

Cholesterol Oxidation in Dried Egg Pasta: Detecting 7-Ketocholesterol Content

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ABSTRACT

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Modern pasta drying cycles often involve heating at temperatures between 70–90°C for periods that often exceed 10 hr. Under these conditions, cholesterol in dried egg pasta can undergo oxidation. Normal phase high-performance liquid chromatography (HPLC) was used to analyze the 7-ketocholesterol content, as this compound is a useful index of the oxidation development process. Egg pasta samples manufactured both industrially and by local shops were analyzed; durum wheat semolina, water, and eggs were the only ingredients. Some of the industrial prod-

ucts, with established high-quality composition and manufacturing technologies, were considered as reference samples. The comparison of analytical results confirmed that cholesterol oxidation can be limited by a careful control of time and temperature parameters employed during drying treatments and by avoiding the use of powdered eggs. Finally, no significant increase in cholesterol oxidation during storage of industrial egg pasta was observed.

Many cholesterol oxidation products are biologically active and may have varying degrees of toxic effects, such as atherogenesis and carcinogenesis (Bischoff 1969, Kandutsch et al 1978, Taylor et al 1979, Smith 1981, Parish et al 1986, Maerker 1987, Watanabe et al 1988, Smith and Johnson 1989, Addis 1990, Addis and Warner 1991, Kumar and Singhal 1991, Smith 1992, Böisinger et al 1993).

Recently, Guardiola et al (1994) and Smith (1993) reviewed the different analytical methods used so far for the determination of oxysterols in foods and in human tissue. Because of the complexity of food matrices and their low oxysterol content, the determination of 7-ketocholesterol has been used as a simple, fast and reliable method to evaluate the extent of oxidation in some food products (Zunin et al 1990, Zunin et al 1995, Penazzi et al 1995). In fact, 7-ketocholesterol forms during the first stages of the oxidation process (Nourooz-Zadeh and Appelqvist 1988a). It is one of the major oxidation products (Kim and Nawar 1993) and can thus be used as a marker of the extent of cholesterol oxidation. Measuring the amounts of other major cholesterol oxides, such as 7-hydroxides, could give a more exhaustive picture of cholesterol oxidation, but the detection of 7-ketocholesterol alone is certainly a useful simplification.

Following our previous study on cholesterol oxidation in baked biscuits and snacks (Zunin et al 1995), this study determines the 7-ketocholesterol content of dried egg pasta made from durum semolina, water, and eggs. The nutritional value is considerably enhanced with respect to common pasta by the presence of egg proteins, which increase protein content and compensate for the shortage of lysine characteristic of wheat proteins. Unfortunately, the use of considerable quantities of eggs leads to the presence of significant quantities of cholesterol, which could undergo oxidation during drying. Modern pasta drying cycles often involve heating at temperatures between 70 and 90°C for periods that often exceed 10 hr. Under these conditions, cholesterol oxidation should be limited (Kim and Nawar 1993). Nonetheless, it could lead to the formation of significant amounts of oxidation products because of the high quantities of cholesterol involved.

A wide-ranging study was conducted on samples of commercial egg pasta, aimed at determining the oxidation level reached by cholesterol. Possible differences between industrial egg pasta and egg pasta manufactured by local shops were also investigated.

Finally, because an increase in cholesterol oxidation products during storage was recorded in many food products (Nourooz-Zadeh and Appelqvist 1987, 1988a,b; Zunin et al 1995), this study tried to determine variations in the extent of oxidation during storage for industrial egg pasta, which has a shelf life of more than one year.

MATERIALS AND METHODS

Samples

Two groups of commercial samples were purchased in local stores and analyzed: IP, 32 samples of industrial dried egg pasta ($\leq 12.5\%$ mc) in sealed packages, representative of the major producers and the most common shapes; SP, 12 samples of dried egg pasta manufactured by local shops ($\leq 12.5\%$ mc) and sold loose.

Moreover, three samples of industrial egg pasta were analyzed repeatedly over an entire storage period (18 months). For each of these samples, the company provided 12 packages, sealed at the time of manufacture, which were stored at room temperature and analyzed each month during the first six months, and every two months during the following year.

Reagents

Cholesterol (5-cholesten-3 β -ol) and 7-ketocholesterol (5-cholesten-3 β -ol-7-one) standards were purchased from Sigma Chemical Co. (St. Louis, MO); 7-ketopregnenolone (5-pregnen-3 β -ol-7,20-dione) was supplied by Steraloids (Wilton, NH); α -cholestanol by Fluka (Buchs, Switzerland).

Determination of 7-Ketocholesterol Content

Extraction of lipids. Extraction of lipids was done on a 5-g sample of finely ground pasta. Distilled water (20 ml) was added to each sample to obtain the conditions described by Bligh and Dyer (1959). According to their method, ≈ 40 ml of organic extract was obtained. The solvents were then evaporated under a stream of nitrogen at $< 30^\circ\text{C}$.

Enrichment of 7-ketocholesterol and high-performance liquid chromatography (HPLC) analysis. The 7-ketocholesterol was enriched by solid-phase extraction (SPE) on a Bond Elut Florisil cartridge (Varian, Harbor City, CA) and then detected by normal phase HPLC (Penazzi et al 1995). Identification of 7-ketocholesterol was confirmed by spectral analysis and peak purity check. Quantification of 7-ketocholesterol was performed by internal standard method, with multiple level calibration ($r^2 = 0.9987$). The minimum quantifiable amount of 7-ketocholesterol was 1×10^{-9} g/injection. Results were expressed as ppm of 7-ketocholesterol in lipids.

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Three individual measurements per sample were performed. Standard deviation was 3.0×10^{-1} and standard error was 1.7×10^{-1} .

Determination of Cholesterol Content

Isolation and HRGC analysis of sterol fraction. Sterols were isolated as proposed by Fascioli et al (1994) and then derivatized to trimethylsilyl ethers and analyzed by high-resolution gas chromatography (model 8600, Perkin Elmer, Beaconsfield, Buckinghamshire, England) according to EC regulation 2568/91. Results were expressed as mg of cholesterol/100 g of pasta. Three individual measurements were performed per sample: standard deviation was 2.9 and standard error was 7.3.

RESULTS AND DISCUSSION

The method for the determination of 7-ketocholesterol has already been validated (Penazzi et al 1995) and avoids saponification of the lipidic fraction. If saponification occurs even at the mild conditions necessary to avoid destruction of 7-ketocholesterol, it might lead to the formation of cholesterol oxidative artifacts (Rose-Sallin et al 1995).

Figure 1 shows the amount of 7-ketocholesterol (ppm in lipids) determined in the samples of commercial egg pasta, separating the IP samples from the SP samples. Note that SP samples are dried egg pasta ($\leq 12.5\%$ mc), although the most common locally produced egg pasta is fresh egg pasta ($\leq 30\%$ mc). The latter products were not analyzed in this study because they are not subject to cholesterol oxidation. In fact, they do not undergo prolonged drying treatments and they must be consumed within three to four days after production.

Amounts of 7-ketocholesterol determined in IP samples showed a wide scatter (standard deviation = 10.87), ranging from a minimum value of 6.3 to a maximum value of 57.0 (ppm in lipids). Most samples (87.5%) showed amounts of < 20 ppm in lipids, with a particularly high incidence between 8 and 18 ppm. Only three samples had 7-ketocholesterol amounts < 8.0 ppm. Two other samples showed a significant difference, with 7-ketocholesterol contents of 57.0 and 47.9 ppm, respectively. These samples, however, had different shapes but were produced by the same company. It can be assumed that they were produced with powdered eggs. Previous studies (Zunin et al 1995) showed that the use of powdered eggs could enhance the oxidation process

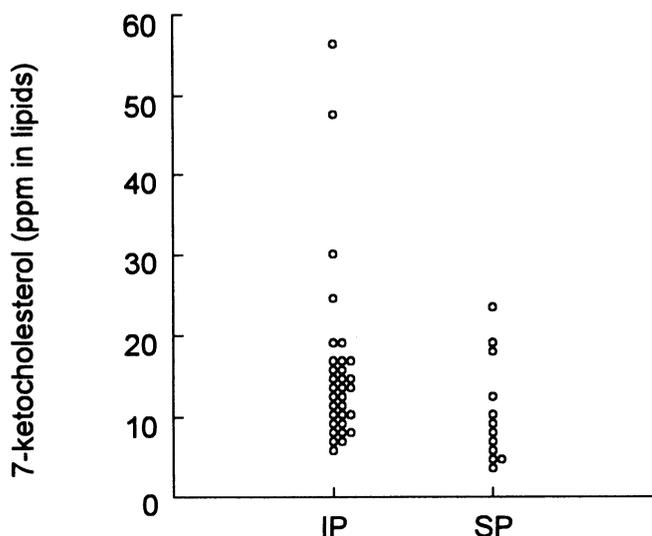


Fig. 1. 7-Ketocholesterol content in analyzed dried egg pasta produced industrially (IP) or by local shops (SP).

because cholesterol oxides already present in egg powder (Chicoye et al 1968; Tsai and Hudson 1984, 1985, Missler et al 1985; Naber and Biggert 1985; Nourooz-Zadeh and Appelqvist 1987; Morgan and Armstrong 1989; Caboni et al 1991) raise the oxidative instability of cholesterol. The amount of cholesterol oxides, thus could markedly increase during storage.

As the lipidic fraction of the analyzed IP was $\approx 4\%$, the recorded values of 7-ketocholesterol ranged between 10^{-7} and 10^{-8} moles per 100 g of pasta.

Figure 2 shows a two-dimension plot of the 7-ketocholesterol and cholesterol values recorded in the analyzed IP. Ten of these samples (indicated with solid circles) came from one company that uses only fresh eggs, not less than four hen eggs/kg of semolina (the minimum quantity required by Italian law), and the most advanced production technologies to guarantee the quality of its products (75°C for 20 min, then 65°C for 8 hr). Because they were manufactured with the most suitable ingredients and the most modern technologies, they were a useful reference standard for determining the extent of cholesterol oxidation in egg pasta.

The cholesterol content recorded in IP usually ranged between 150 and 200 mg/100 g of pasta, with the exception of two samples with cholesterol contents considerably lower (127.1 and 115.7 mg, respectively). However, in these two samples 7-ketocholesterol content was not particularly high (15.1 and 8.6 ppm in lipids, respectively), and we cannot assume that wide-ranging oxidative destruction of cholesterol took place. Therefore, these products probably contain less than 4 eggs/kg of semolina. As expected, the 10 reference samples had the highest cholesterol amounts. In these samples, however, a close control over drying technologies efficiently limited cholesterol oxidation; 7-ketocholesterol amounts did not exceed 15 ppm in lipids. On the other hand, considering all IP samples, the linear correlation between cholesterol and 7-ketocholesterol content was practically nonexistent ($r^2 = 0.010$). This confirms that manufacturing methods have a significant influence on cholesterol oxidation, irrespective of its quantity.

By examining the two-dimensional plot, we note that the reference samples tend to form a cluster and that the surrounding area comprises products that we may define as good-quality because of the number of eggs used in the dough and because of the level of cholesterol oxidation. The few samples that are located far from this area with low cholesterol content were probably produced with fewer eggs, while the samples with higher 7-ketocholesterol

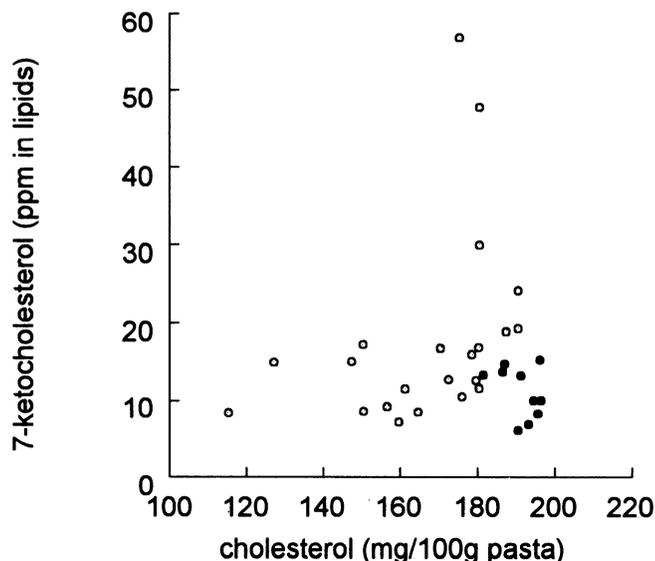


Fig. 2. Two-dimensional plot of 7-ketocholesterol and cholesterol content of analyzed industrial egg pasta. • = Samples produced by one manufacturer using known ingredients and manufacturing technologies.

content were produced by drying treatments that could not safeguard the integrity of cholesterol or with powdered eggs.

The analyzed SP samples (Fig. 1) showed more homogeneous results (standard deviation = 6.48) than did the IP samples, with a minimum value of 4.3 ppm, a maximum value of 23.9 ppm, and a mean value $x_{SP} = 10.76$ ppm. Mean values recorded in the two groups of samples ($x_{IP} = 15.98$, $x_{SP} = 10.76$) appeared to be quite different, but the *t*-test with pooled variances (experimental $F = 2.86 < \text{critical } F_{31,11} = 3.31$) showed no significant differences ($T = 1.56$, $P > 0.05$) between the two groups.

In the SP group, the two samples with the highest levels of 7-ketocholesterol (19.3 and 23.9 ppm) also came from the same manufacturer. However, these samples, together with a third sample with 18.3 ppm of 7-ketocholesterol, also had very low amounts of cholesterol (133.2, 124.1, and 115.3 mg/100 g of pasta, respectively). The simultaneous presence of high levels of 7-ketocholesterol and low levels of cholesterol justifies the assumption that these products were subjected to drying treatments that led to a significant cholesterol oxidation. However, we cannot rule out the possibility that single producers may have disregarded manufacturing regulations and even used fewer eggs. This assumption is supported by the low content of fatty substance extracted from these samples, which was to be ascribed mostly to egg yolks and amounted to $\approx 3\%$; significantly lower than the content found in all other analyzed samples (4%). Even if all SP analyzed samples had been produced less than 15 days before, it is interesting to note that the three samples in question were the only ones that can be stored up to six months, while all other samples had a maximum storage period of one month. This confirmed a reduced attention to the product quality and the use of more drastic drying treatments to guarantee longer conservation of the product itself.

For SP samples, it was impossible to choose reference samples of proven good quality. However, apart from the three samples discussed above, the other samples were comparable with the best IP samples. Some of them even had a cholesterol content over 200 mg/100 g of pasta and a 7-ketocholesterol content < 7 ppm in lipids.

To follow cholesterol oxidation during IP storage, one manufacturer provided three groups of pasta samples to be analyzed during the course of their entire shelf life. In this case, too, ingredients and manufacturing technologies were well known. Each sample was provided in 12 sealed packages, all belonging to the

same batch, that were stored at 20–24°C, avoiding exposure to direct sunlight, reproducing conditions normally encountered during marketing and home storage. Sample moisture was $8.01 \pm 0.05\%$. Unlike other food products (Nourooz-Zadeh and Appelqvist 1987, 1988a,b; Zunin et al 1995), 7-ketocholesterol amounts in all three samples did not increase regularly with time (Fig. 3) but remained close to initial values.

Results of analysis of variance (ANOVA) showed that changes in 7-ketocholesterol content were significant in all three samples ($P = 0.05$), but such changes were considerable only in sample 1. This sample had a 7-ketocholesterol content of 10.1 ppm at the time of production, which reached a maximum value of 20.1 ppm after one month, then dropped to 9.2 ppm after five months. During the following months there was a renewed increase in 7-ketocholesterol content, which was still slightly higher than the initial value after a year.

In samples 2 and 3, there were less marked variations of 7-ketocholesterol content (7.1 and 6.3 ppm, respectively at the time of production). In these two samples, there was also a decrease of 7-ketocholesterol content to 4.5 ppm after the first two months, then a new increase during the last six months. However, 7-ketocholesterol content remained lower than the initial value until the 15th month.

As is well known, 7-ketocholesterol tends to form quite rapidly during cholesterol oxidation, and it is one of the oxides found in the highest amounts (Park and Addis 1985; Pie et al 1990, 1991; Nourooz-Zadeh and Appelqvist 1988a; Zunin et al 1995). However, several authors (Nourooz-Zadeh and Appelqvist 1987, 1988b; Van de Bovenkamp et al 1988) observed that 7-ketocholesterol content may decrease upon long-term storage of different foods. Thus, we may assume that, at all times during storage of dried egg pasta, the amounts of 7-ketocholesterol are the sum of the one that forms and the one that breaks down. Such quantity does not increase with time, so we must conclude that cholesterol oxidation is not favored. Therefore, we may assume that the presence of natural antioxidants such as a carotenoids, both in semolina and eggs, inhibits oxidation by means of a competitive mechanism.

The analytical results of this study allow us to confirm that, in spite of variations of cholesterol oxidation levels, products manufactured either industrially or by local shops do not show significant differences. As far as industrially produced pasta is concerned, we could also determine the quantity of 7-ketocholesterol that should be found when these products are manufactured with state-of-the-art industrial techniques. The presence in both groups of a vast majority of samples with limited cholesterol oxidation confirms that product quality can be ensured by a careful control of time and temperature parameters employed during drying treatments and by avoiding the use of powdered eggs. Because few authors (Emanuel et al 1991) have studied the modifications of plasma oxysterol levels as a consequence of their content in food, further studies are needed to accurately determine which values are cause for alarm for long-term toxicity.

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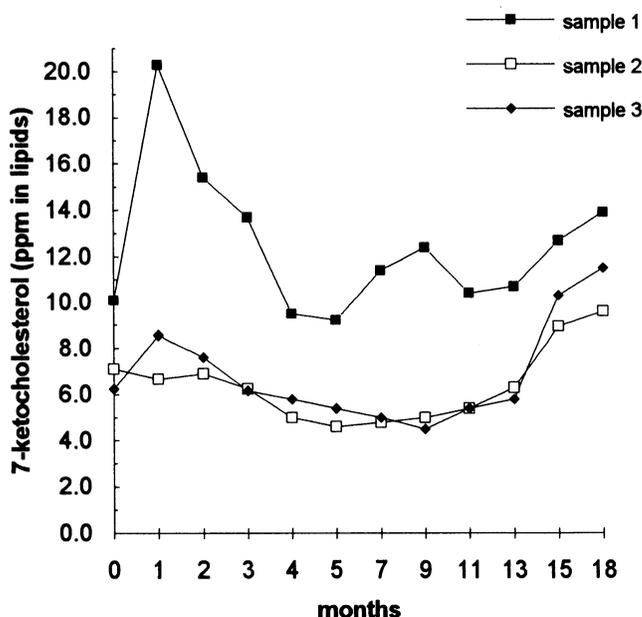


Fig. 3. Changes in 7-ketocholesterol content during storage of industrial pasta from known ingredients and manufacturing technologies.

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