p–ISSN: 2723 - 6609 e-ISSN: 2745-5254
Vol. 5, No. 8 August 2024 http://jist.publikasiindonesia.id/

Effect of Fermentation Duration and Glucose Concentration on Organoleptic Characteristics of Hard Candy Glucose Substitution of Coffee Pulp by Fermentation Method

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Keywords: coffee pulp, hard candy, fermentation, liquid sugar.

ABSTRACT

Coffee pulp is the outer layer of coffee pulp which is one type of waste from the coffee processing process that increases every year without maximum utilization. Coffee pulp contains cellulose (63%) which can be utilized in the manufacture of semi-finished products in the form of liquid sugar through the fermentation method. Phenol in coffee pulp causes a sour and astringent taste in processed products, so a fermentation process is needed to degrade phenol compounds during aerobic fermentation. The purpose of this study was to determine the effect of the length of fermentation of coffee pulp on the phenol content of liquid sugar produced and to analyze the effect of the concentration of liquid sugar fermented from coffee pulp on the organoleptic characteristics of candy in the market. The test of phenol content using the Folin Ciocalteu method and the test of reducing sugar using the Nelson Somogyi method showed that the longer the fermentation process took place, the lower the levels of total phenol and reducing sugar in the juice and coffee pulp sugar produced. Sensory testing with the ranking test on 30 panellists showed that the variation of adding 20% coffee pulp fermented liquid sugar concentration was overall most liked by panellists because it was close to candy products in general with the highest sucrose concentration compared to other candy formulas.

Introduction

Coffee is one of the leading commodities with an increasing amount of production in line with the increase in coffee consumption in the world. Coffee consumption in Indonesia has increased, from 3.5 million sacks (60 kg/sack) in 2010 to 5 million sacks in 2021 (Lyu & Huang, 2024). The Central Statistics Agency (BPS) noted that coffee production in Indonesia in 2022 increased by 1.10%, namely 794,800 tons from 786,191 tons in the previous year. The increase in coffee production and consumption will have an impact on increasing by-products in the form of coffee waste. The most produced

coffee waste is solid and liquid in the form of pulp, leather, mucilage or mucus, and stretcher bark (Maghfirah, Priscila, Sahara, & Istiqomah, 2023). The utilization of coffee waste in the food sector is currently not optimal. Coffee pulp still contains cellulose (63%), hemicellulose (2.3%), and lignin (17%) (Nurfitriyani, 2022).

One of the studies shows that the high level of cellulose in the coffee pulp can be used in the manufacture of semi-finished products in the form of liquid sugar through the fermentation method (Kresnadipayana et al., 2022). However, liquid sugar from coffee pulp processing is not widely used in the process of making finished products. So that further processing is needed to produce finished products that can be accepted in the market (Wati, Suriati, & Semariyani, 2023). This liquid sugar can be used as a raw material for making candies, drinks, cakes, ice cream, and so on. Among some of these liquid sugar products, candy is a product that requires high glucose levels in the manufacturing process. Thus, glucose produced from the fermentation process can be used as raw materials for making hard candies in this research (Annie, Iman, & Anoraga, 2023).

On the other hand, the use of coffee pulp as a raw material for making liquid sugar produces a sour and spicy taste (Kurniawan, Tawali, & Fitri, 2024). The sour and sour taste is produced by phenol compounds found in coffee pulp (Asmak, Bima Lathif, Yuli, & Ardiyan, 2023). Phenol levels can be degraded during the aerobic fermentation process (Mahardani & Yuanita, 2021). Therefore, a fermentation process with different variations in time is needed to determine the right formulation to reduce the sour and sour taste of liquid sugar. In addition, in the manufacture of candies, variations of different concentrations of liquid sugar are carried out to produce organoleptic characteristics that are close to the taste of candies on the market. Efforts to utilize glucose in liquid sugar in candy products can add economic value to coffee pulp and become one of the efforts to prevent organic waste pollution due to the coffee production process (Zarwinda, Nadia, & Rejeki, 2022). Therefore, it is proposed to make hard candies with liquid sugar substitution as a result of the fermentation process of coffee pulp with fermented yeast. This study aims to determine the effect of coffee pulp fermentation time on the phenol content of liquid sugar produced and analyze the effect of liquid sugar concentration from coffee pulp fermentation on the organoleptic characteristics of candies on the market.

Research Methods

Time and Place

The research activities were carried out offline in March-June 2024 at the Food Microbiology and Biotechnology Laboratory of FP UNS, the Laboratory of Food Processing and Agricultural Product Process Engineering FP UNS, and the Laboratory of Food Chemistry and Biochemistry FP UNS.

Tools and Materials

The tools used in this research include digital scales, vortex, hotplate, glass funnels, cutting boards, knives, measuring flasks, Erlenmeyer, droppers, measuring cups, volume pipettes, filter cloths, stirrers, UV-Vis spectrophotometers, stoves, and blenders. In

addition, media are needed, including basins, closed containers, heat-resistant glass containers, and candy moulds. Meanwhile, the materials used in this research are coffee fruits, sucrose equates, NKL tapai yeast, and distilled water. Nelson A reagent, Nelson B reagent, Arsenomolibdate reagent, Folin Ciocalteu reagent 10%, anhydrous glucose, gallic acid and sodium carbonate 7.5% are also needed.

Working Procedure

Flow Diagram

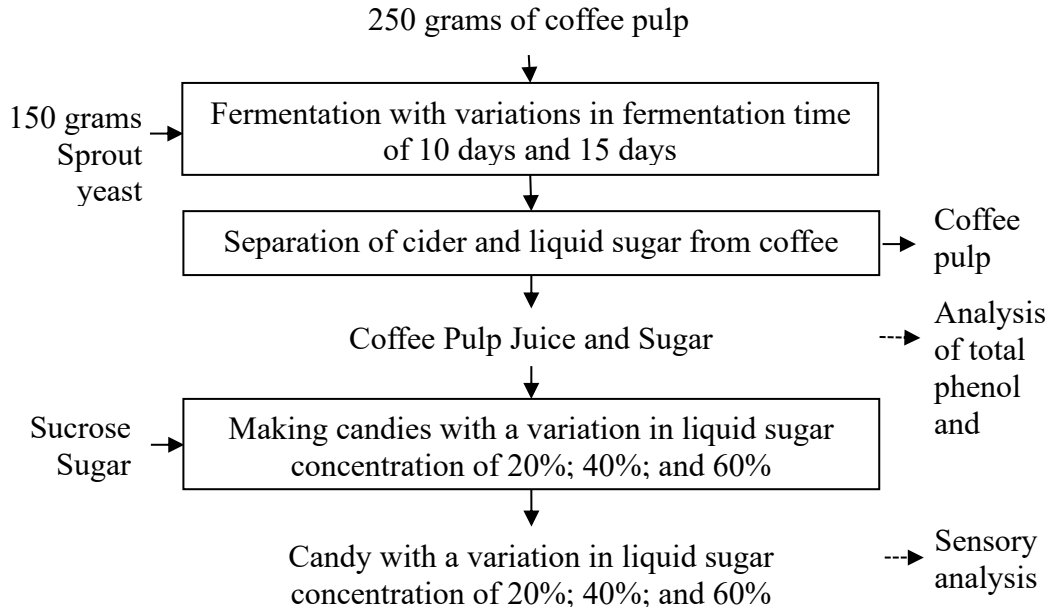

Figure 1. Flow Chart of Research Stages

Based on Figure 1. Flow Diagram of Research Stages, it can be seen that there are several stages carried out in this research:

Fermentation of Coffee Pulp

Coffee pulp is fermented with the addition of NKL tapai yeast 150 grams per 250 grams of coffee pulp starch. Then mixing is carried out until evenly distributed and put in a closed container. The fermentation process is carried out in a place protected from direct sunlight for 10 days and 15 days at a temperature of 30°C (Setiawan, Mahardika, Wardhana, & Werdyani, 2023).

Coffee Juice and Pulp Sugar

The fermented coffee pulp is added to water in a ratio of 1:1 and then refined using a blender. Coffee pulp pulp is put into a filter cloth to separate the juice and sugar of coffee pulp from coffee pulp pulp. The coffee pulp solution is kept in a closed container for a few minutes until a precipitate forms. Furthermore, the juice and coffee pulp sugar separated from the sediment are taken for the next process.

Candy Making

Table 1. Coffee Pulp Candy Formulation

Material	Unit	10-day fermentation	Fermentation 15 days

		356	240	718	162	693	934
Cider and sugar pulp coffee	Ml	20	40	60	20	40	60
Sucrose sugar	Grams	80	60	40	80	60	40
Water	Ml	100	100	100	100	100	100

Based on Table 1. Formulation of Coffee Pulp Candy, the juice and starch sugar of coffee pulp produced from 10 days and 15 days of fermentation are heated to a temperature of 110°C and 80%, 60%, and 40% sucrose sugar is added with a ratio of 20%, 40%, and 60% coffee pulp starch sugar, and is equipped with the addition of the same amount of water in each formulation, namely 100 ml. Then it is reheated to a temperature of 140°C with a continuous stirring process. The end of cooking is determined by a mixture of sweets that have thickened. The hot, thick candy mixture is placed in a mould and allowed to sit until it cools and hardens (Zarwinda et al., 2022).

Analysis of Total Phenol Levels of Products

The analysis of total phenol levels was carried out using the Follin-Ciocalteu method. Samples that have been dissolved in water are reacted with 10% Folin Ciocalteu reagent and incubated for 20 minutes at room temperature. Then 7.5% sodium carbonate was added with an absorbance value read at λ 750 nm. The absorbance data of the galic acid solution was processed using the Microsoft Excel program to create a standard curve of the galic acid solution so that a linear equation was obtained that was used to convert the absorbance data of the sample into the total phenol content in the sample. The total phenol content is calculated by the linear regression equation formula of the gallic acid, namely:

$y = ax + b$ ..... (1)

(Wibawa and Saraswaty, 2023)

The data obtained in this research was analyzed using the IBM SPSS program. Data analysis using the Paired Sample T-Test to determine whether the variation in fermentation time is different or affects the total phenol level produced at the level of significance (α) = 0.05.

Product Reduced Sugar Content Analysis

The analysis of reduced sugar content was carried out using the Nelson-Somogyi method. This test uses the principle of oxidation reaction between the Nelson reagent and glucose with the arsenomolibate reagent (Zia, Aisyah, Zaidiyah, & Widayat, 2019). Testing of this method was carried out at a wavelength of 540 nm with a Uv-Vis Spectrophotometer. The colour produced from the test is blue-greenish. The absorbance data of the standard glucose solution was processed using the Microsoft Excel program to create a curve of the standard glucose solution and a linear equation was obtained which was used to determine the amount of reduced sugar dissolved in the sample. Furthermore,

calculations are carried out to determine the reduced sugar content of the sample with the following formula:

Reduced sugar content (%) = $\frac{X}{berat\ sampel(mg)} \times FP \times 100\%$ ..... (1)

The data obtained in this research was analyzed using the IBM SPSS program. Data analysis using the Paired Sample T-Test to determine whether the variation in fermentation time is different or affects the reduced sugar content produced at the significance level (α) = 0.05.

Product Sensory Analysis

Sensory analysis was carried out using the hedonic test method, namely the ranking test. This test consists of observing the colour, aroma, texture, taste, and overall attributes of coffee pulp hard candies. This test was carried out by 33 panellists who were randomly taken on 6 scales. Organoleptic testing is important to support consumer acceptance of coffee pulp candy products (Fitriyah & Wardani, 2022). The data obtained in this research was analyzed using the IBM SPSS program. Data analysis using the Friedman Test to determine whether the number of ranks of each treatment differed markedly at the level of significance (α) = 0.05. In this test, a ranking was also obtained for each sample where a value of 1 was interpreted as the most preferred formulation by the panelists. The results of the test and data analysis were discussed and the best formulation was determined based on the level of preference of the panelists in the ranking test.

Conclusions of Research Results

The reduction of bitterness and bitterness is characterized by a decrease in the total phenol level after the fermentation process of coffee pulp at different fermentation time variations. The formulation of the best sugar solution is characterized by no decrease in the reduced sugar content or the reduced sugar content does not exceed the specified limit. The best coffee pulp candy formulation is shown with the highest number in the ranking order obtained from the results of the ranking test on 6 formulas and 6 assessment scales.

Results and Discussion

Results of Total Phenol Level Analysis

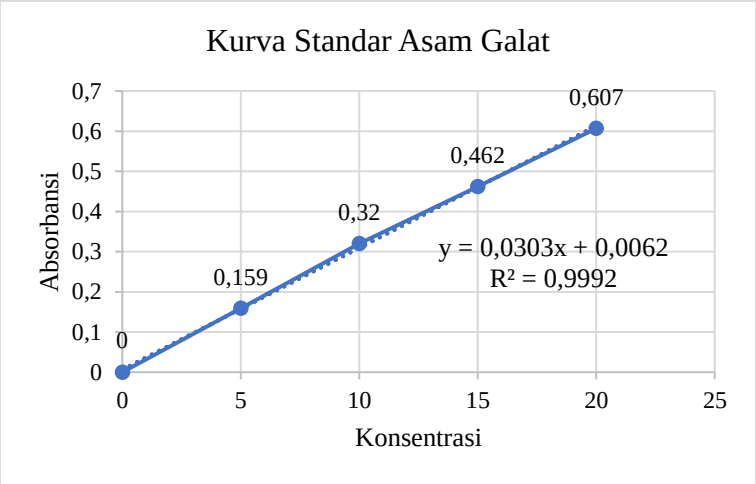

Figure 2. Acid Error Standard Curve

Based on the data that has been obtained in Figure 2. The standard curve of Galic Acid was determined by using a standard solution of Galic acid was carried out using solution of Galic acid with concentrations of 0, 5, 10, 15, and 20 mg/L which obtained absorbance values of 0, 0.159, 0.320, 0.462; and 0.607 Å respectively. Based on this data, the linear regression equation y = 0.0303x + 0.0062 with R2 is 0.9992.

Table 2. Total Levels of Phenol Juice and Coffee Pulp Sugar

Fermentation Time (days)	Absorbance (Å)	Total Phenol Levels (mg GAE/g)	Average Total Phenol Levels (mg GAE/g)
10	0,023	0,5545	0,6315
	0,024	0,5875	
	0,029	0,7525	
15	0,004	-0,07261	0,1749
	0,011	0,1584	
	0,012	0,1914	

Source: Test Results

Based on Table 2, absorbance data were obtained from the results of 10-day and 15-day fermentation in the form of coffee juice and pulp sugar with three repeats. The absorbance data was substituted as the value of y in the linear regression equation that had been formed from the standard curve of the error acid, namely y = 0.0303x + 0.0062 so that the total levels of phenol juice and sugar pulp of fermented coffee for 10 and 15 days were obtained from three repetitions.

Table 3. Normality Test Results

	Fermentation Duration	Kolmogorov-Smirnova			Shapiro-Wilk		
		Statistics	Df	Sig.	Statistics	Df	Sig.
Result	10-day absorbance	0,328	3	.	0,871	3	0,298
	15-day absorbance	0,343	3	.	0,842	3	0,220

	10-day total phenol levels	0,328	3	.	0,871	3	0,298
	15-day total phenol levels	0,343	3	.	0,842	3	0,220
a. Lilliefors Significance Correction							

Source: SPSS Data Acquisition Results

The absorbance data and data on the total phenol levels in the three replicates obtained in Table 2, will be tested for normality to determine whether the data used is distributed normally. Based on Table 3. The results of the normality test showed that the values of all absorbance data and the total phenol levels obtained by 3 repetitions in the juice and sugar pulp of 10 and 15 days of fermented coffee were distributed normally, which was indicated by a P-value of more than 0.05.

Table 4. Paired Sample T-Test Results

Long Treatment of Fermentation	Paired Differences					t	Df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Pair 1 10 Days - 15 Days	0,5391	0,1008	0,0582	0,2887	0,7896	9,262	2	0,011

Source: SPSS Data Acquisition Results

Based on Table 3 which shows that the data is normally distributed, the Paired Sample T-Test is carried out to find out if there is a difference in the average of the two paired samples. The results of the Paired Sample T-Test in Table 4 showed that the comparison of fermentation treatment affected the total phenol content in the juice and sugar pulp of the coffee produced, characterized by a Sig. (2-tailed) (0.011) value of less than 0.05. Based on the average total phenol content of the two samples, it was found that the longer the fermentation process lasts, the lower the total phenol content in the juice and sugar pulp of the coffee produced. The decrease in the total phenol levels in the juice and sugar of coffee pulp as the fermentation takes place can occur because the cultural activity that occurs during the fermentation process of coffee pulp aerobically can degrade phenol compounds (Mahardani & Yuanita, 2021).

Results of Reduced Sugar Content Analysis

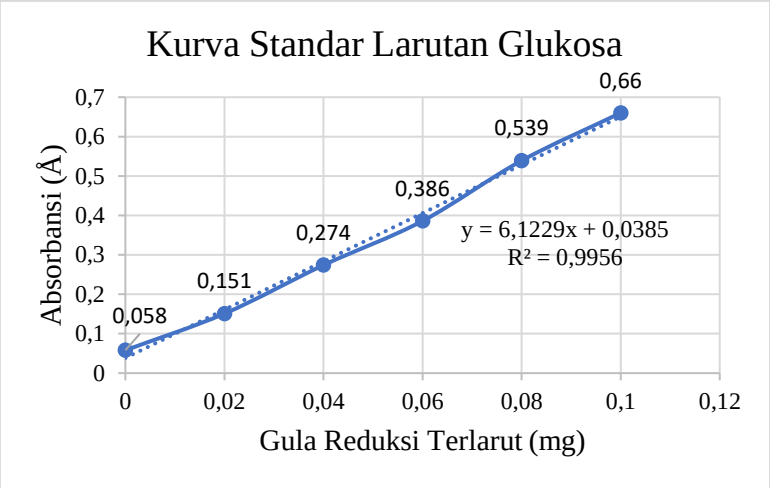

Figure 3. Glucose Solution Standard Curve

Based on the data that has been obtained in Figure 3. The Standard Curve of Glucose Solution was determined by using a standard glucose solution of 0; 0.2; 0.4; 0.6; 0.8; 1 ml with dissolved reduced sugar of 0; 0.02; 0.04; 0.06; 0.08; and 0.1 mg the absorbance values were obtained respectively of 0.058; 0.151; 0.274; 0.386; 0.539; and 0.66 Å. Based on this data, the linear equation y = 6.1229x + 0.0385 with R2 is 0.9956.

Table 5. Reduced Sugar Levels of Cider and Coffee Pulp Sugar

Fermentation Time (days)	Absorbance (Å)	Dissolved Reduced Sugar (mg/ml)	Reduced Sugar Content (%)	Average Reduced Sugar Content (%)
10	0,350	0,0509	0,1271	0,1417
	0,385	0,0566	0,1415	
	0,422	0,0626	0,1566	
15	0,141	0,0167	0,0419	0,0534
	0,194	0.0254	0,0635	
	0,173	0,0219	0,0549	

Source: Test Results

Based on Table 5, absorbance data were obtained from the results of 10-day and 15-day fermentation in the form of coffee juice and pulp sugar with three repetitions. The absorbance data is substituted as a y value in the linear regression equation that has been formed from the curve of the standard glucose solution, namely y = 6.1229x + 0.0385 so that the dissolved reduction sugar of juice and 10 and 15 days of fermented coffee pulp sugar are obtained from three repetitions. The dissolved reduced sugar data obtained was substituted on the reduced sugar content formula with a dilution factor of 250 and a sample mass of 10000 mg, which was then obtained data on the reduced sugar content of cider and fermented coffee pulp sugar for 10 and 15 days which were produced from three repetitions.

Table 6. Normality Test Results

	Fermentation Duration	Kolmogorov-Smirnova			Shapiro-Wilk		
		Statistics	Df	Sig.	Statistics	Df	Sig.
Result	10-day absorbance	0,177	3	.	1,000	3	0,969
	15-day absorbance	0,221	3	.	0,986	3	0,772
	Reduced sugar content 10 days	0,177	3	.	1,000	3	0,974
	Reduced sugar content 15 days	0,220	3	.	0,986	3	0,776

a. Lilliefors Significance Correction

Source: SPSS Data Acquisition Results

Absorbance data and reduced sugar content data in three repetitions obtained in Table 5, will be tested for normality to determine whether the data used is distributed normally. Based on Table 6. The results of the normality test showed that the values of all absorbance data and the total phenol levels obtained by 3 repetitions in the juice and sugar pulp of 10 and 15 days of fermented coffee were distributed normally, which was indicated by a P-value of more than 0.05.

Table 7 Paired Sample T-Test Results

Long Treatment of Fermentation	Paired Differences					t	Df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Pair 1 10 Days - 15 Days	0,0883	0,0122	0,0070	0,0581	0,1185	12,587	2	0,006

Source: SPSS Data Acquisition Results

Based on Table 6 which shows that the data is normally distributed, the Paired Sample T-Test is carried out to find out if there is a difference in the average of the two paired samples. The results of the Paired Sample T-Test in Table 7 showed that the comparison of fermentation treatment affected the reduced sugar content in the juice and coffee pulp sugar produced, characterized by a value of Sig. (2-tailed) (0.006) less than 0.05. Based on the average reduced sugar content of the two samples, it was found that the longer the fermentation process lasts, the lower the reduced sugar content in the juice and sugar pulp of the coffee produced. This is because the sugar formed during fermentation is broken down by yeast to produce alcohol (Finallika & Widjanarko, 2015)

so the sugar content produced in the fermentation of coffee pulp with a longer duration will cause a decrease in reduced sugar content.

Results of Sensory Analysis

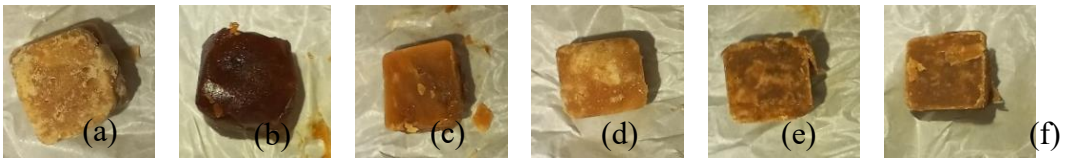

Figure 4. Hard candy with added sugar from 10-day fermentation of coffee pulp (a) 20% (356), (b) 40% (240), (c) 60% (718) and 15-day fermentation (d) 20% (162), (e) 40% (693), (f) 60% (934)

Hard candies resulting from the addition of fermented sugar solution of coffee pulp used for sensory testing can be seen in Figure 4, it can be seen that there are 6 types of candies with different formulations with an average brownish colour. The candy will be tested by panellists to assess related 5 attributes, namely colour, aroma, texture, taste, and overall.

Table 8 Normality Test Results

	Attribute	Kolmogorov-Smirnova			Shapiro-Wilk		
		Statistics	Df	Sig.	Statistics	Df	Sig.
Result	Colour	0,172	198	0,000	0,908	198	0,000
	Aroma	0,182	198	0,000	0,913	198	0,000
	Texture	0,174	198	0,000	0,913	198	0,000
	Taste	0,193	198	0,000	0,908	198	0,000
	Overall	0,176	198	0,000	0,912	198	0,000

a. Lilliefors Significance Correction

Source: SPSS Data Acquisition Results

Sensory test data on each attribute is obtained in Table 8, a normality test will be carried out to determine whether the data used is distributed normally. Based on **Table 8.** The results of the normality test, it was found that the values of all sensory test data on the five attributes in the juice and sugar pulp of coffee fermented for 10 and 15 days were not distributed normally, which was shown by a P-value of less than 0.05.

Table 9 Results of the Coffee Pulp Candy Ranking Test

Formulation	Colour	Aroma	Texture	Taste	Overall
356	3,24	3,12	3,48	3,73	3,20
240	3,33	3,36	3,95	3,61	3,24
718	3,61	3,98	2,92	3,09	3,53
162	2,86	3,15	2,97	3,14	3,30
693	3,58	3,77	3,56	3,50	3,64
934	4,38	3,61	4,11	3,94	4,09

Source: SPSS Data Processing Results

Based on Table 9, it was found that the data obtained from each formulation was not normally distributed with a P-value of less than 0.05, so the Friedman Test was carried out so that the results of the ranking test were obtained for each attribute in each candy formulation. Based on Table 9. The results of the Coffee Pulp Candy Ranking Test were obtained in the order of the panellists' preferences for different formulations in colour, aroma, texture, taste, and overall attributes. The results of the Ranking Test showed that formulation 356 with 10-day fermentation treatment and a concentration of 20% coffee pulp sugar addition was the most preferred in colour and aroma attributes. Meanwhile, the formulation of 718 with a 10-day treatment and a concentration of 60% coffee pulp sugar is most preferred in terms of texture and taste attributes. Overall, the panellists liked the 356 formulations the most. The 356 formulation is preferred because it has the brightest colour and a hard texture close to that of a typical hard candy. This is because the 356 formulation contains the most sucrose sugar compared to other coffee pulp candy formulations.

Conclusion

Fermentation time influences the total phenol content and reduced sugar content in coffee cider and pulp; extended fermentation results in decreased phenol and reduced sugar levels due to the degradation of phenol compounds during aerobic fermentation and the breakdown of sugar by yeast into alcohol. Additionally, a higher concentration of glucose derived from the fermentation of coffee pulp enhances the texture and taste of hard candy products, making them more favourable to panellists, although the colour and aroma attributes may be less preferred.

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