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Effect of Variety and Maturation of Cheese on Supercritical Fluid Extraction Efficiency

JESSICA L. YEE,[†] JOHN WALKER,[§] HANY KHALIL,[#] AND RAFAEL JIMÉNEZ-FLORES*,[†]

Department of Dairy Science, Dairy Products Technology Center, Department of Science and Mathematics, and Department of Food Science and Nutrition, California Polytechnic State University, San Luis Obispo, 1 Grand Avenue, San Luis Obispo, California 93407

Supercritical fluid extraction (SFE) has been utilized by the food industry in many applications to extract, fractionate, and recover compounds from various food matrices. However, little research has been conducted using SFE as an alternative process for producing reduced-fat cheese. Lipids in cheeses may be selectively extracted due to the nonpolar properties of supercritical carbon dioxide (SC-CO_2), without leaving residual chemicals as is the case in solvent extraction. The objective of this study was to evaluate the influence on the extraction process due to cheese variety and protein breakdown by age. A Latin square design was utilized to test the extractability of lipids from Parmesan and Cheddar cheeses, aged young (9−10 months) or old (24 months). Extraction took place in a 500 mL SFE vessel using 100 g of grated cheese samples. The SFE parameters of the extraction were 350 bar, 35 °C, and supercritical carbon dioxide at a flow rate of 20 g/min for 55 min. Compositional analysis measured all treated samples and controls of total lipids, lipid profiling, total protein, protein/peptide analysis, moisture, ash, and pH. Cheese type was a major variable in fat extraction. The extraction in Cheddar showed an average fat reduction of 53.56% for young cheese, whereas that in old Cheddar was 47.90%. However, young Parmesan was reduced an average of 55.07%, but old Parmesan was reduced at 68.11%, measured on a dry basis. SFE extracted triglycerides and cholesterol, but did not remove phospholipids. This investigation introduces the observations of the effect of Cheddar and Parmesan varieties on SFE, offering data on the important parameters to consider in the design of SFE processes to reduce fat in cheese.

KEYWORDS: Supercritical fluid extraction; carbon dioxide; cheese; variety; maturation

INTRODUCTION

The demand for more low-fat product choices in the marketplace has accelerated manufacturing of low-fat cheeses (*1*). The past two decades have presented the dairy industry with a challenge in cheese technology to develop healthier, low-fat cheeses with sensory attributes similar to those of full-fat cheeses. However, when fat, one of the most essential and economically valuable components from the formulation (*2*), is removed, the ratios of the components shift, and essential biochemical changes do not occur in the same manner (*1*). Fat removal from cheese alters the textural and flavor profiles compared to the full-fat counterparts (*3*). Numerous approaches have been researched to overcome these types of problems with variable success (*1, 4–7*).

There has been little exploration of manufacturing a full-fat, flavorful, matured cheese and then removing the fat from the product. One exception is the work by Barbano and collaborators in which the cheese is grated and heated, the fat is centrifuged out, and the cheese is re-formed (*8, 9*). The lack of more processes on fat extraction from mature cheeses may be due to the traditional lipid extraction methods in which the use of solvents is essential. However, supercritical fluid extraction (SFE) technology offers a more attractive and innocuous alternative to fat extraction.

Fat removal using CO_2-based SFE in food matrices is based on lipid solubility in the solvent, without any harmful chemical residues (*10*). SFE technology has been used to extract, fractionate, and recover numerous compounds (*11*). Previous research of lipid removal in food matrices includes buttermilk powders to concentrate polar milk fat globule membrane lipids, reduction of cholesterol in milk and butter, obtaining vitamins A and E by defatting meat (pork and beef), powdered and fluid milk, treating nuts and seeds to prolong shelf life, ω-3 fatty acid extraction from brown seaweed for functional foods, and defatting potato chips, as just a few examples (*11–18*). Supercritical carbon dioxide (SC-CO_2) is an ideal solvent for removing lipids such as triglycerides and cholesterol from cheese matrices

* Corresponding author [telephone (805) 756-6103; fax (805) 756-2998; e-mail rjimenez@calpoly.edu].
[†] Department of Dairy Science, Dairy Products Technology Center.
[§] Department of Science and Mathematics.
[#] Department of Food Science and Nutrition.

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due to its behavior as a nonpolar solvent and its low critical temperature, which allows for the lipids to be removed without thermally degrading the integrity of the cheese microstructure (*18–20*). When compressed to liquid-like densities, the carbon dioxide has an excellent solvent power due to the controllable density of the supercritical fluid, having gas-like diffusivity and viscosity, no surface tension, and liquid like-density. For example, at approximately 383 bar and 40 °C, the density of SC-CO_2 would be 0.95 g/mL, very similar to liquid densities resulting in high solvent power (*21*). Typically, the solvent power is more affected by pressure; thus, increasing pressure allows for more lipids to be removed (*11*). In addition, it is of interest to determine if the duration of cheese ripening may also play a role in the efficiency of lipid extraction.

One of our major objectives is to develop a process for reduced-fat cheeses that offers a decrease in dietary fat while retaining similar texture and flavors characteristic of the original cheese. The application of SFE on cheese may be an advantageous alternative for reduced-fat cheese technology and is considered as one step toward our goal. The specific purpose of this work was to determine how the variety and maturation level of the cheese affects fat removal by SFE.

MATERIALS AND METHODS

Sample Preparation. Commercial Cheddar cheeses aged over 9 months and over 2 years were purchased in 2 lb blocks. Commercial Parmesan cheese aged over 10 months and Parmigiano-Reggiano cheese aged over 2 years were purchased in approximately 0.75 lb wedges. The surface areas of the cheeses were normalized to the same fine grate for each cheese to give a consistent sample size and to prevent the sample size from being a confounding variable in the study. For each cheese sample, the entire block or wedge of cheese was hand grated into a plastic bag using the smallest grate pore size (2 cm) and thoroughly mixed to evenly distribute the sample. Approximately 100 g of the hand-grated cheeses was portioned by random assignment into two samples, control (no SFE treatment) and SFE treated.

Experimental Design. A Latin square was used for the experimental design to ensure that each treatment occurred each day and only once each time of day. Because the experiment was run over 4 days, day and time of day are used as blocking factors in the analysis. There were three treatment factors in the experiment: cheese, age, and SFE. Each of the four combinations of cheese and age was randomly assigned to one time period during each day. Two cheese samples of the same type were treated at each time period, one with SFE and one without (control). The SFE unit used was a laboratory-scale system (model SFE 500; Thar Technologies, Inc., Pittsburgh, PA) operated in dynamic mode, which continuously provided fresh supercritical fluid during extraction (*22*). Parameters were selected on the basis of previous work conducted by Yee et al. (*20, 23*).

The experimental unit consisted of the filter bag surrounding each cheese sample. The controls and SFE treatments were paired together and run simultaneously. The treatment condition for SFE-treated cheeses was 350 bar, 35 °C, and 1000 g of CO_2 for 55 min. The control samples were held in an incubator (VWR Scientific Inc., West Chester, PA) set at 35 °C at atmospheric pressure for the same 55 min period.

Cheese Compositional Analysis. Cheese samples were prepared by blending the entire sample (model 400829005, Sears Roebuck & Co., Chicago, IL) in plastic blender cups on the crumb setting for 5−10 s increments, until the sample was in small homogeneous pieces. Care was taken to ensure that there was no oiling off of the cheese during sample preparation.

Fat Content and Characterization. The fat content was measured for all full-fat and SFE-treated Cheddar and Parmesan cheese samples, determined by the Mojonnier fat analysis as per Standard Methods for the Examination of Dairy Products (*10*). All samples were analyzed in duplicate, and the average grams of fat was taken as the sample value.

The lipid profiles of cheeses and extracted lipids were characterized using thin layer chromatography (TLC); refer to Yee et al. for the full procedure (*23*). Briefly, lipid samples were diluted to a 10 mg/mL concentration with chloroform/methanol (2:1) solvent mixture. Polar standards phosphatidylethanolamine (PE), phosphatidylcholine (PC), and sphingomyelin (SM) (Sigma Chemicals Co., St. Louis, MO) were made into 2 mg/mL concentration. Cheese lipid samples were applied in 25 μL amounts (250 μg of lipid) for all plates using a 25 μL syringe (Hamilton Co., Reno, NV). Precoated silica gel plates 20 × 20 cm in size were used for separation (EMD Chemicals Inc., Gibbstown, NJ). A polar solvent system was made up of chloroform/methanol/water (130:50:8, v/v). A nonpolar solvent system was made consisting of petroleum ether/ethyl ether/glacial acetic acid (170:30:4, v/v). After the plates were developed with iodine (Sigma Chemicals Co.), they were imaged using a ChemiDoc XRS imaging system consisting of a camera and hood (model 765107236, Bio-Rad Hercules, CA).

Protein Content and Profile. The percent protein in the cheese and water-soluble nitrogen samples in all full-fat and SFE-treated Cheddar and Parmesan cheese samples were determined by the Kjeldahl method in the AOAC International methods for cheese, 920.123 Nitrogen in Cheese (*24*). Alkaline urea−polyacrylamide gel electrophoresis (PAGE) gels were utilized to examine the proteolytic breakdown products in the cheese samples, according to the procedure of Andrews (*25*). Cheese samples were standardized to 0.400 mg of protein using 1× concentrated urea buffer, and 27 μL (8 μg) of standardized sample was loaded in each well. A casein sodium salt standard from bovine milk (C-8654, Sigma Chemical Co.) was used for comparison to the cheese, applied at 8 μL (8 μg) in each gel. The gels were photographed using a ChemiDoc XRS imaging device consisting of a camera and hood (model 765107236, Bio-Rad).

Moisture Content. The moisture content was measured using the AOAC International method for cheese, 955.30, and a vacuum oven (*26*) (Isotemp Vacuum Oven, model 281A, Fisher Scientific, Tustin, CA).

Ash Content. The ash content was determined according to the AOAC International method for cheese, 935.42 Ash of Cheese Gravimetric Method (*27*).

pH. Approximately 10 g of cheese sample and 10 mL of deionized water (1:1, cheese/water) was added to a small 50 mL capacity mortar (Coors porcelain, Sigma-Aldrich, St. Louis, MO). Utilizing the mortar and pestle, a cheese slurry was made, until homogeneously blended. The pH-meter (Orion model 410A+) was then calibrated, and the samples were tested in duplicate for their pH levels.

Statistical Methods. An analysis of variance (ANOVA) was computed using the general linear model function in Minitab (*28*) to analyze the differences in the average responses of fat and moisture based on changes in experimental variables cheese, age, and SFE treatment and their two-way or three-way interactions. Day and time period were used as random, blocking factors in the model. To reduce overall type I error, the significance of treatments and their interactions was evaluated using a 1% significance level due to the large number of terms of interest in each model and the presence of four response variables. For significant model terms, specific differences between levels were evaluated using Tukey's pairwise comparisons with a 99% simultaneous confidence level to control the type I error from multiple comparisons of the different factor levels at no more than 1%.

RESULTS AND DISCUSSION

Cheese Compositional Analysis. The objective of this study was to investigate the influence of cheese variety and age on SFE efficiency. For each untreated cheese (control) and cheese treated by SFE, the cheese matrix composition was analyzed for before and after levels of fat, protein, moisture, ash, and pH (**Table 1**). Data analysis was performed by taking two measurements of the same sample; the pairs were averaged, and the differences in the averages (after SFE minus before SFE) gave one response. The response (fat or moisture) was the mean difference between two measurements before and the mean of the two measurements after treatment. Protein and ash levels were not statistically analyzed because they did not change in composition before and after treatment on a gram basis, when

Table 1. Compositional Data for 100 g of Cheddar and Parmesan Cheeses before and after SFE Treatment

		Cheddar				Parmesan			
		young		old		young		old	
		control	SFE	control	SFE	control	SFE	control	SFE
fat (g)	before	35.42 ± 0.23[a]	35.53 ± 0.33	35.64 ± 0.18	35.48 ± 0.19	27.41 ± 0.48	27.31 ± 0.79	28.32 ± 1.88	28.39 ± 1.66
	after	35.10 ± 0.17	10.57 ± 1.02	35.47 ± 0.28	11.35 ± 0.85	27.42 ± 0.50	8.83 ± 0.89	28.47 ± 2.02	6.71 ± 1.18
protein (g)	before	25.41 ± 0.85	24.66 ± 0.84	23.19 ± 0.20	23.49 ± 0.36	30.85 ± 1.07	30.62 ± 0.78	33.11 ± 0.94	33.31 ± 0.88
	after	24.48 ± 0.63	24.23 ± 0.81	23.01 ± 0.51	22.68 ± 0.65	30.28 ± 0.65	30.11 ± 0.56	33.04 ± 0.76	33.07 ± 0.97
moisture (g)	before	34.58 ± 0.31	34.46 ± 0.32	35.04 ± 0.12	35.10 ± 1.06	33.75 ± 0.99	33.79 ± 0.73	30.04 ± 0.83	29.88 ± 0.78
	after	32.97 ± 0.33	31.78 ± 0.32	33.08 ± 0.12	31.57 ± 0.59	31.82 ± 0.85	30.85 ± 0.77	28.08 ± 2.08	26.85 ± 1.71
ash (g)	before	3.76 ± 0.03	3.74 ± 0.03	3.82 ± 0.04	3.82 ± 0.02	5.80 ± 0.07	5.75 ± 0.09	4.37 ± 0.14	4.42 ± 0.19
	after	3.76 ± 0.33	3.73 ± 0.32	3.82 ± 0.02	3.67 ± 0.02	5.71 ± 0.10	5.68 ± 0.10	4.37 ± 0.10	4.33 ± 0.13
pH	before	5.07 ± 0.00	5.12 ± 0.00	5.05 ± 0.01	5.05 ± 0.01	5.33 ± 0.01	5.33 ± 0.01	5.11 ± 0.00	5.11 ± 0.00
	after	5.10 ± 0.01	5.14 ± 0.01	5.05 ± 0.00	5.06 ± 0.01	5.33 ± 0.00	5.35 ± 0.01	5.12 ± 0.01	5.22 ± 0.01

[a] Standard deviation calculated from the four average responses for each cheese.

Table 2. *P* Values of Statistical Tests for Fat and Moisture ($\alpha = 0.01$)

	response variable[a]	
model term	fat	moisture
cheese	(<0.001*)	0.935
age	(0.182)	0.175
SFE	(<0.001*)	<0.001*
cheese × age	0.015	0.275
cheese × SFE	<0.001*	0.558
age × SFE	0.096	0.553
cheese × age × SFE	0.016	0.639
day	0.780	0.049
time	0.159	0.429

[a] Parentheses indicate that the data are not interpretable due to significant higher order interaction. An asterisk indicates significance at the 0.01 level.

calculated on the basis of initial and final total weights of the cheese before and after treatments. **Table 2** summarizes the *P* values of the statistical tests for fat and moisture at the 1% significance level.

Efficiency of Fat Extraction. The main factors that determined the extractability of lipid were the analyte solubility with supercritical carbon dioxide, analyte−matrix interactions, analyte location in the matrix, and porosity of the matrix. In general, the smaller the particle size of the samples, the more rapid and complete the extraction because the diffusion path length is shorter, enabling the solvents to penetrate and solute to move out of the matrix (*21*).

We hypothesized that lipids would be removed more efficiently from older cheeses due to the breakdown of the casein matrix, allowing less analyte and matrix interaction and thus easier extraction. However, the removal of fat was not significantly affected by the age of cheeses, but rather the type of cheese. On the basis of the different manufacturing practices for Cheddar and Parmesan type cheeses, the matrix and chemical and physical compositions of the cheeses are different and will change how SFE will extract fat from the cheese matrix. The combination of cheese type and pressure, temperature, and carbon dioxide treatment will determine how efficiently fat can be removed. Parmesan cheese is a hard, grainy textured cheese, which is typically classified by moisture content and length of ripening. However, the moisture content is relatively low due to the extended length of ripening and storage conditions. The particular Cheddar cheeses used in this experiment were packed and cured in air- and water-tight shrinking film and therefore have no firm rind and are more homogeneous in composition due to minimal moisture loss (*29*). Because the packaging allowed for similar moisture content of the cheese, it was possible to determine the effect of maturation level on fat extraction, although age had no significant difference in lipid removal according to this experiment.

Table 3. Change in Mean Fat by Cheese Type, Age, and SFE Treatment

cheese sample	mean fat change (g)	group[a]
Parmesan control	0.08	A
Cheddar control	−0.24	A
Parmesan treatment	−20.08	B
Cheddar treatment	−24.55	C

[a] Treatments are not significantly different from each other at the 1% significance level.

Table 3 displays the change in mean fat by cheese type and SFE treatment. A significant difference could not be detected between any of the controls. However, Parmesan cheeses treated by SFE had a significantly lower loss of fat after SFE than Cheddar cheeses treated by SFE. Parmesan cheese treated by SFE cheese is 2.56−6.38 g lower than the mean change in fat for the Cheddar cheeses treated by SFE. When the control and SFE-treated cheeses were compared, there was more fat loss after SFE compared to the control cheese for every type of cheese. On average, for the young Cheddar cheese samples, there was an average fat reduction of 70.25%. Old Cheddar cheese samples experienced an average fat reduction of 68.29%. Young Parmesan cheese had an average fat reduction of 67.67, and old Parmesan had a reduction of 76.37%. The average change in fat was interactively affected by cheese type and SFE treatment ($P < 0.001$).

The relevance of this level of fat extraction can be evaluated by considering a commercial scenario. According to the U.S. Food and Drug Administration definitions of fat descriptors in food products (CFR title 21, section 101), the term "reduced fat" may be used on a label provided that the food contains at least 25% less fat per reference amount. By this definition, all of the SFE-treated cheeses meet this claim (*30*). The level of fat extraction is dependent on cheese type and SFE parameters, for different cheese varieties, and the parameters would need to be optimized and further researched to determine fat extraction efficiencies. In summary, the efficiency of SFE in fat extraction is impressive. Using only a single extraction, Cheddar and Parmesan cheeses experienced a reduction in fat of roughly between 48 and 68% on a dry basis.

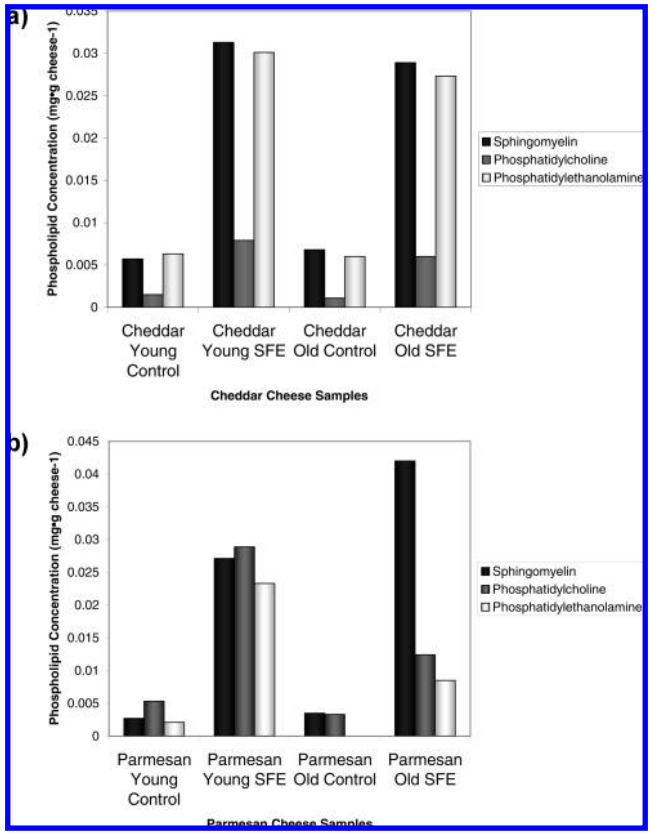

Figure 1. Average phospholipid concentration of sphingomyelin, phosphatidylcholine, and phosphatidylethanolamine in (**a**) Cheddar cheese samples and (**b**) Parmesan cheese samples from thin layer chromatography polar plates.

pH. The pH in all cheese samples treated with SFE slightly increased. This moderate increase in pH may be explained by free fatty acids being removed from the cheese matrix during extraction. Free fatty acids contribute to the total acidity in the cheese, and the removal of these acidic compounds may result in an increase in the pH of the cheese. Typically, when sample matrices are treated with SFE, the interaction of water and carbon dioxide yields carbonic acid, thereby lowering the pH of some samples depending on the initial pH, but this was not observed in the SFE-treated cheeses (*31*). Therefore, it is not completely understood why this increase occurs, and it is suggested that this observation be further explored.

Lipid Profiling. Nonpolar lipids were reduced, and the polar phospholipids were concentrated per gram of cheese as shown in **Figure 1**. The SFE process produced young Cheddar cheese that contained on average 0.0693 mg of phospholipids/g of cheese, compared to full-fat cheese containing on average 0.014 mg of phospholipids/g of cheese. This process therefore increased on average 4.82 times the available phospholipids. Similar results were found with old Cheddar cheeses as the SFE-treated samples showed an increase of 4.33 times the phospholipids. Young Parmesan cheese showed a 6.02 times increase and old Parmesan an increase of 4.00 times. This is important because phospholipids may provide health benefits. For example, sphingolipids have been reported to provide essential functions in intracellular signaling in various biological functions, such as cell growth, development, and apoptosis (anticancer benefits) and play a part in aging and age-related diseases (*2, 12*).

The nonpolar lipid Cheddar and Parmesan cheese TLC plates confirmed that only nonpolar lipids and cholesterol were extracted during the process. SFE removal of cholesterol is the subject of several patents beyond the scope of this work. The overall result of this lipid removal is a cheese product that is reduced in fat content, especially cholesterol, and has a higher concentration of health beneficial phospholipids, providing a unique alternative to any other cheese variety and means of cheese manufacturing.

Protein Profiling. It was very interesting to observe that in this experiment there was no correlation between the degrees of proteolysis (correlated with age) and the removal of fat extraction. Typically, the longer the maturation level, the more breakdown of caseins occurred in the cheese matrix (*32*). However, the effect of the degree of proteolysis in textural changes in elasticity, fracturability, and hardness may not be as significant for the efficiency of SFE of lipids between similar varieties. The overall methods of cheesemaking and curing are vastly different for Cheddar and Parmesan cheese, with the only commonality being the rennet coagulation for curd formation. Considering the final product and its textural and compositional characteristics, the matrix will naturally be different for each variety and will affect the extraction efficiency.

This study may serve as a benchmark for future work in SFE technology in developing reduced-fat cheese products. Cheese products are potentially an excellent matrix for fat removal by SFE, due to cheesemaking being a concentration process of proteins and lipids. With these preconcentrated components, it has been shown that the SFE process is a relatively quick and easy method to reduce nonpolar lipids and further concentrate polar lipids.

Effect of Moisture. When the changes in moisture before and after SFE treatment were observed, the mean change in moisture was affected only by SFE treatment ($P < 0.001$), but not cheese type or age (**Table 2**). Cheeses treated with SFE had a mean loss of 3.05 g of moisture, compared to a 1.87 g loss in mean moisture for the control cheese, with a mean loss of moisture for SFE cheese 0.51−1.85 g higher than the mean loss of moisture for the control. The overall trend observed was that, on the basis of grams of water present in the cheese before and after extraction, there was a decrease in the amount of water, which may be due to the carbon dioxide physically drying the cheese during the SFE process.

Because initial moisture could not be controlled in the experiment, we cannot tell whether the differences in fat removed from the samples were due to differences in moisture or differences in cheese types or ages. The only way to distinguish the effect of moisture on the SFE of lipids from the effect of the cheese matrix is to have the same cheese with various levels of moisture. The conclusion from our statistical analysis is that there is some attribute of the cheese that causes SFE to extract the lipids differently; however, if the moisture is to be properly analyzed for its effect on extraction, we must have several different levels of initial moisture for each type and age of cheese. This process also resulted in retaining the phospholipids in the cheese matrix. It must be emphasized that the type of cheese extracted will result in different lipid extraction efficiencies. In addition, the moisture content in the cheeses may have a role in SFE of lipids. In the processing of different varieties of cheeses, each processing parameter would need to be optimized to obtain the desired fat reduction levels for different varieties of cheese.

Our study demonstrates that SFE is a useful tool for analyzing the extraction efficiencies in various cheeses. Through these experiments we have shown that SFE can be applied to any low-moisture or hard-type cheeses. This process can especially be applied to any ripened, flavorful cheese.

ABBREVIATIONS USED

SFE, supercritical fluid extraction; SC-CO_2, supercritical carbon dioxide; TLC, thin layer chromatography; PE, phosphatidylethanolamine; PC, phosphatidylcholine; SM, sphingomyelin; alkaline urea-PAGE, alkaline urea polyacrylamide gel electrophoresis; CFR, *Code of Federal Regulations*.

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