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Utilization of Super Red Dragon Fruit Peel (*Hylocereus Costaricensis* (F.A.C. Weber) Britton & Rose) in the Making of Fermented Beverage

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Abstract. Utilization of super red dragon fruit (*Hylocereus costaricensis*) are often limited to its flesh, whereas the peel as much as 30-35% weight of fruit was rich in antioxidants. Further processing is needed to increase the consumption of the dragon fruit peel. Effort achievement was made through fruit peel powder application on fermented beverage. Fermentation process (42°C within 14 hours) was conducted with 6.0% (v/v) of *Streptococcus thermophilus*: *Lactobacillus plantarum*: *Lactobacillus bulgaricus* = 1:1:1, skim milk (5% and 10%), and the peel powder of red dragon fruit (1.0, 1.5, 2.0%). Skim milk (1%) and dragon fruit peel powder (5%) were the best formulation based on pH value (4.05-4.08), total of titrated acids [TTA] (0.74%), and total of lactic acid bacteria [LAB] (Log 8.77-8.91 CFU/ml). The best formulation was then used to determine LAB culture ratio (*S. thermophilus*: *L. plantarum*: *L. bulgaricus* (1:1:1, 1:1:2, 1:2:1, 2:1:1). The ratio of 1:1:1 is selected as the best ratio based on pH (4.14), TTA (0.69%), total LAB (Log 9.41 CFU/ml). The product with preferred formulation contains phenolic 483.67 mg GAE/ml, flavonoid 315.59 mg QE/ml, IC₅₀ 39.94x10⁴ mg/l (increased 241% [very strong level]), declared microbiologically safe (free of coliform), and still acceptable by consumers in hedonic (4.69 of 7.0).

Keywords: antioxidant, fermentation, *Hylocereus costaricensis*, peel, lactic_acid_bacteria

1. Introduction

Super red dragon fruit can be found in Indonesia as a preferred fruit [33]. However, the utilization of dragon fruit is usually only limited to its fruit, even though according to Megawati and Ulinuha (2014) and Waladi, *et al.* (2015), the peel of dragon fruit gives 30-50% from overall weight before utilized. Stinzing, *et al.* (2002), Jamilah, *et al.* (2011), and Saati (2010) research also reveal that the peel of dragon fruit is rich in polyphenol, anthocyanin, and betacyanin that are potential as antioxidant. Jaafar, *et al.* (2009) and Nurliyana, *et al.* (2010) added that antioxidant component such as vitamin C and polyphenol compound in red dragon fruit peel is higher than its fruit.



The utilization of dragon fruit peel can be done through application of dragon fruit peel on fermented drinks. Lactic acid bacteria (LAB) such as *Streptococcus thermophilus*, *Lactobacillus plantarum*, and *Lactobacillus bulgaricus* have the potential to increase antioxidant activity during fermentation process [25]. This research used dragon fruit peel powder as a raw material that has a longer shelf life than the juice form. Dragon fruit peel powder (1.0, 1.5, and 2.0%) with skim milk concentration (5 and 10%) under fermentation condition 42°C for 14 hours assisted by 6.0% (v/v) LAB mixed culture. Formula that gave the best fermented drink according to national-international standards based on a number of analyzes (pH, Total Titratable Acidity, and total LAB), furthermore was used to determine the ratio of *S. thermophilus*: *L. plantarum*: *L. bulgaricus* culture (1:1:1, 1:1:2, 1:2:1, and 2:1:1). pH, Total Titratable Acidity, and total LAB analysis were done and compared with national-international standard to determine the best LAB ratio. Furthermore, total phenolic, total flavonoid, antioxidant activity and microbiology safety quality (coliform) were performed on product with selected formula and LAB ratio. Fermented drink product with antioxidant activity from dragon fruit peel was expected to give solution for the utilization of unused fruit parts, as well as increasing product diversification and consumer health.

2. Materials and methods

2.1 Material

Materials used are: super red dragon fruit peel (*Hylocereus costaricensis*), skim milk powder, sucrose, aquades, *Streptococcus thermophilus*, *Lactobacillus plantarum*, and *Lactobacillus bulgaricus* culture, Eosin Methylene Blue Agar (EMBA), Man Rogosa Sharpe Agar (MRSA), and Man Rogosa Sharpe Broth (MRSB). Analysis materials used are: NaOH, NaCl, AlCl₃, HCl, selenium, boric acid, DPPH, dye solution, standard ascorbic acid, HPO₃-HOAc, phenolphthalein, pH 4 and pH 7 buffer, Folin-Ciocalteu, Na₂CO₃, ethanol, and 70% alcohol.

2.2 Methods

Research phase consisted of the production of dragon fruit peel powder phase, phase 1, and phase 2. The production of dragon fruit peel powder phase involved sorting and cleaning; incision (~2~3 mm); drying (cabinet dryer 60°C, 10 hours); size reduction (blender); and sifting (60 mesh). Dragon fruit peel powder according to its concentration used in research phase I. Research phase I (Figure 1) begun with dissolution of peel powder (1.0, 1.5, 2%) inside water and filtering. Extract gained then added 5% (b/v) sugar and skim milk (5 and 10%) (b/v). Pasteurization is done (80°C, 15 minutes) and cooled down until 42°C. As much as 6% *S. thermophilus* (10 hours), *L. plantarum* (12 hours), and *L. bulgaricus* (10 hours) starter with ratio of 1:1:1 were inoculated and incubated for 14 hours at 42°C. Red dragon fruit peel powder fermentation drink then analyzed pH (AOAC, 2005), total titratable acidity (AOAC, 2005), and total LAB (Wehr and Frank, 2013). Selected formulation dragon fruit peel powder to water ratio and skim milk concentration was used on the next step of research through the comparison of every test parameter against BSN (2009), CAC (2003), U.S. Food and Drug Administration/USDA (2013), Food Standards Australia New Zealand (2014), Japan External Trade Organization/JETRO (2011), and Kenya Bureau of Standards/KEBS (2013).

Phase II research (Figure 1) was done by inoculating *S. thermophilus* (10 hours), *L. plantarum* (12 hours), and *L. bulgaricus* (10 hours) starter with ratio of 1:1:1, 1:1:2, 1:2:1, and 2:1:1. Incubation condition in fermentation process and analysis parameter was done just like the previous phase. Culture ratio that can gave results according to BSN (2009), CAC (2003), U.S. Food and Drug Administration/USDA (2013), Food Standards Australia New Zealand (2014), Japan External Trade Organization/JETRO (2011), and Kenya Bureau of Standards/KEBS (2013) was then determined as selected ratio. Chemical test such as antioxidant activity [23], phenolic [16], flavonoid [16], and microbiological test such as coliform [4] was performed on fermentation drink from selected formulation and ratio. Furthermore, it was evaluated proximately [2], toxicity [18], and hedonic (1-7 range) of 70 trained panelists, most of them were students from semester 5 to semester 8 of the UPH Food Technology study program.

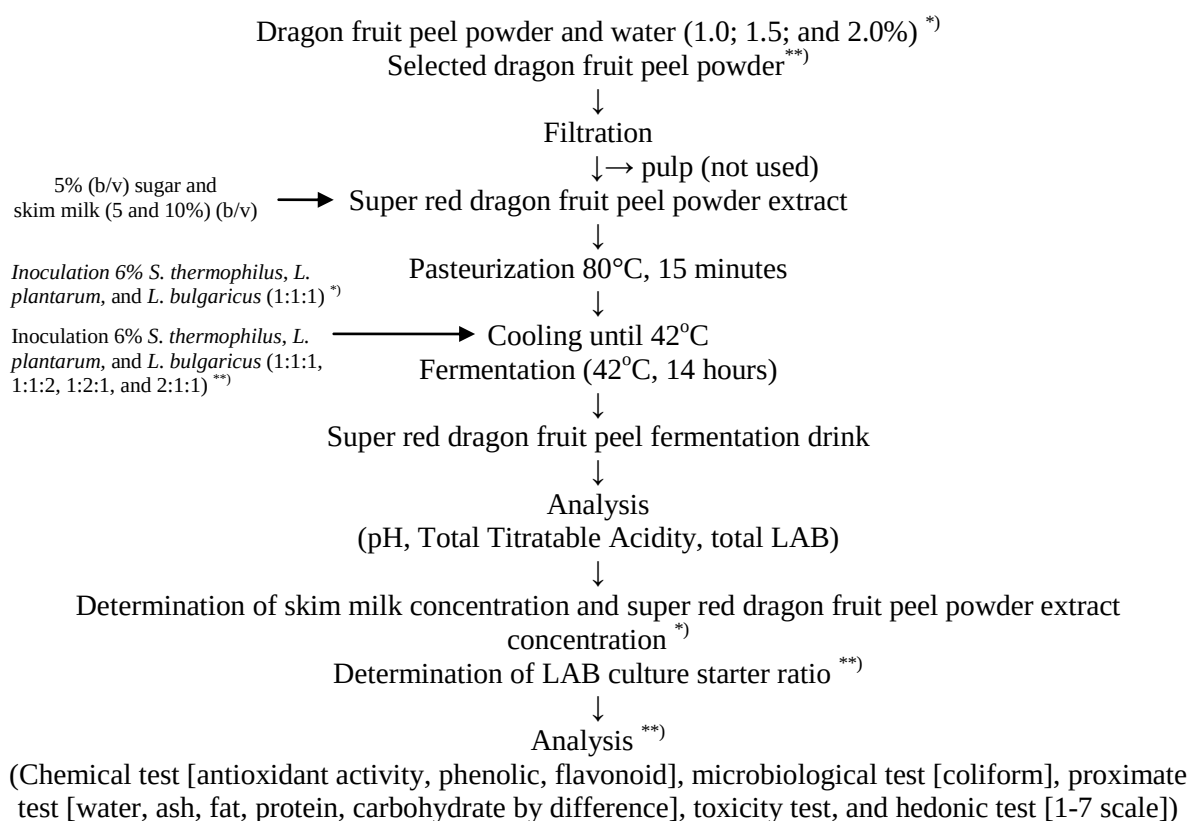


Figure 1. Flow Chart of Research Phase I *) and Phase II **)

2.3 Experimental design

Experimental design in phase I research was Completely Randomized Design with two factors. The first factor (skim milk concentration) consisted of two-level (5% [B1] and 10% [B2]) with three repetitions. The second factor (dragon fruit peel powder concentration) consist of three level (1.0% [A1], 1.5% [A2], 2.0% [A3]) with three repetition. Phase II research used Completely Randomized Design with one factor which is the ratio of *S. thermophilus*, *L. plantarum*, and *L. bulgaricus* culture consist of four level (1:1:1 [A1], 1:1:2 [A2], 1:2:1 [A3], and 2:1:1 [A4]) with three repetition.

3. Result

3.1 Phase I

Phase I research was done to determine skim milk concentration (5 and 10%) and red dragon fruit peel powder (1.0, 1.5, and 2.0%) through pH value, total titratable acidity, and total LAB analysis and comparing their results with BSN (2009), CAC (2003), USDA (2013), Food Standards Australia New Zealand (2014), JETRO (2011), and KEBS (2013). *S. thermophilus*, *L. plantarum*, and *L. bulgaricus* culture as much as 6% that were inoculated had optimum age of 10 hours (1.8×10^8 cfu/ml), 12 hours (1.6×10^9 cfu/ml), and 10 hours (3.7×10^8 cfu/ml).

Statistical test results between skim milk concentration and dragon fruit peel powder concentration did not show any interaction ($p > 0.05$) against fermented drink pH, while skim milk concentration gave effect ($p < 0.05$) and dragon fruit peel powder concentration did not give any effect ($p > 0.05$) against pH of fermented drink. Table 1 shows that the fermentation process decreases the pH value, and the higher the concentration of skim milk, the higher the pH value. Fermentation process gave LAB a chance to produce lactic acid that was going to be decomposed into H^+ ion and $CH_3CHOCOO^-$. However, milk as buffer would minimize changes in pH due to lactic acid that was produced from fermentation process [36]. Increase in dragon fruit peel powder concentration was not

enough to become a good substrate for LAB so that the number of H^+ ion from low organic acid did not give significant effect in pH value.

Table 1. Phase I Test Result (pH, Total LAB)

	pH	Total LAB (Log)
Control (unfermented) ***)	6.39	5.6
Skim milk concentration		
5	4.05±0.08 ^a	8.8±0.14 ^a
10	4.12±0.03 ^b	8.9±0.16 ^a
Super red dragon fruit peel powder concentration		
1.0	4.08±0.00 ^a	8.9±0.17 ^a
1.5	4.07±0.08 ^a	8.9±0.19 ^a
2.0	4.10±0.01 ^a	8.7±0.10 ^a

Note: - Different notation showed there was significant difference ($p < 0.05$)
 - No comparison parameter analysis and treatment ***)
 *** not compared to other data (as supporting data)

Table 2. Phase I Test Result (TTA)

Skim Milk (%)	Super Red Dragon Fruit Peel Powder (%)	Total Titratable Acidity (%)
Control (unfermented) ***)		0.24
5	1.0	0.74±0.04 ^b
	1.5	0.59±0.03 ^a
	2.0	0.63±0.03 ^a
10	1.0	1.04±0.04 ^e
	1.5	0.93±0.04 ^d
	2.0	0.83±0.04 ^c

Note: - Different notation showed there was significant difference ($p < 0.05$)
 *** not compared to other data (as supporting data)

Statistical test result between skim milk concentration and dragon fruit peel powder showed that both of them interacted ($p < 0.05$) against total titratable acidity value, likewise with skim milk concentration and dragon fruit peel powder that had significant effect ($p < 0.05$) against total titratable acidity value. Table 2 shows that fermentation process increases total titratable acidity value, the increase of skim milk concentration will increase total titratable acidity value, and the increase of dragon fruit peel powder concentration tend to decrease total titratable acidity value. During fermentation, LAB degrades skim milk lactose and broken down through Embden-Meyerhoff Parnas (EMP) glycolysis pathway so that the increase of total titratable acidity happened because of lactic acid produced [17]. Muljatma (2016) said that dragon fruit peel has flavonoid content that can denature bacteria cell protein and terpenoid which is an antibacterial. Inhibited LAB growth could decrease total titratable acidity.

Statistical test result between skim milk concentration and dragon fruit peel powder concentration did not show any interaction ($p > 0.05$), as well as for each factor, did not give significant effect ($p > 0.05$) against total LAB. Table 1 shows that total LAB was increasing while fermentation process happened, total LAB did not have significant difference because of the increase of skim milk concentration and dragon fruit peel powder. Fermentation process could increase total LAB because of the skim milk and sugar used by LAB for its growth.

Based on pH value, total titratable acidity, and total LAB analysis results that were compared with BSN (2009), CAC (2003), USDA (2013), Food Standards Australia New Zealand (2014), JETRO (2011), and KEBS (2013) in Table 3, the best fermented drink from super red dragon fruit peel formulation was fermented drink with 1.0% dragon fruit peel powder and 5% skim milk. Table 2 shows that this formulation could produce fermented drink with lower pH, total titratable acidity that met the KEBS (2013) standard, and total LAB that was higher than CAC (2003) and JETRO (2011) standard. This formula was used for phase II research to determine the best culture ratio between *S. thermophilus*, *L. plantarum*, and *L. bulgaricus*.

Table 3. Fermented drink standard

Standard	pH Value	Total Titratable Acidity (%)	Total LAB (CFU/ml)
BSN (2009)	-	0,2-0,9	> 10 ⁶
CAC (2003)	-	> 0,6	> 10 ⁷
FDA (2013)	-	> 0,6	> 10 ⁶
FSANZ (2014)	< 4,5	-	> 10 ⁶
JETRO (2011)	-	-	> 10 ⁷
KEBS (2013)	-	0,7-0,9	-
Selected Formulation	4.05-4.08	0.74	~10 ⁸
Selected Ratio	4.14	0.69	~10 ⁹

3.2 Phase II

Phase II research was done to determine the culture ratio of *S. thermophilus*, *L. plantarum*, and *L. bulgaricus* with ratio of 1:1:1, 1:1:2, 1:2:1, and 2:1:1 through pH, total titratable acidity, and total LAB analysis and compare the results with BSN (2009), CAC (2003), U.S. Food and Drug Administration/USDA (2013), Food Standards Australia New Zealand (2014), Japan External Trade Organization/JETRO (2011), and Kenya Bureau of Standards/KEBS (2013). In this phase fermented drink analysis from selected formulation and ratio such as chemical test (antioxidant activity, phenolic, flavonoid), microbiology test (coliform), proximate test (water, ash, fat, protein, carbohydrate by difference), toxicity, and hedonic (1-7 scale) were also done.

Table 4. Phase II Test Result (pH, Total LAB, TTA)

LAB <i>S. thermophilus</i> : <i>L. plantarum</i> : <i>L. bulgaricus</i> Ratio	pH (%)	TTA (%)	Total LAB (Log)
Control (unfermented)	6.39	0.24	5.6
1:1:1	4.14±0.03 ^a	0.69±0.03 ^c	9.4±0.02 ^c
1:1:2	4.20±0.03 ^a	0.62±0.08 ^{bc}	9.1±0.05 ^b
1:2:1	4.16±0.14 ^a	0.57±0.04 ^{ab}	8.9±0.02 ^a
2:1:1	4.24±0.03 ^a	0.47±0.06 ^a	8.9±0.05 ^a

Note: - Different notation showed there was significant difference (p<0.05)

- No comparison parameter analysis and treatment

Statistical test result of culture ratio did not give significant effect (p>0.05) against pH value. Table 4 shows that pH value from different culture ration combination did not show any difference. The addition of skim milk that contains lactose was useful as nutrition source for lactic acid bacteria [9]; however, because milk is a buffer, there was only a slight change in pH [36].

The statistical test result of culture ratio gave a significant effect (p<0.05) against total titratable acidity. Table 4 shows that 1:1:1 ratio gave the highest total titratable acidity (0.69%). Vinderola, *et al.* (2001) research showed that *L. bulgaricus* has a role in giving peptide for *S. thermophilus* growth. Along with the usage of peptide by *S. thermophilus*, *L. bulgaricus* growth will slow down. In that research, it was also said that the addition of several *L. bulgaricus* strain will inhibit *S. thermophilus*, and vice versa. This would cause the 1:1:2 and 2:1:1 culture ratio gave lower total titratable acidity compared to fermented drink with 1:1:1 culture ratio. Vinderola, *et al.* (2001) added that the presence of *L. plantarum* can inhibit LAB growth (*L. bulgaricus* and *S. thermophilus*) and decrease lactic acid which caused total titratable acidity value decreased. 1:2:1 culture ratio compared to 1:1:1 culture ratio confirmed that statement

Statistical test result of culture ratio gave significant difference (p<0.05) towards total LAB that was produced during fermentation process. Table 4 shows that 1:1:1 culture ratio gave the highest total

LAB compared to another culture ratio (log 9.41 cfu/ml). Peptide provided by *L. bulgaricus* for *S. thermophilus* for its growth will cause *L. bulgaricus* growth slowed down. This result confirmed the number of LAB in fermented drink with 1:1:2 culture ratio was less than 1:1:1 culture ratio. As well as the presence of *L. plantarum* that could inhibit both of those LAB could confirm the total LAB of 1:2:1 culture ratio was less than 1:1:1 culture ratio.

Based on the analysis of pH, total titratable acidity, and total LAB that was compared with BSN (2009), CAC (2003), USDA (2013), Food Standards Australia New Zealand (2014), JETRO (2011), and KEBS (2013), LAB culture ratio that could give the best fermented drink was the 1:1:1 culture ratio. Table 4 shows that this culture ratio could produce fermented drink with lower pH, total titratable acidity that met the CAC (2003) and FDA (2013) standards, was the closest to KEBS (2013) standard, and total LAB that was a little higher than CAC (2003) and JETRO (2011) standards.

Several analyses were carried out on the fermented drink from selected formula and ratio such as: chemical test (antioxidant activity, phenolic, flavonoid), microbiology test (coliform), proximate test (water, ash, fat, protein, carbohydrate by difference), toxicity, and hedonic (1-7 scale). Antioxidant activity was done in order to know the antioxidant potential in fermented drink that can inhibit free radical. The analysis was done by determining IC₅₀ that is correspondent to the sample concentration and can reduce 50% DPPH. The smaller the IC₅₀ value shows higher antioxidant inhibition [26]. Results showed that IC₅₀ of fermented drink from dragon fruit peel was 39.94×10⁴ ml/l. this value increased 241% from dragon fruit peel extract IC₅₀ (94.70×10⁴ mg/l). According to Primurdia and Kusnadi (2014), IC₅₀ value is grouped in 4 categories that are very strong (IC₅₀<50 mg/l), strong (IC₅₀ 50-100 mg/l), medium (IC₅₀ 101-150 mg/l), and weak (IC₅₀>150 mg/l), so that fermented drink from dragon fruit peel antioxidant was categorized as very strong.

Phenolic and flavonoid test was done in order to know their presence in antioxidant activity increment. Total phenolic content was 483.67 mg GAE/l, increased 315.63% compared to dragon fruit peel extract before fermentation (116.37 mg GAE/l); while flavonoid content increased 238.14% from 93.33 mg GAE/l (before fermentation) to 315.59 mg GAE/l (after fermentation). Total phenolic and flavonoid increment was caused by the increase in LAB metabolism that hydrolyzes glucoside and caused a change in phenolic composition [28]. Moreover, LAB also produces enzyme to break down sugar and free up phenolic compound that contributes in adding phenol group in flavonoid compound [6, 31, 10]. Miller and Wolin (1996) added that fermentation process can increase flavonoid content because LAB can degrade fiber into simpler compound so that the covalent bond in insoluble fiber can release phenolic compound and increase its bioavailability.

Coliform bacteria test was done as a sanitary indicator of drink product. Generally, bacteria that is categorized as coliform are *Escherichia*, *Enterobacter*, *Citrobacter*, *Hafnia*, *Serratia*, *Edwardseilla*, *Proteus*, *Arizona*, *Pseudomonas*, *Bacil paracolon*, *Providencia*, *Yersinia*, and *Klebseilla* (Benito, *et al.*, 2010). Fermented drink from dragon fruit peel test gave negative result and met the maximum limit of SNI (10 MPN/ml). According to Alakomi *et al.* (2006), several lactocin (one form of bacteriosin) can be produces by LAB so that it can inhibit the growth of negative gram bacteria.

Proximate test consists of water content, ash content, fat, protein, and carbohydrate (by difference) gave results 89.21%, 0.39%, 0.12%, 1.80%, and 8.48%. Every proximate parameter showed that the fermented drink met the SNI standard. Ash content maximum 1.00%, protein minimum 1.00%, fat maximum 0.50% for fermented drink without fat and minimum 0.60% normal fermented drink [3].

Toxicity test on the product was done by Brine Shrimp Lethality Test (BSLT) method in order to know the toxic limit of fermented drink from dragon fruit peel. BSLT is a fast-preliminary test to know the presence of biochemical activity with toxic properties. Advanced test that was needed to know the toxic dose for human was done by testing on mice and the result will be extrapolated so that

it would match human based on OECD No. 425 (2011) procedure. Therefore, BSLT couldn't become the basic guidelines in determining dose that could be toxic to human. BSLT use shrimp larva that is counted based on its death percentage. Toxicity value is stated in LC_{50} , that is the product concentration needed to cause 50% death of shrimp larva. Toxicity limit based on LC_{50} was grouped in four categories that are $LC_{50} < 30$ ppm: very strong toxic, LC_{50} 30-100 ppm: toxic, LC_{50} 100-1000 ppm: light toxic, and $LC_{50} > 1000$ ppm: not toxic (Juniarti, 2009). Toxicity test result showed that fermented drink from dragon fruit peel is categorized as light toxic ($LC_{50} = 484,98$ ppm). According to Muaja, *et al.* (2013), the presence of flavonoid and phenolic compound can cause toxic properties.

Hedonic test of fermented drink from red dragon fruit peel was done in order to know the product acceptance level. The test scale consisted of 7: very dislike (1), dislike (2), rather dislike (3), neutral (4), rather like (5), like (6), very like (7). Average result of every parameter is color 5.96, flavor 3.83, taste 4.14, viscosity 4.63, and overall acceptance 4.69 (neutral), which mean that the product was still acceptable to a panelist.

4. Conclusions

The production of dragon fruit peel fermentation drink with 1% dragon fruit peel powder addition, 5% skim milk, *S. thermophilus* : *L. plantarum* : *L. bulgaricus* ratio of 1:1:1 produce pH, total titratable acidity, and total LAB which meet the standard. Sequentially the pH, total titratable acidity, and total LAB were 4.14, 0.69%, and Log 9.41 CFU/ml. Product from selected formulation and culture ratio had 89.21% water content, 0.39% ash content, 0.12% fat, 1.80% protein, and 8.48 carbohydrate (by difference), total phenolic 483.67 mg GAE/ml, total flavonoid 315.59 mg QE/ml. The product had an increase in antioxidant activity by 241% during the fermentation process, with IC_{50} value after fermentation as big as 94.70×10^4 ml/l (very strong). Hedonically, dragon fruit peel fermentation drink was still acceptable to consumers (4.69 of 7.0), and was declared safe for consumption with very low toxicity and free from coliform microorganism.

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