

Fat and Cholesterol Content of Beef Patties as Affected by Supercritical CO₂ Extraction

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ABSTRACT

Beef patties (raw, raw freeze-dried, cooked, and cooked freeze-dried) were prepared for treatment with supercritical carbon dioxide extraction (SC-CO₂). Each type of patty was then assigned to one of four treatments: control, static extraction at 170 atm/50°C, dynamic extraction at 170 atm/50°C and dynamic extraction at 544 atm/40°C. Freeze drying of the patties prior to SC-CO₂ extraction improved removal of fat and cholesterol. Freeze drying enhanced ($P < 0.01$) cholesterol extraction; however, precooking had limited effects ($P > 0.05$) on cholesterol extraction. Supercritical fluid extraction could be effective to reduce the fat and cholesterol content of preformed meat products, without requiring comminution of the sample.

Key Words: supercritical, carbon dioxide, extraction, beef, cholesterol

INTRODUCTION

CONSUMER CONCERN over dietary intake of fat and cholesterol is an important issue. Lowering fat content of meat products has been achieved commercially by trimming external fat from fresh meat and by use of added water or other components in processed products. However, substantially reducing the amount of cholesterol in fresh and processed products remains a challenge to the meat industry.

Supercritical carbon dioxide (SC-CO₂) extraction provides a potential method for substantial reduction of cholesterol from meat products such as ground beef patties. SC-CO₂ extraction was effective in removal of triglyceride-based oils from dehydrated ground fish muscle (Yamaguchi et al., 1986) and various oil seed flakes (Snyder et al., 1984; Pubols et al. 1985; List et al., 1989). SC-CO₂ is used commercially in decaffeination of coffee (Zosel, 1978) and processing of hops (Sharpe and Crabb, 1980). The feasibility of SC-CO₂ extraction for processing foods had been summarized by Rizvi et al. (1986).

SC-CO₂ extractions are performed under high pressure above the critical temperature of the solvent (31°C for CO₂). The intense pressure densifies the CO₂ which solubilizes a portion of the lipid components and removes them from the food matrix. Chao et al. (1991) achieved up to at 40% reduction in cholesterol content of ground beef using SC-CO₂. However, such extractions had limited effects on fat extraction of meat products containing high levels of fat (Clarke, 1991). King et al. (1989) reported that reducing the water content of meat products made SC-CO₂ extraction much more efficient, resulting in almost complete delipidation at high pressures and SC-CO₂ flow rates. Research has also indicated that SC-CO₂ was very effective in reducing the cholesterol concentration and/or fat content of dehydrated beef powder (Wehling, 1991). Demand for beef in the dehydrated form is relatively low, however.

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SC-CO₂ extraction may be more effective with precooked meat products because of their reduced moisture content compared to fresh products. In many countries, demand for convenient precooked products is increasing. A precooked convenience item low in cholesterol and fat may further increase the demand for such products by making them more attractive to diet-health-conscious consumers.

Limited research has been conducted on the effectiveness of SC-CO₂ in reducing fat and cholesterol content of precooked or fresh beef. Our objectives were to compare the effectiveness of SC-CO₂ in reducing cholesterol and fat content of precooked, fresh and dehydrated beef patties. Extraction conditions were chosen to maximize removal of cholesterol relative to fat (Chao et al., 1991) and to minimize thermal alteration of meat samples. In addition, relatively high flow rates were used in dynamic extractions to hasten processing.

MATERIALS & METHODS

Meat preparation

A meat block was formulated at 20% fat from lean (90% lean) and fat (50% lean) beef chuck trimmings. The composite meat sample had the proximate composition: moisture-63.1%, fat-20.1%, protein-20.4%, and ash-1.0%. Trimmings were coarse ground, mixed and reground through a 0.3 cm plate. Patties were formed using a commercial patty maker. Samples were prepared by removing a 5 cm core from the center of the patties (due to limited size of the extraction vessel).

Patties for the precooked treatments were cooked to internal temperature 70°C in a commercial convection oven and liquids liberated during cooking were allowed to drain from the patties. Temperature was monitored with copper-constantan thermocouples using a Campbell Scientific recorder. Half of both the precooked and raw patties were then freeze-dried in a commercial freeze-drier with a 3.5 m³ chamber (Vacudyne Corp., Chicago, IL; operating pressure <1 mm Hg; chamber condenser temp -40°C), and all samples were vacuum packaged in Cryovac bags (W.R. Grace, Simpsonville, SC) and frozen (-34°C) for subsequent extraction.

Extraction apparatus

The extractions were performed on the apparatus shown in Fig. 1. Carbon dioxide from a cylinder was fed through an electronic flow meter (Model D12 H-SF, Micro Motion, Inc., Boulder, CO) that monitored the mass of CO₂ used during extraction. The CO₂ then

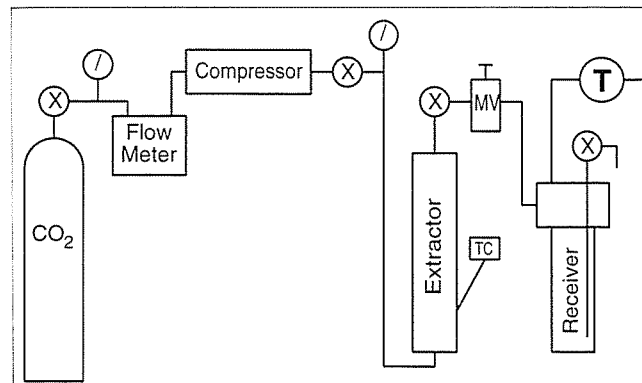


Fig. 1.—SC-CO₂ meat extractor.

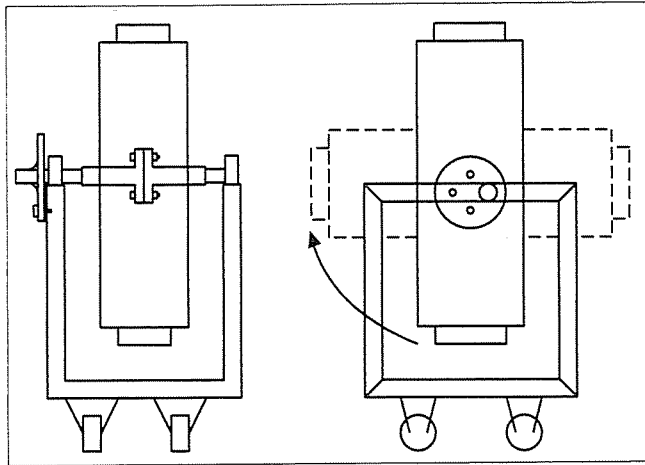


Fig. 2.—Adjustable extraction vessel.

flowed to a gas booster compressor (Model AGT-62/152, Haskel Eng. Corp., Burbank, CA). After pressurization, the fluid was introduced into a double ended, 2L extraction vessel (316 stainless steel pressure rated at 720 atm) with dimensions: 15.2 cm o.d., 7.6 cm i.d., 45.7 cm length (Autoclave Engineers, Erie, PA). Extraction pressure was set to the desired value by adjusting the air intake valve setting to the compressor. Temperature of the extraction vessel was controlled by a custom-designed heating mantle (Glas-Col Corp, Terre Haute, IN) coupled to a Barber-Colman Model 560 microprocessor controller (Barber-Colman Co., Rockford, IL).

The extract fluid was next passed through a micro-metering valve (MV) to a receiving vessel. The receiver, a modified 300 mL Magne-dash autoclave (Part No. 70-1395, Autoclave Engineers), was heated with electrical resistance tape and fitted with both a thermocouple and dip tube for sampling. The total volume of CO₂ passed through the system was determined with a dry test meter (Model DTM-200A, Singer-American Meter Division, Philadelphia, PA), in Fig. 1.

The extractor vessel was mounted on a stand with a pivotal bearing assembly to facilitate extraction with meat patties held in either horizontal or vertical position (Fig. 2). By swinging the extraction vessel at an angle of 90°, either position could be accommodated. Some patties were held in the horizontal position on a preformed stainless steel screen tray (41 cm in length, 7 cm in width, 1.5 cm in depth), to allow contact with the CO₂. Extraction of patties in a vertical configuration was accomplished by using a cylindrical multi-tiered stand (42 cm height), with 4 stainless steel screens (7.5 cm diameter) mounted at intervals of 10.5 cm, to hold the patties. Either of the sample holders could hold three meat patties inserted into the vessel for extraction.

Extraction procedure

Both a dynamic and static mode were used. In the static mode, the tray containing the hamburger patties was inserted into the extraction vessel, in a horizontal position. The system was gradually brought to desired temperature and pressure. For the static extractions, a pressure of 170 atm and 50°C were maintained for 3 hr before the vessel, which was held in the horizontal position, was depressurized. For each static extraction, a constant amount of CO₂ (1.8 kg) was used.

In the dynamic extraction mode, two pressures (170 and 544 atm) were applied at 40°C for both extractions. Dynamic extractions were conducted using an expanded gas flow rate between 10–12 L/min with the extraction vessel in a vertical position. A total of 5.54 kg CO₂ was used for all dynamic runs. Rapid reductions in fat content could be realized when operating in the dynamic mode due to the high CO₂ flow rates.

The coupling of extractor position (horizontal vs vertical), with static and dynamic modes of extraction, was not done to allow a comparison of extraction efficiencies. Conducting static SFE in the vertical mode could yield variable results due to the density gradient of extraction fluid from the bottom to the top of the extraction vessel. Likewise, dynamic extraction of samples, placed in the horizontal position, would provide less contact of the sample matrix with the flow of the SC-CO₂. Samples held in the vertical position, inside the vessel, were more thoroughly contacted by the extraction fluid.

Composition analysis

Duplicate samples from each patty were analyzed for moisture and lipid content as described by Novakofski et al. (1989) using chloroform and methanol (87:13). Mean lipid content for each treatment was calculated using duplicate measurements from each of three patties exposed to identical pretreatment and extraction conditions. Protein content was determined from duplicate samples of each patty using a Kjeldahl procedure (Method 24.027, AOAC, 1984). Duplicate samples (3g freeze-dried, 5g nonfreeze-dried) were placed in crucibles and dry ashed in a 550°C muffle furnace for 24 hr. Combusted samples were allowed to cool in sealed desiccators and dry sample weight was recorded as sample ash content.

Cholesterol determination

A modified Folch et al. (1957) procedure was used to obtain lipid extracts of duplicate samples (1.0–1.3g for nonfreeze-dried and 0.1–0.3g for freeze-dried samples), using chloroform and methanol (2:1). The mixture (sample, chloroform, methanol and water) was centrifuged (192G, 10°C, 5 min) and the process repeated. Chloroform layers were combined and chloroform was evaporated under N₂ gas. Samples were saponified with 5 mL of 15% (w/v) KOH (in 90% ethanol). Upon cooling, water (5 mL) was added and nonsaponifiable material was extracted twice with hexane (10 mL). Dried samples were analyzed for cholesterol concentration using the procedure of Zak et al., (1954). Sample absorbance, measured at 560 nm using a spectrophotometer (Spectronic 21, Bausch and Lomb, Rochester, NY), was compared to a standard curve and cholesterol content (mg/100g) was calculated. The assay was validated by adding cholesterol to samples and evaluating the recovery (Park, 1991).

Data were analyzed using a SAS statistical program (SAS Institute, Inc. 1985). General linear model procedures were used to determine least square means and to evaluate contrasts for effects of precooking, freeze drying, extraction and pressure. The statistical model for contrasts of precooked vs raw and freeze dried vs nonfreeze-dried were method (static or dynamic extraction), pressure (544 atm and 170 atm), freeze drying (freeze-dried or nonfreeze-dried) and precooking (raw or precooked). The model for contrast of control vs extracted included both method and pressure, and the model for contrast of 544 atm vs 170 atm pressure included only extraction pressure.

RESULTS & DISCUSSION

ALL OF THE HAMBURGERS were weighed before and after extractions. All meat patties retained their shape when removed from the extraction vessel and holding trays. The lipid extract was also collected after SFE from the receiver vessel. The fatty extract was brown in color and had a rich meaty aroma. Static extractions of the hamburger patties resulted in a 23 and 13% weight loss on the raw and cooked patties, respectively. Static extraction of the freeze dried analogues yielded a 4 and 2% reduction in weight, respectively. Approximate initial weight of both the raw and cooked hamburgers was 40–41g. Freeze dried patties averaged 17–19g. From these results, apparently static extraction was not effective for removal of lipid material, primarily due to the limited amount of CO₂ used and the low solubility of fat in SC-CO₂ (0.2 wt %) under these conditions (Friedrich et al., 1982). Collection of the lipid extract was difficult under these conditions, since upon decompression, most of the solubilized lipid precipitated on the extraction vessel wall or in the interconnecting tubing.

Dynamic extraction, using slightly different conditions (170 atm/40°C), gave similar weight losses for hydrated patties (Fig. 3). However, the freeze-dried patties lost more weight in the dynamic mode than in the static mode, indicating a greater amount of lipid material had been extracted when the CO₂ supply to the extractor was continually replenished. Lipid could also be collected in the receiver using this mode of extraction, ranging from a total of 3.0 g from three raw hamburger patties in one experimental run to 7.6 g for the dynamic extraction of three cooked hamburgers.

Dynamic extraction at 544 atm and 40°C showed greater weight losses in the meat patties, particularly for those that were freeze-dried before extraction. Weight losses for raw,

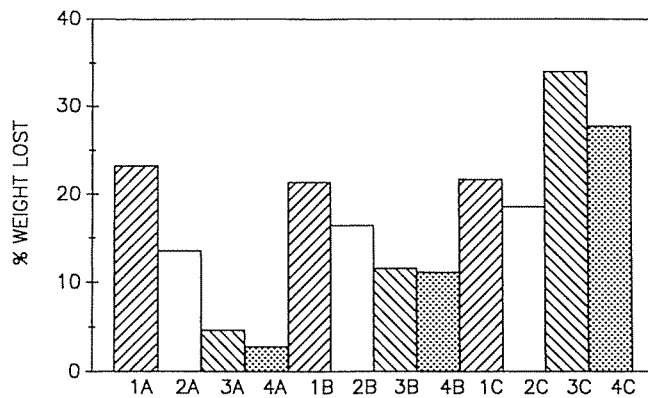


Fig. 3.—Percent weight loss for SC-CO₂ extraction of hamburgers at different conditions. (1=raw, 2=cooked, 3=raw, freeze dried, 4=cooked, freeze-dried, A=static, 170 atm, 50°C, B=dynamic, 170 atm, 40°C, C=dynamic, 544 atm, 40°C).

Table 1—Proximate composition of control and supercritical CO₂ extracted beef patties (Dry matter basis)

	Extraction pressure (atm)	Protein (%)	Ash (%)	Fat (%)	Cholesterol (mg/100g)
Raw Control		49.19	2.38	48.42	177.09
Static ^a	170	56.10	2.11	41.79	193.80
Dynamic	170	55.24	2.24	42.52	196.56
Dynamic	544	56.82	2.30	40.88	194.38
Raw FD ^b Control		44.78	2.03	53.19	167.48
Static	170	48.00	2.22	49.79	154.07
Dynamic	170	52.19	2.35	45.45	145.79
Dynamic	544	70.49	3.16	26.34	102.97
Cooked Control		59.78	1.98	38.25	189.33
Static	170	71.20	2.56	26.23	186.55
Dynamic	170	72.89	2.62	24.49	182.95
Dynamic	544	77.17	2.77	20.06	176.24
Cooked FD Control		62.59	2.20	35.22	194.34
Static	170	62.91	2.33	34.76	163.12
Dynamic	170	69.13	2.58	28.30	134.98
Dynamic	544	83.00	3.15	13.85	100.54

^a Extraction method: Static = CO₂ is not replenished; Dynamic = CO₂ is replenished.
^b Freeze-dried.

Table 2—Difference (Δ) in percentage fat and cholesterol of supercritical CO₂ extracted patties vs controls

Extraction pressure (atm)	Moisture-retained basis		Dry matter basis		
	Δ% Fat ^a	Δmg/100 g Cholesterol ^b	Δ% Fat	Δmg/100g Cholesterol	
Raw Static ^c	170	- 1.44	13.00	- 6.63	16.71
Dynamic	170	- 0.72	16.16	- 5.90	19.47
Dynamic	544	- 2.51	10.20	- 7.54	17.29
Raw FD Static	170	- 2.87	- 11.72	- 3.40	- 13.41
Dynamic	170	- 6.78	- 18.82	- 7.74	- 21.69
Dynamic	544	- 26.70	- 63.29	- 26.85	- 64.51
Cooked Static	170	- 6.61	- 5.20	- 12.02	- 2.78
Dynamic	170	- 7.49	- 7.38	- 13.76	- 6.38
Dynamic	544	- 9.76	- 11.54	- 18.19	- 13.09
Cooked FD Static	170	- 1.19	- 36.29	- 0.46	- 31.22
Dynamic	170	- 8.24	- 66.39	- 6.92	- 59.36
Dynamic	544	- 23.12	- 103.35	- 21.37	- 93.80

^a Δ% Fat = mean percentage fat of extracted sample - mean percentage fat of control sample (raw, FD, cooked or cooked and FD).
^b Δ% Cholesterol = mean percentage cholesterol of extracted sample - mean percentage cholesterol of control sample (raw, FD, cooked or cooked and FD).
^c Extraction method: Static = CO₂ is not replenished; Dynamic = CO₂ is replenished.
^d Freeze-dried.

freeze-dried patties were about 35 wt % and for the cooked, freeze-dried patties, 27 wt %. For these two cases, total fat extracted from each run on the three patties, yielded over 8 g of collected fat per extraction. Note that the quantity of CO₂ used in both dynamic extractions corresponded to two volumetric turnovers of CO₂ in the extractor vessel.

The effects of SC-CO₂ extraction on the proximate analysis of the extracted hamburgers were compared (Tables 1 to 3).

Table 3—Contrasts of extraction parameters on a dry matter and moisture-retained basis

Variable	Contrast Comparisons			
	Raw vs Cooked	FD ^a vs NFD ^b	Control vs Extracted	170 atm vs 544 atm
Dry matter basis				
Δ Fat ^c	0.13	0.86	***	***
Δ Cholesterol ^d	***	***	0.29	0.12
Cholesterol	0.46	***	0.28	0.11
Fat	***	0.43	**	***
Protein	***	0.35	0.08	***
Ash	***	0.09	**	***
Moisture-retained basis				
Δ Fat ^e	0.12	***	**	***
Δ Cholesterol ^f	***	***	0.23	0.09

^a Freeze-dried.
^b Not freeze-dried.
^c Difference in percentage fat from control on a dry matter basis.
^d Difference in percentage cholesterol from control on a dry matter basis.
^e Difference in percentage fat from control on a moisture-retained basis.
^f Difference in percentage cholesterol from control on a moisture-retained basis.
 ** = Significant difference (P < 0.05).
 *** = Significant difference (P < 0.01).

Some general trends for each type of beef patty are apparent from Table 1. Higher extraction pressures seemed to increase the protein content due to reduction in fat and moisture content. Cholesterol content was also reduced at higher CO₂ pressures, with exception of the raw hamburger. This confirmed the observations of King, et al. (1989) that lipid extraction was inhibited by moisture in the meat matrix.

Differences in percentage of fat and cholesterol as a function of extraction conditions for each patty type, (moisture-retained and dry basis) were also compared (Table 2). Differences tended to substantiate trends noted in Table 1. The large reduction in cholesterol content of the cooked, freeze-dried patties was probably due to denaturing of the protein and membranes, which would allow more of cholesterol to be removed.

The interrelationship between all of the meat treatments and the extraction conditions could be statistically related through the use of contrast analysis. Precooking significantly increased (P < 0.01) protein and ash content (Table 3) and decreased (P < 0.01) percentage fat. Freeze drying enhanced (P < 0.01) cholesterol extraction, but had no effect on sample fat, protein, or ash content. Clarke (1991) and Wehling (1991) also reported SC-CO₂ was effective in reducing cholesterol content of freeze dried meat products.

The contrast of control vs extracted (both static and dynamic) was not significant (P > 0.05) for cholesterol but was significant (P < 0.05) for fat and ash content. The contrast of extraction pressure was significant (P < 0.01) for sample fat, protein, and ash content and was not significant (P > 0.11) for cholesterol reduction.

The contrast of raw vs cooked was significant (P < 0.01) for difference in cholesterol content on a dry matter (DM) and moisture-retained (MR) basis (Table 3). Freeze drying increased (P < 0.01) the difference in percentage cholesterol on a DM and MR basis and fat on a MR basis (largely due to differences in moisture content). Differences in percentage cholesterol were not significant (P > 0.05) for the contrast of control vs extraction, but the difference in percentage fat was greater (P < 0.05) for extracted samples. The contrast of extraction pressure was significant (P < 0.01) for differences in percentage fat on a MR and DM basis.

CONCLUSION

FREEZE-DRYING was an effective means of enhancing extraction of cholesterol using supercritical CO₂. However, the effects of precooking meat patties on cholesterol extraction were limited. A process which combines freeze drying with supercritical fluid extraction could produce a very lean meat product.

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REFERENCES

AOAC. 1984. *Official Methods of Analysis*, 14th ed. Association of Official Analytical Chemists, Washington, DC.

Chao, R. R., Mulvaney, S. J., Bailey, M. E., and Fernando, L. N. 1991. Supercritical CO₂ conditions affecting extraction of lipid and cholesterol from ground beef. *J. Food Sci.* 56: 183.

Clarke, A. D. 1991. Supercritical fluid extraction technology for fat reduction. Missouri Agric. Exp. Sta. J. Series No. 11, 427. *Reciprocal Meat Conference Proc.*, Vol. 44.

Folch, J., Lees, M., and Sloane Stanley, G. H. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 226: 497.

Friedrich, J. P., List, G. R., and Heakin, A. J. 1982. Petroleum-free extraction of oil from soybeans with supercritical CO₂. *J. Am. Oil Chem. Soc.* 59: 288.

King, J. W., Johnson, J. H., and Friedrich, J. P. 1989. Extraction of fat tissue from meat products with supercritical carbon dioxide. *J. Agric. Food Chem.* 37: 951.

List, G. R., Friedrich, J. P., and King, J. W. 1989. Supercritical CO₂ extraction and processing of oil seeds. *Oil Mill Gazetteer*, 95(6): 28-34.

Novakofski, J., Park, S., Bechtel, P. J., and McKeith, F. K. 1989. Composition of cooked pork chops: effect of removing subcutaneous fat before cooking. *J. Food Sci.* 54: 15.

Park, S. E. 1991. A surimi-like material made from beef and pork and a novel protease in bovine and porcine skeletal muscle. Ph.D. thesis, University of Illinois, Urbana.

Pubols, M. H., McFarland, D. C., Eldridge, A. C., and Friedrich, J. P. 1985. Feed efficiency and pancreatic enzymes of chicks fed soybean meal extracted with supercritical carbon dioxide. *Nutr. Rep. Int.* 31: 1191.

Rizvi, S. S. H., Daniels, J. A., Benado, A. L., and Zollweg, J. A. 1986.

Supercritical fluid extraction: operating principles and food applications. *Food Technol.* 40(7): 57-64.

SAS Institute, Inc. 1985. *SAS[®] User's Guide: Statistics, Version 5 ed.* SAS Institute, Inc., Cary, NC.

Sharpe, F. R. and Crabb, D. 1980. Pilot plant extraction of hops with liquid carbon dioxide and the use of these extracts in pilot and production scale brewing. *J. Inst. Brew.* 86: 60.

Snyder, J. M., Friedrich, J. P., and Christianson, D. D. 1984. Effect of moisture and particle size on the extractability of oils from seeds with supercritical CO₂. *JAOCs* 61: 1851.

Wehling, R. L. 1991. Supercritical fluid extraction of cholesterol from meat. Ch. 10. In *Fat and Cholesterol Reduced Foods*, C. Haberstroh and C. E. Morris (Ed.), p. 133. The Portfolio Publishing Co., The Woodlands, TX.

Yamaguchi, K., Murakami, M., Nakano, H., Konosu, S., Kokura, T., Yamamoto, H., Kosaka, M., and Hata, K. 1986. Supercritical carbon dioxide extraction of oils from Antarctic Krill. *J. Agric. Food Chem.* 34: 904.

Zak, B., Dickenman, R. C., White, E. G., Burnett, H., and Cherney, P. J. 1954. Rapid estimation of free and total cholesterol. *Am. J. Clin. Pathol.* 24: 1307.

Zosel, K. 1978. Separation with supercritical gases: Practical applications. *Angew. Chem. Int. Ed. Engl.* 17: 702-709.

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