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Effect of Ethanol (Sophia) Concentrations on Physicochemical Quality and Lipid Oxidation of Na'an Maran (Dried Timorese Beef)

Gemini E.M. Malelak[1]*, Carmelita De Araujo Ximenes[1], Arnol E. Manu[1], Sulmiyati Sulmiyati[1], I Gusti N Jelantik[1]

[1] Department of Animal Science, Faculty of Animal Husbandry, Marine and Fisheries, University of Nusa Cendana, Kupang, 85228, Indonesia.

ABSTRACT

Na'an maran is a traditional Timorese dried beef product. This experiment investigated the effects of adding Sophia (an ethanol-containing beverage) at different alcohol concentrations on the quality and potential shelf-life of Na'an maran. A completely randomized design (CRD) 4 × 5 (four treatments and five replicates each treatment). The treatments were: S_0 (no added alcohol/ control): S_1 (Sophia with 10% alcohol); S_2 (Sophia with 12% alcohol); and S_3 (Sophia with 14% alcohol). Quality characteristics assessed included pH. texture (hardness), meat microstructure, water, protein and lipid contents, TBA values, and ethanol residue. Data were analyzed using analysis of variance (ANOVA). The results showed that the addition of Sophia at 10–14% significantly reduced pH, yield, and hardness ($P < 0.05$). The lipid content was significantly lower in the 12–14% treatments ($P < 0.05$). and TBA values decreased with alcohol treatment ($P < 0.05$). indicates improved oxidative stability. The protein content and ethanol residue were not significantly affected. Microstructural observations revealed that alcohol-treated samples had reduced diameters of muscle fibers and collagen. Overall. The study suggests that Sophia at 10–14% alcohol concentration can improve tenderness and may help prolong the shelf life of Na'an maran by lowering pH and water-related properties and reducing lipid oxidation (TBA). Importantly. All measured ethanol residues were below the Indonesian Ulema Council (MUI) recommended limits.

KEYWORDS: Na'an maran, Alcohol concentration, pH, lipid oxidation, microstructure

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Corresponding Author:
Gemini E.M. Malelak

INTRODUCTION

Na'an maran is a dried. boneless beef product prepared by the Timorese. The beef is sliced into rope-shaped pieces. seasoned with salt. and dried in the sun. This dried meat has a tough texture and a dark red color. Na'an maran can be eaten directly after drying. but long-term storage causes the color to turn black. the texture to harden. and the flavor to fade. If it is to be consumed. it must be ground first. As a result. this dried meat product is rarely sold commercially. even though it can command high economic value.

In the world. dried meat products are known by various regional names. such as: pastirma (Turkey), jerky (North America), carne-de-sol (Brazil), biltong (South Africa), kaddid (North Africa) and cecina (Spain) (Mediani et al., 2022). In general, dried meat is referred to as biltong. Biltong-like products do not require rehydration or cooking, as they are ready-to-eat and can serve as a snack in certain diets (Jones et al., 2017). The stability of the final product and the sensory quality of ready-to-eat biltong can be influenced by differences in the type of meat used, the composition of the spice mixture, the use of food additives (Petit et al., 2014), the level of salting and drying ((Engez et al., 2012). Food additives that are often used include apple cider vinegar, sugar, black pepper, coriander, nitrite, sugar and/or cumin (Kaban, 2013). The purpose of using various food additives includes improving flavor, enhancing meat texture/tenderness, preventing microbial damage and slowing lipid oxidation which can help maintain the color of

na'an maran. Color change occurs due to the non-enzymatic oxidation of myoglobin (Fe^{2+}) to form metmyoglobin (Fe^{3+}); this reaction generally occurs parallel to the rancidification process (Han et al.. 2024)

The use of food additives (ADTs) in meat preservation offers producers the opportunity to improve the quality of traditional meat products. Various natural ADTs found in the environment can be used according to established recommendations including alcoholic beverages. For example, the use of different 4% alcoholic beverages in the production of semi-smoked sausages affected the aroma and flavor but did not alter the protein. lipid. or water content (Dolgosheva et al., 2022). Utilizing red wine in meat marinades can increase tenderness by reducing myofibrillar protein fragmentation (Istrati et al.. 2015) and inhibiting protein oxidation of beef during aging (Arcanjo et al.. 2019). Marinating steaks with beer. however. reduced aroma and overall sensory values; the steaks exhibited a red/brown color. an astringent taste and a peculiar aroma (Melo et al.. 2008).

The appearance and overall quality scores of wine-marinated steaks were lower than those of beer-marinated steaks and control samples. Alcohol also acts as an antioxidant and its antioxidant capacity depends on the total phenols. production method. and raw materials used to produce the alcohol (Polak et al.. 2020).

On Timor Island, there are many types of alcoholic beverages known as Sopi, produced by distilling sap from Borassus flabellifer (tal) or *Corypha utan lamk* (Naiola. 2008). After scientific research in 2019, this sopi drink was upgraded to Shopia, which is now commercially produced and sold legally in accordance with applicable regulations. The resulting Sophia (original Sopi) has an alcohol content of 45%. When using alcohol in meat processing, it is important to consider the residual ethanol (C_2H_5OH) concentration in the product. During cooking ethanol can evaporate leading to lower concentrations in the finished food (Snitkjær et al., 2017). Some foods including boiled beef treated with alcohol have final ethanol concentrations that are so low they are undetectable (Ryapushkina et al.. 2016). The Commission of the Indonesian Ulema Council (MUI) has set a permissible yield limit of 0.5%–0.78% in food (*Fatwa Majelis Ulama Indonesia*. 2018)

Hard dried meat can be treated with alcohol to soften its texture and alcohol may also slow lipid oxidation, potentially extending shelf life. Ethanol content in the final product added with alcohol needs to be known, because consumer groups such as those with young children, pregnant women and drivers should consume foods with low alcohol levels. It was reported that *Na'aan maran* is a traditional meat product from Timor. It is characterized by a hard texture, black appearance, and little to no flavor, thus using alcohol beverage like Sophia to enhance the characteristic of *na'an maran*/ dried meat. The purpose of this study was to determine the effect of varying alcohol concentrations on the pH value, yield, microstructural state of na'an maran (dried meat). texture. water content. protein. lipid. lipid oxidation (TBA) values and ethanol residue.

MATERIALS AND METHODS

2.1. Materials and Research Design

Twenty one Kgs of Balinese beef from the topside was purchased from the Bimoku Slaughterhouse in Kupang. Sophia (Grade C) alcoholic beverage with an alcohol content of 45% and table salt (NaCl) were used. The meat was divided into four groups; S_0 = Control (Without Sophia); S_1 = Sophia with 10% alcohol concentration; S_2 = Sophia with 12% alcohol concentration; S_3 = Sophia with 14% alcohol concentration. with each treatment repeated five times.

2.2. Alcohol Dilution

Sophia with a 45% alcohol content was diluted to 10%. 12%. and 14% alcohol concentrations. The alcohol concentration was diluted using distilled water as a diluent. as follows: 10% concentration = 334 ml alcohol + 1.166 ml distilled water

12% concentration = 400 ml alcohol + 1.100 ml distilled water

14% concentration = 467 ml alcohol + 1.033 ml distilled water

2.3. Preparation of Beef Samples and Drying

The beef was trimmed of excess lipid and connective tissue and sliced lengthwise (rope-shaped) approximately 3 cm in thick. The slices were divided into four groups according to the treatment to be applied. each consisting of 5 kg of meat (according to the number of replicates for each treatment). resulting in 20 experimental units. each consisting of 1 kg of meat. Adding 2% salt (W/W) and 350 ml of alcohol at the appropriate concentrations (10%. 12%. and 14%) for each treatment. then marinated for approximately 5 minutes. The meat was then dried in the sun until completely dry. characterized by a more compact texture. a darker color. a non-sticky feel in hand. Then samples were taken for analysis.

2.4. Parameters Measured and Measurement Method

2.4.1. pH Value

A 10-gram meat sample was manually pulverized using a mortar and mixed with 10 ml of distilled water. The pH meter probe/tip was calibrated with buffer solutions 4 and 7. Dip the electrode of the Hanna H 199192 pH meter into the meat extract. Allow the pH meter to stabilize and record the pH value. Each sample was read in duplicate.

2.4.2. Yield

Yield is obtained by comparing the final weight resulting from the process with the initial weight before processing. Yield = (Final Weight)/(Initial Weight) × 100%.

2.4.3. Texture/Hardeness

Hardness (N) values of the samples were determined by using a Textur Profile analysis (TPA) Shimadzu EZ Test Tipe SM-500N-168.

2.4.4. Observation of Meat Structure

Meat slides were prepared in the Pathology and Anatomy Laboratory. Prof. Dr. W.Z. Johanes Kupang Regional General Hospital. The steps in preparing meat slides include tissue preparation. dehydration and clarification. paraffin infiltration (paraffinization) and blocking. tissue sectioning. and staining (Wahyuni et al.. 2012).

Samples were cut sufficiently and fixed in 10% formalin for 24 hours for tissue microscopy using the paraffin method with hematoxylin-eosin (HE) staining. Dehydration and clarification were performed on meat tissue that had been immersed in fixative solution. washed with running water for 60 minutes. then immersed in 70% alcohol for 60 minutes. and drained. The meat tissue was then immersed in 80% alcohol for 60 minutes. The immersion in 80% alcohol was repeated twice. then drained. The tissue was then placed in xylene. The immersion in xylol was repeated once more until the tissue appeared transparent. Paraffin infiltration was carried out by melting the paraffin and placing it in four measuring cups in an incubator at a temperature of 58 to 60°C for 24 hours. The tissue that had previously appeared transparent was placed in the 1st. 2nd. and 3rd liquid paraffin in the incubator for one hour each. The blocking process was carried out by pouring the 4th paraffin into a mold placed on a heating furnace so that the paraffin remained in a liquid state. The tissue in the 3rd paraffin was then placed in the mold. The molds. previously placed on a heating furnace. were removed. air-dried. and then stored in a refrigerator.

The sectioning process was carried out using a microtome. This process began with the paraffin block containing the cooled tissue being removed from the mold and then placed back in the refrigerator. The microtome was prepared. and the paraffin block was placed on the microtome for sectioning. The paraffin block was cut to a thickness of 5 to 6 µm and placed in a water bath containing a mixture of water and gelatin. The sections were then transferred to glass slides and dried. The paraffin block was stained using hematoxylin and eosin (HE). The slides were first deparaffinized and placed in xylene for 2 minutes. then repeated once more. The slides were drained and dipped in 90% alcohol for 1 minute. The slides were then drained and immersed in hematoxylin for 10 minutes. The slides were then drained and washed under running water for 2 minutes. The slides were immersed in eosin for 5 minutes. The slides were then drained and immersed in absolute alcohol solution four times. The preparation was placed back in xylene for 2 minutes. then repeated once more. and then drained. The dried preparation was dripped with a mixture of xylene and vaseline and covered with a cover slip. Photographs of the meat preparation were taken using a light microscope at 100 x magnification

2.4.5. Water Content

Water content measurement followed the (National Standardization Agency of Indonesia, 2013) procedure. The samples were oven-dried at 125°C for 1 hour.

Water content (%) = [(initial weight – weight after drying) / initial weight] × 100

2.4.6. Protein

Protein content was determined following the (National Standardization Agency of Indonesia, 2013) procedure. The sample was extruded to release nitrogen from the protein as ammonium salts. These ammonium salts were broken down into NH_3 during distillation using NaOH. The liberated NH^3. bound to boric acid. produced ammonium phosphate. which was quantitatively titrated with a standard acid solution to obtain total nitrogen. Protein content was obtained from N x 6.25.

2.4.7. Lipid

Lipid content was determined following the (National Standardization Agency of Indonesia, 2013) procedure. The sample was treated with HCl and then extracted with petroleum ether. The resulting petroleum ether extract was evaporated to dryness. and the lipid content was calculated gravimetrically.

Lipid content (%) = (weight of empty lipid flask and lipid - weight of empty lipid flask / sample weight) x 100%

2.4.8. Thiobarbituric Acid (TBA) Values

Ten grams of na'an maran were blended with 100 mL of distilled water for 2 minutes. To reach a pH of 1.5. a few drops of 4 M HCl were added gradually to the homogenate. The mixture was then heated. and a 5 mL aliquot was subjected to distillation. with the distillate collected in a conical flask. A blank was prepared following the same procedure using 100 mL of distilled water instead of the sample (Mohd-esa et al.. 2010). TBARS (thiobarbituric acid reactive substances) values were reported as milligrams of malonaldehyde per kilogram of sample.

2.4.9. Ethanol

The ethanol content was calculated using the procedure of (Yulianti, 2014). A 10 mL solution was measured. placed in an Erlenmeyer flask. and added with 50 mL of PP indicator solution and distilled water. The ethanol content was determined by titration with 0.1 N NaOH.

% ethanol content = (a x M x Mr C_2H_5OH) x (sample weight x 100) - 1 x 100%

Where:

a = Average titration result (mL)

M = Molarity of NaOH (0.1 N)

Mr = Relative mass of C_2H_5OH = 46.

Data analysis

Data were analyzed using one-way analysis of variance (ANOVA) if there was a significant difference between treatments ($p<0.05$). continued with Duncan's test with a significance level of 5% (SPSS 25).

RESULTS AND DISCUSSION

3.1. pH value of naa'an maran

Statistical analysis showed that adding Sophia at different alcohol concentrations (10%–14%) reduced the pH of na'an maran ($P < 0.01$) and all alcohol concentrations had a similar effect on its pH in which ranged from 5.45-5.77 (Table 1). The pH value of Na'an Maran was found to be within the same range as commercial dried meat. specifically 5.22–5.76 (Veselá et al..2025) and 5.64 - 5.73 (Shi et al.. 2020).

Table 1. pH value, yield and hardness (N) of Na'an Maran

Parameter	Alcohol concentration				
	S_0 (0%)	S_1 (10%)	S_2 (12%)	S_3 (14%)	*P value*
pH	6,22±0,46[b]	5,6±0,15[a]	5,45±0,18[a]	5,77±0,12[a]	0,001
Yield (%)	47,80±2,17[b]	32,34±2,06[a]	32,14±253[a]	30,7±1,96[a]	0,001
Hardenss (N)	45,23±2,33[b]	36,54±2,03[a]	35,78±2,11[a]	35,42±2,13[a]	0,031

Note: Different superscripts in the same row indicate significant differences ($P<0.05$), ± standard deviation

The drying procedure significantly influences the pH value of dried meat products. This is important because a low pH is crucial to prevent protein denaturation in meat. which directly impacts its quality and shelf life (Mishra et al.. 2017). Alcohol penetrates meat tissue and causes protein denaturation. which damages protein structures and opens cellular membranes. This accelerates the drying process and fluid evaporation. The study shows that alcohol concentrations of 10 – 14% have the same ability to lower the pH of na'an maran.

3.2. Yield

The yield of na'an maran decreased significantly ($P<0.01$) following the addition of Sophia with varying alcohol concentrations. The yields for all concentrations tested (10%–14%) fell within a narrow range of 30.71% to 32.43% compared to control 47.80% (Table. 1). Alcohol dehydrate tissues by replacing the water within them. This action contributes to weight reduction during a drying process. due to water removal. Decreasing in moisture because of ethanol evaporation (Kupaeva et al.. 2024).

3.3. Texture (Hardness)

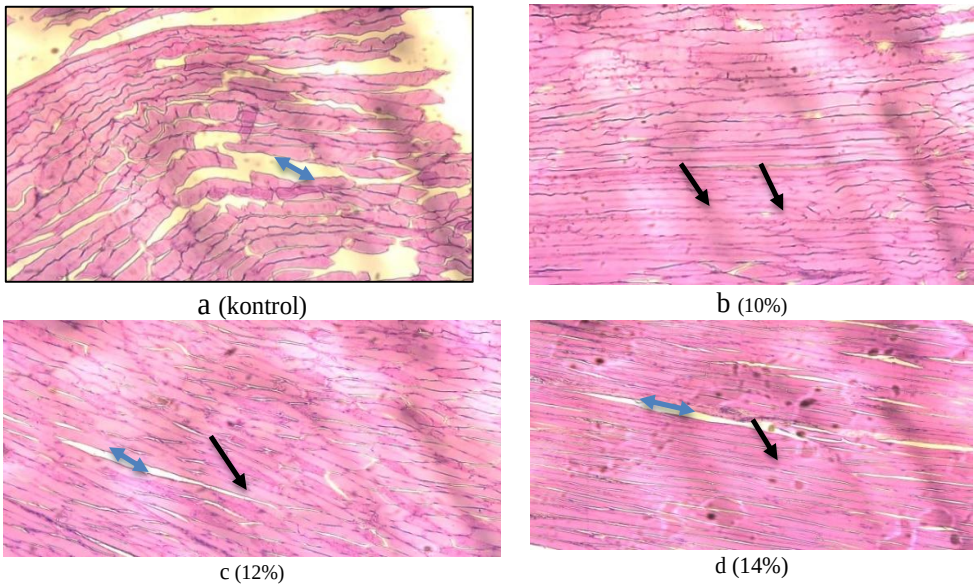
The highest hardness value of 45.23 N was found in the control sample (without alcohol). while samples given alcohol concentrations (10-14%) had lower hardness values. namely 35.78–36.54 N ($P<0.05$). This indicates that the administration of 10-14% alcohol concentrations had the same effect in reducing the hardness value or improve texture of na'an maran. Alcohol in improving the texture of processed meat was also reported by (Gradinarska et al.. 2022) where the use of redwine increased the texture of dry fermented sausages.

This increase in sample texture. indicated by a decrease in hardness value. is due to alcohol's ability to degrade the protein structure of meat. particularly the filamentous proteins that form the myofibrils of the meat mass (Ikonić et al.. 2016). Meat hardness is also related to its water content; low water content results in high hardness values or a tougher texture (Spaziani et al.. 2009). In this study. although the water content decreased as a result of the addition of alcohol (Table 2). the hardness value also decreased. indicating that the hardness value was more influenced by the protein structure than the water content. Table 2 also shows that the total protein content did not change. indicating that alcohol was only able to degrade the filament protein without changing the total amount of protein. The range of hardness values for na'an maran was the same as that reported by (Engez et al.. 2012) namely 34.81 N - 44.13 N.

3.4. Microstructure

Meat microstructure consists of muscle fibers. tissue. and lipid. The microstructure of processed meat is positively correlated with meat firmness (texture) (Momchilova et al.. 2023). The main factor influencing meat texture is structural changes related to the relationship between protein and water molecules (Khalid et al.. 2023). It is important to understand the physical changes of meattexture during cooking. in fact. duration and temperature.

The microstructure of na'an maran meat in this study visually demonstrated a reduction in the diameter of muscle fibers and collagen tissue due to alcohol treatment. In the control (without alcohol) (Figure 1A). the meat structure. consisting of muscle fibers (in red) and connective tissue protein (collagen) (in white). had larger diameters of muscle fibers and collagen compared to the alcohol treatment (Figures 1b. c. and d). The size of muscle fibers and connective tissue/collagen decreased. indicating that alcohol treatment caused muscle tissue and collagen to undergo damage/fracture or protein denaturation. Degradation of meat protein structure by proteolytic enzymes is a well-known phenomenon that greatly enhances texture profile of dry-fermented meat products (Khalid et al.. 2023). In this study. the tenderness value of dry-fermented meat increased as a result of the addition of alcohol. Alcohol degrades muscle fibers and connective tissue. resulting in improved meat structure.



a (kontrol) b (10%) c (12%) d (14%)

Figure 1. *Na'an Maran* Structure. Mucle fiber (➚), Connective tissue collagen (⟷)

The decrease in muscle and collagen fiber diameter indicates that alcohol causes dehydration and protein denaturation. which can result in smaller and denser fibers. However. in this study. alcohol administration did not cause changes in the protein content of na'an maran (Tabel.2). This indicates that administration of alcohol up to a concentration of 14% only affects the spatial structure of proteins. such as collagen and muscle. thus affecting texture. but not the total protein content.

3.5. Water Content

Table 2 showed that Na'an Maran's moisture content dropped to 41.82% at the highest alcohol concentration 14% ($P<0.05$). This water content range is lower than the 39-45% water content of dried meat reported by (Engez et al., 2012) and the 21.5-25.3% reported by (Petit et al., 2014). Generally. the drying process will cause a decrease in meat water content of between 4-5% (Yulianti. 2014). Variable water content reductions in dried meat can be caused by different drying techniques (Engez et al., 2012) and the addition of food additive (Ferreira et al.. 2013).

Table 2. Chemical composition of Na'an Maran treated with alcohol at different concentrations

Parameters	Alcohol Consentration				p-value
	S_0 (0%)	S_1(10%)	S_2 (12%)	S_3 (14%)	
Water content (%)	51.14±1.70^a	48.34±3.41^a	46.21±2.83^a	41.82±0.22^b	0.021
Protein (%)	50.02±1.87	49.97±2.27	50.51±3.81	50.68±0.49	0.967
Lipid (%)	5.16±1.68^a	4.86±2.26^a	4.02±0.76^b	3.27±0.46^b	0.031
TBA(mg.MAD/kg)	1.94±0.06^b	1.84±0.29^b	1.45±0.67^a	1.20±0.06^a	0.04
Ethanol (%)	0.15±0.04	0.11±0.02	0.11±0.02	0.11±0.03	0.100

$^{a.b}$ Different superscripts in the same row indicate significant differences ($P<0.05$). ± standard deviation

The total water content of Na'an maran classifies it as a dried meat product according to standards for dried meat (Biltong) from South Africa. which specify 20–50% moisture. and Charqui from Brazil. where Brazilian legislation sets dried meat moisture at 40–50% (Jones et al., 2017). It is known that decreasing water content during the drying process is key to limiting microbial proliferation and the production of hazardous substances.

3.6. Protein content

The protein content of dried meat in this study ranged from 50.02 to 50.68%. It was not affected by the administration of alcohol (P>0.05). This protein content was almost the same as that reported (Engez et al.. 2012) which was 44.21 to 49.69%. A decrease in pH causes meat proteins to denature. or changes in the structure of myofibrillar protein (MFP) during freezing. However. increasing the freezing rate can reduce the pH drop. which is conducive to maintaining MFP stability (Tan et al.. 2021). In this study. although alcohol concentrations of 10-14% can cause a decrease in pH and alter MFP. resulting in a softer texture. it does not alter the overall protein content. It can be said that alcohol concentrations of 10-14% have a greater impact on the spatial structure of proteins thus. affecting only texture but not total protein.

The addition of different strong alcohols (40% ABV or higher) at 4% in semi-smoked sausage production does not affect nutritional value. with sausage protein values ranging from 23.37% to 25.28%. lipid 11.23% to 15.27%. and water 53.12% to 58.25% (Dolgosheva et al,. 2022). the addition of ethanol extract did not lead to significant changes in the protein composition and microstructural characteristics of meat pates (Kupaeva et al., 2024).

3.7. Lipid content

The lipid content of dried meat decreased when alcohol was added at concentrations of 12- 14% ($P < 0.05$). Table 2 shows that the lipid content declined by approximately 1–2%. with a range of 3.27–5.16%. This range is higher than the 1.32–2.07% reported by (Engez et al., 2012). The reduction in lipid could be caused by some lipid melts and is lost as drip. Alcohol penetrates meat tissue and damages tissue structures and opens cellular membrane thus lipid melts and is lost easily. This accelerates the drying process and fluid evaporation. In general. drying lowers water content by approximately 4–5% and total lipid content by roughly 3–4% (Yulianti, 2014). Differences in drying techniques; air drying. sun drying. or oven drying. have different effects on the composition of dried meat. such as water content and total lipid. Air drying has a lower total lipid content than sun drying and oven drying. while the water and total lipid content in sun drying and oven drying techniques are relatively the same (Mediani et al.. 2022).

3.8. Lipid oxidation

The acceptable TBA level for consumable meat products is 2 mg MDA/kg (Campo et al., 2006). TBA value for lamb meat is 1 mg MDA/Kg while the TBA limit per SNI No. 2352-1991 is 3 mg/kg. Using Sophia contributed to a statistically significant reduction in malondialdehyde (MDA) content ($P < 0.05$) in Na'an Maran. The TBA values in this experiment was ranging from 1.20 mg MDA/kg to 1.94 mg MDA/kg. It is showed that adding 12% and 14% alcohol concentration had a lower TBA value namely 1.45 mg MDA/kg and 1.20 mg MDA/kg respectively. compare to 1.84 mg MDA/kg and 1.94 mg MDA/kg (Table 2). All TBA values meeting the 3 mg MDA /kg criterion however only at a 12% and 14% alcohol concentration could reduce the TBA value. TBA value of dried meat was 0.93 mg MDA/kg was reported by (Zioud et al., 2023). Role of alcohol reduce the MDA content also reported in dried fermented sausage (Gradinarska et al., 2022). It has been identified that total phenolic content (TP) has a direct influence on the antioxidant properties of the alcoholic beverages (Polak et al., 2020).

3.9. Residual ethanol

One important consideration when using alcohol in meat processing is the final ethanol (C_2H_5OH) concentration in the product. as it is related to consumer health. Ethanol concentration generally decreases when food is heat-treated. forexample during drying or boiling (Snitkjær et al., 2017).

In this study. the ethanol residu was 0.11% - 0.15% (Table 2) and there is no different among treatment and control (P>0.05). According to the Fatwa Commission of the Indonesian Ulema Council (MUI) that the highest limit for ethanol consumption is 0.78%. but for the sake of caution. adopts a safer limit of 0.5% (*MAJELIS ULAMA INDONESIA*, 2018). The results of this study showed that naan maran added with 10-14% alcohol concentration is safe for consumption.

CONCLUSION

This experiment shows that using Sophia alcohol at 10%–14% can reduce the pH. yield and hardness of Na'an maran. Microstructure images indicate that alcohol treatment reduces the diameter of muscle fibers and collagen. Water content decreased only at 14% alcohol concentration while fat content and lipid oxidation decreased as 12%–14% alcohol concentraton. All ethanol residues were below the Indonesian Ulema Council (MUI) recommended limits.

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CONFLICT OF INTEREST

This article has no reported conflicts of interest. and all authors approve the manuscript.

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