1 **Effects of soy hull pectin and insoluble fiber on**
2 **physicochemical and oxidative characteristics of fresh**
3 **and frozen/thawed beef patties**

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24 **Abstract**

25 The objective of this study was to determine the effects of pectin and insoluble
26 fiber isolated from soy hulls on water-holding capacity (WHC), texture, color, and lipid
27 oxidation of fresh and frozen/thawed beef patties. Beef patties were formulated with no
28 dietary fiber (control), 1% soy hull pectin, insoluble fiber, or their mixture (1:1),
29 respectively. The addition of soy hull pectin significantly decreased display weight loss
30 and increased cook yield of both fresh and frozen/thawed beef patties. In addition, no
31 significant difference in hardness between fresh and frozen/thawed beef patties was
32 observed for all dietary fiber treatments. However, incorporation of insoluble soy hull
33 fiber decreased color and lipid oxidation stabilities of both fresh and frozen/thawed
34 beef patties. Our results indicate that the incorporation of soy hull pectin could be an
35 effective non-meat ingredient to minimize water loss and hardness defects of frozen
36 beef patties.

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38 **Keywords:** beef patty, dietary fiber, freezing, pectin, soy hulls

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46 **1. Introduction**

47 Freezing has extensively been practiced in the meat industry as well as in
48 households, since it is one of the most effective ways to preserve meat and meat
49 products. According to the consumer survey conducted through the National
50 Cattlemen's Beef Association (NCBA, 2011), 44% of beef patties were sold fresh,
51 whereas the remaining 56% were sold frozen. Moreover, the survey found that 47% of
52 the beef patties that consumers purchased fresh from retail stores actually ended up
53 being stored in household freezers. This survey result implies that a majority of the beef
54 patties undergo the freezing/thawing process in the food supply chain from
55 manufacturers to consumers.

56 Despite the positive impact of freezing on the extension of shelf life, however, the
57 quality deterioration related to freezing/thawing, such as a decrease in water-holding
58 capacity (WHC), discoloration, texture, and/or lipid oxidation has been reported. In
59 particular, the decline of WHC due to the damage of muscle structure by ice crystal
60 formation causes the excessive moisture loss during thawing/cooking and the decrease
61 of tenderness of frozen/thawed beef patties (Bhattacharya, Hanna, & Mandigo, 1988;
62 Berry & Leddy, 1989; Georgantelis et al., 2007; Hanenian, Mittal, & Usborne, 1989).

63 Dietary fiber obtained from several plants has been substantially used as a functional
64 non-meat ingredient, and their physiological and technological benefits largely differ
65 from its solubility in water (Talukder, 2015). Considering positive effects of dietary
66 fiber on WHC, freeze/thaw stability, and texture of processed meat products, in general,
67 it could be expected that dietary fiber incorporation can minimize the quality changes
68 of frozen meat products due to freezing/thawing process. In this regard, some fiber-rich
69 sources, such as oat's soluble fiber (Piñero et al., 2008), wakame (López-López et al.,

2010), and walnut (Jiménez Colmenero et al., 2003) have been applied. However, there has been little information on the comparison on insoluble and soluble fibers as a functional non-meat ingredient in frozen beef patties.

Pectin, which is one of water-soluble hydrocolloids, has been previously utilized as an effective fat replacer, texture modifier in meat products (Cardoso, et al., 2013; Thakur et al., 1997), and a cryo-protectant in fish surimi (Sych et al., 1990). Recently, pectin and insoluble fiber (residues after pectin extraction) from soy hulls, which are the seed coats of soybeans, has been evaluated for its potential efficacy for the meat application. Kim, Lee, and Kim (2015) found that the addition of both soy hull pectin and insoluble fiber improved WHC and textural properties of meat emulsions. Furthermore, Kumar et al. (2011, 2013) reported that soy hull flour could delay the lipid oxidation of chicken nuggets.

Given the observed functional characteristics of dietary fiber from soy hulls, it could be postulated that the incorporation of soluble pectin and insoluble fiber from soy hulls can improve some quality attributes of frozen/thawed beef patties. Therefore, the aim of this study was to determine the effects of pectin and insoluble fiber isolated from soy hulls on WHC, texture, color, and lipid oxidation of fresh and frozen/thawed beef patties.

2. Materials and methods

2.1. Preparation of soy hull pectin and insoluble fiber suspension

The isolation of pectin (by acid extraction and alcohol-precipitation) and insoluble fiber (by alkali hydrolysis after pectin extraction) from soy hulls was conducted with the method described by Kim, Lee, and Kim (2015). Dietary fiber suspensions of the

pectin, insoluble fiber, and their mixture(1:1),wereprepared by mixing with 5 volumes of deionized distilled water (1:5, v/w), respectively, and stirred at room temperature for 1 h.

2.2. Beef patty manufacture

Beef rounds (*M. semimembranosus*) and beef back fat from same beef carcasses were obtainedat the Purdue University Meat Laboratory at 7 days postmortem, vacuum-packaged, and stored at -40 °C until use.Thefrozen beef muscle and fat were thawed in a 2 °C cooler for 24 h.After the removal of visible connective tissue and excess fat from the beef muscle, thebeef muscle and fat were ground through a 3/8 inch plate using a meat grinder (M-12-FS, Torrey, Monterrey, NL, Mexico). Beef patties were formulated with 80.75% beef lean, 14.25% beef fat, and 5% cold water as a control, whereasdietary fiber treatments were prepared with 79.75%% beef lean portion,14.25% beef fat, and each 6% dietary fiber suspension prepared (1% dietary fiber and 5% cold water based on total weight), respectively. All ingredients were manually mixed by hand for 5 min, and then, re-ground through a1/4 inch plate to be mixed uniformly. A total of eighteen patties (approximately 100 g each) per treatment was manufactured using a round shape patty maker (4.5 inch × 2.75 inch). Nine patties were analyzed as a fresh treatment on the manufacture day, whereasthe other nine patties as a frozen treatment were weighed, placed on a wax paper, vacuum-packaged, and stored at -40 °C for 1 month.All frozen patties were thawed in a 2 °C cooler for 24 h and re-weighed after blotting with a blot paper to calculate a thaw loss. To assess display weight loss and lipid oxidation,two patties from each fresh and frozen/thawed treatment were placed on a Styrofoam tray, over-wrapped with PVC film, and

118 displayed in a 2 °C cooler for 5 days under continuous fluorescent natural white light
119 (3,500 K).

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121 2.3. Physicochemical and textural analysis

122 The pH value of raw beef patties was determined in duplicate from two patties
123 using an electronic pH meter (Sartorius Basic Meter PB-11, Sartorius AG, Germany).

124 Surface color of raw beef patties was determined using a Hunter MiniScan EZ
125 colorimeter (Hunter, Reston, VA, USA). CIE L* (lightness), a* (redness), and b*
126 (yellowness) values were recorded at 1, 3, and 5 day retail display from five random
127 spots from three patties (15 readings per treatment). Hue angle was calculated using
128 the following expression; $\text{hue angle} = \tan^{-1}(b^*/a^*)$ (AMSA, 2012).

129 Thaw loss from all frozen patties was calculated as the weight difference between
130 initial and frozen/thawed samples, which was expressed as a percentage loss.
131 Cook yield was determined in quadruplicate by the weight differences between raw and
132 cooked patties. Four beef patties per treatment were cooked in a pre-heated electric
133 grill (135 °C) until the targeted core temperature reached 71 °C monitored by using a
134 digital thermometer equipped with a data logger. Display weight loss was calculated in
135 duplicate as the weight difference of displayed beef patties between 0 and 5 days.

136 Texture profile analysis was performed in quadruplicate from four patties
137 according to the method of Bourne (1978) described by Claus and Sørheim (2006)
138 using a TA-XT Plus Texture Analyzer (Stable Micro Systems Ltd., Surrey, UK).

139 On 0 and 5 days of display, lipid oxidation of fresh and frozen/thawed beef patties
140 was assessed in duplicate by 2-thiobarbituric reactive substances (TBARS) assay
141 described by Buege and Aust (1978).

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143 2.4. Statistical analysis

144 Experimental design was a completely randomized design with a total of three
145 independent batches. The dietary fiber, freezing/thawing, and display time (for TBARS
146 and color stability) were considered as the main effects. All data were analyzed using
147 the PROC MIXED procedure of SAS 9.4 software (SAS Institute Inc.). If there was no
148 significant interaction between the main effects ($P > 0.05$), pooled data were used to
149 compare the main effect means (display weight loss and TBARS). Least squares means
150 for all traits were separated (F test, $P < 0.05$) by using least significant differences.

151

152 3. Results and discussion

153 3.1. pH and water-holding capacity (WHC)

154 The effects of soy hull pectin and insoluble fiber on pH and WHC of fresh and
155 frozen/thawed beef patties are shown in Table 1. A significant interaction between
156 dietary fiber and freezing/thawing effects on pH and cook yield of beef patties was
157 found. Fresh beef patties containing soy hull pectin (pectin and mixture treatments)
158 had a lower pH than the other fresh beef patties ($P < 0.05$). Similar results were
159 observed in previous studies, in which the acidic pH of pectin source decreased the pH
160 of meat products (Kim, Lee, & Kim, 2015; Méndez-Zamora et al., 2015; Sariçoban et
161 al., 2010). However, among frozen/thawed treatments, such difference in pH was not
162 observed ($P > 0.05$). The acidic pH of pectin is related to the presence of
163 polygalacturonic acid and acidic sugar (Sariçoban et al., 2010). Thus, the release of
164 these acidic components during thawing, as included in thaw drip, might be
165 responsible for the observed no differences in pH among frozen/thawed beef patties.

Although not significant, thaw loss of dietary fiber treatments (2.62-2.97%) was slightly lower than that of the control (3.76%). In fresh beef patties, dietary fiber treatments (68.4-69.0%) had a significantly higher cook yield than the control (64.7%). The cook yield of frozen/thawed control (61.7%) was lower than that of fresh control ($P < 0.05$). However, dietary fiber treatments exhibited similar cook yield between fresh and frozen/thawed beef patties ($P > 0.05$). In particular, pectin treatment showed the highest cook yield among all treatments, regardless of freezing/thawing ($P < 0.05$). Display weight loss of dietary fiber treatments (2.0-2.2%) was significantly lower than that of control (2.8%). In general, it has been known that frozen/thawed meat has inferior WHC due to structural damage and/or muscle protein denaturation by ice crystal formation (Leygonie, Britz, & Hoffman, 2012). Previously, it was reported that the addition of dietary fiber into frozen beef patties could lead to the improvement of WHC due to water and fat retention abilities in the fiber matrix (López-López et al., 2010; Piñero et al., 2008). Our result was also in agreement with the previous finding. Furthermore, it has been suggested that pectin could be directly involved in the ice crystal formation process by affecting size and crystal growth in frozen foods (Thakur et al., 1997). For this reason, pectin has been particularly used as a cryo-protectant in fish surimi. Sych et al. (1990) suggested that 1% pectin addition could stabilize myofibrillar proteins, mainly myosin. In addition, according to Kim, Lee, and Kim (2015), soy hull pectin had a higher water binding capacity than insoluble fiber fraction. Thus, the notable effect of pectin for improving WHC of frozen/thawed beef patty might be associated with the superior water binding capacity of pectin and/or its cryo-protective effect. Further studies would be needed to elucidate the underlying mechanisms of the positive effect of soluble and insoluble fiber has on

190 WHC of frozen meat products and determine the cryo-protective effect of pectin on
191 meat proteins, particularly.

192

193 3.2. Texture

194 In textural properties (Table 1), a significant interaction between dietary fiber and
195 freezing/thawing effects was only found in hardness ($P < 0.05$). Meanwhile,
196 springiness, gumminess, and chewiness of beef patties were not affected by dietary
197 fiber addition or freezing/thawing ($P > 0.05$). Dietary fiber treatments, particularly the
198 pectin and mixture treatments, exhibited lower hardness than the control in both the
199 fresh and the frozen/thawed beef patties ($P < 0.05$). Similarly, previous studies
200 reported that dietary fiber incorporation reduced instrumental hardness of beef patties
201 (Aleson-Carbonell et al., 2005; López-López et al., 2010; Troutt et al., 1992).
202 According to López-López et al. (2010), the reduction in hardness could be associated
203 with the improvement of moisture retention ability by adding dietary fiber. Thus, our
204 finding on hardness could be attributed to the improved moisture retention, since beef
205 patties formulated with soy hull pectin and/or insoluble fiber had significantly higher
206 cook yield compared to control. Control exhibited a significant difference in hardness
207 between fresh and frozen/thawed beef patties. However, similar hardness between
208 fresh and frozen/thawed treatments was observed for all dietary fiber treatments ($P >$
209 0.05). Previously, numerous studies reported that a loss of excessive water during
210 thawing and the myofibrillar protein damages (e.g. denaturation and oxidation) during
211 frozen storage could result in the toughening of frozen/thawed beef patties (López-
212 López et al., 2010; Utrera, Morcuende, & Estévez, 2014). Thus, in this study, no
213 difference in hardness of dietary fiber treatments between fresh and frozen/thawed

214 beef patties might be associated with the decreased thaw loss and/or increased cook
215 yield due to dietary fiber addition.

216

217 3.3. Color stability

218 Changes in CIE a^* (redness, Fig. 1A) and hue angle (the degree of discoloration,
219 Fig. 1B) of fresh and frozen/thawed beef patties during 5 days retail display were
220 determined. The redness of beef patties was affected by display period, dietary fiber,
221 freezing/thawing, and an interaction between dietary fiber and freezing effects ($P <$
222 0.05). Between fresh beef patties, insoluble fiber and mixture treatments had a lower
223 redness than control during the entire display period ($P < 0.05$). However, there was
224 no difference in redness between frozen/thawed beef patties ($P > 0.05$). During 5 day
225 retail display, the fresh beef patties formulated with insoluble soy hull fiber discolored
226 rapidly ($P < 0.05$), whereas the degree of discoloration was similar
227 among frozen/thawed beef patties, regardless of the addition of dietary fiber ($P > 0.05$).
228 The initial difference in redness might be explained by the unique brown color of
229 insoluble fiber from soy hulls. As similar results, Kumar et al. (2011) and Kim et
230 al. (2015) reported that soy hull flour decreased the redness of meat products. In
231 general, the color stability of frozen/thawed meat is affected by the loss of myoglobin
232 as a drip and/or myoglobin oxidation. In particular, myoglobin oxidation is related to a
233 decrease in endogenous reducing systems and/or coupling with oxidative condition
234 including lipid oxidation (Leygonie, Britz, & Hoffman, 2012). The results from the
235 current study indicate that insoluble soy hull fiber might negatively affect the color
236 stability of fresh beef patties. However, the observed discoloration due to dietary fiber
237 addition was more prevalent in fresh beef patties compared to their frozen/thawed

238 counterparts. This observation was likely associated with overriding impacts of
239 freezing/thawing on surface color darkening (Kim et al., 2015), which might mask the
240 extent of discoloration of frozen/thawed beef patties in the display conditions with
241 dietary fiber.

242

243 3.4. Lipid oxidation

244 Regarding 2-Thiobarbituric acid reactive substances value (TBARS) of beef
245 patties on 0 and 5 days of display (Fig. 1C), there were no significant interactions
246 between the main effects, dietary fiber, freezing/thawing, and display time. Thus, the
247 results were expressed using pooled data, which was affected by each dietary fiber
248 and freezing/thawing effects ($P < 0.05$). Insoluble fiber treatments showed a
249 significantly higher TBARS value compared to the other treatments, which indicates
250 the addition of insoluble soy hull fiber might negatively affect lipid oxidation of both
251 fresh and frozen/thawed beef patties during display time. This result did not agree with
252 the previous findings of Kumar et al. (2011, 2013) who reported that soy hull flour
253 could prevent the lipid oxidation of chicken nuggets. Previous studies reported that
254 soybean has remarkable antioxidant activity, resulting from the presence of
255 polyphenolic components (Prakash et al., 2007). However, potential prooxidant effects
256 of soyhulls due to lipoxygenase (Baker & Mustakas, 1973) and ferrous iron (Laszlo,
257 1988) were also reported. Thus, the increased lipid oxidation might be affected by the
258 residual prooxidant components in insoluble fiber derived from soy hulls. Further
259 studies would be warranted to determine this postulation, as well as, the effects of
260 addition of other anti-oxidants and/or different retail packaging systems on oxidation
261 stability of beef patties with dietary fiber incorporation.

262

263 **4. Conclusion**

264 In conclusion, 1% dietary fiber incorporation (both soy hull pectin and insoluble
265 fiber) improved WHC of both fresh and frozen/thawed beef patties. In particular, soy
266 hull pectin could eliminate the differences in cook yield and hardness between fresh
267 and frozen/thawed beef patties without diverse impacts on color and lipid oxidation.
268 Our results indicate that soy hull pectin could be an effective non-meat ingredient to
269 minimize water loss and texture defects of frozen beef patties.

270

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273

274 **Conflicts of interest**

275 The authors declare that there are no conflicts of interest.

276

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Figure Captions

Fig. 1. Effects of soy hull pectin and insoluble fiber on color stability (A, redness and B, hue angle) and lipid oxidation (C) of fresh and frozen/thawed beef patties. Pooled means for fresh and frozen/thawed beef patties were used for the TBARS results of the initial (0 d) and end of display (5 d). Control, without dietary fiber; Insoluble fiber, replacement of 1% beef lean with 1% insoluble soy hull fiber; Pectin, replacement of 1% beef lean with 1% soy hull pectin; Mixture, replacement of 1% beef lean with the mixture of 0.5% soy hull pectin and 0.5% insoluble soy hull fiber. Error bars indicate standard errors of the mean. a, b the different letters between each treatment are significantly different ($P < 0.05$).

Fig. 1. Effects of soy hull pectin and insoluble fiber on color stability (A, redness and B, hue angle) and lipid oxidation (C) of fresh and frozen/thawed beef patties. Pooled means for fresh and frozen/thawed beef patties were used for the TBARS results of the initial (0 d) and end of display (5 d). Control, without dietary fiber; Insoluble fiber, replacement of 1% beef lean with 1% insoluble soy hull fiber; Pectin, replacement of 1% beef lean with 1% soy hull pectin; Mixture, replacement of 1% beef lean with the mixture of 0.5% soy hull pectin and 0.5% insoluble soy hull fiber. Error bars indicate standard errors of the mean. a, b the different letters between each treatment are significantly different ($P < 0.05$).

Table legend

Table 1. Effects of soy hull pectin and insoluble fiber on pH, WHC, and texture of fresh and frozen/thawed beef patties

Table 1. Effects of soy hull pectin and insoluble fiber on pH, WHC, and texture of fresh and frozen/thawed beef patties

Treatments ^A	pH	WHC (%)			Texture profile analysis				
		Thaw loss	Cook yield	Display weight loss ^B	Hardness (N)	Springiness	Cohesive -ness	Gumminess (N)	Chewiness (N)
<i>Fresh beef patties</i>									
Control	5.60b	-	64.74c	2.79a	4.32b	0.75	0.67	2.07	1.55
Insoluble fiber	5.61b	-	68.98b	2.15b	4.24b	0.73	0.67	2.08	1.54
Pectin	5.47c	-	69.02a	2.03b	3.65c	0.73	0.67	1.75	1.26
Mixture (1:1)	5.54c	-	68.35ab	2.22b	3.61c	0.71	0.66	1.71	1.24
<i>Frozen/thawed beef patties</i>									
Control	5.76ab	3.76	61.72d	-	5.99a	0.68	0.59	2.54	1.87
Insoluble fiber	5.82ab	2.75	66.49bc	-	5.23b	0.69	0.62	2.20	1.62
Pectin	5.84a	2.62	70.09a	-	4.03c	0.67	0.61	1.67	1.17
Mixture (1:1)	5.83a	2.97	67.65b	-	4.59c	0.68	0.61	1.91	1.37
SED ^C	0.044	0.537	0.805	0.132	0.356	0.036	0.014	0.089	0.652
<i>Significance of P value</i>									
Dietary fiber	0.3062	0.2460	0.0002	0.0018	<0.0001	0.4944	0.5173	0.0737	0.0815
Freezing/thawing	0.0559	-	0.1480	<0.0001	0.0854	0.3982	0.0224	0.4635	0.6076
Interaction	0.0042	-	0.0466	0.0832	0.0189	0.4370	0.2944	0.0918	0.1079

^ATreatments: control, without dietary fiber; Insoluble fiber, replacement of 1% beef lean with 1% insoluble soy hull fiber; Pectin, replacement of 1% beef lean with 1% soy hull pectin; Mixture, replacement of 1% beef lean with the mixture of 0.5% soy hull pectin and 0.5% insoluble soy hull fiber.

^BPooled data from both fresh and frozen/thawed beef patties.

^cSED: standard errors of difference.

a-dLeast squares means within a column lacking a common superscript letter indicate significant differences ($P < 0.05$).