Antioxidant, antimicrobial and curing potentials of micronized celery powders added to pork sausages

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Abstract

Meat industries utilize plant material such as celery in cured meat products. Extraction of valuable bioactive compounds, nitrates and nitrites often involves processes that increase cost or lack sustainability. Thus, this study investigated the effect of ball-milled celery powders on the physicochemical, antioxidant, and antimicrobial properties along with curing efficiency in comminuted meat product. Pork sausages loaded with celery powders with different average particle sizes: 265 µm (T1), 68 µm (T2) and 7 µm (T3) were compared to those added without and with sodium nitrite (150 ppm). a* values were increased for sausages with larger particle size. The L* values decreased for all celery powders. Residual nitrite for all particle sizes increased in the earlier stages and decreased at the end of storage period. The curing efficiency also increased for larger size particles with an increase until day 9 followed by a gradual decrease. Superfine celery powder had a tendency to improve the antioxidant activities. The antimicrobial effect of celery powders was not comparable with nitrite added sausages. The textural parameters remained unaffected by particle size. Thus, instead of extracts or juices, micronized celery powders could be used to improve the antioxidant activities and curing efficiency of label friendly reformulated meat products.

Keywords: Celery powder, Ball-mill, Natural, Nitrite, Pork sausage

Introduction

Nitrate (NO_3) and nitrite (NO_2) found naturally in the environment are also often utilized in several food products (Moorcroft et al., 2001). These incidentally discovered ions have been used to cure meats for centuries (Pierson and Smoot, 1982). Before the discovery of the curing process, salt was used to preserve meat. However, improved preservation and reddish color development was noticed with a certain type of salt that contained an impurity known as saltpeter, which is a common name for nitrates (Binkerd and Kolari, 1975). Apart from cured meat color, nitrite, which is formed by reduction of nitrate, is also well known for improving aroma, flavor, antioxidant effect, and antimicrobial activity, particularly against pathogen *Clostridium botulinum* (Shahidi and Pegg, 1992; Xi et al., 2012). Despite these advantages, the use of nitrites in meat has been associated with carcinogenic nitrosamines (Sindelar and Milkowski, 2011; Sebranek et al., 2012). Concerns are further raised by occasional reports that link addition of nitrite into meat with cancer. Due to the lack of equivalent substitute for conventional sodium nitrites and nitrates (Gray et al., 1981), natural plant materials are being studied. Among various plants, vegetables such as lettuce, beets, radishes, spinach, and celery are known to contain high concentrations of nitrates as precursors of nitrite (Sindelar et al., 2012). Meat industry has exploited such rich natural sources of nitrate to produce cured meats.

To reduce nitrate to nitrite, starter cultures are included to result in the characteristic cured meat color and flavor (Moler et al., 2003; Sebranek et al., 2012). However, the reduction of nitrate to nitrite by microbes is both time-consuming and cost intensive. Moreover, traditionally cured meats were produced with age-old method using naturally present nitrate reducing bacteria to convert nitrate to nitrite (Cassens, 1990). For several

years, celery (*Apium graveolens* var. dulce) due to its high nitrate and nitrite contents that has been utilized as a curing and flavoring agent in meat products (Bacus, 1984). Processors usually make use of celery powders as a natural source of nitrate and nitrite in meat products since celery also has low vegetable pigment content along with mild flavor (Sebranek and Bacus, 2007; Horsch, 2014). Furthermore, other constituents of celery such as proteins, fibers, and carbohydrates can positively or negatively influence the formation of nitric acid (Djeri, 2010). Nitric acid in turn can affect the growth of pathogens such as *Clostridium botulinum* and *Listeria monocytogenes* (Horsch, 2014). Celery is also known to contain phenolic acids (such as ellagic acid and pyrogallol) and flavonoids (such as quercetin and hesperitin) that contribute to its antioxidant activities (Sorour et al., 2015). Therefore, this plant with its various constituents can serve many purposes in food applications. Another major consideration is the high demand for natural and organic products because they are perceived to be healthy (Djeri and Williams, 2014).

Extraction of bioactive compounds, nitrites and nitrates is commonly achieved via physical and chemical methods that increase production costs. Furthermore, chemical methods such as solvent extraction have demerits that include low extractability and toxic chemical remnants (Huang et al., 2008). Planetary ball-mill treatment is an environment friendly milling technique wherein materials are placed in a container along with balls (Khadka et al., 2014; Ramachandraiah and Chin, 2016). While various sizes and types of container and balls are available, stainless steel and ceramic are commonly used (Khadka et al., 2014). This low cost milling method is known to reduce the particle size of dietary fibers, thereby influencing its physicochemical properties (Liu et al., 2016). Particle size reduction also has several advantages that include improved extractability, solubility and

bioavailability of important constituents (Khadka et al., 2014). Particle size reduction also affects other properties such as flowability, water holding capacity, and absorptivity, thus decreasing the amount of ingredients needed (Merkus and Meesters, 2014; Khadka et al., 2014). Although most studies involve the use of concentrate, extracts, or juices, no study has focused on the effect of particle size of celery powders on physicochemical properties or antioxidant activities of meat products. Therefore, the objectives of study were: i) to produce celery powders of different particle size distributions by routine grinding and ball-milling, and ii) to evaluate the effect of particle size on physicochemical properties, curing efficiency, antimicrobial activity, and antioxidant activity.

Materials and Methods

Plant materials

Fresh celery (*A. graveolens L. var. dulce*) stalks were obtained from a local market, Gwangju, South Korea. Cut celery stalks were cleaned and placed in a hot air oven at 100 °C (Labtech, LDO-250F, Namyangju-city, South Korea). Drying temperature of 100 °C was decided based on our earlier studies (Ramachandraiah and Chin, 2017), which yielded higher antioxidant activities at this temperature compared to lower temperatures (50 and 75°C).

Ball-mill treatment and particle size analysis (PSA)

Celery stalks dried at 100 °C were pulverized using a 650 W kitchen (dry) grinder, passed through a sieve of 500 mesh to obtain coarse powder (C), and then passed through a sieve of 300 mesh to obtain micro-powders (M). These micro-powders were then subjected

112 to ball-mill (planetary) treatment (Pulversette No. 6; Fritsch, Darmstadt, Germany) by
113 placing powders with ZrO_2 balls (6-mm diameter) and milling at 400 rpm for 24 h to form
114 superfine powders (S). The weight of the balls was four times the weight of the sample.
115 Intermittent milling was undertaken to avoid any overheating of the sample in the container.
116 Particle size distributions of persimmon by-product powders were determined using a
117 Malvern Mastersizer 2000 Particle Size Analyzer (Malvern Instrument Ltd., Malvern, UK)
118 based on laser diffraction. These powders were dispersed in ethanol prior to size
119 distribution measurement.
120
121 **Preparation of porcine sausages**
122 Pork sausages were manufactured using celery powders (1%) with different
123 granularity. Table 1 shows the formulation used in this study for the preparation of pork
124 sausage batter. Porcine muscles were ground with a blender (K55, Crypto Peerless, UK) for
125 thirty seconds. Reference sausages were prepared by mincing the meat with sodium nitrite
126 ($NaNO_2$), salt (NaCl), and ice water. Similar to conventional sausages, a synthetic
127 antioxidant, butylated hydroxytoluene (BHT), was also added to the reference sausages.
128 Likewise, control sausages were prepared by homogenizing meat without nitrite and
129 treatment sausages were added with celery powders. Celery powders with different particle
130 size added to the formulation were: coarse (T1), micro (T2), and superfine powder (T3).
131 1 % of celery powders were used as preliminary studies showed that increasing this amount
132 led to unfavorable results. These homogenized meat batters were then packed under
133 vacuum and held at 4 ^{o}C for 2 h for proper equilibration of batters (Sebranek *et al*., 2012).
134 Later, these batters were stuffed in casing (polyvinylidene chloride). Sausages were heated

to have an internal temperature of 72 °C using a water bath (WB-22, Daihan Scientific, Korea). Cooked sausages were immediately chilled for 30 min. These sausages were then held at 4 °C for 6 h before storing at 20 °C. Quality analysis of vacuum-packed sausages stored at 20 °C was performed every 3 days from 0 to 15 days.

Color and pH quantification

A pH-meter (MP-120, Mettler–Toledo, Schwarzenbach, Switzerland) was used to measure pH values of meat samples. Sausage samples (10 g) were added to distilled water (90 ml), blended 30 s and pH measurement was undertaken. Colorimetric assessment of the surface of each sausage was done using a model CR-10 color reader (Minolta, Tokyo, Japan). To determine the color values of the sausages, each sample was cut into 2 cm sections. Color determination was undertaken by placing the colorimeter perpendicular to the cut surface. Parameters analyzed were L* (lightness), a* (redness), and b* (yellowness) values. At least six measurements of each sausage were done for these parameters.

Curing efficiency and Pigment analysis

Residual nitrite analysis

Quantification of residual nitrite was done by using the AOAC method (AOAC, 1990). Approximately 5 g of comminuted sample was initially added with some hot (80 °C) distilled water. Lumps were broken up and the final volume was made to 300 mL. These samples were then placed in a hot (100 °C) water bath for 1 h, filtered with Whatman No. 2 paper, and added with water to reach a final volume of 500 mL. Then 25 mL of filtrate was

added to 2.5 mL of sulfanilamide and incubated for 5 min. After the incubation, 2.5 mL of N-(1-naphthyl) ethylene diamine (NED) was added and incubated for 15 min. These steps were repeated for nitrite standard solutions. A 0.5 g of Sulfanilamide was dissolved in 150 mL of acetic acid (15% v/v). 0.2 g of N-(1-naphthyl)-ethylenediamine·2HCl was dissolved in 150 mL of acetic acid solution (15% v/v). The concentration of nitrite was measured using the standard curve.

Pigment analysis for cured samples

Pigment contents of cured sausages were analyzed as described by Pietrasik et al. (2016). To approximately 2.0 g meat sample, 9.0 mL of acetone solvent (acetone diluted to 92.5% using distilled water) was added and mixed for a minute using a glass rod. Tubes containing the samples were incubated at room temperature for 10 min. Later, samples were filtered using Whatman paper No. 42 and the filtrate was analyzed at wavelength of 540 nm. A mixture of acetone and water (80:20) was used as blank. Quantification of nitrosyl hemochrome concentration was based on the obtained absorbance (A540 × 290) and recorded in parts per million (ppm).

Total pigment analysis

Similar to pigment test for cured samples, 9.0 mL of acidified acetone solvent was added and mixed with 2.0 g meat sample. Acidified acetone solvent was prepared by mixing diluted acetone (92.5% in water) and concentrated HCl at a ratio of 2 to 1. Tubes containing samples were set aside at room temperature for 90 min. Following filtration with Whatman No. 42 paper, absorbance of the filtrate was measured at 640 nm. The blank was

prepared the same as for pigment analysis of cured samples. Total pigment concentration was calculated as A640 × 680 and recorded in ppm.

Thiobarbituric acid reactive substances (TBARS)

TBARS determination was performed as described by Shinnhuber and Yu (1977). To tubes containing 2 g of test materials, 3 mL of thiobarbituric acid solution (1 g/100 mL) and 17 mL of trichloroacetic acid (2.5 g/100 mL) were added and mixed. Tubes containing the mixture were then heated to 100°C for 30 min. Subsequently, 5 mL aliquot was carefully added to each tube containing 5 mL chloroform, mixed, and centrifuged at 200 g for 5 min. Three mL of the solution from each tube was then transferred to another tube containing 3 mL of petroleum ether. After mixing, the mixture was centrifuged at 200 g for 10 min. Malondialdehyde as the reactive substance was quantified at 532 nm using a spectrophotometer (UV-1601; Shimadzu, Co. Kyoto, Japan) and expressed as mg malondialdehyde kg^{-1}.

Microbiological analysis

Total bacterial counts were assessed using total plate count (TPC) agar medium and those of *Enterobacteriaceae* were assessed using violet red bile (VRB) agar medium as described by Chin et al. (2006).

Texture profile analysis (TPA)

TPA was performed for sausages using a Universal testing machine 3344 (Instron, Canton, USA). Sausages were cut to dimensions of 1.3 cm (height) and 1.25 cm (diameter) with a puncturing apparatus. Textural parameters such as hardness (gf) and springiness (cm) were

204 analyzed along with machine derived data of gumminess (hardness × cohesiveness), chewiness
205 (hardness × cohesiveness × springiness), and cohesiveness (ratio of active work done under the
206 second compression curve to that done under the first compression curve). The analysis was
207 undertaken using published procedures (Bourne, 1978).
208
209 **Statistical analyses**
210 In this study, three replications were performed and quantification of all parameters were
211 made in duplicate. Data are presented as mean ± standard derivations. SPSS 20.0 for
212 Windows (SPSS Inc., Chicago, IL, USA) was used for all data analyses. Statistical
213 comparisons were carried out with two-way analysis of variance (treatment and storage time),
214 and *p*-values < 0.05 were considered significant.
215
216 **Results and Discussion**
217 **Physicochemical properties: pH and cut surface color**
218 Average particle sizes of celery powders evaluated by laser diffraction instrument were 265
219 µm for coarse powder, 68 µm for micro powder, and 7 µm for ball-milled or superfine
220 powder. Effects of celery powders (CP) on pH and L* are shown in Table 2. As main
221 factors, treatments and storage time had significant effects ($p < 0.05$). However, no
222 interaction was found between the two factors ($p > 0.05$). The pH values of samples with
223 all treatments were lower than those of reference sausages ($p < 0.05$). The pH values of
224 sausages added with CP of higher particle size (T1 and T2) were lower than those of T3 (p
225 < 0.05). Concentration of various constituents including carbohydrates, proteins and
226 polyphenols observed in celery concentrates could possibly influence pH and nitrite

227 reactions (Djeri, 2010; Horsch et al., 2014). It is likely that the amount of phytocomponents
228 in the powders with different particle sizes (T1, T2, & T3) could have affected the pH
229 values.

230 Among color parameters, L* was affected by treatment and storage ($p < 0.05$). No
231 interaction was found between these factors ($p > 0.05$). Sausages added with CP (all
232 particle sizes) had lower L* values compared to other sausages ($p < 0.05$). The lowest L*
233 value was found for sausages added with ball-milled CP (T3). This could be attributed to
234 the increased surface area and in turn to well-dispersed powder. This could be attributed to
235 the increased surface area of powder that can cause particles to be well dispersed in the
236 sausage. Furthermore, it is possible that ball-milling could have enhanced the extraction of
237 pigments from celery stalk. In T3, owing to its lower mean particle size of 7µm, celery
238 particles were not visible compared to the other two treatments. Decreased L* values with
239 addition of plant materials have also been reported in other studies (Naveena et al., 2008).
240 CIE redness (a*) and yellowness, (b*) are shown in Table 3. The two factors, treatment
241 (particle size of celery powder), storage days (time), and their interaction (treatment and
242 storage days) had significant effects ($p < 0.05$). Sausages added with celery powders had
243 lower redness values but higher yellowness values compared to reference sausages. This
244 might be due to pigments present in celery powders, in agreement with results of Horsch et
245 al. (2014). They suggested that the color differences between natural and conventional
246 nitrite hams was due to plant pigments present in celery concentrates. However, among
247 samples added with celery powders at different particle sizes, redness values decreased
248 with decreasing particle size (granularity). Increasing fineness of powder caused lower
249 redness but higher yellowness (b*). Interestingly, the redness and yellowness remained

stable throughout the storage time for sausages added with sodium nitrite while redness increased and yellowness decreased with storage time for sausages loaded with celery powder. A relatively large increase in the redness of sausages was noticed for the sample added with CP at the largest particle size (265 μm) on day 3 after storage. This indicated that powders with relatively larger particle sizes retained higher nitrite content, which was released gradually during storage.

Microbial growth

Results for the effect of celery powder on microbial growth in sausages are presented in Table 2. Similar to pH and L* values, interaction between treatment and storage time was not significant. Addition of celery powder decreased total bacterial count of sausage compared to control sausage, indicating that celery powder might have antimicrobial activity. *Enterobacteriaceae* counts for all treatments were also lower than those of control sausages. Increasing storage time resulted in increased microbial growth. Previous studies have shown that nitrite can retard the growth of microbes such as *L. monocytogenes* without completely stopping their growth even at concentrations used in conventional curing (Xi et al., 2012). Therefore, microbial counts increased with increasing storage time. Moreover, the effect of celery powder as an antimicrobial agent is dependent on the concentration of ingoing nitrite (Xi et al., 2012). Variability in antimicrobial effect of celery powder at different sizes could also be partially due to difference in concentration of nitrite present in celery powders with various particle sizes.

TBARS

Results for the ability of celery powder to retard lipid oxidation are summarized in Table 2. Although celery-loaded sausages had lower TBARS values than control sausages, their antioxidant effects were not comparable to those of reference sausages. Higher antioxidant activity observed in reference sausages is mainly due to sodium nitrite and BHT, which are commonly added to meat products. However, in this study, celery powder with the smallest particle size (T3) had higher tendency to decrease TBARS than CP with the other two particle sizes. Some studies have shown that celery powder (CP) is comparable to sodium nitrite for limiting lipid oxidation over time (Sindelar et al., 2007). Variations in nitrite and polyphenolic contents can often lead to different antioxidant and curing effects. It is important to note that antioxidant properties of CP can greatly affect its applicability in food. Nevertheless, CP with different particle sizes had lower TBARS values than control sausages, indicating its antioxidant capacity.

Residual Nitrite

For residual nitrite concentrations, significant ($p < 0.05$) interaction between treatment and storage time was found (Table 3). During the initial stage of storage, residual nitrite levels for reference sausages were the highest among all samples. However, with increasing storage time, residual nitrite level decreased as reported in other studies on nitrite added meat products (Xi et al., 2012). CP loaded sausages had progressively higher residual content than reference sausages. Residual nitrite content in celery added ham has been reported to be higher than conventional nitrite added ham (Horsch et al., 2014).

Sausage added with CP at particle size of 265 μm had the highest residual nitrite, followed by sausage added with CP at particle size of 68 μm and that at 7 μm. In addition,

296 sausages added with CP at the largest particle size had higher residual nitrite levels in
297 comparison with sausages added with CP at the other two particle sizes throughout the
298 storage time. This is related to increased a* values of sausages added with these powders.
299 Even with enhanced extraction of ball-milling, sausages added with superfine powders did
300 not have high a* values. The relatively lower particle size might have exposed larger
301 quantities of nitrite at the initial stage, thereby improving the curing efficiency at the
302 earliest stage. On the other hand, relatively larger particles might have acted as reservoir for
303 nitrates. Larger particles might have retained more nitrates within plant fibers and such
304 nitrate might have been gradually released during storage. Similar variations have also been
305 noticed in other studies (Sindelar et al., 2007). Furthermore, the process of curing through
306 nitrite is a complex one. The addition of nitrite into meat product can result in the
307 formation of an intermediate pigment, nitrosylmetmyoglobin (NO-MMb), which is
308 unstable. It auto reduces with storage time due to the presence of endogenous or exogenous
309 reductants in muscle tissue (Pegg and Shahidi, 2000). In the present study, residual nitrite
310 level of reference sausage decreased with increasing storage time.
311
312 **Total and cured pigment analysis**
313 Effects of celery powders on cured and total pigments are shown in Table 3. The
314 concentration of cured pigment was higher for reference sausage than that for any other
315 treatment. However, the concentration decreased with increasing storage time. At the initial
316 stage of storage, the concentration of cured pigment was higher for lower particle size
317 amongst celery powders. With increasing storage time, the concentration of cured pigment
318 increased. These results are contrary to other studies showing that naturally cured meat

products have comparable cured pigments to conventionally cured products (Pietrasik et al., 2016). Such variation in results could be attributed to the lack of uniformity in sample composition (particles retaining varying amounts of nitrates). Similar variation has also been observed in previous studies (Sindelar et al., 2007).

The curing efficiency is the ratio between cured pigment and total pigment in percentage (AMSA, 2012). These results indicated that the efficiency was higher initially for reference sausage than any other sausage, although it decreased with storage time. The curing efficiency for celery powder with the lowest particle size (7 µm) was higher than that of other particle sizes until 3 days after storage. After 3 days, curing efficiencies of samples added with CP at relatively larger particle sizes (T1 and T2) were higher than those added with CP at the lowest particle size (T3). At 6^{th} day, the curing efficiency of sausage added with celery powder was higher than that of reference sausage. Such increase in curing efficiency for celery powder loaded sausages during storage might be due to gradual release of nitrite from celery particles. The presence of some endogenous bacteria might have also led to the gradual conversion of nitrate to nitrite.

Textural parameters

Textural parameters of sausages prepared with CP of different particle sizes are shown in Table 4. Regardless of particle size, CP did not significantly affect texture parameters such as hardness, springiness, chewy, gumminess, or cohesiveness of sausages. Even with decreased water-holding capacity of ball-milled celery powders (Ramachandraiah and Chin, 2016), textural properties of sausages were unaffected when compared to sausages added with coarse powders. Cell wall components of plant parts possess the ability to retain water

342 during cooking. Previous studies have shown improved textural properties upon addition of
343 some plant materials (Pietrasik et al., 2016). This is often attributed to cell wall
344 polysaccharide such as pectin. In the current study, no such enhanced ability to retain
345 moisture content was noticed. CP with larger particle size (T1) indicating higher
346 particulates (fibers, carbohydrates, and minerals) did not retain moisture better than CP
347 with lower particle size powders. This is different from a previous study showing that the
348 springiness is improved and cohesiveness is lower for CP added ham (Pietrasik *et al.,* 2016).
349

350 **Conclusions**

351 Sausages loaded with celery powder at the largest particle size had higher redness (a*)
352 values but lower yellowness (b*) than those loaded with CP at lower particle sizes during
353 storage. Sausages added with CP at the largest particle size also had higher residual nitrite
354 content than those added with CP at smaller particle sizes. Textural properties of sausages
355 were unaffected by particle size of CP. Thus, celery powders with larger particle size would
356 be preferable for improving physicochemical properties and antioxidant activities of meat
357 products intended for longer storage. Although celery powders had antimicrobial effect,
358 they were not comparable to conventional nitrite. These results emphasize the need for
359 further investigations on the efficiency of conversion from nitrate to nitrite for different
360 particle sizes with the use of starter cultures. It could also be worthwhile to evaluate the
361 effect of an additional natural antimicrobial to reach antimicrobial quality comparable to
362 conventional sausages. Nevertheless, celery powder with different particle sizes could serve
363 as an alternative for developing natural organic label friendly meat products.
364

Conflicts of Interest

The authors declare that they have no conflict of interest.

Author Contributions

Conceptualization: Ramachandraiah K, Chin KB. Data curation: Ramachandraiah K, Chin KB. Formal analysis: Ramachandraiah K, Chin KB. Methodology: Ramachandraiah K, Chin KB. Software: Ramachandraiah K, Chin KB. Validation: Ramachandraiah K, Chin KB. Investigation: Ramachandraiah K, Chin KB. Writing - original draft: Ramachandraiah K. Writing - review & editing: Ramachandraiah K, Chin KB.

Ethics Approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

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464 and sensory attributes of naturally-cured frankfurters. Meat Sci 90*:*130–138.
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468

Table 1. Formulation of pork model sausages incorporated with celery powders

Treatments: CTL (No Nitrite), Ref (Nitrite 150 ppm; BHT 100 ppm), T1 (Celery powder 265 µm), T2 (Celery powder 68 µm), T3 (Celery powder 7 µm).

Ingredients (%)	**CTL**	**REF**	**T1**	**T2**	**T3**
Lean meat	55.0	55.0	55.0	55.0	55.0
Back Fat	20.0	20.0	20.0	20.0	20.0
Water/Ice	23.1	23.1	22.1	22.1	22.1
Salt (NaCl)	1.50	1.50	1.50	1.50	1.50
Sodium tripolyphosphate (STPP)	0.40	0.40	0.40	0.40	0.40
BHT	-	0.010	-	-	
Sodium Nitrite ($NaNO_2$)	-	0.015	-	-	-
Celery powder	-	-	1.0	1.0	1.0
Total	100.0	100.0	100.0	100.0	100.0

Treatments: CTL (No Nitrite), Ref (Nitrite 150 ppm; BHT 100 ppm), T1 (Celery powder 265 µm), T2 (Celery powder 68 µm), T3 (Celery powder 7 µm).

Table 2. Physicochemical and microbiological properties of pork sausages as affected by celery powder particle sizes

	pH	L*	TPC	VRB	TBARS
Treatment	*	**	*	*	*
Storage	**	**	*	*	**
(Treatment x Storage)	NS	NS	NS	NS	NS
Treatment †					
Control	6.08^{bc}	78.2^{a}	3.06^{a}	2.44^{a}	0.40^{a}
Ref	6.12^{a}	75.6^{b}	2.21^{d}	1.21^{d}	0.24^{c}
T1	6.07^{bc}	69.6^{cd}	2.40^{c}	1.52^{c}	0.32^{b}
T2	6.05^{c}	67.7^{d}	2.48^{c}	1.46^{c}	0.33^{b}
T3	6.10^{ab}	65.3^{e}	2.70^{b}	1.71^{b}	0.28^{bc}
SEM	0.013	0.13	0.09	0.10	0.025
Storage (days)					
0	6.16^{a}	71.8^{a}	0.74^{e}	0.51^{e}	0.20^{c}
3	6.08^{bc}	71.9^{a}	1.76^{d}	0.57^{e}	0.25^{bc}
6	6.04^{c}	71.6^{ab}	2.41^{c}	0.85^{d}	0.30^{b}
9	6.06^{bc}	71.2^{bc}	2.94^{b}	1.82^{c}	0.38^{a}
12	6.09^{b}	71.3^{bc}	3.36^{a}	2.21^{b}	0.40^{a}
15	6.07^{bc}	71.0^{c}	3.45^{a}	2.81^{a}	0.45^{a}
SEM	0.014	0.15	0.06	0.08	0.027

†Treatment: same as in Table 1; L*, CIE lightness; TPC, total plate count (total bacteria); VRB, violet red bile (*Enterobacteriaceae*). T1, 265 µm, T2,68 µm, T3, 7 µm. NS = not significant; *p<0.05, **p<0.001.

[a-e] Means with different superscripts in the same column (treatment) are different ($p < 0.05$).

[a-e] Means with different superscripts in the same column (storage day) are different ($p < 0.05$).

SEM, Standard error of the mean.

Table 3. CIE a*, b*, residual nitrite, cured and total pigments of pork sausages as affected by different particle sizes

		Storage days					
		0	3	6	9	12	15
a*	CTL	6.60 ± 0.1^{Ac}	5.80 ± 0.1^{Bd}	5.76 ± 0.26^{Bd}	5.83 ± 0.25^{Bd}	5.97 ± 0.2^{Be}	5.93 ± 0.25^{Be}
	REF	11.23±0.25^{Aa}	11.15± 0.12^{Aa}	11.31± 0.21^{Aa}	11.33± 0.26^{Aa}	11.36± 0.21^{Aa}	11.25± 0.21^{Aa}
	T1	7.60 ± 0.26^{Cb}	9.73 ± 0.38^{Bb}	10.10± 0.10^{Bb}	10.20± 0.10^{Bb}	10.37±0.15^{ABb}	10.23±0.06^{ABb}
	T2	5.40 ± 0.10^{Bd}	6.77 ± 0.45^{Ac}	7.35 ± 0.34^{Ac}	7.17 ± 0.15^{Ac}	7.20 ± 0.10^{Ac}	7.00 ± 0.10^{Ac}
	T3	4.77± 0.15$_{Ce}$	6.00 ± 0.82$_{Bd}$	6.67 ± 0.51$_{ABc}$	6.73 ± 0.40$_{ABc}$	6.60 ± 0.36$_{ABd}$	6.40 ± 0.10$_{Bd}$
b*	CTL	9.30 ± 0.10^{Ac}	9.27 ±0.06^{Ac}	9.33 ±0.21^{Ab}	9.00 ±0.26^{Ac}	9.30 ±0.10^{Ab}	9.03 ±0.15^{Ac}
	REF	5.30 ±0.10^{Ad}	5.40 ± 0.17^{Ad}	5.50 ± 0.36^{Ac}	5.57 ± 0.38^{Ad}	5.67 ± 0.25^{Ac}	5.77 ± 0.35^{Ad}
	T1	14.50±0.20^{Aa}	12.97±0.21^{Bb}	12.70±0.20^{Ba}	12.70±0.10^{Bb}	12.97±0.15^{Ba}	12.80±0.10^{Bab}
	T2	13.97±0.38^{Ab}	12.87±0.31^{Bb}	12.47±025^{Ba}	12.70±0.36^{Bb}	12.60±0.20^{Ba}	12.63±0.06^{Bb}
	T3	14.73±050^{Aa}	13.67±0.38^{Ba}	12.93±0.76^{Ba}	13.27±0.15^{Ba}	13.17±0.31^{Ba}	13.27±0.40^{Bab}
Residual nitrite PPM (µg/g)	CTL	0.00^{Ac}	0.00^{Ac}	0.00^{Ae}	0.00^{Ab}	0.00^{Ae}	0.00^{Ad}
	REF	29.83±1.70^{Aa}	28.36±0.49^{ABb}	26.63±0.75^{Bbc}	21.50±1.21^{Ca}	17.60±0.61^{Da}	8.21±0.17^{Ea}
	T1	11.30±0.91^{Db}	36.56±3.66^{Aa}	33.90±4.06^{Ba}	23.13±1.22^{Ca}	12.53±0.42^{Db}	7.44±0.10^{Eb}
	T2	9.63±0.64^{Cb}	29.96±1.85^{Ab}	28.26±1.05^{Ab}	22.96±2.60^{Ba}	7.43±0.20^{DEc}	6.67±0.06^{Ec}
	T3	8.73±0.44^{Db}	29.10±2.13^{Ab}	25.93±0.52^{Bc}	21.7±0.66^{Ca}	6.8±0.15^{Ed}	6.56±0.07^{Ec}
Cured Pigments (mg/kg)	CTL	22.33±0.46^{Bb}	19.63±0.12^{Ce}	24.00±0.17^{Ab}	10.07±0.23^{Ed}	10.70±0.35^{Dc}	7.37±0.23^{Fe}
	REF	48.23±0.29^{Aa}	25.47±0.58^{Cd}	32.80±0.35^{Ba}	15.63±0.29^{Dd}	14.97±0.06^{Eb}	13.27±0.40^{Fc}
	T1	11.27±0.06^{Fe}	38.23±0.06^{Aa}	30.77±0.58^{Bc}	24.83±0.81^{Ca}	15.67±0.40^{Eb}	17.37±0.06^{Da}
	T2	13.33±0.12^{Fd}	35.07±0.23^{Ac}	24.03±0.29^{Bb}	22.20±0.69^{Cb}	17.33±0.46^{Da}	16.13±0.29^{Eb}
	T3	14.97±0.23^{Ec}	36.23±0.98^{Ab}	23.03±0.29^{Bd}	17.70±1.39^{Cc}	11.43±0.23^{Fc}	15.97±0.12^{Db}
Total Pigments (mg/kg)	CTL	98.17±0.12^{Aa}	97.00±0.17^{Ba}	83.23±0.75^{De}	83.37±0.81^{De}	95.37±0.23^{Cb}	84.13±0.06^{Ea}
	REF	91.47±0.58^{Cd}	65.53±0.64^{Fe}	94.37±0.23^{Bb}	75.57±0.46^{Eb}	83.03±0.23^{Dd}	101.17±0.23^{Ab}
	T1	95.70±0.17^{Bb}	79.80±0.35^{Dc}	88.67±0.58^{Cd}	70.50±0.35^{Ed}	66.73±0.23^{Fe}	115.83±0.64^{Aa}
	T2	88.87±0.23^{Ce}	78.47±0.46^{Dd}	91.50±0.52^{Bc}	74.00±0.17^{Ec}	98.47±0.23^{Aa}	98.33±0.46^{Ac}
	T3	93.07±0.06^{Bc}	84.40±0.69^{Db}	136.60±1.21^{Aa}	69.57±0.46^{Ee}	86.23±0.12^{Cc}	84.23±0.12^{Dd}

†Treatment: same as in Table 1: a*, CIE redness; b*, CIE Yellowness

$^{a-e}$ Means with the same letter and in the same column are not different ($p > 0.05$).

$^{A-F}$ Means with the same letter and in the same row are not different ($p > 0.05$).

504 **Table 4. Textural properties of pork sausages as affected by different particle sizes**
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Treatment		Texture Profile Analysis				
		Hardness (gf)	**Springiness (mm)**	**Gumminess**	**Chewiness**	**Cohesiveness**
Control	Mean	3144[a]	4.43 [a]	25.23 [a]	117.8 [a]	0.0073 [b]
	SD	49	0.76	0.53	18.7	0.001
Ref	Mean	3365[a]	4.40 [a]	30.45 [a]	131.3 [a]	0.009[ab]
	SD	75	0.52	8.31	17.08	0.002
T1	Mean	3612 [a]	4.46 [a]	29.41 [a]	131.8 [a]	0.0082[ab]
	SD	213	0.55	2.1	22.2	0.0008
T2	Mean	3399 [a]	4.13 [a]	35.08 [a]	143.3 [a]	0.0103[a]
	SD	474	0.55	6.74	21.5	0.0009
T3	Mean	3372 [a]	4.96 [a]	28.58 [a]	140.4 [a]	0.0084[ab]
	SD	281	1.15	5.58	34.1	0.0008

506 [†]Treatment: same as in Table 1
507 [a] Means with the same letter in the same column are not different ($p > 0.05$).
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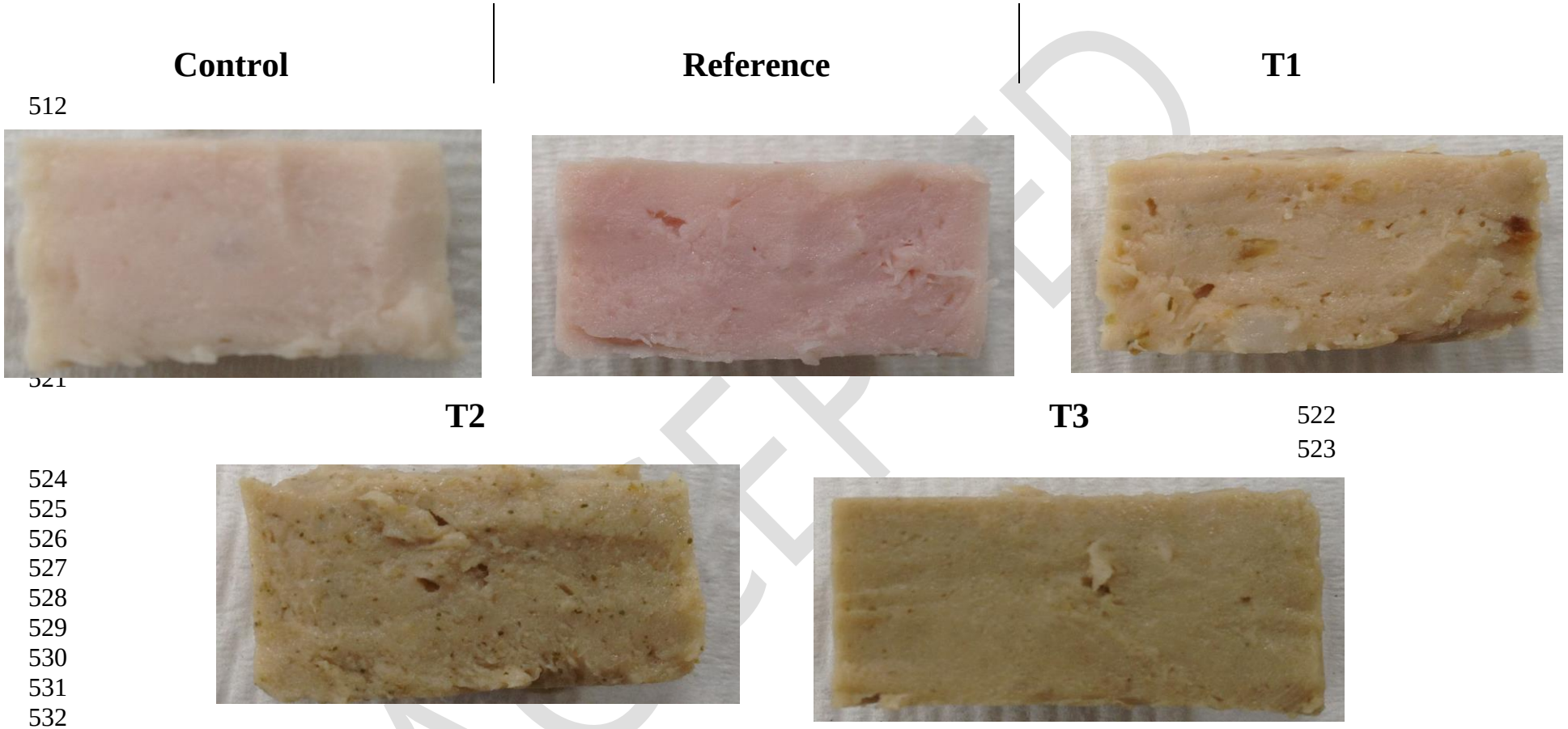

Figure 1. Cut surfaces of pork sausages at day 0. Control (No nitrite added), Reference (150 ppm Nitrite), T1 (Celery powder; 265 µm), T2 (Celery powder; 70 µm), T3 (Celery powder; 7 µm).