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Edy Kurnianto kurniantoedy17@gmail.com lewat@ejournal.undip.ac.id kepada saya

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Mrs Irdha Mirdhayati:

Thank you for submitting the manuscript, "Chemical characteristic, lactic acid bacteria population, and angiotensin converting enzyme inhibitory activity of traditional fermented beef "cangkuk" by spontaneous fermentation with the addition of bamboo shoot" to Journal of the Indonesian Tropical Animal Agriculture. With the online journal management system that we are using, you will be able to track its progress through the editorial process by logging in to the journal web site:

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If you have any questions, please contact me. Thank you for considering this journal as a venue for your work.

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Dear Author,

Your manuskrip has been reviewed by reviewer. Please revise it. We give you 2 weeks for revising.

Best regards,

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Irdha Mirdhayati <mirdhayati@gmail.com> kepada JITAA

Sen, 1 Jun 2020, 12.19

Dear Sir,

Thank you very much for your email. I will improve the manuscript properly as soon as possible.

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REVIEWER'S COMMENT

Reviewer comment to the manuscript entitled:

Chemical characteristic, lactic acid bacteria population, and angiotensin converting enzyme inhibitory activity of traditional fermented beef "cangkuk" by spontaneous

GENERAL COMMENT

The title of the research is interesting, because the content elaborates a traditional animal food product. The data is sufficient. However, the manuscript needs some revisions and additional up to date references.

RUNNING HEAD

Comment:

Suggestion:

Good

TITLE

Comment :

Suggestion :

Good

ABSTRACT AND KEYWORDS

Comment :

Suggestion :

Good

INTRODUCTION

Comment :

Need revisions.

Suggestion :

State of the art of this research is not clearly written yet in the Introduction. Please add the new paragraph that explain about the importance of ACE inhibitory and some other parameters. Why these parameters must be evaluated? Please add supporting references in this paragraph. Needs additional references.

REVIEWER'S COMMENT

MATERIALS AND METHODS	
Comment :	Suggestion :
Good enough, but need revisions	Please add reference in this processing method. Please give more explanation about the process of fermentation
RESULTS AND DISCUSSION	
Comment :	Suggestion :
Need revisions	The Results and Discussions much better to be divided into some groups, for example: chemical characteristics, total LAB, and ACE inhibitory activity. This suggestion is accordance with the title of the research. Please add some references.
CONCLUSIONS	
Comment :	Suggestion :
Good enough.	

Eligibility to Publish (Use ✓)

Based on the comment above, this manuscript is:

- A. eligible to publish in JITAA without revision.
- B. eligible to publish in JITAA with minor revision.
- C. eligible to publish in JITAA with major revision. ✓
- D. No eligible to publish with reason:
 1. duplication of other article
 2. no suitable among the title, hypothesis and conclusions.
 3. weak in methodology :

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4. Miscelenaous:

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COMMENT DOCUMENTATION

REVIEWER'S COMMENT**(THIS DOCUMENT IS SAVED FOR MANAGEMENT OF JITAA)**

Date of received manuscript :
Date of completed review :
Name Reviewer :
Institution :
University :
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1 Chemical characteristic and ACE inhibitory activity of cangkuk

2

3 **Chemical characteristic, lactic acid bacteria population, and angiotensin**
4 **converting enzyme inhibitory activity of traditional fermented beef "cangkuk" by**
5 **spontaneous fermentation with the addition of bamboo shoot**
6

7

8

ABSTRAK

9 Penelitian ini bertujuan untuk menguji sifat kimia, populasi bakteri asam laktat (BAL)
10 dan aktivitas inhibitor *angiotensin converting enzyme* (ACE) daging sapi yang
11 difermentasi dengan penambahan rebung bambu. Penelitian menggunakan Rancangan
12 Acak Lengkap Faktorial dua faktor. Faktor A yakni metode preparasi rebung : dicacah,
13 digiling, dan diekstrak. Faktor B adalah rasio daging dan rebung (B) yakni 1;0,75 ; 1:1,
14 1:1,25. Parameter kimia yang diamati adalah protein total, protein terlarut, derajat
15 hidrolisis, pH, total asam tertitrisasi. Data kimia dan jumlah BAL dianalisis dengan sidik
16 ragam, sedangkan aktivitas inhibitor ACE dianalisis deskriptif. Hasil penelitian
17 menunjukkan bahwa metode preparasi rebung dengan cara digiling dan diekstrak mampu
18 menurunkan total protein dan nilai pH. Rasio daging sapi dan rebung 1:0,75 dan 1:1
19 mampu menurunkan protein total dan meningkatkan total asam tertitrisasi. Interaksi faktor
20 A dan faktor B hanya terjadi pada peningkatan derajat hidrolisis. Faktor A dan B tidak
21 nyata terhadap jumlah bakteri asam laktat. Aktivitas inhibitor ACE yang dihasilkan
22 berkisar 36,5-79,6%. Dapat disimpulkan bahwa metode preparasi rebung bambu dengan
23 cara digiling dan rasio daging : rebung 1:0,75 dan 1:1 dapat meningkatkan derajat
24 hidrolisis dan memiliki aktivitas inhibitor ACE yang tinggi.

25 *Kata Kunci : cangkuk, daging fermentasi, rebung, sifat kimia, inhibitor ACE*

26

27

ABSTRACT

28 This research was conducted to examine the chemical properties, lactic acid bacteria
29 (LAB) population and Angiotensin Converting Enzyme (ACE) inhibitory activity of
30 fermented beef meat by the addition of bamboo shoots. This research used factorial
31 completely randomized design. Factor A was the preparation method of bamboo shoots:
32 chopped, ground, and extracted. Factor B was the ratio of meat and bamboo shoots (B)
33 which was 1:0.75, 1:1, 1:1,25 ratios. The chemical properties observed were total protein,
34 soluble protein, hydrolysis degree, pH and titratable acidity value. The results showed
35 that bamboo shoot preparation method by ground and extracted were able to reduce total
36 protein, and pH value. The ratio of meat and bamboo shoot (1:0.75 and 1:1) were able to
37 decreased total protein but increased total titratable acidity. The interaction of the factors
38 A and factor B only occurred in increasing hydrolysis degree. Both of two factors (A and
39 B) could not significantly effect to the number of LAB. The ACE inhibitory activity
40 ranged from 36.5 to 79.6%. It can be concluded that the preparation methods of bamboo
41 shoots by ground and 1: 0.75 and 1: 1 of beef : bamboo shoot ratio could increased
42 hydrolysis degree and had high ACE inhibitory activity.

43

44 *Keywords: cangkuk, fermented meat, bamboo shoot, chemical properties, ACE*
45 *inhibitory activity*

46

47

INTRODUCTION

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48 Fermented food products have long been known to Indonesian people. These
49 foods made from various sources such as vegetable, grain, milk and meat. The traditional
50 types of fermented products from buffalo milk known as “dadih” originally from West
51 Sumatra and danke from South Sulawesi (Soenarno *et al.*, 2013). Indonesian local
52 fermented products derived from meat are not widely known. One of the traditional
53 fermented meat products is “cangkuk”.

54 According to Salahuddin (2004), “cangkuk” is a traditional fermented product
55 derived from buffalo meat added with *betung* bamboo shoot (*Dendrocalamus asper*) and
56 salt, originally from Sorolangun District, Jambi Province. “Cangkuk” was made for
57 special events such as in the holy month of Ramadan, a family celebration and feast days.
58 Cangkuk is also known by the people in Kuantan Singingi District, Riau Province. With
59 the same designation, cangkuk was processed using meat, particularly beef. The
60 processing and basic materials used are also similar to those in Sorolangun District,
61 Jambi.

62 Bamboo shoot was prepared by people in Riau Province before consumed in two
63 types of processing methods, namely boiling and fermentation. Bamboo shoot was
64 fermented by using indigenous microbe of its self and added salt at 1-5 % w/w. In
65 processing cangkuk, both of bamboo shoot and beef meat were fermented together with
66 added rice and salt in certain concentrations. Bamboo shoot and rice contribute in supply
67 nutrient for indigenous microbe during fermentation hence they had high carbohydrate.
68 Salt in lower concentration contributes in supply mineral. Choudhury *et al.*, (2012), said
69 that bamboo shoot had high moisture and carbohydrate content, protein and mineral.

70 Bamboo shoot had an active material such as vitamin, amino acids, antioxidant and
71 steroids.

72 Recent research on fermented meat products has been widely reported. Dry-cured
73 ham is a type of fermented pork, seasoned and followed by spontaneous fermentation for
74 10-11 months. Fermented meat products named according to the country or place of
75 origin, such as Spanish dry-cured ham originating from Spain (Escudero *et al.*, 2012),
76 Jinhua Ham (Li *et al.*, 2003) and Xuanwei Ham from China (Zhou and Zhao, 2007).
77 Fermented sausage known as salami, such as salami Milano from Itali (Ruiz *et al.*, 2014),
78 Chorizo (Broncano *et al.*, 2012) and Sremska sausage from Serbia (Zivkovic *et al.*, 2012).

79 Based on these studies, it can be seen that fermented meat has the advantage as a
80 source of bioactive peptides, free amino acids with a unique flavor, do not contain
81 pathogenic bacteria, and better sensory properties. The two physiological functions
82 shown are as antioxidants and antihypertensive (Escudero *et al.*, 2012; Albenzio *et al.*,
83 2017).

84 In Southeast Asia, fermented meat products are known as sour meat which is
85 processed to increase consumers' needs in choosing the variety of foods they want. Meat
86 products are inoculated with microbes at controlled time and processing conditions to
87 produce the desired properties. Local producers generally use natural fermentation
88 without inoculation or controlled conditions. The microorganisms found in products
89 come from the meat itself or the environment (Singh *et al.*, 2012). A number of active
90 components physiologically including bioactive peptides have been found in traditional
91 fermented food. Therefore, traditional fermented meat is an interesting target for a new
92 functional meat product (Arihara, 2006).

93 Based on a direct survey in the field conducted recently, "cangkuk" remain
94 produced and consumed by the community in certain age groups (> 50 years) in Riau
95 Province. The presumption of the community consuming "cangkuk" is in accordance
96 with the tastes of the elderly, a tender texture, a specific aroma and taste is a little sour
97 that is preferred. Meanwhile, there has been no scientific evidence that has been studied
98 and reported in bioactivity of "cangkuk". Furthermore, research on Indonesian traditional
99 fermented meat products is very limited. "Cangkuk" that produced by spontaneous
100 fermentation of beef meat with addition of bambeoo shoot could generated new source
101 of bioactive peptide.

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102 MATERIALS AND METHODS

103 The materials used in processing beef meat fermented "cangkuk" were fresh beef
104 meat (brisket) from male Bali cattle with 2.5-3 years old, green bamboo shoot, distilled
105 water, rice and salt. The chemical used were sulphuric acid, hydrogen chloride, sodium
106 hydroxide, boric acid, catalyst selenium, trichloroacetic acid, Lowry reagent, Bovine
107 Serum Albumin (BSA), Folin-ciocalteau, angiotensin converting enzyme from rabbit
108 lung (Sigma), hippuryl-hystidyl-leucyne substrate (Sigma), sodium chloride, sodium
109 borate buffer pH 8,3, ethyl acetat and MRSA (*de Man Rogosa Sharpe agar*), (Merck).
110 All of chemical and reagent were analytical grade.
111

Commented [U3]: After this paragraph, please add the new paragraph that explain about the importance of ACE inhibitory and some other parameters. Why these parameters must be evaluated? Please add supporting references in this paragraph.

Commented [U4]: Please write the places where meat and bamboo shoot were obtained?

Commented [U5]: Is this ACE which was used in the determination of ACE inhibitory activity? Please its confirmed in the determination procedures.

112 The equipmet that used to prepare bamboo shoot were knife, plastic ware,
113 analytical balance, blender, glassware, volumetric flask, tube centrifuge, and high speed
114 centrifuge. The instrument that used for analysis of "cangkuk" were mortar, pH meter,

115 Kjeltec set, burette flask, spectrophotometer UV-Vis, waterbath, magnetic stirrer,
116 micropipette, oven, petri dish, autoclave and colony counter.

117

118 **Preparation of Bamboo Shoot**

119 Bamboo shoots were harvested from local farms in Kampar Regency and were
120 brought to the laboratory in order to remove their skin and smooth hair. Bamboo shoot
121 washed and drained, followed by thinly sliced. There were three preparation method of
122 bamboo shoot (factor A) in this experiment, namely : chopped (A1), ground (A2) and
123 extracted (A3). A1 was prepared with this following: bamboo shoot was chopped into
124 small pieces by using a knife. A2 was prepared by mixed the bamboo shoot and distilled
125 water in 1:1 ratio, homogenized within blender during 3 minutes. A3 was prepared by
126 mixed bamboo shoot and distilled water with 1 : 3 ratio, homogenized within blender for
127 3 minutes. Then, water soluble extracts were obtained by centrifugation at a speed 6000
128 rpm for 30 minutes. Supernatant was taken as a water soluble extract. Factor B was
129 prepared by weighing bamboo shoot based on their ratio of meat weight, 1:0.75; 1:1 and
130 1:1.25, respectively.

131

132 **Preparation of Beef Meat**

133 The fresh beef meat, part of the brisket, was purchased from Animal Slaughtering
134 House in Pekanbaru city which was put in the ice box and brought to the laboratory in the
135 cold condition. Further processing conducted by separating the fat and connective tissue
136 of the meat and washed with distilled water and then drained at room temperature. The
137 meat was cut into small piece with size 5 x 5 x 2 cm³.

138

139 **Processing of the Cangkuk**

140 This stage was aimed to conduct fermentation of "cangkuk" according to the
141 general method in Riau society. Fermented beef meat "cangkuk" was initially by prepared
142 300 g of small cut of beef meat for each experimental unit. Then, bamboo shoot was
143 prepared based on their preparation methods (chopped, ground, a water soluble extract)
144 and ratios to meat weight, i.e: 1:0.75; 1:1 and 1:1.25 (225 g, 300 g and 375 g). All of the
145 ingredients were put into each of polypropylene plastic boxes based on their treatments.
146 Bamboo shoot based on their treatments was mixed with beef meat, salt and rice. Salt and
147 rice were added at 1% from total weight beef meat and bamboo shoot. All of the
148 ingredient was mixed homogeneously and prior to an aerobic fermentation during seven
149 days in a room temperature. After the fermentation process, fermented beef meat was
150 separated from another ingredient and was packed in aluminium-plastic laminated , then
151 kept in freezer at-20°C until analyzed.

152

153 **Analysis of Total Protein by Micro Kjeldahl Method**

154 Analysis total protein of the sample was conducted by micro-Kjeldahl method
155 (AOAC 960.52). The samples analyzed consisted of fresh beef before fermentation, and
156 the fermented beef meat ("cangkuk") from each treatment.

157 **Analysis of Soluble Nitrogen (Protein) in Trichloroacetic Acid (SN-TCA)**

158

159 An amount of 20 mL of fermented beef meat was added with 20% TCA (w/v) of
160 20 mL. The mixture is then allowed to stand for 30 minutes for sedimentation and then
161 centrifuged (speed of 7,800 x g, for 15 minutes). The supernatant was then analyzed for
162 nitrogen content using the Lowry method (Waterborg, 2002). An aliquot of 0.5 mL of the

Commented [U6]: Is this ACE that was used for determination of ACE inhibitory activity? Please add reference in this processing method.

Commented [U7]: Please give more explanation about the process of fermentation in this research.

163 sample was mixed with 5.5 mL of an alkaline-copper reagent and incubated for 20 min
164 at room temperature. The mixture was added to 0.5 mL Folin-Ciocalcetau's phenol
165 reagent at 8-fold dilution with distilled water and allowed to stand for 30 min. Absorbance
166 was measured at 750 nm using spectrophotometer. Bovine serum albumin (BSA) was
167 used as standard.

168

169 **Degree of Hydrolysis**

170 The degree of hydrolysis measured according to procedures of Hasnaliza *et al.*,
171 (2010), which is calculated based on the percentage ratio of soluble protein in
172 trichloroacetic acid (TCA) to total protein content of the fermented beef meat. The degree
173 of hydrolysis can be calculated by the following formula:

174 % Degree of Hidrolysis = [Soluble Nitrogen in TCA 20% (w/v)] / Total protein x 100 %

175

176 **pH analysis**

177 An amount of five grams of finely ground sample of fresh beef meat before
178 fermentation, and fermented beef meat from each treatment were prepared. Then, add the
179 distilled water 10 ml and stir until evenly distributed. The pH value is measured using a
180 pH meter that has been calibrated with a buffer of pH 4.0 and pH 7.0.

181

182 **Total Titratable Acidity**

183 Total titratable acidity was measured using the principle of lactic acid base.
184 Amount of ten mg sample was put into the Erlenmeyer flask, then added 2-3 drops of 1%
185 phenolphthalein indicator. Samples were titrated with 0.1 N NaOH solution until they

186 formed a pink color and did not disappear within 30 seconds (Rahman *et al.*, 1992). The
187 total titratable acidity calculated by the formula:

188
$$\text{Total titratable acid (\%)} = \frac{V \text{ NaOH} \times N \text{ NaOH} \times \left(\frac{90}{1000}\right)}{\text{sample volume}} \times 100\%$$

189

190 **The Number of Lactic Acid Bacteria**

191 The number of lactic acid bacteria colonies was determined by pour plate method
192 and analyze using the Standard Plate Count (SPC). The deMan Rogosa Sharpe Agar
193 (MRS) used as the propagation medium of lactic acid bacteria. A sample of 1 mg of
194 inoculant pipetted into a sterile Petri dish and then the MRS medium which has cooled
195 (temperature about 37°C) is poured into sterile Petri dish as much as 12-15 mL. The
196 mixture is homogeneous by moving the Petri dish. Petri dishes were incubated upside
197 down after hardening, at 37°C for 24-48 hours. All analyses were done in duplo. The
198 number of colonies per gram (as log₁₀ of CFU/g for each sample) is calculated using the
199 formula:

200
$$\text{Number of colonies / g} = \text{Number of colonies per Petri dish} \times \frac{1}{\text{dillution factor}}$$

201

202 **Measurement of ACE inhibitory activity**

203 The ACE inhibitory activity was measured according to the method of Chusman
204 and Cheung (1971) with modification by Arihara *et al.*, (2001). This assay based on the
205 liberation of hippuric acid from Hip-His-Leu catalyzed by ACE. A sample solution (15
206 μL) was mixed with 125 μL of 100 mM sodium borate buffer (pH 8.3) containing 7.6
207 mM Hyp-Hys-Leu and 608 mM NaCl and then pre incubated for 5 min at 37°C. The

208 reaction was initiated by the addition of 50 μ L of ACE dissolved in distilled water and
209 the mixture was incubated for 30 min at 37°C. For the blank, distilled water was used
210 about 50 μ L. The reaction was stopped by adding 125 μ L of 1 N HCl. The hippuric acid
211 liberated by ACE was extracted by adding 750 μ L ethyl acetate to the mixture with
212 vigorous shaking. After centrifugation at 12,000 rpm for 10 min, 500 μ L of the upper
213 layer was collected and then dried at 90°C for 60 min. Hippuric acid was dissolved with
214 1 mL distilled water and photometrically was determined at 228 nm. ACE inhibitory
215 activity was calculated using the equation:

$$216 \quad \text{Inhibitory activity (\%)} = [(C-A) / (C-B)] \times 100$$

217 where A : absorbance of sample reaction, B : absorbance of blank , C: absorbance of
218 control (distilled water for sample).

219

220 **Data Analysis**

221 Analysis of total protein, soluble protein, degree of hydrolysis, pH value, total
222 titratable acidity, and the number of lactic acid bacteria were statistically analyzed by
223 analysis of variance with completely randomized factorial with 3 replications. Factor A
224 is the preparation method of bamboo shoots: chopped, ground, and extracted. Factor B is
225 the ratio of meat and bamboo shoots (B) which is 1, 0.75; 1: 1, 1: 1,25 ratios. Post hoc
226 analysis was carried out by the Duncan Multiple Range Test (DMRT) to see the difference
227 between treatments (Steel and Torrie, 1993). Bioactivity data of ACE inhibitory activity
228 were analyzed by calculating the mean and standard deviation.

229

230

RESULTS AND DISCUSSIONS

Commented [U8]: The Results and Discussions much better to be divided into some groups, for example: chemical characteristics, total LAB, and ACE inhibitory activity. This suggestion is accordance with the title of the research.

231 Total protein of "cangkuk" were presented in Table 1. Total protein of raw beef
232 meat before fermentation process was 18.95 ± 0.30 (g/100 g). This research showed that
233 preparation methods of bamboo shoot (factor A) by ground and extracted could decrease
234 the total protein of "cangkuk" greater than by chooped during spontaneous fermentation
235 for one week ($P < 0.01$). In term of the ratio of meat and bamboo shoot (factor B), 1:0.75
236 and 1:1 ratios could decrease the total protein greater than 1:1 and 1:1.25 ratios ($P < 0.01$).
237 There were no interaction between factor A and factor B to decrease the total protein.
238 This research showed that addition of bamboo shoot with different preparation method
239 on spontaneous fermentation beef meat contribute in change total protein value after
240 fermentation. This phenomenon similar with Zhou *et al* 2017, studied on fermentation of
241 solid beef inoculated with two starter cultures (*Lactobacillus curvatus* and *Pediococcus*
242 *pentosaceus*) in different inoculums concentration, fermentation time, and fermentation
243 temperature. An increase of inoculums concentration, fermentation times and
244 fermentation temperatures could decrease protein composition in term water-soluble
245 protein and salt soluble protein after fermentation, while insoluble protein increased.

246 Bamboo shoot became nutrient source for indigenous microbe during spontaneus
247 fermentation in this research due to had high content of moisture, carbohydrate, protein
248 and mineral. Bamboo shoot had an active material such as vitamin, amino acids, and
249 steroids (Choudhury *et al.*, (2012). According to Table 1, both of the preparation method
250 of bamboo shoot and bamboo shoot ratios could decrease the total protein in the different
251 way. It is shown that the preparation method by using ground and extracted could
252 decrease total protein greater than by chopped. The size reduction of bamboo shoot using
253 blender facilitated fermentation progress more compared to the chopped method.

Commented [U9]: What the meaning spontaneous fermentation?

254 Furthermore, meat and bamboo shoot ratios showed that decreased the total protein was
255 greater at 1:0,75 and 1: 1 than 1:1 and 1:1.25. This finding showed that the bamboo shoot
256 ratio must be lower and similar with than beef meat, at the high ratio 1,25 the total protein
257 decreased more smaller due to fermentation progress was lower.

258 The main component of meat is a protein (range from 15-22 g/100 g) which
259 consist of high essential amino acid percentage. Beef protein composition of red meat
260 with adipose tissues is about 18%. During “cangkuk” processing, the fermentation occurs
261 spontaneously with the help of mix salt and rice as additional ingredients on anaerobic
262 condition. Fermented meat according to Hui (2006) is a fermentation process which
263 consists in the growth and development of the microbial flora. This process naturally
264 present in the meat during spontaneous fermentation or by the addition of starter culture.
265 During the fermentation process, protein of beef meat was hydrolyzed by indigenous
266 proteolytic enzymes from spontaneous microbe within the meat and bamboo shoot
267 generated low molecular weight of protein (i.e:water-soluble protein), bioactive peptides
268 and free amino acid (Steinkraus, 2002; Ockerman and Basu, 2017).

269 The preparation method of bamboo shoot by ground indicated that decreased of
270 total protein during fermentation in line with increasing the soluble protein and
271 percentage of hydrolysis degree. Since peptide is a lower molecular weight protein, it
272 could be the one of the components of soluble protein which was measured by SN-TCA
273 methods. The soluble protein of fermented beef ”cangkuk” with different preparation
274 methods and a ratio between meat and bamboo shoot was shown in Table 1.

275 Based on the analysis of variance, the differences of preparation method of
276 fermented beef “cangkuk” has a very significant effect to increase the soluble protein

Commented [U10]: Is any comparable references?

277 value ($P < 0.01$), while the ratio of meat and bamboo shoot has no significant effect on the
278 value of soluble protein ($P > 0.05$) of fermented beef "cangkuk". And also, there were no
279 interactions between preparation methods and ratio of meat and bamboo shoot to soluble
280 protein value.

281 The preparation methods of bamboo shoot by using chopped and blended produced
282 the soluble protein content higher than by using extraction methods. The lower soluble
283 protein content in extraction method was caused by water soluble extracts had reduction
284 in the nutritional components of bamboo shoots due to some carbohydrate (starch, fiber)
285 and water-insoluble compound (lipid) were wasted on the separation of water insoluble
286 bamboo shoot components at centrifugation process. This directly caused the lower
287 progress of fermentation as evidenced by the low level of hydrolysis degree, low content
288 of soluble protein, but total protein still high. Starch, fiber and lipid were important
289 nutrient for the microbe during fermentation (Kampen, 2014).

290 Degree of hydrolysis describes the percentage of peptide bond that cleaved during
291 hydrolysis by the proteolytic enzyme of microbe indigenous on fermentation of beef
292 meat. Degree of hydrolysis of fermented beef with different preparation method and ratio
293 between meat and bamboo was shown in Table 1. Analysis of variance showed that the
294 interaction of preparation method and ratio of meat and bamboo shoot had a significant
295 effect to increase the degree of hydrolysis of fermented beef. The combination of
296 treatment A2B2 (ground method and ratio meat : bamboo shoot 1:1) had the highest
297 degree of hydrolysis. The increasing of hydrolysis degree in line with the decreasing of
298 total protein and increasing the soluble protein. In this research, ground method of
299 bamboo shoot and 1:1 ratio in "cangkuk" processing could decrease higher in total protein

300 and increase soluble protein more greater, consequently the degree of hydrolysis became
301 higher (3.38 %).

302 Degree of hydrolysis is a key parameter of hydrolysis reaction and used for
303 monitoring progress of hydrolysis, describes the percentage of peptide bond that cleaved
304 during hydrolysis, then generated smaller peptide with lower molecular weight (Nielsen
305 *et al.*, 2001). Soluble protein measured in this research based on soluble nitrogen
306 compound that in trichloro acetic acid 20%, they could be smaller peptide (Rutherford,
307 2010). During hydrolysis, a wide variety smaller peptide and free amino acid were
308 generated, depending on enzyme specificity (Faithong *et al.*, 2010).

309 Degree of hydrolysis from fermented beef meat in this research had lower (2.0 -
310 3.3%) percentage than the degree of hydrolysis of fermented egg shell membrane by using
311 lactic acid bacteria culture starter (25.1%) (Jain and Anal, 2017). According to Faithong
312 *et al.*, (2010), degree of hydrolysis of Thailand traditional fermented shrimp and krill
313 product, *Jalo*, *Kong-shom* and *Kapi* were 25%, 25-40% and 20-22%, respectively. The
314 degree of hydrolysis of local fermented food was varied according producer, raw material,
315 ingredien additive, fermentation time and type proteolytic microbial enzyme.

316 Variance analysis resulted that different preparation methods of bamboo shoot
317 was significantly effect ($P < 0.05$) to decrease the pH of fermented beef "cangkuk",
318 meanwhile the different ratio of meat and bamboo shoot and interactions between the two
319 factors showed a non significant effect ($P > 0.05$) to pH value.

320 Fermented beef "cangkuk" prepared by addition of water soluble extract of
321 bamboo shoot had lower pH value than the "cangkuk" that produced by addition of
322 ground and chopped bamboo shoot, 4.40 contrasted with 4.57 and 4.67, respectively. The

323 successive growth of microorganisms during meat fermentation was dominated by lactic
324 acid bacteria which play an important role in the process. The lactic acid bacteria consume
325 sugar primarily and carbohydrates and convert it into lactic acid, which lowers the pH
326 (Ockerman and Basu, 2017). This psycho-chemical change called acidification, which
327 has a preservative effect because its ability to inhibit spoilage and
328 pathogenic microorganism (Bover-Cid *et al.* 2000).

329 Owens (2015) reported an indigenous fermented pork sausage from Thailand
330 called *Nham* which fermented at room temperature for 3 - 4 days reached ultimate pH
331 4.6. In comparison to fermented meat cangkuk with different preparation methods of
332 bamboo shoot, the pH value range from 4.40 - 4.67. The same studies indicated that the
333 primary microorganism during fermentation were associated to *Lactobacilli* and
334 *pediococci*. These certain *lactobacilli* identified as *Lactobacillus plantarum*,
335 *Lactobacillus pentosus* and *Lactobacillus sakei*. While the *pediococci* observed as
336 *Pediococcus acidilactici* and *Pediococcus pentosaceus*.

337 Another acidification process can be conducted by measuring the titratable acidity
338 in fermented beef "cangkuk". The data in Table 1. showed that the titratable acidity
339 calculated as a lactic acid percentage. Variance analysis resulted that different ratio of
340 meat and bamboo shoot was very significantly effect ($P < 0.01$) decreasing the titratable
341 acidity of fermented beef "cangkuk", as different preparation methods and interactions
342 between the two factors showed a non significant effect ($P > 0.05$).

343 The titratable acidity in this study was expressed as lactic acid because it is
344 assumed that the bacteria were dominantly from lactic acid bacteria which perform the
345 metabolic product of the carbohydrate breakdown into lactic acid. Related to pH of

346 fermented beef "cangkuk" which indicated decrease value in line with the increasement
347 of the titratable acidity.

348 Fermented beef "cangkuk" made with different ratio between meat and bamboo
349 shoot was significantly decreased the titratable acidity percentage, which range from,
350 1.03%; 0.84% and 0.70%, respectively. Fermented beef "cangkuk" having the 1.25
351 bamboo shoot ratio was significantly decreased compared to 0.75 ratio of bamboo shoot.
352 This probably addressed by the presence of lactic acid.

353 Ockerman and Basu (2017) adduced the characteristics of different types of semi-
354 dry fermented sausages has reduced pH to 4.7 - 5.3 (lactic acid 0.5 - 1.3%, total acidity
355 1%), with a processing period of 1 - 4 week. This finding has the same result with the
356 titratable acidity of fermented beef "cangkuk" as shown in Table 1.

357 Lactic acid bacteria play important role of lactic acid food fermentation, especially
358 the lactic acid fermentation type of food. Variance analysis resulted that either the
359 preparation method differences and different ratio of meat and bamboo shoot did not
360 significantly affect ($P>0.05$) the lactic acid bacteria cell count.

361 The average cell count of lactic acid bacteria of fermented beef "cangkuk" in this
362 research was $7 \log_{10}$ cfu/g. It means that about 10 million of lactic acid bacteria were
363 assumed to be present in the product. According to Kameník *et al.* (2013) the preliminary
364 population of $6.5 \log$ cfu/g showed an increasement to 8.0–9.0 \log cfu/g of the lactic acid
365 bacteria population until the peaking day of the 7 days of dry fermented sausage samples.
366 Although the fermentation process of fermented beef "cangkuk" was conducted
367 spontaneously, it reaches a high cell count of lactic acid bacteria.

368 Angiotensin converting enzyme (ACE) inhibitory activity of fermented beef
369 "cangkuk" from combination of preparation method and different ratio of bamboo shoot
370 was seen in Figure 1. The combination of A2B1 had highest angiotensin converting
371 enzyme activity (80%).

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372 This research emphasized that addition bamboo shoot as an ingredien additive in
373 spontaneous fermentation of beef meat to produce "cangkuk" which had potent ACE
374 inhibitory activity. This indicated that local fermented product from Indonesia also had a
375 functional effect as well as meat fermentation products typical of other countries.

376 The ACE inhibitory activity was influenced by peptide sequence derived from
377 hydrolysis process during fermentation and ability to bind the active site of ACE (Ryan
378 *et al.*, 2011; Choe *et al.*, 2019). The inhibitory activity of ACE peptide is thought to be
379 due to the high residual hydrophobic amino acids such as proline, alanine and aliphatic
380 amino acids such as glycine. According to Manoharan *et al.* (2006) peptides that have
381 proline or aromatic residues at the C-terminal end and hydrophobic amino acid residues
382 at the N-terminal end have potential ACE inhibitory activity. The same author stated that
383 ACE has an important role in regulating the blood pressure. While in this research, high
384 ACE inhibitory activity in A2B1 treatment is the basis for the second phase of this
385 research to study the antihypertensive activity in Spontaneous Hypertensive Rats.

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387

CONCLUSION

388 The results of this study show that bamboo shoot prepare through the ground
389 process with the ratio of meat and bamboo shoot 1 : 0.75 has protrude chemical

390 characteristics and bioactivity. It is recommended to conduct further research using these
391 results.

392

393 ACKNOWLEDGMENTS

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396 by the Programm Applied Global Research 2019.

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515 **Table and Figure**

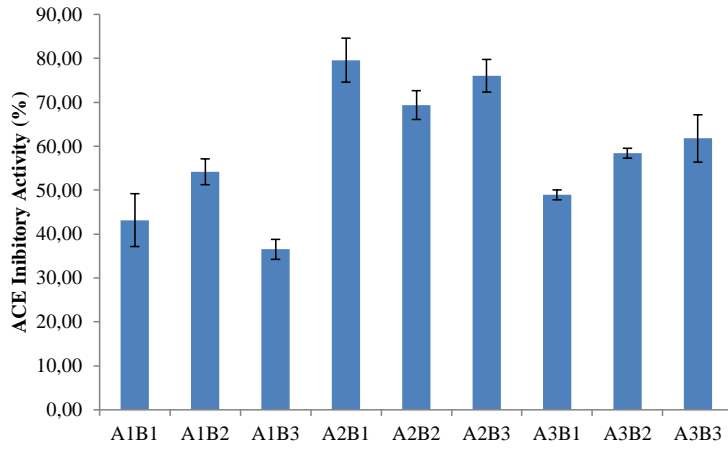
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516
 517 Table 1. Preparation Method, Meat and Bamboo Shoot Ratios and Their Interactions on Total
 518 Protein, Soluble Protein, Degree of Hydrolysis, pH Value, Titratable Acidity and Lactic Acid
 519 Bacteria of Fermented Beef Meat “cangkuk”

Parameters	Preparation Method	Meat : Bamboo Ratios	Significance
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	C	G	WSE	1:0.75	1:1	1:1.25	PM	MBR	INT
Total Protein (g/100 g)	16.62	15.02	15.25	14.85	15.61	16.43	**	**	ns
Soluble Protein (g/100 g)	0.46	0.44	0.32	0.39	0.40	0.44	**	ns	ns
Degree of Hydrolysis (%)	2.83	2.97	2.13	2.66	2.61	2.66	*	ns	*
pH Value	4.67	4.57	4.40	4.58	4.45	4.59	*	ns	ns
Titrateable Acidity (%)	0.79	0.84	0.94	1.03	0.84	0.70	ns	**	ns
Lactic Acid Bacteria Total (Log ₁₀ CFU/g)	7.11	7.11	7.05	7.17	7.07	7.03	ns	ns	ns

520 C = Chopped, G = Ground, WSE = Water Soluble Extract, PM = Preparation Method, MBR = Meat
521 and Bamboo Shoot Ratio, INT = Interaction between Preparation Method and Meat and Bamboo
522 Shoot Ratio, **= highly significant (P<0.01); * = significant (P<0.05); ns = non significant (P>0.05)
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Figure 1. Angiotensin converting enzyme inhibitory activities of beef meat fermentation from combination of preparation method (A) and ratio of meat and bamboo shoot (B). A1: Chopped, A2: Ground, A3: Extracted. B1 : 1:0.75, B2 : 1:1, B3 : 1:1.25. data presented in mean±standard deviation from 2 replications. Sample concentration 10 ml contain 15 g/100 g protein.

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Revised manuscript

Irdha Mirdhayati <mirdhayati@gmail.com>
kepada JITAA

Dear Editor **JITAA**

Hereby we enclosed the revised manuscript entitle "**Chemical characteristic, lactic acid bacteria population, and angiotensin converting enzyme inhibitory activity of traditional fermented beef "cangkuk" by spontaneous fermentation with the addition of bamboo shoot**".

Thank you very much for your attention.

Best regards,

Irdha Mirdhayati

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Demikian maksud kami. Atas perhatian Bapak, kami ucapkan terima kasih.

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