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Inhibition of Lipid Oxidation in Pork Bundles Processing by Superheated Steam Frying

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The effect of superheated steam treatment on the oxidative stability of lipids in packaged Zousoon (pork bundles) was investigated. The aroma quality of Zousoon samples was evaluated by sensory analysis and chromatographic analysis of volatiles. Results of this study indicated that oxidation of lipids occurred in pan-fried Zousoon after prolonged storage. Significant amounts of highly volatile compounds such as formaldehyde, acetaldehyde, acetone, and hexanal in Zousoon were identified by a modified method of cysteamine derivatization followed by gas chromatography−mass spectrometry (GC-MS) analysis. Superheated steam was found to be effective in suppressing lipid oxidation in canned Zousoon as compared with Zousoon fried by the conventional method in a frying pan. The superheated steam-fried samples had relatively low thiobarbituric acid reactive substance (TBARS) and peroxide (POV) values before and after storage, whereas samples prepared by pan frying had relatively high TBARS and POV values before and after storage. Superheated steam-fried Zousoon had superior lipid stability to that prepared by the conventional pan-frying method.

KEYWORDS: Superheated steam; oxidative stability; Zousoon (pork bundles)

INTRODUCTION

Zousoon (pork bundle), a popular intermediate moisture pork product found in Taiwan and China, is produced by roasting threaded pork ham muscle using a pan fryer. Zousoon is now produced in a variety of textures and flavors and is prized for its high nutritional quality and stable shelf life (*1*). The physicochemical and microbiological properties of Zousoon have been intensively investigated (*2*). An a_w range of 0.20−0.59 and moisture content from 2 to 12% was encountered in commercial products. They concluded that Zousoon and other Chinese intermediate moisture meats are biologically stable and safe products. They are shelf stable without refrigeration or thermal processing and can be eaten without rehydration.

Increased awareness of the adverse biological effects of some oxidation products has caused concern over the possible formation and presence of biologically active compounds in fat-based food. Despite its importance to consumer acceptability, very little information is currently available regarding the oxidative stability of intermediate moisture pork products. Huang et al. (*3*) utilized a headspace gas chromatography−mass spectrometry (GC-MS) technique to analyze the volatile compounds in Zousoon. A close relationship between a_w and lipid oxidation in a typical Brazilian intermediate moisture meat product was found (*4*). Igene (*5*) reported that kilishi stored for 60 weeks under ambient conditions (in cellophane bags) was analyzed for lipid and fatty acid profiles and oxidative stability. Total lipid content was 25%; 89.42% was triglyceride (TG), and 10.57% was phospholipid (PL). The thiobarbituric acid (TBA) number reached 2.01 in 60 weeks.

Lipid oxidation is one of the major causes of quality deterioration in raw and cooked meat products (*6*). Processes involved in meat products including cutting, grinding, mixing, and cooking can enhance the degradation of polyunsaturated fatty acid hydroperoxides into secondary products. During cooking, thermal and oxidative degradation of depot TG and tissue PLs occur simultaneously. Directly heating the meat sample under acidic (pH 3 or lower) conditions enhances the degradation of the existing lipid hydroperoxides and generates additional degradation products. Ahn et al. (*7*) reported a high correlation coefficient of propanal, pentanal, hexanal, and total volatiles with thiobarbituric acid reactive substances (TBARS) of cooked pork meat from *Longissimus* (L.) *dorsi*, *Longissimus psoas*, and *Rectus* (R.) *femoris* muscle. Ahn et al. (*8*) showed that oxygen contact with meat was the most important factor in the development of lipid oxidation.

Superheated steam has been used in preparing soaked rice with a high level of moisture (*9*), sequential heat−cool peeling of tomatoes (*10*), sterilization of packaging materials (*11*), reheating a chilled dish of mashed potatoes (*12*), drying beet pulp (*13*), bacterial reduction in spices (*14*), and various food

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processing applications (*15*). Recently, superheated steam at a temperature between 175 and 200 °C was applied directly into an automatically rotating drum fryer to replace the conventional frying pan used for Zousoon processing (*1*).

Although intermediate moisture products are microbially more stable than raw or cooked meat, they are still subject to deterioration through chemical and physical processes including oxidation, protein denaturation, cross-linkage, and browning, which can reduce their nutritive value and eating quality (*16*). Because lipid oxidation in a Chinese style intermediate moisture meat product can be reduced using vacuum or modified atmosphere packaging, we hypothesized that exclusion of oxygen right from the beginning of the frying process, by using superheated steam, could inhibit the initiation of peroxidation, thus minimizing lipid oxidation in Zousoon. The objective of this study is to investigate the effects of superheated steam on inhibiting lipid oxidation in Zousoon.

MATERIALS AND METHODS

Materials. Cysteamine, TBA, propyl gallate, ethylenediaminetetraacetic acid (EDTA), 1,1,3,3-tetraethoxypropane, ammonium thiocyanate, and ferrous chloride were purchased from Sigma Chemical Co. (St. Louis, MO). Methanol and chloroform were LC grade and purchased from Aldrich Chemical Co. (Milwaukee, WI). Authentic standards, 2-methylpropanal, 2-methylbutanal, 3-methylbutanal, formaldehyde, acetaldehyde, acetone, propanal, butanal, pentanal, hexanal, and tributylamine were purchased from Acros Organics (Geel, Bergium).

Manufacturing and Packaging of Zousoon. Two independent industrially manufactured samples of Zousoon were prepared. Each sample was composed of cooked prerigor (<3 h postmortem) ham (1000 g), sucrose (100 g), salt (15 g), and starch cells (40 g). The samples were either pan-fried following the method described by Huang et al. (*3*) or treated by a specially designed rotary superheated steam dryer as done by Chang et al. (*1*), and a product with a final moisture content of about 10% was obtained. In preparation, the prerigor muscle was cut into pieces parallel to the direction of the muscle fibers to allow heat penetration and yet still maintain the integrity of the long muscle fibers. The raw prerigor muscle was boiled in water in a large shallow gas-fired kettle for about 80 min to solubilize the collagenous materials and to evaporate some of the moisture, until the leached-out solids were reabsorbed by the muscle. The heated muscle was then manually pressed with a paddle to loosen the fibers from the large muscle bundles, to facilitate drying, and to yield long fibers. The partially disintegrated bundles of fibers were transferred to either a gas-fired fry pan equipped with a continuous mechanical scraper or the rotary superheated steam dryer to aid in concomitant drying and disintegration of the muscle bundles into long fibers. The average temperatures for the pan-fried samples were approximately 50, 70, and 85 °C at the surface, center, and bottom, respectively, for 30 min. Superheated steam (150 + 5 °C) was applied and resulted in the average temperature of samples to be about 90 °C for 10 min. Hot melted lard (at a ratio of 20% of the cooked prerigor meat) was added, and the material was heated until the long fibers reached a a_w range of 0.60−0.65. The finished product had a sweet meaty flavor, a chewy texture, and a light brown color. Fried Zousoons (250 g per can) were then packed (303 cans, three piece steel with tinplate) using a Semiautomatic Vacuum Seamer (S-C17V, Shin I Machinery Works Co., Ltd., Taichung, Taiwan). All of the canned Zousoon samples were stored in an incubator at 25 ± 3 °C for 1 year.

Sensory Evaluation. An eight member panel consisting of students and staff was trained to become familiar with the products and to evaluate the warmed over (off) aroma of Zousoon following the method of Poste et al. (*17*). Panelists scored the rancid flavor attributes using a 150 mm line scale, which was anchored at 0, 10, 42.5, 75, 107.5, and 140 mm corresponding to intensities of none, slight, moderate, strong, and extremely strong, respectively. Panelists evaluated the samples in partitioned booths under white lights. Duplicate evaluations for each of the two replicate runs were conducted. Judges were provided with an external reference for the warmed over aroma consisting of sunlight-treated Zousoon with an oxidized flavor. Panelists evaluated warmed over Zousoon by three short sniffs from a slightly opened sample cup containing 20 g of Zousoon.

Table 1. Warmed Over Aroma Intensity of the Pan-Fried and Superheated Steam-Fried Zousoon[a]

storage period (month)	frying method	
	pan frying	superheated steam
0	$37^a \pm 7$	$45^a \pm 4$
12	$107^a \pm 9$	$57^b \pm 5$

[a] Mean values in raws within groups with the same superscript letters are not significantly different ($p > 0.05$). Number of judgments (eight panelists). A 150 mm line scale with anchor points at 0 = none, 10 = slight, 42.5 = moderate, 75 = strong, and 107.5 and 140 mm = extremely strong.

Volatile Compound Analysis. A selective purge-and-trap method (*18*) was used to collect carbonyls in Zousoon. Powered sample (100 g) was soaked with 500 mL of water in a two arm round-bottom flask in a water both of 25 ± 2 °C. Volatile compounds were purged with nitrogen and trapped with a modified cysteamine solution (100 mL of 0.3 M cysteamine in phosphate buffer, pH 7.2) in an Erlenmeyer flask, following the method of Huang et al. (*19*), and trapped volatiles were then extracted with 10 mL of dichloromethane. An internal standard, tributylamine (1 mM), was added to the extract. The extract was fluxed with nitrogen to concentrate to about 1 mL to quantify the volatiles in the stored Zousoon samples.

GC. An HP 5800A gas chromatograph (Hewlett-Packard, Palo Alto, CA) equipped with a fused silica column (60 m × 0.32 mm i.d., film thickness, 0.25 μm; SPB-1 Supelco Co.) and a flame ionization detector was used to analyze the chloroform extracts. The operation conditions were as follows: injector and detector temperatures, 250 °C; extra pure helium carrier flow rate, 1.0 mL/min; temperature program, 70−180 °C increased at 4 °C/min.

GC-MS. GC-MS analysis was accomplished using a HP 5890A gas chromatograph coupled to a 5972 Mass Selective Detector. Mass spectra were obtained by electron ionization and chemical ionization at 70 eV and a source temperature of 250 °C. The filament emission current was 1 mA, and the spectra were recorded and analyzed with HP G111034C MS Chemstation Software installed in an IBM Personal Computer. The operation conditions were the same as those used in the GC analysis described above.

Peroxide Values (POV) Determination. Total lipid was extracted from powdered sample (0.5 g) with 5 mL of chloroform/methanol mixture (2:1 v/v) according to the procedure of Toschi et al. (*20*). Total lipid extract was analyzed for POV using the International Dairy Federation method as described by Shanta and Decker (*21*).

TBARS Assay. A distillation method was used with the addition of propyl gallate and EDTA at the blending step (*22*). Results were expressed as mg malonaldehyde equivalent/kg sample.

Statistical Analysis. All sample analyses were run in triplicate. Statistical analysis was done using a SAS package (version 6.03, 1998) developed by SAS Institute Inc. (Cary, NC). Analyses of variance (ANOVA) using the ANOVA were conducted. Differences between the sample means were analyzed by Fisher's least significant difference test at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Sensory Qualities of Zousoon. An eight member panel consisting of students and staff was trained to become familiar with the products to evaluate the warmed over aroma of Zousoon. There is no significant difference ($p < 0.05$) in warmed over aroma scores between pan-fried and superheated steam-treated Zousoon samples right after the frying process (**Table 1**). However, the warmed over notes for the pan-fried Zousoon were much higher than that of the superheated steam-treated samples after 12 months of storage ($p < 0.05$). The

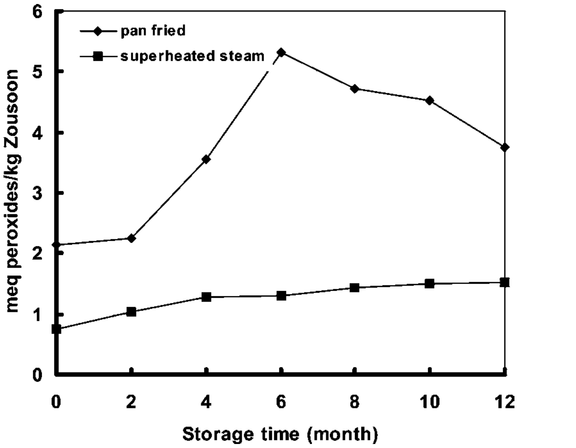

Figure 1. Effects of frying method on POV development in fried Zousoon.

scores for pan-fried and superheated steam-treated Zousoon were 107 and 57 mm, respectively, after 6 months, storage as shown in **Table 1**. The pan-fried Zousoon generated a warmed over aroma after 12 months storage, even when stored in a sealed tin can, whereas the superheated steam-treated samples developed only a slight off aroma.

Lipid Peroxidation Measured by POV. The stability of lipids in both the pan-fried and the superheated steam-treated samples was investigated. POV was significantly higher in the pan-fried Zousoon than in the superheated steam-treated samples immediately following the frying process, with the pan-fried samples having a 2-fold higher value (**Figure 1**). We postulated that autoxidation occurred in the pan-fried samples causing a significant increase in POV values and, therefore, the rate of oxidation was accelerated during storage. These data were consistent with the finding of Wang et al., (*23*) who reported that remarkable lipid peroxidation was observed in raw Chinese style sausages exposed to oxygen and high temperature when being dried at 55 °C in a hot air shelf-drying oven. Hydroperoxides are the major initiation products of autocatalytic oxidation of unsaturated lipids and may be catalyzed by mechanical processing and heating (*24*). Exposure of pork muscle fiber for a long period of time (2 h) in the presence of oxygen may initiate the free radical reaction (*25*).

POV number in pan-fried samples increased with increasing storage time from 2.1 to 5.2 mequiv peroxides/kg Zousoon until 6 months of storage, after which there was a slight decrease, possibly due to the transformation of hydroperoxides into volatile carbonyl compounds. Samples treated with superheated steam and stored in tin cans were less oxidized, as indicated by sensory scores and POV values than those fried in a conventional frying pan. Lipid peroxidation is a free radical chain reaction that can be described in terms of initiation, propagation, and termination processes. Oxygen species and activated catalysts seem to be the main initiators of polyunsaturated fatty acid oxidation via three different pathways: hydrogen abstraction, free radical attack on double bonds, and singlet oxygen "ene reaction" (*26*). The chain reaction of lipid peroxidation in pan-fried Zousoon is likely to be propagated even in sealed tin cans. However, lipid peroxidation in superheated steam-treated Zousoon was inhibited significantly during 1 year's storage.

Lipid Oxidation Measured by TBARS Method. Because the rancidity of animal fat correlated better with malondialdehyde (MDA) content than POV (*27*), the changes in TBARS values of Zousoon prepared either by pan frying or by superheated steam treatment with the various storage periods were determined and shown in **Figure 2**. Immediately following

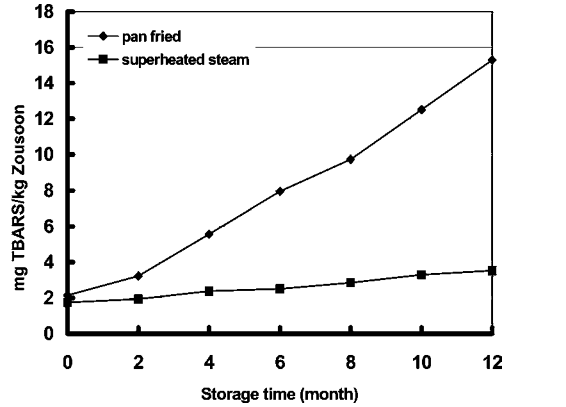

Figure 2. Effects of frying method on TBARS development in fried Zousoon.

Table 2. Identification of Thiazolidine Derivatives in Zousoon

peak no.	thiazolidine derivative	original carbonyls	ID[a]
1	thiazolidine	formaldehyde	A,B
2	2-methylthiazolidine	acetaldehyde	A,B
3	2,2-dimethylthiazolidine	acetone	A,B
4	2-ethylthiazolidine	propanal	A,B
5	2-isopropylthiazolidine	2-methylpropanal	A,B
6	2-propylthiazolidine	butanal	A,B
7	2-(2-methylpropyl)thiazolidine	3-methylbutanal	A,B
8	2-isobutylthiazolidine	2-methylbutanal	A,B
9	2-butylthiazolidine	pentanal	A,B
10	2-pentylthiazolidine	hexanal	A,B

[a] Compounds were characterized on the basis of the following criteria: A, retention index was consistent with those of an authentic standard; B, mass spectrum was consistent with that of Wiley mass spectrum database.

the process, pan-fried Zousoon showed a higher TBARS value than that of the superheated steam-treated sample. The TBARS values of Zousoon prepared by pan frying and superheated steam frying were increased up to 7.5- and 1.5-fold, respectively, after storage at 25 °C for 12 months. Dahle et al. (*28*) postulated that peroxide, which possessed α- or β-unsaturations to the peroxide group, was capable of undergoing cyclization to form MDA. The TBARS value has been widely used and well-accepted as a measure of oxidation rancidity in fat foods, particularly in meat products. The increase in TBARS values of pan-fried Zousoon may be attributed to the presence of oxygen and resulted in a greater degree of lipid oxidation. The transitory nature of the peroxides that are intermediate products resulted in the formation of carbonyl compounds. The TBARS values continued to rise over time, indicating further oxidation, catalyzed by the peroxides generated during the prolonged storage period.

Strecker Aldehydes in Pan-Fried Zousoon Samples. In addition to the MDA level in stored Zousoon, Strecker aldehydes were analyzed. A significant amount of Strecker aldehydes was identified using GC, GC-MS, and authentic standards from the Zousoon stored for 12 months, as listed in **Table 2**. A modified cysteamine derivatization reagent (*19*) was used to collect the volatile aldehydes extracted from the Zousoon samples. Quantitative analyses data are listed in **Table 3**. Detectable amounts of formaldehyde (62.16 ppm), acetaldehyde (69.14 ppm), 2-methylpropanal (18.26 ppm), 3-methylbutanal (32.83 ppm), and 2-methylbutanal (30.69 ppm) formed in pan-fried Zousoon stored for 12 months. Strecker aldehydes, formaldehyde (mouse urine, esterlike), acetaldehyde (sharp, penetrating, fruity), 2-methylpropanal (penetrating, green), 3-methylbutanal (malty, green), and 2-methylbutanal (etheral, bitter, almond, green) may

Table 3. Carbonyls in Pan-Fried and Superheated Steam-Fried Zousoon Stored for 12 Months[a]

carbonyls	superheated steam control (ppm)	superheated steam stored (ppm)	pan-fried control (ppm)	pan-fried stored (ppm)
formaldehyde	7[a]	14[b]	11[b]	62[a]
acetaldehyde	6[a]	18[b]	16[b]	69[c]
acetone	36[a]	51[b]	49[b]	321[c]
propanal	1[a]	3[a]	1[a]	3[a]
2-methylpropanal	8[a]	9[a]	18[b]	18[b]
butanal	1[a]	3[a]	1[a]	7[b]
3-methylbutanal	21[a]	21[a]	30[b]	33[b]
2-methylbutanal	18[a]	19[a]	28[b]	31[b]
pentanal	1[a]	8[b]	1[a]	26[c]
hexanal	26[a]	51[b]	28[a]	321[c]

[a] Control is the canned freshly prepared Zousoon sealed in a tin can and stored under 25 °C for 12 months. Mean values in raws within groups with the same superscript letters are not significantly different ($p > 0.05$).

have been derived from glycine, alanine, valine, leucine, and isoleucine, respectively. These data were in good agreement with our previous study (*3*). It was found that a significant decrease in the concentration of nonpolar amino acids, glycine, alanine, valine, leucine, and isoleucine occurred during the prolonged frying process (150 °C, at least 2 h). Methyl-branched aldehyde, produced by microbial catabolism or by Strecker degradation of amino acid, has been related to the aged and cured flavor of Iberian ham (*29*). No significant increase of Strecker aldehydes in both pan-fried and superheated steam-fried Zousoon was found (**Table 3**), indicating that thermal treatment, rather than autoxidation, may be responsible for the generation of Strecker aldehydes. One exception is acetaldehyde, which may form either by Strecker degradation or lipid autoxidation. The level of acetaldehyde in superheated steam-fried Zousoon is 3.9-fold higher than that in the pan-fried samples after 12 months of storage.

Lipid-Derived Aldehydes in Zousoon Samples. A series of straight chain aldehydes such as formaldehyde, ethanal, propanal, butanal, pentanal, and hexanal were characterized as shown in **Table 2**. Decomposition of hydroperoxides may lead to the formation of these volatile aldehydes. The volatiles from the pan-fried Zousoon samples were dominated by acetone, followed by the short chain aldehydes, formaldehyde, propanal, butanal, pentanal, and hexanal. A 6.3-, 4.5-, 3.9-, 1.1-, 2.2-, 3.4-, and 6.3-fold higher level of acetone, formaldehyde, acetaldehyde, propanal, butanal, pentanal, and hexanal, respectively, was observed in the pan-fried samples as compared to those prepared by frying with superheated steam (**Table 3**). Significant amounts of aliphatic aldehydes were identified in heated pork (*30*). They proposed that these aldehydes, namely, heptanal, nonanal, hexanal, and butyraldehyde, were thermally derived from the corresponding fatty acids, e.g., palmitoleic acid, oleic acid, linoleic acid, and linolenic acid found, respectively, in pork lipids. Straight chain aldehydes, originating from oxidation of polyunsaturated fatty acids of cell membranes, make up the major class of volatiles in traditional French dry-cured ham (*31*). Because free radical reaction belongs to the category of chain reaction, autoxidation occurs even in a sealed container. The fatty acids of dried pork fiber may degrade during the storage period that leads to a formation of volatile aldehydes. Volatile carbonyl analyses indicated that lipid in superheated steam-treated Zousoon underwent only slight oxidative changes even under prolonged storage.

The increase in volatile aldehydes, from the pan-fried Zousoon samples during the conventional frying process, may be attributed to the amount of possible polyunsaturated fatty precursors in pork PL. These short chain volatile aldehydes may mask the characteristic roasty aroma of pan-fried Zousoon. The PL fraction has been identified as primary substrates in development of oxidative deterioration in muscle foods (*32*). Arachidonic acid constitutes greater than 50% of the polyunsaturated fatty acids in meat PLs (*33*). Autoxidation of arachidonic acid may have a role in the development of off-flavors in meat products. The major products included hexanal, methyl 5-oxopentanoate, pentane, methyl butanoate, and 2,4-decadienal, which could be important to off-odor development in oxidized food systems containing arachidonate (*34*). Retro-aldol degradation of (*E*,*Z*)-2,4-decadienal, leading to the formation of 2-octenal, hexanal, and acetaldehyde, has been demonstrated to be thermally driven at neutral pH (*35*).

Volatile aliphatic aldehyde analysis confirmed only trace amounts of the secondary derivative in superheated steam-treated Zousoon as compared with that in pan frying, in which Zousoon has direct contact with hot air, whereas superheated steam contains a very limited amount of oxygen. A substantial amount of highly volatile compounds formaldehyde, acetaldehyde, and acetone rather than the long chain aliphatic aldehydes was characterized in this experiment. Yasuhara and Shibamoto (*30*) recognized significant amount of aldehydes, valeraldehyde, hexanal, heptanal, and octanal in the headspace of heated pork fat (purified pork fat was heated at 250 °C for 2 h). Mottram et al. (*36*) reported that the addition of pork fat to lean pork resulted in a substantial increase in hexanal, valeraldehyde, octanal, decanal, and tetradecanal but only small changes in most other volatiles. Guadagni et al. (*37*) noticed that the C6−C10 aldehydes could be expected to play some role in the overall aroma of the headspace from cooked meats. They purged the headspace volatiles into a trap at 60 °C for 2 h. A possible reason for the variation may come from the temperature for sample preparation. A higher temperature tends to generate volatile aldehydes with higher carbon numbers during sample preparation. In determination of volatiles from a meat sample, distillation was thought to produce additional aldehyde (*38*). The distillation method involves almost the same conditions as the direct heating procedure. The current modified purge and trap method may be considered as a better method for estimating the volatile aldehyde content in meat than either direct heating or distillation, because the meat itself is not exposed to heat treatment. The modified cysteamine derivatization method used in the experiment obviously favors the collection of highly volatile, short chain aldehydes.

Effectiveness of Superheated Steam Treatment in Inactivating Lipid Oxidation in Zousoon Processing. To prevent or minimize the occurrence of autoxidation of Zousoon lipid, several approaches are possible. One of the most practical methods involves the exclusion of molecular oxygen. Lipid oxidation of Zousoon, fried by superheated steam, as measured by POV, TBARS, and volatile compounds, was suppressed during frying and storage, as expected. The superheated steam (150 °C) in the rotary fryer was postulated to exploit most of the oxygen in a way similar to that described by Jenson and Anderson (*39*). They reported that during the drying of pulp by superheated steam, there is no oxygen in the superheated steam dryer and no oxidation occurs in the dried beet pulp, which results in no loss of dry substance and no half burned particles in the dried products.

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