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Development of ready-to-drink tea with *Lactobacillus plantarum* Dad-13 based on the vulnerability aspects of the production process

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Abstract

Tea is one of the plantation crops which contributes to 3.27% of the Gross Domestic Product (GDP) in Indonesia. As one of Indonesia's export commodities, tea plays a big role in foreign exchange revenue. Among various kinds of processed tea drinks, 14.36% of tea consumers in Indonesia have a preference for consuming ready-to-drink (RTD) tea, implying its market potential in Indonesia. Furthermore, the increasing trend of healthy products resulted in the rise of the probiotic beverage market globally. Therefore, an RTD tea product with the addition of *Lactobacillus plantarum* Dad-13 has been developed, named ProbioTea. This study discusses the development of the ProbioTea product and vulnerability analysis of the two processes used: cold brew and hot brew. The study aimed to determine the level of vulnerability at each stage of ProbioTea manufacturing to reduce the risk of failure in large-scale production. This study also investigated antioxidant contents, cell viability, pH, and the visual appearance of ProbioTea. The result showed that the mixing and storage process has a high vulnerability to both cold and hot brew methods. The result of the assessment of risk vulnerability in the ProbioTea production process showed no risk with an extreme level of vulnerability. Thus, it indicated that the risk in the ProbioTea production process can be managed properly. The percentage of antioxidants in the cold brewing method was 51.25 - 71.76%, while the hot brewing method shows 44.89 - 54.74%. After storage, ProbioTea with the cold brew method experienced an increase in antioxidants because the low temperature was able to protect the bioactive compounds in tea. Meanwhile, there was no significant difference in the cell viability of ProbioTea processed on the cold and hot brew. After storage, the cell viability contained in ProbioTea was more than 6.8 CFU/mL. The acidity of ProbioTea processed in both cold and hot brew was not significantly different. This research concluded that the ProbioTea product is good.

1. Introduction

Tea (*Camellia sinensis*) plays an important role in economic activity in Indonesia (DePlantation, 2021), as it contributes to approximately 3.27% of the Gross Domestic Product (GDP). Tea also plays relatively a big role in the foreign exchange revenue of Indonesia, in addition to oil and gas. In 2019, the export value of tea was US$ 92.3 million (BPS, 2019), as 55 countries imported tea from Indonesia.

The most common type of tea in Indonesia can be classified into green tea, black tea, and white tea (Rohdiana, 2015). The difference between those types arises during the production process. The processing of green tea does not go through the enzymatic oxidation process or the process of incubation, whereas black tea undergoes an enzymatic oxidation process. Unlike green and black tea, the simplest processing of white tea is withering and drying (Rohdiana, 2015). Black tea has a higher export volume than other types. In 2019, it was

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36,368 tons or 84.95% of the total volume of tea exports (BPS, 2019). The growth of black tea production in Indonesia and the world is also higher than in green tea (DePlantation, 2021). Therefore, many processed black-tea products have been developed, one of them being the ready-to-drink product.

Ready-to-drink (RTD) tea is a readily prepared tea, which generally uses black tea as the ingredient. RTD tea is usually consumed in cold temperatures either sweetened, unsweetened, or with other flavour additives (Galanakis, 2019). Several types of RTD tea are available in the market such as instant tea powder, batch brewed tea and brewed tea extract. Based on research by the Ministry of Industry in 2020 regarding tea consumption behaviour in Indonesia, shows that 14.36% of consumers have more consumption preferences for RTD tea (Prasetia *et al*., 2020). Data also shows that 56.2% of RTD tea consumers in Indonesia frequently consumed RTD tea 2 to 3 times a week until once a day (Snapcart, 2017), indicating that RTD tea is one of the most popular drinks in Indonesia.

The global probiotic drink market is estimated to increase by 6.1% from 2020 to 2027, while in 2019 the sales value of probiotic drinks was US$ 13.65 billion (Triandafyllidou and McAuliffe, 2019). This increase is due to the high demand from consumers who currently are more aware of healthy food and beverage for reducing the risk of disease (Meybodi *et al*., 2021). Probiotics are mixed or single live cultures of microorganisms applied to products that can enhance the benefits of the product. Probiotics are restricted to products containing live microorganisms, such as freeze-dried cells or in fresh or fermented product (Stanton *et al*., 2001). Probiotics can also be applied to beverages that provide health benefits from the nutritional and nutraceutical properties, the beneficial effects of the fermentation process, as well as the substantial effect of loading probiotics. The health benefits of probiotic drinks are influenced by the number of microorganisms that remain viable as they pass through the digestive tract (Meybodi *et al*., 2021). Based on those reasons, an RTD tea product with *L. plantarum* Dad-13 thus was developed. The product is a probiotic tea namely ProbioTea, as the combination of both probiotics and tea is expected to be a ready-to-drink product that provides many health benefits.

Lactobacillus plantarum Dad-13 is a probiotic strain sourced from curd, which is a fermented food made from buffalo milk, where the fermentation process takes place spontaneously. *Lactobacillus plantarum* Dad-13 has fulfilled several probiotic requirements, including resistance to gastric juice, bile salt, and has anti-bacterial activity against pathogens (Rahayu *et al*., 2015). *Lactobacillus plantarum* Dad-13 also can attach to intestinal epithelial cells and cannot transfer antibiotic-resistant genes. In addition, the probiotic *L. plantarum* Dad-13 added to dairy products is also able to provide health effects such as a reduction in diarrhoea and free (Niken Tari *et al*., 2016). *Lactobacillus plantarum* Dad-13 probiotic powder has also been tested on Indonesian consumers including school-age children in Pakem, Yogyakarta, and Samosir (Banin et al., 2019; Liwan et al., 2020). In general, the results imply that the consumption of probiotic powder *L. plantarum* Dad-13 can increase the number of *L. plantarum* and reduce the number of *E. coli* and non-*E. coli* coliforms in the subject. In addition, the consumption of *L. plantarum* Dad-13 probiotic powder can improve the frequency of bowel movements and stool quality.

This research explored the development of the ProbioTea product and the vulnerability analysis onofach method of the production process. The production process methods used are cold brew and hot brew. The purpose of this study was to determine the level of vulnerability at each stage of the production process. This vulnerability assessment is required because this ProbioTea product is considered a high-risk product in regard to quality. Additionally, various tests were carried out on the development of ProbioTea including antioxidant testing, cell viability testing, pH testing, and product visual appearance testing.

2. Materials and methods

2.1 Materials

The main ingredients used in the production of ProbioTea are dry tea powder, water, liquid sugar, and *L. plantarum* Dad-13 probiotic. The quality of dry tea powder used for ProbioTea products is Broken Orange Pekoe, Broken Orange Pekoe Fanning, and Pekoe Fanning. The appearance characteristic of Broken Orange Pekoe type is particularly short tea leaves, rather small, twisted black, slightly curly, derived from young leaves, and containing a small amount of twisted leaf bone. Meanwhile, the appearance characteristic of Broken Orange Pekoe Fanning is short, smaller, black, twisted, and slightly curly. The Pekoe Fanning type shows short, black, twisted, slightly curly particles, and larger than the Fanning type (passes the 18 mesh sieve) (Badan Standarisasi Nasional, 2016). In more detail, this study used five types of tea quality samples with the sample codes shown in Table 1.

The probiotic used in ProbioTea is *L. plantarum* Dad -13. This probiotic is included in the category of Lactic Acid Bacteria (BAL) which has nutraceutical properties and provides health benefits, therefore this probiotic has

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a Generally Recognized as Safe (GRAS) status. One of the advantages of *L. plantarum* Dad-13 is a local probiotic fermented from buffalo milk (curd).

Table 1. Types of tea quality sample

Sample Code	Type of tea quality
S1	Broken Orange Pekoe (BOP)
S2	Broken Orange Pekoe Fanning (BOPF)
S3	Pekoe Fanning (PF)
S4	Pekoe Fanning 1
S5	Pekoe Fanning 2

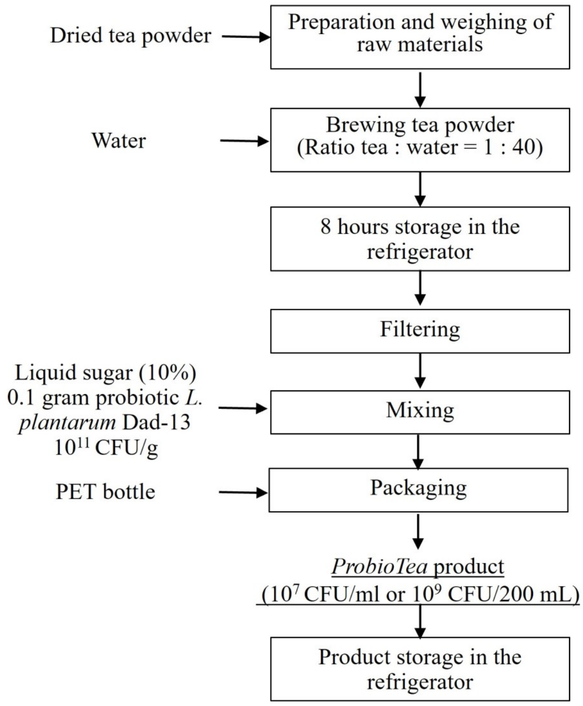

Figure 1. Production process ProbioTea: cold brew method

2.2 Cold brew process

A total of 35 g of dry tea powder was weighed and brewed with 1.4 L of water (ratio 1:40), and then it was stored in the refrigerator for 8 hrs. After 8 hrs, it was filtered to separate the tea powder from the brewed tea water. In addition, 0.1 g of probiotic *L. plantarum* Dad-13 10^{11} CFU/g and 20 g of liquid sugar were put into a PET bottle which was used as packaging. Following that, 200 mL of tea was placed into a PET bottle. ProbioTea product was stored in the refrigerator

2.3 Hot brew process

A total of 35 g of powdered tea was weighed and boiled in 1.4 L of water, then it was left to cool for three minutes until it subsequently reached a temperature of 70°C. It was then brewed and let sit for two minutes. After the brewing process, filtering was carried out to separate the tea powder from the brewed tea water. Similar to the cold brew, 0.1 g of probiotic *L. plantarum* Dad-13 10^{11} CFU/g and 20 g of liquid sugar were placed into PET bottles. After that, 200 mL of brewed tea was placed into a PET bottle. After the tea temperature dropped to 35°C, the lid of the PET bottle was closed,

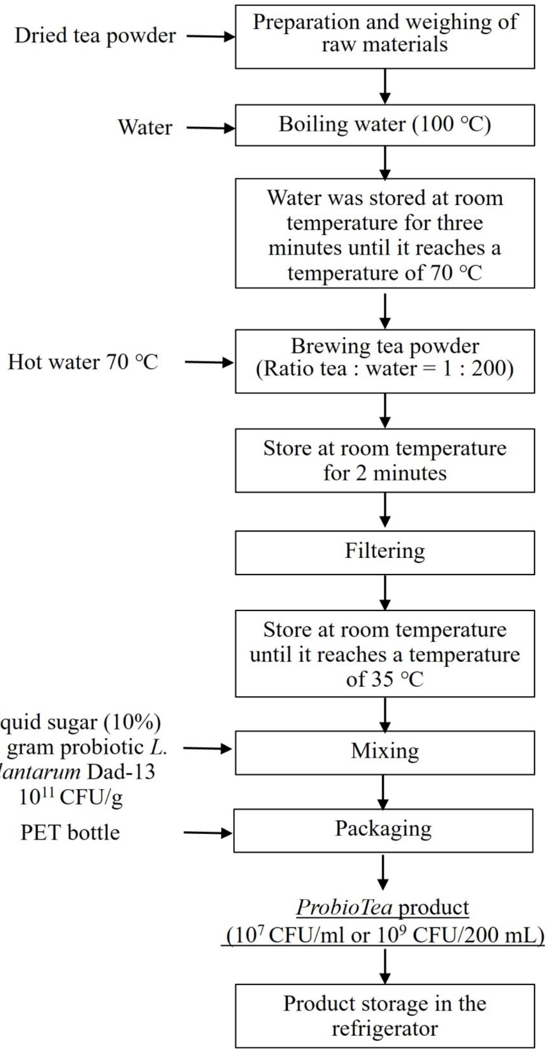

Figure 2. Production process ProbioTea: hot brew method

and the product was stored in the refrigerator. The detailed production process of ProbioTea in both cold brew and hot brew methods is shown in Figures 1 and 2, respectively.

3. Results and discussion

3.1 Vulnerability process analysis

The risk management process generally consists of three critical stages (Khan and Burnes, 2007; Waters, 2007), risk identification, risk analysis, and risk evaluation. According to Raz and Hillson (2005), risk management should facilitate the identification of major risks and the implementation of appropriate measures for their prevention. Based on the two methods of the ProbioTea production process, a vulnerability analysis is performed in each process. Vulnerability analysis is carried out by identifying the risks of the ProbioTea production process, mapping the risks, and mapping the level of risk vulnerabilities that may arise. The analysis of each stage is explained as follows:

3.1.1 Risk identification

Risk identification is executed in the both cold and

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hot brew, as explained in Tables 2 and 3. In the seven stages of the production process in the cold brew method, eight risks are identified. There is a risk of decreasing the quality of raw materials (RC.1.1) caused by the less tight packaging seal and the storage of the tea powder in a humid place that produces the mouldy tea powder. Meanwhile, risks are not identified in the brewing process. In the process of 8-hours storage in the refrigerator, the risk of different concentrations of tea in brewed water for each production batch (RC.3.1) is identified. This was occurred due to unstable refrigerator temperatures as a result of power failure, so the taste of ProbioTea was often inconsistent. There is also a risk of contaminants in the filter (RC.4.1) due to the lack of a rinsing process with hot water resulting in the contamination of ProbioTea. Moreover, there are two identified risks in the mixing process. First, the risk of incompatibility of weighing equipment (RC.5.1) causes the inconsistent weighing of probiotic powder and different cell viability in ProbioTea. The second risk is the risk of insoluble probiotics (RC.5.2) because the probiotics are mixed in cold conditions which then causes sediment in the ProbioTea product. Both risks are also found in the hot brew method. Further, the risk in the packaging process is indicated by the appearance of contaminants in the PET bottle (RC.6.1) due to skipping the sterilizing process using UV irradiation on the bottle which then resulting ProbioTea contamination. In the final stage, two risks are recognized during the storage of ProbioTea in the refrigerator, specifically the risk of decreasing quality in the taste (RC.7.1) and the appearance (RC.7.2) of the product. The former risk occurs due to the probiotic activity in the product causing a decrease in the pH, thereby resulting in a more acidic taste in the product. Besides that, the decrease in the viability of probiotic cells during storage contributes to the short shelf life of ProbioTea products. Meanwhile, the latter risk is caused by the failure of probiotic powder to completely dissolve contributing to the unappealing appearance of ProbioTea (i.e., sediment).

In the hot brew method, there are 10 identified risks from nine stages of the production process as explained in Table 3. Alike in the cold brew method, the decreasing quality of the tea powder (RH.1.1) is also found in the raw material preparation in the hot brew method as the

Table 2. Risk identification in the cold brew method

Process	Risk Code	Detail of Risk	Impact
Preparation of material	RC.1.1	Decreasing the quality of raw materials (i.e., tea powder)	The tea powder becomes moldy, making it indecent for consumption
Storage in refrigerator	RC.3.1	Differences in the concentration of tea in brewed water for each batch	The taste of the product is inconsistent among the batch
Filtering	RC.4.1	Contaminants in the filter	Product contamination
Mixing	RC.5.1	Incompatibility of weighing equipment	Cell viability in each product is different
	RC.5.2	Insoluble probiotics	The appearance of the product is not attractive because of the sediment
Packaging	RC.6.1	Contaminants in the bottle	Product contamination
Product storage	RC.7.1	Decreasing product quality in the taste aspect	The product cannot be stored for a long time
	RC.7.2	Decreasing product quality in the appearance aspect	The appearance of the product is not appealing because of sediment in the bottom of the bottle

Table 3. Risk identification in the hot brew method

Process	Risk Code	Detail of Risk	Impact
Preparation of material	RH.1.1	Decreasing the quality of raw materials	The tea powder becomes moldy and indecent for consumption
Cooling down the boiling water	RH.3.1	The water temperature is still too high	The concentration of tea extract is too thick and the taste of the tea extract is too bitter
Brewing	RH.4.1	Too long brewing time	The concentration of tea extract is too thick and the taste of the tea extract is too bitter
Filtering	RH.5.1	Contaminants in the filter	Product contamination
	RH.6.1	The water temperature is still too high	The probiotic cells may die
Mixing	RH.7.1	Incompatibility of weighing equipment	Cell viability in each product is different
Mixing	RH.7.2	Insoluble probiotics	The appearance of the product is not attractive because of the sediment
	RH.8.1	Contaminants in the bottle	Product contamination
	RH.9.1	Decreasing product quality in the taste aspect	The product cannot be stored for a long time
	RH.9.2	Decreasing product quality in the appearance aspect	The unappealing appearance of the product due to sediment in the bottom of the bottle

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consequence of the packaging seal problem and the humid storage. During 3 mins of the cooling down process, there is a risk that the water temperature is still too high (RH.3.1) due to improper thermometer conditions causing the concentration of tea extract too thick and the taste too bitter. At the brewing stage, there is a risk caused by too long brewing time (RH.4.1). This risk gives a similar impact as the risk RH.3.1. Moreover, the risk of contamination of the filter (RH.5.1) in the filtering process is identified as the consequence of washing without using hot water. This thus resulted in the contamination of the ProbioTea product. The next risk is identified in the cooling process of the tea extract to a temperature of 35°C in which the water temperature is considered too high (RH.6.1), causing the probiotic cells to possibly die. Meanwhile, two risks in the mixing process of the cold brew method have also been identified in the hot brew method: the risk of incompatibility of the weighing equipment (RH.7.1) and the risk of insoluble probiotics (RH.7.2). The impact of both risks are similar in both brew methods. The process of packaging and final storage for both cold and hot brew methods are substantially similar, so the type of identified risks is also similar. The risk of the existing contaminants in the packaging bottle (RH.8.1) is identified in the packaging process. Meanwhile, the decreasing product quality in regard to taste (RH.9.1) and appearance (RH.9.2) of the products are found during the final storage.

3.1.2. Risk priority mapping

The risk priority mapping considers the level of likelihood and impact of risk on deciding the priority level. The likelihood and impact scale are classified into five levels, with the higher number representing the higher degree of likelihood and impact. In the cold brew method as shown in Figure 3a, three risks are categorized as Priority 1, four risks are categorized as Priority 2, and one risk is categorized as Priority 3. Meanwhile, in the hot brew method, three risks are categorized as Priority 1 and seven risks are categorized as Priority 2. It indicates that risks in the production process of ProbioTea using the hot brew method are higher than that of the cold brew method due to the long process in the hot brew method.

The risk categorized as Priority 1 shows the risk that occurs most often with a large impact, while Priority 2 falls for risk with moderate loss impact and Priority 3 for the risk with low loss impact (Jaffee *et al.*, 2008). In both tea-brewing methods, the risks classified as Priority 1 arise at the same process stages of mixing and final storage. Those risks have a high possibility to occur and have a high impact on the production process. The risk

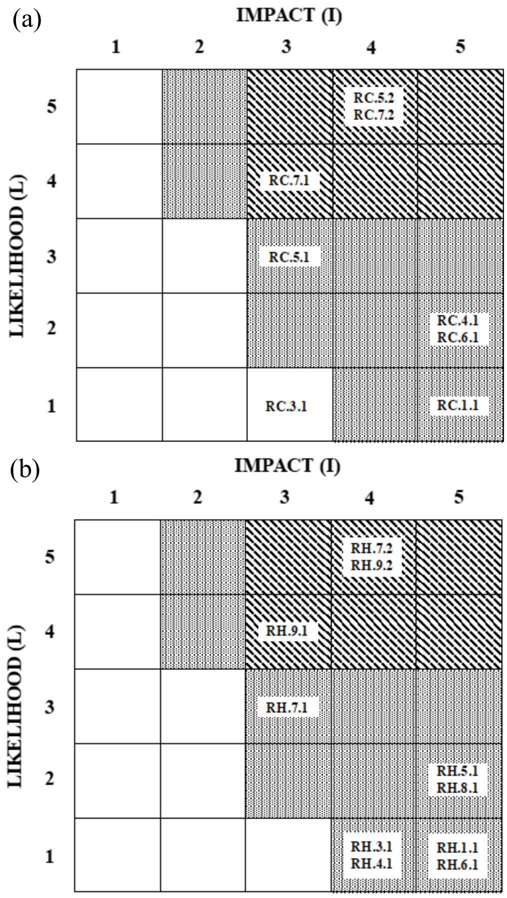

Figure 3. Risk priority mapping on the production process of probiotea in (a) cold brew method and (b) hot brew method

of insoluble probiotics in the mixing process plays a large impact on causing the appearance of the product less attractive and the occurrence of sediment. In addition, this sediment is formed as a result of the mixing process under cold temperature conditions. Another priority risk is the risk of decreasing product quality in the aspect of taste and appearance. The decrease in quality in taste causes a shorter storage period for the product. It is because the activity of probiotic cells that produce organic acids leads to a decrease in the pH and an increase in the acidity of the product. In addition, the sediment is also relatively visibly reducing the appealing value of the ProbioTea product.

According to Kiran (2017), the risk priority number (RPN) is a function of the three parameters including the severity of the effect of failure (or impact), the probability of occurrence (or likelihood), and the ease of detection for each failure mode. Meanwhile, in this risk analysis, an RPN is calculated to prioritize risks that must be managed early based on three priority risk that has been defined (Raab *et al.*, 2013). RPN is calculated by multiplying these three parameters as per the formula (1) and the RPN value for each prioritized risk is shown in Table 4.

$$RPN = L \times I \times D \quad (1)$$

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Table 4. Risk priority number calculation

Risk	L	I	D	RPN
Risk of insoluble probiotics i.e., RC.5.2. and RH.7.2	4	5	1	20
Risk of decreasing product quality (taste) i.e., RC.7.1. and RH.9.1	3	4	2	24
Risk of decreasing product quality (appearance) i.e., RC.7.2. and RH.9.2	4	5	1	20

where L is the likelihood of risk, I am the impact of risk, and D is the detection of risk.

Table 4 shows that the risk of decreasing quality in the taste aspect generates the highest RPN value among others. Therefore, the risk with the highest RPN value must be prioritized and managed further.

3.1.3 Risk vulnerability mapping

The risk vulnerability mapping is the further and deeper risk analysis after determining the risk priority. This risk vulnerability mapping consists of the value of expected loss and capacity-to-manage, in which the expected loss value for each risk is calculated based on the value of likelihood (L) and impact (I). From all 18 identified risks in both cold and hot brew methods, the identified risks are grouped into a risk vulnerability map with five levels of vulnerability (i.e., extremely, highly, moderate, low, and limited) based on the possibility of loss and the capacity to manage risks. Moreover, risk management is inevitably associated with the additional costs of dealing with these risks. Focusing on the risk with high vulnerability is thus necessary to control this associated cost. This vulnerability can be seen in the ability of the risk owner to manage the likelihood and impact of the risk. Thus, the function of likelihood, impact, and capacity to manage the risk should be further considered.

Figures 4a and 4b present that the risk of decreasing the quality of raw materials, i.e., RC.1.1 and RH.1.1, is classified as the risk with high vulnerability, as if the tea is mouldy then it can no longer be used for production. Another risk with the high level of susceptibility is the risk of insoluble probiotics and the risk of quality deterioration in the taste and appearance aspect. Among these four risks, three of them have a high level of vulnerability and are categorized as risk Priority 1. It indicates that these three risks must be immediately managed to reduce their vulnerability and increase the ability to manage risk if a disruption occurs (Nowakowski *et al*., 2015). Detailed risk vulnerability mapping is shown in Figures 4a and 4b.

In addition, after prioritizing the highly vulnerable risk, the risk that falls into moderate vulnerable risk also should be managed. The risk of different concentrations of tea in brewed water in the cold brew method (RC.3.1) is categorized into this level. However, to deal with moderate, low, and limited vulnerable risks, the risk owner should indeed consider the cost associated with the effort to manage this risk.

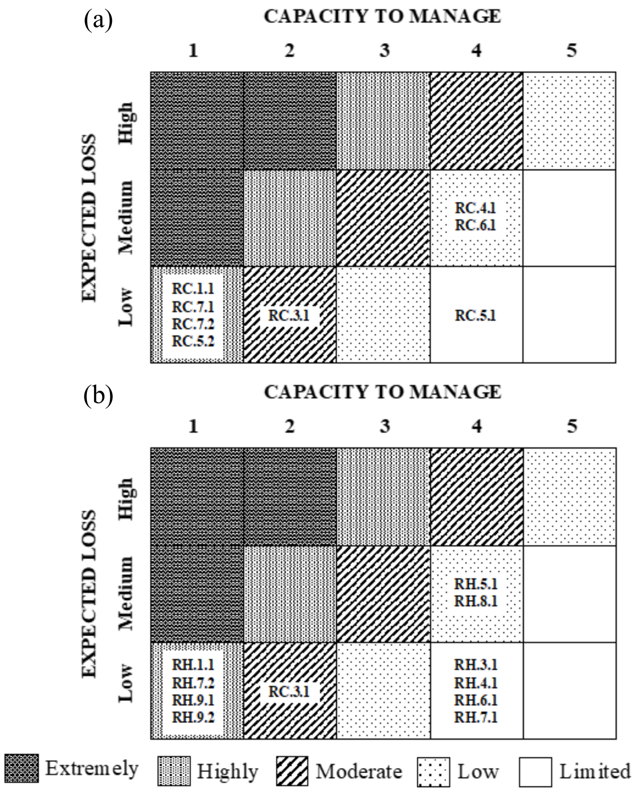

Figure 4. Risk vulnerability mapping on the production process of ProbioTea in (a) cold brew method and (b) hot brew method.

3.2 Quality test analysis

Quality testing is important for the development of new products. Based on the results of the risk and vulnerability analysis, four specific risks give an impact on the product quality, including the risk of decreasing raw materials, the risk of insoluble probiotic powders, the risk of decreasing quality which results in a more acidic taste on the product during storage, and the risk of sediments after storage. In this study, quality tests carried out included antioxidant testing, cell viability calculations, product acidity measurement, and appearance testing.

3.2.1 Antioxidant test

Tea is one of the most popular drinks, globally. Besides its distinctive aroma and taste, tea drink has many health benefits due to the presence of polyphenolic compounds, flavonoids, alkaloids, tannins, and several other volatile components. In addition, the presence of antioxidants in tea drinks plays an important role in protecting body cells from free radical damage. The

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antioxidant content in tea drinks is influenced by several factors, such as the dose of tea leaves being brewed, the dose and temperature of the water used for brewing, and the length of the brewing process. In this study, the probiotic tea drink was brewed using the cold brew method with a 1:40 ratio of tea leaves: water and the hot brew method using a 1:200 ratio of tea leaves: water. The percentage of antioxidants yield resulting from the ratio used is shown in Table 5.

Table 5. Antioxidant percentage (%) of probiotic tea drink *L. plantarum* Dad-13 using cold and hot brewing methods during storage

Sample (S)	Brewing method	First-day storage (Day 0)	Last day storage (Day 28)
S1	Cold	69.21±0.00*	74.80±0.00
	Hot	48.01±0.00*	37.09±0.00
S2	Cold	71.76±0.00*	79.89±0.00
	Hot	54.74±0.08*	41.05±0.00
S3	Cold	59.48±0.00*	73.48±0.00
	Hot	45.89±0.00*	40.02±0.00
S4	Cold	60.98±0.00*	57.44±0.00
	Hot	53.12±0.00*	47.65±0.00
S5	Cold	51.25±0.00*	65.27±0.00
	Hot	44.89±0.00*	38.70±0.00

Values are presented as mean±SD of two replications.
*Significant difference between cold and hot brew sample groups if the p-value $\alpha < 0.05$ on the t-test.

The percentage of antioxidants in probiotic tea drinks brewed with cold and hot brewing methods is significantly different. The percentage of antioxidants obtained by the cold brewing method was 51.25 - 71.76%, while the hot brewing method had a percentage of 44.89 - 54.74%. All samples of the probiotic tea drinks brewed with the cold brew method had a higher percentage of antioxidants than that of the hot brew method. This difference is caused by the higher dose of tea leaves used in the cold brew method than that in the hot brew method to achieve the desired sensory attributes.

According to research by (Hajiaghaalipour *et al.*, 2016), the highest antioxidant activity for white tea can be obtained by taking a long time-hot brewing method, green tea by the long time-cold brewing method, and black tea by the short time-hot brewing method. Besides the time and temperature of the brewing process, the variety and amount of tea used also affect the total flavonoids obtained. Further based on the research by Das and Chatterjee (2018), using the same dose of tea and water, the total phenolic content and antioxidant activity of hot brewed black tea is higher than that of cold-brewed black tea, which is 1:50.

After the storage, the percentage of antioxidants in ProbioTea drinks brewed with the cold brew method was increased due to the activity of *L. plantarum* Dad-13 utilizing sugar as a substrate in the product, although the activity was slow due to cold storage conditions. According to a report by Hassmy and Abidjulu (2017), there is a degradation of complex flavonoid compounds into simpler molecules such as free phenolics by the formed enzymes during the kombucha fermentation process. It can thus be concluded that antioxidant activity was positively correlated with a phenolic compound produced in the product.

However, the opposite happened to the ProbioTea drinks with the hot brew method which decreased the percentage of antioxidants after the storage period. During the kombucha fermentation process, an acidic atmosphere is formed which makes it difficult for phenolic compounds to release protons (Hassmy and Abidjulu, 2017). These protons can bind with DPPH compounds so that the antioxidant activity detected is lower. The use of the hot brewing method is thought to have contributed to the degradation of complex flavonoid compounds into simpler molecules and to the inactivation the heat-sensitive antioxidant compounds. Therefore, in the probiotic tea drink with the hot brewing method, the formation of free phenolic compounds did not occur. In addition, the use of low temperatures is also able to protect the bioactive compounds found in tea from the degradation process during brewing (Magammana *et al.*, 2019).

3.2.2 Cell viability test

Viability testing needs to be done for a ready-to-drink product with added probiotics as it aims to ensure that the cells are still in the drink. The following data in Table 6 shows the viability of probiotic cells of *L. plantarum* Dad-13 in five samples of probiotic tea drinks that were brewed in both cold and hot with a storage period of 28 days.

Based on the results obtained (Table 6) on five samples of probiotic tea drinks, the differences in the tea samples used did not significantly affect the initial cell viability of the probiotic tea drink product. Besides, the use of different brewing methods (i.e., cold and hot) significantly affected the initial cell viability of the product. It is also found in both brewing methods that the probiotic tea drinks stored for 28 days tended to experience a slight decrease in the number of viable cells after 14th days of storing.

Based on Tewari *et al.* (2018), the amount of *L. acidophilus* in green tea also decreased slightly from 8.45 to 7.74 log CFU/mL during 28 days of storage at 4°

C. This is presumably because the survival of probiotics can be better maintained in cold temperatures. However, there was an increase in the number of viable cells in the probiotic tea drink which was brewed using the cold brew method on the 14^{th} day of storage. It occurred due to the growth activity of *L. plantarum* Dad-13 which uses sugar as a substrate in the product, although the activity is running slowly due to cold storage conditions.

Table 6. Cell viability (log CFU/mL) of probiotic tea drink *L. plantarum* Dad-13 using cold and hot brewing methods during storage

Sample (S)	Brewing method	Day		
		0	14	28
S1	Cold	6.97±0.01*	7.41±0.00	7.13±0.00
	Hot	7.21±0.00*	7.15±0.01	7.01±0.00
S2	Cold	6.80±0.00*	7.11±0.00	6.83±0.01
	Hot	7.40±0.00*	7.25±0.00	6.96±0.00
S3	Cold	6.86±0.01*	7.05±0.00	6.96±0.00
	Hot	7.35±0.01*	7.24±0.01	6.84±0.00
S4	Cold	6.88±0.00*	7.21±0.01	6.88±0.00
	Hot	7.15±0.01*	7.26±0.00	7.02±0.01
S5	Cold	6.68±0.01*	7.16±0.00	6.91±0.00
	Hot	7.55±0.01*	7.18±0.00	7.04±0.00

Values are presented as mean±SD of two replications.
*Significant difference between cold and hot brew sample groups if the p-value $\alpha < 0.05$ on the t-test.

Moreover, the decline of viable cells continues until the 28^{th} day of storage which is thought to be due to the influence of organic acids produced by *L. plantarum* Dad -13 itself. Although there is a decrease in the number of viable cells during the storage period, the cell viability of this probiotic tea drink is still considered high which is further able to provide health benefits for people who consume it. As the standards of the International Dairy Federation explained that probiotic products must contain a minimum of 10^6 CFU per gram of product at the time of consumption to continue providing functional benefits (Daneshi *et al*., 2012).

3.2.3 pH measurement

In addition, to measure cell viability, pH measurements were also carried out to determine the acidity level of ProbioTea drinks. The use of different brewing methods resulted in significant differences in the pH value of probiotic tea drinks at the beginning of the storage period. Whereas the use of five different tea samples did not cause a significant difference in the pH of the probiotic tea drinks being produced, as shown in Table 7. The process of tea powder brewing at a higher temperature will increase the solubility of the components of polyphenolic compounds and catechins, which these components contribute to the taste and aroma of the tea beverage products (Palanivel *et al.*, 2018)

During the 28^{th} day of storage on the cold and hot brewed probiotic tea drinks, there was a decrease in the pH of probiotic tea drinks. The cold brewing method had an initial pH of 5.62 - 6.00 and then was decreased to 3.57 - 3.84 at the end of the storage period. Meanwhile, the pH of probiotic tea drinks using the hot brew method had an initial pH of 4.46 – 5.52 and at the end of storage, it decreased to 3.50 – 3.85. This is presumably due to the activity of the probiotic cells of *L. plantarum* Dad-13 which produce organic acids, causing a decrease in pH during the storage period. This decrease in pH can be seen more clearly in Table 7.

This result is in agreement with the research by Tewari *et al.* (2018) stating that in green tea drinks added with *L. acidophilus*, there is a decrease in the pH value from 4.85 to 3.92 under cold storage for 28 days

Table 7. pH of probiotic tea drink *L. plantarum* Dad-13 using cold and hot brewing methods during storage

Sample (S)	Brewing method	Day		
		0	14	28
S1	Cold	5.62*	3.89	3.57
	Hot	4.46*	3.72	3.50
S2	Cold	5.87*	4.36	3.84
	Hot	5.39*	4.02	3.80
S3	Cold	5.85*	4.43	3.80
	Hot	5.17*	3.95	3.85
S4	Cold	6.00*	4.02	3.62
	Hot	5.52*	3.95	3.76
S5	Cold	5.77*	3.95	3.76
	Hot	5.51*	4.01	3.73

Values are presented as mean±SD of two replications.
*Significant difference between cold and hot brew sample groups if the p-value $\alpha < 0.05$ on the t-test .

3.2.4 Visual appearance

The addition of probiotic powder *L. plantarum* Dad-13 approximately 10^{11} CFU/ml in probiotic tea drinks makes the product considered less attractive in the appearance for both cold and hot brew methods. The presence of probiotic powder in this ProbioTea product increases the cloudiness of the water as more clearly seen in Figure 5 and Figure 6.

The storage period of 28 days caused the appearance of the probiotic tea drink to look less appealing due to the separation and sedimentation of probiotic powder on the bottom of the bottle as seen in Figure 7. This sedimentation began to occur on the 14th day of storage due to the addition of probiotic powder when the brewing conditions were cold. The decision to add the probiotic powder during the cold brewing condition was based on an effort to anticipate the cell death of

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(a) (b) (c)

Figure 5. The appearance of cold brewing probiotic tea drink on (a) day 0, (b) day 14, and (c) day 28

(a) (b) (c)

Figure 6. The appearance of hot brewing probiotic tea drink on (a) day 0, (b) day 14, and (c) day 28

probiotics. Therefore, the added probiotic powder does not completely dissolve in the probiotic tea drink and over time it will form sediment.

Regarding the results of the feasibility study for the development of this ProbioTea product through a quality test analysis, it can be concluded that the overall development of ProbioTea can provide health benefits and can continuously be developed with some improvements in taste and appearance. In addition, risk analysis through risk priority mapping and risk vulnerability mapping helps to focus on high impact risks discovered during the production process in both brewing methods. The elaboration between this risk analysis and quality analysis provides a thorough and specific evaluation of the development of ProbioTea products before proceeding to large-scale production.

Figure 7. The sedimentation of probiotic powder on the bottom of the bottle

4. Conclusion

The development of ready-to-drink ProbioTea is based on the potential of domestic consumption and the efforts to provide ready-to-drink that offer health benefits for consumers. ProbioTea is considered a high-risk product in regard to quality due to the use of *L. plantarum* Dad-13 as the source of probiotics because of the risk of insoluble probiotics in the mixing process, the risk of taste degradation, and the risk of appearance degradation during storage. Therefore, storing in cold storage, brewing method, time and temperature of brewing should be considered to reduce the risk of ProbioTea products.

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