Supplementation of PUFA extracted from Microalgae for the development of Chicken Patties

Short title: Chicken Patties with PUFA

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Abstract

In recent years, there has been a growing interest in developing a diverse range of foods enriched withOmega-3 fatty acids. It is widely recognized that through dietary interventions, the lipid fraction of food can be modified to enhance its nutritional content. This study is aimed to develop chicken patties enriched with polyunsaturated fatty acids (PUFAs) extracted from microalgae at a concentration of 0%(T0), 1%(T1), 2%(T2), and 3%(T3). All the treatments were stored at -18℃ for one month and analyzed at 0, 10, 20, and 30 days to assess the effect of PUFAs supplementation on the physicochemical, oxidative, microbiological, and organoleptic properties of chicken patties.

 The results revealed that moisture content was significantly increased during the storage; the maximum moisture was observed in T0 (67.25%±0.03) on day 0, while the minimum was found in T3 (64.69%±0.04) on day 30. Supplementation of PUFAs in chicken patties significantly enhanced the fat content of the product. The highest fat content was observed for T3 (9.7%±0.06. . An increase in PUFAs concentration led to a significant rise in Thiobarbituric acid reactive substances (TBARS). TBARS were increased from 1.22±0.43 at 0 Days to 1.48±0.39 at 30 days

34 of storage. The PUFAs incorporation negatively affected the sensory acceptance of the product

35 ranging from (8.41±0.17 to 7.28±0.12). Nevertheless, the sensory evaluations of the enriched

36 patties were within an acceptable range compared to the control sample. Among all the treatments,

37 T3 exhibited the most elevated nutritional composition. The sensory and physiochemical analysis

38 of supplemented patties suggested that PUFAs extracted from microalgae can be used as a

39 functional ingredient in preparing various meat products, particularly chicken meat patties.

40 However, antioxidants should be added to prevent lipid oxidation in the product.

41 **Keywords:** PUFAs, Microalgae, Patties, Extraction, Supplementation, Functional food

42 **Introduction**

43 Consumer needs relating to the food production zone have changed in the past few years. Now a

44 days, foods are preferred not only to satisfy hunger and source of nutrient provision for humans

45 but also to treat nutritional conditions and enhance both physical and mental health (Akram et al.,

46 2022). Fat is one of the three types of macronutrients responsible for providing energy. Chemically

47 speaking, dietary fats may be broken down into three categories: saturated fatty acids,

48 monounsaturated fatty acids, and polyunsaturated fatty acids (PUFAs) (Saini et al., 2022). Fatty

49 fish like salmon and fish oils are considered the only good sources of PUFAs. There is an urgent

50 need to provide alternatives to foods high in omega-3 fatty acids because most people in Western

51 countries consume relatively little fish (Chinaraket al., 2022). Eggs and chicken meat are among

52 the foods most commonly incorporated in meals for nutrition enhancement worldwide. For

53 instance, in the United Kingdom, PUFAs are consumed primarily from chicken meat (73 %) (Dal

54 Boscoet al., 2022).

55 Saturated fatty acids are the most important contributor to heart disease among all other types of

56 dietary fat (Kyselová et al., 2022). A nationwide restriction on the consumption of saturated fats

57 has been implemented. However, there has been no significant decrease in the incidence of heart

58 disease over time. (Jadhav and Annapure, 2021). The Mediterranean diet, which is quite popular,

59 contains many unsaturated fatty acids, typically called "good" fatty acids. PUFAs are chains of

60 hydrocarbons with two or more double bonds. Depending on where the first double bond is

61 positioned, these fatty acids can be classified as omega-3, omega-6, or omega-9 (Glaser et al.,

62 2010). The two simplest fatty acids (FAs) members of the omega-6 and Omega-3 families are

63 linoleic acid, also referred to as 18:2n-6 linoleic acid, and alpha-linolenic acid, also recognized as

64 18:3 Omega-3 linoleic acid (Calder and Yaqoob, 2009).

65 The long-chain PUFAs with 20 carbons, such as Dihomo-γ-linolenic acid (DGLA), Arachidonic

66 acid (AA), and Eicosapentaenoic acid (EPA), are precursors for the biologically active eicosanoids

67 known as prostaglandins, leukotrienes, and thromboxane. The long chain PUFAs with 22 carbons,

68 such as Docosahexaenoic acid (DHA), are precursors of decosanoids known as resolvins and

69 protectins (Calder, 2010). In addition to these functions, PUFAs play a crucial part in the formation

70 and function of cell membranes (Calder, 2015), as well as in the proper operation of the nervous

71 system (Singh, 2005). Due to their participation in the inflammatory process and the impacts of

72 eicosanoids and decosanoids, PUFAs are also important for normal functioning of immune system

73 (Hadley et al., 2016). Moreover, proper fetus development depends on the presence of PUFAs in

74 the mother's diet throughout pregnancy as AA and DHA Omega-3are considered essential for the

75 growth of retina and nervous system in the fetus (Molloy et al., 2012). The literature reported by

76 Fukaya et al. (2007) demonstrated that the antioxidant activity of PUFAs not only imparts a

77 protective effect against oxidative stress but also plays a role in preserving and functioning of the

78 hippocampus.

79 Meat patties are a popular meat product across the globe among all age groups because of their

80 low price, high nutritional value, convenient use, and sensorial satisfaction (Mizi et al., 2019).

81 Generally, patties are made using beef or mutton however, chicken meat preferred due to its high-

82 value proteins, mineral content, and lower lipid content (Longato et al., 2017). Meat companies

83 traditionally use synthetic compounds to prevent oxidative deterioration and microbial spoilage,

84 prolonging meat products' shelf life. Current studies have focused on utilizing natural compounds,

85 particularly plant-based bioactive compounds, as an alternative to harmful synthetic antioxidants

86 for meat products (Passos et al., 2022). Various natural extracts and functional ingredients have

87 been used in meat products, but little research has been performed on supplementing PUFAs

88 isolated from microalgae in chicken patties.

89 Previous studies revealed that the levels of DHA and EPA in the thigh meat increased to 2.4 and

90 1.3% of the total fatty acids when supplemented with fish oil at a dosage of 4% (konieczka et al.,

91 2017). Another research found that adding 8.2% fish oil to chicken diets increased levels of all

92 polyunsaturated fatty acids in the breast, particularly EPA (6.4%), DPA (3.1%), and DHA (6.4%).

93   (7.8%) (Güzet al., 2017). Considering the importance of PUFAs and chicken meat in our daily

94   diet, the current study was designed to develop PUFAs (Omega-3) enriched chicken patties and to

95   evaluate their physicochemical and sensory characteristics to predict the storage stability of the

96   developed product.

97   **Materials and Methods**

98   **Raw material procurement**

99   Raw materials (meat and dry ingredients) were procured from the local supermarket of Lahore and

100   fetched to the functional food lab, department of food sciences, University of the Punjab, Lahore,

101   Pakistan. The chicken meat was packed, labelled, and stored at -18°C, whereas all other dry

102   ingredients were stored at room temperature. Extracted PUFAs were obtained from the

103   biotechnology laboratory, National University of Medical Sciences (NUMS), Rawalpindi,

104   Pakistan. All the chemicals used in the research experiment were of analytical grade obtained from

105   ISI Pakistan ltd.

106   **Extraction of PUFA from Microalgae**

107   Polyunsaturated fatty acids (PUFA) from algae were determined based on the methodology

108   described by Ikaran et al., (2015) with slight modifications. Initially, an appropriate culture volume

109   containing 30-40 mg of dry biomass was subjected to centrifugation (5500 ×g, 10 min) and

110   subsequently washed with deionized water to eliminate salts from the medium. The biomass was

111   then subjected to freeze-drying. The extraction process was initiated by adding 0.7 mL of a mixture

112   of chloroform: methanol (2:1) to the cell pellet, followed by 24 hours of incubation at 50°C with

113   agitation. After centrifugation, the supernatant containing PUFA was transferred into a clean tube.

114   The residue was re-extracted under identical conditions with 0.3 mL of chloroform: methanol

115   (2:1), and the process was repeated until a whitish pellet was obtained. The supernatants obtained

116   from successive extractions were combined, washed with 0.88% KCl solution, and centrifuged for

117   phase separation. The organic phase was transferred into a pre-weighed vial and evaporated using

118   a rotational vacuum concentrator (RCV 2-18 CD, Christ, Germany) until a constant weight was

119   achieved.

120   **Supplementation of PUFAs for development of chicken patties**

121 Chicken patties were prepared according to the treatment plan elucidated in Table 1. Skinned
122 chicken was properly ground using a meat grinder (8-mm diameter die plate) (MCR08 3.0, Arbel,
123 Brazil) and combined with seasonings and PUFAs, whereas control was prepared without PUFAs
124 Patties were shaped by hand, and each patty was approximately 1cm thick, weighed 80 g and was
125 packed in LDPE bags. The product was stored at freezing temperature (-18°C) analyzed on days
126 0, 10, 20 and 30 to evaluate compositional and physiochemical changes. Fat retention, cooking
127 loss, and sensory evaluation were performed on fried patties, whereas proximate composition,
128 TBARS, and microbiological assessment was conducted on raw chicken patties.

129 **Physicochemical properties of PUFAs supplemented chicken patties**

130 **Nutritional profile of PUFAs supplemented chicken patties**

131 The proximate composition of the chicken patties was analyzed using the standard methods of
132 AOAC (2005). Mashed samples of chicken patties were oven dried at 110℃ for 2 hours, cooled in
133 a desiccator, and moisture content was calculated as weight loss. Fat was extracted applying
134 solvent extraction, ethanol was used as a solvent. Kjeldahl method was used to assess crude protein
135 in samples. Samples were subjected to muffle furnace at a temperature of 550℃ for ashing, and
136 the percentage of ash was calculated. Defatted samples were digested with acid and base to
137 determine the amount of crude fibre. The remaining material was weighed and ashed after
138 digestion. The difference in sample weight was used to compute the crude fibre.

139

140 **Determination of cooking loss of PUFAs supplemented chicken patties**

141 The product was shallow fried in a De'Longhi fryer (Westpoint, Pakistan) with canola oil at a
142 temperature of 155 °C for 25 mins. The product was weighed before and after frying, and cooking
143 loss was calculated as weight difference according to the equation used by Akwetey and Knipe
144 (2012).

145
$$Cooking\ loss\ (\%) = \frac{\text{final weight-initial weight}}{Initial\ Weight} \times 100$$

146 **Determination of fat absorption in PUFAs supplemented chicken patties**

147 Fat Absorption was calculated using the methodology described by Serdaroglu (2006), with

148 modifications. Samples were weighed before and after frying and the given formulae was used to

149 calculate the amount of fat absorbed by each sample. All the samples were run in triplicates, and

150 results were recorded in percentage (%).

151 Fat absorption= % fat in fried meat patties - % fat in raw meat patties

152 Determination of pH of PUFAs supplemented meat patties

153 pH was measured using the method Soltanizadeh et al. described (2014). 45 g of distilled water

154 was used to homogenize the meat patty samples (5 g). The pH meter (JENWAY, USA) recorded

155 the analysis results.

156 Assessment of TBARS in PUFAs supplemented meat patties

157 TBARS were assessed using the method of Rahman et al. (2021) with slight modifications. 50 ml

158 of 20% trichloroacetic acid (TCA) was used to blend with 20g meat patty sample for 2 min. 50ml

159 water was used to rinse the blender content and filtered through a Whatman # 1 filter paper. After

160 carefully combining 0.01 M, 5 ml of 2-thiobarbituric acid with the 5 ml TCA aliquot extract, the

161 mixture was heated at 100 °C for an hour. The absorbance of the brown-orange color solution was

162 determined in a spectrophotometer at a 532 nm wavelength. All samples were run in triplicates,

163 and the amount of malonaldehyde in each sample was recorded as mg of malonaldehyde/kg.

164 Microbiological evaluation of PUFAs supplemented meat patties

165 The total Plate count technique was used to evaluate the microbial load of all samples. For each

166 treatment upto four serial dilutions were made, and nutrient agar was used as growth media. The

167 Streak Plate method was used to inoculate the plates with samples and incubate at 37ºC for 48

168 hours (Prommachart et al., 2020). After incubation, colonies were counted, and results were

169 recorded as CFU x 10^4/g. The yeast and mold count of patties was measured using malt extract

170 agar (MEA), plates were inoculated with diluted samples, incubated at 25ºC for five days with a

171 constant supply of oxygen and results were recorded (Manea et al., 2017).

172   Sensory evaluation of PUFAs supplemented meat patties

173 The nine-point hedonic scale was used to record sensory scores for organoleptic attributes of

174 PUFAs supplemented meat patties. The product was served hot to 20 untrained and 10 trained

175 panelists; sensory was performed in closed chambers with suitable sensory conditions such as

176 proper light, no odors and water for rinsing. The product was evaluated against color, odor, taste,

177 and overall acceptability; scores were recorded and statistically analyzed as described by Antonini

178 et al. (2020) to evaluate the effect of PUFAs supplementation on sensory attributes of meat patties.

179

180 **Statistical Analysis**

181 Descriptive statistics (mean, standard deviation) and inferential statistics (t-test, ANOVA) were

182 used to analyze the primary objective of designed research. Data were examined using the

183 statistical analysis and comparison programme SPSS version 25.0. For multiple comparisons, two-

184 way ANOVA and LSD's Post Hoc analysis were performed. Outcomes were considered

185 statistically significant, showing a p-value higher than 0.05.

186 **Results**

187 **Proximate composition of PUFAs supplemented chicken patties**

188 The results observed for the proximate composition of the control sample and PUFAs enriched

189 meat patties during one month of storage are elucidated in Table 2. Moisture content varied

190 significantly *(p<0.05)* by adding PUFA to the chicken. Moisture content increased dramatically

191 during the storage interval but decreased with the addition of PUFA. It is evident from Table 1 that

192 the highest moisture content was obtained for T0 at 30 days of storage (68.07±0.04%), while the

193 lowest moisture content was obtained for T3 at 0 Days of storage (64.16±0.08%). Regarding

194 overall moisture content among treatments, the highest moisture content was obtained for T0,

195 while the lowest moisture content was obtained for T3. In the case of overall moisture content

196 during storage interval, the highest moisture content was obtained at 30 Days of storage while the

197 lowest overall moisture content was received at 0 Days of storage. Protein content also decreased

198 significantly *(p<0.05)* with the increasing concentration of PUFA and storage interval. The highest

199 protein content was obtained for T0 at 0 Days of storage which was 16.94±0.06%, while the lowest

200 protein content was observed for T3 at 30 Days of storage at 16.01±0.01%. Fat content

201 significantly increased with the addition of PUFA and storage days in the final product. Maximum

202 crude fat was observed for T3 (26.62±0.08%), while minimum content was obtained for T0

203 (26.26±0.12%). At the same time, the content during storage interval showed the highest values

204   for the product at 0 Days of storage (26.26±0.12%), while the lowest overall Fat content was

205   obtained at 30 Days of storage (26.34±0.12%). The mineral content evaluation revealed a non-

206   significant ($p>0.05$) effect of PUFA and storage days on the meat patties. It was similar for all

207   treatments, and no significant changes occurred in the ash content during 30 days of storage.

208   **Cooking Characteristics**

209   All the samples of patties were assessed for their cooking loss among treatments and during storage

210   intervals. The results of the cooking loss in control and microalgae extracted PUFA samples are

211   shown in Table 3. Results showed a non-significant ($p>0.05$) variation in cooking loss of chicken

212   patties supplemented with microalgae-extracted PUFAs. The maximum cooking loss was

213   observed for the T3 sample,30.52±0.44%, while the lowest was observed in the control sample,

214   T0, 29.14±0.64%. Similarly, during the storage time of one month, the highest cooking loss was

215   noticed at 30th day of storage (29.56±0.35%), whereas the lowest cooking loss of (29.15%±0.64)

216   was seen at the start of the study.

217   **Fat Absorption**

218   All the samples of patties were analyzed for their Fat absorption among treatments and during

219   storage interval. The results observed for fat absorption analysis of samples supplemented with

220   microalgae-extracted PUFA samples during one month of storage are provided in Table 3. Results

221   depicted a significant variation in the fat absorption values of meat patties. Fat absorption increased

222   significantly *($p<0.05$)* with the concentration of PUFA and decreased during the storage interval

223   at freezing temperature. In the overall Fat absorption among all treatments, the highest fat

224   absorption was observed for T3 (5.87±0.03%), while the lowest Fat absorption was obtained for

225   treatment T0 (4.90±0.02%). In the case of overall Fat absorption during the storage interval, the

226   highest Fat absorption (4.90±0.02%) was obtained at 30 Days of storage. The lowest overall Fat

227   absorption was received at 0 Day of storage (4.77±0.02%).

228   **pH of microalgae extracted PUFA supplemented chicken patties**

229   the pH of the chicken patties samples supplemented with PUFA during 30 days of storage is

230   provided in Table 4. A significant decrease in pH value was observed *($p<0.05$)* with the rising

231   concentration of Polyunsaturated fatty acids in meat patties and with the increasing storage time

232   at -18°C. It was observed maximum for the control sample with no PUFA, which was 5.94±0.01

233   and minimum for the sample with the highest concentration of PUFA, which was 5.14±0.16.

234   **Thiobarbituric Acid Reactive Species (TBARS)**

235   The results observed for TBARS analysis of meat patties supplemented with PUFA during the

236   specified storage time are provided in Table 4. Significant variations were observed in the TBARS

237   data of samples during storage. The addition of polyunsaturated fatty acids (PUFA) significantly

238   impacted the meat patties. Their TBARS increased from 0.65±0.02 to 1.71±0.01 for the control

239   sample and T3 with the highest PUFA concentration, respectively, for fresh samples. While during

240   storage time maximum value was observed for the T3 sample on day 30, which was 1.90±0.07 and

241   the minimum was observed on day 0, given as 1.02±0.02.

242   **Microbiological Evaluation**

243   The results for the total plate count analysis for meat patty samples are provided in Fig 1. PUFA

244   displayed a non-significant ($p > 0.05$) effect on the meat patties; similar results were observed for

245   the control and PUFA-supplemented samples. However, the bacterial count increased significantly

246   during the 30 days of storage. For the control sample, results revealed a bacterial count of

247   6.94±0.96 CFU x 10^4 /g, while for sample T3, it was observed at 6.70±0.29CFU x 10^4/g. During

248   storage, the bacterial number increased from 6.44±0.96CFU x 10^4/g to 6.58±0.09CFU x 10^4/g for

249   the control sample. Similar trends were observed for samples treated with polyunsaturated fatty

250   acids extracted from microalgae. Overall maximum plate count values were found for the T3

251   sample at 30 days of storage and minimum for the control sample at 0 days of storage.

252   All the samples of patties were analyzed for their Yeast and Mold among treatments and during

253   storage interval. The results of the analysis are shown in Figure 2. Analysis of variance revealed a

254   significant *($p < 0.05$)* effect of storage time on meat patties, while a non-significant effect ($p > 0.05$)

255   of PUFA was observed. It can be seen from the Figure that the highest Yeast and Mold was

256   obtained for T3 at 30 Days of storage. At the same time, the lowest Yeast and Mold were obtained

257   for T0 at 0 Days of storage. Similar results were observed for samples supplemented with PUFA

258   and control samples (T0). Yeast and mould count found at 0 days of storage was 0.71±0.01, while

259   it increased to 0.86±0.08 on the 30th day of storage for the control sample.

260   **Sensory evaluation**

261   All samples were analyzed for color, aroma, texture, taste, and overall acceptability. All the scores

262   observed for samples are provided in Table 5. All the parameters were significantly affected by

263   the supplementation of PUFA and storage time of 30 days. The color score of samples was reduced

264   slightly by adding PUFA in meat samples and was found to be the minimum for the T3 sample at

265   30 days of storage. A similar trend was observed for the taste of samples supplemented with

266   microalgae-extracted PUFA samples. Odour scores were observed to be reduced from 8.6 for the

267   control sample to 7.5 for T3 on 0 days of storage. This score reduced from 8.35 to 7.26 on the 30th

268   day of storage for the control and sample with maximum PUFA concentration, respectively. The

269   texture of the samples was also significantly affected by PUFA addition and was observed

270   maximum for the control sample at 0 days of storage. It was observed at 7.66 for the T3 and 8.56

271   for the control samples. In the overall acceptability scores among treatments, the highest scores

272   were obtained for treatment T0, while the lowest were obtained for T3. In the overall acceptability

273   score during storage interval, the highest was obtained at 0Day of storage while the lowest was

274   obtained at 30 Days of storage.

275   **Discussion**

276   During storage, increased moisture content of patties could be associated with released

277   intracellular moisture and moisture gained from environment, similar findings were reported by

278   Dias et al. (2022), who incorporated canola and olive oil gel emulsions as pork fat replacers in beef

279   patties. However, the decreased moisture content among the treatments could be interlinked to

280   enhanced PUFAs, analogous results were reported by Bavitha et al. (2016) in fish burgers.

281   Serdaroğlu et al. (2018) also reported increased moisture content during storage in beef patties

282   formulated with dried pumpkin pulp and seed. The decrease in protein content during storage could

283   be because there was an increase in moisture content that may have been subjected to the overall

284   reduction in protein content. The decrease in protein among the treatments could be because of an

285   increased polyunsaturated fatty acid addition. Analogous results were reported by Bavitha et al.

286   (2016) in fish burgers during storage. The decrease may be attributed to the leaching out of the

287   water-soluble nitrogenous components during storage along with moisture. Similar results were

288   also reported in these studies when results revealed no significant change in ash content of fish

289   burger patties during storage. In another study by Serdaroğlu et al. (2018) it was reported that no

290   effect on ash content during storage in beef patties formulated with dried pumpkin pulp and seed

291   powder was observed. During storage fat content decreases as there was an increase in moisture
292   content that may have subjected to overall decrease in fat content. The increase in fat among the
293   treatments could be because of increase in polyunsaturated fatty acid addition among the
294   treatments. Very similar results were reported in a study on fish burgers during storage. Liang et
295   al. (2020) also reported decreased fat content during storage in PUFA-enriched eggs due to
296   increased oxidation and TBARS values.

297   An increase in cooking loss was also observed during the one-month meat patties' storage time
298   could be attributed to increased product moisture content during storage, which is the main
299   component reduced during cooking procedures. Cortinas et al. (2004) also reported similar results
300   to the current study in which increasing the PUFA content diet of broiler chicken did not affect the
301   fatty acids content of breast meat while cooking and revealed a non-significant effect. As patties
302   lose their freshness over time, there is an overall increase in fat absorption during storage.
303   Contradictory results displayed reduced fat absorption reported by Soltanizadeh and Ghiasi-
304   Esfahani (2015) when Aloe Vera was added to low-meat beef burgers as a functional ingredient
305   objective of improving their nutritional value.

306   There is a decrease in pH value among the treatments because of increased polyunsaturated fatty
307   acid addition. Troegeler-Meynadier et al. (2006) also reported similar results in their study of the
308   effect of pH and concentration of Linoleic and Linolenic Acids on the Extent and Intermediates of
309   Ruminal Bio hydrogenation *in-vitro*. The increase in TBARS among the treatments was due to an
310   increase in polyunsaturated fatty acid addition among the treatments. Akarpat (2008) also reported
311   that increase in TBARS during storage in beef patties. De Sousa et al. (2020) reported similar
312   results: TBARS increased in beef burgers enriched with PUFAs during storage in the refrigerator.

313   Among treatments, a significant trend was observed for total plate count, so it may be said that
314   adding polyunsaturated fatty acids did not affect the total plate count in chicken meat patties.
315   However, it depicted a rise during 30 days of storage time. Karabagias (2018) also reported similar
316   results in chopped lamb meat during storage when bacterial count showed a significant surge
317   during storage time. The increase in yeast and mold with storage patties can be the loss of their
318   freshness and the increase in microbial load that may have been subjected to an increase in yeast
319   and mould. Among treatments no significant trend was observed for TPC, so it may be said that
320   adding polyunsaturated fatty acids did not affect the yeast and mold in patties. Similar results were

321   also reported by another study performed on chopped lamb meat during storage (Ibrahimet al.,
322   2010).

323   The decrease in texture scores during storage, the reason with storage is that patties lose their
324   freshness, which may have resulted in an overall reduction in texture scores. The decrease in
325   texture scores among the treatments could be because of increased polyunsaturated fatty acid
326   addition. Novello (2019) reported that adding golden flaxseed and by-product in beef patties
327   increased their sensorial acceptance as Golden flaxseed added increased Omega-3 fatty acids.
328   Valenzuela-Melendres et al. (2018) also reported that adding PUFA sources to beef patties
329   increases sensory acceptance. The decrease in color scores during storage could be because storage
330   patties lose their freshness which may have subjected to an overall reduction in color scores.
331   Enhanced concentration of PUFAs is associated with negative sensory scores for patties' colour.
332   The decrease in taste scores during storage could be because storage patties lose their freshness
333   which may have subjected to an overall reduction in taste scores. The decline in taste scores among
334   the treatments could be because of increased polyunsaturated fatty acid addition. Valencia et al.
335   (2006), reported similar results when Omega-3 PUFAs were supplemented to create nutrition-
336   enriched fermented sausage. Kolanowskiet al., 2007 also reported that their sensorial scores were
337   reduced with fish oil powder as a PUFAs source in instant food products.

338   **Conclusion**

339   Polyunsaturated fatty acids are mainly taken from fish and fish products. They have a long history
340   of being good for health. Some people do not typically eat fish, so other products are being
341   produced with the artificial addition of PUFA. The current study revealed that adding indigenous
342   polyunsaturated fatty acids sources can raise the PUFA content in chicken products. According to
343   the findings of the study, moisture content increased considerably *(p<0.05)* during storage while
344   decreasing significantly *(p<0.05)* across the treatments. Under retention, the protein content fell
345   considerably *(p<0.05)*, and non-significant results were found among treatments. Ash content
346   remained unaffected (P>0.05) during storage or among the treatments. Fat content significantly
347   *(p<0.05)* decreased among the days while significantly *(p<0.05)* increased during storage. TBARS
348   significantly *(p<0.05)* increased during storage and among the treatments. Highly significant
349   results were obtained for the overall acceptability scores of patties with PUFA supplementation
350   and during the storage period. Significant results were obtained from their interaction of storage

351   time and treatments. The current study concludes that PUFA extracted from microalgae could be

352   used as a supplement for the patties.

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Table 1 *(on next page)*

Composition of Chicken Meat Patties Supplemented with PUFAs Extracted from Microalage

Treatments composition

Table 1: Composition of Chicken Meat Patties Supplemented with PUFAs Extracted from Microoalage.

Ingredients	Treatments			
	T0	T1	T2	T3
Minced Chicken Meat(g)	48	47.4	46.8	46.2
Breadcrumbs(g)	10	10	10	10
Seasoning(g)	1	1	1	1
Ginger & Garlic(g)	1	1	1	1
PUFAs Oil(ml)	0	0.6 (1%)	1.2(2%)	1.8(3%)

Composition of Chicken Patties Supplemented with PUFA Extracted from Microalgae

Mean Values ± S.D with different letter in individual parameters are significantly different (P< 0.05). T0(control)= without PUFA; **T1=** Chicken Patties containing 1% PUFA; **T2=** Chicken Patties containing 2% PUFA **; T3=** Chicken Patties containing 3% PUFA.

1 **Table 2: Proximate Composition of Chicken Patties Supplemented with PUFA Extracted**
2 **from Microalgae.**

Proximate Composition (%)		PUFA enriched chicken patties			
		T0	T1	T2	T3
Moisture	0 day	$67.25^d \pm 0.03$	$66.29^h \pm 0.04$	$64.82^l \pm 0.05$	$64.16^p \pm 0.08$
	10 days	$67.52^c \pm 0.04$	$66.50^g \pm 0.08$	$65.01^k \pm 0.16$	$64.30^o \pm 0.01$
	20 days	$67.63^b \pm 0.11$	$66.80^f \pm 0.23$	$65.29^j \pm 0.02$	$64.40^n \pm 0.06$
	30 days	$68.07^a \pm 0.04$	$67.14^e \pm 0.01$	$66.02^i \pm 0.25$	$64.69^m \pm 0.04$
Protein	0 day	$16.94^a \pm 0.06$	$16.60^e \pm 0.03$	$16.19^i \pm 0.01$	$16.12^k \pm 0.02$
	10 days	$16.86^b \pm 0.04$	$16.50^f \pm 0.01$	$16.17^i \pm 0.01$	$16.09^l \pm 0.01$
	20 days	$16.72^c \pm 0.11$	$16.37^g \pm 0.02$	$16.15^j \pm 0.01$	$16.06^m \pm 0.01$
	30 days	$16.64^d \pm 0.01$	$16.31^h \pm 0.05$	$16.14^{jk} \pm 0.01$	$16.01^n \pm 0.01$
Fat	0 day	$26.26^l \pm 0.12$	$26.28^k \pm 0.16$	$26.32^j \pm 0.09$	$26.62^a \pm 0.08$
	10 days	$26.37^g \pm 0.12$	$26.49^c \pm 0.16$	$26.41^e \pm 0.31$	$26.39^f \pm 0.38$
	20 days	$26.57b \pm 0.11$	$26.36^h \pm 0.07$	$26.37^g \pm 0.17$	$26.14^m \pm 0.08$
	30 days	$26.34^i \pm 0.12$	$26.41^e \pm 0.33$	$26.48^d \pm 0.23$	$26.41^e \pm 0.34$
Ash	0 day	$2.15^c \pm 0.12$	$2.16^b \pm 0.16$	$2.15^c \pm 0.09$	$2.16^b \pm 0.08$
	10 days	$2.15^c \pm 0.12$	$2.15^c \pm 0.16$	$2.17^{bc} \pm 0.31$	$2.18^a \pm 0.38$
	20 days	$2.17^{ab} \pm 0.11$	$2.16^b \pm 0.07$	$2.16^b \pm 0.17$	$2.18^a \pm 0.08$
	30 days	$2.15^c \pm 0.12$	$2.14^c \pm 0.33$	$2.15^c \pm 0.23$	$2.16^b \pm 0.34$

3 **Note:** Mean Values ± S.D with different letter in individual parameters are significantly different ($P < 0.05$).
4 T0(control)= without PUFA; **T1**= Chicken Patties containing 1% PUFA; **T2**= Chicken Patties containing
5 2% PUFA ; **T3**= Chicken Patties containing 3% PUFA.

Cooking Characteristics of Chicken Patties Supplemented with PUFA Extracted from Microalgae

Mean Values ± S.D with different letter in individual parameters are significantly different (P< 0.05). T0(control)= without PUFA; T1= Chicken Patties containing 1% PUFA; T2= Chicken Patties containing 2% PUFA ; T3= Chicken Patties containing 3% PUFA

3 **Table 3: Cooking Characteristics of Chicken Patties Supplemented with PUFA Extracted from**
4 **Microalgae.**

Cooking Characteristics (%)		PUFA enriched chicken patties			
		T0	T1	T2	T3
Cooking Loss	0 day	$29.15^k\pm0.64$	$29.10^l\pm0.01$	$30.34^f\pm0.01$	$30.52^e\pm0.44$
	10 days	$29.50^i\pm0.23$	$29.11^l\pm0.01$	$30.66^d\pm0.07$	$30.15^g\pm0.44$
	20 days	$29.54^h\pm0.44$	$29.18^j\pm0.04$	$30.75^c\pm0.01$	$30.14^g\pm0.64$
	30 days	$29.56^h\pm0.35$	$29.55^h\pm0.01$	$31.75^a\pm0.06$	$31.12^b\pm0.23$
Fat Absorption	0 day	$4.90^l\pm0.02$	$5.25^h\pm0.04$	$5.57^e\pm0.15$	$5.87^a\pm0.03$
	10 days	$4.87^m\pm0.01$	$5.19^i\pm0.01$	$5.42^f\pm0.03$	$5.82^b\pm0.01$
	20 days	$4.82^n\pm0.04$	$5.12^j\pm0.05$	$5.29^g\pm0.01$	$5.80^c\pm0.01$
	30 days	$4.77^o\pm0.02$	$4.98^k\pm0.09$	$5.28^g\pm0.01$	$5.72^d\pm0.07$

5 **Note:** Mean Values ± S.D with different letter in individual parameters are significantly different ($P< 0.05$).
6 T0(control)= without PUFA; T1= Chicken Patties containing 1% PUFA; T2= Chicken Patties containing
7 2% PUFA ; T3= Chicken Patties containing 3% PUFA.

Physiochemical Characteristics of Chicken Patties Supplemented with PUFA Extracted from Microalgae

Mean Values ± S.D with different letter in individual parameters are significantly different (P< 0.05). T0(control)= without PUFA; T1= Chicken Patties containing 1% PUFA; T2= Chicken Patties containing 2% PUFA ; T3= Chicken Patties containing 3% PUFA

1   **Table 4: Physiochemical Characteristics of Chicken Patties Supplemented with PUFA Extracted**
2   **from Microalgae.**

Physiochemical Characteristics		PUFA enriched chicken patties			
		T0	**T1**	**T2**	**T3**
pH	0 day	$5.94^a\pm0.01$	$5.78^b\pm0.39$	$5.56^d\pm0.06$	$5.14^k\pm0.16$
	10 days	$5.64^c\pm0.19$	$5.56^d\pm0.13$	$5.50^e\pm0.09$	$5.40^h\pm0.39$
	20 days	$5.55^d\pm0.02$	$5.51^e\pm0.38$	$5.51^e\pm0.31$	$5.50^e\pm0.05$
	30 days	$5.45^f\pm0.3$	$5.42^g\pm0.10$	$5.38^i\pm0.06$	$5.33^j\pm0.31$
TBARS	0 day	$0.65^p\pm0.02$	$1.10^l\pm0.02$	$1.45^h\pm0.08$	$1.71^d\pm0.01$
	10 days	$0.83^o\pm0.11$	$1.15^k\pm0.01$	$1.50^g\pm0.01$	$1.82^c\pm0.04$
	20 days	$0.96^n\pm0.04$	$1.22^j\pm0.04$	$1.58^f\pm0.02$	$1.88^b\pm0.01$
	30 days	$1.02^m\pm0.02$	$1.29^i\pm0.01$	$1.62^e\pm0.01$	$1.90^a\pm0.07$

3   **Note:** Mean Values ± S.D with different letter in individual parameters are significantly different ($P< 0.05$).
4   T0(control)= without PUFA; T1= Chicken Patties containing 1% PUFA; T2= Chicken Patties containing
5   2% PUFA ; T3= Chicken Patties containing 3% PUFA.

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Sensory Evaluation (scoring) of Chicken Patties Supplemented with PUFA Extracted from Microalgae

Mean Values ± S.D with different letter in individual parameters are significantly different (P< 0.05). T0(control)= without PUFA; **T1=** Chicken Patties containing 1% PUFA; **T2=** Chicken Patties containing 2% PUFA**; T3=** Chicken Patties containing 3% PUFA.

2 **Table 5: Sensory Evaluation (scoring) of Chicken Patties Supplemented with PUFA**

3 **Extracted from Microalgae.**

Organoleptic Attributes		PUFA enriched chicken patties			
		T0	T1	T2	T3
Colour	0 day	$8.68^a\pm0.02$	$8.11^e\pm0.01$	$7.73^i\pm0.04$	$7.48^m\pm0.06$
	10 days	$8.41^b\pm0.01$	$8.20^f\pm0.01$	$7.68^j\pm0.04$	$7.33^n\pm0.03$
	20 days	$8.28^c\pm0.01$	$7.92^g\pm0.07$	$7.58^k\pm0.02$	$7.29^o\pm0.02$
	30 days	$8.21^d\pm0.03$	$7.82^h\pm0.03$	$7.54^l\pm0.01$	$7.21^p\pm0.11$
Aroma	0 day	$8.60^a\pm0.03$	$8.28^e\pm0.01$	$7.82^i\pm0.01$	$7.56^l\pm0.02$
	10 days	$8.53^b\pm0.04$	$8.22^f\pm0.06$	$7.51^m\pm0.02$	$7.51^m\pm0.03$
	20 days	$8.47^c\pm0.01$	$7.97^g\pm0.04$	$7.72^j\pm0.02$	$7.39^n\pm0.12$
	30 days	$8.35^d\pm0.03$	$7.86^h\pm0.05$	$7.70^k\pm0.01$	$7.26^o\pm0.02$
Texture	0 day	$8.56^a\pm0.01$	$8.21^d\pm0.07$	$7.85^g\pm0.01$	$7.66^k\pm0.01$
	10 days	$8.55^a\pm0.02$	$8.03^e\pm0.03$	$7.83^h\pm0.01$	$7.59^l\pm0.04$
	20 days	$8.53^b\pm0.03$	$7.91^f\pm0.01$	$7.78^i\pm0.04$	$7.43^m\pm0.06$
	30 days	$8.43^c\pm0.01$	$7.93^f\pm0.02$	$7.72^j\pm0.01$	$7.23^n\pm0.17$
Taste	0 day	$8.52^a\pm0.21$	$7.91^e\pm0.14$	$7.69^i\pm0.01$	$7.22^m\pm0.01$
	10 days	$8.31^b\pm0.08$	$7.86^f\pm0.03$	$7.64^j\pm0.01$	$7.21^n\pm0.01$
	20 days	$8.18^c\pm0.04$	$7.82^g\pm0.06$	$7.51^k\pm0.01$	$7.18^o\pm0.02$
	30 days	$8.13^d\pm0.02$	$7.72^h\pm0.02$	$7.35^l\pm0.04$	$7.15^p\pm0.01$
Overall Acceptability	0 day	$8.59^a\pm0.02$	$8.11^e\pm0.01$	$7.85^i\pm0.01$	$7.41^m\pm0.01$
	10 days	$8.53^b\pm0.04$	$8.06^f\pm0.01$	$7.74^j\pm0.07$	$7.34^n\pm0.01$
	20 days	$8.42^c\pm0.03$	$7.99^g\pm0.04$	$7.64^k\pm0.01$	$7.24^o\pm0.05$
	30 days	$8.16^d\pm0.02$	$7.92^h\pm0.01$	$7.58^l\pm0.06$	$7.12^p\pm0.02$

4 **Note:** Mean Values ± S.D with different letter in individual parameters are significantly different ($P < 0.05$).

5 T0(control)= without PUFA; **T1**= Chicken Patties containing 1% PUFA; **T2**= Chicken Patties containing

6 2% PUFA; **T3**= Chicken Patties containing 3% PUFA.

Figure 1

Total Palte Count (10^4 CFU/g) of Chicken Patties Supplemented with PUFAs Extracted from Microalage

Storage study with respect to TPC
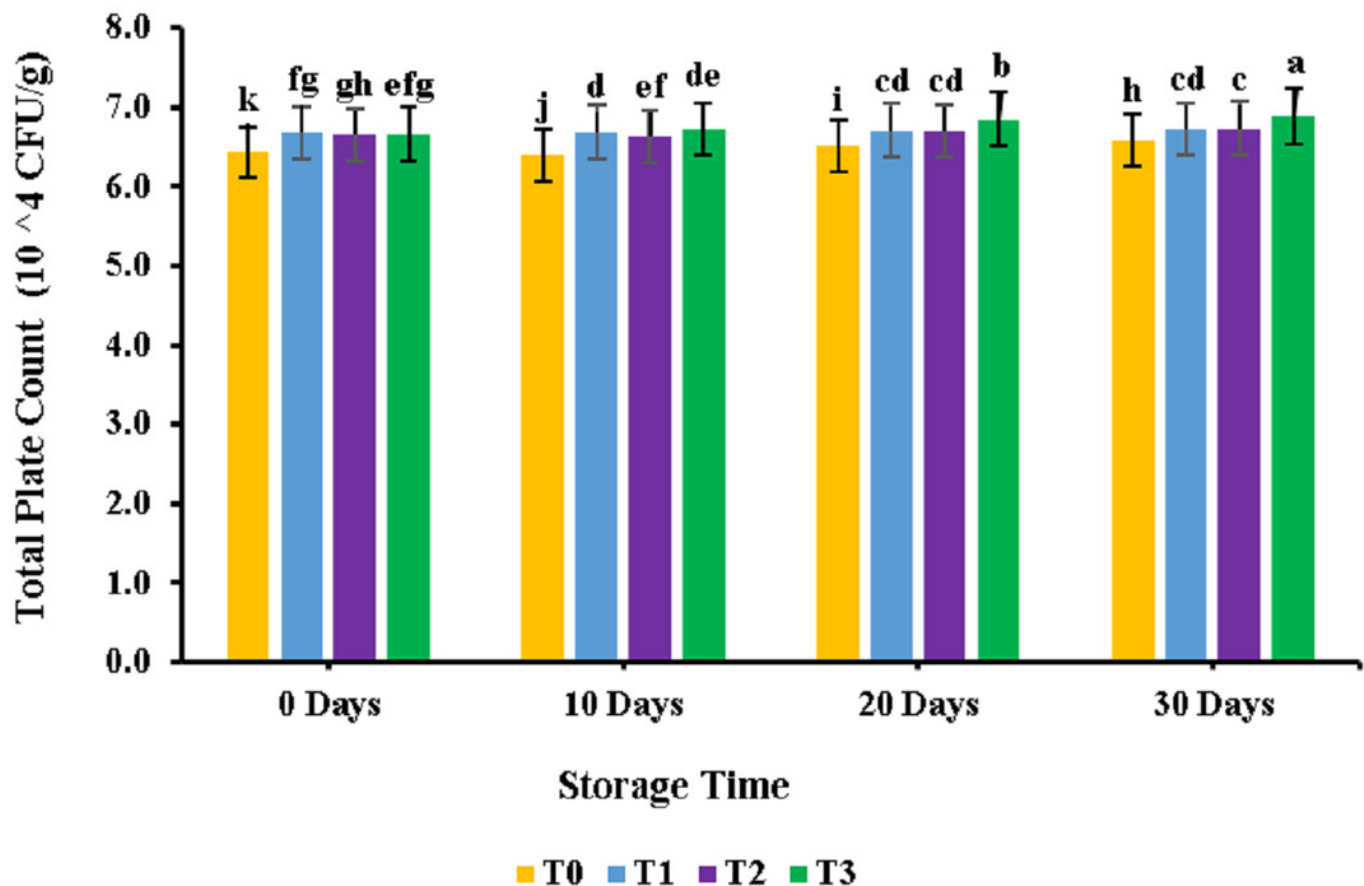
Figure 2

Yeast and Mold Count of Chicken Patties Supplemented with PUFAs Extracted from Microalage

Storage study against yeast and mold