RESEARCH ARTICLE

Heat Resistance of Probiotic Candidate *Enterococcus faecalis* R22B in Different Matrices

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Abstract

Fermented fish, including *rusip* and *bekasam*, contains lactic acid bacteria, some of which have probiotic properties. Considering the beneficial effect, the count of viable cells in probiotic carrier foods must be at least 10^6 CFU/g throughout the product shelf-life. The processing and storage condition significantly affect the viability of probiotics in carrier foods. This research was conducted to determine the heat resistance of probiotic candidate *Enterococcus faecalis* R22B isolated from *rusip* in three different media: *bekasam, rusip,* and physiological saline solution (NaCl 0.85%). *E. faecalis* R22B was heated in each medium at a temperature of 70 °C for 0, 2, 4, 8, and 12 min. The media characteristics showed differences within the parameters included pH values of 4.41-6.99; a_w 0.67-0.92; moisture content 72.56-98.09%; ash content 0.69-12.32%; protein content 0-14.7%; fat content 0-0.84%; and carbohydrate content from 0-4.93%. The results showed that the difference in the heating medium had no significant effect (p>0.05) on the heat resistance of the bacteria, as indicated by the D_{70} value. The required average heating time at 70 °C to decrease *E. faecalis* R22B cells by 1$\log_{10}$ (D_{70} value) in *bekasam, rusip,* and physiological saline media were 3.17, 3.29, and 3.55 min, respectively. Despite having a lower D_T-value, the reduction of bacteria cells in three media heated at 70 °C showed no difference (p>0.05); therefore, *bekasam* and *rusip* are suitable for probiotic carrier food.

Keywords: *bekasam*, D value, heat treatment, pasteurization, *rusip*

Introduction

Oral probiotics are living microbes that will provide health benefits beyond the basic nutrients they contain (Guarner & Schaafsma, 1998). Probiotic culture has been widely used in the dairy industry and its derivatives in probiotic carrier foods/beverages. However, people start to reduce their milk-based products due to the high cholesterol content. Moreover, people with lactose intolerance could not consume these products (Sornplang & Piyadeatsoontorn, 2016). Therefore, it is necessary to develop and study the suitability of probiotic carrier foods/beverages from non-dairy sources.

Fermented fish products such as *bekasam* and *rusip* are known as the sources of lactic acid bacteria (LAB) (Desniar, Rusmana, Suwanto, & Mubarik, 2013; Nurnaafi, Setyaningsih, & Desniar, 2015; Yuliana, Koesoemawardani, Susilawaty, & Kurniati, 2018). In addition to their potential health benefit as probiotic sources, bekasam and rusip are also promising functional foods since they contain bioactive peptides with anticholesterol and antioxidant properties (Rinto, Lestari, & Putri, 2019). In previous studies, we have succeeded in obtaining *E. faecalis* strain B32B and R22B; the LAB isolates from *bekasam* and *rusip* of silver rasbora fish are potential as probiotics (Lestari, Ridhowati, & Nopianti, 2017).

The required daily probiotic doses are no less than 10^6-10^7 CFU/mL (Shah, Ali, & Ravula, 2000) and 10^8-10^{10} CFU/mL (Donnet-Hughes, Rochat, Serrant, Aeschlimann, & Schiffrin, 1999) to provide physiological and therapeutic effects to the host. To ensure the number of viable counts meets this requirement, the probiotics in the food matrices must survive during processing operations, storage, and gastrointestinal digestion. *Bekasam* and *rusip* are fermented products that require a cooking process

before consumption. This condition will certainly affect the survival of the probiotics in the products. Survival and viability are important parameters considering that viable probiotics are more effective in providing health benefits than non-viable ones. In the development of probiotic carrier foods, it is necessary to conduct an initial study to evaluate the viability of probiotic cells during processing. One of the preservation methods which has been largely used in food processing is heat treatment. Understanding the physiological aspects of microorganisms' survival or inactivation due to heat treatment could help develop more effective processing techniques. Furthermore, consumers will obtain functional and beneficial end products.

Heat resistance studies have been carried out on LAB isolated from cooked sausages (Pérez-Chabela, Totosaus, & Guerrero, 2008), cheese (Christiansen, Nielsen, Vogensen, Brogren, & Ardo, 2006; Jordan & Cogan, 1999), and fermented vegetable (Savedboworn et al., 2014). In addition, studies of LAB stress response to high temperatures have also been carried out on *Lactobacillus plantarum* (Ferrando, Quiberoni, Reinhemer, & Suarez, 2015; De Angelis & Gobbetti, 2004). However, no studies have examined the heat resistance of *E. faecalis* in different heating media/ food matrices. Heat resistance analysis for LAB is usually carried out using synthetic media, such as Maximum Recovery Diluent (Ahmad, Smith, & Mahboob, 2002), Brain Heart Infusion (Flahaut, Frere, Boutibonnes, & Auffray, 1997), and modified de Man, Rogosa, Sharpe broth (Haddaji, Krifi, Lagha, Khouadja & Bakhrouf, 2015). The use of *bekasam* and *rusip* as heating media in this research is the first step to assess the suitability of the two fermented fish products as ready-to-eat probiotic carrier foods. Therefore, this research aimed to determine the chemical characteristics of the medium due to heat intervention on *E. faecalis* R22B. Also, to suggest a heat treatment condition that encourages its viability.

Material and Methods

Preparation of Heating Media

Three types of heating media: *bekasam, rusip,* and physiological saline solution, were used in this study. The physiological saline was selected as heating menstruum due to its isotonic nature and minimal impact on osmotically driven-cell rupture. Snakeskin gourami *bekasam* was purchased from the local market in Palembang, while the anchovy *rusip* was obtained from Bangka Island (Figure 1). A total of 500 g of each fermented fish were blended using laboratory blender 8010G (Waring, USA) with the addition of 100 mL distilled water to facilitate homogenization. Physiological saline was prepared by dissolving 0.85 g NaCl (Merck) in 100 mL of distilled water (USFDA, 2001). All media were sterilized in an autoclave (Hirayama HL36AE, Japan) at 121 °C for 15 min before being chemically characterized and used as heating media.

Figure 1. Snakeskin gourami *bekasam* (left) and anchovy *rusip* (right).

Characterization of Heating Media

Chemical profiling included a_w, pH, moisture content, ash content, carbohydrate content (Luffscrool method), protein content (Kjeldahl method), and fat content (Soxhlet method) were carried out on sterilized media following the procedures adopted from the Association of Official Analytical Chemists (AOAC) as described in Indonesian National Standard SNI 01-2891-1992 (BSN, 1992).

E. faecalis R22B Culture Preparation

Cultures stock of *E. faecalis R22B* were maintained in cryovials (HIMEDIA Viabank 80, India) containing a cryoprotective agent. A total of two glass beads containing a pure strain of *E. faecalis* R22B culture were removed from cryovials, transferred to a test tube containing 10 mL of sterile Man Ragosa Sharpe Broth (MRSB) (Merck), and then incubated at 37 °C for 24 h. Five mL of the growing culture was subsequently inoculated into 45 mL of sterile MRSB and incubated for 40 h. Before being added to the heating medium, the number of viable cells present in the culture was enumerated using the total plate count (TPC) method according to SNI 01-2897:2008 (BSN, 2008) and serially diluted with sterile buffered peptone water. A respective volume of 1 mL aliquots of the diluents were poured with Plate Count Agar (PCA), and the bacterial colonies were counted after 24 h incubation at 37 °C. The suitable colony counting range was 25-250 colonies.

Heat Resistance Test

The assessment of heat resistance was carried out by referring to the method proposed by Jordan & Coogan (1999) with modifications. A total of 50 mL culture were homogenized with 450 mL of *bekasam* and *rusip* using a stomacher (Interscience Bag Mixer 400W, USA) for 30 s. The mixture was then transferred

into a sterile 10 mL test tube and closed tightly. Each tube was heated at 70 °C for 0, 2, 4, 8, and 12 min using a closed water bath (Memmert WNB14, Germany). Moreover, the tubes were cooled in ice for 10 min, serially diluted in Maximum Recovery Diluent (MRD), and plated on a sterile petri dish (Sterilin 90, Thermo Scientific) containing MRSA + $CaCO_3$ (Merck) media. After 36 h incubation at 37 °C, the number of viable cells from each dilution was counted.

Determination of D_{70} Value

The data obtained were processed using linear regression (Microsoft® Excel 2019). The D_{70} value was determined based on the regression curve slope between heating time (x) as independent variables and the number of living cells (y) as the dependent variable.

Data Analysis

The data obtained were analyzed for variability (ANOVA) using parametric statistics with a randomized block design (RBD) using a 95% confidence interval. Statistical differences between treatments were analyzed using Minitab 19 (Minitab LLC, USA).

Results and Discussion

Heating Medium Characteristics

The main factors that affect the heat resistance of a microbe are water activity (a_w), nutrient content, pH of the heating menstruum, the microbes growth phase, the growth temperature, variations in genus, species, and even strains within the same species (Huertas et al., 2016). Therefore, media characterization is an integral part of microbial heat resistance study. The chemical characteristics of the three heating media used in this study are presented in Table 1.

Based on the chemical analysis, the highest water content and water activity (a_w) value were found in 0.85% NaCl media. The low a_w value in *rusip* is related to salt and sugar molecules that naturally have a high affinity to water and bind the available free water molecules in the material. The low a_w value of *rusip* is in line with Rimadhini, Sumardianto and Romadhon (2020) results. This explains the addition of 10% liquid palm sugar resulted in a_w value of 0.77. In this study, the a_w value of the *rusip* was 0.68, higher than the commercial *rusip* that contains more salt and palm sugar. In general, the salt concentration in *bekasam* is 10-15%, while in *rusip*, the addition is around 20-30%. The relation between NaCl concentration and a_w has been described by Chirife and Resnik (1984).

The concentration of NaCl can be used to predict the a_w and vice versa. The authors suggest that variation of predicted value due to temperature changes at 15-50 °C is very low. The high salt concentration will also affect the protein content of the ingredients. *Bekasam* contains higher dissolved and total carbohydrates measured by the Luffscrool than those of *rusip*. This is related to the difference in the proportion of carbohydrates used. The source of carbohydrate in the fermentation of *bekasam* is 15% (w/w) rice; while in *rusip*, the carbohydrate is 10% (v/w) liquid brown sugar. The presence of rice and brown sugar as carbohydrates promotes the production of organic acids during the carbon metabolism in bacterial cells. The accumulation of acids leads to increased acidity and lower pH in the final products. Among the three media, *bekasam* had the lowest pH, with a value of 4.41, while physiological saline had a nearly neutral pH of 6.99.

Bekasam is a fermented fish product made by mixing fish, salt, and cooked rice. Although the fish to rice ratio may vary, the salt concentration of 10% is generally used during *bekasam* preparation (Nuraini, Ibrahim, & Rianingsih, 2014; Wikandari, Suparmo, Marsono, & Rahayu, 2012). On the other hand, higher salt concentration (20-30%) is used in *rusip* products combined with 10% of palm sugar (Huda, 2012; Putri, Budiharjo, & Kusdiyantini, 2014). This high salt concentration corresponds to the lowest a_w of the *rusip*

Table 1. Chemical characterization of heating media

Parameters	*Bekasam*	*Rusip*	NaCl 0.85%
Moisture (%)	72.56 ± 0.48^{a}	72.57 ± 1.09^{ab}	98.93 ± 0.15^{c}
Ash (%)	12.32 ± 0.37^{c}	9.26 ± 0.07^{b}	0.69 ± 0.03^{a}
Fat (%)	0.85 ± 0.04^{c}	0.73 ± 0.04^{b}	0^{*a}
Dissolved carbohydrate (%)	4.93 ± 0.45^{c}	1.66 ± 0.37^{b}	0^{*a}
Total carbohydrate (%)	13.82 ± 0.19^{c}	11.16 ± 0.26^{b}	0^{*a}
Protein (%)	14.70 ± 0.46^{c}	10.90 ± 0.59^{b}	0^{*a}
pH	4.41 ± 0.23^{a}	4.86 ± 0.30^{ab}	6.99 ± 0.19^{b}
a_W	0.87 ± 0.03^{b}	0.68 ± 0.01^{a}	0.93 ± 0.01^{c}

Note:
Data represent the mean value of three replicates ± SD. Means within each row with different lowercase letters are significantly different ($p<0.05$). * fat, carbohydrate, and protein were not detected in the medium.

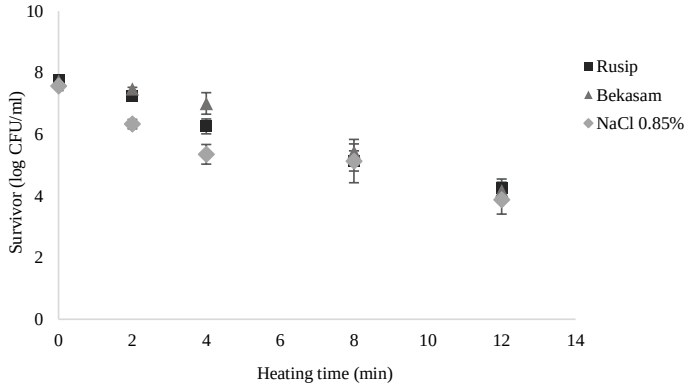

Figure 2. Effects of heating time (min) on the number of survivor cells (log_{10} CFU/mL) in three different matrices [(*bekasam*); (*rusip*); (NaCl 0.85%)], all values are of triplicates.

medium. The time required for autochthonous microbes to ferment the fish is generally shorter, approximately 4-14 days. Various *bekasam* fermentation time has been reported, ranging from 5-7 days (Wikandari et al., 2012) to 14 days (Desniar et al., 2013). As for *rusip*, a two-week fermentation is commonly practiced (Huda, 2012), even though the product can also be consumed after seven days of fermentation (Putri et al., 2014).

E. faecalis R22B Viability Due to Thermal Treatment in Different Media

Figure 2 depicts the number of surviving *E. faecalis* R22B cells in various matrices heated at 70 °C. Each point represents the average number of survivors of three replicates. Based on the results, it can be interpreted that in all media, the number of viable cells of *E. faecalis* R22B decreased along with the longer heating time at 70 °C. The isothermal microbial survival curves are not always linear (Allwood & Russell, 1970; Corradini, Normand & Peleg, 2007). In this study, each different heating medium provides specific survival patterns at the same temperature. When the physiological saline medium was heated, the mortality rate of cells was relatively high. This suggests that high a_w value has a high proportion of free water molecules. Furthermore, it will promote the convectional heat transfer from medium to bacterial cells. After few minutes, the lower rate of cell death was observed, which was presumably due to the *de novo* synthesis of heat shock proteins and a loss of cytoplasmatic water during heating, reducing heat convection (de Jonge, 2019). The slightly skewed survivor cell's pattern of *E. faecalis* heated in *bekasam* medium indicates the presence of cell clumping or the necessity for a certain amount of heat damage to occur before the cells lose their ability to recover (Allwood & Russell, 1970). In this study, fat and protein molecules of *bekasam* and *rusip* were assumed to provide the heat transfer barrier, which delayed the cell's damage, reflecting in the high number of viable cells up to four min of heat treatment.

The D_T value represents the time required to reduce the number of microbial cells at a certain temperature and substrate type by 90% and decrease the population by $1log_{10}$. The D_T value of *E. faecalis* is derived from linear regression equations using the formula $D_T = 1 / (- slope)$. Based on the average number of survival cells from three experiments as indicated in Figure 2 and the respective linear regression curve (Figure 3), the D_{70} values of *E. faecalis* on *bekasam*, *rusip*, and physiological saline were 3.17, 3.29, and 3.55 min, respectively. The coefficient of determination (R^2) shows 98% variability in the number of survivors on *bekasam* and *rusip* was due to heating. On the other hand, the R^2 value of the NaCl medium was only 91.82%. This indicates that only 91.82% variability of surviving cell numbers is determined by heating time, while the rest is due to other factors.

Domestically in household kitchens, *bekasam* and *rusip* undergo heat treatment prior to serving. Both products are usually sauteed with chilies and onions in a short time between 1-2 min. Sauteing is defined as cooking foods in a thin film of hot oil in a skillet set on a hot burner (Fabbri & Crosby, 2016). The sauteing temperature in-home preparation can be as low as 93 °C (Lombard, Peffley, Geoffriau, Thompson, & Herring, 2005) to 120 °C (Lozano-Castellón et al., 2020). The heat transferred to food products through thermal processing is influenced by heating techniques, the type of heating medium, the physical properties of food such as shape, size, and viscosity, and chemical properties related to the composition of the foodstuff (den Besten, Wells-Bennik & Zwietering, 2018). Analyzing the heat resistance of a certain bacteria using sauteing process as a model is considered less reliable due to a higher chance of temperature variation. Therefore, in this study, the pasteurization approach was used to assess the heat effect on the survival of *E. faecalis* R22B. Moreover, this approach may serve as a basis for the development of pouched or canned pasteurized fermented fish as probiotic carrier foods.

Pasteurization is categorized as the mild/moderate heat treatment with a temperature of <100 °C.

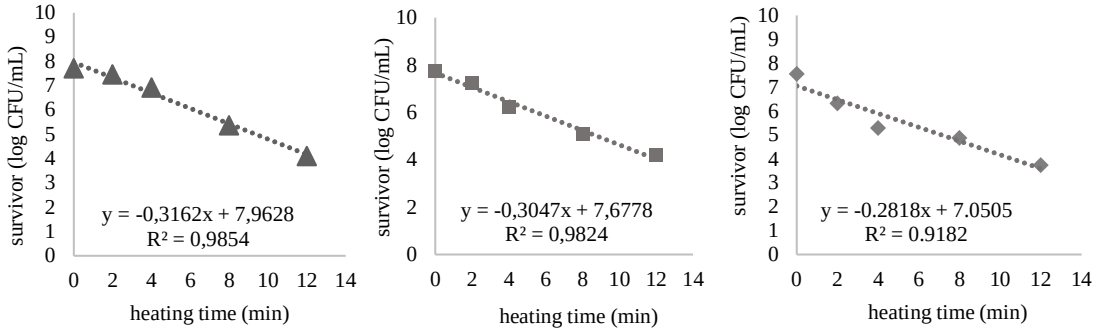

Figure 3. Linear regression of survivor cells (log_{10} CFU/mL) versus heating time (min) in three different media [(*bekasam*); (*rusip*); (NaCl 0.85%)], all values are of triplicates.

Pasteurization in fishery products is commonly carried out on hermetically packed crab meat products to eliminate *C. botulinum* spores type E, also non-proteolytic B and F (USFDA, 2020). The process is also carried out to reduce the number of pathogens such as *L. monocytogenes* and *V. vulnificus*, also putrefactive bacteria through the inactivation of vegetative cells (Horn, Olsen, Hasell & Cook, 2015). It is suggested that fish and fishery products have to be adequately pasteurized up to 10 min at 90 °C (USFDA, 2020). However, a lower pasteurization temperature of 50 °C was found effective in reducing *Vibrio* sp. pathogens in raw oyster from > 100,000 to non-detectable levels in less than 10 min equal to 2-3 logs reduction of spoilage bacteria (Andrews, Park, & Chen, 2000). Heat treatment at 70 °C to 100 °C can reduce microbial pathogens and kill the vegetative forms of microorganisms. However, this has little or no effect on the spores of bacteria (USFDA, 2018). The temperature of 70 °C was used following the method proposed by Martinez, Lopez, and Bernardo (2003), who studied the thermal inactivation of *Enterococcus faecium*.

The heat resistance of bacteria can be determined by D- and z-values (de Jonge, 2019). The estimation of D_T value has been used to determine the heat tolerance of certain bacteria in respective food matrices, such as *Salmonella* Enteritidis in liquid egg products (Kang et al., 2018) and *L. monocytogenes* and *Salmonella* Typhimurium in turkey bologna (McCormick, Han, Acton, Sheldon & Dawson, 2003).

Table 2. D_{70} value of *E. faecalis* R22B in different heating matrices

Experimental no.	*Bekasam*	*Rusip*	NaCl 0.85%
1	3.27	3.52	2.86
2	2.97	3.30	3.63
3	3.26	3.05	4.17
Average	3.17 ± 0.17[a]	3.29 ± 0.23[a]	3.55±0.66[a]

The low D_T value reflects the bacterial population that is more vulnerable to heat treatment. The D_T value of non-spore bacteria such as Pseudomonas, Achromobacter, Enterobacter, Micrococcus, Lactobacillus at 85 °C and 65.5 °C, ranged from 0.008-0.01 seconds and 1-3 min respectively (Hackney, Rippen & Ward, 1991).

Data on Table 2 was compiled based on the D_{70} mean of each treatment in a respected experiment. The difference in the type of heating medium had no significant effect on the D_{70} value of *E. faecalis* R22B (p >0.05). However, the highest D_{70} value of *E. faecalis* R22B was achieved when the bacterial cells were heated in a 0.85% NaCl medium.

The factors that trigger the decline in the value of D_T include the pH value (too low or high pH) and the presence of additive compounds (Horn et al., 2015). The difference in chemical characteristics of the heating medium contributes to a different rate of microbial inactivation at the same temperature (McAuley, Gobius, Britz, & Craven, 2012). In the current study, physiological salts with a neutral pH (6.99 ± 0.19) resulted in a higher D_T value than those of other treatments. Despite containing fat and protein that could provide thermal stability and low a_w due to the sugar (carbohydrates) and salt content, the low pH of *bekasam* and *rusip* ranges from 4.41 ± 0.23 and 4.86 ± 0.30, respectively, may trigger microbial sensitivity against heat. The possible mechanism is through the progressive breakdown of cell wall components (Wu, 2008). Heat processes can inactivate vegetative cells due to damage to cell wall components, cytoplasmic membranes, ribosomal RNA, protein denaturation, including ribosomal proteins and enzymes that play an important role in cell metabolism (Wu, 2008). Although the high a_w tends to decrease microbial resistance to heat, the D_{70} value of *E. faecalis* R22B in 0.85% NaCl medium, which had an a_w of 0.93, was insignificantly higher than those in *bekasam* and *rusip* media. The neutral pH value of the corresponding physiological saline solution was predicted to be the

dominant factor in determining heat resistance. The pH of heating media is the most critical factor and, at the same time, has a significant influence on the heat resistance of bacteria (Palop & Martinez, 2012). Microorganisms show maximum heat resistance at neutral pH, and this resistance decreases with increasing acidity of the media (Doyle, Mazzotta, Wang, Wiseman, & Scott, 2001). Heating bacterial cells at acidic pH raise their sensitivity due to prompt cytoplasmic acidification and rapid protein denaturation (Cebrián, Condón, & Mañas, 2017).

The presence of sugar as additives in the heating menstruum would increase the cell's vulnerability to heat (Horn et al., 2015; Bayles, 2004). Both *bekasam* and *rusip* contain glucose as they are prepared by adding cooked rice and liquid palm sugar. The combination effects of glucose and low pH are presumed to impact bacterial cells negatively, contributing to lower D_T values. This result was consistent with that of Bayles (2004). The presence of glucose significantly decreased the heat resistance of *L. monocytogenes* Scott A challenged at pH 4.8 compared to those treated at pH 7. The use of sterile TSB as the heating medium led the acid-adapted cells at pH 4.8 more heat-sensitive than those tested at pH 7. Therefore, the interaction between pH and the nutrient composition of the heating matrices may determine cross-protection. Ultimately, it generates the heat resistance response. The acid and salt stress combination plays important role in reducing the heat tolerance of bacterial cells, with acid stress being the major determiner of cells' sensitivity to heat (Kang et al., 2018). All previously described mechanisms explain how the D-values were higher in NaCl 0.85% than in *bekasam* and *rusip*.

During the interpretation of D-values, a broad spectrum of variables should be addressed. It is due to the D-value is eminently affected by the combination of several factors. In general, the outer and inner membrane, peptidoglycan cell wall, cytoplasmic membrane, nucleoid, RNA, ribosomes, and multiple types of enzymes are among the cellular targets affected by heat. Therefore, they lead to bacterial death or inactivation (Cebrián et al., 2017). *E. faecalis* and *E. faecium* are known as heat resistance. This characteristic has been reviewed by Sorqvist (2003) after compiling the results of many heat resistance studies of the respective species. Most of the studies used liquids as a heating medium with pH values of 6-8. The D_{60} of *E. faecalis* ranged from 66-2,593 s, equal to 1.1-43.2 min. *E. faecalis* 2350p1 has been reported to have a D_{63} value of 49 min and a D_{72} value of 2.7 min (McAuley.,2012). The range indicates the significant variation in heat resistance exists between strains of this species. However, the test procedures and type of heating medium in each experiment also play an important role. Apart from strain variation, heat resistance is also affected by the age of microorganisms, previous growth and test conditions, exposure to heat shock, acid, and the heating medium composition. Some species are more resistant to heat when tested in foods than in laboratory media (Doyle et al., 2001).

The exposure of bacterial cells to higher temperatures than the optimum growth temperatures could induce the formation of heat-shock proteins that increases their thermal tolerance (McCormick et al., 2003). However, this may occur in the absence of certain limiting factors such as unsuitable pH. It is assumed that during the heating in an acid condition of *bekasam* and *rusip*, the formation of the heat-shock proteins was inhibited. This was due to the adaptation of cells to unfavorable acidic conditions. Therefore, the D_T value of *E. faecalis* was lower in these media than that in physiological saline solution. Meanwhile, the exposure of heat shock to the cells in physiological saline solution supposedly stimulates the formation of those specific proteins. The unfolding of the proteins resulting from the sudden rise of temperature appears to be the primary signal that initialized the heat shock response (Schumann, 2017). The increase of temperature also promotes physiological changes that extensively enhance the ability of bacterial cells to endure such harmful environmental conditions (Shin, Kang, Kim & So, 2018), as indicated by high D_T values.

Horn et al. (2015) formulated the relationship between the chemical characteristics to microbial heat resistance as reflected by the D_T value. Some of the factors that increase the D_T value include anaerobic conditions during heating, competition with other microbes in the media that trigger changes in the oxidation-reduction potential (Eh) of the material, the fat content, which increases thermal stability, and a decrease in the a_w value. A study on Salmonella's resistance to heat using organic wheat flour as food matrices indicates that during the thermal treatment, the a_w of food matrices increase simultaneously, resulting in a reduction of thermal resistance of bacterial cells as reflected by low D_T value (Tadapaneni, Syamaladevi, Villa-Rojas & Tang, 2017). The extent of such change depends on the composition of foods, including the concentration of salts, lipids, proteins, and other components in the food matrices. The same phenomenon is suggested to take place in *bekasam* and *rusip* media. The increase in a_w as the compensation of increasing temperature made *E. faecalis* cells more susceptible to heat.

In summary, *E. faecalis* R22B can survive the heat treatment of 70 °C in *bekasam* and *rusip* media, with

the respective D_{70} value of 3.17 and 3.29 min. To achieve $>10^6$ surviving cells in the pasteurized food matrices and support its function as a probiotic, the number of *E. faecalis* R22B added as adjuncts have to be considered. The addition of 10^{10} -10^{12} CFU/mL of this strain during *bekasam* or *rusip* fermentation is estimated to result in the surviving cell number of 10^6 -10^8 after heat treatment at 70 °C for 6.5 and 7 min, respectively. Other cooking methods with higher temperatures lead to further study to observe the heat tolerance improvement of the strain, i.e., stress adaptation and microencapsulation effect. Adapting the cells to high temperature at a slower rate as a pre-treatment prior to their application in referred food matrices is known as one factor that improves heat tolerance. It is known that slow heating processes would allow some adjustment by the cells (Shin et al., 2018, Kim et al., 2019). This result is important as primary data to address technological challenges in sustaining the viability of the probiotic culture in the food product during processing and storage.

Conclusion

This study demonstrated the chemical characteristics of food matrices regarding the heat resistance of *E. faecalis* R22B as indicated by the D_T value. Heat resistance was insignificantly higher for *E. faecalis* challenged in physiological saline solution than that in *bekasam* and *rusip* media. Among many factors that influence heat resistance, the pH of the heating medium plays the most important role. The addition of a minimum 10^{10} CFU/mL of *E. faecalis* R22B during fermentation of *bekasam* and *rusip* is suggested to acquire the minimum concentration of probiotic bacteria after heat treatment at 70 °C. Further studies are still needed to determine the combination of processing and heating techniques to ensure the loss of pathogenic and putrefying microbes from the material without reducing the viability of the *E. faecalis* as a probiotic candidate.

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