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8

9 **Abstract**

10 This study investigated the changes in volatile compounds in chicken flesh after boiling at
11 various pHs (6.0–9.0) and after chilling storage ($4.0\pm 1.0^{\circ}\text{C}$) for 7 d. The volatile compounds
12 were assessed qualitatively and quantitatively by using a headspace GC–MS analysis. Twenty-
13 one volatile compounds were discovered and categorized as amine, aldehyde, alcohol, ketone,
14 acid, and furan. One type of amine, (2-aziridinyethyl) amine, was the most prevalent volatile
15 component, followed by aldehyde, ketone, aldehyde, acid, ester, and furan. The results showed
16 that the quantity and quality of the volatile compounds were influenced by a pH of the boiling
17 medium. Additionally, the types and volatile profiles of the chicken were altered during chilling.
18 In particular, in the chicken that was boiled at a pH of 8.0, the hexanal (an aldehyde) content
19 increased the most after 7 d of chilling. Moreover, various alcohols formed after the 7 d of
20 chilling of the chicken that was boiled at pHs of 8.0 and 9.0. Because of the oxidation and
21 degradation of fat and proteins, the most altering volatile compounds were the reducing amines
22 and the increasing aldehydes.

23 **Keywords:** volatile compounds; headspace GC-MS; thermal treatment; chicken

24 **1. Introduction**

25 Poultry meat is one of the most widely consumed meats worldwide, with no religious
26 restrictions, in contrast to pork and beef, which are not consumed by Muslims and Hindus,
27 respectively. Chicken is the most common type of poultry in the world. It contains less fat than
28 the other types of meat and provides high-quality protein as well as other nutrients (Connolly et
29 al., 2022). It can be processed in a variety of ways; nevertheless, the processing influences its
30 customer acceptance (Maughan et al., 2012). Sensory perception of food encompasses aspects
31 such as appearance, odor, flavor, taste, and texture; additionally, odor has a considerably strong

32 effect on preference (Nanda et al., 2021). Food contains various volatile compounds (Ayseli et
33 al., 2014), each of which has a unique olfactory profile that is determined by specific chemical
34 combinations. The volatile compounds or flavors are mainly formed by degrading and reacting
35 with non-volatile precursors such as fat, protein, peptides, fatty acids, amino acids, and reducing
36 sugar (Madruga et al., 2010). As the processing and storage affect the reaction and the changes in
37 the food compositions and then affect the volatile compound changes (Calkins and Hodgen,
38 2007), different heating conditions influence the products, giving them a specific aroma and
39 bringing about changes in their volatile profiles during storage.

40 Boiling is a typical method for preparing or cooking chicken. Chicken is cooked in a variety
41 of broths with varying flavors. The components change pH, which affects a variety of processes,
42 including lipid oxidation and the Maillard reaction (Niu et al., 2016). Boiling at 95°C is a viable
43 heating treatment for chicken, according to Wang *et al.* (2020), because it helps to retain the
44 fresh odor and texture while safeguarding food safety. This stands in contrast to the stewing
45 method, which involves slowly heating the meat in the broth to liberate the fatty acids and amino
46 acids and produce the desired flavors or volatiles (Guan et al., 2023). Boiled and pasteurized
47 chicken meat has a shelf life of up to 7 d under mild chilling conditions with acceptability of
48 flavor and odor (Montero-Prado and Morales, 2022) and customer safety (Hasani et al., 2023).
49 There have not been many studies, as far as we know, on how pH affects the volatile flavor of
50 cooked chicken after heat treatment. Therefore, the volatile components of chicken that were
51 boiled at various pH levels as well as the volatile alterations that occurred during chilling storage
52 were examined in this study. This research's findings can be used in the manufacture of chicken
53 products and the design of the corresponding process, including the cooking and pre-cooking
54 steps. Furthermore, understanding the volatile changes that occur during chilling can help design
55 storage settings to limit product alterations.

56 2. Materials and Methods

57 2.1. Sample preparation

58 Chicken breast meat (Ross308 strain, 42–45 d of age, storage at 5–8°C) 9.04% protein,
59 0.74% fat, and 0.57% carbohydrate) was purchased from the local market. It was diced into
60 cubes measuring 1 cm × 1 cm × 1 cm and then boiled in water with pH adjustments of 6.0, 7.0,
61 8.0, and 9.0 made using sodium bicarbonate and citric acid until the core temperature of the
62 chicken reached 95°C for 5 min. The ratio of chicken and water was 1:10. The samples were
63 divided into two parts: 1) to evaluate the volatile compound immediately, and 2) to analyze the
64 volatile compound after 7 d of storage at 4.0 ± 1.0°C (chilling).

65 2.2. Headspace volatile analysis

66 The headspace volatile compounds in the chicken were analyzed using the Thermo
67 Scientific Trace 1300 gas chromatography–mass spectrometry (GC–MS; Thermo Fisher
68 Scientific, UK). The volatile compounds were collected and separated with a TG-5slims column
69 with dimensions of 0.25 mm × 30 mm × 0.25 µm (Thermo Fisher Scientific, UK) using helium
70 (UHP, 99.999%) as a carrier gas at a constant flow rate of 1.6 mL/min. With the modified
71 procedure of Bhadury *et al.* (2021), the initial oven temperature was held at 50°C for 2 min
72 before ramping at 5°C/min until 125°C for 10 min, and then at 10°C/min until 200°C for 2 min.
73 The total run time was 26.50 min. Eluted compounds that exited the GC column were separated
74 by a splitter at a ratio of 2:3 before entry into the mass selective detector (MSD 5975, Agilent
75 Technologies, USA). The MSD was operated in the electron impact mode at 70 eV with 35 to
76 500 scanning m/z. The identification of the separated compounds was performed by comparing
77 the mass spectra to the NIST02 library available in the GC system. The percentage peak area
78 method was used to present the content of each specified component by evaluating it as a
79 proportion of the area of the target component to the total area of all of the detected peaks. Each

80 treatment was examined using 10 g of boiling chicken cubes in triplicate. Every analyte was
81 injected in triplicate.

82 2.3. *Statistical Analysis*

83 The data were analyzed using the Minitab 19 software. A one-way analysis of variance
84 (ANOVA) was used to determine the significant differences between the means, and the Tukey
85 test was used for a comparison, with the significance level set at $P < 0.05$.

86 3. **Results and Discussion**

87 3.1. *Overall volatile profile*

88 The volatile compounds obtained at different pHs of the boiling water, in chicken breast
89 meat were detected using GC-MS. In all, 21 volatile compounds were detected among eight
90 samples (chicken boiled at pHs of 6.0, 7.0, 8.0, and 9.0 and those chilled for 7 d), which could be
91 divided into seven categories. Among all of the volatile compounds were one amine, one ester,
92 one furan, two ketones, eight alcohols, and eight aldehyde compounds. It was found that the pH
93 of the boiling water not only affected the number of volatile compounds but also their relative
94 contents. Moreover, changes were observed in both compound type and quantity during chilling
95 storage. The highest concentration of volatile compounds detected in the chicken was that of
96 amine, followed by that of aldehyde, ketone, alcohol, and acids, respectively, as shown in Fig.
97 1(A). Most of these compounds (except amine) were derived from the Maillard reaction or the
98 thermal degradation of lipids (Ruiz et al., 2002). Amine, alcohol, ketone, alcohol, and acid
99 volatiles were found in chicken boiled at a pH of 6.0, but no acid was found in chicken boiled at
100 pHs of 7.0, 8.0, and 9.0. The free OH^- in the medium with pHs of 7.0, 8.0, and 9.0 most likely
101 neutralized the free H^+ in the medium; moreover, a number of acids were destroyed by heat
102 during boiling. Furthermore, alcohol was not detected in chicken cooked at a pH of 7.0. The

103 volatile compounds changed after 7 d of chilling, as indicated in Fig. 1(B). The concentration of
104 amine in the boiled chicken reduced; in contrast, that of aldehydes and alcohols increased. Acid
105 was only found in the chicken boiled at a pH of 6.0 as 0.2% and increased after storage for 7 d as
106 0.4%. In addition, ester (n-caproic acid vinyl ester) was not found in the chicken boiled at any
107 pH, but it was detected in the chicken boiled at any pH studied after 7 d of chilling storage. It
108 was probably produced from the esterification of the existing alcohol and carboxylic acid
109 (Hwang et al., 2020). In addition, 2-pentyl furan, a non-carboxyl compound derived from linoleic
110 acid oxidation (Wall et al., 2019), lipid oxidation (Zhao et al., 2022), and the Maillard reaction
111 (Niu et al., 2016), was identified to be responsible for fatty and cooked-meaty aromas (Niu et al.,
112 2016; Zhao et al., 2022), which had a very low aroma threshold (Wang et al., 2020).

113 **[Figure 1]**

114 The only amine found in the chicken after boiling and chilling was (2-aziridinyethyl) amine
115 with a fishy flavor (Xu et al., 2019). As it contained nitrogen, it was possibly related to the amino
116 acid degradation during boiling. A reduction in its relative content was found after storage at a
117 chilling temperature (lower than 10°C), but the relative contents of the other products,
118 particularly the aldehydes, increased. The volatile compounds from chicken boiled at a pH of 9.0
119 included the least amount of (2-aziridinyethyl) amine and the most aldehydes; in contrast, those
120 boiled at a pH of 7.0 exhibited an increase in the formation of (2-aziridinyethyl) amine while
121 inhibiting the aldehyde release. However, after 7 d of chilling, the amount of (2-aziridinyethyl)
122 amine in all of the samples (chicken boiled at each pH tested) decreased while that of the
123 aldehydes increased. The aldehyde content in the chicken boiled at pHs of 7.0, 8.0, and 9.0
124 became not significantly different ($P < 0.05$) after chilling for 7 d. It was indicated that the
125 boiling medium pH of 7.0 inhibited the aldehyde production in the fresh boiling but enhanced the
126 generation during storage up to 5.5 times (from 9.9 to 54.6%). This change altered the aroma in

127 the chicken from fishy to aldehyde-type. The changes in type and contents of volatiles during
128 chilling storage were because the pH condition of the boiling process affected the oxidation rate
129 of the volatiles (Hassanzadeh et al., 2022a; Hassanzadeh et al., 2022b), fatty acids (Ba et al.,
130 2013), and lipids (Kim et al., 2016).

131 Prior research (Petrucci et al., 2016; Jung et al., 2023) indicates that heating provided
132 molecular energy to break atom-to-atom bonds, resulting in smaller molecules or volatiles, but it
133 also led to volatile degradation (Yuan et al., 2023). Moreover, Yang et al. (2017) found that the
134 pH of the solution affected the volatility of the compounds through two processes: 1)
135 decomposition, in which a low or high pH caused some compounds to break down into smaller,
136 more volatile molecules, and 2) acid and base reactions, which result in charged ions, which are
137 less volatile than neutral molecules. This investigation included the effects of dissolving the
138 molecules, releasing the volatile molecules through heat, and enhancing the volatile stability
139 under pH conditions.

140 3.2. Aldehydes

141 Aldehydes were the second-most-abundant volatile compounds in the chicken, both after
142 boiling and chilling. They significantly contributed to the overall aroma of cooked chicken
143 because of their low threshold values (Ba et al., 2010). As shown in Fig. 2, after boiling, five
144 aldehydes, namely hexanal, pentanol, nonanal, heptanal, and octanal, were detected in the
145 chicken boiled at pHs of 6.0, 8.0, and 9.0, but three aldehydes, namely hexanal, pentanol, and
146 nonanal, were detected in the chicken boiled at pH 7.0. Among them, nonanal and heptanal had
147 pleasant meat flavors, and octanal had a sweet orange flavor. Benzaldehyde, an organic
148 compound formed by replacing a hydrogen of benzene with an aldehyde group, contributed an
149 almond smell (Zhao et al., 2021).

150

[Figure 2]

151 However, more types of aldehydes were found after chilling for 7 d. Butanal was detected
152 in the chicken boiled at a pH of 6.0 after chilling for 7 d, while acetaldehyde and benzaldehyde
153 were found in the chicken boiled at pHs of 7.0, 8.0, and 9.0 and chilled for 7 d. It was found that
154 acetaldehyde formed in a high concentration in the chicken boiled at a pH of 8.0 and chilled for 7
155 d as the 6.42% relative content increased, as shown in Fig. 3; it caused a pungent odor, which
156 was unpleasant, in the chicken (Luttrel, 2009). A small amount of benzaldehyde (~0.06% relative
157 content increase) was detected in the chilled chicken, which was boiled at pHs of 7.0, 8.0, and
158 9.0; however, its high threshold (Acree and Heinrich, 2024) presented no effect on the sensory
159 reception. The relative contents of hexanal and pentanal clearly increased in the chicken boiled at
160 every pH of water after chilling for 7 d. The highest increase in the relative concentration of
161 hexanal after 7 d of chilling in the chicken boiled at a pH of 8.0 (40.29%), followed by that
162 boiled at pHs of 7.0 (36.34%), 6.0 (23.99%), and 9.0 (15.17%), respectively. Aldehydes were
163 identified as the primary odor components in cooked chicken because of their high volatility and
164 low threshold (Shi et al., 2019). Previous research (Yang et al., 2017) has demonstrated that
165 aldehydes are mostly produced via lipid oxidation. Hexanal and heptanal, known for their
166 unpleasant rancidity and green odor (Kobayashi et al., 2016; Li et al., 2020), are mostly formed
167 by the oxidation of linoleic acid and arachidonic acid, whereas octanal and nonanal are formed
168 through the oxidation of oleic acid (Watanabe et al., 2015), which is high in chicken meat (Kim
169 et al., 2020). Moreover, pentanal, nonanal, and octanal have been reported to be formed from the
170 oxidation of unsaturated fatty acids and the thermal oxidative decomposition of fat (Sampaio et
171 al., 2012).

172

[Figure 3]

173 3.3. *Alcohols*

174 According to Fig. 4, alcohol was not produced in the chicken boiled at a pH of 7.0, but two
175 alcohols, namely 1-pentanol and 1-octen-3-ol, were found in the chicken boiled at a pH of 6.0.
176 Both 1-pentanol and 1-octen-3-ol are the oxidation products of fatty acids composed of linoleic
177 acid (Pratt et al., 2011) and arachidonic acid (Jerkovie et al., 2021), respectively. 1-pentanol and
178 2-hexyl-1-octanol were also found in the chicken boiled at pHs of 8.0 and 9.0, while 1-octen-3-
179 ol, a product of the auto-oxidation of linoleic acid that supplied a mushroom aroma (Feng et al.,
180 2019) and green flavor (Zhang et al., 2023), was detected in the chicken boiled at a pH of 9.0 but
181 not at a pH of 8.0. Because alcohols are the main products of fat oxidation and decomposition
182 and generate the aroma volatiles (Miks-Krajnik et al., 2016), the difference in the type and
183 concentration of the detected volatile compounds in the boiled chicken meat was attributed to the
184 differences in the fat decomposition at different pHs.

185 **[Figure 4]**

186 After storage under chilling conditions for 7 d, 1-pentanol and 1-octen-3-ol detected in the
187 chicken boiled at all of the studied pHs increased, while 2-hexyl-1-octanol detected in the meat
188 boiled at pHs of 8.0 and 9.0 decreased by a relative concentration of 0.24, as shown in Fig. 5. It
189 was possible that 2-hexyl-1-octanol was a substance for some reactions or a substrate for
190 bacterial growth during the chilling storage. The increases in 1-pentanol and 1-octen-3-ol in the
191 chicken boiled at pHs of 8.0 and 9.0 were quite similar, with relative concentrations of around
192 0.45–0.49 and 0.39–0.40, respectively. As both 1-pentanol and 1-octen-3-ol were formed during
193 the oxidation of the fatty acids, the results indicated that there was still fatty acid oxidation
194 during storage, particularly in the chicken boiled at pHs of 7.0 and 8.0. A new volatile
195 compound, 1-hexanol, contributing an oily odor (Niu et al., 2016), was detected in the chicken
196 boiled at pHs of 6.0, 7.0, and 8.0. Even if alcohols were not found in the chicken after boiling at
197 a pH of 7.0, as shown in Fig. 4, four alcohols, namely 1-pentanol, 1-octen-3-ol, 1-hexanol, and 2-

198 butyl-1-octanol, were detected after chilling for 7 d, as shown in Fig. 5. Moreover, various
199 alcohols were present in the chicken boiled at a pH of 8.0 after chilling for 7 d: 1-octen-3-ol, 1-
200 hexanol, 2-hexyl-1-octanol, cyclopropyl carbinol, ethanol, and cyclobutanol. Most of them, such
201 as 1-pentanol, 1-hexanol, and 2-hexyl-1-octanol, gave off fatty odors. Ethanol, which was
202 detected only in the chicken boiled at a pH of 8.0 and chilled for 7 d with a relative content of
203 $0.30 \pm 0.03\%$, indicated the spoilage of the boiled chicken (Miks-Krajnik et al., 2016; Klein et
204 al., 2018). This implied that the chicken boiled at pHs of 6.0, 7.0, and 9.0 had a longer shelf life
205 for chilling storage.

206 **[Figure 5]**

207 3.4. *Ketones*

208 2-butanone compounds, having a pungent odor (Zhao et al., 2021), were detected in the
209 chicken boiled at pHs of 6.0, 7.0, and 8.0, respectively, as shown in Fig. 6. D-limonene, one of
210 the most common terpenes in nature found in beef (Canedo et al., 2009) and chicken
211 (Ramaswamy and Richards, 1982), which was probably from the feed (Bampidis et al., 2005),
212 was found in the chicken boiled at pHs of 6.0 and 8.0. No ketone was found in the chicken after
213 boiling at a pH of 9.0, but both 2-butanone and D-limonene were detected after chilling for 7 d.
214 In contrast, D-limonene found in the chicken after boiling at a pH of 6.0 disappeared after 7 d of
215 chilling due to its low zeta potential at low temperatures in the chilling condition (Li and Lu,
216 2016). Moreover, it was found that the 2-butanone volatile was reduced in the chicken boiled at
217 pHs of 6.0, 7.0, and 8.0 after chilling for 7 d. It was implied that the pungent odor from 2-
218 butanone reduced during chilling, similar to the citrus odor from D-limonene, which also
219 reduced after chilling.

220 **[Figure 6]**

221 **4. Conclusion**

222 The volatile development and stability of boiled chicken were investigated using a
223 headspace GC-MS after boiling and during the chilling storage period. The findings revealed that
224 boiling chicken flesh at various boiling medium pH levels affected the generation of volatiles
225 and alterations that occurred after storage. Amines, aldehydes, alcohols, ketones, acids, esters,
226 and furans all responded significantly to the pH of the boiling medium and chilling storage (7
227 days). The maximum concentration of amine ((2-Aziridinylethyl) amine, boiled at pHs of 6.0–
228 8.0) was found after boiling. In contrast to the alterations observed after 7 days of chilling,
229 amines showed the greatest decline, while aldehydes showed the greatest increase. In specifics,
230 after the chicken was chilled for 7 days, it was found to include n-caproic acid vinyl ester (ester),
231 2-pentyl furan, butanal, acetaldehyde, benzaldehyde, cyclopropyl carbinol, ethanol, and
232 cyclobutanol, depending on the pH of the boiling medium. This study showed that heat in the
233 boiling medium with varying pH levels changed the production of distinct volatiles in the boiled
234 chicken meat. The primary causes of the volatiles' altered composition during chilling were
235 Maillard and oxidation reactions. Various types of volatiles in chicken were generated after
236 boiling it in a medium with a low pH (a pH of 6.0), but the off-flavors (aldehydes) created after
237 chilling were found mostly in the chicken meat boiled at a high pH (pHs of 8.0 and 9.0). The pH
238 of the boiling medium should be taken into account in order to improve the changes in volatiles
239 or flavor in cooked chicken during chilling storage.

240

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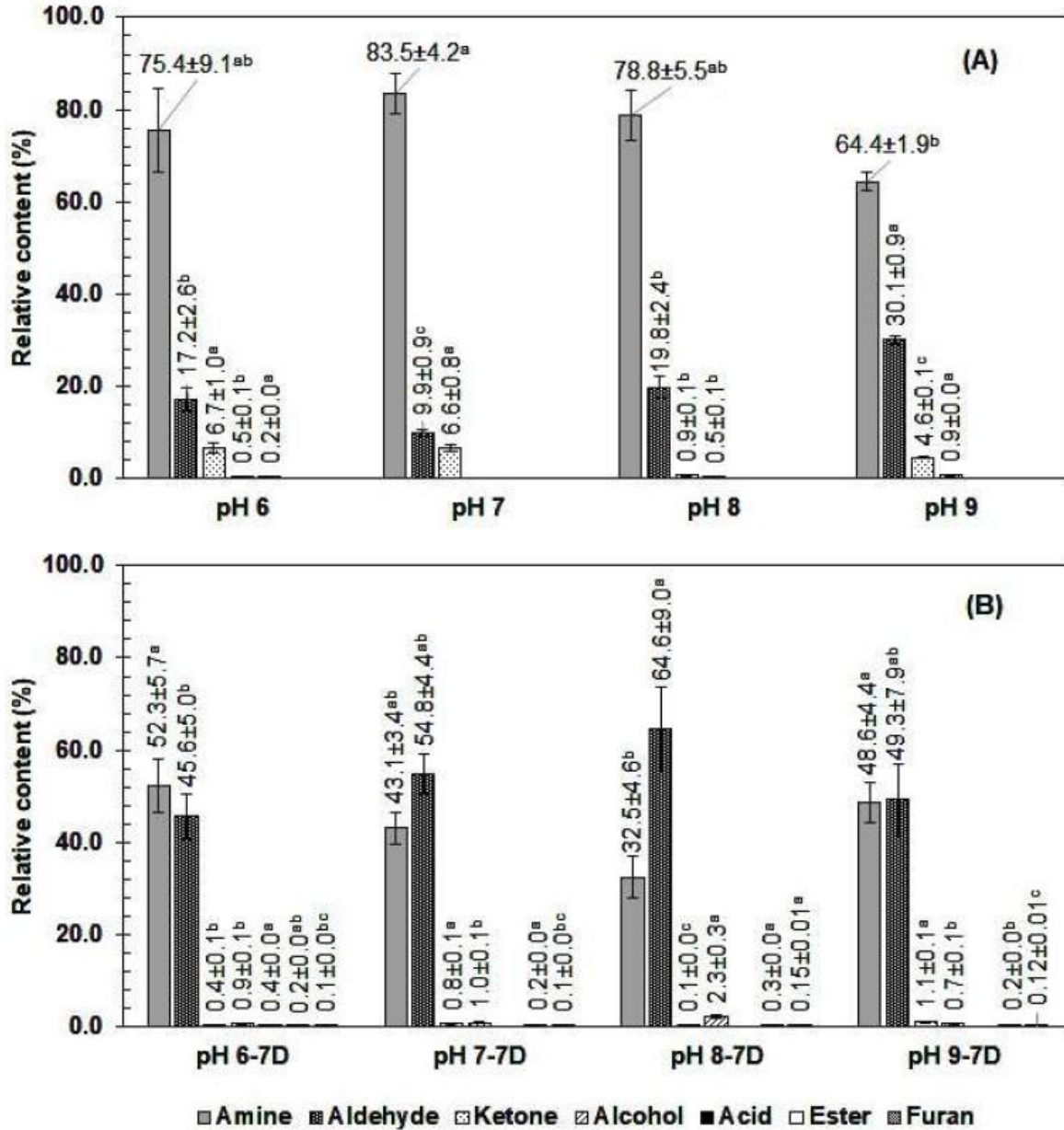
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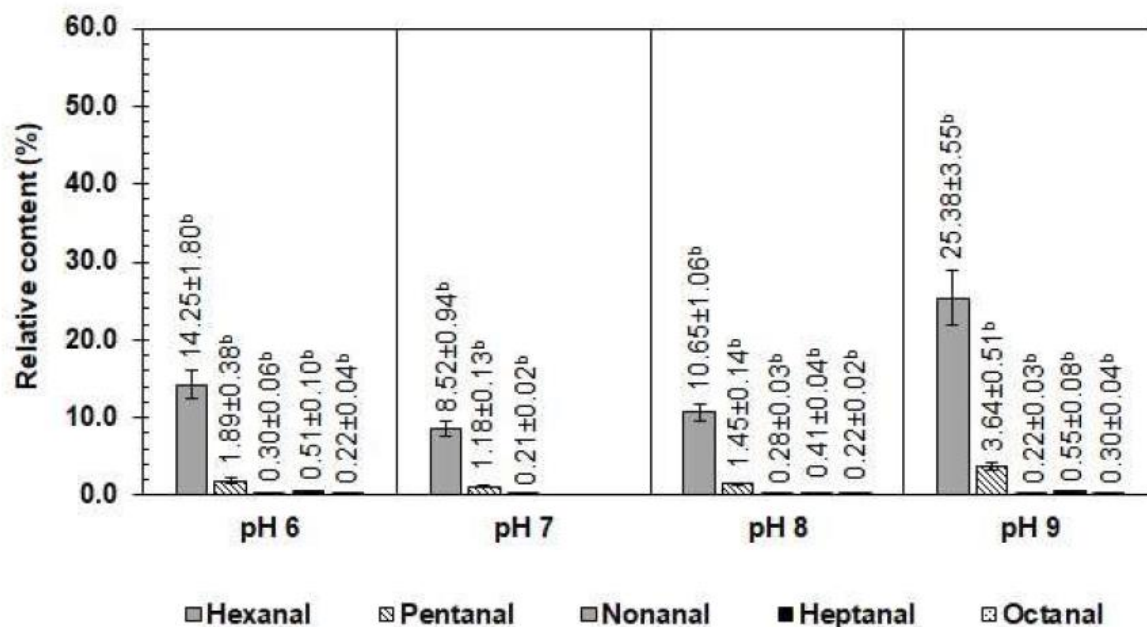
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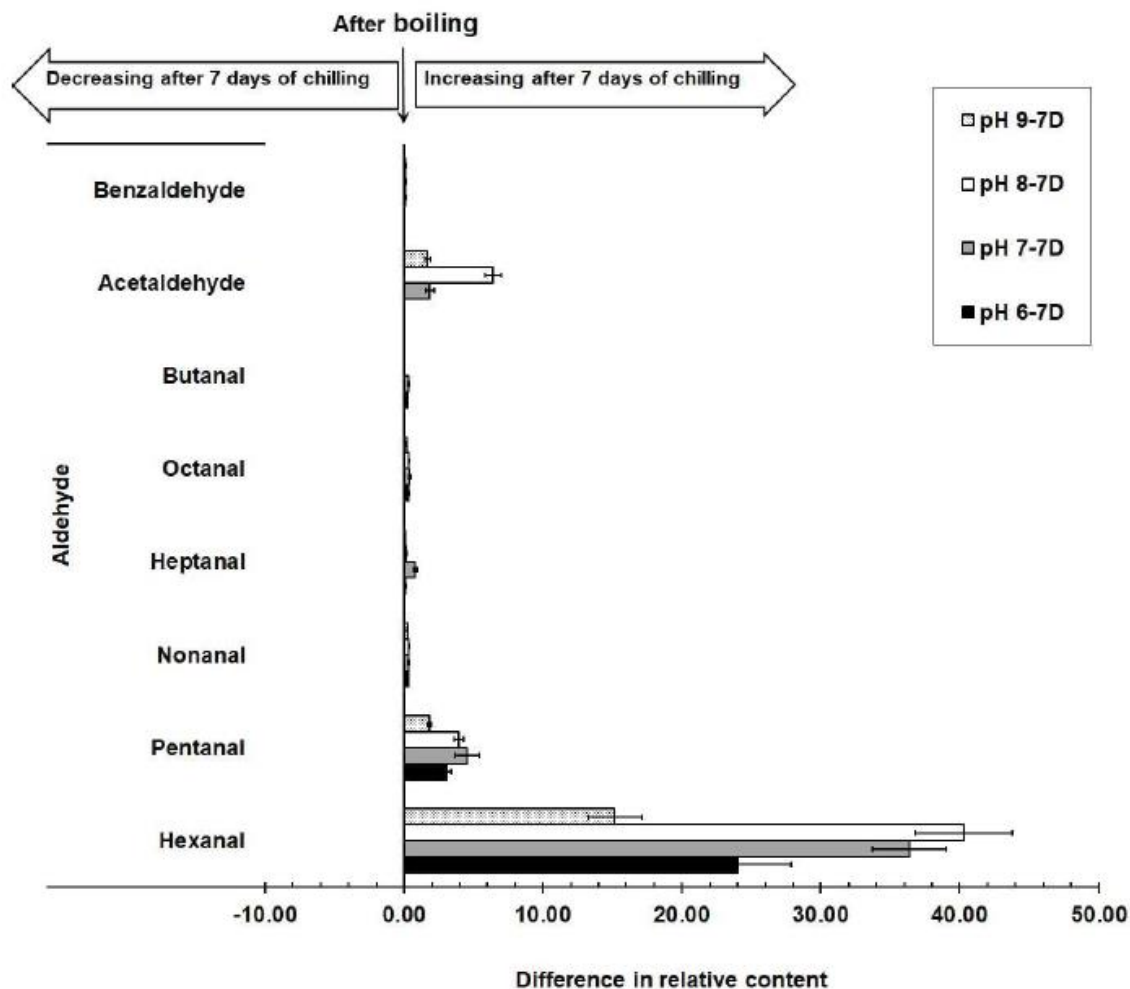
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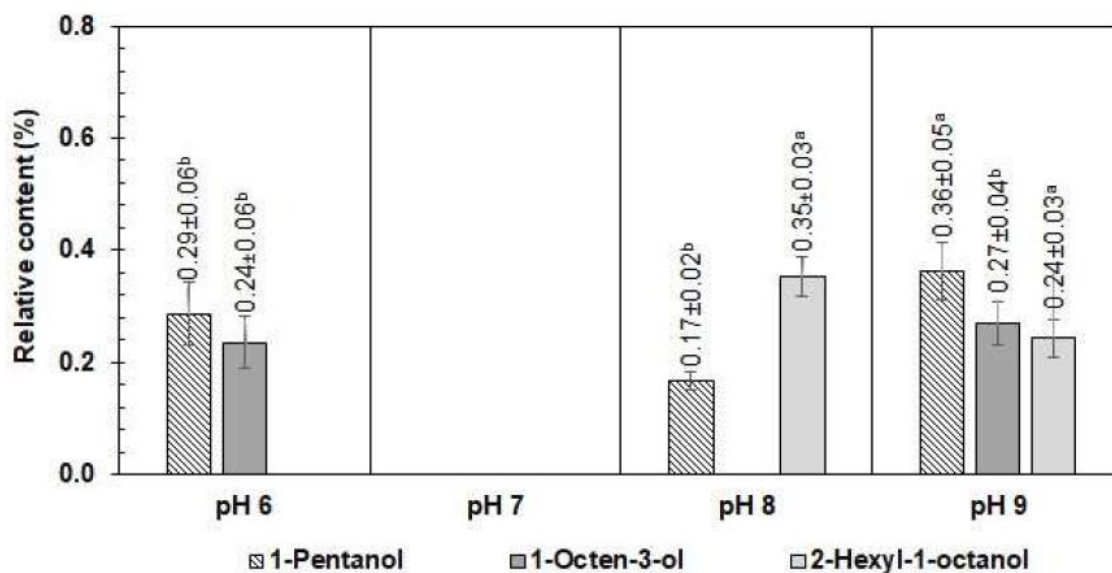
394
 395 **Figure 1** Relative contents (%) of volatile compounds obtained from the GC-MS of chicken (A)
 396 boiled at different pH values and (B) chilled for 7 d. pH 6, pH 7, pH 8, and pH 9 are the chicken
 397 boiled at pH values of 6.0, 7.0, 8.0, and 9.0, respectively. pH 6-7D, pH 7-7D, pH 8-7D, and pH
 398 9-7D are the chicken boiled at pH values of 6.0, 7.0, 8.0 and 9.0, and chilled at $4.0 \pm 1.0^\circ\text{C}$,
 399 respectively. Different superscript letters showed a significant difference ($P < 0.05$) in the same
 400 volatile compounds boiled at different pH values and storage time (0 and 7 d).
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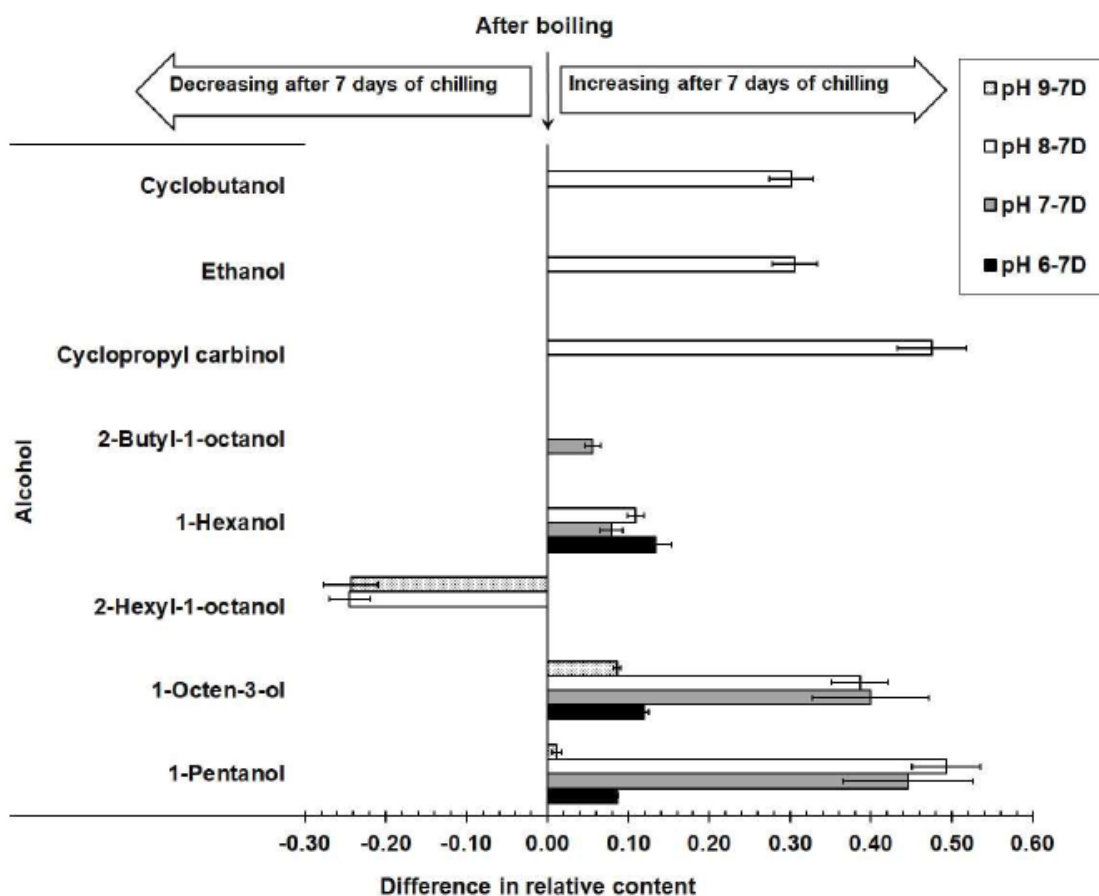
402
 403 **Figure 2** Relative contents (%) of aldehyde volatile compounds obtained from the GC-MS of
 404 chicken boiled at different pH values. pH 6, pH 7, pH 8, and pH 9 are the chicken boiled at pH
 405 values of 6.0, 7.0, 8.0, and 9.0, respectively. Different superscript letters showed a significant
 406 difference ($P < 0.05$) in the same volatile compounds boiled at different pH values.
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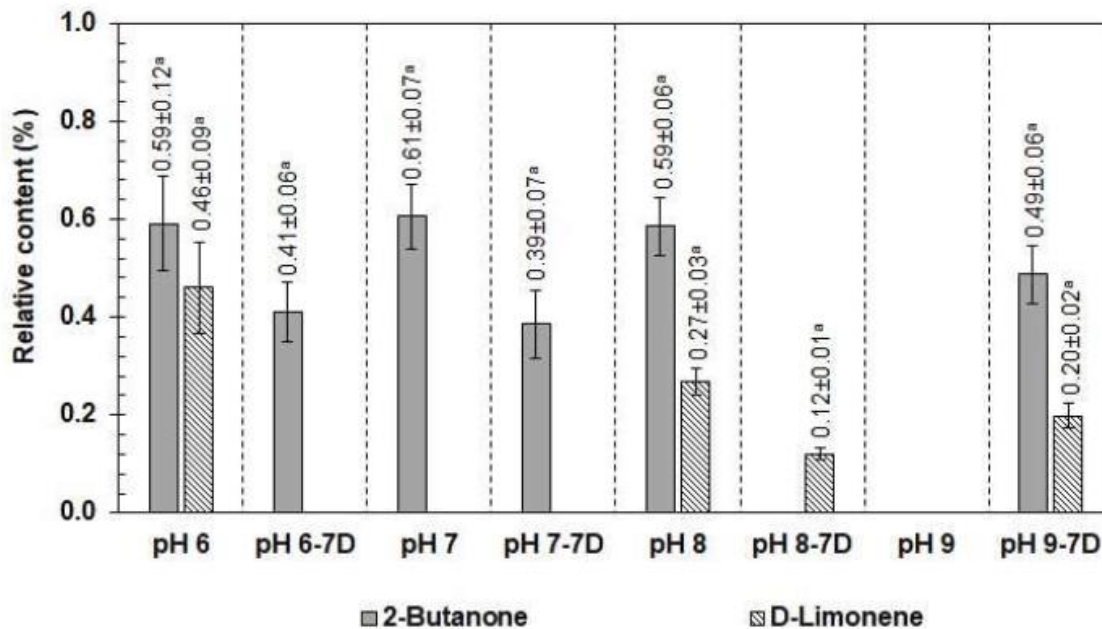
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 409 **Figure 3** Changes in relative contents (%) of aldehyde volatile compounds obtained from the GC-
 410 MS of chicken boiled at different pH values and chilled for 7 d. pH 6-7D, pH 7-7D, pH 8-7D, and pH
 411 9-7D are the chicken boiled at pH of 6.0, 7.0, 8.0, and 9.0, and chilled at $4.0 \pm 1.0^\circ\text{C}$, respectively.
 412



413
 414 **Figure 4** Relative contents (%) of alcohol volatile compounds obtained from the GC–MS of
 415 chicken boiled at different pH values. pH 6, pH 7, pH 8, and pH 9 are the chicken boiled at pH
 416 values of 6.0, 7.0, 8.0, and 9.0, respectively. Different superscript letters in the same substance
 417 showed a significant difference ($P < 0.05$).
 418



419
 420 **Figure 5** Changes in relative contents (%) of alcohol volatile compounds obtained from the GC-MS
 421 of chicken boiled at different pH values of water and chilled for 7 d. pH 6-7D, pH 7-7D, pH 8-7D,
 422 and pH 9-7D are the chicken boiled at pH values of 6.0, 7.0, 8.0, and 9.0, and chilled at $4.0 \pm 1.0^\circ\text{C}$,
 423 respectively.
 424



425
 426 **Figure 6** Relative contents (%) of ketone volatile compounds obtained from the GC–MS of
 427 chicken boiled at different pH values and chilled for 7 d. pH 6, pH 7, pH 8, and pH 9 are the
 428 chicken boiled at pH values of 6.0, 7.0, 8.0, and 9.0, respectively. pH 6-7D, pH 7-7D, pH 8-7D,
 429 and pH 9-7D are the chicken boiled at pH values of 6.0, 7.0, 8.0, and 9.0, and chilled at $4.0 \pm$
 430 1.0°C , respectively. Different superscript letters showed a significant difference ($P < 0.05$) in the
 431 same volatile compounds boiled at different pH values and storage time (0 and 7 d).