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Combination of Salt and Rice Ratio on The Nutritional and Microbiological Qualities of Ronto, a Shrimp Fermented Product from Kalimantan, Indonesia

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Abstract

Ronto is a fermented product of lactic acid bacteria made from rebon shrimp, salt, and rice. This product is a traditional food in the form of side dishes, chili sauce, and flavoring in the coastal communities of South Kalimantan, Indonesia. This research has been conducted on the combination of salt and rice to enhance the quality of shrimp fermentation products. Various studies have reported that salt in the process of shrimp fermentation affect the product quality. However, the impact from combination of salt and rice in various concentrations to the quality of shrimp fermentation products was not yet known. The purpose of this research was to study the effect of the combination of salt and rice on the quality of shrimp fermented products. The experimental was designed with a Completely Randomized Design Pattern, two factors salt and rice. The three concentrations of salt (10%, 11%, and 12%) and two concentrations of rice (20%, 30%,). The results showed that the concentration of salt and rice was significantly influencing on pH, total volatile bases (TVB-N), water content, ash, and microbiological properties. The condition for the best quality result is a combination of 11% of salt and 30% of rice.

Keywords: fermented shrimp product, quality, rice, Ronto, salt

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INTRODUCTION

There are thousands of species of shrimp worldwide (Ortea et al. 2009), 20 species had economic value, one of the species is Penaeus monodon. However, this is categorized as economically worthless shrimp due to its low price compared to others. Shrimp is occasionally sold for fresh consumption because of its tiny size so a poor appetite arises when is to be consumed. Base on this reason, fresh products are processed top reserve with a various method such as salting, drying, fermentation, pickling, smoking, and/or combinations of those methods (Fernandes 2009).

The fermentation method is preferred by fishermen because it is a simple process and inexpensive and resulted in specific distinctive flavors. The fermentation method is one of the oldest techniques in food preservation as it not only extends the shelf-life but also enhances the flavor, nutrition and quality of the product (Visessanguan et al. 2004). Previous research has been done for ronto production, it stated that the color of ronto is pink to brownish purple fermented shrimp paste (porridge-like) and has a distinctive taste with a prominent aroma of acid. The fermented shrimp is widely known in the coastal area of South Kalimantan, Indonesia, specifically in Tanah Bumbu, Kotabaru, and Tanah Laut (Khairina et al. 2016). Ronto is commonly consumed as the main dish, side dish, spicy sauce, or added to vegetable dishes as seasonings due to its rich flavor. Before being used as the main dish, ronto is sautéed with oil and seasonings. As a spicy sauce, ronto is processed by adding chili so that ronto sauce with distinctive flavor is obtained. As for seasonings, ronto is added to cooked food as well as using of tauco.

Soetikno et al (2018) stated that giving different salt and rice to ronto made from rebon shrimp (Acetes japonicus) showed differences in the sensory, chemical, and color properties of the ronto. The best combination of salt and rice was 11% and 30%, and the results showed a pH value of 5.03, TVB-N 180 mg N/100 g of, water content 70.74%, and ash 9.51%. Some sauce and fermented products with main ingredients of shrimp and fish which are used to consuming by Indonesians, Malaysians, Thai people, and Koreans are reported as the product of lactic acid bacteria. It is well-known the fermented shrimp products called balao-balao at Phillippines (Hall 2002), cencalok/cincaluk at Malaysia (Hajep and Jinap 2012; Hajar and Hamid 2013), jaloo & kong-som at Thailand (Faithong et al. 2010), cincaluk at West Kalimantan (Dyastuti et al. 2013), bekasam (Wikandari et al. 2012), rusip (Koesoemawardani et al. 2013) , plaa-som (Paludan-Müller et al. 2002), and ronto (Khairina et al. 2013, 2016).

People are consumed ronto because it hasa a typical flavor that is impossibly found in other fermented shrimp products. Flavors that appeared from ronto are assumed as a result of breaking down the process of complex compounds (carbohydrate, protein, and lipid) which are mainly established in products into some simple compounds. This process is performed by the microbe that has an important role during fermentation. The degradation of the compound is as followed: starch is broken down into simple sugars and organic acids, such as lactic acid and alcohol; protein is broken down into peptides and amino acids; and lipid is break down into fatty acids. The reaction between organic acid and alcohol produces esters that are responsible for flavor and aromas in the product (Steinkraus 2002). Therefore, the acceptability for ronto consumption is highly influenced by sensory attributes, such as color, aroma, flavor, and appearance.

The high quality of ronto by fermentation is depending on salt and rice concentrations. The traditional method is used uncertain concentrations of two main materials during fermentation for ronto production, so that the product will be produced in different quality. Presently, no research has been conducted that surveyed about ronto production. To improve the quality of ronto, this experiment was observed the optimization and evaluated the effects of the combination of salt and rice concentration. The products from a combination of salt and rice were analyzed for pH, TVB-N, water content, ash, and microbiological properties (total bacteria, proteolytic bacteria, halophilic bacteria, lactic acid bacteria) and also product content such as protein, lipid, crude fiber, and carbohydrate.

RESEARCH METHOD

Materials

Shrimp (Penaeus monodon) was collected from fishermen at Aluh-Aluh, Banjar District, South Kalimantan, Indonesia. Salt and rice were obtained from the local market.

Experimental design

A completely randomized design with two factors was used in this study, the first factor is the variation of salt concentration at three level concentrations; 10% w/w (S1), 11% w/w (S2), and 12% w/w (S3) and the second factor is the variation of rice concentration at two concentration level; 20% w/w (R1) and 30% w/w (R2). Control treatment (C) was used to exemplify fishermen's traditional recipe (shrimp: salt: rice = 7:1:2). All combinations of treatments were carried out in triplicate experiments.

Preparation of Ronto

The shrimps was mixed with varying of the salt base on variable design and keep for 2 h, and then mixed with rice until homogenized. The mixed shrimps, salt and rice were placed into hygiene sealed bottles and allowed to ferment for 14 days at ambient temperature. Furthermore, the samples were analyzed for sensory evaluation (color, aroma, texture, and appearance), chemical analysis (proximate, pH, and TVB-N, and microbiological (total bacteria, proteolytic bacteria, halophilic bacteria, and lactic acid bacteria).

Sensory evaluation

A sensory property of ronto was evaluated using 10 trained panelists. The panelists are residents in a coastal area, which are familiar with and consumes the ronto regularly. Panelists were asked to evaluate each attributes using a five-point hedonic scale ranging from 1 dislike to 5 like extremely. Four different attributes were used to evaluate the qualities which were color, aroma, texture, and appearance. Each sample (20 g) was served in a cup and three digits random numbers were used for coding the samples. The evaluation was held in a sensory laboratory equipped with individual tasting booths in a conducive environment for testing. The treatments were arranged in completely randomized.

Chemical analysis

Crude protein content was calculated by converting the nitrogen content determined by Kjeldahl's method (6.25xN), pH, moisture, ash, fat, and crude extract determined (Sudarmadji et al. 1989). The TVB-N contents were determined using the Conway micro-diffusion method (Conway & Byrne, 1933). The amounts of TVB-N were calculated and expressed as mg N/g sample.

Microbiological analysis

Shrimp (25 g) was transferred into a stomacher bag containing 225 mL of 0.85% of NaCl solution. The sample was blending in a Stomacher 400 Lab Blender (Seward Ltd., Worthing, UK) at high speed for 3 min. Dilution water was used for diluting the samples. After that, the sample diluted in serial 8-folds steps was used for analysis by the pour plate technique. Total bacteria used plate count agar (PCA-Merck), the halophilic bacteria used Nutrient Agar-Merck add 5% of NaCl, proteolytic bacteria used Nutrient Agar-Merck with 2% of skim milk, and lactic acid bacteria used MRS-A with 1% of CaCO3. They were incubated at 35 °C for 48 h.

Statistical analysis

The data were analyzed using analysis of variance (ANOVA), then followed by mean comparison using Fisher's LSD test at significance level 5%. Statistical analysis was performed using Statistical Analysis System (SAS) program v9.3.

RESULTS AND DISCUSSION

Sensory evaluation

Fourteen days after fermentation, samples were collected from the storage box and their chemical, microbiological, and sensory properties were immediately analyzed and evaluated. The results showed that the ronto had various qualities under different salt and rice concentrations. Samples were evaluated by 10 panelists trained that were randomly chosen. Attributes recognized by the sensory panel as describing the sensory properties of ronto were color, aroma, texture, and appearance. In general, Fig. 1 showed the photographs of ronto under different salt and rice concentration.

Figure 1. The images of ronto production under different salt and rice concentration.

The addition of salt concentration 10%, 11%, and 12% in 20% of rice was not able to improve the yield and quality of ronto. Black color and unpleasant odor were observed from the samples (S1R1 and S1R2). On the other hand, the quality of ronto produced from salt 10%, 11%, and 12% mixed with rice 30% were better than control. As shown in Fig. 2, the sensory, chemical, and microbiological properties of ronto produced from a combination of 11% salt and 30% rice (S2R2) was the best product. The S2R2 received high acceptance from the sensory panel, the color of the product is orange. The other researcher showed the same the sensory panel of fermentation products (Pongsetkul et al. 2015).

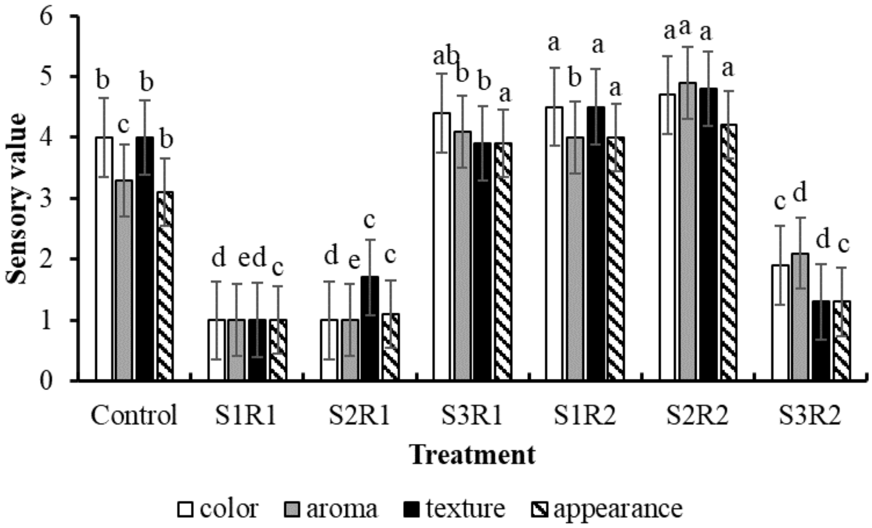

Figure 2. The sensory evaluation of ronto respect to color, aroma, texture, and appearance under different salt and rice concentration (S1R1: salt 10%, rice 20%; S2R1: salt 11%, rice 20%; S3R1: salt 12%, rice 20%; S2R1: salt 10%, rice 30%; S2R2: salt 11%, rice 30%; S3R2: salt 12%, rice 30%). Different notations of Anova results in the treatment showed significant differences in the same parameters.

Chemical analysis of Ronto

According to ANOVA analysis, the significant interaction between salt and rice concentration was not found in all proximate composition, including water content, ash, protein, lipid, crude fiber, and carbohydrate. Proximate composition values of ronto under different salt and rice concentrations can be seen in Fig. 3. It is well known that the objective of the addition of salt in shrimp fermentation is to restrict proteolytic enzymes and to inhibit the growth of pathogenic and spoilage bacteria. Salt is a substance that works as a preservative because it reduces the water activity of foods. Salt's ability to decrease water activity is due to the ability of sodium and chloride ions to associate with water molecules. As a result, this process causes osmosis. Thus, the addition of salt to ronto in the primal step of fermentation is solely a selection of microbes in natural ways.

Fig.3 and Fig. 4 are shown chemical quality represented the mixture of different products, diverse raw materials, and various process methods. The freshness of shrimp chosen, the amount of salt added, and the type of carbohydrates used indeed determine the quality of the product.

The difference in salt and rice concentration in the shrimp fermentation process affects the quality and success of the fermentation process. The amount of salt used must be able to be the initial selection of spoilage and pathogenic bacteria, while the amount of rice must be sufficient to stimulate the growth of acid-forming bacteria. The role of both of them will affect the physical, chemical, microbiological, and sensory changes in the fermented material. Proteolysis and acidity events during fermentation result in changes in biochemical properties that produce shrimp fermentation products that change color, aroma, taste and appearance (Killinc et al., 2006; Khairina et al. 2017).

Ronto water content for all treatments is almost the same. This is due to the use of salt 10% - 12% has a similar ability to draw water from shrimp meat. Ronto ash content is a mineral content derived from salt and shrimp shells, so the ash content is relatively no different. The content of ronto protein with a combination of 10% salt and 20% rice is the highest value. This shows that 10% salt increases the value of alkaline nitrogen with the highest TVB-N value of 430 mg N / 100 g sample at pH 6.5. This situation indicates the occurrence of rot. The combination of 10% salt and 20% rice has not been able to inhibit the growth of spoilage bacteria and stimulate the growth of acid-forming bacteria. Different conditions were seen when 11% and 12% salt were combined with 30% rice, a significant decrease in the value of TVB-N and pH showed that the fermentation process took place perfectly so that it could reduce the pH to less than 5. Rice served as a substrate for bacterial growth lactic acid (Figure 5) and was shown with pH values 4.6 and 5.0 for the treatment of S1R2 and S2R2. The growth of lactic acid bacteria was higher in the sample by giving 30% rice. TVB-N value and nitrogen content significantly increased during fermentation, and the condition is closely related to protein degradation in fish meat due to the activity of halophilic bacteria which are proteolytic. Therefore, some things that influence the growth of bacteria during the fermentation process of fishery products are the provision of salt, sugar, pH, high temperature, the predominance of proteolytic enzymes, and the availability of nutrients (Khairina et al., 2017; Zaog et al., 2019; Puspita et al., 2017).

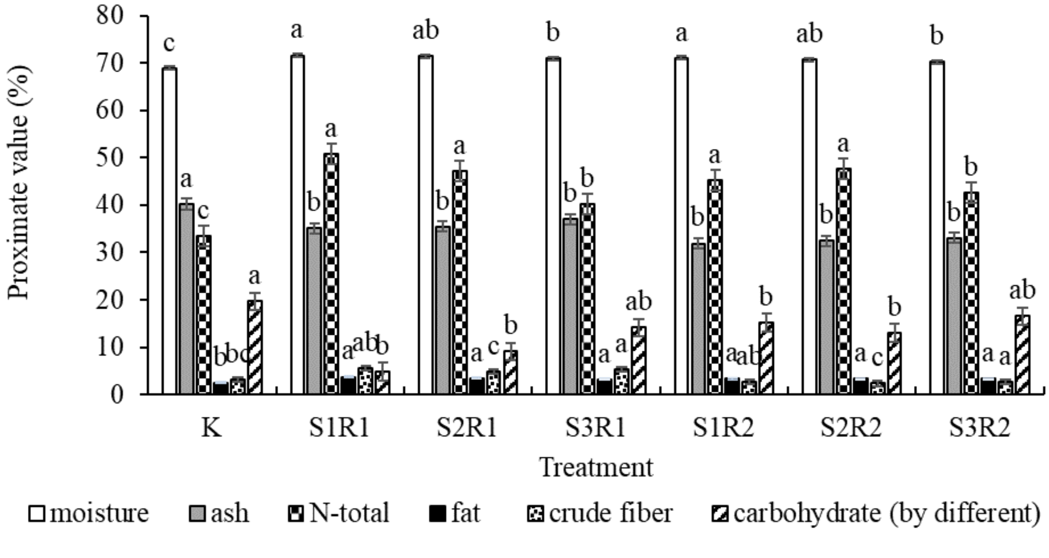

Figure 3. Proximate value of ronto under different salt and rice concentrations (S1R1: salt 10%, rice 20%; S2R1: salt 11%, rice 20%; S3R1: salt 12%, rice 20%; S2R1: salt 10%, rice 30%; S2R2: salt 11%, rice 30%; S3R2: salt 12%, rice 30%). Different notations of Anova results in the treatment showed significant differences in the same parameters.

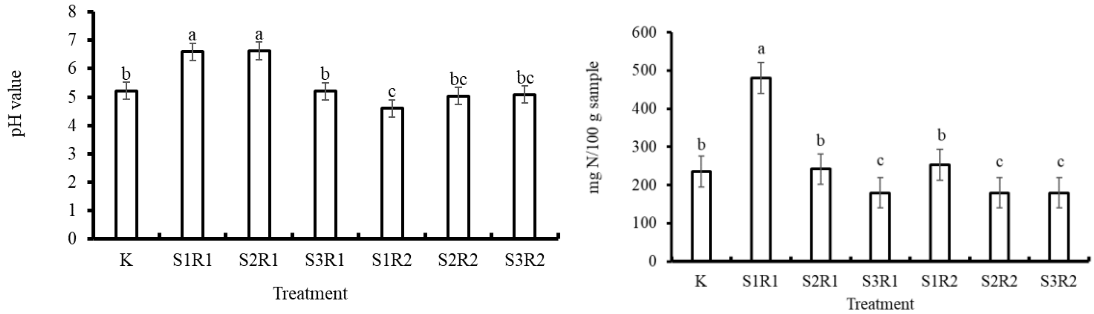

Figure 4. The pH and TVB-N value ronto under different salt and rice concentration (S1R1: salt 10%, rice 20%; S2R1: salt 11%, rice 20%; S3R1: salt 12%, rice 20%; S2R1: salt 10%, rice 30%; S2R2: salt 11%, rice 30%; S3R2: salt 12%, rice 30%). Different notations of Anova results in the treatment showed significant differences in the same parameters.

The acceptable limit of TVB-N set for the salted fish products is 200 mg N/100 g sample (Connell 1990). In association with pH values shown in Fig. 4, pH of control (C), S3R1, S1R2, S2R2, and S3R2 were 5.2, 5.2, 4.6, 5.0, and 5.1, respectively. Those range of pH values of ronto were similar to some products sold in traditional markets of South Kalimantan (Khairina et al. 2016). Compared to balao-balao from the Phillipines, the pH of ronto is similar to the pH of balao-balao at a value of 5.0. Balao-balao is a fermented shrimp product made from the composition of shrimp, rice, and salt with the ratio of 8:4:1 (Rhee et al. 2011). The pH of ronto is significantly lower than that pH of rusip, a fish-fermented product from Bangka Belitung with a pH of 5.9 (Koesoemawardani et al. 2013). Cincalok is a similar product to ronto, but differs in chemical properties due to the type of carbohydrates added to its product (Dyastuti et al, 2013).

Table 1. Comparison of chemical properties of ronto to other similar products

Chemical Properties	Sample from the market (Khairina et al. 2013)	Sample control*	S_3R_1	S_1R_2	S_2R_2	Kong-som(Faithong et al. 2010)	Cincalok (Mohamed et al. 2012)
Water content (%)	69.72-82.63	68.95	70.95	71.09	70.74	71.35-80.71	74.6
Ash (%) dw	17.92 -52.90	40.22	36.99	31.91	32.50	16.56-25.94	50.79
Protein (%) dw	24.18 -31.49	33.56	40.31	45.33	47.58	23.94-37.37	35.83
Lipid (%) dw	1.32-3.87	2.50	3.18	3.39	3.35	0.40-0.76	3.15
Carbohydrate (%) dw	-	6.12	4.11	4.38	3.82	15.75-6.93	10.24
pH	3.92-5.86	5.22	5.21	4.60	5.03	3.89-3.71	-
TVB (mg N/100 g sample)	44.00-158.00	236.00	180.0	252.8	180.0	-	-

*ronto produced with ratio shrimp:salt:rice = 7:1:2

Different concentration of shrimp, salt, and rice in shrimp fermentation surely affects the quality and successfulness of fermentation. The amount of salt added to the product should be capable to act as a selective agent for spoilage and pathogenic bacteria. The addition of rice in several concentrations should adequately stimulate the growth of acid forming bacteria due to their role in altering the physical, chemical, microbiological, and sensory properties of fermented material. Proteolysis and acidy events during fermentation cause biochemical properties changes. As a result, fermented shrimp product changes color, aroma, and appearance (Kilinc et al. 2006). Table 1 is shown the comparison of the chemical properties of ronto and other products of shrimp fermentation by the previous researcher. According to total volatile bases (TVB), the high quality of ronto was produced from the combination of salt 11% and rice 30%, with a value of 180 mg N/100 sample.

Microbiology activity

The microbiological activity of ronto is shown in Fig 5. The product by treatment S1R2 and S2R2 showed the highest amount of lactic acid bacteria, it was 9.28 and 9.05 log CFU/g, respectively. This result could be an indicator that lactic acid bacteria were well-grown in that environment. During the growth phase, lactic acid bacteria yield organic acids as a secondary metabolite. Organic acids play a role to lower the pH of the products. While the pH range is 3.5 to 5.0, some spoilage and pathogenic bacteria were not optimally grown. In general, bacteria grow fast on pH of 6.0-8.0, yeast on pH of 4.5-6.5, and fungi on pH of 3.5-6.8. For Lactobacilli groups and acetic acid bacteria can grow on pH of 5.0-6.0. This environment is in line with the pH of ronto and similar fermented shrimp products with other carbohydrate materials, such as shikae, cincalok, and kong-som which have a range of pH 3.9-5.8. The observed ronto had a pH of 4.6-5.2 which means that Lactobacilli groups and acetic acid bacteria are capable to grow optimally (Dilbagii and Sharma 2007).

The difference in the concentration of salt and rice used in ronto processing has a very significant effect on the number and types of bacteria that grow during fermentation. Proteolytic, halophilic, and lactic acid bacteria grew well on ronto with the addition of 30% rice, which reached a density of log 10^9. Meanwhile, the ronto using 20% rice was only in the log 10^2 – 10^6 range. Rice acts as a suitable substrate for the growth of proteolytic lactic acid bacteria. The decomposition of rice into glucose during fermentation provides a source of energy for the growth of lactic acid bacteria. Khairina et al (2017) reported that during ronto fermentation with 12% salt and 20% rice, the growth of lactic acid bacteria was log 10^6 on the 6th day of fermentation and then decreased to log 10^3 on the 18th day.

According to Rhee et al (2011), the number of lactic acid bacteria and proteolytic bacteria from sikhae (Korean fermented fish product) fermented for 12 days were log 10^6 and log 10^4. Whereas, the number of lactic acid bacteria and a total of microbe from rusip were 10.40 log CFU/g and 8.68 log CFU/g (Koesoemawardani et al. 2013). Palludan-Muller et al. (2002) stated that important microbes working in shrimp fermentation are aerobic bacteria, lactic acid bacteria, and yeast. The shrimp paste fermentation generated distinctive flavor as a result of breaking down of protein, lipid, and carbohydrate in shrimp body and shell which was activated by proteolytic, lipolytic, and amylolytic bacteria (Mohamed et al. 2012).

The numbers of salt added to fermented food probably affect the population of microorganism. The range of salt concentration was correlated with the growth of bacteria. The salt concentration of 2-2.5% found in fermented products caused proteolytic and spoilage bacteria to live. In contrast, a salt concentration of 3-10% in an anaerobic environment stimulated the growth of lactic acid bacteria. If the salt concentration is increased up to 7%, the growth of spoilage bacteria is completely inhibited (Mackie et al. 1971). The salt concentration used in ronto was 10-12%, however, can be used as strong evidence that spoilage bacteria are fully inactive.

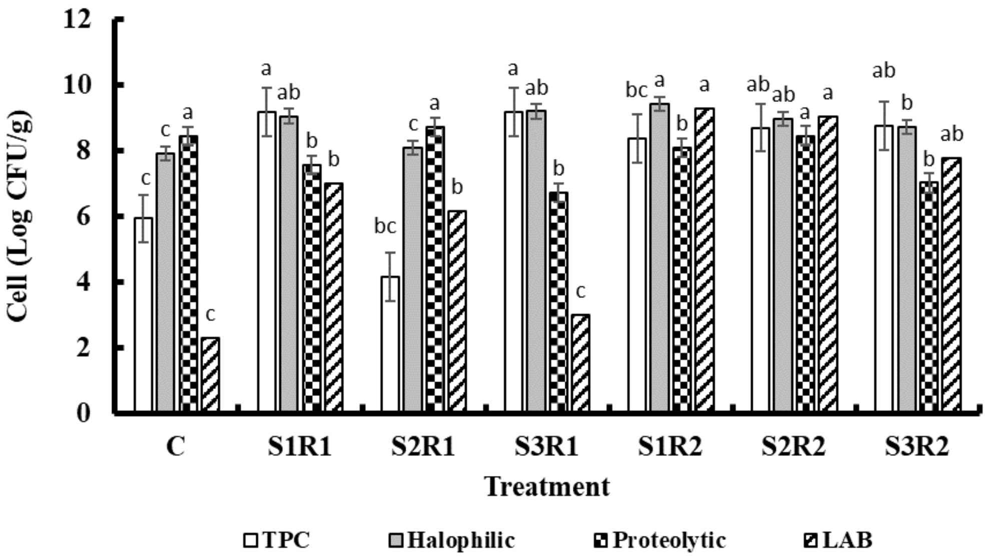

Figure 5. Total bacteria, halophilic bacteria, proteolytic bacteria, and lactic acid bacteria ronto under different salt and rice consentration (S1R1: salt 10%, rice 20%; S2R1: salt 11%, rice 20%; S3R1: salt 12%, rice 20%; S2R1: salt 10%, rice 30%; S2R2: salt 11%, rice 30%; S3R2: salt 12%, rice 30%). Different notations of Anova results in the treatment showed significant differences in the same parameters.

CONCLUSION

In summary, about concerning sensory, chemical, and microbiological properties, treatment S2R2 (salt 11%, rice 30%) produced the best quality ronto, followed by S1R2 (salt 10%, rice 30%) and S3R¬1 (salt 12%, rice 20%). The pH and Total Volatile Bases value were 5.02 dan 180 mg N/100 g sample. Total bacteria, halophilic bacteria, proteolytic bacteria, and lactic acid bacteria of S2R2 ronto were log 8.71 CFU/g, log 8.98 CFU/g, log 8.46 CFU/g, and log 9.05 CFU/g, respectively. The addition of salt 10% and 11% mixed with rice 20% was not able to produce ronto due to spoilage bacteria activity with symptoms possessing black color and unpleasant odor.

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