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Non-Alcohol Rose-Apple Antioxidant Drink with Halal Fish Collagen Hydrolysate

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Abstract. Functional drink is currently an interesting study, especially if they are non-alcoholic based as one of the cornerstones of consumer acceptance in several countries. Roses, which are usually only used as decoration, can actually be processed into functional drink with high antioxidant activity. This study uses the basic ingredients of roses and apples with the addition of fish collagen hydrolysate as a non-alcoholic and halal functional drink. Factorial Randomized Design Group 2 factors were used in this study. The first factor was the Rose-Apple ratio variation consisted of 60:40, 50:50, and 40:60 %v/v. The second factor was Fish Hydrolysate Collagen concentration variation consisted of 0, 0.3, 0.5, and 0.7 %w/v. The data then analyzed with ANOVA at $\alpha = 5\%$ and Duncan's Multiple Range Test. This study result that rose-apple extract 50 (%v/v) with fish hydrolysate collagen 0.7 (%w/v) is the best formulation. The characteristic of the best formulation is pH 2.88 (match with SNI standard about fruit extract which pH maximal 4), total dissolved solids is 29.75°Brix (suitable with SNI standard which minimal 10.5°Brix), vitamin C is 5.18%, and antioxidant activity is 91.26%. Comparison result with commercial collagen drink appeared that the best formulation (89.9%) has higher antioxidant activity than commercial product (19.53%). This study aims that rose-apple drink with fish hydrolysate collagen is a non-alcoholic functional drink that is good for health.

Keywords: anthocyanin, collagen, functional drink, halal, rose

INTRODUCTION

Functional drink is currently an interesting study, especially if they are non-alcoholic based as one of the cornerstones of consumer acceptance in several countries [1]. Roses, which are usually only used as decoration, can actually be processed into functional drink with high antioxidant activity. Several studies related to roses have been carried out, some of them using local roses from Batu, Indonesia. The antioxidant activity of rose pigment extract with citric acid solvent is 79.07% [2], and it has physiological abilities to decrease SGOT (Serum Glutamic Oxaloacetic Transaminase) in white mouse from 117.542 U/L to 18.267 U/L [3]. The source of rose's antioxidant is in its anthocyanin pigment and vitamin. Rose's anthocyanin comprises malvidin and cyanidin glucoside with synergistic characteristics with citric acid [4]. The results of previous study indicate that rose drink has the potential to be developed as a non-alcoholic functional drink with the addition of several other ingredients to enhance its activity.

Another Batu city's local commodity known for high antioxidant and vitamin content is *Rome Beauty* apple. *Rome Beauty* apple has high antioxidant activity because contains of 11.42 mg/100 g vitamin C, notably higher than

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other apple varieties [5]. Vitamin C has a correlation with collagen. Vitamin C in foods has function as a collagen forming agent on the body [6, 7]. Vitamin C has a role in the hydroxylation process of procollagen at chemical bond with lysine and proline amino acid. It adds hydroxy to proline and lysine, so it becomes hydroxylysine and hydroxyproline through hydroxylases [7-10].

Collagen in the body helps to enhance muscle, bones, and joints, and it also helps to maintain healthy skin. In consonance with the great benefits of collagen, the addition of collagen is believed will produce functional effects on foods and drink. Collagen can be added to foods and drink to enhance its nutrition and functional traits without developing technical effects on the process. According to the previous clinical study, collagen has good bioavailability on drink [11, 12].

Collagen's commonly used source as a food additive is scale, skin, and bones from cows, pigs, or fish [13, 14]. Collagen from fish is known as a halal alternative source [15, 16]. In addition, collagen from fish has similarities with collagen that is naturally produced in the human body. Fish collagen undergoes hydrolysis with help from an enzyme (hydrolysate) to have lower molecular mass and viscosity also easier to dissolve in water. Hydrolysates contain eight essential amino acids, glycine amino acid, and proline 20 times higher than other protein resource foods [17]. Based on the earlier data, it is essential to study rose-apple antioxidant drink formulation using fish hydrolysate collagen to develop drink with many benefits, nutritious, and fit for consumption.

METHODS

Factorial Randomized Design Group 2 factors were used in this study. The first factor was the Rose-Apple ratio variation consisted of 60:40, 50:50, and 40:60 %v/v. The second factor was Fish Hydrolysate Collagen concentration variation consisted of 0, 0.3, 0.5, and 0.7 %w/v. The data then analyzed with ANOVA at $\alpha = 5\%$ and Duncan's Multiple Range Test.

Rose Extracts Production

Rose was sorted and analyzed its total dissolved solids, pH, water content, protein, vitamin C, antioxidant, and anthocyanin. Sorted rose (45 g) and solvent (water: citric acid, 95: 5 % w/v) with ratio rose (1): solvent (3) was blended and stored in the refrigerator (10 – 12^{o}C). After 30 minutes, the blended rose was filtered and produced the extract or the rose drink [3].

Apple Extracts Production

Rome apple was sorted and analyzed its total dissolved solvent, pH, water content, protein, vitamin Cm antioxidant, and anthocyanin content. Then apple was cleaned, cut, and weighed. Cleaned and cut apple then blanched in 80^{o}C water for 2 minutes. The apple was blended in water with a 1:1 ratio and then strained to extract [18].

Rose-Apple Antioxidant Drink Production

Rose and apple extract were blended with ratio combination (60:40), (50:50), and (40:60) (%v/v). Na Alginate 0.2 and sugar 30% w/v were added, and then pasteurized at 77^{o}C for 1 minute and decreased and stabilized the temperature to 45^{o}C. Hydrolysate collagen was added to the extract with concentration variations 0, 0.3, 0.5, and 0.7 % w/v at 45^{o}C. Rose-Apple antioxidant drink's total dissolved solids, pH, water content, protein, vitamin C, antioxidant activity, and organoleptic were analyzed [19].

RESULTS AND DISCUSSION

Raw Material Analysis

Analysis of raw materials is used to determine the chemical properties of the raw materials used in the produce of antioxidant drink to determine the comparison before and after treatment. Parameters observed in the chemical analysis of raw materials include pH value, total dissolved solids, water content, protein content, vitamin C, antioxidant activity and total anthocyanin. The chemical characteristic of rose and Rome beauty apple can be seen in **TABLE 1.**

TABLE 1. The Chemical Characteristic of Rose and Rome Beauty Apple

Parameter	Rose	Rome Beauty Apple
pH	4.36	3.28
Total Dissolved Solids (ºBrix)	10	8.5
Water Content (%)	83.79	88.93
Protein (%)	2.83	0.39
Vitamin C (mg/100g)	15.29	11.16
Antioxidant Activity (%)	78.09	85.29
Total Anthocyanin (mg/L)	27.35	0.45

Based on **TABLE 1**, the pH value test on Rome Beauty apples, it was found that the pH value was more acidic than that of red roses, which was 3.28 for apples and 4.36 for red roses. Total Dissolved Solids of roses appeared 10 Brix and Rome Beauty 8.5 Brix apples, which means that there are 10 and 8.5 grams of sucrose in 100 grams of roses and apples. The water content in red roses is 83.79%, beside that apple's water content is 88.93%. The protein content in roses after being tested is known to be 28.27 mg/100g while in apples it is 3.91 mg/100g. Vitamin C in red roses is 15.29 mg/100g while vitamin C in Rome Beauty apples is 11.16 mg/100g. antioxidant activity contained in red roses is 78.09%, while Rome Beauty apples have antioxidants of 85.29%. The total anthocyanin test carried out appeared that the roses studied had a total anthocyanin of 27.35 mg/L, while Rome Beauty apples had 0.45 mg/L of anthocyanin.

Rose-Apple Drink with Fish Collagen Hydrolysate

pH Analysis

According to the ANOVA analysis, there was no interaction between rose-apple drink ratio variation and the variation of fish hydrolysate collagen ($p>0.05$).

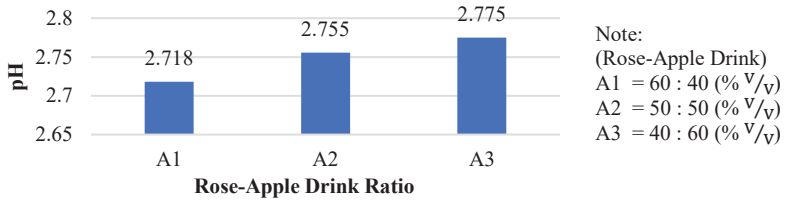

FIGURE 1. pH of Rose-Apple Drink with Ratio Combination Treatment

Based on **FIGURE 1**, the acidity level caused by rose-apple drink ratio treatment appeared that a higher rose extract ratio would cause its pH to decrease, and the drink taste became sourer. Citric acid is a strong organic acid, so citric acid in the extraction process will affect water solvent condition become more acid [20]. Moreover, according to raw material analysis, the rose has higher vitamin C content than the *Rome Beauty* apple. Also, [21]. pH of rose-apple drink with fish hydrolysate collagen concentration treatment appeared in **TABLE 2.**

TABLE 2. pH of Rose-Apple Drink pH with Fish Hydrolysate Collagen Concentration Treatment

Treatment	**pH**
B0 (without collagen)	2.660^{a}
B1 (fish hydrolysate collagen concentration 0.3 (%$^{W}/_{V}$))	2.745^{b}
B2 (fish hydrolysate collagen concentration 0.5 (%$^{W}/_{V}$))	2.778^{bc}
B3 (fish hydrolysate collagen concentration 0.7 (%$^{W}/_{V}$))	2.814^{c}

Note: The column with the same letter stated insignificant result according to DMRT 5%

TABLE 2 appeared that rose-apple drink with fish hydrolysate collagen had higher pH. The higher amount of hydrolysate collagen added would increase pH value. pH value will affect collagen solubility that causes by low

crosslink degree. The difference in pH value can also be affected by solvent variety and acid or base concentration used in the collagen hydrolysis process [22-24].

Total Dissolved Solids Analysis

According to the analysis of variance, no factor affected the interaction and each factor to the rose-apple drink's total dissolved solids ($p<0.05$). Total dissolved solids of rose-apple drink with rose-apple ratio combination treatment showed in **FIGURE 3.**

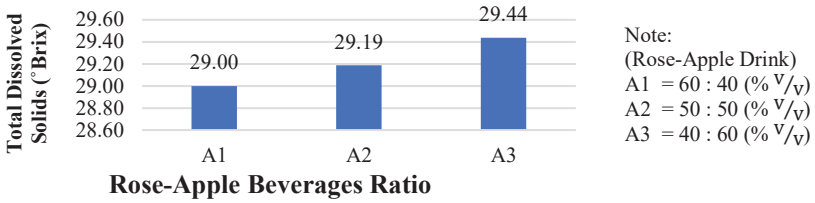

FIGURE 3. Total Dissolved Solids of Rose-Apple Drink with Rose-Apple Ratio Combination Treatment

Total dissolved solids are closely related to the product's total sugar level and organic acid. Higher apple extract's total dissolved solids can be caused by the apple heating process and high organic acid content in Rome Beauty apple. Acid at heating process on apple extract will hydrolyze sucrose in apple and become inverted sugar like glucose and fructose, which is included as reducing sugar that will increase apple extract's total dissolved solids. Components included as total dissolved solids are sucrose, reducing sugar, organic acid, and protein [25]. The values of rose-apple drink total dissolved solids with fish hydrolysate collagen concentration treatment can be seen in **FIGURE 4.**

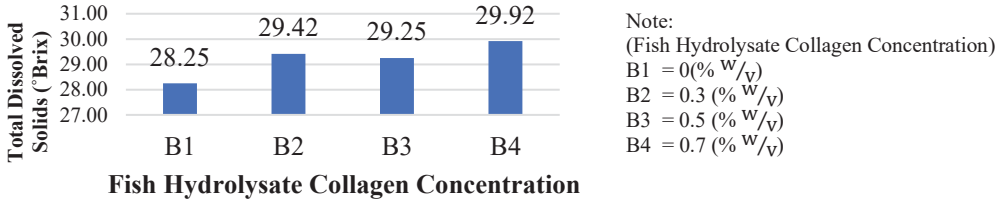

FIGURE 4. Total Dissolved Solids of Rose-Apple Drink with Fish Hydrolysate Collagen Concentration Treatment

Based on **FIGURE 4**, collagen hydrolysates caused smaller collagen molecules and made their water solubility higher. The higher collagen concentration added Rose-Apple drink, and the total dissolved solids would increase. The hydrolysis of collagen will cause the amino acid covalent bond to break and decrease collagen molecule mass and increase its solubility in water.

Vitamin C Content

According to the data, Rose-Apple drink ratio appeared that there was no effect between treatment. Vitamin C rose-apple drink with rose-apple ratio combination appeared in **FIGURE 5.**

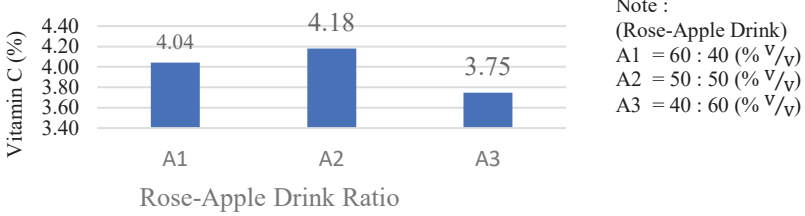

FIGURE 5. Vitamin C of Rose-Apple Drink with Rose-Apple Ratio Combination Treatment

FIGURE 5 appeared that the higher amount of rose extracts would increase its vitamin C content. This was because rose has higher vitamin C content than apple. According to Saati (2014), rose's vitamin C content is 17.23 mg/100 g, moreover according to Estiasih and Khurniyati (2015), Rome Beauty apple's vitamin C content is 11.42 mg/100 g. Furthermore, apple's vitamin C decrease in Rose-Apple drink-making heating process.

TABLE 3. Vitamin C of Rose-Apple Drink with Fish Hydrolysate Collagen Concentration Treatment

Treatment	Vitamin C (%)
B0 (without collagen)	2.98[a]
B1 (fish hydrolysate collagen concentration 0.3 ($\%^{W}/_{V}$))	3.52[ab]
B2 (fish hydrolysate collagen concentration 0.5 ($\%^{W}/_{V}$))	4.50[bc]
B3 (fish hydrolysate collagen concentration 0.7 ($\%^{W}/_{V}$))	4.97[c]

Note: The column with the same letter stated insignificant result according to DMRT 5%

TABLE 3 appeared that the Rose-Apple drink's vitamin C value would increase as fish hydrolysate collagen increased. Collagen has high water affinity power and causes the higher withdrawal of colloid particles and oxygen that cause oxidation in fruit extract. The higher amount of oxidation will decrease flower and fruit's vitamin C

Antioxidant Activity

Antioxidant analysis was done to discover the extract's active compound that can suppress free radicals (DPPH). Figure 6. appeared that the Rose-Apple drink ratio combination did not affect antioxidant activity.

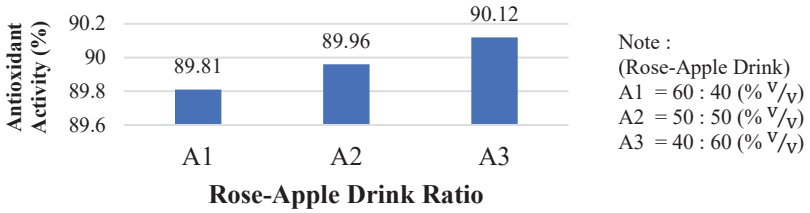

FIGURE 6. Antioxidant Activity of Rose-Apple Drink with Rose-Apple Ratio Combination Treatment

FIGURE 6 appeared that Rose-Apple drink antioxidant activity increased with a higher amount of apple extract that added to the drink. This was because apple contains quercetin as an antioxidant. Rome Beauty apple's quercetin content is 477.96 mg/L [26]. Furthermore, rose contains anthocyanin pigment consisted of pelargonidin and cyanidin that has function as an antioxidant [3]. Low rose extract antioxidant possibly caused by anthocyanin as rose's antioxidant was degraded. Anthocyanin could be degraded because of some factors like temperature, pH, light, structure, oxygen, solvent, enzyme, and metal ion [27]. Antioxidant activity of rose-apple drink with hydrolysate collagen concentration treatment appeared in **TABLE 4.**

TABLE 4. Antioxidant Activity of Rose-Apple Drink with Fish Hydrolysate Collagen Concentration Treatment

Treatment	Antioxidant Activity (%)
B0 (without collagen)	89,43[a]
B1 (fish hydrolysate collagen concentration 0.3 ($\%^{W}/_{V}$))	90,00[a]
B2 (fish hydrolysate collagen concentration 0.5 ($\%^{W}/_{V}$))	90,00[a]
B3 (fish hydrolysate collagen concentration 0.7 ($\%^{W}/_{V}$))	91,47[b]

Note: The column with the same letter stated insignificant result according to DMRT 5%

TABLE 4 appeared that the higher amount of fish hydrolysate collagen added to the drink, its antioxidant activity would increase. Collagen antioxidant activity is affected by factors like structure, composition, amino acid hydrophobicity, and molecule mass. The higher peptide antioxidant activity positively correlated with low molecule mass [28].

Rose-Apple Drink Best Treatment with De Garmo Analysis

De Garmo analysis was done to discover the rose-apple extract ratio and fish hydrolysate collagen concentration treatment with the highest score on the overall parameter. The effectivity score can be seen in **TABLE 5.**

TABLE 5. The Best Treatment Determined with De Garmo Method

Treatment	ES	Rank
A1B0 Rose-Apple Drink 60 : 40 ($\%^{V}/_{V}$) + Fish Hydrolysate Collagen 0 ($\%^{W}/_{V}$)	0.453	11
A2B0 Rose-Apple Drink 50 : 50 ($\%^{V}/_{V}$) + Fish Hydrolysate Collagen 0 ($\%^{W}/_{V}$)	0.590	6
A3B0 Rose-Apple Drink 40 : 60 ($\%^{V}/_{V}$) + Fish Hydrolysate Collagen 0 ($\%^{W}/_{V}$)	0.538	7
A1B1 Rose-Apple Drink 60 : 40 ($\%^{V}/_{V}$) + Fish Hydrolysate Collagen 0.3 ($\%^{W}/_{V}$)	0.499	8
A2B1 Rose-Apple Drink 50 : 50 ($\%^{V}/_{V}$) + Fish Hydrolysate Collagen 0.3 ($\%^{W}/_{V}$)	0,480	9
A3B1 Rose-Apple Drink 40 : 60 ($\%^{V}/_{V}$) + Fish Hydrolysate Collagen 0.3 ($\%^{W}/_{V}$)	0,599	5
A1B2 Rose-Apple Drink 60 : 40 ($\%^{V}/_{V}$) + Fish Hydrolysate Collagen 0.5 ($\%^{W}/_{V}$)	0,427	12
A2B2 Rose-Apple Drink 50 : 50 ($\%^{V}/_{V}$) + Fish Hydrolysate Collagen 0.5 ($\%^{W}/_{V}$)	0,709	2
A3B2 Rose-Apple Drink 40 : 60 ($\%^{V}/_{V}$) + Fish Hydrolysate Collagen 0.5 ($\%^{W}/_{V}$)	0,635	4
A1B3 Rose-Apple Drink 60 : 40 ($\%^{V}/_{V}$) + Fish Hydrolysate Collagen 0.7 ($\%^{W}/_{V}$)	0,686	3
A2B3 Rose-Apple Drink 50 : 50 ($\%^{V}/_{V}$) + Fish Hydrolysate Collagen 0.7 ($\%^{W}/_{V}$)	**0,771**	**1**
A3B3 Rose-Apple Drink 40 : 60 ($\%^{V}/_{V}$) + Fish Hydrolysate Collagen 0.7 ($\%^{W}/_{V}$)	0,460	10

According to **TABLE 5,** the best Rose-Apple drink formulation that has been analyzed is A2B3 treatment (rose-apple drink 50:50 %v/v with 0.7 %w/v fish hydrolysate collagen concentration) with water content 70.33%, vitamin C 5.18%, pH 2.88, total dissolved solids 29.75°Brix, protein content 1.08%, antioxidant 91.26%, and overall effectivity score 0.771. According to Standard Nasional Indonesia (SNI, 2014) about apple extract, Rose-Apple antioxidant drink meet with SNI standard with unique and normal aroma, flavor, and color, maximal pH 4, and total dissolved solids higher than 10.5°Brix.

Comparison between Rose-Apple Drink Best Treatment and Commercial Collagen Drink

Antioxidant activity analysis was done to the best formulation and collagen products in the market using DPPH free radical scavenging method. The antioxidant activity of rose-apple drink (best formulation) and commercial collagen drink appeared in **TABLE 6.**

TABLE 6. The Antioxidant Activity of Rose-Apple Drink (Best Formulation) and Commercial Collagen Drink

Treatment	Antioxidant Activity (%)
Commercial collagen Drink	19.53
The Best Formulation (Rose-Apple Drink Ratio 50 : 50 ($\%^{V}/_{V}$) + Fish Hydrolysate Collagen 0.7 ($\%^{W}/_{V}$))	91.26

According to **TABLE 6**, the best formulation has higher antioxidant activity than commercial product. This was caused by the addition of fish hydrolysate collagen on each product that increases antioxidant activity. Hydrolysate collagen produces a more stable product to free radicals and has high antioxidants as electron donors [29]. Rose-Apple drink high antioxidant activity (89.97-91.26%) caused not only by fish hydrolysate collagen content,

but also from *Rome Beauty* apple phenolic compounds, organic acids, vitamin C, rose anthocyanin pigment, and high vitamin C content in rose. Rose is known to contain anthocyanin pigment even when the rose is not fresh. Rose-apple antioxidant drink with fish hydrolysate collagen were relatively high presumed because of rose used in the study contain 27.35 mg/L anthocyanin, and the best formulation contain 67.48 mg/L. Moreover, market collagen drink antioxidant, as stated in the ingredients, came from ascorbic acid antioxidant (vitamin C), L-glutathione, algae, pomegranate extract, fish hydrolysate collagen, and synthetic antioxidant that added in the products on purpose as a food additive. The other factors that keep anthocyanin's stability, so it will not easily shift and degraded are temperature, pH, oxygen, light, and metal ion [2], co-pigment, enzyme, concentration, and pressure [30]. The anthocyanin of a similar product in the market could not be identified as presumed caused by temperature, time, and pressure in the production process. Usually, a similar product in the market is in a powder that used high temperature. Anthocyanin instability causes the compounds to be hydrolyzed at glycosidic bond, and aglycon ring open also form stable aglycon and colorless carbonyl and chalcon group [31].

CONCLUSIONS

Rose-Apple drink best formulation is A2B3 (rose-apple extract 50 (%v/v) with fish hydrolysate collagen 0.7 (%w/v)) with physical characteristic pH 2.88 (suitable with SNI) and total dissolved solids 29.75°Brix (suitable with SNI), vitamin C 5.18%, antioxidant activity 91.26%. Comparison result with commercial collagen drink appeared that the best formulation 1 (Rose-Apple Drink Ratio 50 : 50 ($\%^{V}/_{V}$) + Fish Hydrolysate Collagen 0.7 ($\%^{W}/_{V}$) has higher antioxidant activity than commercial product. The antioxidant activity of the best formula is is 91.26% and Commercial Collagen Drink is 19.53%. This study aims that rose-apple drink with fish hydrolysate collagen is is a non-alcoholic functional drink that is good for health. The results of this study can be continued for large-scale manufacture and organoleptic tests to determine consumer acceptance in the future.

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