BIODIVERSITAS
Volume 22, Number 8, August 2021
Pages: 3440-3445

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d220841

Evaluation of proximate composition, microbial load and sensory characteristics of instant holat as traditional spice in North Sumatra, Indonesia

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Manuscript received: 6 June 2021. Revision accepted: 25 July 2021.

Abstract. *Maser WH, Sinaga MZE. 2021. Evaluation of proximate composition, microbial load and sensory characteristics of instant holat as traditional spice in North Sumatra. Biodiversitas 22: 3440-3445.* Holat is the name of traditional food from North Sumatra, Indonesia which has astringent flavor derived from the bark of *Phyllanthus emblica*. The aims of this study were to determine the proximate composition and evaluate microbial load, and sensory characteristics of instant holat spice. There were 12 treatments, namely the combination of different amounts of bark (15 g, 25 g, 35 g, 45 g) and drying time (20 h, 22 h, 24 h). The proximate composition (moisture, ash, protein, fat, crude fiber, fat, and total carbohydrate content), microbial analysis (total plate count; yeast and mold count), and sensory characteristics (aroma, color, taste, and overall acceptability) were carried out. The results showed that water, protein, fat, crude fiber, and total carbohydrates contents were all significantly different ($p < 0.05$), except ash content. There was no yeast, mold, and bacterial growth on total plate count. There were no significant differences in all sensory characteristics (aroma, color, taste, and overall acceptability) of instant holat before and after brewing ($p \geq 0.05$). The treatment of F4-24 was the best formula and has the highest score that might be developed as a product of instant holat spice. Further study should be conducted to determine the effect by increasing the amount of bark and evaluating its bioactivity.

Keywords: Instant holat spice, *Phyllanthus emblica* bark, proximate composition, sensory characteristics, North Sumatra

INTRODUCTION

Holat is the name of traditional Indonesian cuisine from Padang Bolak, North Sumatera (Khoiriyah et al. 2015; Paluseri et al. 2017). The word holat is derived from the word chelate, which means astringent, and refers to the taste of the key ingredient (Damanik and Sinaga 2020; Pulungan et al. 2020). The essential ingredient of holat is the bark of the Indian gooseberry (*Phyllanthus emblica*) (Paluseri et al. 2017; Maser and Marsella 2019).

Phyllanthus emblica, known as kimalaka (Indonesian), is widespread in Indonesia (Uji 2007). Southern North Sumatra with a rainfall of 2000-2500 mm/year on mixed dryland agriculture and humic acrisol soil types is one of the regions that are overtaken by this plant, (Khoiriyah et al. 2015). This plant is distributed in tropical and subtropical areas such as Pakistan, Uzbekistan, Burma, Nepal, Bangladesh, Bhutan, Sri Lanka, South East Asia, and China, as well as the Malay Peninsula (Khan 2009; Huabprasert et al. 2012; Dasaroju and Gottumukkala 2014; Priya and Islam 2019).

Phyllanthus emblica (Euphorbiaceae) is a moderate deciduous tree that can grow to a height of 10–18 m (Khan 2009). This plant has a greenish-grey bark and greenish-yellow flowers that grow in axillary clusters. The branchlets reach a length of 40 cm long and have more than 100 leaves clustered in them. The bark is brown and peels off into little uneven flakes (Gaire and Subedi 2014).

Indian gooseberry has been utilized as traditional medicine, functional food, and cosmetic (Sripanidkulchai and Fangkrathok 2014). The fruit is the most commonly used as a food ingredient, while the barks are frequently used as medicine (Kumar et al. 2012; Priya and Islam 2019). The bark contains terpenes, phytosterols, and terpenoids (Deepak and Gopal 2014), as well as polyphenols, flavonoids, tannins, ellagic acid, and gallic acid, which function as antioxidants and radical scavengers, and antimicrobial (Iamsaard et al. 2014; Chaphalkar et al. 2017; Ahmad et al. 2021). Holat has the potential as functional food and nutritious cuisine (Pulungan et al. 2020).

Instant food is a simple food product that may be prepared quickly and easily, with ingredients that have been pre-mixed so that it may be conveniently and readily available (Ali et al. 2012; Monteiro et al. 2014; Munasinghe et al. 2015; Dhiman et al. 2017). The availability of instant holat spice makes cooking easier and can preserve Indonesian traditional food culture. Two formulations have been used to investigate the sensory properties of instant holat spice (Maser and Marsella 2019). However, the proximate composition and microbial analysis have not been performed. Therefore, the purpose of this study was to examine the proximate composition, microbial analysis, and sensory characteristics of instant holat spice.

MATERIALS AND METHODS

Materials

The bark of *Phyllanthus emblica* (collected from plant with stem diameter of 6-9 cm, the bark shaving of 40-60 g/stem) was obtained from the forest of Padang Bolak, North Sumatra. The analytical grade chemicals and solvents were purchased from Merck and Sigma Aldrich, USA.

Sample preparations

Four different compositions of instant holat spice were used in this study (Table 1). Oil (15 mL/50 g onion) was used to fry the smooth onion until it was slightly brown and fragrant. The ginger, lemongrass, and garlic were mashed together. The leeks and celery were cut into small pieces. All ingredients were mixed as in Table 1) and boiled for about 5 minutes in 400 mL of water until the initial boiling. The mixture of spices was dried in a cabinet drier at 60°C for a predetermined time (20 hours, 22 hours, and 24 hours) and refrigerated in an airtight packing.

Proximate composition

The proximate composition of instant holat spice was evaluated in duplicate. The protein content was determined using the Kjeldahl method (AOAC 2011). AOAC methods were used to analyze moisture, fiber, fat, and ash contents (AOAC 2011). Carbohydrate content was determined by subtracting 100 from the total moisture, fat, ash, and protein content (AOAC 2011).

Microbial analysis

Microbial analysis was performed using the conventional plate count.as described by Maturin and Peeler (2001) for total plate count, yeast and mold.

Sensory evaluation

Sensory characteristics were conducted with 80 untrained panelists from staff and students of the Food Science and Technology Study Program, USU. A 7-point hedonic scale (7-like very much and 1-dislikes very much) was used to evaluate four aspects (taste, odor, color, and overall acceptability) of sensory testing (Yusof et al. 2021). Duplicate samples were prepared using non-brewed instant holat spice and brewed with 400 mL of boiling water. Panelists must rinse their mouths with water before every new sample was tested, (Galla et al. 2017).

Statistical analysis

The study was performed in duplicate, and the mean values and standard deviation (SD) were calculated. An analysis of variance (ANOVA) was used to analyze the data.

RESULTS AND DISCUSSION

Proximate composition

The proximate composition of all instant holat spice was presented in Table 2. The moisture content of four formulations varied and differed significantly ($P < 0.05$). Formulation 4 (F4) had the highest moisture content (18.55 ± 0.17%), while F1 had the lowest (9.15 ± 0.11%). It might be due to higher content of *P. emblica* bark in F4; therefore, increasing the bark content increases moisture content of instant holat.

Drying time affected moisture content. The increasing drying time result in decreasing moisture content (Idah et al. 2010; Shalini et al. 2017), as in F3-20 (17.89 ± 0.02%), F3-22 (17.25 ± 0.30%), and F3-24 (16.49 ± 0.33%), which have a significantly different moisture content ($P < 0.05$). However, there was no significant interaction between bark content and drying time on moisture content ($P \geq 0.05$). The moisture content of four formulations of instant holat spice meets the requirements of dried food, with moisture content ≤ 25% (Afolabi 2014).

The ash content of all formulations of instant holat was not significantly different ($P \geq 0.05$). F1-22 has the highest ash content (7.80 ± 0.01%), while F4-24 has the lowest one (7.70 ± 0.01%). The results showed that drying time had no effect on the ash content and previous studies (Delgado et al. 2016; Isik et al. 2019; Bikila et al. 2020). The inorganic residue left after the ignition or complete oxidation of organic matter in biological material is referred to as ash (Liu 2019). Ash is a measure of a food ingredient's total mineral content (Bikila et al. 2020). The drying temperature did not affect the mineral content in the material (Manthey and Hall 2007) as well as drying time. It is based on the concept that minerals are not destroyed by heating because they are less volatile than other compounds (Liu 2019).

Table 2 shows that the protein content of the four holat formulations was significantly different ($P < 0.05$). F1-20 contained the highest protein content (11.21 ± 0.08%) and the lowest protein content was F4-25 (10.42 ± 0.02%). The drying time had no significant effect on protein content as well as interaction of drying time and amount of bark ($P \geq 0.05$). Nevertheless, the crude protein content decreased with increasing the drying air temperature, like previous research (Miranda et al. 2010; Aksoy et al. 2019; Isik et al. 2019) while there was a slight difference in drying time had no effect on protein levels. Protein loss might be caused by denaturation or changes in solubility during drying (Miranda et al. 2010; Aksoy et al. 2019; Isik et al. 2019). In addition, a further possible reason for this decrease is the release of amino acids from proteins after denaturation, which might interact with other compounds like as sugars to create melanoidines through the Maillard reaction (Miranda et al. 2010).

Table 1. Formulation of instant holat spice

Ingredients	Formulations			
	F1	**F2**	**F3**	**F4**
Bark of *Phyllanthus emblica* (g)	15	25	35	45
Onion (g)	50	50	50	50
Oil (mL)	15	15	15	15
Garlic (g)	23	23	23	23
Leeks (g)	10	10	10	10
Celery (g)	10	10	10	10
Lemongrass (g)	7	7	7	7
Ginger (g)	4	4	4	4
Salt (g)	3	3	3	3

Note: F1: Formulation 1; F2: Formulation 2; F3: Formulation 3; F4: Formulation 4

Table 2. Proximate composition of instant holat spice

Formulation-drying time (hours)	Proximate composition					
	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Crude fiber (%)	Total carbohydrate (%)
F1-20	10.15±0.21^{h}	7.78±0.01^{b}	11.21±0.08^{a}	12.14±0.04^{a}	13.07±0.04^{f}	58.72±0.33^{c}
F1-22	9.56±0.16^{h}	7.80±0.01^{a}	11.14±0.04^{ab}	12.09±0.02^{a}	13.06±0.01^{f}	59.42±0.22^{b}
F1-24	9.15±0.11^{g}	7.77±0.01^{b}	11.08±0.01^{b}	11.85±0.05^{b}	13.02±0.01^{f}	60.15±0.07^{a}
F2-20	13.97±0.01^{f}	7.75±0.00^{c}	10.89±0.04^{c}	11.56±0.03^{c}	14.62±0.07^{d}	55.82±0.11^{e}
F2-22	13.82±0.01^{e}	7.75±0.00^{c}	10.84±0.01^{c}	11.36±0.06^{d}	14.55±0.01^{de}	56.23±0.08^{e}
F2-24	12.30±0.37^{e}	7.74±0.00^{cd}	10.82±0.03^{c}	11.15±0.04^{e}	14.48±0.03^{e}	57.99±0.44^{d}
F3-20	17.89±0.02^{b}	7.73±0.01^{de}	10.64±0.04^{d}	11.03±0.01^{f}	15.89±0.05^{c}	52.70±0.01^{hi}
F3-22	17.25±0.30^{c}	7.74±0.01^{de}	10.57±0.02^{e}	10.97±0.02^{fg}	15.83±0.02^{c}	53.49±0.35^{g}
F3-24	16.49±0.33^{d}	7.73±0.00^{de}	10.53±0.01^{e}	10.90±0.00^{g}	15.81±0.01^{c}	54.35±0.34^{f}
F4-20	18.55±0.17^{a}	7.72±0.00^{e}	10.43±0.05^{f}	10.67±0.11^{h}	17.00±0.01^{a}	52.62±0.33^{i}
F4-22	18.42±0.10^{a}	7.71±0.01^{f}	10.44±0.01^{f}	10.57±0.02^{i}	16.95±0.09^{a}	52.87±0.07^{hi}
F4-25	18.14±0.05^{ab}	7.70±0.01^{f}	10.42±0.02^{f}	10.52±0.02^{i}	16.84±0.05^{b}	53.23±0.05^{gh}

Note: The data represent the mean and standard deviation of duplicate samples. Means in the same column with the same letters are not significantly different ($P \geq 0.05$)

The fat content differed significantly ($P < 0.05$). The highest fat content was in F1-20 (12.14 ± 0.04%) and the lowest was in F4-25 (10.52 ± 0.02). The decrease in fat content was caused by a decrease in the oil percentage. The oil percentage in F4 was 8.98%. Drying time had a significant effect on fat content, i.e., F2-20 (11.56 ± 0.03%), F2-22 (11.36 ± 0.06%), and F2-24 (11.15 ± 0.04%). This can be caused by volatile compounds in materials such as essential oils (Adak et al. 2018; Choudhary and Grover 2019), which evaporate during the drying process (Marey and Shoughy 2016). The longer the drying is carried out, the more compounds are lost. A study by Delgado et al. (2016) showed that the fat content had decreased with increasing drying time. Increasing the drying temperature resulted in higher reduction of crude fat (Miranda et al. 2010; Marey and Shoughy 2016). The fat content of instant pindang seasoning powder and seasoning with Tom Yam flavor was between 1-2 % (Phornphisutthimas 2010; Mareta et al. 2019), whilst fat content in instant holat spice was higher due to the higher proportion of oil in formulations.

Crude fiber in the four holat formulas was significantly different due to the amount of bark ($P < 0.05$). F4-20 had the highest crude fiber content (17.00 ± 0.01%), while F1-24 had the lowest one (13.02 ± 0.01%). Drying time did not significantly affect crude fiber content ($P \geq 0.05$). Because bark is used as an ingredient in instant holat spice, therefore crude fiber content was higher than in seasoning with Tom Yam flavor (4.33%) (Phornphisutthimas 2010).

The four holat formulas had significantly different total carbohydrate content ($P < 0.05$) (Table 2.). F4-20 had the lowest total carbohydrate content (52.62 ± 0.33%), and F1-24 had the highest one (60.15 ± 0.07%). The amount of moisture content, ash, fat, and protein in instant holat spice affected the total carbohydrate content. The test results showed low moisture content while high total carbohydrate content, the same thing was also in the study of Miranda et al. (2010).

Microbiological analysis

There was no microbial growth (data not shown) either in total plate count, and yeast and mold count. Antimicrobial compounds in spice ingredients, such as bark of *Phyllanthus emblica* have (Dhale and Mogle 2011; Adak et al. 2018), onion (Ye et al. 2013), garlic (Ross et al. 2001; Liu et al. 2021; Prajapati et al. 2021), and others, can prevent microbial growth. It has been reported that the bark of *Phyllanthus emblica* can inhibit the growth of *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella typhi* which is thought to be caused by the presence of tannins and flavonoids (Dhale and Mogle 2011; Adak et al. 2018). Moreover, onions have also been shown to have antimicrobial activity against bacteria (E. *coli*, *B. subtilis*, and *S. aureus*), yeasts (*R. glutinis*, *S. cerevisiae*, and *C. tropicalis*), and molds (*A. niger*, *M. purpureus*, and *A. terreus*), that is thought to be affected by methyl 5-methylfuryl sulfide (18.30%), methyl 3,4-dimethyl-2-thi (9.72 %) (Ye et al. 2013). In addition, allicin activity is the main activity of garlic constituents, and there is a lot of evidence that allicin can inhibit different organisms like gram-positive and gram-negative bacteria (Ross et al. 2001; Prajapati et al. 2021).

Sensory characteristics

Sensory characteristics of instant holat spice were evaluated before and after brewing. Evaluation before brewing was carried out to determine consumers' first perceptions of instant holat spices, while testing after brewing was carried out to determine the preference of customers. There is some evidence that the temperature and length of the brewing process affect constituent extraction and the brewing process caused different flavors of green tea (Lee and Chambers 2009). Researchers discovered that the amounts of tannin, free sugar, and total nitrogen in green tea enhanced as the water temperature and brewing time risen in research to evaluate how different brewing changes occur in green tea constituents (Lee et al. 1989). The result of this extraction process causes changes in sensory characteristics. Table 3 showed the sensory characteristics of non-brewed instant holat spice, for which aroma, color, and overall acceptability were not significantly different (P ≥ 0.05). Sensory characteristics did not differ significantly due to low concentration of bark in the formulations, this result was also similar in yogurt studies (Noh et al. 2013; Hekmat et al. 2015).

The highest score of aroma, color, and overall acceptability was found at F1-24 (5.25 ± 1.35), F4-22 (5.38 ± 1.14), and F4-24 (5.58 ± 1.03), respectively, while the lowest were found at F2-20 (4.58 ± 1.17), F1-22 (3.56 ± 1.35), and F1-22 (4.48 ± 1.28). Figure 1 showed that the color and overall characteristics of F4 are the most favored by the panelists, whereas F1 is the least preferred. F4 contained the highest amount of *P. emblica* bark in instant holat spice that may has the most astringent flavor (Madhuri et al. 2011; Vasant et al. 2013). Due to its astringent flavor, holat has its own unique flavor and so-called holat (Damanik and Sinaga 2020; Pulungan et al. 2020). Based on the sensory characteristics evaluation of non-brewed holat instant spice, therefore F4-24 is the most preferred formula by the panelists.

Table 4 reveals that the sensory characteristics of brewed instant holat spice were not significantly different in terms of aroma, color, taste, and overall acceptability (P ≥ 0.05). Figure 2 showed the results of the sensory analysis of the aroma, color, taste, and acceptability of the whole sample of brewed instant holat spice.

Table 3. Sensory characteristics of non-brewed instant holat spice

Formulation-drying time (hours)	Sensory characteristics		
	Aroma	Color	Overall acceptability
F1-20	4.58±1.30^{c}	5.03±0.96^{abc}	5.30±0.85^{ab}
F1-22	4.98±1.10^{abc}	3.56±1.35^{g}	4.48±1.28^{d}
F1-24	5.25±1.35^{a}	3.59±1.29^{g}	4.58±1.28^{d}
F2-20	4.58±1.17^{c}	4.77±0.93^{bcd}	4.88±1.11^{bcd}
F2-22	4.75±0.87^{abc}	4.28±1.12^{de}	4.82±1.10^{bcd}
F2-24	4.65±1.03^{bc}	4.74±1.23^{bcde}	4.91±0.98^{bcd}
F3-20	4.93±1.19^{abc}	4.18±1.10^{ef}	4.82±1.18^{bcd}
F3-22	4.78±1.07^{abc}	4.62±1.07^{cde}	4.91±0.63^{bcd}
F3-24	4.88±1.02^{abc}	4.33±1.15^{de}	4.76±1.00^{bcd}
F4-20	5.20±1.18^{ab}	3.69±1.26^{fg}	4.64±1.14^{cd}
F4-22	4.63±1.13^{bc}	5.38±1.14^{a}	5.21±0.99^{abc}
F4-25	4.90±1.06^{abc}	5.23±0.99^{ab}	5.58±1.03^{a}

Note: The data represent the mean and standard deviation of duplicate samples. Means in the same column with the same letters are not significantly different (p ≥ 0.05)

Table 4. Sensory characteristics of brewed instant holat spice

Formulation-drying time (hours)	Sensory characteristics			
	Aroma	Color	Taste	Overall acceptability
F1-20	4.08±1.21^{b}	4.08±1.42^{bcd}	3.03±1.18^{d}	3.63±1.33^{c}
F1-22	4.73±1.06^{a}	3.45±1.13^{e}	4.15±1.18^{ab}	4.35±0.77^{ab}
F1-24	4.60±1.22^{a}	3.98±1.33^{cde}	4.05±1.38^{abc}	4.18±0.90^{ab}
F2-20	4.78±0.83^{a}	4.28±1.06^{bcd}	4.28±1.28^{a}	4.40±0.96^{ab}
F2-22	4.58±0.90^{a}	3.88±0.85^{de}	4.10±1.14^{ab}	4.28±0.96^{ab}
F2-24	4.83±0.98^{a}	4.58±1.11^{ab}	4.21±0.86^{ab}	4.65±0.95^{a}
F3-20	4.73±0.93^{a}	4.13±0.97^{bcd}	4.26±1.12^{ab}	4.53±1.11^{ab}
F3-22	4.65±1.08^{a}	4.58±0.98^{ab}	4.15±1.14^{ab}	4.55±1.15^{ab}
F3-24	4.75±1.06^{a}	4.48±0.88^{abc}	4.36±1.27^{a}	4.58±1.06^{ab}
F4-20	4.68±1.21^{a}	3.53±1.41^{e}	3.64±1.20^{bc}	4.13±1.11^{ab}
F4-22	4.28±1.13^{ab}	4.15±1.14^{bcd}	3.49±1.21^{cd}	4.03±1.29^{bc}
F4-25	4.63±1.13^{a}	4.85±1.08^{a}	3.64±1.40^{bc}	4.68±1.21^{a}

Note: The data represent the mean and standard deviation of duplicate samples. Means in the same column with the same letters are not significantly different (p ≥ 0.05)

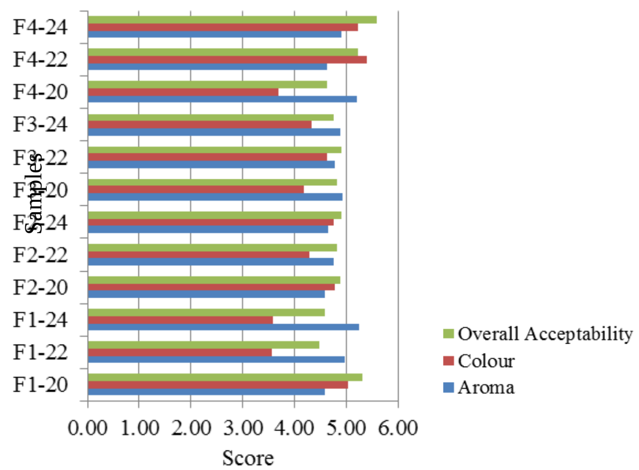

Figure 1. The diagram of the score of sensory characteristics of non-brewed instant holat spice

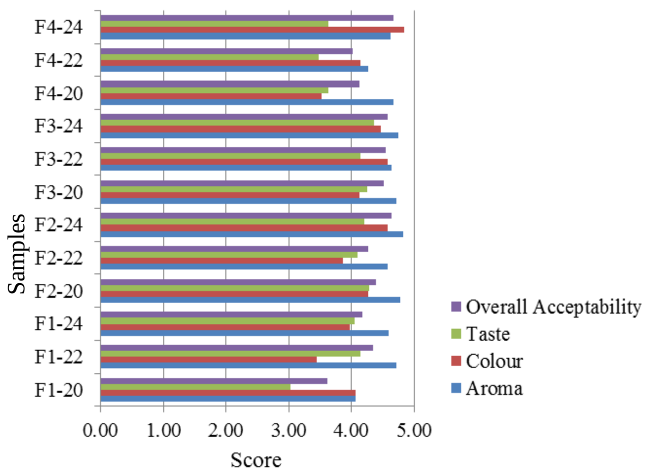

Figure 2. The diagram of the score sensory characteristics of brewed instant holat spice

Figure 3. The product of F4-24 instant holat spice

Samples with high scores of aroma, color, taste, and overall acceptability were F2-24 (4.83 ± 0.98), F4-24 (4.85 ± 1.08), F3-24 (4.36 ± 1.27), and F4-24 (4.68 ± 1.21), respectively, while samples with the low score were F1-20 (4.08 ± 1.21), F1-22 (3.45 ± 1.13), F1-20 (3.03 ± 1.18), and F1-20 (3.63 ± 1.33). In general, F4 which has the highest amount of *P.emblica* bark was the most preferred formula, while F1 was the least preferred. However, the panelists' overall acceptance scores decreased after brewing. F4-24 has a score of 5.58 ± 1.03 before brewing to 4.68 ± 1.21 after brewing. This may be related to the changes of chemical content in instant holat spice after brewing. As in espresso, the higher the brewing temperature and time, the lesser the sensory quality (Batali et al. 2020; Klotz et al. 2020). Based on the average outcomes and the overall sensory analysis, it is concluded that F4-24 (Figure 3) is the most preferred formulation of instant holat spice.

ACKNOWLEDGEMENTS

The study was financially supported by the Research Institute, Universitas Sumatera Utara, Medan, Indonesia.

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