds or change the free radicals that have been formed to become more stable and less reactive by breaking the chain reaction (polymerization) or known as chain breaking (Sayuti and Yenrina, 2015).

The flavonoid group of compounds has been reported to have antioxidant activity. Anthocyanins are included in the flavonoid group, namely compounds that function as antioxidants because these compounds are phenolic compounds with an -OH group attached to the carbon of the aromatic ring. Antioxidants block reactive oxygen species (ROS) that damage DNA and cause mutations. The free radical product of this compound is resonance stabilized and is not reactive when compared to other free radicals so that it can function as an antioxidant (Budiarti et al., 2014).

These anthocyanins can lower blood sugar levels by increasing insulin resistance, protecting beta cells, increasing insulin secretion, and reducing glucose digestion in the small intestine. Its mechanism of action is related to its antioxidant properties, but enzymatic prevention and other means are also relevant (Sancho and Pastore, 2012). Antioxidant activity is able to capture free radicals that cause pancreatic beta-cell damage and inhibit pancreatic beta-cell damage so that the remaining beta cells still function. These antioxidants are thought to be able to protect a number of beta cells that remain normal, thus enabling the regeneration of the remaining beta cells through the process of mitosis or through the formation of new islets by endocrine proliferation and differentiation of ductal and ductular cells (Suryani et al., 2013). However, to achieve an effect in a particular tissue, the bioactive compound must be available i.e. effectively absorbed from the intestine into the circulation and delivered to the appropriate area to reach the target. Fruits rich in anthocyanins, extracts or pure compounds have been shown to be effective in preventing or suppressing diseased areas (Miguel, 2011).

CAROTENOID

Chemical structure

Carotenoids, natural pigments found in an array of different foodstuffs, are the most abundant

pigments present in the human diet (Ngamwonglumlert & Devahastin, 2019). They are lipid-soluble and show a range of reds, oranges, and yellows, and are divided into 2 groups, which are carotenes and xanthophylls. Carotenes are hydrocarbons, with xanthophylls being their oxygenated counterparts (Campbell-Platt and IUFoST, 2017). There are over 600 different carotenoids, but this paper will focus only on 5 of them, that being lutein, β-Carotene, lycopene, zeaxanthin, and astaxanthin.

In the human diet, carotenoids are mainly found in fruits and vegetables, though they also appear in some animal tissues and products, such as salmon and egg yolk. The name itself is a clue as to the most well-known source of carotenoids; that being carrots, where they give carrots their orange coloring. Although, carotenoids are also available from leafy greens such as kale, spinach, and lettuce, and other sources, like in tomatoes, where the red pigment is a result of the presence of lycopene, a red-colored carotenoid (Schieber & Weber, 2016; Ngamwonglumlert & Devahastin, 2019). All of these things are commonly present in the average diet.

The general structure of carotenoids grants them their colorings. They usually have a 40-carbon chain backbone, with 8 isoprene molecules, and multiple double bonds. The differences between the colors occur due to differences in the full structure. (Ellison, 2016) Some examples of the structure of a few carotenoids are shown in Figure 3 below.

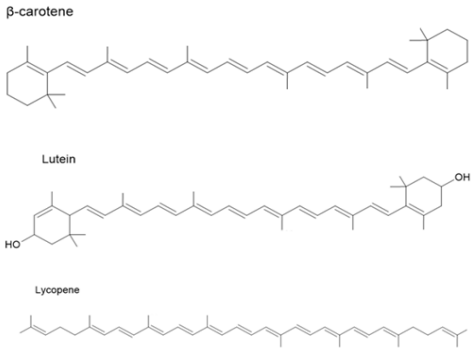

Figure 3. Several carotenoids structures

Carotenoids have been observed to have many positive effects on human health. The most studied of which being their antioxidative properties, and link to eye health. Some of them (such as β-carotene) are provitamin A, meaning they're easily converted into vitamin A, which is essential for the proper function of the immune system (Krinsky & Johnson, 2005). There is even evidence of carotenoids having positive effects against cancer, but this paper will be focused on the 6 mentioned possible effects of carotenoids regarding diabetes and glucose intolerance, and complications that can arise from such.

Carotenoid as antidiabetic

Since carotenoids are antioxidants and diabetes is a disease that is brought on by oxidative stress, the possibility of carotenoids having a role in the fight against diabetes has been speculated on for a while. Limited studies have been done on the subject, and the few that have been done have produced mixed results. Each of the selected carotenoids will be looked at separately.

β-Carotene

β-Carotene, a red-orange pigment found in carrots, sweet potatoes, and other fruits and vegetables, is one of the most common carotenes available, with it being abundant in the human diet (Schieber & Weber, 2016). It is the most commonly studied carotenoid, with health benefits being widely known at this point. Benefits of β-Carotene for the prevention of diabetes have been suggested many times, though until recently, there has not been solid evidence regarding such.

A study (Sluijs et al., 2015) done over 10 years and released in 2015 concluded that, among 37,846 people, higher consumption of β-Carotene does contribute to lowered risk of diabetes. Other, smaller studies have also found an association between β-Carotene and lowered risk of diabetes (Montonen et al., 2004; Ärnlöv et al., 2008; Asemi et al., 2016). β-Carotene is considered a strong antioxidant, which is generally attributed to its benefits against diabetes, as antioxidants reduce oxidative stress, the base cause of diabetes.

There is evidence that β-Carotene is also beneficial for those already afflicted with diabetes. Some studies (Canas et al., 2012; Asemi et al., 2016) have observed that consumption of β-Carotene by individuals with type 2 diabetes has benefits towards insulin metabolism.

Lycopene

Lycopene is another one of the major dietary carotenoids. It exhibits a red color and is most found in tomatoes, watermelon, and other red or orange-red fruits, though it is mostly associated with tomatoes and tomato products (Rao et al., 2006). Unlike β-Carotene, lycopene is a non-provitamin A carotenoid (Ellison, 2016).

Lycopene has been observed to reduce oxidative stress, which is perceived to play a part in the prevention of the development of type 2 diabetes and the alleviation of its complications. A few studies have been conducted to find a solid connection, though results are generally inconclusive. Some studies yielded results suggesting that higher lycopene intake reduces the risk of diabetes (Sugiura et al., 2015), but the margins were not significant. Other studies show that there is no association with lycopene and lowered diabetes risk or prevention at all (Wang et al., 2006). The interest in lycopene's role in human health is more focused towards other diseases such as cancer rather than diabetes, so not many studies have been done.

Lutein

A xanthophyll (that is, an oxygenated carotenoid) carotenoid that is available in egg yolks, corn, carrots, and fish, lutein is one of the major dietary carotenoids. It is yellowish in color, and along with β-Carotene, lycopene, and zeaxanthin, is one of the most common carotenoids present in the human diet (Abdel-Aal et al., 2013).

The role of lutein in eye health is well documented (Koushan et al., 2013; Abdel-Aal et al., 2017; Mitra et al., 2021), though its potential benefits against diabetes itself has limited research, and what research was conducted yielded inconsistent results (Sluijs et al., 2015; Sugiura et al., 2015). However, it has been documented that lutein helps alleviate complications caused by diabetes, particularly when it comes to the eyes, such as with diabetic retinopathy (Hu et al., 2011). A study was also done on diabetic rats that concluded that lutein might also help prevent the development of diabetes related cataracts (Arnal et al., 2009).

Zeaxanthin

Zeaxanthin is a xanthophyll that is yellow in color, found in eggs and squash and an array of other foodstuffs (Tudor & Pintea, 2020). It is not as abundant as some of the other carotenoids mentioned in this paper, but its effect on health has more often been studied thus far compared to other carotenoids, excluding perhaps β-Carotene. Its effects are often studied alongside lutein, as they are isomers of each other, with both being commonly related to protection against several eye diseases, among other things (Ribaya-Mercado & Blumberg, 2004).

The role of zeaxanthin specifically in the prevention of diabetes itself has not been investigated all that often, however more general studies of the association between carotenoids and risk of type 2 diabetes have yielded conflicting results. Some report an inverse relationship between zeaxanthin intake and cases of diabetes (Montonen et al., 2004; Coyne et al., 2005), while others remain inconclusive (Sluijs et al., 2015).

Astaxanthin

Astaxanthin is a xanthophyll that, unlike its counterparts previously discussed, are commonly present in salmonid and crustaceans, where astaxanthin gives them their red orange coloring (Higuera-Ciapara, Félix-Valenzuela and Goycoolea, 2006). It was chosen to be included in this paper because it is a somewhat common animal sourced carotenoid, as well as its associations with diabetes alleviation and prevention.

CONCLUSION

The COVID-19 pandemic, which has been ongoing since December 2019, has caused many deaths.

Diabetes mellitus proved to be a significant risk factor for COVID-19 infection. Studies have shown that natural pigments from plants possess many health benefits especially as diabetes mellitus patients with their phytochemicals. These pigments, namely chlorophylls, anthocyanins, and carotenoids exhibit antidiabetic properties, with different working mechanisms. Furthermore, potential bioactive compounds also containing in these natural pigments are very abundant in nature and human diet. Some researchers are be done with in vitro and in vivo analysis to give the proofs about the effectiveness of natural pigments as antidiabetic substances. This potential should be utilized properly through the exploration of natural pigments for practical applications in food or for commercialization in industrial forms. Although natural pigments have a weakness, namely the color is not uniform, and tends to be expensive. However, natural pigments have the advantage of being food coloring which tends to be safe compared to synthetic dyes and is also beneficial for human health as functional food.

REFERENCES

Abdel-Aal, E.-S. et al. (2013) 'Dietary Sources of Lutein and Zeaxanthin Carotenoids and Their Role in Eye Health', Nutrients, 5(4), pp. 1169–1185. doi: 10.3390/nu5041169.

Abdel-Aal, E.-S. M. et al. (2017) 'Lutein and Zeaxanthin Carotenoids in Eggs', in Egg Innovations and Strategies for Improvements. Elsevier, pp. 199–206. doi: 10.1016/B978-0-12-800879-9.00019-6.

Abdelhafiz, A., & Sinclair. (2021). Diabetes in COVID-19 pandemic prevalence, patient-characteristics and adverse outcomes. Int J Clin Pract, 75, 14112. https://doi.org/10.1111/ijcp.14112

Alenezi K, Tovmasyan A, Batinic-Haberle I, et al. Optimizing Zn porphyrin-based photosensitizers for efficient antibacterial photodynamic therapy. Photodiagnosis Photodyn Ther. 2017; 17 : 154 – 159.

Andersen, O. M.; Jordheim, M. Anthocyanins. In Encyclopedia of Life Sciences; MacMillan: New York, 2010; pp 1–12.

Andersen, O. M.; Jordheim, M. The Anthocyanins. In Flavonoids: Chemistry, Biochemistry, and Applications; Andersen, O. M.; Markham, K. R., Eds.; Taylor and Francis: Boca Raton, Fla., 2006; pp 471–551.

Andersen, O. M.; Markham, K. R., eds. Flavonoids: Chemistry, Biochemistry, and Applications; Taylor and Francis: Boca Raton, Fla., 2006.

Arnal, E. et al. (2009) 'Lutein prevents cataract development and progression in diabetic rats', Graefe's Archive for Clinical and Experimental Ophthalmology, 247(1), pp. 115–120. doi: 10.1007/s00417-008-0935-z.

Ärnlöv, J. et al. (2008) 'Serum and dietary β-carotene and α-tocopherol and incidence of type 2 diabetes mellitus in a community-based study of Swedish men: report from the Uppsala Longitudinal Study of Adult Men (ULSAM) study', Diabetologia 2008 52:1, 52(1), pp. 97–105. doi: 10.1007/S00125-008-1189-3.

Asemi, Z. et al. (2016) 'Effects of beta-carotene fortified synbiotic food on metabolic control of patients with type 2 diabetes mellitus: A double-blind randomized cross-over controlled clinical trial', Clinical Nutrition, 35(4), pp. 819–825. doi: 10.1016/j.clnu.2015.07.009.

Asgary S, Moshtaghian J, Hosseini M, Siadat H: Eff ects of alfalfa on lipoproteins and fatty streak formation in hypercholesterolemic rabbits. Pak J Pharm Sci 21, 460–464 (2008) 8.

Ashok, S. A. (2011). Phytochemical and Pharmacological Screening of Wheatgrass Juice (Triticum aestivum L.)). International Journal of Pharmaceutical Sciences Review and Research, 9(1), 159–164. http://www.wheatgrassevidence.org/Ashok2011.pdf

Budiarti, A.; Ulfah, M.; Oktania, F. A., 2014, Aktivitas Antioksidan Fraksi Kloroform Ekstrak Etanol Daun Sirsak (Annona muricata L.) dan Identifikasi Kandungan Senyawa Kimianya, Fakultas Farmasi, Universitas Wahid Hasyim.

Campbell-Platt, G. and IUFoST (2017) Food science and technology. 2nd edn. Edited by G. Campbell-Platt. Wiley.

Canas, J. A. et al. (2012) 'Insulin Resistance and Adiposity in Relation to Serum β-Carotene Levels', The Journal of Pediatrics, 161(1), pp. 58-64.e2. doi: 10.1016/j.jpeds.2012.01.030.

Castañeda-Ovando, A.; Pacheco-Hernández, Ma. de L.; Paez Hernández, Ma. E.; Rodríguez, J. A.; Galán-Vidal, C. A. Chemical Studies of Anthocyanins: A Review. Food Chem. 2009, 113 (4), 859–871.

Chen M., Schliep M., Willows R.D. et al.: A red-shifted chlorophyll. – Science 329: 1318-1319, 2010.

Chew A.G.M., Bryant D.A.: Chlorophyll biosynthesis in bacteria: The origins of structural and functional diversity. – Annu. Rev. Microbiol. 61: 113-129, 2007.

Choe, E.; Min, D. B. Chemistry and Reactions of Reactive Oxygen Species on Foods. Crit. Rev. Food Sci. Nutr. 2006, 46 (1), 1–22.

Coyne, T. et al. (2005) 'Diabetes mellitus and serum carotenoids: findings of a population-based study in Queensland, Australia', The American Journal of Clinical Nutrition, 82(3), pp. 685–693. doi: 10.1093/ajcn/82.3.685.

Daayf, F.; Lattanzio, V., Eds. Recent Advances in Polyphenol Research; Wiley-Blackwell: Chichester, U.K., 2008.

Ellison, S. L. (2016) 'Carotenoids: Physiology'. doi: 10.1016/B978-0-12-384947-2.00120-3.

Escribano-Bailon, M. T.; Santos-Buelga, C.; Rivas-Gonzalo, J.C. An- ´ thocyanins in Cereals. Review. J. Chromatogr. A 2004, 1054 (1–2), 129–141.

Ferretti, G.; Bacchetti, T.; Belleggia, A.; Neri, D. Cherry Antioxidants: From Farm to Table. Molecules 2010, 15 (10), 6993–7005.

Ferruzzi MG, Blakeslee J. Digestion, absorption, and cancer preventative activity of dietary chlorophyll derivatives. Nutr Res. 2007; 27 : 1 – 12.

Freitas, V. de; Mateus, N. Chemical Transformations of Anthocyanins Yielding a Variety of Colour (Review). Environ. Chem. Lett. 2006, 4 (3), 175–183.

Ghosh, D.; Konishi, T. Anthocyanin and Anthocyanin-Rich Extracts: Role in Diabetes and Eye Function. Asia Pac. J. Clin. Nutr. 2007, 16 (2), 200–208.

Giusti, M. M.; Rodr´ıguez-Saona, L. E.; Griffin, D.; Wrolstad, R. E. Electrospray and Tandem Mass Spectroscopy as Tools for Anthocyanin Characterization. J. Food Agric. Chem. 1999, 47 (11), 4657–4664.

Gould, K.; Davies, K.; Winefields, C., Eds. Anthocyanins, Biosynthesis, Functions and Applications; Springer-Verlag: New York, 2009.

Guan WJ, Liang WH, Zhao Y, et al. Comorbidity and its impact on 1590 patients with Covid-19 in China: A Nationwide Analysis. Eur Respir J 2020; DOI:10.1183/13993003.00547-2020

Harborne, J. B.; Grayer, R. J. The Anthocyanins. In The Flavonoids; Harborne, J. B., Ed.; Chapman & Hall: New York, 1988.

Harborne, J. B.; Williams, C. A. Advances in Flavonoid Research since 1992. Phytochemistry 2000, 55 (6), 481–504.

Hardoko, Febriani A, Siratantri T. 2015. Aktivitas antidiabet secara invitro agaragar, agarosa dan agaropektin dari rumput laut Gracilaria Gigas. Jurnal Pengolahan Hasil Perikanan Indonesia. 18(2): 128-139.

He, J.; Giusti, M. Anthocyanins: Natural Colorants with Health Promoting Properties. Annu. Rev. Food Sci. Technol. 2010, 1 (1), 163–187.

Hu, B.-J. et al. (2011) 'Application of Lutein and Zeaxanthin in nonproliferative diabetic retinopathy.', International journal of ophthalmology, 4(3), pp. 303–6. doi: 10.3980/j.issn.2222-3959.2011.03.19.

He, J.; Giusti, M. Anthocyanins: Natural Colorants with HealthPromoting Properties. Annu. Rev. Food Sci. Technol. 2010, 1 (1), 163–187.

Higuera-Ciapara, I., Félix-Valenzuela, L. and Goycoolea, F. M. (2006) 'Astaxanthin: A Review of its Chemistry and Applications', Critical Reviews in Food Science and Nutrition, 46(2), pp. 185–196. doi: 10.1080/10408690590957188.

Horii S, Fukase H, Matsuo T, et al. Synthesis and alpha-D-glucosidase inhibitory activity of N-substituted valiolamine derivatives as potential oral antidiabetic agents. J Med Chem. 1986;29(6):1038-46. [PMID:3519969]

Hosikian A, Lim S, Halim R, et al. Chlorophyll extraction from microalgae: a review on the process engineering aspects. Int J Chem Eng. 2010; 2010 : 1 – 11.

Hui DS, AZHAR EI, Madani TA, et al. The continuing 2019-nCoV epidemic threat of novel coronaviruses to global health - The latest 2019 novel coronavirus outbreak in Wuhan, China. Int J Infect Dis 2020; 91: 264-6. 2.

Ignat, I.; Volf, I.; Popa, V. I. A Critical Review of Methods of Characterization of Polyphenol Compounds in Fruit and Vegetables. Food Chem. 2011, 126 (4), 1821–1835.

Jelic D, Tatic I, Trzun M, et al. Porphyrins as new endogenous anti-inflammatory agents. Eur J Pharmacol. 2012; 691 : 251 – 260.

Kähkönen, M. P.; Heinonen, M. Antioxidant Activity of Anthocyanins and Their Aglycons. J. Agric. Food Chem. 2003, 51 (3), 628–633.

Kong, JM, Chia LS, Goh NK, Chia TF, Brouillard R (2003) Analysis and biological activities of anthocyanins. Phytochemistry 64: 923-933.

Koushan, K. et al. (2013) 'The Role of Lutein in Eye-Related Disease', Nutrients, 5(5), pp. 1823–1839. doi: 10.3390/nu5051823.

Krinsky, N. I. and Johnson, E. J. (2005) 'Carotenoid actions and their relation to health and disease', Molecular Aspects of Medicine, 26(6), pp. 459–516. doi: 10.1016/j.mam.2005.10.001.

Lanfer-Marquez UM, Barros RM, Sinnecker P. Antioxidant activity of chlorophylls and their derivatives. Food Res Int. 2005; 38 : 885 – 891.

Larkum AWD, Kühl M. Chlorophyll d: the puzzle resolved. Trends Plant Sci. 2005; 10 : 355 – 357.

Lee, D.M. Issue 122 Item 9 Antioxidant Vitamins Helpful in Diabetic Ketoacidosis Treatment. http://www.diabetesincontrol.com/aser ver/adclick.php?n=a97aadea, 2002.

Leopoldini, M.; Rondinelli, F.; Russo, N.; Toscano, M. Pyroanthocyanins: A Theoretical Investigation on Their Antioxidant Activity. J. Agric. Food Chem. 2010, 58 (15), 8862–8871.

Li WT, Tsao HW, Chen YY, et al. A study on the photodynamic properties of chlorophyll derivatives using human hepatocellular carcinoma cells. Photochem Photobiol Sci. 2007; 6 : 1341 – 1348.

Liu, Z.-Q. Chemical Methods to Evaluate Antioxidant Ability. Chem. Rev. 2010, 110 (10), 5675–5691.

Lust JB (1986): The Herb Book. Bantam Books Inc., London 10. Gray AM, Flatt PR: The traditional plant treatment, Sambucus nigra (elder), exhibits insulin-like and insulin-releasing actions in vitro. J Nutr 78, 325–334 (1997) 11.

Kariadi, S.H. K.S. Peranan Radikal Bebas dan

Antioksi dan pada Penyakit Degeneratif Khususnya Diabetes Mellitus. Bagian Penyakit dalam. Fakultas Kedokteran/RS Hasan Sadikin. Bandung, 2001.

Magalhaes, L. M.; Segundo, M. A.; Reis, S.; Lima, J. L. F. C. Methodological Aspects about In Vitro Evaluation of Antioxidant Properties. Anal. Chim. Acta 2008, 613 (1), 1–19.

Marpaung, A.M. 2020. Tinjauan dan Manfaat Bunga Telang (Clitoria ternatea L.) bagi Kesehatan Manusia. J. Functional Food. & Nutraceutical, 1(2), pp.47-69.

Mehranjani MS, Shariatzadeh MA, Desfulian AR, Noori M, Abnosi MH, Moghadam ZH: Eff ects of Medicago sativa on nephropathy in diabetic rats. Indian J Pharm Sci 69, 768–772 (2007) 9.

Miguel, M. G., 2011, Anthocyanins: Antioxidant and/or anti-inflammatory activities, Journal of Applied Pharmaceutical Science, Vol; 1 (6), 7-15.

Min, D. B.; Bolf, J. M. Chemistry and Reaction Of Singlet Oxygen in Foods. Comprehensive Rev. Food Sci. Food Saf. 2002, 1, 58–72.

Mitra, S. et al. (2021) 'Potential health benefits of carotenoid lutein: An updated review', Food and Chemical Toxicology, 154, p. 112328. doi: 10.1016/j.fct.2021.112328.

Montonen, J. et al. (2004) 'Dietary Antioxidant Intake and Risk of Type 2 Diabetes', Diabetes Care, 27(2), pp. 362–366. doi: 10.2337/diacare.27.2.362.

Moss G.P.: Nomenclature of tetrapyrroles. IUPAC-IUB Joint Commission on Biochemical Nomenclature (JCBN). Recommendations 1986. – Eur. J. Biochem. 178: 277-328, 1988.

Motohashi, N., Ed. Flavonoids and Anthocyanins in Plants, and Latest Bioactive Heterocycles I; Springer: New York, 2008.

Neelam, K. et al. (2017) 'Putative protective role of lutein and zeaxanthin in diabetic retinopathy', British Journal of Ophthalmology, 101(5), pp. 551–558. doi: 10.1136/bjophthalmol-2016-309814.

Ngamwonglumlert, L. and Devahastin, S. (2019) 'Carotenoids', in Encyclopedia of Food Chemistry. Elsevier, pp. 40–52. doi: 10.1016/B978-0-08-100596-5.21608-9.

Nurdin et al. (2009). Chlorophyll Level of Various Geen Leaves and Copper-chlorophyll derivatives and its Characterization. Jurnal Gizi dan Pangan, 9(1), 13–19.

Nurulita, Y., Dhanutirto, H., & Soemardji, A. A. (2008). Penapisan Aktivitas dan Senyawa Antidiabetes Ekstrak Air Daun Dandang Gendis (Clinacanthus nutans). Jurnal Natur Indonesia, 10(2), 98. https://www.academia.edu/28880611/Penapisan_Aktivitas_dan_Senyawa_Antidiabetes_Ekstrak_Air_Daun_Dandang_Gendis_Clinacanthus_nutans_

Ohran, D. D., Orhan, N., Demir, O., & Konuklugil, B. (2021). Phenolic Content, Antioxidant and In Vitro Antidiabetic Effects of Thirteen Marine Organisms from Mediterranean Sea. Farmacia, 69(1), 68–74. https://doi.org/10.31925/farmacia.2021.1.9

Oliveira, H., Fernandes, A., F. Brás, N., Mateus, N., de Freitas, V., & Fernandes, I. (2020). Anthocyanins as Antidiabetic Agents—In Vitro and In Silico Approaches of Preventive and Therapeutic Effects. Molecules, 25(17), 3813. doi:10.3390/molecules25173813

Pari and Latha., 2005, Antidiabetic Effect of Scoparia dulcis: Effect on Lipid Peroxidation in Streptozotocin Diabetes, *Gen. Physiol. Biophys*, 24; 13 – 26.

Pereira, D. M.; Valentao, P.; Pereira, J. A.; Andrade, P. B. Phenolics: From Chemistry to Biology. Molecules 2009, 14 (6), 2202–2211.

Pervaiz, T., Songtao, J., Faghihi, F., Haider, M. S., & Fang, J. (2017). Naturally Occurring Anthocyanin, Structure, Functions and

Biosynthetic Pathway in Fruit Plants. Journal of Plant Biochemistry & Physiology, 05(02). https://doi.org/10.4172/2329-9029.1000187

Prangdimurti E. 2007. Kapasitas Antioksidan dan Daya Hipokolesterolemik Ekstrak Daun Suji (Pleomele angustifolia N.E. Brown). Disertasi Doktoral Sekolah Pascasarjana, IPB, Bogor

Qiu, N. W., Jiang, D. C., Wang, X. S., Wang, B. S., & Zhou, F. (2019). Advances in the members and biosynthesis of chlorophyll family. Photosynthetica, 57(4), 974–984. https://doi.org/10.32615/ps.2019.116

Quideau, S.; Deffieux, D.; Donat-Casassus, C.; Pouysegu, L. Plant ´ Polyphenols: Chemical Properties, Biological Activities and Synthesis. Angew. Chem. Int. Ed. 2011, 50 (3), 586–621.

Raghvendra; Sharma, V.; Shakya, A.; Hedaytullah, M. D.; Arya, G. S.; Mishra, A.; Gupta, A. D.; Pachpute, A. P.; Patel, D. Chemical and Potential Aspects of Anthocyanins—A Water Soluble Vacuolar Flavonoid Pigments: A Review. Int. J. Pharm. Sci. Rev. Res. 2011, 6 (1), 28–33.

Rao, A. V., Ray, M. R. and Rao, L. G. (2006) 'Lycopene', in, pp. 99–164. doi: 10.1016/S1043-4526(06)51002-2.

Ribaya-Mercado, J. D. and Blumberg, J. B. (2004) 'Lutein and Zeaxanthin and Their Potential Roles in Disease Prevention', Journal of the American College of Nutrition, 23(sup6), pp. 567S-587S. doi: 10.1080/07315724.2004.10719427.

Saeidnia S, Gohari AR, Shahverdi AR, Permeh P, Nasiri M, Mollazadeh K, Farahani F. 2009. Biological activity of two red algae, Gracilaria salicornia and Hypnea flagelliformis from Persian Gulf. Journal Pharmacognosy Research. 1(6): 428-430

Sales PM, Souza PM, Simeoni LA, Silveira D, αAmylase inhibitors: a review of raw material and FARMACIA, 2021, Vol. 69, 1 74 isolated compounds from plant source. JPPS, 2012; 15(1): 141-183.

Sancho, R. A. S.; Pastore G. M., 2012, Evaluation of the Effects of Anthocyanin in Type 2 Diabetes, Journal of Food Res Int, Vol: 46, 378-386.

Sanger G, Kaseger BE, Rarung LK, Damongilala L. 2018. Potensi beberapa jenis rumput laut sebagai bahan pangan fungsional, sumber pigmen dan antioksidan alami. Jurnal Pengolahan Hasil Perikanan Indonesia. 21(2): 208-217.

Sanger G, Widjanarko SB, Kusnadi J, Berhimpon S. 2013 Antioxidant activity of methanol extract of seaweeds obtained from North Sulawesi. Food Science and Quality Management. 19 (1): 63-70.

Sayuti, K.; Yenrina, R., 2015, Antioksidan Alami dan Sintetik, Andalas University Press, Padang.

Scheer H.: An overview of chlorophylls and bacteriochlorophylls: Biochemistry, biophysics, functions and applications. – In: Grimm B., Porra R.J., Rüdiger W., Scheer H. (ed.): Chlorophylls and Bacteriochlorophylls. Advances in Photosynthesis and Respiration. Pp. 1-19. Springer, Dordrecht 2006.

Schieber, A. and Weber, F. (2016) 'Carotenoids', in Handbook on Natural Pigments in Food and Beverages. Elsevier, pp. 101–123. doi: 10.1016/B978-0-08-100371-8.00005-1.

Schoenhals, K. Prepared Foods. Virgo Publishing. Health & Nutrition Division. http://www.vpico.com, 2005.

Sluijs, I. et al. (2015) 'Dietary intake of carotenoids and risk of type 2 diabetes', Nutrition, Metabolism and Cardiovascular Diseases, 25(4), pp. 376–381. doi: 10.1016/j.numecd.2014.12.008.

Sugiura, M. et al. (2015) 'High-serum carotenoids associated with lower risk for developing type 2 diabetes among Japanese subjects: Mikkabi

cohort study', BMJ Open Diabetes Research & Care, 3(1), p. e000147. doi: 10.1136/bmjdrc-2015-000147.

Suryani, Nani, Endang, Tinny, dan Aulanni'am, 2013, Pengaruh Ekstrak Biji Metanol terhadap Peningkatan Kadar Insulin, Penurunan Ekspresi TNF-α dan Perbaikan Jaringan Pankreas Tikus Diabetes, Jurnal Kedokteran Brawijaya, Vol: 27 (23).

Suryowinoto. S. Mengenal Beberapa Tanaman yang Digunakan Masyarakat Sebagai Antidiabetik untuk Menurunkan Kadar Gula dalam Darah. Badan Pengawas Obat dan Makanan. http://www.pom.go.id/default.asp, 2005.

Tias, Fauziah Naryuning., 2010, Aktivitas Antioksidan dan Komponen Bioaktif dari Keong Pepaya (Melo sp.), Fakultas Perikanan dan Ilmu Kelautan, Institut Pertanian Bogor (Skripsi).

Tiwari, A.K., J.M. Rao. Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospect. Current Science, 2002; vol 83, 1 (30-38). 2.

Tudor, C. and Pintea, A. (2020) 'A Brief Overview of Dietary Zeaxanthin Occurrence and Bioaccessibility', Molecules, 25(18), p. 4067. doi: 10.3390/molecules25184067.

Veitch, N. C.; Grayer, R. J. Flavonoids and Their Glycosides, Including Anthocyanins. Nat. Prod. Rep. 2008, 25 (3), 555–611.

Vidal, S.; Francis, L.; Williams, P.; Kwiatkowski, M.; Gawell, R.; Cheynier, V.; Waters, E. The Mouth-Feel Properties of Polysaccharides and Anthocyanins in a Wine Like Medium. Food Chem. 2004, 85 (4), 519–525.

Vs, S., Ts, S., & Lekshmi S. (2020). In vitro antidiabetic potential of Euphorbia hirta Linn.: A nutritionally significant plant. ~ 1 ~ Journal of Pharmacognosy and Phytochemistry, 9(1), 1–4. http://www.phytojournal.com

Wallace, T. C. Anthocyanins in Cardiovascular Disease. Adv. Nutr. 2011, 2, 1–7.

Wang, L. et al. (2006) 'Plasma Lycopene, Other Carotenoids, and the Risk of Type 2 Diabetes in Women', American Journal of Epidemiology, 164(6), pp. 576–585. doi: 10.1093/aje/kwj240.

Wardatun., 2011, Uji Aktivitas Antioksidan Ekstrak Etanol Akar, Kulit Batang, dan Daun Tanaman Sambiloto (Andrographis paniculata Ness.) Dengan Metode Linoleat-Tiosianat, Fitofarmaka, Vol 1(2): 9-13.

Watanabe J, Kawabata J, Kurihara H, Niki R. Isolation and identification of alpha-glucosidase inhibitors from tochu-cha (Eucommia ulmoides). Biosci Biotechnol Biochem. 1997;61(1):177-8. [PMID:9028049]

Williams, C. A.; Grayer, R. J. Anthocyanins and Other Flavonoids. Nat. Prod. Rep. 2004, 21 (4), 539–573.

Winiarska H, Dworacka M, Borowska M, Bobkiewicz-Kozłowska T, Gorecki P: The eff ects of plant extracts of Medicago sativa and Trigonella foenum-graceum on postprandial glucose levels in type 2 diabetic rats. Herba Polonica 53, 34–44 (2007)

Zoppini G, Fedeli U, Schievano E, et al. Mortality from infectious diseases in diabetes. Nutr Metab Cardiovasc Dis 2018; 28: 444-5.

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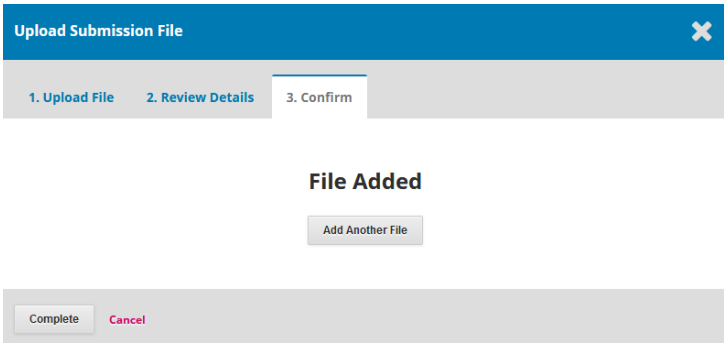
A. Upload your file in the **Upload Submission File** section.

Select **Article Text** in the **Article Component** → **Upload** your **file**. The type of file that accepted is in **.doc** or **.docx** only → 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.

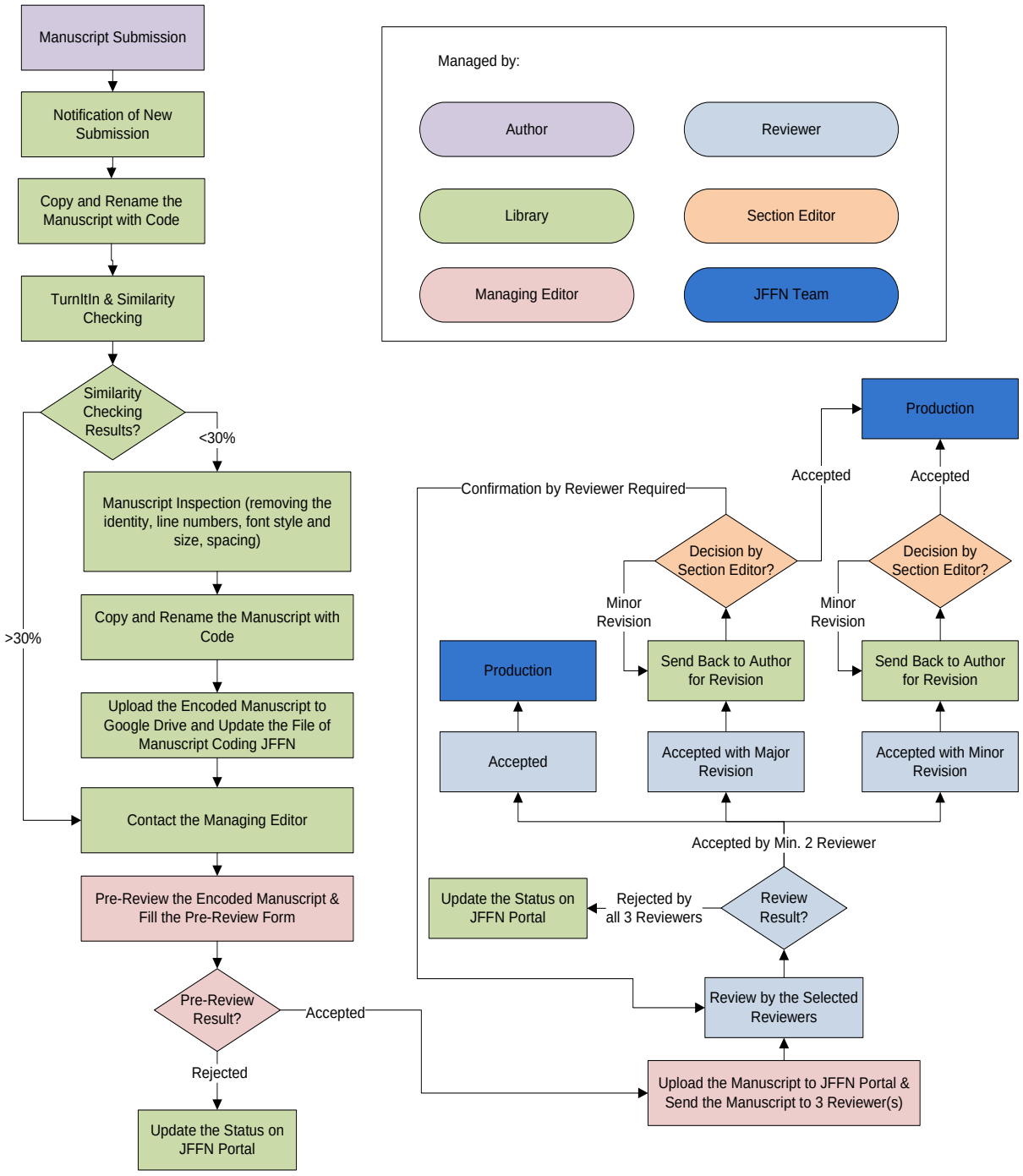
3. You have to enter the **Metadata** of your article.

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 keyword → 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.

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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.

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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.

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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).

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Akademisi, Peneliti non-komersial	Rp 400.000
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