DEVELOPMENT OF CONVENIENT GINGER CUBE: EVALUATION ON BIOACTIVE COMPOUND, ANTIOXIDANT PROPERTIES AND SENSORY ACCEPTANCE

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ABSTRACT

The objective of this research is to develop convenient ginger cube from ginger plant (Zingiber officinale roscoe) with several bioactive compounds that have benefits for health. Ginger has the potential to be applied as a functional food and is considered as the safest herb to be used. The formulation of the cubes was determined by response surface methodology (RSM) method. Ginger powder was mixed together with molasses and icing sugar in a mixing bowl. The mixture then was pressed with a cube maker machine and packed in individual plastic for storage. Antioxidant activity was studied by using total phenolic compound, total flavonoid compound, Radical Scavenging Activity, Ferric Reducing Antioxidant Power and Trolox Equivalent Antioxidant

Capacity. Bioactive compounds (6-gingerol and 6-shogoal) were analysed by using Ultra High-Performance Liquid Chromatography and egg albumin denaturation method was used for anti-inflammatory assay study. Total carotenoid content was measured by using a UV-Vis Double Beam PC Scanning Spectrophotometer at 450 nm wavelength. Microbial test and sensory evaluation also have been studied in order to evaluate the cubes obtained. In spite of all the processing steps carried out on the ginger fiber, results showed that the cubes obtained still have bioactive compounds and the taste can be accepted by sensory panellists.

Keywords: Ginger cube, bioactive compound, antioxidant, total carotenoid content

1. INTRODUCTION

Ginger is a herbaceous tropical perennial plant that comes from the underground stems or rhizomes of *Zingiber officinale* (Rosc.), which belongs to the family Zingiberaceae. In the Zingiberaceae, it belongs to the subfamily *Zingiberoideae*, whose members are aromatic with unbranched aerial stems, open sheaths, distichous leaves, and hypogeal germination (Vasala 2012). Ginger is a tropical plant that thrives in hot and humid environments (Kadam et al. n.d.). It is typically grown annually during cultivation. Ginger is grown all over the world, with India, China, Indonesia, Nepal, Thailand, Nigeria, Bangladesh, Japan, and the Philippines ranking as the top producers. China and India are the two that predominately supply the global market among them (Vasala 2012).

The characteristic of pungency and piquant flavour of ginger rhizome contribute to its widespread uses in beverages and foods. Ginger is known to have anti-inflammatory, antipyretic, anticancer, antiviral, analgesic, anti-helmintic, antioxidant, and antidiabetic properties. 6-gingerol is the most abundant bioactive molecule in ginger, and it has anti-inflammatory, antipyretic, analgesic, and antioxidant properties (Khdary et al. 2023). The US Food and Drug Administration has classified ginger as generally recognised as safe (GRAS) since it has no toxicity. A dose of 0.5-1 g ginger powder taken twice a day for three months to two and a half years had no negative effects (Vasala 2012).

Ginger contains a variety of bioactive phenolics, along with non-volatile pungent substances like shogaols, gingerols, zingerones, and paradols. The ginger rhizome is composed of primarily 60-70% carbohydrates, 9% protein, 3-8% crude fiber, 3-6% fatty oil, 8% ash, and 2-3% volatile oil. The volatile (essential) oils that make up to 3% of fresh ginger's weight, such as zingerone, shogaols, gingerols, and others, are what give ginger its distinctive flavour. The pungency of the fresh ginger rhizome is due to gingerols, of which the major pungent principle is [6]-gingerol (1-[4'-hydroxy-3'-methoxyphenyl]-5-hydroxy-3-decanone), an oily liquid, and the most abundant constituent among the gingerols. The pungency of cooked or dried ginger comes from shogaols which are non-volatile phenylpropanoid-derived compounds from gingerols. The less pungent zingerone which has a spicy-sweet aroma is also produced from gingerols during drying process (Srinivasan 2017). It has also been reported that ginger has anti-inflammatory effects through mechanisms that demonstrate the role of inhibition of pre-inflammatory factors such as prostaglandin and leukotriene biosynthesis, which could decrease pain associated with rheumatoid and osteoarthritic conditions (Kadam et al., n.d.). Consuming fresh ginger showed promising results for reducing pain brought on by arthritis in people (Sultana Sathi 2022).

Processing of ginger cube is an alternative for convenient packaging and lifestyle. Instead of using this ginger cube as drink, it can also be applied as cooking ingredient. It will be easier, economical and less time consuming in preparing drink and food for families.

2. MATERIALS AND METHODS

3.

Cubes consist of 20% ginger powder and 80% sugar (combination of molasses, with or without icing sugar) were prepared by using a cube maker machine. Cubes consist of ginger rhizome powder with skin and root while certain cubes consist of ginger powder with clean rhizome (refer to Table 1). Cubes then were analysed for data of bioactive compounds, antioxidant properties, total phenolic content (TPC), total carotenoid content (TCC), microbial analysis and sensory evaluation test.

Table 1. Samples ratio for ginger cubes

Samples	Clean Ginger Powder (%)	Ginger Powder with Waste (%)
C1	20	0
C2	0	20
C3	20	0
C4	10	10
C5	5	15
C6	20	0
C7	15	5
C8	0	20
C9	10	10

2.1 Bioactive compound

Analysis of bioactive compound in methanol extract was done by using Ultra High Performance Liquid Chromatography (UHPLC, Dionex UltiMate™ 3000 RS) with acetonitrile moving phase solvent and water. Separation of the bioactive compounds was performed using Symmetry® C18 5 µm (4.6 x 250 mm) with the column temperature 25°C and acetonitrile:water (90:10) as the mobile phase with a flow rate of 1.0 mL/min for 30 minutes. Determination of *6-gingerol dan 6-shogaol* was done by using a photo diode array at 228 nm wavelength.

2.2 Antioxidant properties

Samples of ginger cubes underwent extraction with distilled water at a ratio of 24 mg to 10 mL (w/v). Each mixture was placed in a centrifuge tube and vortex for 1 min and agitated at 150 rpm at room temperature in a shaker for 1 h. The mixture was then centrifuged at 8,500 RPM for 10 min using Biofuge Primo (Heraeus). The supernatant was filtered through Whatman No.541 filter paper (Merck) to obtain a clear extract. The filtrates were assayed for their total phenolic content (TPC) and antioxidant activity assay (AOA). All experiments were run in triplicate.

Total phenolic in all samples was determined with Folin–Ciocalteau assay (Singleton and Lamuela-Raventos, 1999) by using gallic acid as a standard phenolic compound. The reaction mixture was kept in dark for 2 h and its absorbance was measured at 765 nm against distilled water as a blank solution using the microplate reader. The TPC was expressed as mg gallic acid equivalents (mg GAE/g dry weight), which was determined from known concentrations of gallic acid standard. Data were reported as a mean ± standard deviation for three replications.

Determination of free radical scavenging activity was done according to this method. The hydrogen atom or electron donation ability of the corresponding extracts and some pure compounds was measured from the bleaching of purple coloured methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). This spectrophotometric assay uses stable radical DPPH as a reagent, according to a slightly modified method of Lu and Yeap Foo (2000). After a 40 min incubation period at room temperature, the absorbance was read against a blank at 517 nm using microplate reader. The percentage of inhibition of free radical DPPH by the extracts was calculated as follow:

$$\text{Inhibition (\%)} = (A_{\text{blank}} - A_{\text{sample}} / A_{\text{blank}}) \times 100$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound.

2.2.1 Extraction of samples

Samples of ginger cubes underwent extraction with distilled water at a ratio of 24 mg to 10 mL (w/v). Each mixture was placed in a centrifuge tube and vortex for 1 min and agitated at 150 rpm at room temperature in a shaker for 1 h. The mixture was then centrifuged at 8,500 RPM for 10 min using Biofuge Primo (Heraeus). The supernatant was filtered through Whatman No.541 filter paper (Merck) to obtain a clear extract. The filtrates were assayed for their total phenolic content (TPC) and antioxidant activity assay (AOA), as described below. All experiments were run in triplicate.

2.2.2 Determination of total phenolic content (TPC)

Total phenolic in all samples was determined with Folin–Ciocalteau assay (Singleton and Lamuela-Raventos, 1999) by using gallic acid as a standard phenolic compound. Fresh weight of each sample was converted into dry weights on the basis of the moisture content. Briefly, 1 g of sample was dissolved in 20 mL of distilled water, then 50 µL of appropriately extracts solutions and standard gallic acid solutions (12.5, 25.0, 50, 100, and 200 µg/mL) were mixed with 50 µL of distilled water in a well of 96-well plate, then 100 µL of Folin-Ciocalteau reagent solution (prediluted 10-fold with distilled water) was added. After 6 min, 100 µL of 7.5% (w/v) Na_2CO_3 was added and mixed gently. The reaction mixture was kept in dark for 2 h and its absorbance was measured at 765 nm against distilled water as a blank solution using the microplate reader. The TPC was expressed as mg gallic acid equivalents (mg GAE/g dry weight), which was determined from known concentrations of gallic acid standard. Data were reported as a mean ± standard deviation for three replications.

2.2.3 Determination of Antioxidant Activity

2.2.3.1 Determination of free radical scavenging activity

The hydrogen atom or electron donation ability of the corresponding extracts and some pure compounds was measured from the bleaching of purple coloured methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). This spectrophotometric assay uses stable radical DPPH as a reagent, according to a slightly modified method of Lu and Yeap Foo (2000). 100 µL of the extracts was added to 200 µL of a 0.007% methanol solution of DPPH. After a 40 min incubation period at room temperature, the absorbance was read against a blank at 517 nm using microplate reader. The percentage of inhibition of free radical DPPH by the extracts was calculated as follow:

$$\text{Inhibition (\%)} = (A_{\text{blank}} - A_{\text{sample}} / A_{\text{blank}}) \times 100$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound.

2.2.3.2 FRAP assay

The ferric reducing antioxidant power (FRAP) assay was performed as previously described by Benzie and Strain (1996). This method was developed to measure the ferric reduction ability of plasma at a low pH. When the ferric 2,4,6-tripyridyl-s-triazine complex (Fe^{3+}-TPTZ) is reduced to the ferrous form (Fe^{2+}-TPTZ), an intense blue colour is developed. Briefly, The FRAP reagent

was prepared by mixing 10 volumes of 300 mM acetate buffer (pH 3.6), with one volume of 10 mM TPTZ in 40 mM HCl and with one volume of 20 mM $FeCl_3 \cdot 6H_2O$ and the mixture then incubated at 37 °C for at least 10 min. A total of 20 µL of extract solution and 80 µL of distilled water were added to 200 µL of freshly prepared FRAP reagent in a well of 96-well plate. After four min, the absorbance was read using a microplate reader at 593 nm against reagent blank, which was prepared by the same procedure described above except that extract solution was substituted by 20 µL of water. The FRAP value was calculated and expressed as miligram of trolox equivalents per 100 g of dry weight sample (mg TE /100 g dw) based on a calibration curve plotted using trolox as a standard at a concentration ranging from 12.5 to 200 µg/mL.

2.2.4 Spectrophotometric measurements

All spectrophotometric measurements were taken using BIOTEK GEN5 Eon Microplate Spectrophotometer (Winooski, Vermont, USA) in a 96 well plate.

2.3 Total carotenoid content

Carotenoid was extracted according to Rojsanga et al. (2014). Samples were inserted in quartz cuvettes and absorption was measured by using a Spectro UV-Vis Double Beam PC Scanning Spectrophotometer (Model UVD-2950) at 450 nm of wavelength. Each sample was extracted (n = 3) as described earlier. The absorption of each extract was performed at 454 nm in triplicate. Total carotenoid content was calculated based on the calibration curve and expressed as mg β-carotene equivalent (BE) in 100 g sample.

2.4 Microbial analysis

The test was carried out for both cube form and drink infusion. Sample for drink infusion was prepared by diluting the cube in 100ml of > 95°C water for 2 minutes. Samples (10 g for solid and 10 ml for liquid) were aseptically weighed/measured and homogenized in 90 ml Ringer's solution (Oxoid, Hampshire, England) using a paddle blender, Stomachers (Seward Model 400, London, UK) for 30 seconds. Serial dilution up to 5 times was carried out using the same diluent.1 ml of each dilution were inoculated into petri dish before mixing it with selected media using pour plate method. Media used in this study were plate count agar (PCA; Oxoid, Hants, UK) for enumeration of total mesophilic aerobic bacteria and potatoes dextrose agar (PDA; Oxoid, Hants, UK) for yeast and mould enumeration. The inoculum was evenly mixed by gently swirling the plate. The agar containing inoculum were left to thoroughly solidify before inverting the plate for incubation. Media used in this study were plate count agar (PCA; Oxoid, Hants, UK) for enumeration of total mesophilic aerobic bacteria and potatoes dextrose agar (PDA; Oxoid, Hants, UK) for yeast and mould enumeration. The enumeration of coliform and Escherichia coli (E.coli) was carried out using compact dry EC (Nissui, Pharmaceutical, Japan). Microbial colonies were counted after 24 h incubation at 37 ± 1ºC for both PCA and Compact Dry EC (ICMSF, 1978) while PDA was incubated at 32 ± 1ºC for 48 h. All microbial count results were expressed in colony-forming units CFU/g.

2.5 Sensory evaluation

Nine sets of coded samples of ginger cubes infusion were served to selected 40 semi-trained panellists. The infusion was prepared by diluting the cube in 250ml of boiling water (> 95°C) for 2 minutes. A sheet of paper with the coded number of the samples was given to the panellist to evaluate their colour, aroma, taste and overall acceptance. They were asked to score their liking according to the 7-point hedonic scale (1=dislike very much, 2=dislike moderately, 3=dislike, 4=neither like nor dislike, 5=like, 6=like moderately, 7=like very much).

4. RESULTS AND DISCUSSION

5.

Cubes (C1, C3 and C6) consist of 20% clean ginger rhizome powder while C2 and C8 were cubes with ginger powder consist of skin and root. Cubes (C4, C5, C7 and C9) were made by combination of clean ginger rhizome powder and ginger powder contained skin and root. Table 2 shows the results of bioactive compound in ginger cubes. Compound of 6-Gingerol and 6-Shogoal still can be detected in these cubes by using UHPLC. From the result obtained, C2 has given higher reading of 6-Gingerol and 6-Shogoal compound compared to other cubes. This showed that ginger skin and root contain more 6-Gingerol and 6-Shogoal compound compared to clean rhizomes.

Table 2. Results of bioactive compounds in ginger cubes

Samples	6-Gingerol (mg/g dw)	6-Shogaol (mg/g dw)
C1	1.85	0.27
C2	2.16	0.31
C3	1.57	0.24
C4	1.60	0.24
C5	1.45	0.24
C6	1.58	0.25
C7	2.03	0.27
C8	2.07	0.31
C9	1.84	0.27

Table 3 showed the results of moisture content, total phenolic content, total carotenoid content and antioxidant properties for ginger cubes that have been developed. C5 has given the lowest value of total phenolic content and C7 has the highest carotenoid compound compared to other cubes. Darker tone in ginger skin and root has contributed to the higher amount of carotenoid in C1, C7 and C8. To determine the antioxidant activity of ginger samples, the development of their scavenging effect of free radicals on the 2.2-diphenil-1-picrylhydrazyl (DPPH) and FRAP were investigated. Comparison of the mean values of antioxidant activities showed that all the cubes in aqueous extracts have high antioxidant activity.

Table 3. Results of moisture content, total phenolic content, total carotenoid content and antioxidant properties for ginger cubes

Samples	MC (%)	TPC	TCC	DPPH	FRAP
C1	4.31^{bc}	5.16^{b}	0.97^{ab}	39.44^{f}	21.51^{b}
C2	4.11^{c}	4.43^{f}	1.05^{a}	50.48^{c}	18.59^{e}
C3	4.48^{ab}	4.76^{d}	0.99^{ab}	55.57^{a}	20.62^{c}
C4	4.39^{ab}	4.67^{e}	0.91^{bcd}	52.21^{b}	18.58^{e}
C5	4.44^{ab}	3.80^{h}	0.74^{e}	55.34^{a}	17.59^{f}
C6	4.62^{a}	4.69^{e}	0.62^{f}	55.26^{a}	20.47^{c}
C7	4.50^{ab}	6.26^{a}	0.83^{d}	40.26^{e}	23.62^{a}
C8	4.36^{abc}	5.00^{a}	0.95^{bc}	40.49^{e}	23.78^{a}
C9	4.61^{a}	3.97^{g}	0.87^{cd}	47.10^{d}	19.49^{d}

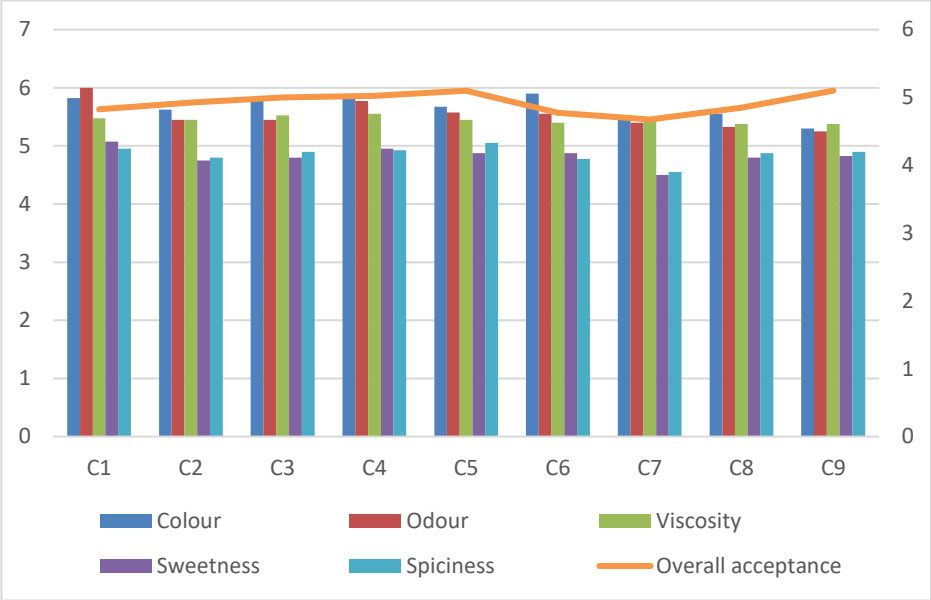
Result (Table 4) showed that both cubes were above the acceptable limits for total viable count and total coliform. Recommended Microbial Limits for Botanical Ingredients, United States Pharmacopeial Convention, USP-NF 35-30 (2012) indicating that maximum limit for Total Viable Count should not exceed 1.0×10^5 cfu/g. and total coliform must be lower than 1.0×10^3 cfu/g. Heat treatment (as stated in methodology) had managed to reduce the count to an acceptable level. The infusion prepared (with temperature >95°C) had lower the count. Meanwhile, for yeast and mould, no growth was detected in this analysis.

Table 4. Results of microbial analysis for ginger cubes

Samples	Total Viable Count	Yeast and Mould	Total Coliform
C1	3.00×10^7	<1.00 x 10	3.54×10^5
C2	1.68×10^6	<1.00 x 10	1.34×10^5
Infusion	7.50×10^3	<1.00 x 10	<1.00 x 10

Figure 1 showed the results for sensory analysis that has been done in this study. Nine sets of coded samples of ginger cubes infusion were served to selected 40 semi-trained panellists. Results showed that the infusion prepared has given overall scores more than 4.7 in 7 hedonic scale. This showed that the taste of these ginger cubes can be accepted by consumers. There is also no significant different between cubes consist of ginger rhizome powder with skin and root and cubes which consist of ginger powder with clean rhizome.

Figure 1. Sensory evaluation of ginger cubes



4. CONCLUSION

Despite all of the processing done, the ginger cubes obtained in this study still have bioactive compounds and maintain its antioxidant activities with an acceptable taste by the sensory panellists. In terms of texture, flavour and taste, panellists also cannot differentiate between clean rhizomes ginger powder and powder with ginger waste.

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