Capabilities of Different Cooking Oils in Prevention of Cholesterol Oxidation During Heating

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ABSTRACT: The potential of various cooking oils to prevent cholesterol degradation and/or oxidation, as measured by the production of 7-ketocholesterol during heating at different temperatures, was studied using a cholesterol model system. In the control group (without cooking oil), cholesterol was relatively stable, and 73% of its initial concentration was present after 30 min of heating at 125°C. Less than 30 and 10% of cholesterol remained at 150 and 175°C after 30 min, respectively, and 10% at 200°C after 10 min. In the treatment group, cholesterol mixed with corn, canola, soybean, or olive oil had significantly improved thermal stability. More than 60 and 40% of cholesterol remained at 150 and 175°C after 30 min, respectively. In the control group, 7-ketocholesterol was produced when samples were heated above 150°C, and levels increased consistently during 30 min of heating. At 175 or 200°C, the level of 7-ketocholesterol did not increase further after reaching the highest level after 10 min of heating. 7-Ketocholesterol is not stable above 175°C, and its degradation rate could be much faster than its production at 200°C. 7-Ketocholesterol was not found in samples of cholesterol mixed with corn oil or laboratory-prepared soybean and rice bran oils until the heating temperature was raised to 175°C for 20 min. The levels of 7-ketocholesterol in those treatment groups were greater than that in the control group at 175°C for 30 min. These oils may increase the thermal stability of 7-ketocholesterol and retard its degradation rate.

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KEY WORDS: Cholesterol, 7-ketocholesterol, oil, oxidation.

Cholesterol is an essential molecule for humans: It is a component of cell membranes and acts as a precursor of steroid hormones and bile acids. Many foods that contain high levels of cholesterol, such as eggs, seafood, milk, cheese, butter, and meat, are important sources of cholesterol. However, cholesterol in foods is readily oxidized to form cholesterol oxidation products (COP) when they are exposed to light, oxygen, active chemicals, and high temperatures (1). High intake of COP from foods can result in increased COP levels in plasma and intestinal cells (2,3). A higher ratio of COP to cholesterol was found in plaque, which is the cause of cardiovascular diseases and formation of certain types of cancers (4). Many reports have confirmed that COP are toxic and harmful to many cells and that they contribute to plaque formation (5,6). Therefore, lowering the COP level in frequently consumed foods may reduce the risk of cardiovascular disease and cancer.

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Heating is the most common and primary causative factor of cholesterol oxidation that produces COP in foods (7). Common COP—7-ketocholesterol, 7-hydroxycholesterol, and 5,6-epoxycholesterol—were produced during heating in model studies with a high level of cholesterol (8,9). All of those models demonstrated that COP were generated in significant quantities during heating from a high concentration of cholesterol (8,9). In food systems, Kumar *et al*. (10) reported that the level of COP in foods can range from 1 to 10% of the total cholesterol. The level of COP in eggs, milk, meat, seafood, and their products has been studied extensively, and heating has been found to accelerate cholesterol oxidation and produce harmful COP in foods.

In many food preparations, cooking oil is applied to the foods before or during heating. Cooking oils from plants are a rich source of tocopherols and tocotrienols, which are important antioxidants for preventing lipid oxidation. Those antioxidants could quench free radicals that are produced during heating and cause lipid oxidation. The antioxidants in plant oils may also be able to prevent cholesterol oxidation and simultaneously reduce COP production during heating. However, information on prevention of cholesterol oxidation by various cooking oils during heating is limited. In this study, the capabilities of different plant cooking oils in inhibiting cholesterol oxidation and COP production during heating were investigated using a model system with a low level of cholesterol mixed with various cooking oils. The results may enhance our understanding of the relationships between cholesterol oxidation and COP production and the role of different cooking oils in prevention of cholesterol oxidation and COP production during heating. This study may provide guidance for preparing cholesterol-rich foods that would minimize COP and reduce the possible risk of heart diseases and cancer.

EXPERIMENTAL PROCEDURES

Chemicals and materials. All solvents were HPLC grade. Hexane was obtained from Fisher Scientific Inc. (Fair Lawn, NJ). Cholesterol, 5α-cholestane, and 7-ketocholesterol were purchased from Sigma Chemical Co. (St. Louis, MO). Corn oil, canola oil, soybean oil, rice bran oil, and olive oil were purchased from a local supermarket. Laboratory-prepared soybean and rice bran oils were produced in our facilities using the method described below.

Laboratory-prepared soybean and rice bran oil. One hundred grams of blended soybean or rice bran oil was mixed with

500 mL of hexane and incubated at 70°C for 30 min. After centrifugation, the upper solvent layer with extracted lipids was collected. The laboratory-prepared soybean and rice bran oils were obtained after the solvent had been evaporated by vacuum drying.

Cholesterol oxidation during heating. A cholesterol solution was made by dissolving 100 mg of cholesterol in 1000 mL of hexane. Cholesterol solution (1.0 mL) was added to a 25-mL test tube. The hexane solvent in the test tube was evaporated under vacuum at 30°C using a CentriVap Mobile System (Labconco, Kansas City, MO). For the control group (without cooking oil), the test tube was incubated in an oil bath at a set heating temperature and time. After the test tube had cooled to room temperature, 1 mL of a 5α-cholestane solution (10 mg in 1000 mL) as an internal standard was added to the test tube and vortexed for 30 s. For the treatment group (cholesterol mixed with each cooking oil), 1 mL of cooking oil solution (1 g oil/100 mL of hexane) was added to the 25-mL test tube to which 1 mL of the cholesterol solution had previously been added and dried. The solution of cooking oil and cholesterol was vortexed for 30 s, and the homogenous solution was dried under vacuum at 30°C using the CentriVap Mobile System. The test tube with cholesterol and cooking oil was heated in an oil bath at a set heating temperature and time. The 5α-cholestane solution (1.0 mL) was added after the test tube had cooled. The concentrations of cholesterol and COP in the control and treatment groups were determined using a GC–MS method.

Analysis of cholesterol and COP using GC–MS. The GC–MS system consisted of a Varian CP3800 gas chromatograph and a Saturn 2000 mass spectrometer (Varian, Inc., Walnut Creek, CA) with a SAC-5 fused-silica capillary column (30 m × 0.25 mm × 0.25 μm film thickness) (Supelco Inc., Bellefonte, PA). Helium was used as a carrier gas at a flow rate of 2 mL/min. The injection port temperature was 300°C. The GC oven temperature was increased by 15°C/min to a final temperature of 250°C from the initial temperature of 200°C. The MS detector was operated at an ionization voltage of 70 eV and an ion source temperature of 200°C.

Statistical analysis. Each of the control and treatment groups was replicated three times at the same heating temperature and time. The standardized *t*-test procedure (Excel Data Analysis; Microsoft Inc., Seattle, WA) was used to compare the loss of cholesterol and production of 7-ketocholesterol between the control and the treatment groups. A significant difference between treatment means was considered at $P < 0.05$.

RESULTS AND DISCUSSION

Figure 1 is the GC–MS chromatogram of a mixture of the internal standard (5α-cholestane), cholesterol, and 7-ketocholesterol. These compounds can be separated readily and determined without chemical derivatization by using the GC column and operational conditions as described. Percentages of retained cholesterol during heating at different temperatures and times are depicted in Figure 2. In the control group, less than 30% of the cholesterol was lost after 30 min of heating at 125°C. At a heating temperature of 150°C, the loss of cholesterol increased to 70% after 30 min. This is in agreement with the result reported by Chien *et al.* (9), in which 64.8% of cholesterol was lost after 30 min with heating at the same temperature (150°C). At a 175°C heating temperature, the losses of cholesterol were 61, 87, and 88% at 10, 20, and 30 min, respectively, in the control (Fig. 2). Only 11% of the cholesterol was retained after heating at 200°C for 10 min. These results indicate that the loss of cholesterol by heating is extremely rapid when the heating temperature is above 175°C.

Major COP—7-hydroperoxycholesterol, 7-hydroxycholesterol, 7-ketocholesterol, and 5,6-epoxycholesterol—were

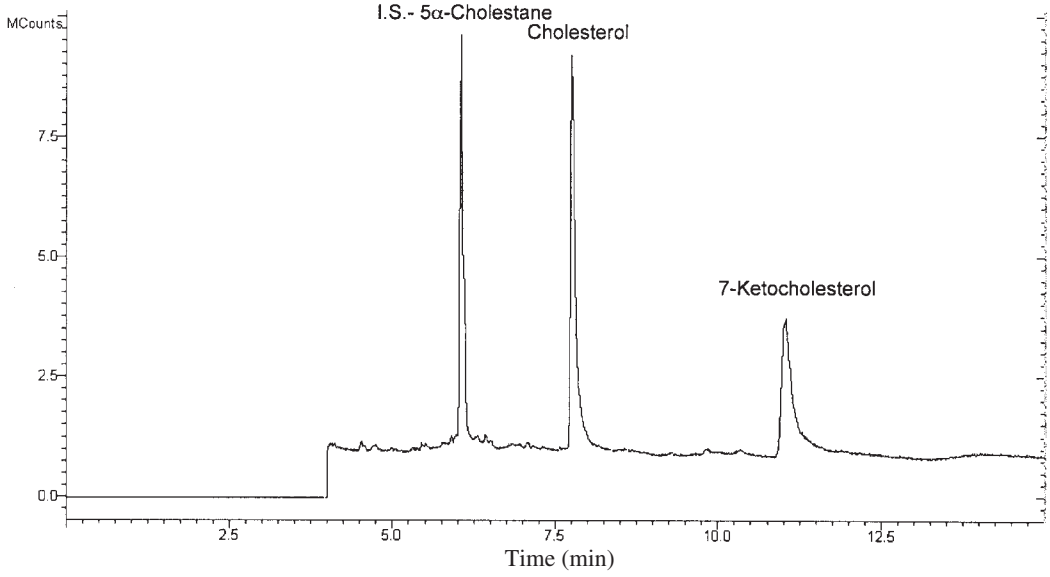

FIG. 1. GC–MS chromatogram of a standard mixture of 5α-cholestane (internal standard), cholesterol, and 7-ketocholesterol.

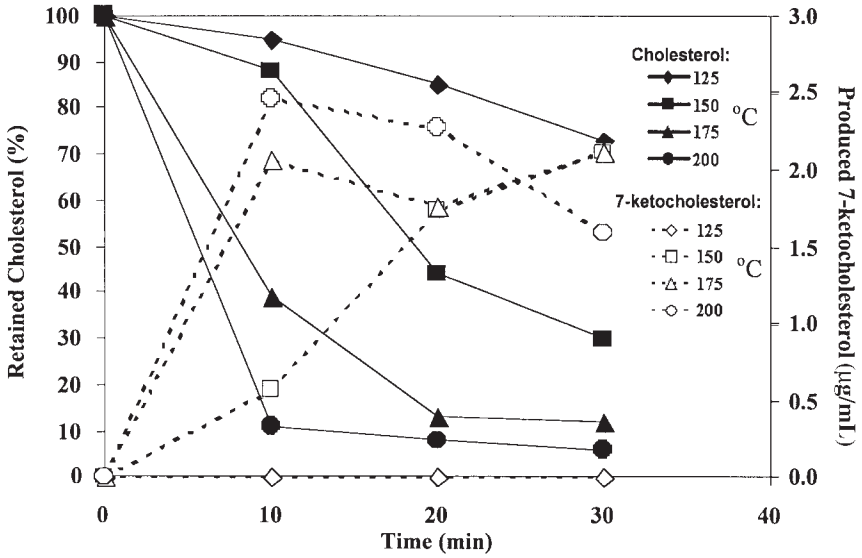

FIG. 2. Changes in retained cholesterol and 7-ketocholesterol produced during heating at 125, 150, 175, and 200°C for the control (without cooking oil).

detected by TLC and HPLC analysis when high concentrations of cholesterol models were used (9,10). The mechanism of cholesterol oxidation is suggested to be similar to that of lipid oxidation, i.e., free radical chain reactions that are accelerated by heating (1,7). 7-Ketocholesterol has been identified as a primary COP in many foods (10–16). It was also the principal COP in this study even in the low-concentration cholesterol model that was used. Figure 2 indicates the changes in 7-ketocholesterol content when the cholesterol was heated at different temperatures and times. 7-Ketochoelsterol was not detected even after cholesterol had been heated at 125°C for 30 min. However, it was found when the temperature was raised to 150°C. At 150°C, the concentration of 7-ketocholesterol was markedly increased to 2.11 μg/mL during 30 min of heating. However, at 175°C, it increased to 2.06 μg/mL in 10 min and then leveled off. Having reached its highest concentration (2.46 μg/mL) after 10 min of heating at 200°C, the 7-ketocholesterol concentration subsequently decreased. The production of 7-ketocholesterol did not correspond to the loss of cholesterol at 200°C, which would suggest that the rate of degradation of cholesterol at 200°C was less than the rate of degradation of 7-ketocholesterol.

These results showed that the production rate of 7-ketocholesterol was greater than the rate of cholesterol degradation at 150°C. When the heating temperature was above 175°C, the 7-ketocholesterol production rate could be suppressed by the cholesterol degradation rate. The 7-ketocholesterol may rapidly

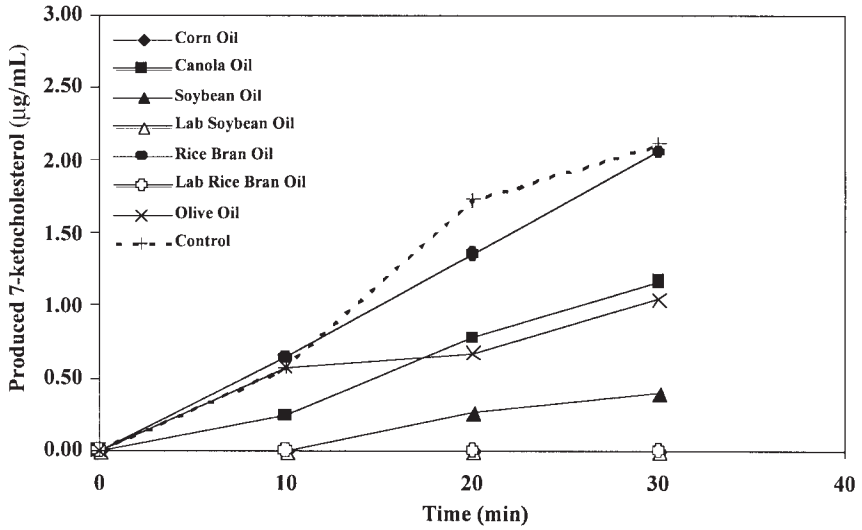

FIG. 3. Changes in retained cholesterol in the control and treatments during heating at 150°C.

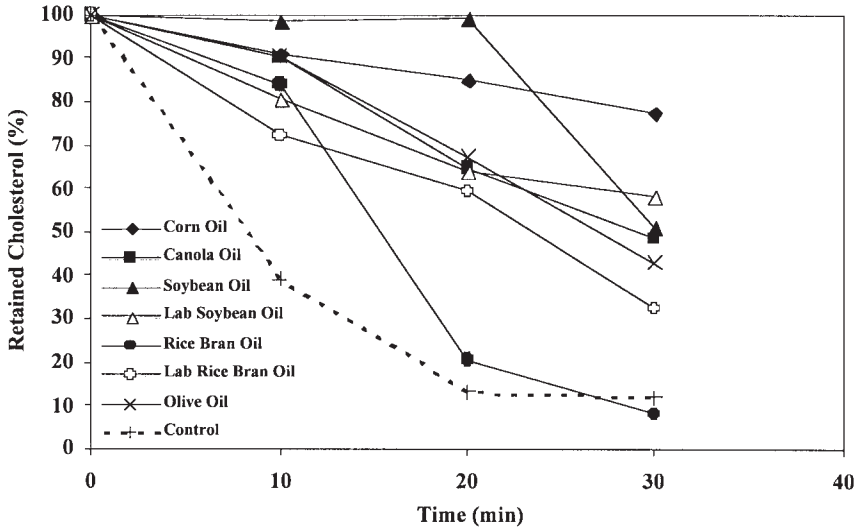

FIG. 4. Changes in retained cholesterol in the control and treatments during heating at 175°C.

degrade to small molecular products that are undetectable or that may elute in the first 4 min, a time when the MS detector was turned off. It appears that 7-ketocholesterol is degraded by heating, with its concentration in dynamic flux through its production and degradation in this cholesterol model. Similar observations have been reported in different model studies (8,9).

Because the changes in cholesterol concentration were pronounced in each 10-min interval at heating temperatures of 150 and 175°C, and because these temperatures are normally applied for roasting, baking, and frying muscle foods and seafoods, they were chosen to evaluate the role of cooking oil in preventing cholesterol oxidation and 7-ketocholesterol production. Seven different cooking oils from plant sources (soybean oil, laboratory-prepared soybean oil, corn oil, canola oil, rice bran oil, laboratory-prepared rice bran oil, and olive oil) were used in this study. The percentages of retained cholesterol during heating at 150 and 175°C in different cooking oil treatments are shown in Figures 3 and 4. After 20 min of heating at 150°C, the percentage of retained cholesterol in samples from each cooking oil treatment was significantly higher than that in the control group (Fig. 3). At 175°C, the cholesterol loss was significantly inhibited after 10 min in each treatment group, except rice bran oil (Fig. 4). The capability of rice bran oil to stabilize cholesterol disappeared after 20 min of 175°C heating. Cholesterol stability with corn, soybean, and laboratory-prepared soybean oil treatments was significantly higher than with canola, olive, rice bran, and laboratory-prepared rice bran oil treatments during 30 min of heating at 150 and 175°C. The

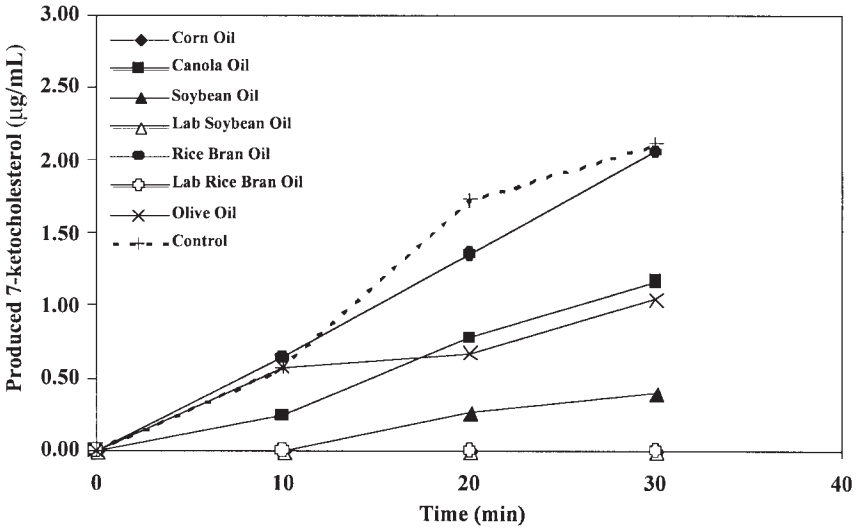

FIG. 5. Production of 7-ketocholesterol in the control and treatments during heating at 150°C.

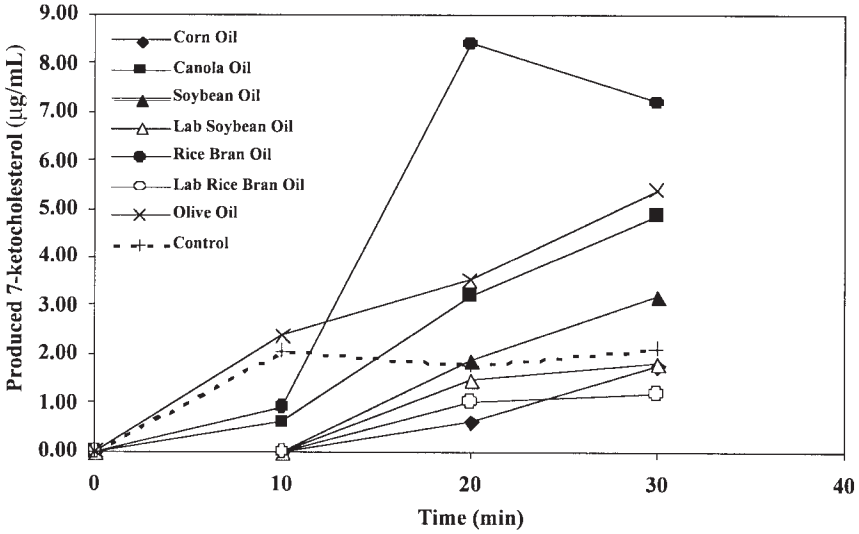

FIG. 6. Production of 7-ketocholesterol in the control and treatments during heating at 175°C.

capabilities of cooking oils to inhibit cholesterol loss were also reported by Echarte *et al*. (15). In that study, higher retention of cholesterol was found in salmon samples fried with olive or soybean oils than in the sample roasted without oil. Also, the retained cholesterol in the salmon sample fried with soybean oil was higher than that fried with olive oil. Their result, that soybean oil had a greater capability to inhibit cholesterol loss, is in agreement with our study.

Unlike the control group, 7-ketocholesterol was not detected in the corn, laboratory-prepared soybean, and laboratory-prepared rice bran oil treatment groups during 30 min of heating at 150°C (Fig. 5). At 150°C, the level of 7-ketocholesterol in the canola, soybean, and olive oil treatments was significantly lower than that of the control group. At 175°C, the concentration of 7-ketocholesterol in each treatment group generally increased consistently during 30 min of heating (Fig. 6). Among the treatment groups, the level of 7-ketocholesterol in corn, laboratory-prepared soybean, and laboratory-prepared rice bran oil treatments was significantly lower than that in canola, soybean, and olive oil treatments. These results were similar to those of Echarte *et al*. (15), who reported that the levels of 7-ketocholesterol in salmon samples fried with soybean oil were two and four times lower than those fried using olive oil and roasted, respectively.

The antioxidants in cooking oils may contribute to the inhibition of cholesterol loss and 7-ketocholesterol production during heating. Enhanced cholesterol stability and reduced COP production in the presence of synthetic antioxidants during thermally induced cholesterol oxidation has been reported (17). Vitamin E is the major antioxidant in common cooking oils from plants. It may play an important role in preventing cholesterol loss and 7-ketocholesterol production during heating. Both vitamin E isomers, α-tocopherol and γ-tocopherol, are abundant in corn and soybean oils (18). Rice bran oil contains a higher level of α-tocopherol and γ-tocotrienol. Compared with other oils, canola and olive oils contain lower levels of γ-tocopherol and α-tocopherol, respectively (18). Higher levels of both α- and γ-tocopherol in cooking oil may effectively inhibit cholesterol oxidation. In this study, the level of 7-ketocholesterol in the laboratory-prepared rice bran oil and soybean oil treatment is much lower than that of rice bran oil and soybean oil treatment, respectively (Figs. 5, 6). In the laboratory-prepared oils, phenolic compounds, most of which are removed during the bleaching step of commercial oil refining, were most likely retained. Those phenolic compounds in the laboratory-prepared oil may have an antioxidative function and reinforce other antioxidants in the oil to inhibit cholesterol oxidation during heating (19,20). Thus, the loss of antioxidants during oil refining may also be a critical factor for those oils having a lower capability to prevent cholesterol oxidation.

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