Research Article

Changes in Sensory, Physicochemical and Microbiological Properties of Ronto During Fermentation

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Abstract

Background and Objective: Ronto is a traditional Indonesian fermented shrimp product; however, there are very few reports on how its properties change during fermentation. The aim of this study was to study the changes in sensory, physicochemical and microbiological properties of ronto during fermentation. Materials and Methods: Shrimp (Acetes japonicus) was mixed with salt and rice to give a mixture that was 11.5% salt and 20% rice. The mixture was put into plastic bottles and incubated at room temperature for 18 days. Samples were taken after every 2 days and sensory, physicochemical and microbiological properties were analyzed. Results: It was found that the fermented shrimp developed a sour, savory flavor and bright pink color after 8 days of fermentation, both of which became stronger after 12 days. After 14 days of fermentation, the texture of the shrimp gradually became that of a porridge-like paste. The acidity of the fermented shrimp increased during fermentation, after 12 days of fermentation, the pH had decreased from 8.3-4.8. The total volatile bases increased from 25-150 mg N/100 g over 18 days of fermentation. Lactic acid, proteolytic and amylolytic bacteria were involved in the fermentation process. The concentrations of these three bacteria increased during the early phases of fermentation and decreased in the later phases. Conclusion: Ronto fermentation ended after 14 days when the color of the fermented material became bright pink and when it had developed a strong, sour and savory flavor. That product had a lightness (53.11), a redness (11.72), a yellowness (15.68), a pH (4.8) and the texture of porridge-like paste and was relatively low in total volatile bases.

Key words: Ronto, fermented shrimp, sensory, physicochemical, microbiology

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Data Availability: All relevant data are within the paper and its supporting information files.
INTRODUCTION

Ronto is a traditional fermented shrimp paste (Acetes sp.) that is widely produced in the coastal areas of South Kalimantan. It is made from a mixture of shrimp, salt and rice in various compositions. To make ronto, most of the processors use a ratio of 7:1:2 shrimp to salt to rice, giving a final product that is 11.5% salt and 20% rice by volume. Fermentation usually proceeds at ambient temperature for 14 days in a closed container. Fermentation is completed when the shrimp paste becomes bright pink and develops a strong, sour and savory flavor.

Many countries have products similar to ronto such as cincalok or cincaluk in Indonesia, cencalok in Malaysia, balao-balao and burong isda in the Philippines, kung-chom or koong-som in Thailand and sik-khæ in Korea. The differences among these products are primarily in the type of raw materials used, salt concentration and carbohydrate type and concentration. Cincaluk found in West Kalimantan uses shrimp (Acetes sp.) with 4% salt and 16% sucrose and is fermented for 8 days. Cencalok is a fermented shrimp product from Malaysia that is made from shrimp (Acetes sp.) combined with 10-20% w/w salt along with some amount of rice powder and is fermented for more than a month. Rusip is a fermented fish product from Sumatera that is made from a mixture of anchovies, 25% salt and 10% rice and is fermented for 2 weeks. Balao-balao from the Philippines uses shrimp, 4-5% salt and 80% rice and is fermented for 7-10 days. Kung-chom or koong-som from Thailand uses 7% salt, 1% garlic and 2% rice with a fermentation time of 7-10 days. Sikhae is a fermented fish dish from Korea made from salt, cooked millet, red pepper powder, garlic and minced ginger, with a fermentation time of 2 weeks.

Over the course of a 7 day fermentation period, the pH of rusip decreased from 6.53-4.89 and the acidity and dissolved proteins increased from 1-2% and 3.0-6.0%, respectively. The concentration of lactic acid bacteria increased until reaching a level of log 10 CFU g⁻¹ after 16 days fermentation, after which the level decreased. According to Hajar and Hamid, cincalok from Malaysia is similar to belacan. The product is a suspension of tiny pink shrimp in a salty sauce with a strong shrimp smell. During the course of a 4 day fermentation, the pH of Balao-balao decreased to 4.08 with titratable acidity reaching 1.32% (as lactic acid). During fermentation of kong-som from Thailand, a sour taste developed, which is a typical flavor for shrimp fermented by lactic acid bacteria in a pH range from 3.7-3.9. Changes in the protein contents of the fermented products vary and are influenced by the proteolytic activity of the microorganisms and enzymes during the fermentation process. Additionally, protein hydrolysis during fermentation is mediated by lactic acid bacteria and indigenous proteases. As reported by Reerueangchai et al., the amount of bacteria increased with fermentation time and was related to the increase in Total Volatile Bases (TVB-N) which also occurred during fermentation of the seasoned shrimp. Adams reported that over the 28 day fermentation period of shik-kae, sensory properties developed and there were changes in the chemical and microbiological properties. The sensory attributes change from the mixture having the odor of fish to the mixture having a sour taste and the optimum sensory values are reached on the 14th day of fermentation. Compression force decreased faster from 160-40 kg force. The pH value decreased from 7-5 over the first 10 days, while total lactic acid increased from 100-2500 mg/100 g during the same period. The TVB value had increased from 30-170 mg% by day 28. According to Chaijan and Panpipat, several reactions might be involved in the development of the color of fermented shrimp paste. A reddish pink to orange color in the shrimp paste might result from astaxanthin, a natural pigment, being released from its protein-bound state during proteolysis.

An understanding of the sensory, physicochemical and microbiological changes that occur during the fermentation of ronto is important for quality control of the process in industry, however, there are very few reports on these changes. Thus, this study was carried out to study the changes in the sensory, physicochemical and microbiological properties during ronto fermentation.

MATERIALS AND METHODS

Materials: The shrimp was obtained from fisherman in Muara Kintap, Kintap District, Tanah Laut Regency, South Kalimantan Province. Salt was obtained from a local market in Muara Kintap. The type of rice used was IR 32. The rice was cooked in a water-to-rice ratio of 3:1.

Ronto fermentation: Seven portions of shrimp and 1 portion of salt were mixed and left to rest for 2 h, after which 2 portions of boiled rice were added. The mixture (350 g) was transferred to a plastic bottle and fermented at ambient temperature for 18 days. Every 2 days, a sample was taken for analysis of the sensory, physical, chemical and microbiological properties.

Sensory description: Descriptions of the sensory properties of ronto were provided by ronto producers. They described the
changes in aroma, color and texture during fermentation and reported if the signs that the fermentation process was complete had developed. They were asked to answer three questions as part of their daily observations: (1) Is the desired aroma of ronto present?, (2) Is the desired color of ronto observed? and (3) Has the desired texture of ronto been obtained?

**Physical analysis:** The physical properties of ronto monitored during fermentation were color and viscosity. The color of ronto was measured using a Chroma meter Minolta CR-400 and the viscosity of ronto was measured using viscometer Brookfield DV-11 Pro. A 50 g sample of ronto was blended for 5 min and then transferred to a cylindrical tube with a diameter of 3 cm and depth of 6 cm until the tube was full. Afterwards, viscosity was measured with viscometer with spindle #64 at 50 rpm. Measurements are reported in dyne cm⁻¹.

**Chemical analysis:** A 200 g sample of ronto was blended with a Miyako BL 101-PL blender at maximum speed for 5 min. The blended sample was used for chemical tests. Water content was determined using gravimetric analysis in an oven for 24 h until a constant mass was obtained. For pH analysis, a 25 g sample of the was combined with 25 mL of distilled water. After that, an Eutech Instrument-pH 150 pH meter was used to measure the pH of the sample. The titratable acidity was measured by titrating the sample with 0.1 N NaOH using phenolphthalein as an indicator. Total volatile base levels were determined using the Conway micro-diffusion method. The amounts of TVB were calculated and reported as mg N g⁻¹ sample. The soluble nitrogen content was determined using the Lowry method. One milliliter of the ronto extract was combined with 1 mL of Lowry reagent and left to react for 15 min. Next, 3 mL of Folin’s reagent was added and the mixture was left to stand for 45 min at room temperature. Absorbance was measured with a spectrophotometer at a wavelength of 600 nM. Soluble N was determined using a standard curve of bovine serum albumin.

**Microbiological analysis:** For microbiological analysis, a 10 g sample of ronto was homogenized using a Stomacher with 90 mL of sterile physiological saline. Serial dilutions (1:10) of each homogenized sample were made in the same diluent and duplicate counting plates were prepared using appropriate dilutions. Bacteria were counted used dilution and the pour plate method. Samples were incubated at 37°C for 48 h in an appropriate medium. The total number of bacteria was determined using Plate Count Agar (Oxoid). The number of lactic acid bacteria was determined using de Man, Rogosa and Sharpe agar with 1% CaCO₃. The proteolytic bacteria and amylolytic bacteria were enumerated on nutrient agar with 2% w/v skim milk and nutrient agar supplemented by 1% starch, respectively. The yeast contents were determined using Potato Dextrose Agar (Merck).

**RESULTS AND DISCUSSION**

**Sensory properties:** A description of the sensory properties of ronto developed during fermentation are presented in Table 1. Initially, the aromas of the samples were dominated by the smells of fresh shrimp and sea water. The sour, savory fermented shrimp flavor began to appear at day 8 of fermentation and increased up to day 12. Furthermore, the strong, sour and fermented shrimp aroma remained unchanged until the last day of observation. A change in the sample color to pale pink was observed after 2 days of fermentation and a bright pink color appeared after 8 days of fermentation. The bright pink color remained unchanged until the last day of fermentation (day 18). The salty taste from the added salt began to be masked by a sour taste that developed by day 12. On day 12, the taste of the sample was predominantly sour compared to the salty, strong savory fermented shrimp flavor the mixture had earlier. This flavor remained until day 18. Changes in appearance were seen in the gradual disintegration of the shrimp and rice. By day 12, approximately 50% of the shrimp and 80% of the rice had disintegrated. Consequently, a porridge-like ronto that was primarily a suspension of disintegrated shrimp and rice was formed.

**Color:** The changes in color were monitored by chromometer and the results are presented in Table 2. Color measurements showed various parameters (lightness, redness and yellowness symbolized with L*, a* and b*) that increased starting on day 4. Ronto producers reported that the red color started to appear clearly on day 8, while based on measurements using Chroma meter, the red color began to develop on day 4. The color of the ronto was determined by the combination of L* values that ranged from 53.11-54.92, a* values that ranged from 10.42-11.72 and b* values that ranged from 14.62-16.90. An L* value (lightness) of 0 means black and a value of 100 means white. Ronto with L* values above of 50 appear as vivid white. Redness value (a*) is located between red and green, positive (+) values are closer to red, negative (-) values are closer to green and a value of 0 means neutral. Ronto with
a positive a* value means it tends to appear red. Yellowness value (b*) is located between yellow and blue, where a positive (+) value is closer to yellow, a negative (-) value is closer to blue and a value of 0 means neutral. Ronto with a positive b* value means it tends to appear yellow. Hence, the color of ronto is a mixture of pink to yellow. According to Hunter lab, who used a CIELAB system, the combination of the 2 colors is between dull red and dull orange. Wade et al.8 and Tume et al.9 stated that color changes in lobster, crab, shrimp and other crustaceans are related to astaxanthin content. Astaxanthin (3 3'-Dihydroxy-β-carotene-4,4'-dione) belongs to the class of carotenoids. This pigment naturally occurs because of hydrolysis and proteolysis of astaxanthin after the protein in shrimp breakdown. Thus, a mixture of red and orange colors appear during shrimp fermentation20. A similar mechanism also causes the color change of terasi. Chaiphan and Panpipat12 stated that the orange and reddish pink colors in shrimp paste are formed during the breakdown of natural pigments liberated from proteins by protease enzymes. Astaxanthin content in shrimp paste was correlated with L*, a* and b* values, supporting the hypothesis that the color change in shrimp paste was influenced by astaxanthin content. Pongsukul et al.21 reported that the ranges of L*, a* and b* values of 3 types of shrimp paste in Thailand were 34.13-51.84, 5.42-15.39 and 4.56-17.59, respectively.

**Viscosity and moisture:** Figure 1 shows changes in viscosity and water content during fermentation. The viscosity of ronto decreased significantly during the first 2 days of fermentation and then decreased gradually until day 6. These data correlate with the disintegration of shrimp and rice during fermentation. The proteins in the shrimp and the carbohydrates in the rice were hydrolyzed by enzymes produced by microorganisms and broken down into simple compounds, which caused the fermented material to be soft and have a lower viscosity. According to Adams19, the extensive proteolytic activity and liquefaction that occur during fermentation are thought to be largely a result of autolytic breakdown of the fish tissue. The physical characteristics of balao-balao, a product similar to ronto, included the liberation of gas, liquefaction, development of a red color and softening of the shrimp shell22. The product turns into a suspension of tiny pink shrimp in a salty tasting sauce. The product has a very strong briny shrimp smell, which stings the nose. The savory flavor of cencalok and ronto appear because of the concentration of the amino acid glutamate23. The moisture content of ronto during fermentation ranged from 67.06-68.03%. This range was similar to commercial ronto sold at traditional markets.
Fig. 1: Changes in viscosity and moisture during ronto fermentation. Vertical bar stands for standard deviation (n = 3).

Table 2: Change of color during ronto fermentation

<table>
<thead>
<tr>
<th>Time (day)</th>
<th>Lightness (L°)</th>
<th>Redness (a°)</th>
<th>Yellowness (b°)</th>
<th>Hue (°)</th>
<th>Chroma (°)</th>
<th>Visual color</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>53.07 ± 0.68</td>
<td>8.46 ± 0.37</td>
<td>8.99 ± 0.00</td>
<td>46.89 ±1.25</td>
<td>12.36 ±0.26</td>
<td>Dull grey, whitish</td>
</tr>
<tr>
<td>2</td>
<td>52.16 ± 0.94</td>
<td>8.05 ± 0.30</td>
<td>8.94 ± 0.42</td>
<td>48.03 ±1.33</td>
<td>12.08 ±0.46</td>
<td>Pale red, whitish</td>
</tr>
<tr>
<td>4</td>
<td>54.25 ± 0.28</td>
<td>10.42 ± 0.16</td>
<td>10.30 ± 0.24</td>
<td>44.82 ±0.79</td>
<td>14.66 ±0.24</td>
<td>Pale red</td>
</tr>
<tr>
<td>6</td>
<td>54.67 ± 0.69</td>
<td>10.81 ± 0.72</td>
<td>12.82 ± 0.41</td>
<td>49.96 ±1.02</td>
<td>16.78 ±0.78</td>
<td>Pale red</td>
</tr>
<tr>
<td>8</td>
<td>54.74 ± 0.18</td>
<td>11.34 ± 0.35</td>
<td>15.31 ± 0.32</td>
<td>53.53 ±0.62</td>
<td>19.05 ±0.43</td>
<td>Pale red</td>
</tr>
<tr>
<td>10</td>
<td>54.82 ± 1.23</td>
<td>10.71 ± 0.52</td>
<td>14.62 ± 0.10</td>
<td>53.84 ±1.27</td>
<td>18.13 ±0.34</td>
<td>Bright pink</td>
</tr>
<tr>
<td>12</td>
<td>53.42 ± 0.41</td>
<td>10.61 ± 0.30</td>
<td>15.34 ± 0.25</td>
<td>49.57 ±6.28</td>
<td>19.16 ±0.33</td>
<td>Bright pink</td>
</tr>
<tr>
<td>14</td>
<td>53.11 ± 0.41</td>
<td>11.72 ± 0.18</td>
<td>15.68 ± 0.04</td>
<td>54.14 ±0.58</td>
<td>19.23 ±0.26</td>
<td>Bright pink</td>
</tr>
<tr>
<td>16</td>
<td>54.92 ± 0.11</td>
<td>11.22 ± 0.11</td>
<td>16.90 ± 0.21</td>
<td>56.44 ±0.12</td>
<td>20.29 ±0.24</td>
<td>Bright pink</td>
</tr>
<tr>
<td>18</td>
<td>53.44 ± 0.30</td>
<td>10.84 ± 0.14</td>
<td>15.60 ± 0.37</td>
<td>55.18 ±0.46</td>
<td>19.00 ±0.36</td>
<td>Bright pink</td>
</tr>
</tbody>
</table>

All values are arithmetic means ± standard deviation (n = 3). Values within columns followed by similar letters were not significantly different by Fisher’s LSD test (p<0.05). CV stands for coefficient of variation.

(69.67%)21, whereas the water content of rusp, which is made from small fish (ten) mixed with 12.5% w/w salt and 5% w/w brown sugar, was 69.36-65.23%2. However, kong som from Thailand has a higher water content (73.35-80.71%)12.

Acidity: Changes in pH and total titratable acid during ronto fermentation can be seen in Fig. 2a and b. As shown in Fig. 2a, the pH value of the sample was initially 8.3. By day 6, the pH had dropped significantly to 5.0, by day 10, it had reached 4.8 and at that point remained constant until day 18. This occurred because the total acid increased from 4.4 mg g⁻¹ (day 0) to 18.5 mg g⁻¹ on day 12 and 23.00 mg g⁻¹ on day 18. The increase in total titratable acid and the decrease in pH value are closely related to enzymatic activity in the raw materials and microorganisms that grow during fermentation, particularly lactic acid bacteria. These results were in line with the sensory evaluation given by ronto producers, who described a sour taste first appearing on day 6 and becoming strong by day 12. Although the total acid increased until day 18, the pH was relatively stable after day 10 of fermentation. This could be due to the buffering capacity of the system during ronto fermentation. Rice was added as a substrate for microorganisms to grow and produce organic acids (such as lactic acid and acetic acid) during fermentation. The acids are products of the breakdown of complex carbohydrates such as the rice that was added into the fermentation of salted fish. The rice is able to control the rate of acid production and maintain product quality22,23. According to Shirai et al.2, pH stability during shrimp fermentation is presumed to occur because of the buffer system, which is the result of the breakdown of organic acids because of the salt, the reaction of organic acids with amino acids, or the neutralization of some organic acids with calcium obtained from chitin in the sediment.

Nitrogen: Changes in total nitrogen and dissolved nitrogen during ronto fermentation are given in Fig. 2b. Changes in total nitrogen and dissolved nitrogen can be used as an indicator of proteolysis. Dissolved nitrogen means that the amount of nitrogen released during proteolysis and is calculated by considering the concentration of proteins and aromatic amino acids26. Figure 2b shows that dissolved nitrogen remained relatively constant during fermentation. Proteins were hydrolyzed by proteases in the raw materials.
and microorganisms producing simpler compounds such as peptides and amino acids. These compounds were used by microorganisms for growth and metabolic activities. Thus, the amount of dissolved nitrogen during ronto fermentation was affected by the rate of protein hydrolysis and the rate of nitrogen uptake by microorganisms.

**Total volatile bases (TVB-N):** The TVB-N is defined as the concentration of volatile bases resulting from the breakdown of proteins. It can be used to measure the quality of fish products and is a chemical indicator for the deterioration of fish products. The quality of fish can be estimated by chemical methods such as measuring volatile compounds, which are important characteristics for the assessment of quality in seafood products. Measurements of volatile compounds are the most common chemical indicator of marine fish spoilage\textsuperscript{27,28}. The level of total volatile bases in ronto during fermentation can be seen in Fig. 2c. The level of TVB-N observed during ronto fermentation was relatively constant at a low level (34.08 mg N/100 g) from 0-8 day. However, after day 8 of fermentation, the TVB-N value increased significantly until day 12, when it reached a level of 150 mg N/100 g, at which point it remained relatively constant until day 18. Connell\textsuperscript{19} reported that the maximum level of TVB-N in fish processed with salt is 200 mg N/100 g. Reaching a TVB-N level of 150 mg N/100 g was a good indicator that the breakdown of proteins into simpler compounds by proteolytic bacteria so that changes in the physical properties of ronto could occur was happening. The main constituents of TVB-N are trimethylamine, dimethylamine and ammonia\textsuperscript{28}. Trimethylamine originates from bacterial decomposition. Its presence in fish is therefore taken as an indication of bacterial growth. Ammonia comes from the decomposition of amino acids, thus reducing the quality of the available protein\textsuperscript{29}. Therefore, based on value of TVB-N in ronto over the 18 days of fermentation, the product was still safe for consumption.

**Starch:** The change in total starch concentration during ronto fermentation is shown in Fig. 2d. Total starch decrease during fermentation was an indicator of the breakdown of starch by amylolytic bacteria. Consequently, the components of ronto disintegrated to give a porridge-like texture. The total starch
value dropped fast between 2-8 day, after which it remained constant until day 18. These changes followed the growth of amylolytic bacteria for approximately 2 log cycles from 0-4 day. Afterwards, it decreased for 2 log cycles until day 10 and the number of viable cells remained constant from 12-18 day (Fig. 3b). Rice is the most common starch source for fermented shrimp in South Asia\textsuperscript{11}. High amounts of glucose are produced from rice starch by amylolytic lactic acid bacteria. They produced an amylolytic enzyme that influences the fermentation process\textsuperscript{10-31}.

**Microbiological changes:** The growth of total bacteria, lactic acid bacteria, yeast, proteolytic bacteria and amylolytic bacteria during ronto fermentation can be seen in Fig. 3. It was assumed that lactic acid bacteria, yeast, proteolytic bacteria and amylolytic bacteria played important roles in ronto fermentation. Early in the fermentation process, yeast grew from 5 log CFU g\(^{-1}\) on day 1-7 log CFU g\(^{-1}\) on day 2 and then gradually decreased to 4.37-3.50 log CFU g\(^{-1}\) on 4 and 10 days, respectively. This could be due to the competition with lactic acid bacteria, which grew quickly between 2-6 days up to 5.48 log CFU g\(^{-1}\). The growth of lactic acid bacteria produced organic acids that acted as preservatives. The availability of simple sugars was a result of the breakdown of rice starch by amylolytic bacteria, which was detected during ronto fermentation (Fig. 3b). It provided simple sugars which were used as a source of energy for microorganisms. The amount of proteolytic bacteria was higher than the amount of amylolytic bacteria, which peaked at 6.88 log CFU g\(^{-1}\) on day 2. The amount of proteolytic bacteria decreased gradually during ronto fermentation to approximately 3.61 log cycles on day 18. Several studies on shrimp and fish fermentations have been published. Rao and Stevens\textsuperscript{31} observed the growth of *L. plantarum* A6, lactic acid and amylolytic bacteria, which worked as inoculum on shrimp waste fermentation with a maximum enumeration value of \(10^4\) CFU mL\(^{-1}\) on 12-13 h of growth. Paludan-Muller et al\textsuperscript{30} reported that at the beginning of shrimp fermentation, yeast growth appeared at 4 log CFU g\(^{-1}\) on day 1, increased to 7 log CFU g\(^{-1}\) by day 3, slowly decreased to 5.5 log on day 5 and remained constant until day 12 in *pla-som* fermentation. Reerueangchai et al\textsuperscript{13} reported that fermented spice made from shrimp waste occurred as a result of proteolytic bacteria activity and in the case of sikhae, the amount of proteolytic bacteria and yeast increased for the first 12 days of fermentation\textsuperscript{10}. Yanglang and Maneerat\textsuperscript{32} monitored glutaminase producing bacteria during the production of kung-som. They successfully isolated lactic acid bacteria and Coagulase-Negative *Staphylococcus* (CNS), i.e., *Lactobacillus rhamnosus*, *Staphylococcus piscifermentans* and *Staphylococcus saprophyticus* respectively. The other types of lactic acid bacteria isolated from bekasam, kung-som and shik-hae were *L. plantarum*, *L. pentosus* and *Pediococcus pentosaceus*\textsuperscript{33}, *Lactobacillus plantarum* sp.,\textsuperscript{34} and *L. mesenteroides* and *Lactobacillus plantarum*\textsuperscript{10}, respectively.

**CONCLUSION**

During ronto fermentation, a strong and sour aroma typical of fermented fish developed and the color changed from a dull gray to bright pink. The breakdown of shrimp and rice resulted in a decrease in the viscosity of the ronto. Successful fermentation was marked by a decrease in pH from 8-5 by day 4, which then remained constant until day 18, the total titratable acid increased from 4.92 mg g\(^{-1}\) to 18.49 by day 12 and the Total Volatile Bases (TVB) increased to
150 mg N/100 g in the sample. Lactic acid bacteria, yeast, proteolytic bacteria and amylolytic bacteria played important roles during the fermentation of ronto.

**SIGNIFICANT STATEMENT**

This study explores the changes in sensory, physicochemical and microbiological properties of ronto that occur during the fermentation process. This research can be beneficial for the development of product processing techniques and aid in the introduction of an indigenous south Kalimantan regional product to a broader audience. This study will help scientists to uncover the critical feature of the time required for optimal fermentation of ronto in a quantitative manner, which has never been done before. With this new information, a new method of ronto fermentation may be developed.

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