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Hydrolysis With Two Enzymes in Edible Fern (*Diplazium esculentum*) as a Source of Flavours

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***Abstract*— Ferns (*Diplazium esculentum*) had high content of proteins, fats, flavonoids, and polyphenols, especially in young fern leaves. Its potential to developed as a source vegetable flavour. Currently, understanding about fern flavor processing technologies and the potential of ferns for health is still relatively limited, especially on local Indonesian fern. This research aims to know about morphological characteristics of Indonesian local fern vegetables (*Mloko Jember*, *Air Jember*, and *Ayam Banyuwangi*), and determined the effect of processing to its chemical characteristics and volatile compound, then hydrolyzed enzymes to produce non-volatile flavours. Ferns are processed by steaming, drying, and control methods and are hydrolyzed by combining two enzymes - bromelain and protease, with a pH of 7 and temperature of 55°C for 1 hour. The result showed that the morphology of the three fern are different in the shape of shoot and plant based on their location. The researchers discovered that enzymatically hydrolyzing the proteins in local fern vegetables using two enzymes (bromelain and protease) produced amino acid components such as aspartic acid and glutamic acid. These have MSG-like flavour.**

***Keywords*— Bromelain enzyme; flavours; glutamic acid; protease (papain)**

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I. INTRODUCTION

Ferns (*Diplazium esculentum*) are a significant play a vital role in the sustainability of local economies and the environment. It is consumed as a vegetable food, especially in Indonesia because of its cheap price, chewy and unique taste. The protein content of ferns is (10.67%±0.05 - 25.39%±0.45), and fat (1.62%±0.04 - 25.39%±0.45) [1]. Each species' genetic makeup determines the unique aroma of ferns.

The aroma of these vegetables is influenced by factors such as specific cultivar, ripeness, and horticultural practices [2]. Also, different raw materials affect the content of non-volatile flavours and light umami flavours [3], and affect the glutamic acid content [4]. The processing affects the taste that high-temperature processing affects the non-volatile flavours content, example the granulator drying treatment at 50°C for 5 hours on the fish paste powder from mackerel scad (*Decapterus* spp.) boiled waste gave the highest value of glutamate content [5]. Processing and storage may result in changes in taste [6].

The environment plays an important role in shaping the taste of vegetables. The nutritional balance of the soil is very important because certain levels of nutrients are required for vegetable synthesis. Soil nutrients are essential for flavours development. Environmental factors such as climate, soil quality, and macro/micronutrients play a significant role in the development of a distinct aroma and photosynthesis [7,8]. Temperature

affects the rate of enzymatic reactions and metabolites, causing changes in taste [9].

The taste of plants is influenced by their genetics, which determines enzyme systems, precursors, and activity in the formation of taste [10]. Poor genetic material can lead to lower crop yields and impact taste [11]. Genetic factors also affect volatile components in tomatoes, with genetics determining 10-12 volatile components and only two components (hexanal and methyl salicylate) being affected by the environment [12]. Finally, the sugar content and specific volatile components in strawberry plants are linked to genetics [13].

Vegetables have been used as a source of flavours was developed protein hydrolysates from *Oyster mushrooms*, *Abalone mushrooms*, and *Shiitake mushrooms* enzymatically using the papain enzyme [14]. Some references, as explained enzymatically hydrolyzed proteins from *Shiitake*, *Oyster*, *Bunashimeji*, and *Enoki mushrooms* using the enzyme bromelain [15], the amino acid cysteine is not found in mushroom, hydrolyzed soybeans with the proteolytic enzyme flavourszyme, hydrolyzed mud clams (*Polymesoda erosa*) with alacalase and fixed them with maillard [16].

The flavours produced are the amino acids aspartate and glutamate which can function as food flavoursing. Flavours-forming amino acids (aspartate and glutamate) are usually available in the form of MSG. Monosodium glutamate (MSG) is widely used as a food additive, but excessive consumption can cause serious health problems. Therefore, efforts are needed to develop natural flavours enhancing ingredients by using vegetables as a substitute for MSG [17]. Free glutamic acid levels vary in foods and natural ingredients [2]. So, in this research, ferns were chosen as a source of healthy flavours sourced from local Indonesian ingredients. Flavours from natural ingredients (vegetables) are in great demand and need to be developed for health reasons, therefore necessary to study. Fern vegetables have of unique volatile flavours and new non-volatile flavours, namely as a source of umami flavours from glutamic acid and aspartate which has MSG-like properties and a fern taste.

This research aims to know about morphological characteristics of Indonesian local fern vegetables (*Mloko Jember*, *Air Jember*, and *Ayam Banyuwangi*) and determine the effect of processing on the chemical characteristics and volatile components of Indonesian local fern vegetables, as well as the effect of enzyme hydrolysis on the production of non-volatile flavours. Non-volatile components are produced by hydrolyzing ferns using a combination of two enzymes, i.e. bromelain and protease, at pH 7 and temperature 55℃ for 1 hour. It has been found that edible ferns have the potential to develop unique flavours during processing, particularly when enzymes are added. It is intended that this research would lead to ferns being used as a natural flavouring source for consumption.

II. MATERIAL AND METHODS

A. Materials

The materials for producing flavours were as follows local Indonesian fern vegetables from Durjo Village, Panti District, Jember Regency (known as *Mloko Jember* and *Air Jember*), and local fern from the Sumber Arum Village, Songgon District, Banyuwangi Regency (known as *Ayam Banyuwangi*), as well the chemicals of analysis were Na_2CO_3, folin ciocalteu, NaOH, glutamic acid, BSA, ninhydrin, gallic acid, quercetin, bromylin enzyme, protease enzyme, ethanol, H_2SO_4, HCl, and aquadest.

B. Experimental design

This study used three factors to conduct the research i.e., three types of local Indonesian fern vegetables named *Mloko Jember*, *Air Jember*, and *Ayam Banyuwangi*. With 3 levels of treatment: the control treatment factor used fresh ferns (control), steamed for 10 minutes, and steamed for 10 minutes then dried it at 60°C for 12 hours. Each treatment was repeated twice, resulting in 18 experiments.

C. Sample preparation

Prepare a sample for paste of local fern

The ferns are washed with water until clean and cut into 1-2 cm pieces. Various treatment were fresh ferns (control), steamed for 10 minutes, and steamed for 10 minutes then dried it at 60°C for 12 hours. Next, the each sample was cooled, blended, and ground. Through this treatment, the resulting sample is in the form of fern paste.

D. Parameters of analyzing fern paste

The fern paste was then analyzed for the content of ash, protein, fat, polyphenols, flavonoids, total sugar, reducing sugar, and sucrose using proximate [19], polyphenol [20], flavonoids method [21], reducing sugar, total sugar, and sucrose [22] .

Analyzing of volatile content of fern paste using GC-MS

For distillation (preparation), the resulting paste is then distilled to determine the volatile components using GC-MS (Shimadzu GCMS-QP2010 Plus, Japan). The formulated sample was weighed as much as 10 g using analytical balance (Matler Toledo 210 Scales, Swiss) and 50 mL of distilled water was added using destillator (Pyrex). Distillation was carried out until the sample boiled for 2 hours, and the distillation results were collected to examine the volatile content using GC-MS.

Identification and determination of volatile component content obtained by GC-MS. Work stages include sample preparation (sample filter with membrane filter syringe-Hamilton). Inject the sample into the GC-MS device, and determine the volatile components are 50% organic and 50% non-organic.

The GC-MS instrument was equipped with a split injector set at a temperature of 250ºC. Samples were injected using the spotless method. MS detector temperature 280ºC. The column used was a Restek Rtx®-50 column (Crossbond® 5% phenyl-50% methyl polysiloxane) with an inner diameter of 0.25 mm, a length of 30 m and a thickness of 0.25 µm. The carrier gas used is helium with a pressure of 63.2 kPa, program oven temperature 80ºC, and final temperature 280ºC. Total flow 5.0 mL/min, column flow 0.98 mL/min, linear velocity 36.4 cm/sec, purge flow 3.0 ml/min. The mass spectrum of each compound peak detected in the chromatogram is compared with known compounds in the data bank from Wiley7.LIB

Analyzing of non-volatile content of fern paste using HPLC

During preparation, samples that are in paste form and have been formulated are hydrolyzed with 2 (two) enzymes, namely bromelain and protease, pH 7, temperature 55°C, for 1 hour to produce the amino acids. Next, the sample was stopped from hydrolysis by heating it to a temperature of 90°C for 10 minutes using hotplate (Selecta, Spain) and drying it in oven (Memmert UN55, Germany) at a temperature of 60°C, for 18 hours.

Weigh the sample as much as 0.1 g and add 10 mL of distilled water. Next, the sample was filtered and 25 µL was taken to be put into a test tube. Dry the samples in a vacuum dryer (DZF-6090, China). Add 25 µL of derivatization solution. Leave for 25 minutes at room temperature. Add 25 of 1 M sodium acetate buffer. Then the sample is injected into the HPLC instrument (Shimadzu 210, Japan).

E. Parameters of fern analyzing

All the chemical characteristic was done in triplicate and expressed as mean ± standard deviation. Data was analyzed using Minitab 19 with two way ANOVA (Analysis of Variance) at the 5% level and followed by Tukey test.

III. RESULT AND DISCUSSION

A. Morphological characteristics of three fern local species

Three types of fern plants were obtained from the Indonesian cities of Jember known as *Mloko Jember* and *Air Jember* and Banyuwangi known as *Ayam Banyuwangi*. These ferns displayed different morphological characteristics in their spores, fibre, and leaves. **Fig. 1** shows the morphology of the three fern types, which differ in the shape of the shoots and the overall shape of the plant based on their location. This suggests that fern vegetables grown in different regions exhibit varying morphological characteristics.

The young leaves of the *Ayam Banyuwangi* type are hairless and do not curl, whereas the young leaves of the *Mloko Jember* and *Air Jember* types are curly and have hair. The content of glutamic acid is impacted by raw material differences [4]. Glutamic acid is a major component of food proteins (both animal and plant) in bound form, while free glutamic acid is present in most foods, providing a mild umami taste. The taste of vegetables is influenced by different factors, including cultivar, ripeness, and horticultural practices [2]. The environment also plays a significant role in shaping the flavours of vegetables, as the nutritional balance of the soil is crucial for vegetable synthesis. Taste development requires soil nutrients [2].

a. Shoots of fern *Mloko Jember* | b. Shoots of fern *Air Jember* | c. Shoots of fern *Ayam Banyuwangi*

d. Plant of fern *Mloko Jember* | e. Plant of fern *Air Jember* | f. Plant of fern *Ayam Banyuwangi*

Fig. 1 (a-f) Shoots and plant of Indonesian local fern

B. Chemical characteristics

The chemical characteristics of ash, protein, fat, flavonoids, polyphenols, total sugars, reducing sugars, and sucrose can change after processing. Drying fern vegetables at 60°C causes an increase in the concentration of flavonoids, polyphenols, reducing sugars, total sugars, and sucrose, while the protein and fat content decreases. As shown in **Table 1**, polyphenolic compounds play a role in the maillard reaction, causing an increase in the reaction [23]. Bitterness and astringency arise from the presence of polyphenols [24]. The taste of dried ferns will be more bitter and astringent if they have a higher polyphenol content.

The colour of the fern leaves also became browner, but steaming made them green. Polyphenols and flavonoids are abundant in fresh fern vegetables, and they also have active enzymes that oxidize them. The enzyme is responsible for the browning of fern leaves when they are squeezed, but they remain green when they are steamed. In the other study [25], enzymes such as polyphenol oxidase, peroxidase, lipoxygenase, and ascorbic acid oxidase favour the oxidation of phenolic compounds, leading to undesirable colour changes. According to reference [26], deactivating the enzyme responsible through processes like blanching or steaming can prevent this effect.

TABLE 1
IMPACT OF PROCESSING AND TYPE OF FERN ON CHEMICAL CHARACTERISTICS

Sample	Code	Ash (%)	Fat (%)	Flavonoid (mg QE/g)	Polyphenol (mg GAE/g)	Protein (%)	Reducing sugar (%)	Total sugar (%)	Sucrose (%)
Mloko Jember	Fresh	13.99±0.64^{b}	3.28±0.06^{d}	0.06±0.00^{f}	0.05±0.00^{g}	2.9±0.08^{f}	0.46±0.04^{f}	0.19±0.00^{e}	-
	Steamed	14.62±0.81^{a}	5.87±0.27^{a}	0.38±0.01^{d}	0.18±0.04^{d}	9.68±0.22^{b}	0.73±0.21^{e}	0.80±0.05^{d}	-
	Dried	13.20±0.18c	1.96±0.06^{e}	2.80±1.14^{a}	0.76±0.35^{b}	3.19±0.00^{f}	0.95±0.09^{cd}	1.52±0.05^{bc}	0.44±0.04^{a}
Air Jember	Fresh	15.59±0.31b	1.81±0.04^{e}	0.08±0.00^{ef}	0.05±0.00^{g}	7.64±0.10^{c}	1.08±0.02^{bc}	0.71±0.03^{d}	-
	Steamed	15.11±0.18a	4.81±0.27^{b}	0.14±0.00^{e}	0.13±0.00^{e}	10.98±2.73^{f}	1.12±0.03^{b}	0.12±0.00^{e}	-
	Dried	14.2±0.14c	0.80±0.03^{f}	1.80±0.05^{b}	0.80±0.02^{a}	2.91±0.03^{a}	0.81±0.55^{de}	1.59±0.00^{b}	0.42±0.00^{a}
Ayam Banyu-wangi	Fresh	10.34±0.46e	4.25±0.04^{c}	0.04±0.01^{f}	0.04±0.00^{g}	5.95±0.19^{e}	0.16±0.01^{g}	1.95±0.19^{a}	-
	Steamed	8.81±0.29f	6.22±0.50^{a}	0.15±0.00^{e}	0.10±0.01^{f}	7.04±0.40^{d}	0.83±0.58^{de}	1.38±0.07^{c}	-
	Dried	11.78±0.20d	2.35±0.11^{e}	0.76±0.04^{c}	0.42±0.00^{c}	1.97±0.00^{g}	1.56±0.67^{a}	1.59±0.00^{b}	0.05±0.05^{b}

Note: Different superscript letters in the same column indicate a significant differences of P≤0.05 in Tukey test

The way fern vegetables are processed can affect their chemical properties. When ferns are steamed and then dried, their protein content can be damaged. According to reference [27], changes in the solvent environment can cause the protein's original structure to break down, which is known as denaturation. Factors such as extreme temperature and pH can lead to protein denaturation, causing a loss of protein properties, such as biological activity, solubility, water-holding capacity, and foam-forming ability. However, denaturation can also increase protein digestibility and lead to the creation of desired compounds, such as flavours.

Processing techniques can have an impact on the nutritional value and taste of vegetables, fruits, and edible mushrooms. It is well known that cooking methods and thermal conditions can affect the sensory aspects of vegetables [28]. Steaming is considered the most effective method for retaining bioactive compounds in vegetables, such as broccoli and spinach [29]. In addition, steaming can activate the amylolytic enzymes present in these vegetables. During the steaming process, the food matrix may soften, and protein denaturation may occur, changing the spatial arrangement of protein molecules without breaking peptide bonds. Importantly, different cooking methods, conditions, and food matrices can lead to varying flavonoid or polyphenol contents in different vegetables [30].

C. Volatile components of three local Indonesian ferns

The three species of ferns differ in the amount, type and concentration of volatile components, as shown in **Tables 2**, **3**, and **4**. Steaming produces the less volatile components in these three species. Processing by drying the Jember species produces more types of volatile components than the Banyuwangi species. The volatile components of the Banyuwangi type of fresh treatment have more types than the Jember type. This is in line with research [2] which stated that the activity of different enzyme systems impacts flavours.

However, food processing can also impact health-promoting compounds. According to reference [31], the processing techniques like steaming can increase food digestibility and nutrient bioavailability by softening the food matrix. Steaming is considered a safer technique as it retains the bioavailability of important vegetable elements [32]. Food processing has several benefits. It destroys unwanted compounds and microorganisms, extends shelf life, and improves food digestibility and nutrient bioavailability. It also creates desired compounds such as flavours compounds, antioxidants, and colouring agents.

Table 5 shown the volatile flavours that remain after processing. Polyphenols cause the bitter and astringent taste of fern. The higher the polyphenol content in dried fern, the more bitter and astringent the taste. Processing and the type of fern can influence the quantity and concentration of volatile components, as shown in **Tables 2**, **3**, and **4**. Methyl-d3 1-dideuterio-2-propenyl ether is considered an identifying component of contol, steamed, and dried Indonesian local fern.

D. Non-volatile components of three local Indonesian ferns

In our research, the bromelain enzyme used had a unit activity of 8.83±0.77 (U/mL) and the protease enzyme had an activity of 31.56±3.4 (U/mL), at pH 7 and a temperature of 55°C, to be used to hydrolyze fern protein, to produce non-volatile components. The results of fern hydrolysis can be seen in **Table 6**.

TABLE 2
VOLATILE COMPONENTS INTERN VEGETABLES BEFORE AND AFTER PROCESSING OF *MLOKO JEMBER* FERN

No	Area %	Fresh *Mloko Jember* fern	Area %	Steamed *Mloko Jember* fern	Area %	Dried *Mloko Jember* fern
1	5.28	5-methoxy-1-aza-6-oxabicyclo(3.1.0)hexane	0.06	Acetamide, 2-fluoro-	3.45	Carbamic acid, monoammonium salt
2	5.57	ethyl 1-hexyl-4-hydroxy-2(1h)-oxo-3-quinoline carboxylate	0.09	2-Heptanamine, 5-methyl-	0.16	(E,E,E)-2,6,10,14-Cyclopentadecatetraen-1-one
3	27.38	Carbamic acid, monoammonium salt	21.49	Carbamic acid, monoammonium salt	0.64	Propanedioic acid
4	1.74	Carbodiimide, bis(trimethylgermyl)-	21.34	Methane, dichloro-	0.68	Acetamide, 2-fluoro-
5	0.24	1-phenoxy-4-methoxybutan-2-one	0.08	1,3,5-Trimethylhexahydro-1,3,5-triazine	1.32	Acetic acid
6	5.06	ethyl 1-hexyl-4-hydroxy-2(1h)-oxo-3-quinolinecarboxylatE	0.91	Methyl-d3 1-Dideuterio-2-propenyl Ether	41.33	Methyl-d3 1-Dideuterio-2-propenyl Ether
7	8.36	Methane, oxybis[chloro	1.85	Acetamide, 2-fluoro-	10.14	Silane, fluoro trimethyl-
8	5.85	Acetic acid	0.99	1-Propanol, 2-methyl-	9.96	5-Hydroxypyrimidine
9	2.82	(E)-2-(4-Methyl-3-isopentyl)-1-propenyl phenyl sulfone	2.95	20-Deethyl-17-oxovincadifformine	2.29	2-Furancarboxaldehyde
10	30.92	Methyl-d3 1-Dideuterio-2-propenyl Ether	0.80	1,4-Dioxane-2,6-dione	3.19	3-Butyn-1-ol
11	0.83	3-Octanone	8.65	Acetic acid	2.95	2-pentadecyl-4,4,5,5-tetradeutero-1,3-dioxolane
12	0.97	methoxy, phenyl-, oxime	4.02	2-Propanone, 1-hydroxy-	2.98	ethyl 1-hexyl-4-hydroxy-2(1h)-oxo-3-quinoline carboxylate
13	1.99	Hexanal, 2-ethyl-	33.81	Methyl-d3 1-Dideuterio-2-propenyl Ether	1.73	nitro acetonitrile
14	2.48	3-Octanol	1.92	Phosphine, bis(1,1-dimethyl ethyl)(1-methyl ethyl)-	14.29	methoxy, phenyl-, oxime
15	0.15	Ethanedioyl dichloride	1.03	Aziridine, 1-methyl-	0.87	sec-bromobutane-1,1,1,3,3-d5
16	0.08	2-Methyl-3(2H)-isothiazolone			0.97	(S)-N, N'-dimethyl-3-phenylpropane-1,2-diamine
17	0.08	4H-6-o-chlorophenyl-3-phenyl-v(1,2,3)-triazolo[1,5-d][1,3,4]oxadiazine			1.24	didecyl 1,4-dihydro-2,6-dimethyl-3,5-pyridine dicarboxylate
18	0.20	5-methyl-2-octyl-(2h)-furan-3-on			0.93	dl-alanyl-dl-alanine
19					0.87	5-methyl-2-octyl-(2h)-furan-3-on

TABLE 3
VOLATILE COMPONENTS INTERN VEGETABLES BEFORE AND AFTER PROCESSING OF *AIR JEMBER* FERN

No	Area %	Fresh *Air Jember* fern	Area %	Steamed *Air Jember* fern	Area %	Dried *Air Jember* fern
1	1.36	3,3-dimethyl-2-phenyl-2-(1-oxo-1,2,3,4-tetrahydronaphthalen-2-yl)azirane	2.53	ethyl 1-hexyl-4-hydroxy-2(1h)-oxo-3-quinoline carboxylate	0.39	Propanoic acid, anhydride
2	3.79	5-methoxy-1-aza-6-oxabicyclo(3.1.0)hexane	51.05	Carbamic acid, monoammonium salt	1.10	trans-.beta.-ionon-5,6-epoxide
3	14.40	Carbamic acid, monoammonium salt	0.07	Methenamine, N-methyl-	7.90	Carbamic acid, monoammonium salt
4	0.01	propanoic acid, 2-oxo- (cas) pyruvic acid	0.34	2-octadic-1"-enyloxy-1,1,2,2-tetradeutero ethanol	0.16	(1's,3s)-3-hydroxy-3-phenyl-n-(1-phenylmethyl)propionamide
5	0.92	ethyl 1-hexyl-4-hydroxy-2(1h)-oxo-3-quinoline carboxylate	0.06	propanoic acid, 2-oxo-	14.10	propanedioic acid
6	5.37	methyl butyric acid	0.34	4,5-difluoromethane isomer	3.96	2-propanone, 1-hydroxy-
7	1.88	(1's,3s)-3-hydroxy-3-phenyl-n-(1-phenylmethyl)propionamide	5.17	ethyl 1-hexyl-4-hydroxy-2(1h)-oxo-3-quinoline carboxylate	3.11	7\ n-(p-anisidinomethyl)-4-methyl phthalimide
8	45.73	Methyl-d3 1-Dideuterio-2-propenyl Ether	1.69	5-Chloro-3-ethoxy-6-methyl-2H-1,4-oxazine-2-one	50.70	Methyl-d3 1-Dideuterio-2-propenyl Ether
9	3.19	3-Octanone	0.70	Acetic acid	6.22	1,3-Dioxolane, 2-heptyl-(CAS) Octanal, cyclic 1,2-ethanediol acetal
10	9.19	butanoic acid, 3-methyl-	36.67	methyl-d3 1-dideuterio-2-propenyl ether	2.55	1-propane sulfonyl chloride, 3-chloro-
11	13.70	methoxy, phenyl-, oxime	0.06	acetic acid, fluoro-, ethyl ester	1.94	ethyl 1-hexyl-4-hydroxy-2(1h)-oxo-3-Quinolinecarboxylate
12	0.17	phenol, 3-methoxy-4-(phenyl methoxy)-	0.05	propane, 2,2-difluoro-	0.93	acetic acid, fluoro-, ethyl ester
13	0.17	benzoi acid, 2-hydroxy-6-methyl-, ethyl ester (CAS) 2,6-crescentic acid, ethyl ester (CAS) ethyl 6-methylsalicylate	0.78	ribitol	1.75	1.75 acetamide, 2-fluoro-
14	0.06	silane, bis(trifluoromethyl)dimethyl-	0.05	propane, 2-fluoro-2-methyl-	0.74	crotonyl chloride
15	0.02	benzenemethanamine, n,n-dimethyl-(cas) n,n-dimethylbenzylamine	0.43	methoxy, phenyl-, oxime	1.73	n-methyl-n-(methyl-d3)aminoheptane
16	0.03	n-(1-(1-allyl-3-butenyl)carbonyl)-n'-methyl			0.26	butanoic acid (cas) n-butyric acid
17					1.90	methoxy, phenyl-, oxime
18					0.29	ethyl 1-hexyl-4-hydroxy-2(1h)-oxo-3-quinoline carboxylate
19					0.20	n,n-dimethyl-o-(1-methyl-butyl)-hydroxylamine
20					0.04	Dichlorofuranone
21					0.04	2-Pyrazoline, 1-butyl-5-methyl-

TABLE 4
VOLATILE COMPONENTS INTERN VEGETABLES BEFORE AND AFTER PROCESSING OF *AYAM BANYUWANGI* FERN

No	Area %	Fresh *Ayam Banyuwangi* fern	Area %	Steamed *Ayam Banyuwangi* fern	Area %	Dried *Ayam Banyuwangi* fern
1	2.02	Docosanoic acid	6.38	Acetamide, 2-fluoro-	13.47	Carbamic acid, monoammonium salt
2	6.90	Methane, tetranitro-	13.19	Methane, tetranitro-	0.04	(2S,3S)-2,3-epoxy butanol
3	0.84	2-propanoic acid	1.20	Ethanol, 2-[2-(2-phenoxy ethoxy)ethoxy]-	0.50	acetic acid, anhydride with formic acid
4	12.52	Methyl-d3 1-Dideuterio-2-propenyl Ether	0.86	Acetic acid	5.29	Methyl-d3 1-Dideuterio-2-propenyl Ether
5	54.90	1,5-Pentanediol, 3-methyl-	10.61	Acetic acid	4.92	2,3-Butanediol
6	10.78	Silane, fluoro trimethyl-	1.77	Propanone, 1-hydroxy-	2.08	di(15)N-Thiourea
7	4.17	Acetic acid, fluoro-, ethyl ester	59.53	Methyl-d3 1-Dideuterio-2-propenyl Ether	0.30	1,3-bis(Trimethylsiloxy0-2,4-bis(t-butyldimethylsilyl)-1,3-dioxo-1.lambda(6).,3.lambda(6).-dithiete
8	1.74	1-(Pent-4-ynyl)pyrano[3,4-b]indol-3-one	0.58	1,3,2-Dioxaborolan-4-one, 2-ethyl-	5.93	2-Butanone, 3-hydroxy-
9	1.28	.beta.-D-Ribofuranosyl-8-nitro-s-triazolo[1,5-a]pyridine	0.49	0.49 1,2,5-Thiadiazole	38.55	Silane, fluoro trimethyl-
10	0.33	Cyclohexane, 1-bromo-3-methyl-	0.46	L-Proline, ethyl ester	1.71	Pentanoic acid, 2-methyl-
11	0.23	0.23 Pentane, 2-methoxy-	4.47	methoxy, phenyl-, oxime	4.95	Ethanedioic acid
12	0.67	2-furan carboxylic acid, tetrahydro-3-methyl-5-oxo-, methyl ester	0.20	1,2-Ethanediamine, N-(2-aminoethyl)-	21.10	iso-valeric acid
13	2.61	3-Heptanol, 4-methyl-	0.25	Methyl 3-Butenyl-1-d2 Ether	0.08	2-Amino-2-methyl-1,3-propanediol
14	0.13	10-(tetrahydro-pyran-2-aryloxy)-tricyclo[4.2.1.1 2,5]decan-9-ol			0.02-	Ethane, 1,1-dimethoxy-
15	0.12	cyclohexanol, 4-[[(1,1-dimethyl ethyl)dimethylsilyl]oxy]-,cis-			0.17	bis(trifluoromethyl)sulphate
16	0.17	2H-Pyran, 2-[(1,1-dimethyl-2-butynyl)oxy]tetrahydro-			0.66	2,3,5-trimethyl pyrazine
17	0.22	endo-7-(3-Carbethoxy-3-butenyl)-2,9-dioxabicyclo[4.3.0]nonane			0.22	Propane, 2-fluoro-2-methyl-
18	0.21	2-Ethyl-2,4,5-trimethyl-2H-imidazole				
19	0.15	0.15 hex-3-ene-1,6-diol				

TABEL 5
RESIDUAL VOLATILE COMPONENTS AFTER PROCESSING OF FERN

Code	Fresh	Steamed	Dried
Mloko Jember	carbamic acid, monoammonium salt (27,38%), acetic acid (5,85%), methyl-d3 1-dideuterio-2-propenyl ether (30,92%). methoxy, phenyl- ,oxime (0,9%)	carbamic acid, monoammonium salt (21,49), methyl-d3 1-dideuterio-2-propenyl ether (0,91%), acetic acid (8,65%), methyl-d3 1-dideuterio-2-propenyl ether (33,81%)	carbamic acid, monoammonium salt (3,45%), methyl-d3 1-dideuterio-2-propenyl ether (41,33), acetic acid (1,32%). methoxy, phenyl- ,oxime (14,29%)
Air Jember	carbamic acid, monoammonium salt (14,4%), propanoic acid, 2-oxo- (cas) pyruvic acid (0,01%), ethyl 1-hexyl-4-hydroxy-2(1h)-oxo-3-quinoline carboxylate (0,92%), methyl-d3 1-dideuterio-2-propenyl ether (45,73%), methoxy, phenyl- ,oxime (13,75%)	carbamic acid, monoammonium salt (51,05%), propanoic acid, 2-oxo- (cas) pyruvic acid (0,06%), ethyl 1-hexyl-4-hydroxy-2(1h)-oxo-3-quinoline carboxylate (5,17%), methyl-d3 1-dideuterio-2-propenyl ether (36,67%), methoxy, phenyl- ,oxime (0,43%)	carbamic acid, monoammonium salt (7,90%), propanoic acid, 2-oxo- (cas) pyruvic acid (0,39%), ethyl 1-hexyl-4-hydroxy-2(1h)-oxo-3-quinoline carboxylate (1,94%), methyl-d3 1-dideuterio-2-propenyl ether (50,70%), methoxy, phenyl- ,oxime (1,90%)
Ayam, Banyuwa-ngi	methyl-d3 1-dideuterio-2-propenyl ether (12,52%)	methyl-d3 1-dideuterio-2-propenyl ether (59,53%), methoxy, phenyl- ,oxime (4,47%)	methyl-d3 1-dideuterio-2-propenyl ether (5,29%)

Steaming fern vegetables aims to deactivate the polyphenol oxidase enzyme so that the vegetables remain green. The steaming process also causes the matrix to become softer and also causes the protein to become denatured, namely a change in the spatial arrangement due to changes in the secondary, tertiary, and quaternary structure of a protein molecule without breaking the peptide bonds (primary structure) or unfolding or protein strands becoming untied. Fern proteins experience denaturation during steaming, thereby breaking covalent, hydrogen, and disulfide bonds causing the protein to strand or change its native properties. Denaturation can also be caused by changes in pH, especially breaking ionic bonds, disrupting ion stability, and thus changing the tertiary structure of proteins. The occurrence of denaturation causes loss of protein properties, namely loss of biological activity (enzymes), solubility, water holding capacity, loss of ability to form foam, increased sensitivity to enzyme activity (increasing protein digestibility), as well as the formation of desired compounds such as flavours.

The presence of denaturation will make it easier for protease and bromelain enzymes to attack proteins on both the outer and inner surfaces. Excessive heating of protein foods can cause effects such as sulfhydryl bonds in natural proteins, usually found on the inside of the protein helical circle, which when heated, the circle opens and can cause a reaction. Sulphihydryl bonds are very sensitive to reactions that can change colour and taste, denaturation can change texture, flavours, colour and taste [33].

Protein hydrolysis involves breaking peptide bonds in the protein structure into simpler bonds through an enzymatic hydrolysis process [34]. Additionally, in other research [35], the hydrolysis process is influenced by substrate concentration, enzymes, temperature, pH, and time, with longer durations leading to a more complete hydrolysis process. Knowing the optimum pH, temperature, and time can be used for hydrolysis to produce amino acid components or umami taste and fern taste. According to reference [36], the bromelain enzyme belongs to the endo-peptidase enzyme group, and protease (papain) belongs to the exopeptidase group. The combination of the two has good synergy to produce peptides and high amino acids. Additionally, the enzymatic hydrolysis using bromelain increased arginine, alanine, glutamic acid, and asparagines [37].

In other research study [38], it was found that hydrolysis using bromelain endopeptidase produces higher hydrophobic amino acids, which are significant in the formation of flavours compounds. Peptides with a high molar ratio of L-glutamic acid peptides and having hydrophilic C-terminal residues are reported to give rise to a strong umami taste. However, peptides containing hydrophobic amino acids in their sequence or at the end of the peptide chain, which exhibit excellent biological properties, are known to impart a distinct bitter taste.

The results of fern hydrolysis by combining the two enzymes in our research can be seen in **Table 6**. There were 17 types of amino acid components detected in ferns, with the most abundant components being glutamic acid with a value of 2.05-2.5%, and aspartic acid with a value of 1.2-1.7%, also amino acid cystein 0.44-0.66%, which is necessary for children who lack protein. As stated by researcher [17], glutamic acid and aspartic acid have MSG-like properties, also the glutamic acid is the source of the most dominant umami (savoury) taste and has an impact on the perfection or authenticity of the taste [39]. This research demonstrated the production of 17 types of amino acids, indicating a nearly perfect hydrolysis enzymatic process on Indonesian local fern. According to reference [40], that the well-executed hydrolysis process results in a high-quality hydrolyzate product with a good flavours.

Ferns contain a lot of protein with a high proportion of hydrophobic amino acids, namely leucine (0.85-1.3%), valine (0.3-0.55%), isoleucine (0.6-0.87%), and histidine (0. 39-

TABLE 6
NON-VOLATILE FERN COMPONENTS THAT ARE HYDROLYZED WITH ENZYMES

Code	Amino Acid	Fresh (%)			Steamed (%)			Dried (%)			Properties of Amino Acids	Source
		Mloko Jember	*Air Jember*	*Ayam Banyuwangi*	*Mloko Jember*	*Air Jember*	*Ayam Banyuwangi*	*Mloko Jember*	*Air Jember*	*Ayam Banyuwangi*		
1	Aspartic Acid (Asp)	1.64	1.7	1.66	1.53	1.57	1.66	1.2	1.33	1.28	amino acid hydroxy-aliphatic	MSG-Like [17]
2	Glutamic Acid (Glu)	2.47	2.32	2.5	2.29	2.2	2.5	2.17	2.05	2.1	amino acid hydroxy-aliphatic	MSG-Like
3	Serine (Ser)	0.65	0.69	0.62	0.6	0.61	0.62	0.48	0.43	0.5	hydroxy-aliphatic	Sweet
4	Glycine (Gly)	0.72	0.76	0.8	0.65	0.69	0.8	0.55	0.51	0.59	simple aliphatic	Sweet [43]
5	Histidine (His)	0.61	0.65	0.62	0.54	0.6	0.62	0.46	0.39	0.44	base	Bitter
6	Arginine (Arg)	0.7	0.64	0.75	0.6	0.68	0.75	0.52	0.56	0.54	base	Sweet
7	Threonine (Thr)	0.55	0.58	0.6	0.48	0.4	0.6	0.38	0.43	0.35	hydroxy-aliphatic	Sweet [43]
8	Alanine (Ala)	0.68	0.61	0.72	0.5	0.52	0.72	0.44	0.48	0.5	hydroxy-aliphatic	Sweet [43]
9	Proline (Pro)	0.81	0.85	0.89	0.77	0.71	0.89	0.65	0.62	0.68	proline	Tasteless
10	Tyrosine (Tyr)	0.53	0.55	0.64	0.43	0.45	0.64	0.4	0.36	0.42	aromatic	Tasteless [43]
11	Valine (Val)	0.49	0.43	0.55	0.38	0.4	0.55	0.33	0.3	0.38	simple aliphatic	Bitter [43]
12	Methionine (Met)	0.39	0.41	0.48	0.3	0.37	0.48	0.29	0.25	0.3	dn sulfur	Bitter [43]
13	Sisteine (Cys)	0.64	0.59	0.66	0.52	0.58	0.66	0.47	0.4	0.44	dn sulfur	Tasteless
14	Isoleucine (Lle)	0.82	0.87	0.85	0.71	0.75	0.85	0.62	0.67	0.6	simple aliphatic	Bitter [43]
15	Leucine (Leu)	1.23	1.28	1.2	1.07	1.1	1.2	0.87	0.93	0.85	simple aliphatic	Bitter [43]
16	Fenilalanine (Phe)	0.66	0.69	0.63	0.56	0.64	0.63	0.41	0.46	0.43	aromatic	Bitter [43]
17	Lysine (Lys)	1.01	1.1	1.06	0.98	1.03	1.06	0.78	0.74	0.81	base	

0.65%) (**Table 6**). Other research [41] showed that the bitter taste is the sum of glycine, histidine, proline, valine, methionine, isoleucine, leucine, phenylalanine; umami taste amount of aspartic acid, glutamic acid; sweet taste sum of serine, arginine, alanine, threonine; salty taste amount of cysteine; and bland or tasteless amounts of tyrosine and lysine. The amino acid cysteine is necessary for children who lack protein and this kind of amino acid is not available on mushroom [14,15].

In a reference [42], it was also mentioned that the amino acid tyrosine (Tyr) has aromatic properties and is tasteless, while phenylalanine (Phe) has a bitter taste. Bitter taste in ferns can also be detected due to the presence of amino acids such as valine (Val), methionine (Met), leucine (Leu), phenylalanine (Phe), and lysine (Lys). Meanwhile in this study, amino acids were produced from three different species of Indonesian local fern (*Mloko Jember*, *Air Jember*, *Ayam Banyuwangi*), and the various processing methods resulted in varying amounts of amino acids. Additionally, differences in species, growing locations, and growing conditions were found to impact the production of amino acids. It is believed that the umami taste is derived from water-soluble or hydrophilic enzymatically hydrolyzed proteins such as aspartate and glutamate, while the fern taste comes from fat-soluble amino acids including Gly, Ala, Val, Leu, Ile, Met, and Pro.

IV. CONCLUSION

The three types of Indonesian local ferns (*Mloko Jember*, *Air Jember*, *Ayam Banyuwangi*) differ in their morphology and chemical characteristics. It is believed that methyl-d3 1-dideuterio-2-propenyl ether is the compound responsible for identifying local ferns. The processing method and type of fern used can impact the amount, type, and concentration of volatile components produced. Processing can affect the chemical composition of ferns, including protein, fat, polyphenols, flavonoids, reducing sugars, total sugars, and sucrose.

The researchers found that enzymatically hydrolyzing the proteins in fern vegetables (*Mloko Jember*, *Air Jember*, *Ayam Banyuwangi*) using two protease enzymes and bromelain produced amino acid components such as aspartic acid and glutamic acid. These amino acids were dominant as non-volatile components, giving fern vegetables a water-soluble umami taste and an oil-soluble fern taste. The amino acids Gly, Ala, Val, Leu, Ile, Met, and Pro are also present and the fern contains the amino acid cysteine, which is necessary for children who lack protein.

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CONFLICT OF INTEREST

Authors declare no conflict of interest to disclose.

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