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SCREENING OF ANTIOXIDANT AND NUTRIENT COMPOUNDS FROM SEVERAL VARIETIES OF ZUCCHINI (*Cucurbita Pepo* L.) AT DIFFERENT HARVEST AGES IN THE MIDDLE PLAINS

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Abstract. In an effort to accelerate the fulfillment of weekly vegetable needs, short-lived vegetable plants are needed. One of the short-lived vegetable plants is zucchini (Cucurbita pepo L), with a harvest age of 28 - 60 days. Zucchini is included in the category of immature fruit vegetables, harvested before they are physiologically ripe in order to obtain the color and size desired by the market. This research aims to determine the types of antioxidant compounds in several varieties of zucchini harvested at different ages in the midlands. The research was carried out from September 2023 to January 2024, in Tawangargo Village, Karangploso District, Malang Regency at an altitude of 624 meters above sea level. Thus, 4 (four) samples were obtained, namely: harvested green zucchini aged 10 days (H1); harvested green zucchini fruit aged 20 days (H2); harvested yellow zucchini fruit aged 10 days (K1); yellow zucchini fruit harvested at 20 days (K2). The fruit obtained was then observed in the laboratory using LC-HRMS analysis. As a result of observing the number of compounds detected in each variety and different harvest ages, there were differences in the number of compounds found, from the groups of phenols, polyphenols, amino acids, fatty acids and glucose. The screening results of zucchini with green fruit detected more diverse types of compounds than yellow zucchini. Fruit harvested young shows more diverse screening results for anti-oxidant compounds and nutrients than fruit harvested old.

Keywords: *Cucurbita Pepo L., antioxidant compounds, zucchini.*

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1. Introduction

Today's society has begun to realize the importance of healthy living. This can be seen from the increase in weekly vegetable consumption in Indonesia. Vegetable consumption in 2018 was 1.0414 kg capita^{-1} week^{-1}, increasing to 1.1209 kg capita^{-1} week^{-1} in 2021 with an increase of 7.63% (Kementerian Pertanian, 2020). In an effort to accelerate the fulfillment of weekly vegetable needs, short-lived vegetable plants are needed. One of the short-lived vegetable plants is zucchini (*Cucurbita pepo* L), with a harvest time of 28 days (Souza *et al*., 2020; Yuan *et al*., 2023) to 60 days after planting. Apart from being short-lived, zucchini fruit is also beneficial for health (Zhong *et al*., 2015). Zucchini fruit contains the secondary metabolite compound cucurbitacin (Houhou *et al*., 2022) which can inhibit the growth of cancer cells (Alghasham, 2013).

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The leaves contain fatty acids that are beneficial for the body and the level of cultivation and consumption is also high in the world (Wadas *et al.*, 2012). Zucchini plants require a certain growing environment for their growth and reproduction. Production is influenced by several factors, namely: variety, climate and cultivation techniques (Caetano *et al.*, 2024; Kader, 2013). Environmental factors, namely temperature and light, are two important factors that regulate the development of this plant. The appropriate daily minimum air temperature ranges from 9-15ºC, while the daily maximum temperature ranges from 25-38ºC (Candian *et al.*, 2021).

Zucchini is included in the category of immature fruit vegetables, harvested before they are physiologically ripe in order to obtain the color and size desired by the market. Several European countries want zucchini when the skin of the fruit is still light green, the skin of the fruit has not yet hardened and the seeds in the fruit have not yet formed (Kader, 2013; Kader *et al.*, 2017). Apart from that, developing zucchini in Indonesia is a challenge in itself. This is because zucchini is suitable if grown in the highlands (Kusumiyati *et al.*, 2019), which have an air temperature range of 12-30.8ºC. Meanwhile, the area of highlands in Indonesia is limited. The area of the highlands planted with various highland vegetables is 154,092 ha (Ritung *et al.*, 2015). Based on this, efforts are needed to develop it extensively by expanding the cultivation area in other plains such as in the medium plains.

More in-depth research regarding the secondary antioxidant content in zucchini with different fruit colors and different harvest ages needs to be carried out. The tool used is mass spectrometry which is often combined with chromatography techniques. Liquid chromatography (LC) is the most widely used, because it is a versatile tool for the analysis of polar and non-polar metabolites, while gas chromatography (GC) is used for the analysis of volatile and primary metabolites after derivatization (Cajka & Fiehn, 2016).

Based on this background, this research aims to determine the types of antioxidant compounds in several zucchini varieties harvested at different ages in the midlands.

2. Material and methods

The research was carried out from September 2023 to January 2024, in Tawangargo Village, Karangploso District, Malang Regency at an altitude of 624 meters above sea level. The materials used are: zucchini varieties consisting of Jacky with green fruit and Zucchini F1 with yellow fruit, manure and NPK. Zucchini are planted in plots measuring 3 x 1 m, a total of 4 plots and maintained until harvest ± 40 – 55 DAT (days after transplanting). Fruit is harvested at ± 10 days after the female flowers appear and at ± 20 days after the female flowers appear. Thus, 4 (four) samples were obtained, namely: harvested green zucchini aged 10 days (H1); harvested green zucchini fruit aged 20 days (H2); harvested yellow zucchini fruit aged 10 days (K1); Yellow zucchini fruit harvested 20 days old (K2).

The fruit obtained was then observed in the laboratory using LC-HRMS analysis using the method Method: ESI Positive Ion (Attached in the link) Solvent: Methanol Mass identification: Compound Discoverer™ 3.2 software with filter peak extraction using the databases of MzCloud and Chemspider with annotation masses ranging from -5 ppm to 5 ppm.

LC-HRMS analysis of ethyl acetate and water fractions was carried out using an Agilent 6520, Accurate Q-TOF Mass Spectrometer equipped with a G1311A quaternary pump, G1329A autosampler and G1315D diode array detector, during the entire process, the column temperature was maintained at 30°C. After passing through the diode array detector flow cell, the elute column is directed to a Q-TOF HRMS equipped with an electrospray interface. Mass spectrum analysis was performed using positive electron spray ionization (ESI positive mode) in the mass range of 100-2000 daltons at a scanning speed of 1.03. Reading the results using Compound Discoverer™ 3.2 software with filter peak extraction using the MzCloud and Chemspider databases with annotation masses ranging from -5 ppm to 5 ppm.

3. Result and discussion

The results of laboratory analysis showed differences in the number of compounds detected in each variety and different harvest ages. There were differences in the number of compounds found as in the table below.

Table 1. Number of compounds obtained from LC-HRMS in zucchini fruit with different harvest ages

Sample	Amount of compound
H1	176
H2	172
K1	170
K2	138

Table 1 shows that zucchini with green fruit color has more detectable compounds both at young harvest ages and older harvest ages, whereas zucchini with yellow fruit color have fewer amounts and types of antioxidant compounds and at harvest ages. At older ages, 132 compounds were found, while at younger harvest ages 170 compounds were found.

These results are in accordance with the statement from Martínez-Valdivieso et al. (2017) that, zucchini has beneficial micronutrient compounds such as minerals, carotenoids, vitamin C, phenolic compounds, etc. Because of its antioxidant and antiradical properties, it has been used in traditional medicine to treat colds and relieve aches, anti-carcinogenic, anti-inflammatory, antiviral, antimicrobial and analgesic (Blanco-Díaz *et al*., 2014; Oloyede *et al*., 2012).

Some compounds are found specifically, namely: only found at the same harvest age or the same fruit color, even at all colors and harvest ages and many compounds are only found at certain harvest ages and/or certain fruit colors. These differences are caused, among other things, by genetics and the growing environment. The influencing growing environment includes altitude, temperature, sunlight and rainfall. Zucchini grows well in sub-tropical areas and develops well in the highlands of Indonesia. When zucchini is planted in the midlands, the temperature increases, which will affect the physiological and biochemical processes of the plant (Lestari, 2008; Lestari *et al*., 2021).

The types of compounds found in young harvested zucchini are presented in Table 2. There are specific compounds that are only found in young harvested zucchini (H1 and K1). The compounds present are from the phenol, polyphenol, flavonoid, glucose, fatty acid and amino acid groups. This is in accordance with research from Wadas et al. (2012) which states that, the pharmacological effects consist of antidiabetes, anti-hypertension, anti-tumor, anti-mutagenic, immuno-modulating, anti-bacterial, anti-hyper-cholesterolemia, anti-intestinal parasite, antalgic, anti-inflammation of various Zuchini species. One of these compounds is Stearidonic acid, which is a better precursor than α-linolenic acid for EPA biosynthesis to increase skin elasticity (Kumar *et al*., 2015; Prasad *et al*., 2021). Another compound found is nicotinic acid which is a source of vitamin B3 and functions as an important part of reduction and oxidation coenzymes, which are involved in energy metabolism, amino acid metabolism and detoxification reactions for drugs and other substances (Rachmayanti *et al*., 2017).

Certain compounds can only be found in fruit of the same color at different harvest ages, such as green zucchini. There are also compounds found in all samples and some are specific to certain types of fruit and harvest ages as shown in Table 3. Screening for anti-oxidant and nutritional compounds in zucchini fruit of different colors and different harvest ages is intended so that as consumers we can have a basis for selection. For food to be consumed and is one of the considerations for farmers or producers to determine the harvest age that provides optimal profits (Seleim *et al*., 2015).

Table 2. Antioxidant compounds found in young harvested fruit

Nama senyawa	Formula	Area max	
		H1	K1
11,12,14-Trihydroxy-7-methoxy-6,20-epoxyabieta-8,11,13-trien-20-one	$C_{21} H_{28} O_6$	113671650,9	108790036,2
Asarone	$C_{12} H_{16} O_3$	43191927,78	45035798,74
Nicotinic acid	$C_6 H_5 N O_2$	25333402,95	19759417,46
2,4,6-Octatriyn-1-ol	$C_8 H_6 O$	58812442,1	37088640,95
Trilaurylamine	$C_{36} H_{75} N$	43191191,12	32673402,88
Boc-Asn-Oh	$C_9 H_{16} N_2 O_5$	33055364,88	28838617,04
Stearidonic acid	$C_{18} H_{28} O_2$	6953018,864	6494935,856
Nicotinic acid	$C_6 H_5 N O_2$	25333402,95	38118162,64
Vicine	$C_{10} H_{16} N_4 O_7$	29211668,53	10459153,72
Fingolimod	$C_{19} H_{33} N O_2$	5121325,942	7112397,287
2,4-Dimethylbenzaldehyde	$C_9 H_{10} O$	4418464,641	4797641,804

Table 3. Screening results for anti-oxidant and nutritional compounds found in more than one sample

Compound	H1	H2	K1	K2
D-(+)-Pyroglutamic Acid	√	√	√	√
Choline	√	√	√	√
L-α-PALMITIN	√	√	√	√
Stearamide	√	√	√	√
1-Stearoylglycerol	√	√	√	√
trans-3-Indoleacrylic acid	√	√	√	√
Oleamide	√	√	√	√
L-(+)-Arginine	√	√	√	√
Meglutol	√	√	√	√

Compound	H1	H2	K1	K2
L-Histidine	√	√	√	√
N,N-Bis(2-hydroxyethyl)dodecanamide	√	√	√	√
Adenosine	√	√	√	√
3-Methylsulfolene	√	√	√	√
navenone A	√	√	√	√
(+/-)-Muscone	√	√	√	√
Methyl isonicotinate	√	√	√	√
(4Z,6E,8Z)-9-(2-Methyl-2H-azepin-7-yl)-4,6,8-nonatrien-3-one	√	√	√	√
2-Amino-1,3,4-octadecanetriol	√	√	√	√
Bis(methylbenzylidene)sorbitol	√	√	√	√
ascopyrone M	√	√	√	√
Bis(4-ethylbenzylidene)sorbitol	√	√	√	√
Benzaldehyde	√	√	√	√
sphinganine	√	√	√	√
Capsi-amide	√	√	√	√
Tapentadol	√	√	√	√
LU3453000	√	√	√	√
Aminopropylcadaverine	√	√	√	√
NP-001346	√	√	√	√
Benzaldehyde	√	√	√	√
nylon cyclic dimer	√	√	√	√
Stearamide	√	√	√	√
LU3453000	√	√	√	√
TRILAURYLAMINE	√	√	√	√
(+/-)12(13)-DiHOME	√	√	√	√
g-Butyrobetaine	√	√	√	√
(8E)-2-Amino-8-octadecene-1,3,4-triol	√	√	√	√
N,N-Diethyldodecanamide	√	√	√	√
NSC 622050	√	√	√	√
Acetophenone	√	√	√	√
Erucamide	√	√	√	√
α-Linolenic acid	√	√	√	√
4-Octyl-1,3-cyclopentanedione	√	√	√	√
(3?)-androsta-5,16-dieno(17,16-b)quinolin-3-ol	√	√	√	√
Tridemorph	√	√	√	√
Dodecylamine	√	√	√	√
Adenine	√	√	√	√
1-(14-methylhexadecanoyl)pyrrolidine	√	√	√	√
MFCD00801046	√	√	√	√
Lauramide	√	√	√	√
C14-Dihydroceramide	√	√	√	√
4-Coumaric acid	√	√	√	√
4-Hydroxybenzaldehyde	√	√	√	√
1-Methyl-1-benzylidene-acetone	√	√	√	√
Docosanamide	√	√	√	√

Compound	H1	H2	K1	K2
Phytosphingosine	√	√	√	√
Myristamide	√	√	√	√
N3,N4-Dimethyl-L-arginine	√	√	√	√
N,N-Dimethyldecylamine N-oxide	√	√	√	√
α-Linolenoyl ethanolamide	√	√	√	√
Diphenamid	√	√	√	√
Homo-anatoxin	√	√	√	√
APM	√	√	√	√
9(Z),11(E),13(E)-Octadecatrienoic Acid methyl ester	√	√	√	√
L-α-PALMITIN	√	√	√	√
Phloionolic acid	√	√	√	√
1-Phenyl-2-hexanone	√	√	√	√
MDPV	√	√	√	√
trans-p-Coumaraldehyde	√	√		
(2S)-2-Piperazinecarboxylic acid	√	√		
(11cis,15cis)-5,5',8,8'-Tetrahydro-5,8:5',8'-diepoxy-beta,beta-carotene	√	√		
L-Alanyl-3-{[(2E)-4-amino-4-oxo-2-butenoyl]amino}-L-alanine	√	√		
4-Amino-4-deoxypentopyranose	√	√		
(3beta,4alpha,5alpha)-3-Hydroxy-4,14-dimethylcholest-8-en-7-one	√	√		
5-Pentylresorcinol	√	√		
DG(18:3(6Z,9Z,12Z)/16:0/0:0)	√	√		
4-Methoxybenzaldehyde	√	√		
2-Aminooctadec-4-yne-1,3-diol	√		√	
11,12,14-Trihydroxy-7-methoxy-6,20-epoxyabieta-8,11,13-trien-20-one	√		√	
asn-pro	√		√	
2,4,6-Octatriyn-1-ol	√		√	
Asarone	√		√	
Boc-Asn-Oh	√		√	
Vicine	√		√	
Ibuprofen	√			√
L-Isoleucine	√	√		√
N-acetyl-L-2-aminoadipic acid	√	√		√
D-ribosylnicotinate	√	√		√
L-Glutathione (reduced)	√	√		√
DL-Homoserine	√	√		√
Valine	√	√	√	
Pentahomoserine	√	√	√	
(2E,4Z,12E)-1-(1-Piperidinyl)-2,4,12-octadecatrien-1-one	√	√	√	
3,4-Diaminopyridine	√	√	√	
Pipecolic acid	√	√	√	
Uracil	√	√	√	
12-oxo Phytodienoic Acid	√	√	√	
Talatizamine	√	√	√	
(2E,4E,16Z)-1-(1-Piperidinyl)-2,4,16-icosatrien-1-one	√	√	√	
Etretinate	√	√	√	

Compound	H1	H2	K1	K2
3-[(3-Hydroxyundecanoyl)oxy]-4-(trimethylammonio)butanoate	√	√	√	
Sedanolide	√	√	√	
Maleamic acid	√	√		√
Gly-l-pro	√	√		√
D-(+)-Proline	√	√		√
1,2-Benzoquinone	√	√		√
Rutin	√	√		√
NP-019498	√	√		√
C16-Dihydroceramide	√	√		√
1-Phenyl-2-hexanone	√	√		√
alpha-methylstyrene	√	√		√

4. Conclusion

The variety and time of harvest determine the nutritional content and anti-oxidant compounds in zucchini. The screening results of zucchini with green fruit detected more diverse types of compounds than yellow zucchini. Fruit harvested young showed more diverse screening results for anti-oxidant compounds and nutrients than fruit harvested old.

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