**Characteristics of *Sie reuboh* with Different Combination Palm Vinegar(*Arenga pinnata*) and Kaffir Lime Leaves (*Citrus hystrix*) Addition**.

Masyitaha\*, I.I. Ariefb, & T.Suryatic

aStudy Program of Animal Production and Technology, Faculty of Animal Science, Graduate School, Bogor Agricultural University

bDepartment of Animal Production and Technology, Faculty of Animal Science, Bogor Agricultural University

Jalan Agatis, Kampus IPB Darmaga Bogor 16680, Indonesia.

\*Corresponding author: [masyitah3181@gmail.com](mailto:masyitah3181@gmail.com)

**ABSTRACK**

*Sie reuboh* is traditional food from Aceh made from beef, buffalo or goat. The use of palm vinegar was recognized to provide sour taste and antibacteria of *sie reuboh.* Kaffir Lime leaves have a distinctive aroma and a good source of natural antioxidants and also have antibacterial properties that may inhibit pathogen bacteria. This research was aimed to analyze of the characteristics of *sie reuboh* with addition of palm vinegar and kaffir lime leaves with different for concentrations of the *sie reuboh* produce quality and to evaluate the stability of sie reuboh with addition of palm vinegar and kaffir lime leaves stored at room temperature. The research was consisted on 2 stages. Stage 1 *sie reuboh* was treated with different levels of both ingredients palm vinegar and kaffir lime leaves. Stage 2 was storage at room temperature for 9 days to *sie reuboh* the best of microbiology analysis in stage 1. *Sie reuboh* were made with the addition of palm vinegar (*Arenga pinnata*) and kaffir lime leaves (*Citrus hystrix*) with three replications. The result showed that different levels of palm vinegar and kaffir lime leaves showed significant effect (P<0.05) on the tenderness of *sie reuboh* was better than other addition without of palm vinegar and kaffir lime leaves was compared *sie reuboh* with the addicted of palm vinegar and kaffir lime leaves and the best *sie reuboh* was obtained from concentration of palm vinegar 120 mL + kaffir lime leaves 20 g, yielding showed antioxidant activity in free radical inhibition marked with ability to maintaining stable TBARS value until the end stored at room temperature and effective to maintained of quality *sie reuboh* marked conformity by limit total plate count and mold growth for 3 days of stored at room temperature.

*Key words: palm vinegar, lime leaves, sie reuboh, room temperature*

**ABSTRAK**

*Sie reuboh* adalah salah satu makan khas tradisional Aceh yang terbuat dari bahan dasar daging sapi, kerbau atau kambing. Cuka aren merupakan bumbu yang memberikan cita rasa asam pada produk *sie reuboh* and bersifat antibakteri. Daun jeruk purut memiliki aroma yang khas dan sumber yang baik sebagai antioksidan alami dan memiliki sifat antibakteri yang dapat menghambat bakteri patogen. Tujuan penelitian adalah menganalisis karakteristik *sie reuboh* yang diberi penambahan cuka aren (*Arenga pinnata*) dan daun jeruk purut (*Citrus hystrix*) dengan konsentrasi berbeda untuk menghasilkan mutu *sie reuboh* yang baik dan mengevaluasi stabilitas *sie reuboh* yang diberi penambahan cuka aren dan daun jeruk purut yang berbeda selama penyimpanan suhu ruang. Penelitian dilakukan terdiri atas 2 tahap. Tahap I melakukan penambahan cuka aren dan daun jeruk purut dengan konsentrasi berbeda. Tahap II penyimpanan suhu ruang selama 9 hari pada *sie reuboh* dengan hasil mikrobioligis yang terbaik pada tahap 1. *Sie reuboh* dengan perlakuan penambahan cuka aren (*Arenga pinnata*) dan daun jeruk purut (*Citrus hystrix*) dengan tiga ulangan. Hasil penelitian menunjukkan cuka aren dan daun jeruk purut dengan konsentrasi yang berbeda menunjukkan berpengaruh nyata (p<0.05) pada keempukan *sie reuboh* yang lebih baik tanpa penambahan cuka aren dan daun jeruk purut dibandingkan dengan *sie reuboh* yang diberikan penambahan cuka aren dan daun jeruk purut dan *sie reuboh* dengan konsentrasi 120 mL cuka aren + 20 g daun jeruk purut menunjukkan aktivitas antioksidan dalam menghambat radikal bebas yang ditandai dengan kemampuan mempertahankan nilai TBARS sampai akhir penyimpanan serta efektif dalam menstabilkan mutu produk *sie reuboh* yang ditandai dengan kesesuaian batas maksimum *total plate count* dan kapang sampai hari ke-3 penyimpanan suhu ruang.

*Kata kunci: sie reuboh, cuka aren, daun jeruk purut, penyimpanan suhu ruang*

**INTRODUCTION**

*Sie reuboh* is a traditional food from Aceh, Indonesia, and processed from meat cow or buffalo meat with addition of such ingredients as palm vinegar, fat, and spices. The meat and these ingredients were cooked to obtain soft texture, Its quality could be maintained and still acceptable for a month at room temperature. The processed meat is reheated prior to consumption.

Palm vinegar is an important ingredient for preparation of *sie reuboh*, which contribute to sour taste. This vinegar involves alcohol fermentation by Saccharomyces, and acetic acid fermentation by Acetobacter aceti, A. pastorianus and A. hansenii (Plessi, 2003). Presence of acetic acid in palm vinegar is responsible for meat preservation. Acetic acid is effective to decontaminate molds and yeast as main target in meat and its derivative products (Lopez *et al*. 2012). Antimicrobial activity of acetic acid is responsible for presence of detrimental effects on microorganism through lowering pH value (Theron dan Lues 2007).

Kaffir lime leaves have long been utilized as traditional medicine and food spices (Copriady *et al*. 2005). They are also recognized to have antioxidant activity that is linked with presence of essential oil, flavonoid, phenolic, saponin, steroid, terpenoid and alkaloid, terpen (Rahmi *et al.* 2013). Lime peels and leaves are good source of antioxidant compounds. Flavonoid was a phenolic compound reported to show high antioxidant activity (Liciana *et al.* 2013). This research was aimed to analyze of the characteristics of *sie reuboh* with addition of palm vinegar and kaffir lime leaves with different for concentrations of the *sie reuboh* produce quality and to evaluate the stability of *sie reuboh* with addition of palm vinegar and kaffir lime leaves stored at room temperature. The use of preservative as natural antioxidant and antimicrobial source in meat products enhanced quality of meat product. Yuliani *et al.* (2010) studied antibacterial activity of essential oils from lime leaves, and the result showed that the compound was able to induce inhibitory and lethal effects on *Staphylococcus aureus* and *Escherichia coli*. In addition, Widaningrum *et al.* (2015) found that the use of vinegar (processed from coconut water and banana peel) was able to extend shelf life of chicken meat stored at room temperature through retarding growth of pathogenic bacteria *Listeria monocytogenes*.

**MATERIALS AND METHODS**

**Sample Preparation**

*Sie reuboh* was prepared from knucle meat 4000 g , additional ingredients used were ginger 20 g, table salt 15 g, fresh turmeric 20 g, galangal 20 g, onion 15 g, fresh chili 25 g, chili powder 10 g, cayenne pepper 10 g, water 300 mL, palm vinegar (100 mL, 120 mL, and 140 mL), and kaffir lime leaves (10 g, 20 g and 30 g). The manufacturing process, the meat was cleaned, cut 40-60 g, washed, and drained for 5-10 min. All spices were pulverized, then mixed water and meat. The mixture was cooked in a soil-made crock. After 15 min, palm vinegar was added, and cooked for 45 min. Kaffir lime leaves were then added and cooked to reduce water. *sie reuboh* was treated with different levels of both ingredients palm vinegar and kaffir lime leaves as follows: P0 (0 mL palm vinegar + 0 kaffir lime leaves), P1 ( 100 mL palm vinegar + 10 g kaffir lime leaves), P2 (120 mL palm vinegar + 20 g kaffir lime leaves) and (140 mL palm vinegar + 30 g kaffir lime leaves)

**Physicochemical Characteristics of *Sie Reuboh***

Acidity of *sie reuboh* was determined using pH meter (HANNA HI 99163 instrument, USA) following the method of Dominigue *et al.* (2015). Previously calibrated in buffer at pH 4 and 7. Water activity (aw) was determined using aw meter Novasina (Novasina, Switzerland). Meat tenderness was assessed using modified method of Bourne (1978) was using texture analyzer TA-XT2i (Stable Micro System, UK).

**Microbiological Analyses (BAM 2001)**

*Sie reuboh* samples 25 g were incorporated in 225 ml of buffer pepton water (BPW) solution and homogenized, then allowed to stand for 30 min to get a 10-1 dilution. As much as 1 mL suspension was pipette-transferred until getting a 10-3 dilution.

**Total plate count (TPC)**. As much as 1 mL suspension from 10-1-10-3 dilutions was transferred into petridishes in duplicate, then added 20 mL Plate Count Agar (PCA) medium and homogenized. The petridishes were incubated at 37°C for 36 h.

***Escherichia coli.*** As much as 1 mL suspension from 10-1-10-3 dilutions were aseptic-pipetted into petridishes in duplicate, then added 20 mL Eosin Methylen Blue Agar (EMBA) medium and homogenized. Petridishes were incubated at 37°C for 36 h.

***Staphylococcus aureus.*** As much as 1 mL suspension from 10-1-10-3 dilutions were aseptic-pipetted in duplicate into petridishes that were already filled with 15–20 mL Baird Parker Agar (BPA) medium + egg yolk and homogenized. The suspension was then well-spread on the media surface using hockey stick and allowed to stand for ± 30 min at room temperature. The media were incubated upside down at 37°C for 36 h.

**Antioxidant Activity using DPPH assay (Tangkanakul *et al.* 2009)**

Sample 2 g was extracted by 5 ml of methanol 100 % at room temperature for 24 h., and filtered. The filtrate was transferred into another tube, and 5 ml of methanol was added. The filtrate solution 10 ml was added by methanol to obtain 10 ml volume. Antioxidant activity was determined by ability scavenge DPPH and the antioxidant capacity was determined by ability scavenge of vitamin C (0, 0.5, 1.0, 1.5, 2.0 and 2.5 ml/mg) to scavenge DPPH. The solution was incubated for 20 min, and the absorbance was detected at 517 nm. Methanol was used for control. Inhibitory activity to DPPH was calculated as follow:

% Scavenging **=**

**Thiobarbituric acid reactive substances (TBARS) (Sorensen dan Jorgensen 1996)**

Standard curve was made from stock solution of 1,1,3,3-tetra oxypropane (TEP) 0.002M at concentration of 2x10-6 M to 8x10-6 M. Minced sample 10 g was homogenized by adding 50 mL distilled water containing 0.1% of propel galate (PG) and 0.1% of ethylene diamine tetraacetic acid (EDTA). The solution was transferred quantitively into distillation tube by adding 47.5 mL of distilled water, and then added with 2.5 mL of HCl 4N and 5 drops of antifoaming agent. The solution was distilled to obtain 50 of distillate for each sample. The distillate was incubated in waterbath at 100ºC for 40 min, and cooled. The absorbance was detected at wavelength of 532 nm using spectrophotometer. Each experiment was performed at duplicate. Malonaldehyde was calculated using this formula:

Malonaldehyde content (MD) =

**Statistical Analysis**

The physicochemical, activity antioksidan, TBARS and microbiological quality data were analyzed using the analysis of variance (ANOVA). The significantly different treatments were further-tested by Duncan’s multiple range test. Data of physicochemical and microbiological properties as a result of various concentrations of palm vinegar and lime leaves were obtained from experiment using grup randomized design. Mean while, data of physicochemical, antioxidant activity, TBARS and microbiological properties during storage at room temperature were obtained using factorial complete randomized design

**RESULTS**

**Characteristics of *sie reuboh* with addition of palm vinegar (*Arenga pinnata*) and kaffir lime leaves (*Citrus hystrix*) with different**

Physicochemical characteristics showed properties of *sie reuboh* as a result of palm vinegar addition significant effects (p<0.05) on tenderness (Table 1). The tenderness ranged from 3607.67±510.15 gs – 6832.33± 860.76 gs. P0 showed the best tenderness 3606.67±510.15 gs, than P1, P2 and P3 produced the lowest tenderness 5750.67±717.38 gs, 6010.33± 615.48 gs dan 6832.33± 860.76 gs.

**Characteristics Properties of *Sie Reuboh* during Storage at Room Temperature**

During storage at room temperature, we found significant difference (p<0.05) in aw (Table 2). The aw value ranged from 0.837±0.00 - 0.837 ± 0.00. At day 3, an increase in aw are 0.860 ± 0.00 was observed, but aw was decreased at day 6 dan 9 are 0.855 ± 0.00 - 0.837 ± 0.00.

Antioxidant capacity and antioxidant activity of *sie reuboh* show significant difference (p<0.05) (Table 2). Antioxidant capacity ranged from 133.65±50.59 EVC mg 100 g-1 - 269.44±39.02 EVC mg 100 g-1, while antioxidant activity ranged from 33.93±2.16 % - 53.34 ± 2.33 %.

Thiobarbituric acid reactive substance (TBARS) value of *sie reuboh* show significant difference (p<0.05) (Tble 2). TBARS was observed from 0.66 ± 0.04 mg MDA/kg 100-1 BK - 0.85±0.07 mg MDA/kg 100-1 BK. Increase in storage time led to reduction of antioxidant capacity and activity, but increased TBARS.

Microbiological characteristics the results show significant effects (p<0.05) on total plate count and mold (Table 2). Total plate count was observed from 3.15±0.58 log cfu/g-1 - 4.84±0.21 log cfu/g-1, while mold ranged from 2.83±0.21 log cfu/g-1 – 4.79±0.14 log cfu/g-1. Both total plate count and mold increased with increase in storage period.

**DISCUSSION**

**Characteristics of *sie reuboh* with addition of palm vinegar (*Arenga pinnata*) and kaffir lime leaves (*Citrus hystrix*) with different**

**Tenderness.** Higher concentration of palm vinegar was associated with lower tenderness. Higher concentration of palm vinegar was associated with lower tenderness. Presence of acetic acid in palm vinegar was able to extract protein, thus weakening protein myofibril bonds in meat, reducing water content of meat, and leading to higher meat dryness. Fardiaz (1983) reported that presence of acid contributed to demolish myofibril bonds, leading to higher release of water. Widiati *et al.* (2002) showed that lower water content of meat was responsible for dry texture, thus higher effort was needed to destruct myofibrillar tissue. Lawrie (2003) stated that most water in mosculorum was located in myofibril, specifically in thick filament from myosin and thin filament from actin/tropomyosin. Degree of tenderness could be linked with 3 proteins in endomysium, myofibrillar, and sarcoplasm. Their contribution was dependent on myofibrillar contraction, types of tendon and cooking temperatur.

**Characteristics of *Sie reuboh* Stored at Room Temperature**

**Water activity (aw**). Enhancement of aw was associated with temperature and moisture of sample storage. Sigh *et al.* (2001) found that increment of aw resulted from addition of top water layer which was induced by storage conditions product. Temperature and moisture remarkably affected water content, since the product would make same moisture as its environment (Purnomo 1995). Higher absorbed water led to increase in aw, leading to more susceptibility to microbial deterioration (Rahayu dan Nuwitri 2012). Temperature and pH also contributed to changes in aw (Lawrie 2003). In day 6 and day 9 of storage, the aw value was 0.855 - 0.837 until the end of storage period. In day 9, presence of hypha growth was observed, but their contamination was not found in all samples. Decreased aw in *sie reuboh* was associated with nutritional degradation, particularly protein, by microorganism, which lead to lower water binding capacity of protein. Arizona *et al*. (2011) found water binding capacity of meat treated with various levels of liquid smoke in initial storage was 32.58 %, where the lowest value 14.6 % was observed in day 4. We observed that longer storage period led to decreased water binding capacity of meat as a result of microbial activity that promoted protein damage. Rahayu and Nurwitri (2012) reported that microorganism required dissimilar aw conditions for their optimum growth such as yeast 0.88, molds 0.80, Gram positive bacteria 0.90, and Gram negative bacteria 0.93.

**Antioxidant capacity and antioxidant activity.** Antioxidant capacity of sie reuboh was affected by interaction of composition and concentration of antioxidant compounds contained in spices, palm vinegar and lime leaves added in *sie reuboh*. Antioxidant activity was decreased, indicating that antioxidant compounds in *sie reuboh* inhibited lipid oxidation through retarding activity of free radicals during storage. Zhang *et al.* (2010) reported that polyphenol, flavonoid, terpene, phenolic, and tannin were responsible for antioxidant activity. Peel and leave extracts of Citrus hystrix showed remarkable antioxidant activity. Flavonoid is a phenolic compound that shows strong antioxidant activity (Licina *et al.* 2013). Murdijati and Gardjito (2013) reported that palm vinegar was used to provide sour taste and avoid changes in color induced by oxidation. According to synergistic properties, antioxidative agent was grouped into 2 categories: high phenolic compound and high acid compound (Kataren 2012). Antioxidant effect from phenolic compound is unable to scavenge oxygen, but able to retard formation of free radicals from lipid, which react with oxygen during lipid oxidation, thus autoxidation was postponed (Pereira *et al*. 2009).

**TBARS**. Decrease in antioxidant activity was associated with increase in TBARS. Initially, the unique flavor of *sie reuboh* was strongly detected, but we found that no rancidity flavor was observed in the end of storage period. Decrease in antioxidant activity was associated with increase in TBARS. Initially, the unique flavor of *sie reuboh* was strongly detected, but we found that no rancidity flavor was observed in the end of storage period. Lipid oxidation occurred until the end of storage period in room temperature with decrease in antioxidant activity to inhibit oxidation by retardation of free radicals. This indicated lipid degradation as a result of free radical autoxidation of unsaturated fatty acids in *sie reuboh* affected by factors during storage, which was associated with higher accumulation of lipid oxidation of unsaturated fatty acids in *sie reuboh*. Presence of antioxidant compounds in spices, palm vinegar, and kaffir lime leaves was able to attenuate oxidation rate of unsaturated fatty acids in meat. The maximum level of TBARS in processed meat that showed no rancidity flavor was 2.28 mg MDA/Kg. TBARS of *sie reuboh* until day 9 showed no rancidity flavor. Compo et al. (2006) found that TBARS of 2.28 was acceptable threshold for oxidation of rancid beef. Increase in TBARS was due to secondary lipid oxidation product such as malonaldehyde (MDA) (Thanonkaew *et al.* 2006). Yuanita (2006) stated that lipid oxidation in food occurred during storage and was influenced by time, temperature, and air exposure. The oxidation was observed in room temperature and high temperature processing. Oxidation was due to presence of unsaturated fatty acid from room temperature to 100 °C (Kataren 2012). Tranggono (1990) reported synergistic effect of acid and antioxidant activity in retarding rancidity and browning on foods containing carbohydrate, protein, fat or oil. Inhibition of lipid oxidation was due to ability of metal bonding. Presence of metal was able to induce initial oxidation (Cahyadi 2009). Retardation of MDA formation and the use of spices with antioxidant properties showed desirable effects on meat products (Li *et al.* 2010).

**Total plate count (TPC)**. *Sie reuboh* was acceptable up to day 3 storage at room temperature. According to Badan Standarisasi Nasional (BSN) Total plate count value for processed meat was 105 cfu/g. Al-Qadiri *et al.* (2008) Longer storage period was correlated with higher number of bacteria, since some nutrients were required for their growth. Lawrie (2003) stated that RH, temperature, and oxygen availability were also responsible for microbial growth. Their growth could be retarded by inclusion of preservative compounds that enable to maintain product quality and assure food safety (Devatkal *et al.* 2010). Widyaningrum *et al.* (2015) observed the effects of vinegar made from banana peel on chicken meat, and the result suggested that the use of this vinegar could destabilize L. monocytogenes in raw chicken meat stored in room temperature and cool storage. Shan *et al*. (2007) reported that antibacterial effects of phenolic compound enabled to degrade cell wall and cytoplasm membrane, causing degradation of cellular components, alteration of fatty acids and phospholipid, and changes in synthesis of DNA and RNA, as well as degradation of translocated protein. Several studies showed that phenolic compounds from plant sources were able to inhibit pathogens in food. Total phenolic compounds are associated with antibacterial activity. Yuliani *et al*. (2011) stated that terpene compounds showed antibacterial effect through deconstruction of wall cell and inhibition of cell growth.

**Mold.** Mold growth was observed until the end of storage at room temperature. Mold growth which is influenced by some factors including temperature (optimum growth at 25– 35°C), water and oxygen availability, pH, and nutrition (Waluyo 2007). Pelczar and Chan (2010) stated that incubation at 37°C was the optimum condition for mold growth, but higher temperature was needed for optimum growth of yeast. Madduluri *et al*. (2013) reported that saponin was effective as antifungal by lowering surface tension of wall cell, and promoting membrane dysfunction that caused migration of protein and enzyme from cells.

**CONCLUSION**

Addition of palm vinegar 120 mL and lime leaves 20 g was effective to maintain the quality of *sie reuboh* until day 3 according to microbiological properties and TBARS until the end of storage period at room temperature.

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**Table**

Table 1 Characteristics of *sie reuboh* with addition of palm vinegar (*Arenga pinnata*) and kaffir lime leaves (*Citrus hystrix*) with different

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Variables | Treatments | | | |
| P0 | P1 | P2 | P3 |
| aw | 0.848± 0.02 | 0.838± 0.02 | 0.835± 0.02 | 0.831± 0.01 |
| pH | 5.86± 0.33 | 5.50± 0.52 | 5.39± 0.56 | 5.31± 0.66 |
| Yield (%) | 47.79± 1.24 | 49.29± 0.62 | 49.5± 1.61 | 51.31± 2.86 |
| Tenderness (*gs*) | 3607.67±510.15b | 5750.67±717.38a | 6010.33±615.48a | 6832.33±860.76a |
| *total plate count*  (log cfu/g -1) | 3.44± 0.72 | 2.85± 1.13 | 2.75± 0.28 | 3.08± 0.66 |
| *E.coli\**(colony) | <25 (1) | <25 (3) | nd | nd |
| *S.aureus\**(colony) | nd | nd | nd | nd |

Note: Different superscripts in the same line showed significant difference (p<0.05); detection of <25 colonies in P0 at dilution 3 and in P1 at dilution 3: nd= not detected (no colony observed)

Table 2 Characteristics of *Sie reuboh* Stored at Room Temperature

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Period of storage  (Day) | Variables | | | | |
| Total Plate Count  (log cfu/g -1) | Mold  (log cfu/g -1) | Antioxidant  Activity  (%) | Antioxidant Capacity  (mg EVC 100 g-1  BK *Sie reuboh)* | TBARS  (mg MDA/KgBK *Sie reuboh*) |
| 0 | 3.15 ± 0.58c | 2.83 ± 0.21b | 53.34 ± 2.33a | 269.44 ± 39.02a | 0.66± 0.04b |
| 3 | 3.97 ± 0.62bc | 3.28 ± 0.60b | 42.19 ± 4.24ab | 193.38 ± 49.42ab | 0.79± 0.05a |
| 6 | 4.69 ± 0.24a | 4.39± 0.45a | 37.29 ±10.08b | 136.53 ± 80.46b | 0.82 ± 0.04a |
| 9 | 4.84 ± 0.21a | 4.79± 0.14a | 33.93 ± 2.16b | 133.65 ± 50.59b | 0.85 ± 0.07a |

Note: Different superscripts in the same line showed significant difference (p<0.05).