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1 **Characterization of fermented Okara powder and its effect on lipid**

2 **oxidation of emulsion-type sausage pork sausage during cold storage**

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21 **Abstract**

22 In the present study, Okara, a soybean by-product from the production of tofu and
23 soymilk, was processed by solid state fermentation (SSF) with *Neurospora crassa* Mai
24 1-1, a filamentous and edible fungus which was firstly isolated in our group. The
25 proximate composition, dietary fiber content, water-soluble polysaccharides and
26 antioxidant activity of Okara was investigated. The crude fat and protein, soluble
27 dietary fiber content and ergosterol content of Okara was significantly increased by SSF
28 with *N. crassa*. And the antioxidant activity (evaluated by the ABTS radical cation
29 scavenging activity and DPPH radical scavenging activity) of Okara was significantly
30 enhanced by SSF with *N. crassa*. In addition, emulsion-type sausage was taken as a
31 model system for farther investigation, sausage with fermented Okara power had
32 reduced values of thiobarbituric acid reactive substances (TBARS) ($p < 0.05$), peroxide
33 value. Therefore, this study demonstrated that fermented Okara showed higher nutrition
34 value and antioxidant activity compared to the non-fermented Okara counterpart, and
35 as an additive has good effect on antioxidant of emulsion-type sausage.

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37 ## Introduction

38 In the last decades, large amounts of agro-industrial residues which lead to the
39 economic loss and socio-environmental problems were produced in the world,
40 especially in developing countries. In recent years, there has been an increasing interest
41 in the more efficient utilization of these agro-industrial residues, which could provide
42 an effective way to reduce the production economic loss and solve many environmental
43 hazards (Li et al., 2013; Vong & Liu, 2016a). Therefore, great global attention has been
44 paid to search for alternative uses and value-add of agro-industrial residues. Okara, the
45 byproduct of soybean curd, soy protein and soymilk manufacturing, is discharged as an
46 agro-industrial waste with little market value (Shi et al., 2012). Okara is a large amount
47 of production, cheap and porous available resource in Asian countries such as Japan
48 and China (Shi et al., 2012; Zhu et al., 2008). For example, the amount of produced
49 Okara in Japan, Korea and China was 800,000, 310,000 and 2,800,000 tons,
50 respectively, during the manufacturing of soybean curd (Vong & Liu, 2016b). Okara is
51 generally discarded and considered as waste, which have aggravated burden of the
52 industry on waste treatment. However, many nutritional constituents such as
53 polysaccharides, monosaccharides, oligosaccharides, dietary fiber, protein, and mineral
54 composition were found in Okara as well, suggesting that it is a potential source of low-
55 cost vegetable nutrition for human consumption (Mateos-Aparicio, Redondo-Cuenca,
56 Villanueva-Suárez, 2010; Vong & Liu, 2016b; Zhu et al., 2008). In the past years, there
57 has been growing interest in ways of reusing the Okara. Several health-stimulating
58 effects of Okara have also been demonstrated in recent years. Many studies have
59 reported that the utilization of Okara to produce value-added food products, such as
60 soluble dietary fiber, polysaccharides, and natural antioxidant sources (Mateos-
61 Aparicio, Redondo-Cuenca, Villanueva-Suárez, 2010; Li et al., 2013; Vishwanathan,

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62 Singh & Subramanian, 2011). In addition, Okara can also be used as an ingredient in
63 various foodstuffs (Vong & Liu, 2016a). Thus, value-added and full utilization of this
64 soybean by-product (Okara) is of interest to the soybean industry.

65 Previous studies have demonstrated that the biochemical modification by
66 submerged and solid state fermentation (SSF) directly contributes to many
67 advantageous properties of products (Shi et al., 2012; Xiao et al., 2015b). In recent
68 years, SSF with filamentous fungi was observed to serve as an efficient approach to
69 enhance the nutritional value and health-stimulating effects of pulse products. During
70 the SSF process, many enzymes were produced by the microbes which could hydrolyze
71 its constituents and contribute to the development of a product with improved nutrition,
72 flavour, and aroma. Therefore, SSF may be an economic and environmental friendly
73 technique to reuse the agro-solid waste Okara (Vong & Liu, 2016a).

74 *N. crassa* is an edible filamentous fungus generally recognized as safe. *N. crassa*,
75 as a model of model microbes for biochemical genetics and molecular biology, has also
76 been seen as a valuable organism for biotechnological applications (Dogaris et al., 2009;
77 Rao et al., 1985). Besides, *N. crassa* is a well-known fungus that has been used for
78 fermentation of agricultural residues substrates, sorghum bagasse, wheat straw,
79 brewer's spent grain and orange peels (Dogaris et al., 2009; Mamma, Kourtoglou &
80 Christakopoulos, 2008; Romero et al., 1999; Xiros et al., 2008) has been investigated
81 previously. However, to the best of our knowledge, no investigation is available on the
82 effect of SSF with *N. crassa* on the production of antioxidant activity, and nutritional
83 properties of Okara.

84 As is known to all, pork is the most favorable meat in China, pork-based emulsion-
85 type sausage is one of major processed pork products, preferred by Chinese consumers,
86 In order to produce the special texture and flavor, pork fat is usually added into the

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87 sausage. However, high animal fats have negative impacts on human health. are
88 gradually associated with several types of obesity, hypertension, cardiovascular
89 diseases and coronary heart diseases (ÖZvural & Vural, 2008; Serrano et al., 2007;
90 Javidipour & Vural, 2002). Fermented Okara with abundant dietary fibers and plant
91 protein, is taken for making a balanced diet in emulsion-type sausage. Adding
92 Fermented Okara power to sausages would supply the requisite quantities of Okara and
93 natural antioxidants, known to prevent tissue damage and "oxidative stress" related
94 diseases (Chen et al., 2013). It may extend the shelf-life of food products (Gedrovica &
95 Karklina, 2013). Therefore, the objective of this study was to investigate effect of
96 fermented Okara power on textural, color and lipid peroxidation properties of pork
97 sausage and provide a novel method for developing the sausage that contains dietary
98 fiber.

99 Materials & Methods

100 Preparation of fermented Okara

101 200 g soybean soaked in water at 25 °C for 10 h was ground with 1.5 L water by
102 a soybean milk machine (FP-4116, Deer Electric Appliance Co., Ltd. Guangdong,
103 China), and filtrated. The fresh raw Okara was sterilized at 108 °C for 15 min in an
104 autoclave, and then cooled and inoculated with 2% (v/w) spore suspension of the sterile
105 Okara. After mixing, aliquots (200 g) of the inoculated Okara were transferred into
106 glass culture dish (15 cm in diameter, 2.5 cm in depth) and incubated at 28 °C for 48 h
107 in an incubator with continuous white light (SPT-P--A, Hefei scientific equipment co.,
108 LTD, Hefei, China). Duration of the solid state fermentation was 48 h and samples were
109 aseptically withdrawn at 0 and 48 h were lyophilized by using a freeze drier (Thermo

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110 Heto Power Dry LL3000, Thermo Fisher Scientific Inc, Massachusetts, the United
111 States). And then dried materials were milled to fine flour passing through a 0.5 mm
112 sieve using a food grinder, the flour was kept at 4 °C for further analyses.

113 **Proximate composition analysis of Okara**

114 The moisture, crude protein, crude fat and ash contents of Okara samples were
115 determined according to the method of AOAC (1990). The total dietary fiber and
116 insoluble dietary fiber were determined using the AOAC methods (Association of
117 Official Analytical Chemists 1994). Soluble dietary fiber was calculated by subtracting
118 the insoluble dietary fiber proportion from the soluble dietary fiber. The results of crude
119 protein, crude fat, ash and dietary fiber contents were expressed as grams per 100 g of
120 dry weight basis. All determinations were made in triplicate.

121 **Ergosterol content measurement**

122 Ergosterol content was determined according to the method of Feng, Eriksson &
123 Schnürer (2005) with minor modification. Briefly, two grams samples of lyophilized
124 Okara was mixed with 37.5 mL methanol, 25 mL 95% ethanol and 5 g potassium
125 hydroxide, and incubating at 100 °C for 15 min. Then the mixture was added with 12.5
126 mL of distilled water, and gently mixed. The extracts were then centrifuged at 5000 g
127 for 15 min at 4 °C (TDL-5-A, Anting science instrument factory, Shanghai, China).
128 After that, 5 ml of supernatant was collected and mixed with 5 mL of pentane using a
129 vortex for 1 min to extract ergosterol. The upper phase was transferred to a clean tube
130 when two clear phases appeared in the mixture. The extraction procedure was repeated
131 twice. The upper phase containing ergosterol was dried through a vacuum rotary
132 evaporation procedure (Heizbad Hei-VAP, Heidolph Instruments GmbH and Co.KG,
133 Germany) (30 °C, 100 r/min) and dissolved in 0.5 mL methanol. The ergosterol

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134 concentration was assayed by HPLC (Waters Separations Module 2695; Japan)
135 equipped with an Agilent Eclipse plus C18 column (4.6×250mm, 5 µm; USA). Samples
136 were eluted with 100% methanol at 1.5 mL/min at 25 °C, and ergosterol was detected
137 by absorption with a photodiode array detector (Waters 2996; Japan) at 282 nm. The
138 injection volume was 20 µL and the retention time was around 7.25 min. The ergosterol
139 was quantified by comparing with an ergosterol standard curve in pure methanol.

140 **Emulsion-type sausage model**

141 Prior to preparation of the sausages, the frozen pork was control thawed overnight
142 in refrigerator (4 °C) immediately, and pork shoulder (70%) and pork back fat (30%)
143 were minced by a 10 mm perforated plate chopper, then according to the formulation
144 given in Table 1 the three kind of raw emulsions were prepared. In this study, 20% of
145 fermented and unfermented Okara was added to research its effects on the emulsion-
146 type sausage. All the ingredients were mixed and cut into a fine emulsion by a bowl
147 cutter below 12 °C at all times. The raw emulsions were then stuffed into 30 mm
148 diameter casings and tied into about 150 mm long using a sausage stuffer, giving each
149 sausage an weight approximate 60 g. At last, sausages were cooked for 30 min and
150 cooled to room temperature rapidly, then stored at 4 °C, and taken out for determination
151 of peroxide value after 0, 7, 14, 21 days.

152 **Determination of antioxidant activity**

153 One gram sample was mixed with 20 mL portion of distilled water and then
154 incubating at 50 °C for 4 h with continuous shaking (150 rpm) in the water bath shaker.
155 After centrifuged at 5000 g for 15 min (4 °C), the supernatant was collected and pellets
156 were re-suspension with 20 mL of distilled water and centrifuged. The supernatants
157 collected from the two centrifugation steps and then were pooled and filtered under
158 vacuum through a 0.45 µm-membrane. The collected filtrates were evaporated using a

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159 rotary evaporator (Heidolph Instruments Co., Ltd., Schwabach, Germany) to dryness
160 under a reduced pressure at 50 °C, and then re-dissolved in a known volume (25 ml)
161 for further analysis.

162 The ABTS radical cation scavenging activity of fermented and non-fermented
163 Okara was evaluated according to the method of Xiao et al. (2015a). ABTS radical
164 cation solution was generated by mixing with 7 mM aqueous solution of ABTS radical
165 cation with 2.45 mM aqueous solution of $K_2S_2O_8$. Before used, ABTS radical cation
166 solution was conducted in the dark place at room temperature for 16 h. The ABTS
167 radical cation solution was diluted to an absorbance of 0.7 ± 0.02 at 734 nm by ethanol
168 and equilibrated at 30 °C. 0.5 mL of Okara samples solution at different concentrations
169 was mixed with 2 mL diluted ABTS radical cation solution and incubated 6 min. The
170 absorbance was read using a spectrophotometer at 734 nm., The capability to scavenge
171 the ABTS radical cation was calculated by using the following equation:

172 ABTS radical cation scavenging activity (%) = $[(A_{blank} - A_{sample})/(A_{blank}] \times 100\%$

173 The DPPH radical scavenging activity of the test samples was examined according
174 to the method of Xiao et al. (2014). Briefly, 1 mL of Okara samples solution at different
175 concentrations was mixed with 1 mL of 0.2 mM DPPH solution. After 30 min of
176 incubation, the absorbance was read at 517 nm (A_{sample}). The absorbance of the DPPH
177 solution (A_{DPPH}) was also read at 517 nm. The capability to scavenge the DPPH radical
178 was calculated in light of the following equation:

179 DPPH radical scavenging activity (%) = $[(A_{DPPH} - A_{sample})/A_{DPPH}] \times 100\%$

180 **Determination of TBARS values**

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181 On days 0, 7, 14 and 21, the thiobarbituric acid reactive substances (TBARS)
182 values of randomly selected sausages (n=3) were analyzed as described by Tarladgis,
183 Watts, & Younathan (1960) and Feng et al. (2013) with minor modification. 5 g samples
184 were homogenized with 35 mL 0.1% EDTA in 7.5% trichloroacetic acid (TCA), The
185 mixture was then shaken (150 rpm) for 30 min for better extraction, and filtered twice
186 by Buchner funnel. After that, 5 mL filtrates were taken out and mixed with 5 mL 0.02
187 mol·L-1 2-thiobarbituric acid solution (TBA) and then incubated at boiling water bath
188 for 40 min, After cooled down to room temperature in cold flowing water, absorbance
189 of the solutions was recorded at 532 nm using a Multiskan GO spectrophotometer
190 (Thermo Fisher Scientific Co., Ltd., Waltham, MA, USA). Using the following
191 equation to calculate the capability to scavenge the thiobarbituric acid reactive
192 substances (TBARS) value:

193 TBARS value (mg/kg) = $A_{532} \times 7.8$

194 The results were expressed as mg MDA per kg sausage.

195 **Determination of peroxide values** (ferric thiocyanate method)

196 The peroxide values (POV) were measured by a method modified from the
197 International Dairy Federation (74A:1991). Samples (2 g) was were homogenized and
198 diluted to a volume of 10 mL with chloroform–methanol (7 + 3, v/v). 1mL homogenate
199 was taken into test tube, and mixed with 50 µL ferrous chloride solution (20 mM in 3.5%
200 HCl), then mixed with 50 µL ammonium thiocyanate solution (300 g/L). After
201 incubated at room temperature for 5 min, the absorbance of the sample was determined
202 at 500 nm against a blank that replaced the sample with chloroform–methanol (7 + 3,

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203 v/v). The contents of lipid hydroperoxides (LOOH) in samples were calculated on the
204 basis of an iron standard.

205 Statistical analysis

206 Each parameter was tested in triplicate samples, results were presented as the average
207 and standard deviation values. The data were analyzed with SPSS version 18.0. One-
208 way analysis of variance (ANOVA) was used to evaluate the difference between means
209 followed by Duncan's test. The graphs were prepared using OriginPro 8 software
210 (OriginLab Corporation, Northampton, MA, USA).

211 Results and discussion

212 Proximate Composition of Okara

213 The changes in the proximate composition of Okara during SSF are presented in
214 Table 2. The moisture content is an important parameter during SSF process. It was
215 found in Table 2 that the moisture content of the Okara increased from 77.90% to 83.36%
216 during the fermentation period, which could attribute to the metabolism of *N. crassa*
217 during fermentation. In addition, Table 2 showed that SSF with *N. crassa* significantly
218 increased crude fat, crude protein and ash content, and decreased the carbohydrate
219 contents of Okara. Previous studies of SSF have also reported a significant increase in
220 protein content as well as a remarkable decrease of the carbohydrate content when
221 compared to the non-fermented samples counterparts (Reyes-Moreno et al., 2004; Xiao
222 et al., 2015b). The increase of protein in the fermented Okara could be attributed to its
223 accumulation during fermentation. Synthesis of new proteins has been reported to occur
224 during fermentation in previous studies, and this probably explains this increase (Obeta
225 1983). In addition, Sparringa & Owens (1999) have reported that fermentation for 72 h
226 significantly (21.7%) increased the protein content, which may reflect an increase in

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227 mould biomass. Additionally, the reduction of carbohydrates content might have been
228 consumed by the *N. crassa* for its growth.

229 The main dietary fiber in Okara are plant cell wall polysaccharides (Li, Qiao & Lu,
230 2012). dietary fiber is an essential component of a healthy diet which has been
231 established by the scientific community. The health effect of dietary fiber includes the
232 regulation of the intestinal transit, and the prevention or treatment of diabetes,
233 cardiovascular diseases and cancer (Macagnan, Silva & Hecktheuer, 2016). The SSF of
234 Okara with *N. crassa* is a process by which insoluble dietary fiber is partially broken
235 down into soluble dietary fiber. Table 2 shows the changes of total dietary fiber,
236 insoluble dietary fiber, soluble dietary fiber after fermentation. The total dietary fiber
237 and insoluble dietary fiber decreased from 66.6% to 46.61% and from 61.02% to
238 40.15%, respectively. However, it was found that soluble dietary fiber increased from
239 2.27% to 5.08%, which indicated that the dietary fiber of Okara was decomposed and
240 consumed by *N. crassa*, and some insoluble dietary fiber was translated into suggested
241 that N. crassa growth have degraded the Okara DF to produce lower molecular-weight
242 soluble dietary fiber. Soluble fiber performs certain important physiological functions
243 and builds up microflora by acting as a substrate food for beneficial microorganisms,
244 therefore, it acts as a prebiotic and improves host health (Chawla & Patil, 2010). The
245 increasing soluble dietary fiber content of Okara achieved by SSF with *N. crassa* is an
246 interesting research work, which can produce sufficient health components.

247 **Growth of *N. crassa* during the SSF process**

248 Ergosterol is a specific indicator to fungi, thus, it was widely used as a sensitive
249 and reliable indicator molecule for the quantitative measure of fungal biomass. In
250 addition, ergosterol is also a precursor for the synthesis of vitamin D_2 with important
251 biological effects to the human health, so it can also provide the nutritional value for

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252 fungal fermented food products (Klamer & Bååth, 2004). In this study, ergosterol
253 content was measured which could use as the biomass quantitative marker of *N. crassa*
254 during Okara fermentation. Ergosterol content of different fermentation time of Okara
255 is shown in Figure 1. There are little ergosterol content in Okara at the beginning of
256 fermentation (before 12 h), which indicated that *N. crassa* grown slowly. However, the
257 ergosterol content was rapidly increased when the fermentation time was longer than
258 12 h. For instance, the ergosterol content was only 17.35 μg/mg when *N. crassa* grown
259 in Okara for 12 h, a significant higher ergosterol content (about 17.3 folds) after 36 h
260 of fermentation. Li et al., (2015) have also reported the similar ergosterol content
261 (maximum value was 375.3 μg/mg) of fermented Okara processed with *P. aemulans*.
262 In addition, it was fond that there was no statistically significant increase of ergosterol
263 content of fermented Okara when the fermentation time was longer than 36 h ($p < 0.05$).
264 Besides, according to the growth status of *P. aemulans*. the mycelium of *P. aemulans*
265 covered Okara completely after 48 h cultivation continually, as well as emitting a
266 uniquely fresh flavor during that period. The obtained results indicated that the biomass
267 of *N. crassa* reached the peak after 48 h of fermentation.

268 **Antioxidant activities of Okara**

269 At present, many different antioxidant assays with various mechanisms are
270 employed to measure antioxidant activity (Shahidi & Zhong, 2015). It is very difficult
271 to compare the results obtained from different assays for the variant in substrates,
272 probes, reaction conditions and quantification methods (Sreeramulu et al., 2013).
273 Therefore, in the present study, the two most commonly antioxidant assays (i.e., DPPH
274 radical scavenging activity and ABTS radical cation scavenging activity) were explored
275 to determine the antioxidant capacity of fermented and unfermented Okara, and the
276 results are shown in Figure 1a-b.

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277 DPPH is a stable nitrogen-centered and lipophilic free radical which is widely used
278 in determining the free radical scavenging activity in a relatively short time as compared
279 to the other assays. Through electron transfer or hydrogen atoms donation, antioxidant
280 compounds neutralize the DPPH free radical character and thus violet colour of the
281 reaction mixture is changed to yellow (Bhanja, Kumari & Banerjee, 2009).

282 In the present investigation, the fermented Okara showed higher DPPH radical
283 scavenging activity than unfermented Okara at different concentration. The
284 concentration of fermented Okara increased from 1 mg/mL from 20 mg/mL resulted in
285 significantly increased from 40.87 % to 82.37 % in DPPH radical scavenging activity,
286 and when the concentration of fermented Okara continue increased to 40 mg/mL, DPPH
287 radical scavenging activity increased insignificantly to 84.83%. But the DPPH radical
288 scavenging activity of unfermented Okara keep in a low value, from 5.4% to 10.97%,
289 did not markedly increase with increasing concentration like with fermented Okara.

290 The ABTS radical cation solution possesses a distinctly blue green color which
291 disappears quickly in the presence of antioxidants. The ABTS radical cation
292 decolourisation assay also showed quite similar results compared to those results
293 obtained in DPPH reaction.

294 As shown in Figure 1a, the ABTS radical cation scavenging activity of fermented
295 Okara is also higher than unfermented Okara, and was observed on the 20 mg/mL of
296 concentration (90.88 %) as compared to 1 mg/mL (14.95%). The results were found to
297 be significant at the $p < 0.05$ level.

298 In the present study, both the ABTS radical cation and DPPH radical scavenging
299 activities were enhanced after SSF with *N. crassa*. Enhanced DPPH radical and $ABTS^{+}$
300 scavenging effects through microbial fermentation has been observed in many previous
301 studies (Bhanja et al., 2009; Xiao et al., 2014). Salar, Certik & Brezova (2012) reported

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302 that maize processed by SSF with the fungus *Thamnidium elegans* for 5 days
303 significantly ($p < 0.05$) enhanced DPPH radical and ABTS radical cation scavenging
304 activity, which might be associated to the release of more soluble bioactive compounds.
305 Basically, SSF is a complex and mysterious biochemical process to increasing
306 antioxidant activity. For instance, Angulo-Bejarano et al., (2008) and Xiao et al.,
307 (2015b) reported that chickpea processed by SSF with *Rhizopus* strains or *Cordyceps*
308 *militaris* improved their nutritional properties with enhanced essential amino acids and
309 in vitro protein digestibility. Li et al. (2013) have demonstrated that Okara processed
310 by SSF with *Morchella esculenta* enhanced the antioxidant activity for the produced
311 potent antioxidant polysaccharides. Shi et al. (2012) found that polysaccharides from
312 fermented Okara showed strong antioxidant activity. In recent, Vong & Liu (2016b)
313 have also reported that the health benefits and nutritional quality of Okara were
314 enhanced by fermentation, and the fermented Okara is also an inexpensive substrate for
315 extraction of bioactive substances. Therefore, fermented Okara with good antioxidant
316 activity can be obtained by SSF.

317 **Change of lipid oxidative of sausages during storage**

318 Meat products are susceptible to lipid oxidation, because the process involves the
319 oxidation of membrane-abundant polyunsaturated fatty acids (Murphy et al., 1998).
320 POV is the most common method to measure hydroperoxides, which are the primary
321 products of lipid oxidation. TBARS is a measure of malonaldehyde (MDA) a secondary
322 product of lipids oxidation (Zaborowska et al., 2001). In this study, the lipid oxidative
323 process of emulsion-type sausage was evaluated by the POV and TBARS values in
324 order to determine how it was affected by the fermented and unfermented Okara powder
325 at different storage time, and the results are shown in Figure 2a-b. The concentration of
326 POV was significant ($p < 0.05$) at each storage day in the different treatments, and was

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327 significant ($p < 0.05$) during storage in the same treatments. The sausage with fermented
328 Okara powder addition has a smallest change in POV value after 21 days of storage,
329 increased from 0.15 to 0.70 meq/kg. During the same period, the POV value of the
330 sausage with unfermented Okara powder addition increased from 0.76 to 1.65 meq/kg,
331 and the POV value of the sausage with no addition increased from 0.42 to 1.54 meq/kg.
332 Similarly, the TBARS values of sausage with fermented Okara powder addition rose
333 rapidly from 1.69 to 2.58 mg/kg, the TBARS value of the sausage with unfermented
334 Okara powder addition increased from 0.90 to 4.55 mg/kg, and the TBARS value of
335 the sausage with no addition increased from 0.85 to 3.79 mg/kg. Both the POV and
336 TBARS value showed that the addition of fermented Okara powder had good effects
337 for preventing lipid oxidation of sausage.

338 Creation of antioxidant foods that can inhibit lipid oxidation, is expected to protect
339 human health from many geriatric diseases. Lipid oxidation is generally induced by
340 reactive free radicals and oxygen species (Zhang et al., 2015). Free radical scavenging
341 antioxidants interfere with the initiation or propagation steps of lipid oxidation
342 reactions by scavenging lipid radicals and forming low-energy antioxidant radicals that
343 do not readily promote oxidation of unsaturated fatty acids (Frankel, 1998). The higher
344 efficiency in prevention of the hydroperoxide formation correlated well with the higher
345 DPPH and ABTS radical scavenging activities (Maqsood & Benjakul, 2010). Okara
346 fermented by microbial prevent lipid oxidation has been observed in previous studies
347 (Masako 1997), Masako reported that the baked goods with addition of fermented
348 Okara showed reduced lipid oxidation and starch retrogradation during storage.
349 Chamorro et al. (2015) has reported that grape pomace extracts contain bioactive
350 antioxidants, can delay lipid oxidation during refrigeration of meat, and extending its

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351 shelf-life. Therefore, fermented Okara with good antioxidant activity can be used as
352 additive to prevent lipid oxidation of emulsion-type sausage.

353 ## Conclusions

354 Results of this investigation could obtain fermented Okara powder with both
355 added nutritive value and good antioxidant activity, have a potential as food ingredient
356 for developing a novel emulsion-type sausage that contains dietary fiber. Since the
357 product with better functionality, lower cost, more nutritional benefit and longer storage
358 life, is very importance for the emulsion processing industry. Meanwhile, the work may
359 also be significantly beneficial toward waste treatment for the tofu processing industry.

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361 ## References

362 Angulo-Bejarano PI, Verdugo-Montoya NM, Cuevas-Rodríguez EO, Milán-Carrillo J,
363 Mora-Escobedo R, Lopez-Valenzuela JA, Garzón-Tiznado JA, Reyes-Moreno, C.
364 2008. Tempeh flour from chickpea (Cicer arietinum, L.) nutritional and
365 physicochemical properties. *Food Chemistry* 106(1):106-112.
366 AOAC. 1990. Official methods of analysis (15th ed.). Washington, DC: Association of
367 Official Analytical Chemists.
368 AOAC. 1994. AOAC Official Method 991.43. Total, soluble, and insoluble dietary fiber
369 in foods. Washington DC: Association of Official Analytical Chemists.
370 Bhanja T, Kumari A, Banerjee R. 2009. Enrichment of phenolics and free radical
371 scavenging property of wheat koji prepared with two filamentous fungi.
372 *Bioresource Technology* 100(11):2861-2866.
373 Chamorro S, Viveros A, Rebolé A, Rica BD, Arija I, Brenes A. 2015. Influence of
374 dietary enzyme addition on polyphenol utilization and meat lipid oxidation of
375 chicks fed grape pomace. *Food Research International* 73:197-203.
376 Chawla R, Patil GR. 2010. Soluble Dietary Fiber. *Comprehensive Reviews in Food
377 Science and Food Safety* 9(2):178-196.
378 Chen F, Long X, Yu M, Liu Z, Liu L, Shao H. 2013. Phenolics and antifungal activities
379 analysis in industrial crop Jerusalem artichoke (Helianthus tuberosus L.) leaves.
380 *Industrial Crops & Products* 47(3):339-345.
381 Dogaris I, Vakontios G, Kalogeris E, Mamma D, Kekos D. 2009. Induction of cellulases
382 and hemicellulases from *Neurospora crassa* under solid-state cultivation for

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PeerJ Preprints NOT PEER-REVIEWED

383 bioconversion of sorghum bagasse into ethanol. *Industrial Crops and Products*,
384 29(2-3):404-411.

385 Feng XM, Eriksson AR, Schnürer J. 2005. Growth of lactic acid bacteria and *rhizopus*
386 *oligosporus* during barley tempeh fermentation. *International Journal Of Food*
387 *Microbiology* 104(3):249-256.

388 Feng T, Ran Y, Zhuang H, Rong Z, Fang Z, Wang Y, Gu Z, Jin Z. 2013. Physicochemical
389 properties and sensory evaluation of *Mesona Blumes* gum/rice starch mixed gels
390 as fat-substitutes in Chinese Cantonese-style sausage. *Food Research*
391 *International* 50(1): 85-97.

392 Frankel EN. 1998. Antioxidants. In Lipid oxidation (1st ed.). Dundee, UK: The Oily
393 Press. p. 303

394 Gedrovica I, Karklina D. 2013. Sensory evaluation of meatballs with Jerusalem
395 artichoke (Helianthus tuberosus L.). *World Academy of Science, Engineering*
396 *&Technology* 7:499-501.

397 International Dairy Federation. 1991. International IDF standards. 74A:1991. IDF-
398 Square Vergote 41, Brussels, Belgium.

399 Javidipour I, Vural H. 2002. Effects of incorporation of interesterified plant oils on
400 quality and fatty acid composition of turkish-type salami. *Molecular Nutrition &*
401 *Food Research* 46(6), 404-407.

402 Klamer M, Bååth E. 2004. Estimation of conversion factors for fungal biomass
403 determination in compost using ergosterol and PLFA 18:2ω6,9. *Soil Biology &*
404 *Biochemistry* 36(1):57-65.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.2636v1 | CC BY 4.0 Open Access | rec: 13 Dec 2016, publ: 13 Dec 2016

405 Li B, Qiao M, Lu F. 2012. Composition, nutrition, and utilization of Okara (Soybean
406 Residue). *Food Reviews International* 28(3):231-52.
407 Li S, Sang Y, Zhu D, Yang Y, Lei Z, Zhang Z. 2013. Optimization of fermentation
408 conditions for crude polysaccharides by *Morchella esculenta* using soybean curd
409 residue. *Industrial Crops and Products* 50:666-672.
410 Li Y, Meng S, Wang L, Zhang Z. 2015. Optimum fermentation condition of soybean
411 curd residue and rice bran by *Preussia aemulans* using solid-state fermentation
412 method. *International Journal of Biology* 7(3).
413 Macagnan FT, Silva LPD, Hecktheuer LH. 2016. Dietary fibre: The scientific search
414 for an ideal definition and methodology of analysis, and its physiological
415 importance as a carrier of bioactive compounds. *Food Reviews International*
416 85:144-54.
417 Mamma D, Kourtoglou E, Christakopoulos P. 2008. Fungal multienzyme production
418 on industrial by-products of the citrus-processing industry. *Bioresource Technology*
419 99(7):2373-2783.
420 Maqsood S, Benjakul S. 2010. Comparative studies of four different phenolic
421 compounds on in vitro antioxidative activity and the preventive effect on lipid
422 oxidation of fish oil emulsion and fish mince. *Food Chemistry* 119(1):123-132.
423 Mateos-Aparicio I, Redondo-Cuenca A, Villanueva-Suárez MJ. 2010. Isolation and
424 characterisation of cell wall polysaccharides from legume by products: Okara
425 (soymilk residue), pea and broad bean pod. *Food Chemistry* 122(1):339-345.
426 Matsuo M. 1999. Application of Okara Koji, Okara Fermented by Aspergillus

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.2636v1 | CC BY 4.0 Open Access | rec: 13 Dec 2016, publ: 13 Dec 2016

427 oryzae,for Cookies and Cupcakes. *Journal of Home Economics of Japan* 50:1029-
428 1034.
429 Murphy A, Kerry JP, Buckley J, Gray I. 1998. The antioxidative properties of rosemary
430 oleoresin and inhibition of off-flavours in precooked roast beef slices. *Journal of*
431 *the Science of Food and Agriculture* 77:235–243
432 Obeta JAN. 1983. A note on micro-organisms associated with the fermentation of
433 African locust bean (Parkia filicoidea) during iru preparation. Plant Foods For
434 Human Nutrition 3:245-250.
435 ÖZvural EB, Vural H. 2008. Utilization of interesterified oil blends in the production
436 of frankfurters. *Meat Science* 78(3):211-216.
437 Rao M, Mishra C, Keskar S, Srinivasan, MC. 1985. Production of ethanol from wood
438 and agricultural residues by *Neurospora crassa*. *Enzyme and Microbial*
439 *Technology* 7(12): 625-628.
440 Reyes-Moreno C, Cuevas-Rodríguez EO, Milán-Carrillo J, Cárdenas-Valenzuela OG,
441 Barrón-Hoyos J. (2004). Solid state fermentation process for producing chickpea
442 (Cicer arietinum L) tempeh flour. Physicochemical and nutritional characteristics
443 of the product. *Journal of the Science of Food and Agriculture* 84(3),:271-278.
444 Romero MD, Aguado J, González L, Ladero M. 1999. Cellulase production by
445 *Neurospora crassa* on wheat straw. *Enzyme and Microbial Technology* 25(3-
446 5):244-250.
447 Salar RK, Certik M, Brezova V. 2012. Modulation of phenolic content and antioxidant
448 activity of maize by solid state fermentation with *Thamnidium elegans* CCF 1456.
449 *Biotechnology and Bioprocess Engineering* 17(1):109-116.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.2636v1 | CC BY 4.0 Open Access | rec: 13 Dec 2016, publ: 13 Dec 2016

450 Serrano A, Librelotto J, Cofrades S, Sánchezmuniz FJ, Jiménezcolmenero F. 2007.
451 Composition and physicochemical characteristics of restructured beef steaks
452 containing walnuts as affected by cooking method. *Meat Science* 77(3), 304-313.
453 Shahidi F, Zhong Y. 2015. Measurement of antioxidant activity. *Journal of Functional*
454 *Foods* 18: 757-781.
455 Shi M, Yang Y, Guan D, Zhang Y, Zhang Z. 2012. Bioactivity of the crude
456 polysaccharides from fermented soybean curd residue by *Flammulina velutipes.*
457 *Carbohydrate Polymers* 89:1268-1276.
458 Sparringa RA, Owens JD. 1999. Protein utilization during soybean tempe fermentation.
459 *Journal of Agricultural and Food Chemistry* 47(10): 4375-4378.
460 Sreeramulu D, Reddy CVK, Chauhan A, Balakrishna N, Raghunath M. 2013 Natural
461 antioxidant activity of commonly consumed plant foods in India: effect of
462 domestic processing. *Oxidative Medicine and Cellular Longevity* (2013):1-12.
463 Tarladgis BG, Watts BM, Younathan MT. 1960. A distillation method for the
464 quantitative determination of malonaldehyde in rancid foods. Journal of American
465 Oil Chemical Society 37:44–48.
466 Vishwanathan KH, Singh V, Subramanian R. 2011. Influence of particle size on protein
467 extractability from soybean and Okara. *Journal of Food Engineering* 102:240-246.
468 Vong WC, Liu SQ. 2016a. Biovalorisation of Okara (soybean residue) for food and
469 nutrition. *Trends in Food Science & Technology* 52:139-47.
470 Vong WC, Liu SQ. 2016b. Changes in volatile profile of soybean residue (Okara) upon
471 solid-state fermentation by yeasts. *Journal of the Science of Food & Agriculture*.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.2636v1 | CC BY 4.0 Open Access | rec: 13 Dec 2016, publ: 13 Dec 2016

472 http://dx.doi.org/10.1002/jsfa.7700.

473 Xiao Y, Wang L, Rui X, Li W, Chen X, Jiang M, Dong M. 2015a. Enhancement of the
474 antioxidant capacity of soy whey by fermentation with *Lactobacillus plantarum*
475 B1–6. *Journal of Functional Foods* 12:33-44.

476 Xiao Y, Xing G, Rui X, Li W, Chen X, Jiang M, Dong M. 2014. Enhancement of the
477 antioxidant capacity of chickpeas by solid state fermentation with *Cordyceps*
478 *militaris* SN-18. *Journal of Functional Foods* 10:210-222.

479 Xiao Y, Xing G, Rui X, Li W, Chen X, Jiang M, Dong M. 2015b. Effect of solid-state
480 fermentation with *Cordyceps militaris* SN-18 on physicochemical and functional
481 properties of chickpea (*Cicer arietinum* L.) flour. LWT-Food Science and
482 Technology 63(2):1317-1324.

483 Xiros C, Topakas E, Katapodis P, Christakopoulos P. 2008. Hydrolysis and fermentation
484 of brewer's spent grain by Neurospora crassa. *Bioresource Technology*
485 99(13):5427-5435.

486 Zaborowska Z, Uchman W, Bilska A, Jeleń H, Rudzińska M, Wąsowicz E, Kummerow
487 FA. 2001. Effect of Storage on Oxidation of Cholesterol and Lipids in Liver Pate
488 Type Sausage. *Electronic Journal of Polish Agricultural Universities*.4(2).

489 Zhang Y. Shen Y, Zhu Y, Xu Z 2015. Assessment of the correlations between reducing
490 power, scavenging DPPH activity and anti-lipid-oxidation capability of phenolic
491 antioxidants. *Lebensmittel-Wissenschaft und-Technologie* 63(1):569-574.

492 Zhu YP, Cheng YQ, Wang LJ, Fan JF, Li LT. 2008. Enhanced antioxidative activity of
493 Chinese traditionally fermented Okara (Meitauza) prepared with various
494 microorganism. *International Journal of Food Properties* 11(3):519-529.

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495 List of Tables:

496 Table 1 Formulation for sausage batters. FS, sausage with fermented Okara； US,
497 sausage with unfermented Okara; CK, sausage without Okara.

498

499 Table 2 The changes of the proximate composition of Okara at different incubation
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501 (a,b) in the same row indicate significant differences from each other ($p < 0.05$). The
502 results of crude protein, crude fat, ash and carbohydrate contents were calculated based
503 on the dry matter and expressed as grams per 100 g of dry basis. The moisture was
504 calculated based on the wet matter and expressed as grams per 100 g of wet basis.

505

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507 Fig. 1 Changes of ergosterol content of Okara fermented by *N. crassa*. The values
508 shown represent means ± SD of triplicate assays (n = 3). Lowercase letters (a−c) over
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510

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515

516 Fig. 3 Changes of lipid oxidation (TBARS) in emulsified pork sausages during 3 weeks

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517 of cold storage. Data are means ± standard error of triplicate assays (n = 3). Different
518 superscripts on the bar indicate significant differences among the treatments (A–C) at
519 each storage day ($p < 0.05$) or storage weeks (a-d) in each treatment ($p < 0.05$). FS,
520 sausage with fermented Okara；US, sausage with unfermented Okara; CK, sausage
521 without Okara.
522

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523 **Table 1**

524 Formulation for sausage batters.

Ingredients	CK(g)	US(g)	FS(g)
Pork shoulder	840	770	770
pork back fat	360	330	330
Unfermented Okara	/	20	/
Fermented Okara	/	/	20
Water	240	320	320
Salt (NaCl)	18	18	18
Sodium nitrite	0.048	0.048	0.048
Sodium tripolyphosphate	2.88	2.88	2.88
Sodium pyrophosphate	1.92	1.92	1.92
Aginomoto	2.4	2.4	2.4

525

526 **Table 2**

527 The changes of the proximate composition of Okara after incubation with *N. crassa.*

	Unfermented Okara	Fermented Okara
Moisture (g/100g)	77.90 ± 0.24^{a}	83.36 ± 0.39^{b}
Crude fat (g/100g)	6.67 ± 0.43^{a}	9.52 ± 0.31^{b}
Crud protein (g/100g)	17.52 ± 0.22^{a}	24.54 ± 0.28^{b}
Ash (g/100g)	2.81 ± 0.05^{a}	3.86 ± 0.08^{b}
Total dietary fiber (g/100g)	66.60 ± 0.94^{b}	49.28 ± 0.87^{a}
Insoluble dietary fiber (g/100g)	64.23 ± 1.03^{b}	44.2 ± 0.7^{a}
Soluble dietary fiber (g/100g)	2.36 ± 0.19^{a}	5.08 ± 0.51^{b}

528 Results are presented as the Mean ± SD (n = 3). Lowercase letters (a,b) in the same row
529 indicate significant differences from each other ($p < 0.05$). The results of crude protein,
530 crude fat, ash and carbohydrate contents were calculated based on the dry matter and
531 expressed as grams per 100 g of dry basis. The moisture was calculated based on the
532 wet matter and expressed as grams per 100 g of wet basis.

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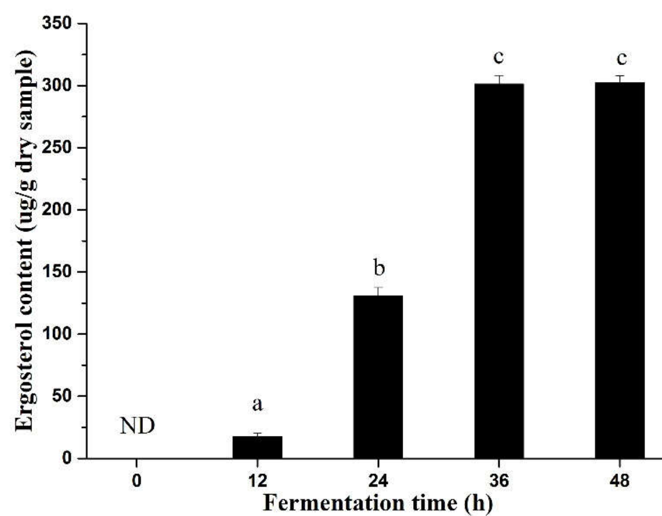

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535 Fig. 1

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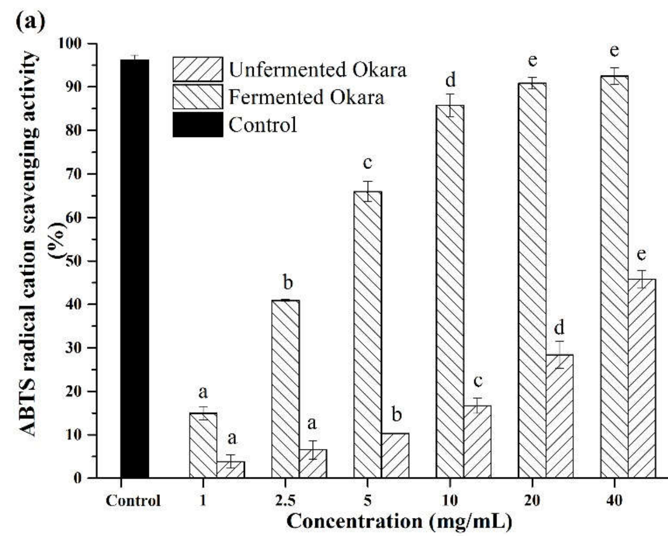

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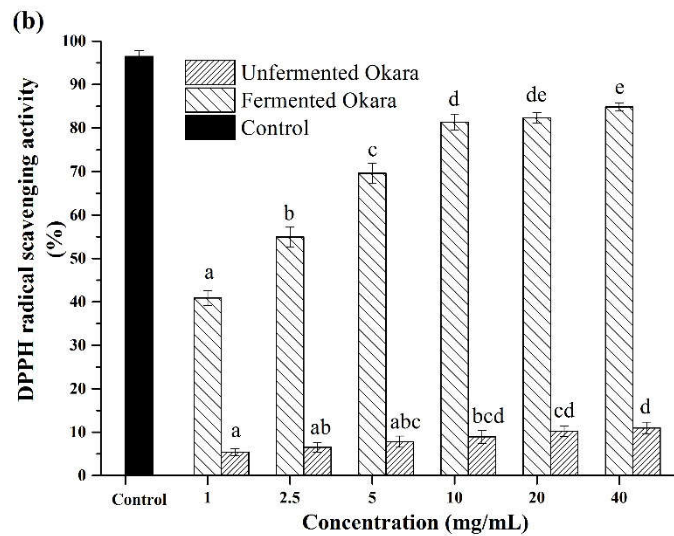

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538 Fig. 2

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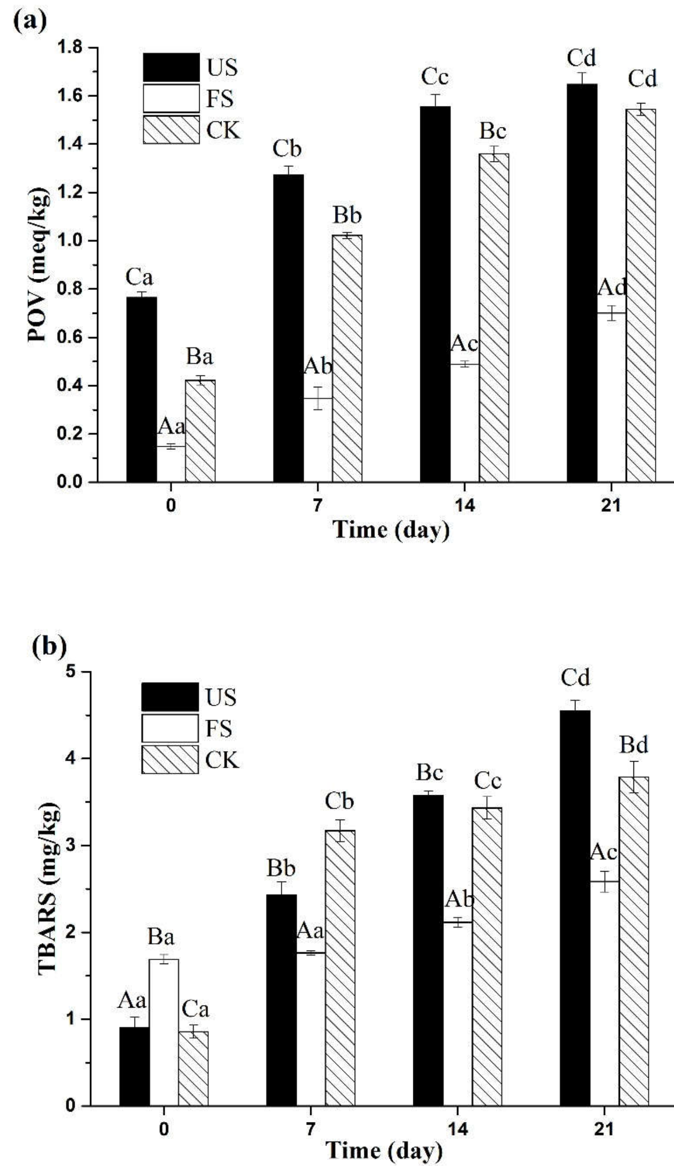

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541

542 Fig. 3

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