Food Research 7 (6) : 35 - 47 (December 2023)
Journal homepage: https://www.myfoodresearch.com

FOOD RESEARCH

RESEARCH PAPER

Application of antioxidant peptides derived from goat milk in ginger coffee formulation

*Lestari, D., Sari, J.P. and Pramudito, T.E.

Food Technology Study Program, Faculty of Biotechnology, Atma Jaya Catholic University of Indonesia, Tangerang, Banten 15345, Indonesia

Article history:
Received: 21 December 2021
Received in revised form: 22 January 2022
Accepted: 5 July 2022
Available Online: 29 November 2023

Keywords:
Antioxidant activity,
Ginger coffee,
Goat milk,
Hydrolysis,
Peptide,
Whey protein

DOI:
https://doi.org/10.26656/fr.2017.7(6).1016

Abstract

Peptides can be obtained from the degradation of goat milk protein by enzymatic hydrolysis method using food-grade papain enzyme. These peptides have antioxidant activity that can be applied to beverage formulations. This study aimed to characterize antioxidative peptides from whey and goat milk and to develop a ginger coffee drink formulation with antioxidant peptides. Protein hydrolysis was performed for 0, 15, and 30 mins and the highest degree of hydrolysis was obtained from protein samples which were hydrolyzed for 15 mins. The whey hydrolysate 15 mins had a protein concentration of 0.54±0.07 mg/mL and one protein band of 17 kDa. Goat milk peptides had a protein concentration of 1.71±0.00 mg/mL and one protein band of 15 kDa. This sample also has the highest antioxidant activity, where Whey peptides had an antioxidant activity of 79.98±0.86% while goat's milk peptides 94.97±0.44%. These two peptides were applied to ginger coffee drink formulation. The formulations with the highest antioxidant activity were ginger coffee with goat milk and whey peptides in a ratio of 4:1 (90.15±1.02%) and ginger coffee with goat milk (85.23±1.15%). Based on results from the organoleptic test, these two formulations were also acceptable to panellists in terms of taste, overall properties, and interest.

1. Introduction

Bioactive peptides are protein fragments that have certain health properties and generally consist of 2 to 20 amino acids (Sarmadi and Ismail, 2010). These peptides are obtained by breaking the peptide bonds present in proteins. The cleavage of this peptide bond can be done through enzymatic hydrolysis (Ahmed *et al.,* 2015). In the enzymatic hydrolysis process, one of the most widely used enzymes is papain. Papain is a proteolytic enzyme that can be found in papaya. This enzyme is sold commercially so it is easier to obtain. Papain also has a higher heat resistance compared to other enzymes (Cohen *et al*., 1986; Amri and Mamboya, 2012).

Proteins that can be used as a source for bioactive peptides are milk protein. Milk protein consists of 80% casein and 20% whey. Casein protein is divided into α-casein, β-casein, and κ-casein, while whey protein is divided into immunoglobulins, lactoferrin, and other peptide fractions (Borkova and Snaselova, 2005). Goat milk is starting to be widely used as a source of bioactive peptides. This is because goat milk is widely consumed as a substitute for cow milk.

Bioactive peptides derived from goat milk protein have many potential health benefits. Through several studies that have been carried out, it is known that bioactive peptides from goat milk have potential health benefits. These benefits include antioxidant properties, antithrombotic properties, antihypertensive properties, and immunomodulatory effects of peptides (Atanasova and Ivanova, 2010). Amino acids in bioactive peptides derived from goat milk protein are claimed to be more effectively absorbed into the body compared to amino acids of bioactive peptides derived from cow milk (Ahmed *et al.,* 2015). These bioactive peptides from goat milk could be utilized further by formulating them into beneficial drinks. In this study, we focused on the antioxidant properties of bioactive peptides.

Coffee is a beverage product that is widely consumed. Coffee consumption in Indonesia has increased since 2016. In 2020, national coffee consumption is estimated to reach 294,000 tons, or an increase of about 13.9% compared to 2019 and is expected to continue to rise in the following years (Global Agricultural Information Network (GAIN), 2019). This increase in coffee consumption is closely

*Corresponding author.
Email: *diana.lestari@atmajaya.ac.id*

eISSN: 2550-2166
Published by Rynnye Lyan Resources

related to the growth of coffee shop businesses in Indonesia (GAIN 2019). Coffee has many flavours one of which is ginger-flavoured coffee. Ginger is a spice that is easily found in Indonesia and is starting to be widely used in combination with food or drinks. Ginger has many benefits, such as anti-inflammatory effects and antioxidant properties (Mosovska *et al.,* 2015).

The addition of more ginger will increase the ginger coffee antioxidant, but too much ginger can affect the sensory. It is necessary to add other sources of antioxidants to maintain/increase the value. Therefore, this study aimed to characterize antioxidant peptides derived from whey protein and goat milk protein and to develop a functional ginger coffee drink formulation with the addition of antioxidant peptides. In addition, this study was also conducted to find out information on nutritional value, the shelf life of beverages, as well as consumer acceptance and interest in ginger coffee products with bioactive peptides.

2. Materials and methods

2.1 Preparation and isolation of goat milk protein

Milk protein isolation was carried out based on Yoshida *et al*. (2000) and Ahmed *et al.* (2015). Fresh goat milk was centrifuged at 2000×*g* at 4°C for 30 mins. After centrifugation, a layer of fat will form on top of the milk, and then this layer of fat is removed. After that, the milk was pasteurized at 72°C for 15 s. Pasteurized milk can be stored at 4°C for hydrolysis. To isolate whey protein, milk that has been skimmed is added with 5% acetic acid until the pH of the milk reaches 4.6. Then the milk was centrifuged again at 7,100×*g* for 30 mins. Whey protein will be in the supernatant. The results of this isolation were stored at -4°C for further testing.

2.2 Whey protein dialysis

Whey protein was dialyzed with a 12 kDa MWCO membrane to separate protein from the acid solution according to the method of Virgen-ortiz *et al.* (2012). Some whey isolates were dialyzed while others were for electrophoresis profile. Dialysis was carried out by immersing the dialysis membrane in distilled water (the ratio of whey protein and distilled water was 20:250 mL) and stored overnight at 4°C.

2.3 Papain activity analysis

Analysis of papain activity was based on the method of Anson (1938). Papain enzyme was the product of Nanning Pangbo Biological Engineering Co., Ltd (China). This enzyme was mixed with 0.05 M phosphate buffer pH 7 and then vortexed for 3 mins. After that, 0.65% (w/v) whey protein was dissolved in 0.05 M phosphate buffer pH 7. Approximately 800 µL of whey protein which had been mixed with phosphate buffer was put into the sample and blank vial. In the sample vial, papain which had been mixed with 200 µL of buffer was added, while 200 µL of distilled water was added to the blank vial. The mixture, both sample and blank, was vortexed and then incubated at 37°C for 10 mins. To stop the reaction, 500 µL of cold TCA was added to each vial. After adding TCA, 200 µL of distilled water was added to the sample vial, while 200 µL of papain was added to the blank vial, then vortexed and incubated at room temperature for 30 mins.

The incubated mixture was then centrifuged at 14,500 x *g* for 10 mins. A total of 400 µL of the supernatant obtained after centrifugation was transferred to a new vial, then 1 mL of 0.4 M Na_2CO_3 was added and vortexed. After that, 200 µL of Folin-Ciocalteu reagent was added to each vial and then vortexed again. The mixture was incubated at 37°C for 30 mins and then centrifuged again at 14,500×*g* for 10 mins. The supernatant formed was measured for absorbance at 660 nm. To determine papain activity, a standard tyrosine curve is needed with tyrosine concentrations of 50 µg/mL, 100 µg/mL, 150 µg/mL, 200 µg/mL, and 250 µg/mL. The enzyme activity was determined using this formula:

$$UA = \frac{[\text{Tirosin}]}{\text{V enzim}} \times \frac{1}{P} \times \frac{1}{T}$$

$$AS = \frac{UA}{[\text{Protein}]}$$

Where UA: amount of tyrosine produced /mL enzyme /min (U/mL), AS: specific enzyme activity (U/mg), [Tyrosine]: tyrosine concentration (µmol), V enzyme: volume of used enzyme (mL), P: dilution factor (volume of supernatant / total volume of reaction), T: incubation time (10 mins), and [Protein]: protein concentration (mg/mL)

2.4 Hydrolysis of goat milk and whey protein

Hydrolysis was done based on the method described by Ahmed *et al*. (2015). There were 2 samples to be hydrolyzed: whey dialyzed protein and goat milk. This hydrolysis process was carried out using food-grade papain enzyme. Papain was added to distilled water with a ratio of 1:5 (w/v). After that, the enzyme was added to the sample with a ratio of 1:200 (v/v). Hydrolysis reaction was carried out at 37°C for 0, 15, and 30 mins with pH 7. Hydrolysis was stopped by heating the samples at 80°C for 15 mins and then centrifuging 2000 ×*g* for 5 mins, the resulting supernatants were used for further testing.

https://doi.org/10.26656/fr.2017.7(6).1016

2.5 Determination of the degree of hydrolysis

The degree of hydrolysis is determined by the concentration of protein present in the sample before and after hydrolysis. Based on the method described by Lestari and Suyata (2020), the Lowry method was used to calculate the protein concentration (Lowry *et al*., 1951). A 1.2 mL of samples (goat milk, whey protein, whey hydrolysate, and goat milk hydrolysate) were added with 6 mL of Biuret reagent (consisting of copper sulfate, sodium hydroxide, sodium potassium tartrate). The mixture was vortexed and incubated for 10 mins at room temperature. After incubation, the mixture was added with a 300 µL Folin-Ciocalteu reagent and vortexed. The mixture was incubated for 30 mins at room temperature and absorbance was measured at 650 nm. The degree of hydrolysis was determined using this formula:

$$\text{Degree of hydrolysis} = \frac{[\text{Initial protein}] - [\text{Final protein}]}{[\text{Initial protein}]} \times 100\%$$

Where [Initial protein]: protein concentration before hydrolysis (mg/mL) and [Final protein]: protein concentration after hydrolysis (mg/mL)

2.6 Protein profile analysis

Analysis of the protein profile was conducted based on the sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS PAGE) method described by Laemmli (1970) and modified by Singh *et al*. (2011). The analyzed samples consisted of goat milk, whey protein, goat milk hydrolysate, and whey hydrolysate. Samples were added with 5% SDS in a ratio of 1:5 (v/v) and heated at 85°C for 1 hr. The heated mixture was centrifuged at 2000×*g* for 5 mins and the supernatant was collected and added to the sample buffer (consisted of 6% Tris-HCl 1 M pH 6.8; 50% glycerol 50%; 20% SDS 10%; 5% β-mercaptoethanol; 10% bromophenol blue 1%; and 9% distilled water) in a ratio of 1:1. After that, the mixture was heated for 2 mins in boiling water.

SDS-PAGE gel consisted of 4% stacking gel and 20% separation gel. A total of 18 µL of whey hydrolysate and 15 µL of whey protein were inserted into the SDS-PAGE gel well. For goat milk, a total of 8 µL was added and 10 µL of goat milk hydrolysate was added. The Low Molecular Weight (LMW) marker (PageRuler™, Thermo Scientific, USA) was used as a standard for protein molecular weight. Electrophoresis was carried out at 70 Volts for 3 hrs. The gel was stained for 15 mins with a dye solution consisting of 0.1% Coomassie Brilliant Blue R-250 (Merck, Germany), 45% methanol, 44.9% distilled water, and 10% glacial acetic acid. After that, the dye was removed with a destaining solution consisting of 10% methanol, 10% glacial acetic acid, and 80% distilled water.

2.7 Antioxidant activity and IC_{50} analysis

Antioxidant activity analysis was carried out using DPPH (1,1-diphenyl-2-picrylhydrazyl) (Smart-Lab, Indonesia) referring to the method of Zhang *et al.* (2013) and Umayaparvathi *et al.* (2014). About 0.1 mM DPPH solution in ethanol was prepared. After that, 600 µL of the peptide from each treatment was added with 600 µL of DPPH and vortexed. After being vortexed, the solution was incubated for 30 mins in a dark room. For blank preparation, the peptide was substituted with ethanol. The absorbance was measured at 517 nm, then the antioxidant activity could be calculated using the formula:

$$\text{Antioxidant Activity (\%)} = \frac{\text{Blank absorbance} - \text{Sample absorbance}}{\text{Blank absorbance}} \times 100\%$$

The IC_{50} value of the peptide with the highest antioxidant activity was determined by freeze-drying each sample. The calculation of the IC_{50} value was based on Abubakr *et al.* (2013). The dried samples were dissolved in distilled water at various concentrations (100 – 600 µg/mL). Then each sample concentration was measured for its antioxidant activity. The antioxidant activity was plotted in a graph, and then the regression equation (y = ax + b) on the graph was used to calculate the IC_{50} value. Furthermore, the peptide with the highest antioxidant activity was added to the ginger coffee formulation. After that, the antioxidant activity of each product was measured.

2.8 Peptide fractionation with membrane filter

Peptide samples with the highest antioxidant activity were filtered using a filter membrane. This peptide fractionation method refers to Chang *et al*. (2013). The membranes used are 30 kDa (Sartorius, UK) and 10 kDa cut-off (Amicon®, Irlandia). The peptides were filtered using a 30 kDa cut-off membrane then peptides that could pass through the membrane were filtered again using a 10 kDa cut-off membrane. After that, peptides with sizes larger than 30 kDa, between 30 - 10 kDa, and smaller than 10 kDa were measured for their antioxidant activity. The filtered peptides were also analyzed by SDS -PAGE electrophoresis.

2.9 Formulation of ginger coffee

A total of 100 g of ginger was cleaned and then cut into medium-sized pieces. After that, 600 mL of water was added and then heated. The ginger water was boiled for 15 mins. Next, 20 g of coffee, 3 g of creamer, and 60 g of sugar were brewed with 300 mL of ginger water. As for the control formulation, this coffee was added with 200 mL skim goat milk. The coffee used in this formulation was instant coffee derived from robusta coffee beans. After the ginger coffee temperature

https://doi.org/10.26656/fr.2017.7(6).1016

reached 50°C, peptides were added according to the formulation (Table 1).

Table 1. Ginger coffee formulation for 500 mL.

Ingredients (mL)	Samples					
	C	W	GM	GMW1	GMW2	GMW3
Ginger Coffee	300	300	300	300	300	300
Goat milk peptide	-	-	200	160	100	40
Whey peptide	-	200	-	40	100	160
Goat milk	200	-	-	-	-	-

C: control only using goat milk, W: with whey peptide, GM: with goat milk peptides, GMW1: with goat milk and whey peptides in a ratio of 4:1, GMW2: with goat milk and whey peptides in a ratio of 1:1, GMW3: with goat milk and whey peptides in a ratio of 1:4.

2.10 Organoleptic test of ginger coffee product

The hedonic test was carried out based on Li *et al.* (2015) with 35 untrained panellists. Panellists were asked to rate the taste, aroma, colour, and overall attributes of the ginger coffee formulation. They could rate the products on a scale of 1 to 5. A scale of 1 indicates dislike very much, a scale of 2 indicates dislike slightly, a scale of 3 indicates like slightly, a scale of 4 indicates like, and a scale of 5 indicates like very much.

An interesting test was conducted based on Rebollar *et al.* (2012). A total of thirty-five panellists were asked to rate how much they wanted to buy the product. They could rate the sample on a scale of 1 to 5. A scale of 1 indicates not being interested, while a scale of 5 indicates very interested.

2.11 Shelf life analysis

The shelf life analysis was carried out based on the method of Nicoli *et al.* (2009). The most favoured ginger coffee products were stored at 4°C. These samples were kept for 12 days and changes that occurred in sensory attributes were observed on days 0, 1, 2, 3, 4, 7, 8, 9, 10, and 11. The observed attributes included colour (using a colorimeter), aroma, appearance, and pH in each sample.

2.12 Proximate analysis

Proximate analysis was carried out by sending the samples to PT. Saraswanti Indogenetech. The proximate analysis included tests for water content, protein content, total fat content, ash content, total calories from fat, total calories, and carbohydrate content. The method used for water content analysis is based on SNI 8773:2019 appendix A.3, for protein content the method used is Kjeldahl, for fat content the method used is Weibull-Stoldt, a muffle furnace is used to determine the ash content, and for carbohydrate content, the method used is based on 18-8-9/MU/SMM-SIG. The total calories and calories from fat were obtained by calculation. In this analysis, the samples were the most favoured ginger coffee. The results of this proximate analysis were used to arrange a table of the nutritional value of ginger coffee products.

2.13 Statistics analysis

All analyses were conducted at least in triplicate. After that, all of the data were analyzed using Statistical Package for the Social Sciences (SPSS) software. The statistical analysis used was one-way ANOVA and Duncan's multiple range test at a significant value of 0.05.

3. Results

3.1 Enzyme activity

Based on the enzyme activity test results, the papain enzyme can hydrolyze whey protein. The papain enzyme used in this research had an activity of 32.94 U/mL, while its specific activity was 1,049.08 U/mg. These results can be obtained from the calculation formulas listed in the previous chapter.

3.2 Degree of hydrolysis

The degree of hydrolysis was determined by calculating the protein concentration before and after the samples were hydrolyzed. Determination of protein concentration was carried out by the Lowry method. Based on the data in Table 2, the whey protein sample had the largest protein concentration and was significantly different ($P<0.05$) compared to whey hydrolysate samples. The three whey hydrolysate samples also had significantly different protein concentrations and degrees of hydrolysis ($P<0.05$). The

Table 2. Degree of hydrolysis of whey and goat milk hydrolysate

Sample	Protein concentration (mg/mL)	Degree of hydrolysis (%)
Whey protein	2.35 ± 0.04^{a}	-
Whey hydrolysate 0 min	1.21 ± 0.02^{b}	48.31 ± 0.65^{c}
Whey hydrolysate 15 mins	0.54 ± 0.07^{d}	77.02 ± 2.80^{a}
Whey hydrolysate 30 mins	0.68 ± 0.02^{c}	71.14 ± 0.72^{b}
Goat milk	5.17 ± 1.56^{a}	-
Milk hydrolysate 0 mins	2.51 ± 0.11^{b}	51.42 ± 2.15^{b}
Milk hydrolysate 15 mins	1.71 ± 0.00^{c}	66.86 ± 0.07^{a}
Milk hydrolysate 30 mins	1.73 ± 0.14^{c}	66.47 ± 2.75^{a}

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different ($P<0.05$).

https://doi.org/10.26656/fr.2017.7(6).1016

largest degree of hydrolysis was in the 15 mins whey hydrolysate sample. This sample also had the lowest protein concentration.

In goat milk samples, the highest protein concentration was goat milk without hydrolysis. On the other hand, samples that had the lowest protein concentration were the 15 and 30 mins milk hydrolysate samples. The two samples are not significantly different (P>0.05). The degree of hydrolysis of the two samples is also not significantly different. However, the 15 mins milk hydrolysate had a slightly higher degree of hydrolysis than the 30 mins milk hydrolysate.

3.3 Protein profile from whey and goat milk

Protein profile analysis for the whey sample can be seen in Figure 1. Lane 1 is a sample of non-dialyzed whey protein. This sample had 4 bands with sizes of 13 kDa, 16 kDa, 28 kDa, and 64 kDa. On the other hand, lane 2 is a sample of dialysis whey protein. This sample had three bands with a molecular weight of 13 kDa, 16 kDa, 28 kDa, and 63 kDa, which is similar to the non-dialyzed whey protein. After hydrolysis, there was a reduction in the band of the samples. Samples of whey dialysis hydrolysate 0 mins, 15 mins, and 30 mins (lanes 6-8) only had one band with the same molecular weight, which is 17 kDa.

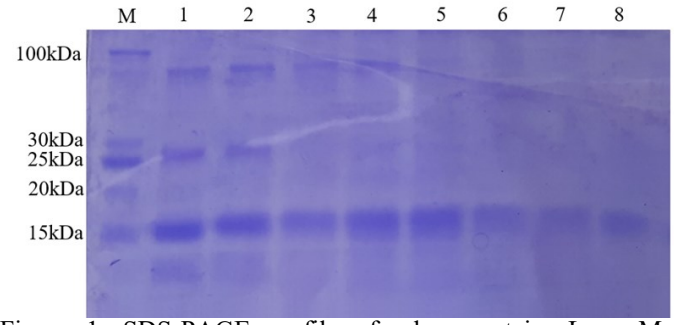

Figure 1. SDS-PAGE profile of whey protein. Lane M: Marker, Lane 1: whey isolate, Lane 2: whey dialysis isolate, Lane 3-5: whey hydrolyzed 0, 15 and 30 mins respectively, Lane 6—8: whey dialysis hydrolyzed 0, 15 and 30 mins respectively.

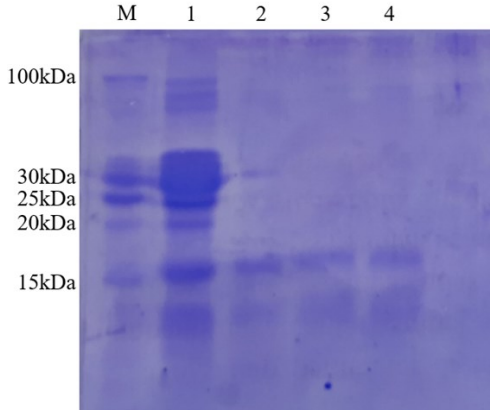

Figure 2. SDS-PAGE profile of goat milk. Lane M: Marker, Lane 1: goat milk, Lane 2-4: goat milk hydrolyzed 0, 15 and 30 mins respectively.

In the protein profile for the goat milk sample (Figure 2), non-hydrolyzed goat milk (lane 1) had nine bands with sizes of 15 kDa, 19 kDa, 25 kDa, 31 kDa, 36 kDa, 41 kDa, 80 kDa, 88 kDa, and 103 kDa. The goat milk sample had more bands than the whey protein sample because goat milk also contained casein. However, these bands were reduced after the goat milk was hydrolyzed. Goat milk hydrolysate 0 min (lane 2) had two bands with a size of 15 kDa and 30 kDa. While goat milk hydrolyzate for 15 and 30 mins, each only had one band with a size of 15 kDa.

3.4 Antioxidant activity

The antioxidant activity of the whey and goat milk hydrolysate samples can be seen in Table 3. The highest antioxidant activity was found in the 15 mins hydrolysate sample, both for whey and goat milk. The antioxidant activity of the 15 mins hydrolysate was significantly different from that of the 0 and 30 mins hydrolysate (P<0.05). In the whey sample, the lowest antioxidant activity was the 0 min hydrolysate sample. In the case of the goat milk sample, the lowest antioxidant activity was in the 30 mins hydrolysate. Between the two samples, goat milk hydrolysate had higher antioxidant activity than whey hydrolysate.

Table 3. Antioxidant activity of whey and goat milk peptide

Sample	Antioxidant activity (%)
Whey hydrolysate 0 min	76.60±0.77^{c}
Whey hydrolysate 15 mins	79.98±0.86^{a}
Whey hydrolysate 30 mins	78.13±0.79^{b}
Milk hydrolysate 0 mins	92.63±1.18^{b}
Milk hydrolysate 15 mins	94.97±0.44^{a}
Milk hydrolysate 30 mins	88.95±0.57^{c}

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different (P<0.05).

The hydrolysate sample with the highest antioxidant activity was used as a sample for calculating the IC_{50} value. For whey samples, the hydrolysate used was 15 mins hydrolysate with a concentration variation of 100 - 600 µg/mL. The increase in sample concentration also increases its antioxidant activity. The highest antioxidant activity was at 600 µg/mL then the lowest antioxidant activity was at 100 µg/mL. The IC_{50} value of the whey hydrolysate 15 mins is 525.81 µg/mL. Similar to the whey hydrolysate sample, the 15 mins goat milk hydrolysate was also varied in concentration from 100 to 600 µg/mL. The concentration of 600 µg/mL had the highest antioxidant activity and the lowest was in the sample with a concentration of 100 µg/mL. The IC_{50} value of the goat milk hydrolysate 15 mins is 105.98 µg/mL.

https://doi.org/10.26656/fr.2017.7(6).1016

Table 4. Antioxidant activity of ginger coffee product containing peptides.

Sample	Formulation (mL)				Antioxidant activity (%)
	Ginger coffee	Goat milk	Whey peptide (15 mins hydrolysate)	Goat milk peptide (15 mins hydrolysate)	
Control	300	200	-	-	15.87±1.57[f]
W	300	-	200	-	78.42±0.95[e]
GM	300	-	-	200	85.23±1.15[b]
GMW 1	300	-	40	160	90.15±1.02[a]
GMW 2	300	-	100	100	81.10±0.95[c]
GMW 3	300	-	160	40	79.87±0.99[d]

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different (P<0.05). C: control only using goat milk (no peptide added), GM: with goat milk peptides (15 mins hydrolysate), W: with whey peptide (15 mins hydrolysate), GMW1: with goat milk and whey peptides in a ratio of 4:1, GMW2: with goat milk and whey peptides in a ratio of 1:1, GMW3: with goat milk and whey peptides in a ratio of 1:4.

Other than the antioxidant activity of the hydrolysate, the antioxidant activity of the ginger coffee formula was also obtained (Table 4). Overall, each formulation of ginger coffee had an antioxidant activity that was significantly different from the others (P<0.05). The GMW1 formulation had the highest antioxidant activity, followed by the GM formulation. On the contrary, ginger coffee without the addition of peptides (control) had the lowest antioxidant activity.

3.5 Antioxidant activity of filtered peptides and their profile

Peptides or hydrolysates with the highest antioxidant activity (whey hydrolysate 15 mins and goat milk hydrolysate 15 mins) were fractionated by molecular weight. The antioxidant activity based on the sample molecular weight can be seen in Table 5. Peptides that were less than 10 kDa had the highest antioxidant activity and were significantly different from other samples. On the other hand, peptides with a size of 30 kDa or more had the lowest antioxidant activity. In goat milk peptide samples, peptides with a size of 30 kDa or more did not have antioxidant activity.

Table 5. Antioxidant activity of filtered whey and goat milk peptides.

Samples	Antioxidant Activity (%)
W >30 kDa	0.82±0.82[c]
W 30-10 kDa	9.45±3.21[b]
W <10 kDa	68.13±1.98[a]
GM >30 kDa	-
GM 30-10 kDa	0.44±0.17[b]
GM <10 kDa	90.79±1.06[a]

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different (P<0.05). W: Whey hydrolysate 15 mins, GM: Goat milk hydrolysate 15 mins.

The filtered peptides were also analyzed for their profiles using SDS-PAGE (Figure 3). Lanes 1 to 3 show a filtered sample of whey peptides. At lane 1, whey peptides with a size of 30 kDa or more had 1 band with a size of 16 kDa.A saturated filter may be the cause of protein retention of less than 30 kDa. Lane 2 represents whey peptides with a size of 30 to 10 kDa. There was only 1 band in this sample with a molecular weight of 19 kDa. Meanwhile, lane 3 is a whey peptide sample with a size of less than 10 kDa. This sample had only 1 band of 16 kDa which is thinner than other samples.

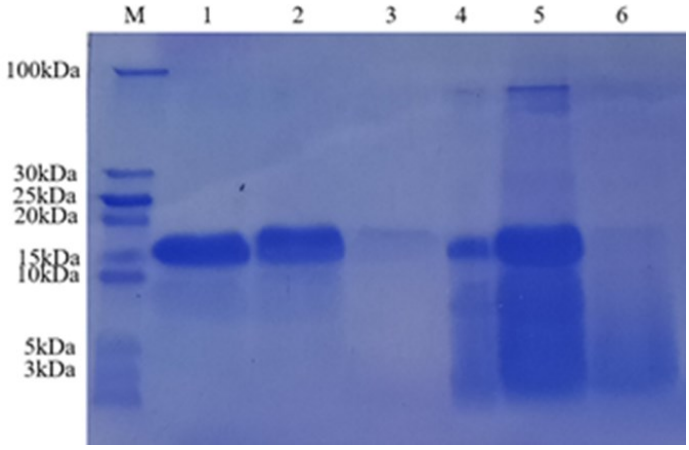

Figure 3. SDS-PAGE profile of whey peptide. Lane M: Marker; Lane 1-3: whey peptides >30 kDa, 30-10 kDa, <10 kDa; Lane 4-6: milk peptides 30-10 kDa, >30 kDa, <10 kDa.

Milk peptide samples are shown in lanes 4 to 6. Lane 4 is a milk peptide with a size of 30 to 10 kDa. There were 2 bands in this sample with molecular weights of 18 kDa and 92 kDa. Lane 5 had 2 bands of 21 kDa and 102 kDa, and a smearing band below 21 kDa. Lane 6 showed 1 band with a size of 17 kDa, the band was thinner than the other samples and also smearing band below 5kDa.

3.6 Organoleptic test

All of the samples can be seen in Figure 4 and the results of the organoleptic test are shown in Table 6. The largest average value obtained is a scale of 3, which means the ginger coffee products are quite liked by the panellists. On the attributes of colour and aroma, all

https://doi.org/10.26656/fr.2017.7(6).1016

Table 6. Organoleptic results of ginger coffee formulation.

Attribute	Control	W	GM	GMW 1	GMW 2	GMW 3
Color	3.69±0.93^{a}	3.46±0.70^{a}	3.46±0.70^{a}	3.46±0.61^{a}	3.57±0.70^{a}	3.40±0.70^{a}
Aroma	3.63±0.77^{a}	3.31±0.93^{a}	3.43±0.91^{a}	3.57±0.74^{a}	3.20±0.90^{a}	3.40±0.88^{a}
Taste	3.40±1.06^{a}	2.34±0.83^{d}	3.29±0.96^{ab}	3.17±0.79^{abc}	2.83±0.99^{bcd}	2.71±1.23^{cd}
Overall	3.43±0.95^{a}	2.69±0.76^{c}	3.37±0.77^{a}	3.31±0.76^{a}	3.17±0.86^{ab}	2.89±0.93^{bc}
Interest	3.14±0.87^{a}	2.23±0.88^{d}	3.03±1.04^{ab}	2.91±0.82^{abc}	2.57±0.92^{bcd}	2.51±0.95^{cd}

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different (P<0.05). C: control only using goat milk (no peptide added), GM: with goat milk peptides (15 mins hydrolysate), W: with whey peptide (15 mins hydrolysate), GMW1: with goat milk and whey peptides in a ratio of 4:1, GMW2: with goat milk and whey peptides in a ratio of 1:1, GMW3: with goat milk and whey peptides in a ratio of 1:4.

samples did not have a significant difference (P>0.05). However, on the other attributes there were significant differences between samples (P<0.05). Preferred samples based on taste attributes were control formulation, GM, and GMW1 formulations. These results are also seen in the overall attributes and interest.

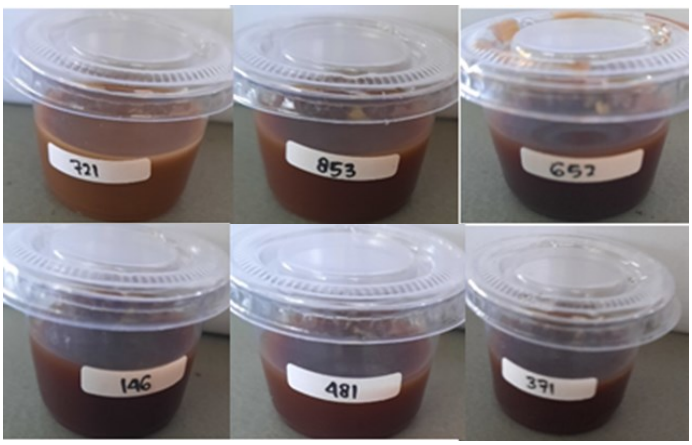

Figure 4. Organoleptic test samples. (a) C: control only using goat milk, (b) W: with whey peptide, (c) GM: with goat milk peptides, (d) GMW1: with goat milk and whey peptides in a ratio of 4:1, (e) GMW2: with goat milk and whey peptides in a ratio of 1:1, and (f) GMW3: with goat milk and whey peptides in a ratio of 1:4.

3.7 Shelf life analysis

For this test, the samples used were the GM and GMW1 formulations. The results for the GM formulation can be seen in Tables 7 and 8. Based on the colourimetric test, the length of storage time could change the colour of the sample. The longer storage time made ginger coffee darker, this was marked by the decrease in L* value. On the contrary, the a* value obtained by the GM samples fluctuated with increased storage time. The value of b* obtained by GM samples tends to decrease with increased storage time. The value of ΔE increases with increased storage time.

In addition to changing colour, GM samples also change in pH and aroma. During storage time, the pH value decreased. This value also had a significant difference (P<0.05) from one another. The changes in the aroma attribute were not very noticeable. On the last day of storage, there were a few brownish precipitates on the bottom of the bottle, but the sample did not thicken.

Shelf life test results of the GMW 1 sample can be seen in Tables 9 and 10. In the colour attribute, the results obtained were similar to the GM colour test result. As the storage time increased, L*, a*, and b* values tended to decrease. For the value of ΔE, the longer the storage time, the greater the colour change against the D-0 sample. The pH and aroma attributes of the GMW 1 sample also had similar results as the GM sample. However, the initial pH of GMW1 had a lower value than the GM sample. The aroma also became slightly sour on the 10th and 11th days of storage. On both days there was also a small amount of brownish precipitates.

3.8 Proximate analysis

The proximate test of the GM and GMW1 samples

Table 7. The color difference in GM formulation for 9 days of storage.

Days	L*	a*	b*	ΔE
0	28.02±0.01^{a}	4.46±0.00^{c}	4.44±0.01^{b}	-
1	27.49±0.00^{c}	4.51±0.02^{b}	4.34±0.01^{c}	0.54±0.00^{f}
2	27.58±0.01^{b}	4.30±0.01^{e}	4.01±0.01^{d}	0.64±0.01^{e}
3	27.16±0.04^{e}	5.17±0.07^{a}	5.15±0.03^{a}	1.33±0.05^{d}
4	27.20±0.01^{d}	4.10±0.01^{g}	3.36±0.02^{e}	1.40±0.01^{c}
7	24.25±0.01^{f}	4.35±0.01^{d}	2.88±0.02^{f}	4.08±0.01^{b}
8	23.64±0.00^{g}	3.98±0.01^{h}	2.39±0.01^{h}	4.86±0.00^{a}
9	23.48±0.01^{h}	4.19±0.01^{f}	2.64±0.01^{g}	4.89±0.01^{a}

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different (P<0.05).

https://doi.org/10.26656/fr.2017.7(6).1016

Table 8. The pH and aroma difference in GM formulation for 11 days of storage.

Days	pH	Aroma	Description
0	5.99±0.02[a]	Very strong aroma of coffee and ginger	Brownish black coffee without any precipitates. This coffee was still watery
1	5.96±0.02[b]	Very strong aroma of coffee and ginger	Brownish black coffee without any precipitates. This coffee was still watery
2	5.88±0.01[c]	Very strong aroma of coffee; strong ginger aroma	Brownish black coffee without any precipitates. This coffee was still watery
3	5.81±0.02[d]	Strong aroma of coffee; slightly strong ginger aroma	Brownish black coffee without any precipitates. This coffee was still watery
4	5.70±0.02[e]	Strong aroma of coffee; slightly strong ginger aroma	Brownish black coffee without any precipitates. This coffee was still watery
7	5.12±0.02[f]	There's a coffee aroma but there's no more ginger aroma	The coffee became darker but still had no precipitates and the coffee was not thick
8	4.92±0.01[g]	Slightly coffee aroma, no more ginger aroma	The colour of the coffee is the same as day 7, and still no precipitates
9	4.81±0.02[h]	Slightly coffee aroma, no more ginger aroma	The coffee becomes darker but still no precipitates
10	4.00±0.00[i]	Slightly coffee aroma and a very slightly acid aroma	The coffee becomes darker but still no precipitates
11	4.00±0.00[i]	Slightly coffee aroma and a very slightly acid aroma	The coffee became darker but the coffee still watery and there was a little bit of brown precipitates at the bottom

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different (P<0.05).

Table 9. The colour difference in GMW1 formulation for 9 days of storage.

Days	L*	a*	b*	ΔE
0	29.78±0.01[a]	4.46±0.01[d]	5.33±0.01[a]	-
1	28.80±0.01[b]	4.65±0.01[a]	5.28±0.02[b]	1.00±0.01[g]
2	28.44±0.00[c]	4.54±0.00[c]	4.97±0.01[c]	1.39±0.00[f]
3	28.34±0.02[d]	4.44±0.02[d]	4.39±0.01[d]	1.72±0.02[e]
4	27.76±0.01[e]	4.32±0.02[e]	3.94±0.01[e]	2.45±0.01[d]
7	23.87±0.07[g]	4.59±0.03[b]	3.03±0.02[f]	6.34±0.06[c]
8	23.93±0.01[f]	4.33±0.02[e]	2.75±0.03[g]	6.39±0.02[b]
9	23.83±0.01[g]	3.96±0.01[f]	2.52±0.01[h]	6.60±0.01[a]

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different (P<0.05).

Table 10. The pH and aroma difference in GMW1 formulation for 11 days of storage.

Days	pH	Aroma	Description
0	5.89±0.02[a]	Very strong aroma of coffee and ginger	Brownish black coffee without any precipitates. This coffee was still watery
1	5.88±0.01[a]	Very strong aroma of coffee and ginger	Brownish black coffee without any precipitates. This coffee was still watery
2	5.78±0.01[b]	Very strong aroma of coffee; strong ginger aroma	Brownish black coffee without any precipitates. This coffee was still watery
3	5.74±0.01[c]	Strong aroma of coffee; slightly strong ginger aroma	Brownish black coffee without any precipitates. This coffee was still watery
4	5.59±0.02[d]	Strong aroma of coffee; slightly strong ginger aroma	Brownish black coffee without any precipitates. This coffee was still watery
7	5.02±0.01[e]	There's a coffee aroma but there's no more ginger aroma	The coffee became darker but still had no precipitates and the coffee was not thick
8	4.81±0.02[f]	Slightly coffee aroma, no more ginger aroma	The color of the coffee is the same as day 7, and still no precipitates
9	4.68±0.02[g]	Slightly coffee aroma, very slightly acid aroma	The coffee becomes darker but still no precipitates
10	4.00±0.00[h]	Slightly coffee aroma and slightly acid aroma	The coffee became darker, there was a little bit of brown precipitate at the bottom
11	4.00±0.00[h]	Slightly coffee aroma and slightly acid aroma	The coffee becomes darker but the coffee still watery and there's a little bit of brown precipitates at the bottom

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different (P<0.05).

https://doi.org/10.26656/fr.2017.7(6).1016

can be seen in Table 11. The tests of these samples had similar results. For the protein content results, the GMW1 sample had a higher yield. The total calories of the GMW1 sample were higher than the GM sample. On the contrary, the water content of the two samples had the same yield.

Table 11. Proximate analysis of GM and GMW1 formula.

Parameter	GM	GMW1
Protein content (%)	1.20	1.40
Ash content (%)	0.56	0.55
Calories from fat (kcal/100 g)	2.66	2.84
Total fat (%)	0.30	0.32
Moisture content (%)	79.72	79.27
Total calories (kcal/100 g)	80.38	82.28
Carbohydrate (%)	18.24	18.46

4. Discussion

Whey protein and goat milk can be hydrolyzed into peptides with the activity of enzymes. The enzyme used is a food-grade papain enzyme with an activity of 32.94 U/mL. Whey protein and goat milk were hydrolyzed for 0, 15, and 30 mins. Proteins that have been hydrolyzed will have a decrease in concentration because the papain enzyme can cut peptide bonds in the protein chain. Papain is a proteolytic enzyme that belongs to the endopeptidase group, which means that this enzyme will break down proteins in certain places. Papain will cut peptide bonds that have basic amino acids, especially arginine, lysine, and residues after phenylalanine (Menard *et al.,* 1990).

Prolonged hydrolysis time will result in more protein being broken down. However, based on the analysis by the Lowry method (Table 2) the protein concentration in the sample hydrolyzed for 30 mins was higher than in the sample hydrolyzed for 15 mins. This could happen because the Lowry method will only detect proteins that can form a complex with copper. The formation of this complex will occur in an alkaline condition. The presence of acid can interfere with the formation of the protein-copper complex. In this research, acid interference may occur due to the use of acetic acid when isolating whey this acid may be still present in the whey protein, and because of this the detection of protein concentration is less accurate (Lu *et al*., 2010). Other than that, the Lowry method is more accurate in detecting colour if the sample contains amino acids tryptophan and tyrosine. The content of tryptophan or tyrosine can support the reaction for the formation of a hetero-polymolybdenum blue complex. This complex is formed because oxidation of the aromatic group catalyzed by the protein-copper complex (Lowry *et al*., 1951).

Decreasing protein concentration during hydrolysis can also be visualized with SDS-PAGE (Figures 1 and 2). In the whey sample, there was a reduction of 3 bands after the sample was hydrolyzed. Whey protein is dominated by β-lactoglobulin and α-lactalbumin. The molecular weight of β-lactoglobulin is in the range of 20.1 to 18 kDa while the molecular weight of α-lactalbumin is in the range of 15 to 14.4 kDa. High molecular weight bands represent immunoglobulins in whey (El-Hatmi *et al.,* 2015). Similar to whey, goat milk samples also had a reduction in bands. The hydrolyzed milk only had 1 to 2 bands out of 9 bands. Visualization of protein in goat milk showed more bands than whey protein. Thick bands in goat milk samples ranging in size from 40 kDa to 30 kDa visualize casein proteins in milk ranging from α-casein, β-casein, and κ-casein (Jovanovic *et al.,* 2007). According to Figure 3, peptides with sizes of >30 kDa, 30 – 10 kDa, and <10 kDa have similar bands. This may be caused by the filtration method that was carried out not optimally. As a consequence, some proteins did not separate according to the size of the membrane, then visualized on SDS-PAGE. Therefore, the method should be improved in future studies.

Hydrolyzed protein samples will produce peptides, these peptides can act as antioxidants (Khan *et al.,* 2019). The highest antioxidant activity was shown in the 15 mins hydrolysate (Table 3) this is compatible with the result of protein concentration analysis (Table 2). The 15 mins hydrolysate had the lowest protein concentration of the other samples, meaning that more protein was broken down into peptides. The higher number of peptides, the higher the antioxidant activity produced. Whey hydrolysate obtained an IC_{50} value of 525.81 µg/mL, meaning that it takes about 500 µg/mL hydrolysate to neutralize 50% of DPPH radicals. This value can be classified as weak antioxidant activity. Milk hydrolysate 15 mins has an IC_{50} of 105.98 µg/mL, meaning that it takes about 100 µg/mL hydrolysate to neutralize 50% of DPPH radical. This IC_{50} value can be classified as moderate antioxidant intensity (Jadid *et al*., 2016). It is important to note that the antioxidant properties of peptides are influenced by the sequence and length of the peptide (Zou *et al*., 2016).

Peptides with antioxidant activity are generally dominated by hydrophobic amino acids or sulfur-containing amino acids. Peptides derived from whey protein are dominated by sulfur-containing amino acids, such as methionine and cysteine (Khan *et al.,* 2019). A large number of amino acid residues will increase the antioxidant activity of whey peptides. In goat milk peptide samples, the antioxidant activity produced was higher than in whey peptides. This happens because when goat milk is hydrolyzed, the casein protein in it is

https://doi.org/10.26656/fr.2017.7(6).1016

also hydrolyzed. Casein peptides are dominated by hydrophobic amino acids. These peptides will promote antioxidant activity therefore antioxidant activity is not only obtained from the whey peptides alone (Chang *et al.,* 2013).

Besides the amino acid composition, peptide antioxidant activity is also influenced by its molecular weight. Based on the results in Table 5, peptides with sizes below 10 kDa have higher antioxidant activity. The results of this analysis also follow the research of Chang *et al.* (2013) which states that peptides <10 kDa and <3 kDa have high antioxidant activity. Unfortunately, the very small size of the protein can be a problem when visualized with SDS-PAGE. SDS-PAGE electrophoresis is more accurate for measuring proteins larger than 10 kDa (Shi and Jackowski, 1998).

Peptides with the highest antioxidant activity were formulated in ginger coffee drinks, therefore these drinks have antioxidant activity. The highest antioxidant activity was found in the GMW1 formulation (Table 4). The antioxidant activity of GMW1 is higher than the GM formulation, although Table 3 shows that goat milk peptides have higher activity than whey peptides. According to Nagasawa *et al.* (2001), the addition of 2 peptides from different sources could increase peptide sequence variation and this variation could increase antioxidant activity. The formulation with the lowest antioxidant activity was the control formulation. This formulation was not added with peptides at all, only non-hydrolyzed goat milk was added. This non-hydrolyzed goat milk contains casein and whey proteins, but of course, these two proteins do not have as much antioxidant activity as the protein that has been broken down into peptides.

Antioxidant activity in the control formulation can be obtained from the addition of ginger because ginger also contains antioxidants. Phenolic compounds in ginger, such as shogaol and gingerol, can act as antioxidants. But is important to know that heating, like what is done in the process of making ginger coffee, can reduce the antioxidant activity of ginger (Mosovska *et al.,* 2015). However, the antioxidant activity of peptides is not significantly affected by the heating process because the peptides were added when the ginger coffee temperature decreased to 50°C. In addition, peptides are also more heat resistant, especially peptides derived from whey (Korhonen *et al.,* 1998).

All formulations of ginger coffee were sampled in the hedonic test. There are 5 attributes that are assessed in this test as listed in Table 6. The colour and aroma attributes have similar values from all formulations. This is because the colour of the peptide added is not too strong and can be covered with the coffee colour. For aroma attributes, whey peptides have a slightly sour aroma but this aroma also can be covered by the aroma of coffee and ginger. Based on Table 6, it can be stated that the formulations with the highest antioxidant activity, GM and GMW1 formulations, were also favoured by the panellists. Of all the attributes assessed, the GM and GMW1 formulations were preferred in taste, overall, and more desirable to buy. Formulations with whey peptides were less favoured by panellists because of the sour taste. This sour taste can come from the acetic acid used when isolating whey. Besides the sour taste, panellists are stating that ginger coffee with whey peptide has a stronger bitter taste. The bitter taste can be obtained from certain peptides (Liu *et al.,* 2013).

Several attributes were changed during the storage time of GM and GMW1 formulas. The colour of the drink will get darker with increasing storage time (Tables 7 and 9). This can be seen from the L* value, the lower the L* value, the darker the colour. A* value indicates a red-green colour component, positive a* value indicates a red colour while a negative value indicates a green colour. Then the value of b* indicates the yellow-blue colour component. Positive values indicate yellow and negative values indicate blue. ΔE value signifies a change in the colour of the sample. The greater the value of ΔE, the greater the colour change (Ly *et al.,* 2020). This change was measured against the colour of the sample on day 0. This attribute change can indicate the shelf life of coffee drinks. However, the attribute change that is widely used to determine the shelf life of coffee drinks is the pH value (Tables 8 and 10). The longer the storage time, the lower the pH value. Decreased pH value of coffee drinks can happen because of the hydrolysis of lactone or the growth of microorganisms that break down the sugar and produce acid (Nicoli *et al.,* 2009). Microorganisms contamination can occur during the processing and packaging of coffee drinks. Coffee drinks can be contaminated by *Enterobacteriaceae*, lactic acid bacteria, and *Aspergillus carbonarius* (Juvonen *et al*., 2011; Claassen *et al*., 2021). Coffee drinks are still suitable for consumption if the pH value is in the range of 5. Besides pH, the other attribute that changes during the storage time is aroma. The intensity of the coffee aroma will also decrease during storage. This decrease in aroma intensity could occur because the thiol aroma-active compound will interact with melanoidin in coffee (Nicoli *et al.,* 2009).

In addition to shelf life analysis, proximate analysis was also carried out for the GM and GMW1 formulations (Table 11). Based on these proximate results, the nutrition facts of each formulation can be arranged (Figures 5 and 6). This nutrition fact was

https://doi.org/10.26656/fr.2017.7(6).1016

arranged regarding BPOM rule number 9 of 2016 (BPOM, 2016). The fat content obtained in the GM and GMW1 formulations was lower than ready-to-drink (RTD) coffee products on the market (2.5 g/200 mL). On the other hand, the protein content of commercial RTD coffee is 3g/200 mL. The GM formulation has a lower protein content, but the GMW1 formulation has the same protein content as the commercial products. For total carbohydrates, both formulations had higher carbohydrate content than RTD coffee (23 g/200 mL). The sugar content used in both formulations is the same number as RTD coffee products.GM and GMW1 formulations have acceptable nutrient content because their nutrient does not exceed the recommended intake. Daily intake of fat is 65 g for women and 75 g for men, daily intake of protein is 60 g for women and 65 g for men, and daily intake of carbohydrates is 360 g for women and 430 g for men (Menteri Kesehatan Republik Indonesia (Menkes RI), 2019). The requirements for the quality of coffee drinks are that they have a normal odour, taste, and colour, and do not contain artificial sweeteners such as saccharin and cyclamate. Coffee drinks are also required to have a minimum of 200 mg/ kg of caffeine (BSN 1996).

Nutrition Facts		
Serving Size		200 mL
Serving per container		1
Amount per serving		
Calories		161 kcal
Calories from fat		5 kcal
		% Daily Value*
Total Fat	**1 g**	**1%**
Protein	**2 g**	**3%**
Total Carbohydrate	**36 g**	**11%**
Sugar	**24 g**	

Figure 5. Nutrition fact of ginger coffee with goat milk (GM) formulation for 200 mL product

Nutrition Facts		
Serving Size		200 mL
Serving per container		1
Amount per serving		
Calories		165 kcal
Calories from fat		6 kcal
		% Daily Value*
Total Fat	**1 g**	**1%**
Protein	**3 g**	**5%**
Total Carbohydrate	**37 g**	**11%**
Sugar	**24 g**	

Figure 6. Nutrition fact of ginger coffee with goat milk and whey peptides in a ratio of 4:1 (GMW1) formulation for 200 mL product.

5. Conclusion

Whey protein and goat milk can be hydrolyzed by the papain enzyme. After being hydrolyzed, the protein concentration decreased compared to the sample that was not hydrolyzed. The protein samples that were hydrolyzed for 15 mins had the highest antioxidant activity. Based on this antioxidant activity, peptides derived from whey can be classified as weak antioxidants, while peptides from goat milk could be classified as strong antioxidants. Both of these peptides could be applied to the formulation of ginger coffee. The best formulations obtained were GMW1 and GM, these two formulations had high antioxidant activity and were acceptable to the panellists. Based on the shelf life test on the pH attribute, the GM and GMW1 formulations could be stored for 7 days at 4°C.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This research was sponsored by Atma Jaya Catholic University of Indonesia through Decentralization Research Funds.

References

Abubakr, M.A.S., Hassan, Z. and Salem, G. (2013). Antioxidant activity of milk fermented with *Lactobacillus plantarum* 1 and *Leuconostoc mesenteroides* isolated from non-dairy sources. *Asian Journal of Pharmaceutical Research and Development*, 1(2), 71-83.

Ahmed, A.S., El-Bassiony, T., Elmalt, L.M. and Ibrahim, H.R. (2015). Identification of potent antioxidant bioactive peptides from goat milk proteins. *Food Research International*, 74(1), 80-88. https:// doi.org/10.1016/j.foodres.2015.04.032

Amri, E. and Mamboya, F. (2012). Papain a plant enzyme of biological importance: a review. *American Journal of Biochemistry and Biotechnology*, 8(2), 99-104. https://doi.org/10.3844/ ajbbsp.2012.99.104

Anson, M.L. (1938). The estimation of pepsin, trypsin, papain, and cathepsin with hemoglobin. The *Journal of General Physiology*, 22(1), 79-89. https:// doi.org/10.1085/jgp.22.1.79

Atanasova, J. and Ivanova, I. (2010). Antibacterial peptides from goat and sheep milk proteins. *Biotechnology and Biotechnological Equipment*, 24 (2), 1799-1803. https://doi.org/10.2478/V10133-010-0049-8

Borkova, M. and Snaselova, J. (2005). Possibilities of different animal milk detection in milk and dairy products. *Czech Journal of Food Science,* 23(2), 41-50. https://doi.org/10.17221/3371-CJFS

Badan Pengawas Obat dan Makanan Republik Indonesia (BPOM). (2016). Peraturan kepala badan pengawas obat dan makanan Republik Indonesia nomor 9 tahun 2016 tentang Acuan Label Gizi. Retrieved from website: https://asrot.pom.go.id/img/Peraturan. [In Bahasa Indonesia].

Badan Standardisasi Nasional (BSN). (1996). Minuman kopi dalam kemasan (SNI 01-4314-1996). Retrieved from website: https://pdfcoffee.com/minuman-kopi-dalam-kemasan-18808sni-01-4314-1996pdf-pdf-free.html. [In Bahasa Indonesia].

Chang, O.K., Seol, K.H., Jeong, S.G., Oh, M.H., Park, B.Y., Perrin, C. and Ham, J.S. (2013). Casein hydrolysis by Bifidobacterium longum KACC91563 and antioxidant activities of peptides derived therefrom. *Journal of Dairy Science*, 96(9), P5544-5555. https://doi.org/10.3168/jds.2013-6687

Claassen, L., Rinderknecht, M., Porth, T., Rohnisch, J., Seren, H.Y., Scharinger, A., Gottstein, V., Noack, D., Schwarz, S., Winkler, G. and Lachenmeier, D.W. (2021). Cold brew coffee – pilot studies on definition, extraction, consumer preference, chemical characterization and microbiological hazards. *Foods*, 10(4), 865. https://doi.org/10.3390/foods10040865

Cohen, L.W., Coghlan V.M. and Dihel L.C. (1986). Cloning and sequencing of papain-encoding cDNA. *Gene*, 48(2-3), 219-227. https://doi.org/10.1016/0378-1119(86)90080-6

El-Hatmi, H., Jrad, Z., Salhi, I., Aguibi, A., Nadri, A. and Khorchani, T. (2015). Comparison of composition and whey protein fractions of human, camel, donkey, goat, and cow milk. *Mljekarstvo*, 65 (3), 159-167. https://doi.org/10.15567/mljekarstvo.2015.0302

Global Agricultural Information Network (GAIN). (2019). Indonesia coffee annual report 2019. Retrieved from website: https://apps.fas.usda.gov/newgainapi/api/report/downloadreportbyfilename?filename=Coffee%20Annual_Jakarta_Indonesia_5-15-2019.pdf.

Jadid, N., Hidayati D., Hartanti, S.R., Arraniry, B.A., Rachman, R.Y. and Wikanta, W. (2016). Antioxidant activities of different solvent extracts of Piper retrofractum Vahl. Using DPPH assay. AIP Conference Proceedings, 1854, 020019 . https://doi.org/10.1063/1.4985410

Jovanovic, S., Barac, M., Macej, O., Vucic, T. and Lacnjevac, C. (2007). SDS-PAGE analysis of soluble proteins in reconstituted milk exposed to different heat treatments. *Sensors*, 7(3), 371-383. https://doi.org/10.3390/s7030371

Juvonen, R., Virkajarvi, V., Priha, O., Laitila, A. (2011). Microbiological Spoilage and Safety Risks in Non-beer Beverages. Kuopio, Finland: VTT.

Khan, I.T., Nadeem, M., Imran, M., Ullah, R., Ajmal, M. and Jaspal, M.H. (2019). Antioxidant properties of milk and dairy products: a comprehensive review of the current knowledge. *Lipids in Health and Disease*, 18, 41. https://doi.org/10.1186/s12944-019-0969-8

Korhonen, H., Pihlanto-Leppala, A., Rantamaki, P. and Tupasela, T. (1998). Impact of processing on bioactive proteins and peptides. *Trends in Food Science and Technology*, 9(8-9), 307-319. https://doi.org/10.1016/S0924-2244(98)00054-5

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227, 680-685. https://doi.org/10.1038/227680a0

Lestari, P. and Suyata. (2020). Aktivitas antioksidan protein hidrolisat dari kasein susu kambing etawa hasil hidrolisis bromelin dari daun nanas madu. *Jurnal Gizi dan Pangan Soedirman*, 4(1), 1-13. https://doi.org/10.20884/1.jgps.2020.4.1.2454 [In Bahasa Indonesia].

Li, X.E., Lopetcharat, K. and Drake, M.A. (2015). Parents' and children's acceptance of skim chocolate milks sweetened by monk fruit and stevia leaf extracts. Journal of Food Science, 80(5), 1083-1092. https://doi.org/10.1111/1750-3841.12835

Liu, X., Jiang, D. and Peterson, D.G. (2013). Identification of bitter peptides in whey protein hydrolysate. *Journal of Agricultural and Food Chemistry*, 62(25), 5719-5725. https://doi.org/10.1021/jf4019728

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein Measurement with the folin phenol reagent. *Journal of Biological Chemistry*, 193 (1), 265-275. https://doi.org/10.1016/S0021-9258(19)52451-6

Lu, T.S., Yiao, S.Y., Lim, K., Jensen, R.V. and Hsiao, L.L. (2010). Interpretation of biological and mechanical variations between the Lowry versus Bradford method for protein quantification. *North American Journal of Medicine and Science*, 2(7), 325-328.

Ly, B.C.K., Dyer, E.B., Feig, J.L., Chien, A.L. and Bino, S.D. (2020). Research techniques made simple: cutaneous colorimetry: a reliable technique for objective skin color measurement. *Journal of Investigative Dermatology*, 140(1), 3-12. https://

doi.org/10.1016/j.jid.2019.11.003

Menard, R., Khri, H.E., Plouffe, C., Dupras, R., Ripoll, D., Vernet, T., Tessier, D.C., Laliberte, F., Thomas, D.Y. and Storer, A.C. (1990). A protein engineering study of the role of aspartate 158 in the catalytic mechanism of papain. *Biochemistry*, 29(28), 6706-6713. https://doi.org/10.1021/bi00480a021

Menteri Kesehatan Republik Indonesia (Menkes RI). (2019). Peraturan Menteri kesehatan Republik Indonesia nomor 28 tahun 2019 tentang angka kecukupan gizi yang dianjurkan untuk masyarakat Indonesia. Retrieved from website: http://hukor.kemkes.go.id/uploads/produk_hukum/PMK_No__28_Th_2019_ttg_Angka_Kecukupan_Gizi_Yang_Dianjurkan_Untuk_Masyarakat_Indonesia.pdf [In Bahasa Indonesia].

Mosovska, S., Novakova, D. and Kalinak, M. (2015). Antioxidant activity of ginger extract and identification of its active components. *Acta chimica slovaca*, 8(2), 115-119. https://doi.org/10.1515/acs-2015-0020

Nagasawa, T., Yonekura, T., Nishizawa, N. and Kitts, D.D. (2001). In vitro and in vivo inhibition of muscle lipid and protein oxidation by carnosine. *Molecular and Cellular Biochemistry*, 225(1), 29-34. https://doi.org/10.1023/A:1012256521840

Nicoli, M.C., Calligaris, S. and Manzocco. (2009). Shelf-life testing of coffee and related products: uncertainties, pitfalls, and perspectives. *Food Engineering Reviews*, 1(1), 159-168. https://doi.org/10.1007/s12393-009-9010-8

Rebollar, R., Lidon, I., Serrano, A., Martin, J. and Fernandez, M.J. (2012). Influence of chewing gum packaging design on consumer expectation and willingness to buy an analysis of functional, sensory and experience attributes. *Food Quality and Preference*, 24(1), 162-170. https://doi.org/10.1016/j.foodqual.2011.10.011

Sarmadi B.H. and Ismail, A. (2010). Antioxidative peptides from food proteins: a review. *Peptides*, 31 (10), 1949-1956. https://doi.org/10.1016/j.peptides.2010.06.020

Shi, Q. and Jackowski, G. (1998). One-dimensional polyacrylamide gel electrophoresis. In Hames, B.D. (Ed). Gel electrophoresis of proteins, p. 1-52. New York, USA: Oxford.

Singh, P., Benjakul, S., Maqsood S. and Kishimura, H. (2011). Isolation and characterization of collagen extracted from the skin of striped catfish (*Pangasianodon hypopthalmus*). *Food Chemistry*, 124, 97-105. https://doi.org/10.1016/j.foodchem.2010.05.111

Umayaparvathi, S., Meenakshi, S., Vimalraj, V., Arumugam, M., Sivagami. G. and Balasubramanian, T. (2014). Antioxidant activity and anticancer effect of bioactive peptide from enzymatic hydrolysate of oyster (*Saccostrea culcullata*). *Biomedicine and Preventive Nutrition*, 4(3), 343-353. https://doi.org/10.1016/j.bionut.2014.04.006

Virgen-ortiz, J.J., Ibarra-Junquera, I., Osuna-Castro, J.A., Escalante-Minakata, P., Mancilla-Margalli, N.A. and Ornelas-Paz, J.D.J. (2012). Method to concentrate protein solutions based on dialysis-freezing-centrifugation: enzyme applications. *Analytical Biochemistry*, 426(2), 4-12. https://doi.org/10.1016/j.ab.2012.03.019

Yoshida, S., Wei, Z., Shinmura, Y. and Fukunaga, N. (2000). Separation of lactoferrin-a and -b from bovine colostrum. *Journal of Dairy Science*, 83(10), 2211-2215. https://doi.org/10.3168/jds.S0022-0302(00)75104-6

Zhang, Q.X., Wu, H., Ling, Y.F. and Lu, R.R. (2013). Isolation and identification of antioxidant peptides derived from whey protein enzymatic hydrolysate by consecutive chromatography and Q-TOF MS. *Journal of Dairy Research*, 80(3), 367-373. https://doi.org/10.1017/S0022029913000320

Zou, T.B., He, T.P., Li, H.B., Tang, H.W. and Xia, E.Q. (2016). The structure-activity relationship of the antioxidant peptides from natural proteins. *Molecules*, 21(1), 72-85. https://doi.org/10.3390/molecules21010072

