Effect of *Citrus aurantium* juice as a disinfecting agent on quality and bacterial communities of striped catfish steaks stored at -20 °C

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Sodium hypochlorite is generally used as a disinfectant in washing of freshwater fishes where the safety aspect of health is of concern. Although plant-based essential oils and synthetic chemical agents have been applied, they might contain toxic substances, are expensive and can cause undesirable quality. This research aims to fill the knowledge gap necessary to validate *C. aurantium* juice as a disinfecting agent for preserving striped

catfish steaks at -20 °C for 28 days. Fifty (50) ppm sodium hypochlorite was used as a commercial disinfectant (control). The results showed that a negative color characteristic (higher a* and increased b*) was found in the control but not in striped catfish steaks immersed in *C. aurantium* juice (TM) on days 14 and 28. No significant differences were found in the peroxide value among the treatments on days 14 and 28 (P>0.05). A lower accumulation of trichloroacetic acid soluble peptides was detected in TM but not in control, while total volatile basic nitrogen of all treatments was up to standard of fish quality during storage. Contrastingly, the total viable count of both treatments increased to > 7.0 log CFU/g on day 28 which did not meet the edible limit of standard for freshwater fishes. The spoilage microbial community was observed on days 0 and 28 of storage which showed a decrease in relative abundance of *Acinetobacter*, *Pseudomonas*, *Brochothrix*, *Lactococcus*, *Carnobacterium*, *Psychrobacter*, and *Vagococcus* as found in TM on day 28. Thus, these results implied that *C. aurantium* juice could replace sodium hypochlorite as an alternative disinfecting agent to control the microbiological spoilage and physico-chemical quality of striped catfish steaks.

1 **Effect of *Citrus aurantium* juice as a disinfecting agent on quality and**

2 **bacterial communities of striped catfish steaks stored at -20 ºC**

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22   **Abstract**

23   Sodium hypochlorite is generally used as a disinfectant in washing of freshwater fishes

24   where the safety aspect of health is of concern. Although plant-based essential oils and synthetic

25   chemical agents have been applied, they might contain toxic substances, are expensive and can

26   cause undesirable quality. This research aims to fill the knowledge gap necessary to validate *C.*

27   *aurantium* juice as a disinfecting agent for preserving striped catfish steaks at -20 $^{\circ}$C for 28 days.

28   Fifty (50) ppm sodium hypochlorite was used as a commercial disinfectant (control). The results

29   showed that a negative color characteristic (higher a* and increased b*) was found in the control

30   but not in striped catfish steaks immersed in *C. aurantium* juice (TM) on days 14 and 28. No

31   significant differences were found in the peroxide value among the treatments on days 14 and 28

32   ($P>0.05$). A lower accumulation of trichloroacetic acid soluble peptides was detected in TM but

33   not in control, while total volatile basic nitrogen of all treatments was up to standard of fish

34   quality during storage. Contrastingly, the total viable count of both treatments increased to > 7.0

35   log CFU/g on day 28 which did not meet the edible limit of standard for freshwater fishes. The

36   spoilage microbial community was observed on days 0 and 28 of storage which showed a

37   decrease in relative abundance of *Acinetobacter, Pseudomonas, Brochothrix, Lactococcus,*

38   *Carnobacterium, Psychrobacter*, and *Vagococcus* as found in TM on day 28. Thus, these results

39   implied that *C. aurantium* juice could replace sodium hypochlorite as an alternative disinfecting

40   agent to control the microbiological spoilage and physico-chemical quality of striped catfish

41   steaks.

42   **Keywords:** *Citrus aurantium* juice; Frozen; Quality; Striped catfish steak; Microbiota

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46   **Introduction**

47   *Pangasianodon hypophthalmus* or Striped catfish (named Pla Sawai in Thai), is a large

48   freshwater catfish that can be found in Thailand, Vietnam, Malaysia, China, and Indonesia.

49   Striped catfish usually prefers a tropical climate where the pH values of freshwater are

50   approximately 6.5-7.5 and the temperatures about 22-26 ^{o}C (Roberts and Vidthayanon, 1991;

51   Singh et al., 2011). This fish is an economically significant aquaculture species because of its

52   high yield percentage and value as a product in food industry (Singh et al., 2011). During

53   processing, striped catfish can be converted from fresh whole fish to produce various

54   convenience foods such as fish fillets, dresses, sticks, balls, and steaks (Food and Agriculture

55   Organization of the United Nations, 2006; Islami et al., 2014; Rathod et al., 2018).

56   In general, freshwater fish are highly susceptible to spoilage from microbial growth and

57   biochemical products due to their intrinsic (water content, pH, nutrient properties) and extrinsic

58   factors (temperature and package conditions) which provide suitable conditions for the growth of

59   spoilage bacteria (Taormina, 2021). Thi et al. (2013) evaluated the microbiota of catfish

60   (*Pangasius hypophthalmus*) and revealed that spoilage bacteria including

61   *Aeromonas, Acinetobacter, Lactococcus and Enterococcus* were prevalent at various processing

62   steps on the processing lines. As previously reported, a range of chilling temperatures has been

63   used to preserve parts of fish products, even though these conditions might negatively affect the

64   sensory characteristics, microbiological and biochemical parameters, resulting in a short shelf

65   life of products (Binsi et al., 2014; Deepitha et al., 2021; Rao et al., 2013). To extend the shelf

66   life, Thong Thi et al. (2016) suggested that vacuum packaging and freezing could be used to

67   control the microbiota of stored catfish.

68   A retardation of deterioration of fresh fish has been considered, especially chlorine-based

69   sanitizers, which are implemented as disinfecting agent in frozen fish and fishery products

70   (World Health Organization, 2009). The use of food additives produced from synthetic chemical

71   agents, especially sodium hypochlorite which is commonly used for preserving fish products,

72   must be limited based on the safety concerns of foods and health. A previous report on residual

73   chlorine toxicity in aquatic systems and environment considered the need for close scrutiny of

74   disinfection procedures in aquatic life (Brungs, 1973). In the same way, another study revealed

75   that the use of chlorinated washing water had no effect on the spoilage microbiota in *Pangasius*

76   *hypophthalmus* fillets (Thi et al., 2013). To overcome the hazard of chemical preservatives,

77   increased research has emphasized the use of plant-based and/or natural preservative agents (Li

78   et al., 2020b; Zhuang et al., 2019). Furthermore, lemon juice, tamarind pulp, lemon grass, and

79   banana leaves have been used as washing agents for minimizing muddy taint associated with fish

80   tissue (Bakar and Hamzah, 1997; Mohsin et al., 1999).

81   *Citrus aurantium*, known as sour orange or bitter orange, grows in semitropical and

82   temperate areas. It can be found in parts of Thailand and has high commercial value. In addition,

83   it has been revealed that *Citrus* flavonoids have wide spectrum of antimicrobial, antidiabetic,

84   anticancer and antioxidant activities. Parts of *C. aurantium,* including flower, leaf, ripe and

85   unripe peel essential oils have been recently investigated based on the chemical and

86   antimicrobial activities in extending shelf-life, which contributes significantly to the quality of

87   food products (Azhdarzadeh and Hojjati 2016; Değirmenci and Erkurt, 2020; Pratama et al.,

88   2019; Wen et al., 2021). Previous studies have indicated that essential oils extracted from plants

89   including oregano, sweet bay, thyme, garlic, clove, cumin, spearmint, and *Allium* species have

90   been used as preservative agents for fishes stored under chilling temperature (Attouchi and

91   Sadok, 2012; Cai et al., 2015; Erkan, 2012; Vatavali et al., 2013; Xu et al., 2015). Viji et al.

92   (2015) showed that ethanolic extract of *C. aurantium* peel enhanced the storage stability and

93   extended the shelf life of mackerel (*Rastrelliger kanagurta*) by 2 days during storage at 0-2ºC.

94   Yerlikaya et al. (2015) presented citrus peel extracts incorporated ice cubes use in controlling the

95   biochemical indices in common pandora (*Pagellus erythrinus*). Besides, *Oncorhynchus mykiss*

96   fillets coated with chitosan enriched with fenugreek essential oil and immersed in *C. aurantium*

97   juice concentrate were investigated by Tooryan and Azizkhani (2020). However, plant essential

98   oils obtained by chemical solvent extraction have high cost, may have toxic substances, and can

99   cause unstable product quality. Consumers are avoiding food raw materials treated with synthetic

100   chemical preservatives and thus the natural choice implemented at the household level and

101   industry is required (Chemat et al., 2012; Płotka-Wasylka et al., 2017). In an attempt to prevent

102   the risk of short shelf-life and to reduce the cost of production, *C. aurantium* juice could be

103   further applied as a new alternative for natural sanitizers owing to its antimicrobial activity

104   (Karabıyıklı et al., 2014).

105   To the best of our knowledge, there are no previous studies on the effect of *C. aurantium*

106   juice as disinfecting agent on the quality of striped catfish during frozen storage. Besides, the

107   potential effect of *C. aurantium* juice on the bacterial community in striped catfish has not been

108   revealed yet in academic databases. Therefore, the aim of this study was to validate the *C.*

109   *aurantium* juice as a preservation agent for striped catfish steaks at -20 °C. The changes in

110   microbiological and physico-chemical quality in samples during a 28-day frozen storage were

111   characterized. Additionally, the bacterial community was assessed by Illumina-MiSeq high

112   throughput sequencing to evaluate the preservative effects of *C. aurantium* juice.

113

114   **Materials and Methods**

115   **2.1 Preparation of *C. aurantium* juice and fish samples**

116   Fresh *C. aurantium* was obtained from a local agricultural farm in Khamthaleso Sub-district,

117   Nakhon Ratchasima province, Thailand. It was rinsed with tap water and sliced. The juice extract

118   was prepared by mechanical machine (Severin, Germany). Subsequently, the juice was filtered

119   by sterile gauze for further experiment.

120   Striped catfish steak was purchased from Sura-Nakhon processing line, Nakhon Ratchasima

121   province, Thailand. The mean length, weight, and thickness of steaks were 10-13 cm, 80-105 g,

122   and 0.5-1.0 cm, respectively. The steaks were washed twice with reverse osmosis water and

123   dried on a cleaned tray for 10 min. The striped catfish steaks were divided into two groups: the

124   first group (control) was immersed in a commercial sanitizer, 50 ppm sodium hypochlorite for 10

125   min, and the second group (TM) was immersed in a natural sanitizer, *C. aurantium* juice for 10

126   min. Each steak was packed in a vacuum sterile bag and stored at -20 °C. The samples of each

127   group were randomly selected for physico-chemical quality analysis during storage on (0, 7, 14,

128   21, and 28 days). Bacterial communities were analyzed on days 0 and 28.

129   **2.2 Physico-chemical analysis**

2.2.1 Color measurement

The colors of each treatment were analyzed using a Chroma meter CR-410 (Konica

minolta, Japan). The color parameters, including L* (brightness), a* (redness/greenness), and b*

(yellowness/blueness), were observed and their mean values were then used to represent the

color values.

2.2.2 Texture analysis

The texture profiles were analyzed by using a texture analyzer (CT3 10K, BROOKFIELD,

USA), equipped with a specific cylindrical probe (TA41). Each sample was then compressed

under the following conditions: a pre-test speed of 2.0 mm/sec, a test speed of 1.0 mm/sec, a

post-test speed of 2.0 mm/sec, a compression of 25%, and a trigger force of 5 g.

2.2.3 Determination of peroxide value (PV)

The PV was measured following the method described by AOAC (2008). The sample (0.5 g)

was mixed with 10 mL of glacial acetic acid-chloroform mixture (3:2, v/v). Saturated potassium

iodide solution (0.5 mL) was then added and the mixture allowed to stand for 15 min in

darkness. After that, 10 mL of distilled water was added, and the free iodine was titrated with

0.01 mol/L sodium thiosulfate solution with the addition of 1 % (w/v) starch solution as an

indicator. Results were also expressed as meq/g sample.

2.2.4 Analysis of pH values and total volatile basic nitrogen (TVB-N)

One gram of each treatment was homogenized with 10 mL distilled water for 1 min. The

mixture was then centrifuged at 4000×g for 10 min, and the pH value of the supernatant was

measured by pH meter (Fisher scientific model AB15).

151   The TVB-N was measured following the method described by Malle and Poumeyrol (1989)

152   with slight modifications. Briefly, 200 mL of 7.5 % (w/v) trichloroacetic acid solution was added

153   to 100 g of fish sample. After homogenization, the mixture was centrifuged at 400×g for 5 min

154   and then filtered by Whatman No. 1 filter paper. Distillation was then performed using Kjeldahl

155   apparatus (Gerhardt, Vapodest 30s). Ten milliliters of filtrate were loaded into distillation tube

156   followed by 6 mL of 10% (w/v) sodium hydroxide. A beaker containing 10 mL of 4% (w/v)

157   boric acid and 0.04 mL of methyl red and bromocresol green indicator was used under the

158   condenser for titration of ammonia. Distillation was started and steam distillation further

159   continued until a final volume of 50 mL was obtained in its beaker (40 mL of distillate). The

160   boric acid solution turned green when alkalinized by the distilled TVB-N which was titrated with

161   aqueous 0.01 mol/L hydrochloric acid solution. Complete neutralization was obtained when the

162   color turned pink on the addition of a further drop of hydrochloric acid. Results were expressed

163   as mg/ 100 g sample.

164   **2.2.5 Measurement of trichloroacetic acid (TCA)-soluble peptides**

165   The measurement of TCA-soluble peptides was performed by the method of Jia et al.

166   (2019) with slight modifications. Two grams of each sample were homogenized with 18 mL of

167   cold 5% (w/v) TCA and stored in an ice bath for 30 min. The mixture was centrifuged at

168   10,000×g for 10 min at 4°C. The TCA-soluble peptides were analyzed by Lowry method (Lowry

169   et al., 1951). Samples were mixed with C solution (1 mL; mixture of 50 mL of A solution, 0.5

170   mL of B_1 solution and 0.5 mL of B_2 solution). D solution (0.1 mL) was then added after 10 min.

171   The absorbance was recorded at 750 nm after 30 min against a blank sample. The TCA-soluble

172   peptides were also expressed as μg tyrosine/g sample.

173 **2.3 Microbiological analysis**

174 **2.3.1 Microbial enumeration**

175 The samples of each group were selected for microbial enumeration during storage on (0, 7,

176 14, 21, and 28 days). Briefly, 25 g of each sample was homogenized with 225 mL sterile saline

177 to produce the first dilution, and 10-fold dilutions were then made. Spread plate method was

178 done. Then, total viable count (TVC) was determined on plate count agar incubated at 37 °C for

179 24 h.

180 **2.3.2 Bacterial community by Illumina-MiSeq high throughput sequencing**

181 To analyze bacterial community, the control and TM kept for 0 and 28 days, respectively,

182 were chosen. Metagenomic DNA of each treatment was isolated using DNeasy Blood & Tissue

183 Kits (Qiagen, Hilden, Germany) according to the manufacturer's protocols. Briefly, 25 mg of

184 pooled sample taken from six steaks of each treatment were randomly extracted and the quality

185 of the extracted DNA was determined by DeNovix QFX Fluorometer. The prokaryotic 16S

186 rRNA gene at V3-V4 region was performed using the Qiagen QIAseq 16S/ITS Region panel.

187 The PCR program was as follows: 95 °C for 2 min, 12 cycles at 95 °C for 30 s, 50 °C for 30 s,

188 72 °C for 2 min and 72 °C for 7 min. The DNA amplicon was then purified with QIAseq beads

189 at 1.1X volume to clean up contaminated PCR products. The 16S rRNA amplicons were labeled

190 with different sequencing adaptors using QIAseq 16S/ITS Region Panel Sample Index PCR

191 Reaction, with the PCR program as follows: 95 °C for 2 min, 19 cycles at 95 °C for 30 s, 60 °C

192 for 30 s, 72 °C for 2 min and 72 °C for 7 min. The DNA libraries were purified with one round

193 of QIAseq beads at 0.9X volume and eluted in 25μl of nuclease-free water. The quality and

194 quantity of approximately 630-bp of DNA libraries were evaluated using QIAxcel Advanced and

195   DeNovix QFX Fluorometer, respectively. The 16S rRNA libraries were sequenced using an

196   Illumina Miseq 600 platform (Illumina, San Diego, CA, USA).

197   Bioinformatics analysis was done while the raw sequences were categorized into groups

198   based on the 5′ barcode sequences. The sequences were processed following DADA2 v1.16.0

199   pipeline (https://benjjneb.github.io/dada2/). The DADA2 pipeline describes microbial diversity

200   and community structures using unique amplicon sequence variants (ASVs). Microbial taxa were

201   classified from Silva version 138 as a reference database.

202

203   **2.4 Statistical analysis**

204   The experiment was conducted in triplicate. Differences in means of physico-chemical

205   parameters and microbial enumeration were subjected to Analysis of Variance (ANOVA)

206   followed by Duncan's Multiple Range Test (DMRT), using IBM SPSS statistics 23. A

207   probability level of $P<0.05$ was considered statistically significant. In terms of bacterial

208   community, Principle Co-ordinate Analysis (PCoA) plots including unweighted UniFrac,

209   weighted UniFrac, GUniFrac with alpha 0.5, and Bray-Curtis distance were used to calculate

210   distances between samples based on the similarity of their members.

211

212   **3. Results and discussions**

213   **3.1 Color characteristics**

214   The parameters of color, such as L* (lightness), a* (redness-greenness), and b* (yellowness-

215   blueness) of striped catfish steak during storage at -20 ℃ were evaluated and the results shown

216   in Fig. 1. The values of a* and b* of the control treatment were significantly higher ($P<0.05$)

217   than those of TM on days 14 and 28. In the case of day 21, a decrease in a* and b* was shown in

218   both treatments when compared to day 14 (P<0.05). The oscillatory pattern was found in all

219   color parameters. The differences observed in color parameters in this study were related to the

220   storage times in that they became redder (higher a*) and more yellow (increased b*). This

221   phenomenon might have been due to the lipid oxidation, which represents a commercial

222   disadvantage during storage at cold conditions, in agreement with previous study in fish (Cakli et

223   al., 2006). Similar characteristics were found in this work and that of Álvarez et al. (2008) who

224   evaluated the L* and a* on the ventral side of *Sparus aurata* during ice storage and reported that

225   those color parameters displayed a very significant negative correlation with storage time

226   resulting in the discoloration of gilthead seabream skin. Likewise, Sáez et al. (2015) studied the

227   effects of vacuum and modified atmosphere on color changes during cold storage of

228   *Argyrosomus regius* fillets and found the values of a* were consistently negative, indicating lipid

229   oxidation. Regarding the disadvantage of chlorine treated fish characteristic, Chuesiang et al.

230   (2020) found that an increase in the b* value of sodium hypochlorite-treated Asian seabass

231   (*Lates calcarifer*) fillets was observed during storage because of the interference from the

232   sodium hypochlorite solution. Naha et al. (2019) clarified that the sodium hypochlorite-bleaching

233   ability may interfere with the visible light absorption of the fish samples due to breaking of the

234   chemical bonds of the colored compounds contained in the samples. Consequently, this negative

235   characteristic may affect the consumer buying decision. The discussion of lipid oxidation will be

236   presented based on the peroxide values in a later section.

237

238   **3.2 Texture profiles**

239   Texture parameters such as hardness, adhesiveness, springiness, and cohesiveness of the

240   striped catfish steak were monitored and results revealed in Fig. 2. The control treatment was

241   observed to have a higher adhesiveness and springiness on day 28 (P<0.05) when compared to

242   day 0. However, the hardness of TM was lower than that of the control treatment on day 28

243   (P<0.05). This might be attributed to the alteration of protein structure. The change of protein

244   oxidation and denaturation (disulfide (SS), carbonyl contents, salt-soluble protein (SSP) and

245   Ca^{2+}-ATPase activity) might have occurred during storage at -20 °C (Lu et al., 2021). However,

246   on days 0-21, all samples had no significant difference (P>0.05) in all textural parameters

247   implying that *C. aurantium* juice immersion did not significantly change the textural parameters

248   (P>0.05) during storage for 21 days as compared to sodium hypochlorite immersion. Despite the

249   sodium hypochlorite not affecting the texture characteristics of striped catfish steak during

250   storage, the safety aspect of antimicrobial agents has been of concern in the European Union due

251   to lingering toxic residues at the food consumption stage. In fact, the use of sodium hypochlorite

252   in the presence of organic matter promotes trihalomethane formation by an oxidation reaction,

253   and it has been extensively discussed as a great disadvantage, especially due to carcinogenic

254   properties (European Food Safety Authority, 2006; World Health Organization, 2009). In the

255   same manner, Hernández-Pimentel et al. (2020) who aimed to reduce the use of sodium

256   hypochlorite revealed that neutral electrolyzed water could be applied as an alternative

257   antimicrobial agent during chicken meat processing because of safe handling, high availability,

258   low toxicity, low corrosion capacity, and no by-product generation.

259

260   **3.3 pH, peroxide values, TVB-N, and TCA-soluble peptides**

261   The change in pH value of each treatment is illustrated in Fig. 3A. On day 0, the pH of the

262   control and TM was 6.68±0.01 and 5.71±0.25, respectively. This result indicated that the pH

263   value of TM was lower than that of the control (P<0.05) due to the low pH condition of *C.*

264   *aurantium* juice. No significant differences (P>0.05) were then indicated in the pH values

265   between the control treatment and TM during storage on days 14 and 21. After that, a decrease in

266   pH was found in both treatments at the end of storage (day 28) due to the ATP decomposition,

267   lactic acid, glycolysis, and pyrophosphate accumulation in fish muscle during storage (Li et al.,

268   2020b). Similarly, the report of Li et al. (2022) showed that the pH value of *Micropterus*

269   *salmoides* decreased slightly up to the 14 days (pH < 7.00) during frozen storage at -30 $^{\circ}$C which

270   might have been caused by microbial fermentation of carbohydrates resulting to organic acids

271   production in fish muscle (Khan et al., 2005). However, the variations in the pH value between

272   these investigations are probably owing to the differences in the geographical location, catching

273   season, water composition, and fish size (Malik et al., 2021).

274   Peroxide value (PV) is a major chemical method which indicates oxidative rancidity in fish

275   as shown in Fig. 3B. The PV of the control and TM increased after 7 days and then decreased up

276   to 21 days (P<0.05). In addition, on days 14 and 28, no significant difference (P>0.05) was found

277   among the treatments. Our findings are in general agreement with the article of Li et al. (2020a)

278   who found that the PV of Blunt snout bream (*Megalobrama amblycephala*) increased before the

279   8th day and subsequently decreased, without significant differences. This might have been due to

280   the degradation of ketones, alcohols, aldehyde, and peroxides producing off-flavors in fish

281   product.

282   TVB-N is related to a change in microbiological and biochemical activities (Kyrana et al.,

283   1997). In fact, 20 mg N/100 g has been used as a point of rejection limit for fish products

284   (Sikorski et al., 2020). Considering our results (Fig. 3C), surprisingly, the TVB-N of all

285   treatments were relevant to standard quality of fishes. The TVB-N of both treatments increased

286   slightly on days 21 and 28, while there were significant differences (P<0.05) at the end of storage

287   (day 28) when compared to the initial date (0 day). An increase in TVB-N corresponded to a

288   change in TCA-soluble peptides (Fig. 3D). A sharp increase in TCA-soluble peptides was found

289   after 14 days, which indicated protein degradation. This activity was catalyzed by endogenous

290   cathepsin during storage and by microbial proteolytic enzymes (Xu et al., 2015), which was

291   related to the growth of total bacterial count (Fig. 4). However, it significantly decreased in both

292   treatments on day 28 (P<0.05). In the case of days 7-28, a lower accumulation of TCA-soluble

293   peptides was shown in striped catfish steak immersed in *C. aurantium* juice. This might be

294   interpreted that *C. aurantium* juice had the potential to retard the activity of endogenous

295   cathepsin, and could inhibit protein-degradation microorganisms, which will be further discussed

296   in the section of Illumina-MiSeq high throughput sequencing. In addition, major phenolic

297   compounds of *C. aurantium* juice were found including flavonoids, *p*-Coumaric, ferulic acids,

298   etc., (Marzouk, 2013). Jongberg et al (2011) and Tang et al (2016) indicated that quinones

299   oxidized from phenolic compounds could react with cysteine in proteins, including myofibrillar

300   protein, which weakened the protein degradation. This also paves the strategy for industrial fish

301   processing and preservation.

302

303

304   **3.4 Microbial enumeration**

305   The results of TVC are displayed in Fig. 5. On day 0, the TVC of the control treatment and

306   TM were approximately 6.89±0.04 and 6.06±0.05 log CFU/g, respectively. There were

307   significant differences (P<0.05) in the initial pH values among treatments, which correlated to

308   the changes in pH (Fig. 3A). This indicated that the *C. aurantium* juice immersion could

309   decrease microbiota in striped catfish steaks due to its acidic stress condition. On day 14, the

310   TVC of the control was higher than those of TM (P<0.05) and reached 6.98±0.07 log CFU/g.

311   Finally, the TVC of both treatments increased to > 7.0 log CFU/g on day 28; which did not meet

312   the edible limit of standard for freshwater fish (ICMSF, 1986). This phenomenon could indicate

313   that the mesophilic bacteria were found in the late period of spoilage process on the striped

314   catfish steaks sample under cold stress, which was consistent with the investigation of Gram and

315   Huss (1996) who indicated the mesophilic microorganisms are dominant on tropical fish. This

316   work agreed with the increasing trend in mesophilic bacteria during freezing storage which has

317   also been observed in previous studies. It has been reported by Ehsani and Jasour (2014) that

318   total viable count of silver carp (*Hypophthalmichthys molitrix*) increased after 30 days at -24 ºC

319   which did not reach the critical maximum levels of food standards (< 7.0 log CFU/g). However,

320   their findings lead us to believe that the striped catfish steaks immersed in *C. aurantium* juice

321   should be stored at < -20 ºC to prolong the shelf-life during storage.

322   **3.5 Bacterial community**

323   To observe the scientific insight of the preservative effects of *C. aurantium* juice immersion,

324   the control and TM were chosen at days 0 and 28, and their bacterial community was then

325   identified by Illumina-MiSeq high throughput sequencing. The relative abundance of different

326   phylum is shown in Fig 5. Five hundred and ninety taxa at the phylum level obtained in this

327   study were demonstrated. *Proteobacteria* (>60%) was a predominant microbiota in all samples.

328   On day 0, *Proteobacteria* (64.55%), *Bacteroidota* (24.49%), *Firmicutes* (5.20%),

329   *Actinobacteriota* (2.71%), and *Fusobacteriota* (1.59%) were the top five phyla of the control,

330   while the dominant phyla responsible for top four phyla microbiota in TM were *Proteobacteria*

331   (60.26%), *Bacteroidota* (19.65%), *Firmicutes* (9.50%), and *Fusobacteriota* (5.68%). At the end

332   of storage time (day 28), *Firmicutes* (22.16%), *Bacteroidota* (0.37%), and *Actinobacteriota*

333   (0.08%) were much more abundant in the control than in the TM treatment. In addition, a

334   relative abundance (0.01%) of *Verrucomicrobiota, Myxococcota, Deinococcota,* and *Chloroflexi*

335   was detected in the control treatment, but not in TM. Fig. 6 displays the relative abundance of

336   di□erent genera in each treatment. One hundred taxa at the genera level were specifically

337   identified. On day 0, *Acinetobacter* (39.63%), *Soonwooa* (8.47%), *Chryseobacterium* (6.62%),

338   *Enhydrobacter* (4.16%), *Aeromonas* (4.15%), *Flavobacterium* (4.01%), *Comamonas* (3.43%),

339   *Pseudomonas* (3.36%), *Psychrobacter* (3.24%), and *Methylobacterium-Methylorubrum* (3.15%)

340   were the top ten dominant genera in the control treatment, while the TM treatment had decreased

341   relative abundance of *Acinetobacter, Soonwooa, Chryseobacterium, Pseudomonas,*

342   *Psychrobacter,* and *Methylobacterium-Methylorubrum*. However, *Acinetobacter, Aeromonas,*

343   and *Comamonas* became the top three dominant genera in TM at day 0, which accounted for

344   26.27%, 12.28%, and 6.56%, respectively. On day 28, *Acinetobacter, Aeromonas, Pseudomonas,*

345   *Macrococcus, Shewanella, Brochothrix, Lactococcus, Carnobacterium, Psychrobacter*, and

346   *Vagococcus* were the top ten dominant genera in all samples. The dominant phyla and genera of

347   freshwater fish reported in this study were consistent with those reported by Gonzalez et al.

348   (2000), Sousa and Silva-Souza (2001), Jia et al. (2018), and Silbande et al. (2018). *Aeromonas,*

349   *Pseudomonas, Lactococcus* were the major microbiota found in *Cyprinus carpio* during storage

350   at 4 ºC and -20 ºC (Li et al., 2018). Likewise, Zhang et al. (2015) revealed that the indigenous

351 microbiota of fresh carp fillets had *Acinetobacter* as the major microbiota, representing 52.8%

352 of the total isolates, while *Aeromonas* were the second most common microbiota, accounting for

353 21.7% of the total isolates. The *Brochothrix, Carnobacterium, Pseudomonas*, *Shewanella*, lactic

354 acid bacteria were also identified as the dominant spoilage flora in stored striped catfish fillets

355 (Noseda et al., 2012). Surprisingly, a decrease in relative abundance of *Acinetobacter,*

356 *Pseudomonas, Brochothrix, Lactococcus, Carnobacterium, Psychrobacter*, and *Vagococcus* was

357 found in TM sample on day 28 accounting for 25.48%, 9.78%, 1.98%, 2.55%, 0.73%, 0.65%,

358 and 0.62%, respectively, when compared to the control. This phenomenon was directly related to

359 the decrease in the accumulation of TCA-soluble peptides (Fig. 3D) in TM owing to a decrease

360 in the spoilage microorganisms. Furthermore, *C. aurantium* juice treatment could inhibit the

361 growth of dominant spoilage bacteria, resulting in the different levels of relative abundance in

362 the two treatments. According to a previous study, *C. aurantium* juice exhibited microbial

363 activity because of the potential effect of bioactive compounds such as flavonoid and phenolic

364 compounds. Generally, the antimicrobial properties of phenolic compounds have been revealed.

365 The mechanism of action induces the alteration of cytoplasmic membrane, the inhibition of ion

366 transportation and enzyme activity and then causes bacterial cell damage (Haraoui et a., 2020).

367   Figs. 7A-D present the PCoA plots that originated from unweighted UniFrac, weighted

368 UniFrac, GUniFrac with alpha 0.5, and Bray-Curtis distance, respectively. These demonstrated

369 the microbial community differences among the samples. On day 0, the composition of

370 microbiome was clearly distinct between control and TM, suggesting that microbiomes among

371 treatments were different. This might have been due to the fact that the pH condition and

372 bioactive compounds of *C. aurantium* juice affected the microbial community of striped catfish

373 steaks, resulting in di□erent microbial community in each treatment. On day 28 of TM, although

374   the relative abundance of microbial spoilages including *Acinetobacter, Pseudomonas,*

375   *Brochothrix, Lactococcus, Carnobacterium, Psychrobacter*, and *Vagococcus* decreased

376   compared to those of control (Fig. 5B), the microbiota compositions of both treatments were

377   clustered in the same group (Figs. 7A-D). This reason could imply that the application of *C.*

378   *aurantium* juice as a disinfectant could replace the use of sodium hypochlorite.

379   **Conclusions**

380   The preservative effect of each disinfectant agent including sodium hypochlorite

381   (commercial disinfectant) and *C. aurantium* juice on microbial community, and physico-

382   chemical quality were revealed. Striped catfish steaks immersed in *C. aurantium* juice showed a

383   lower level of TCA-soluble peptides during storage. Moreover, *C. aurantium* juice affected the

384   relative abundance of *Acinetobacter, Pseudomonas, Brochothrix, Lactococcus, Carnobacterium,*

385   *Psychrobacter*, and *Vagococcus* on day 28. Based on the new findings, this study successfully

386   presented *C. aurantium* juice as a feasible alternative disinfectant option for use in the

387   preparation stage of striped catfish steaks prior to storage at -20 ºC.

388   Further studies are recommended to consider the preservative effect of specific compounds

389   in *C. aurantium* juice on the sensorial evaluation. The specific mechanism of *C. aurantium* juice

390   on fungi should be elucidated. Meanwhile, the effect of combining *C. aurantium* juice with

391   other natural citrus family should be tested in striped catfish steaks to improve the color values

392   and sensory characteristics.

393

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400   **Author Contributions**

401   Kajonsak Dabsantai performed the experiments, analyzed the data, authored or reviewed

402   drafts of the article, and approved the final draft.

403   Thitikorn Mahidsanan conceived and designed the experiments, analyzed the data, prepared

404   figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.

405   **Data Availability**

406   The raw data are available in the Supplemental Files. The sequences are available at the

407   National Center of Biotechnology Information (NCBI) Sequence Read Archive (SRA):

408   PRJNA914840.

409   **Declaration of competing interest**

410   The authors declare that they have no conflict of interest.

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633

634   **Figure captions**

635   Fig. 1. Changes in L* (A), a* (B), and b* (C) of the control and *C. aurantium* juice-immersed

636   striped catfish steaks during storage at -20 °C for 28 days. Results are presented as mean ±

637   standard deviation. Means in each parameter followed by different lowercase letters are

638   significantly different (P<0.05) according to DMRT.

639

640   Fig. 2. Changes in hardness (A), adhesiveness (B), springiness (C), and cohesiveness (D) of the

641   control and *C. aurantium* juice-immersed striped catfish steaks during storage at -20 °C for 28

642   days. Results are presented as mean ± standard deviation. Means in each parameter followed by

643   different lowercase letters are significantly different (P<0.05) according to DMRT.

644

645   Fig. 3. Changes in pH values (A), peroxide values (B), TVB-N values (C), and TCA-soluble

646   peptides (D) of the control and *C. aurantium* juice-immersed striped catfish steaks during storage

647   at -20 °C for 28 days. Results are presented as mean ± standard deviation. Means in each

648   parameter followed by different lowercase letters are significantly different (P<0.05) according

649   to DMRT.

650

651   Fig. 4. Changes in total viable count (TVC) of the control and *C. aurantium* juice-immersed

652   striped catfish steaks during storage at -20 °C for 28 days. Results are presented as mean ±

653   standard deviation. Means in each parameter followed by different lowercase letters are

654   significantly different (P<0.05) according to DMRT.

655

656

657   Fig. 5. Relative abundance of bacterial composition of each sample at phylum level. (CT0,

658   control: striped catfish steak immersed in 50 ppm sodium hypochlorite on day 0; CT28, control:

659   striped catfish steak immersed in 50 ppm sodium hypochlorite on day 28; TM0: striped catfish

660   steak immersed in *C. aurantium* juice on day 0; TM28 striped catfish steak immersed in *C.*

661   *aurantium* juice on day 28.)

662

663   Fig. 6. Relative abundance of bacterial composition of each sample at genus level. (CT0, control:

664   striped catfish steak immersed in 50 ppm sodium hypochlorite on day 0; CT28, control: striped

665   catfish steak immersed in 50 ppm sodium hypochlorite on day 28; TM0: striped catfish steak

666   immersed in *C. aurantium* juice on day 0; TM28 striped catfish steak immersed in *C. aurantium*

667   juice on day 28.)

668

669   Fig. 7. Principle Co-ordinate Analysis (PCoA) plots based on unweighted UniFrac (A), weighted

670   UniFrac (B), GUniFrac with alpha 0.5 (C), and Bray-Curtis distance (D), showing clustering of

671   the bacterial communities from each treatment. (CT0: striped catfish steak immersed in 50 ppm

672   sodium hypochlorite on day 0; CT28: striped catfish steak immersed in 50 ppm sodium

673   hypochlorite on day 28; TM0: striped catfish steak immersed in *C. aurantium* juice on day 0;

674   TM28 striped catfish steak immersed in *C. aurantium* juice on day 28.)

Figure 1

Figure 1

Fig. 1. Changes in L* (A), a* (B), and b* (C) of the control and *C. aurantium* juice-immersed striped catfish steaks during storage at -20 °C for 28 days. Results are presented as mean ± standard deviation. Means in each parameter followed by different lowercase letters are significantly different (P<0.05) according to DMRT.
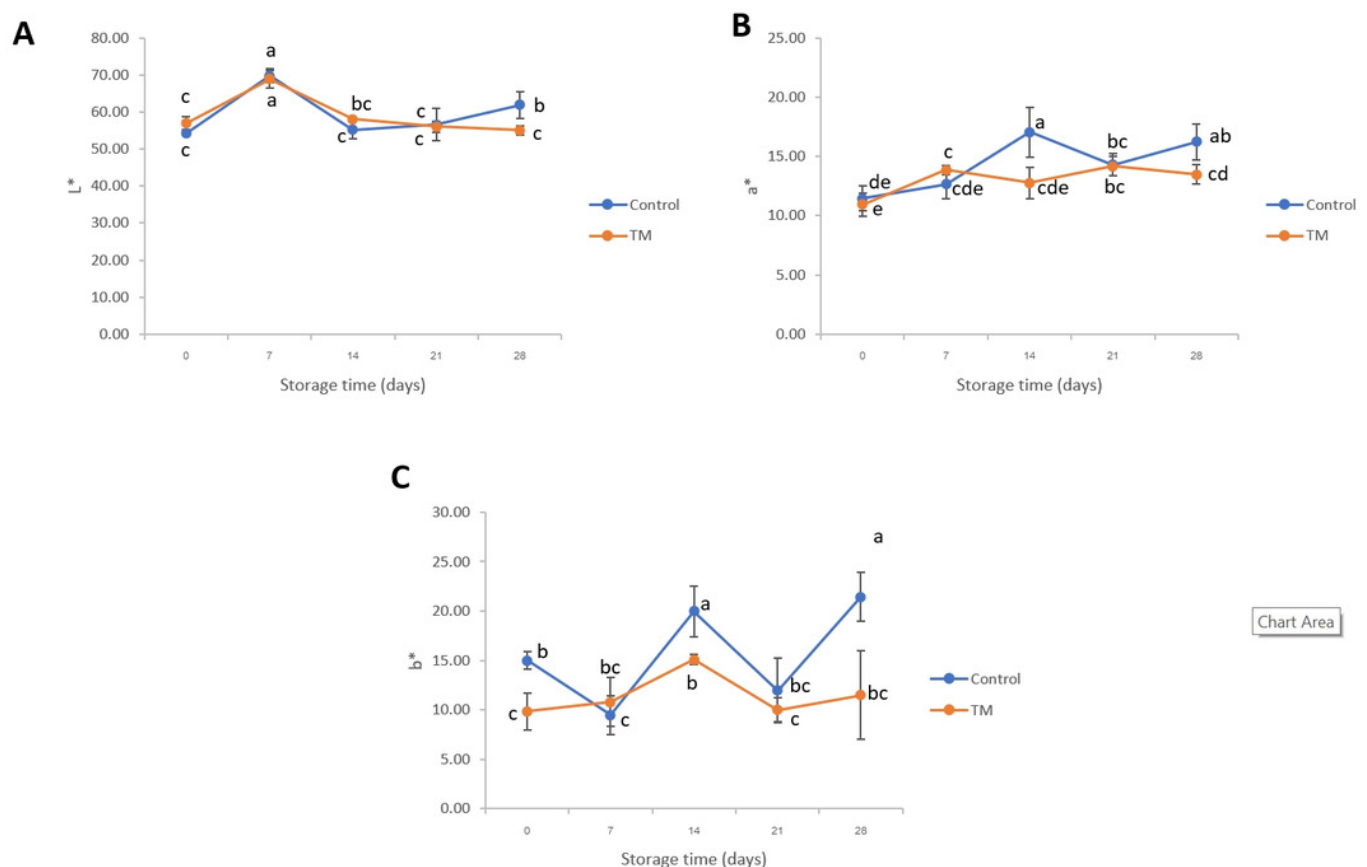
Figure 2

Figure 2

Fig. 2. Changes in hardness (A), adhesiveness (B), springiness (C), and cohesiveness (D) of the control and *C. aurantium* juice-immersed striped catfish steaks during storage at -20 $^{\circ}$C for 28 days. Results are presented as mean ± standard deviation. Means in each parameter followed by different lowercase letters are significantly different (P<0.05) according to DMRT.
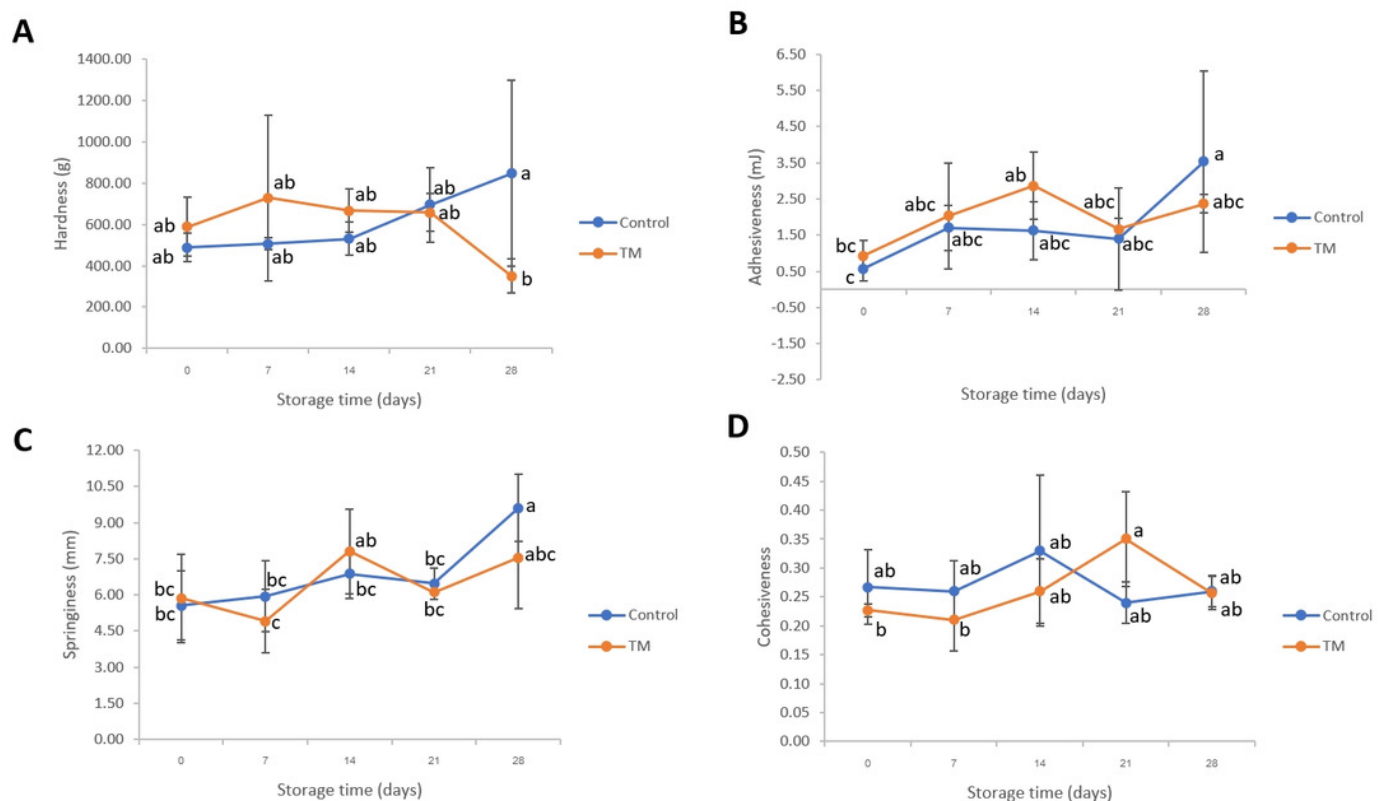
Figure 3

Figure 3

Fig. 3. Changes in pH values (A), peroxide values (B), TVB-N values (C), and TCA-soluble peptides (D) of the control and *C. aurantium* juice-immersed striped catfish steaks during storage at -20 °C for 28 days. Results are presented as mean ± standard deviation. Means in each parameter followed by different lowercase letters are significantly different (P<0.05) according to DMRT.
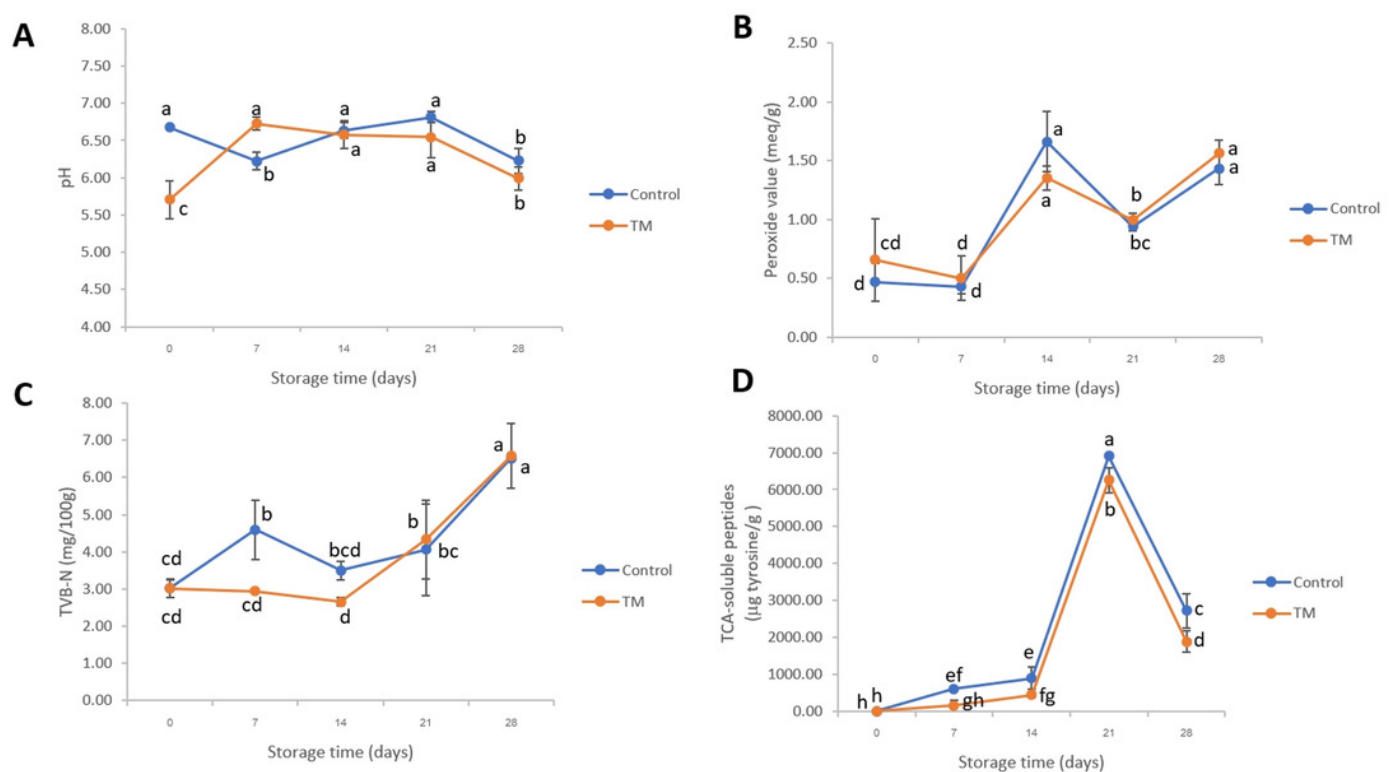
Figure 4

Figure 4

Fig. 4. Changes in total viable count (TVC) of the control and *C. aurantium* juice-immersed striped catfish steaks during storage at -20 °C for 28 days. Results are presented as mean ± standard deviation. Means in each parameter followed by different lowercase letters are significantly different (P<0.05) according to DMRT.

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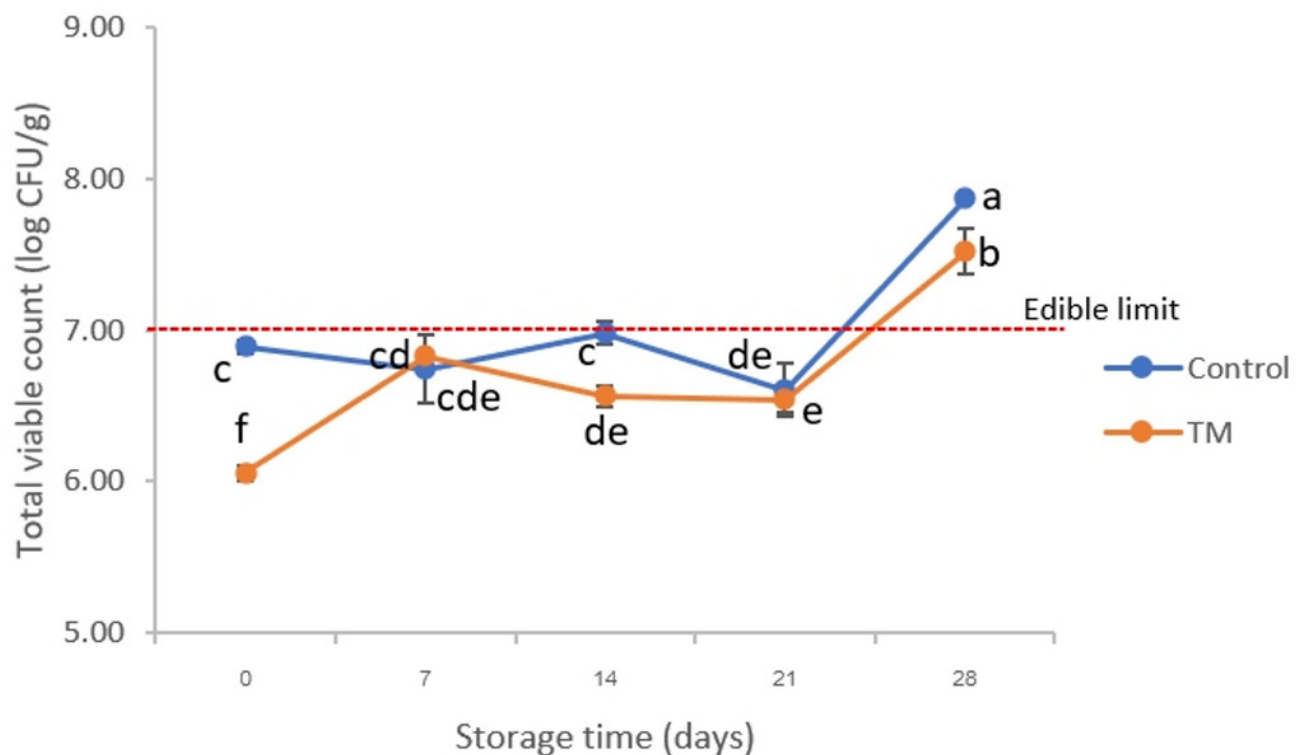
Figure 5

Figure 5

Fig. 5. Relative abundance of bacterial composition of each sample at phylum level. (CT0, control: striped catfish steak immersed in 50 ppm sodium hypochlorite on day 0; CT28, control: striped catfish steak immersed in 50 ppm sodium hypochlorite on day 28; TM0: striped catfish steak immersed in *C. aurantium* juice on day 0; TM28 striped catfish steak immersed in *C. aurantium* juice on day 28.)
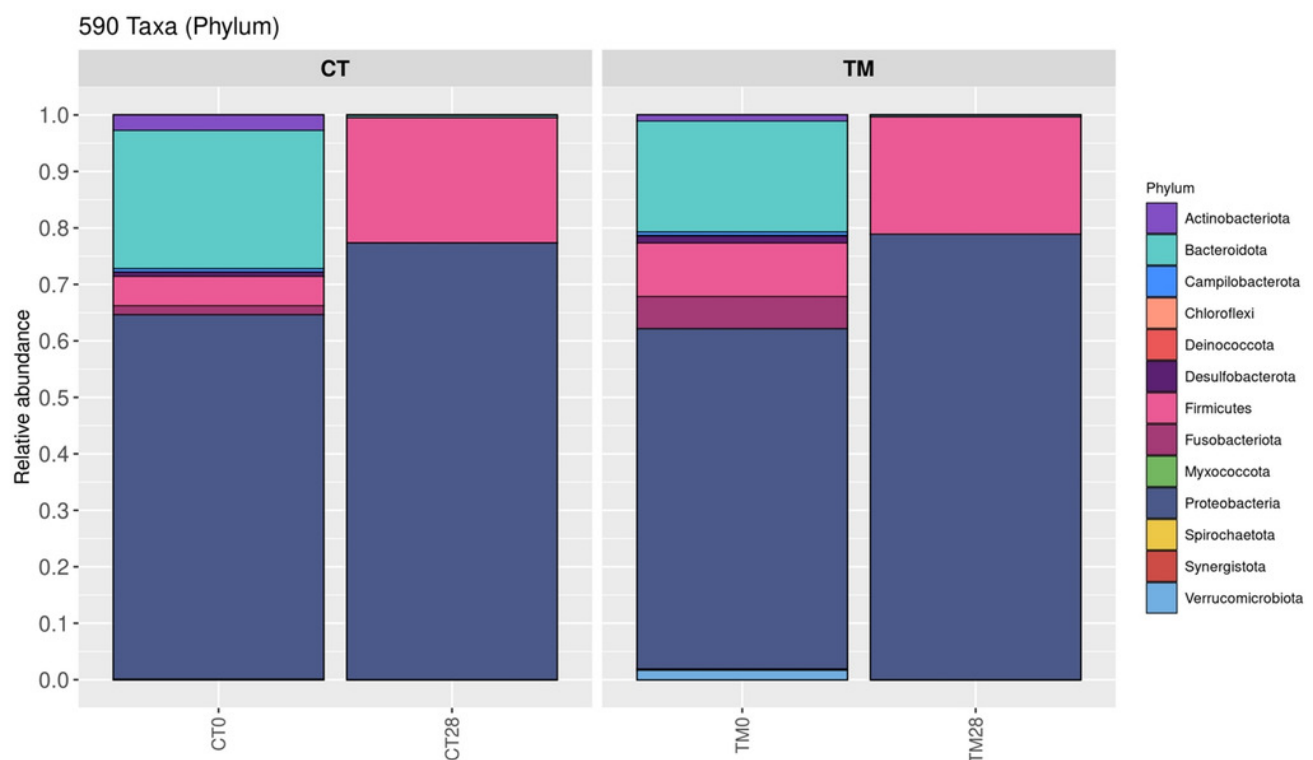
Figure 6

Figure 6

Fig. 6. Relative abundance of bacterial composition of each sample at genus level. (CT0, control: striped catfish steak immersed in 50 ppm sodium hypochlorite on day 0; CT28, control: striped catfish steak immersed in 50 ppm sodium hypochlorite on day 28; TM0: striped catfish steak immersed in *C. aurantium* juice on day 0; TM28 striped catfish steak immersed in *C. aurantium* juice on day 28.)
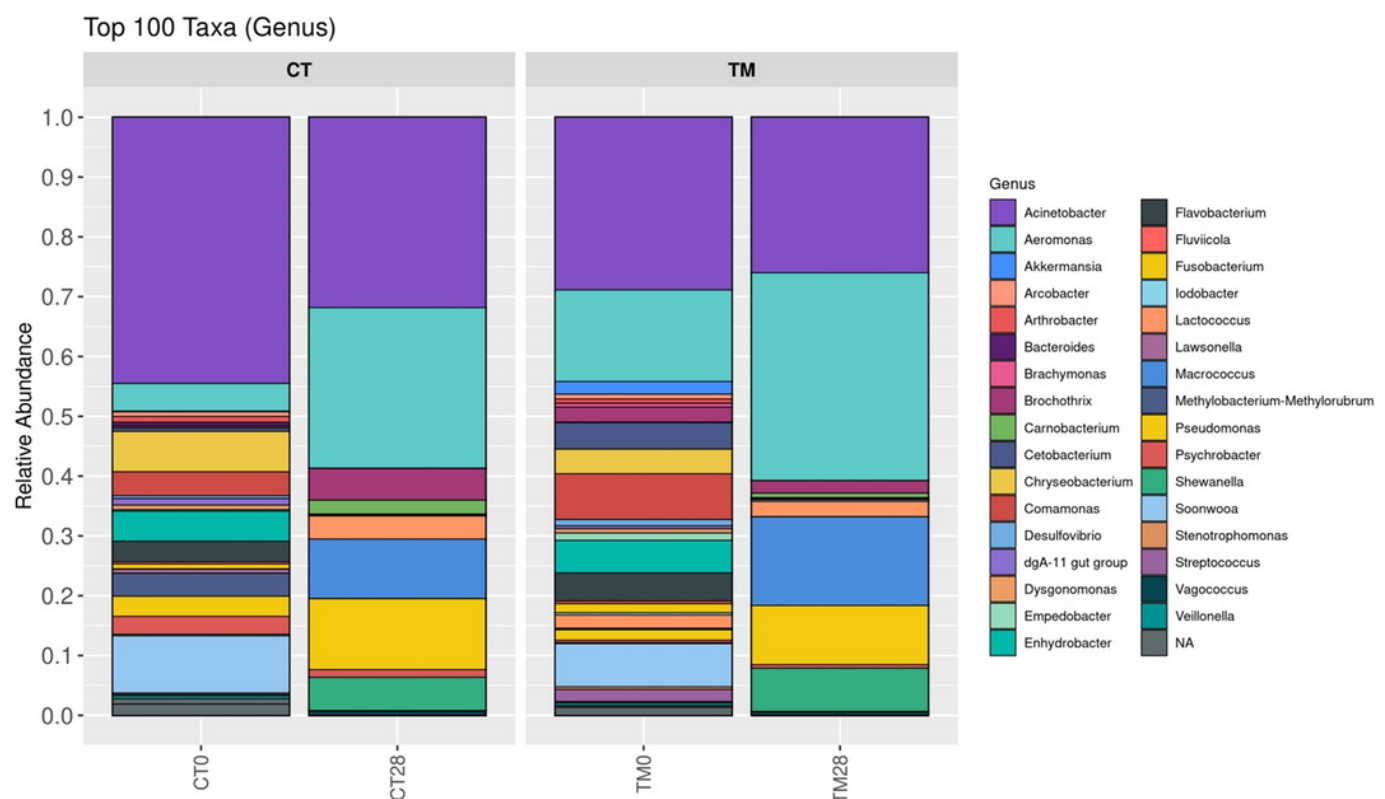
Figure 7

Figure 7

Fig.7.Principle Co-ordinate Analysis (PCoA) plots based on unweighted UniFrac (A), weighted UniFrac (B), GUniFrac with alpha 0.5 (C), and Bray-Curtis distance (D), showing clustering of the bacterial communitiesfrom each treatment. (CT0: striped catfish steak immersed in 50 ppm sodium hypochlorite on day 0; CT28: striped catfish steak immersed in 50 ppm sodium hypochlorite on day 28; TM0: striped catfish steak immersed in *C. aurantium* juice on day 0; TM28 striped catfish steak immersed in *C. aurantium* juice on day 28.)