Determination of the Country of Origin of Pistachio Nuts by DSC and HPLC

Susan M. Dyszel* and **Bruce C. Pettit**
U.S. Customs Service, 1301 Constitution Ave., NW Rm. 7113, Washington, D.C. 20229

The laboratories of the U.S. Customs Service frequently encounter the major analytical problem of determining the country of origin of imported commodities. This is particularly challenging with natural products. This paper will describe the process for one such commodity —pistachio nuts.

Two approaches were taken, high performance liquid chromatography (HPLC) and Differential Scanning Calorimetry (DSC). It was found that the results of these two techniques were complimentary and confirmatory. The HPLC data was obtained on the petroleum ether soluble fraction of the pistachio nuts while the DSC runs evaluated both this fraction and the whole nut. The HPLC results were further investigated by collecting fractions from the eluting peaks, derivatizing them to form the methyl ester of the fatty acids, and profiling by gas chromatography to confirm the triglycerides originally present. The U.S. Customs Service is currently using DSC area ratios to screen imported pistachio nuts for country of origin.

KEY WORDS: County of origin, DSC, GC, HPLC, pistachio nuts, triglycerides.

The laboratories of the U.S. Customs Service frequently encounter the major analytical problem of determining the country of origin of imported commodities. This is particularly challenging with natural products. This paper will describe the development of the country of origin determination for pistachio nuts. While pistachio nuts are grown in several areas of the world, the bulk of the pistachio nuts in commerce are grown in either California, Turkey or Iran. There was a concern that Iranian pistachios would be falsely labelled as being of Turkish origin because Executive Order 12613 (1) established an embargo against importations of Iranian products. Domestic pistachios were included in this project for two reasons. California is one of the major producers of pistachios and a significant portion of its crop is sent overseas for processing before re-entry into the U.S. As a further complication, virtually all Californian pistachios are descendants of Iranian stock from the Kerman region (2).

Differences in the fatty acid profiles among pistachios of different origins and varieties have been reported in the literature (3-7). It was felt that these variations would be expressed more clearly in the triglyceride profiles of the nut oils. This has been shown to be true in the case of olive oils (8).

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*To whom the correspondence should be addressed.

EXPERIMENTAL PROCEDURES

Sample preparation. Samples of California pistachios were obtained from the California Pistachio Growers Association. Samples of Iranian and Turkish pistachios were obtained in those countries by the Customs Attache, Rome. Three varieties of Turkish pistachios were encountered in this work. The first, Siirt, is the premium variety commanding the highest prices. The other two, Uzun and Kirmizi (U&K), are normally sold as a mixture and are, therefore, treated as a single type in this work. Unfortunately, information about the varietal makeup of the Iranian samples was unobtainable. All solvents were HPLC grade (Fisher Scientific, Fairlawn, NJ).

Samples of the pistachio nuts were taken which, when shelled, yielded about 4 grams of nutmeats. The nutmeats were chopped and then ground into fine fragments. The nut composite analytical specimens for DSC were taken at this point in triplicate, weighing between 5 and 15 mg, and were sealed in standard aluminum DSC sample pans. The remaining ground nut composite was then extracted with 20 milliliters of petroleum ether and shaken for one hour. The extract was filtered, the solids discarded, and the solvent removed from the filtrate under flowing nitrogen. The resulting oil fraction was then stored frozen until analysis. Defrosted oil fractions were sampled for DSC at time of analysis. Specimens weighing between 5-10 milligrams were sealed in DSC sample pans for liquids just prior to being run.

HPLC Conditions. The HPLC samples were run using the following conditions: Columns, 2-15 cm, C18, 3 micron particle size (Supleco Inc., Bellefonte, PA); columns were in series; mobile phase, acetone/acetonitrile (55:45, v/v); flow, 1.4 mL/min; column temperature, 40°C (thermostatted water jacket at internal temperature of RI detector); detector, refractive index (Waters Model 410, Waters Associates, Milford, MA); integrator, Hewlett Packard Model 3390A (Hewlett-Packard, Palo Alto, CA); sample prep, 160 mg of oil dissolved in 3.5 mL acetone, solution was filtered through 0.2 micron disposable filter; and injection, 10 microliter, sample loop injector.

GC conditions. Six HPLC injections and collections from the analytical HPLC column were necessary to provide sufficient sample for fatty acid analysis. One fraction was collected for each major HPLC peak. Each fraction was transesterified with BCl_3/methanol (Supelco, Inc.). The resulting methyl esters were separated on a SP-2330 fused silica capillary (30 m × 0.2 mm, Supelco, Inc.) column at 185°C, isothermal in a Varian 3700 GC (Varian Associates,

Palo Alto, CA), with a flame ionization detector with quantitation by relative peak area.

DSC Conditions. The DSC runs were made using a Perkin Elmer DSC-2 (Perkin Elmer, Norwalk, CT), in the heating mode for 233 to 300K at a rate of 5° per min under a dynamic Nitrogen atmosphere, flowing at 25 milliliters per min. The encapsulated specimens were taken at room temperature and placed directly into the cold DSC cell for shock cooling. They were allowed to equilibrate at 233K before the heating run was initiated.

The resulting DSC data was collected and processed by the PE TADS system using the Partial Areas program. This software assumes that a flat baseline exists between the designated endpoints. For the pistachio nuts and their oils, the selected endpoints were 245K and the return to baseline after the thermal peaks. This generally occurred between 280 and 285K. In evaluating the resulting DSC data, it was assumed that the area under the thermal curve could be divided into three distinct thermal events, labeled A, B, and C. Of these, the ratio of area A to B was used as the indicator of the country of origin.

RESULTS AND DISCUSSION

Figure 1 shows pistachio nuts from four representative origins as highlighted by their HPLC curves. Peak identification was by relative retention time, using an olive oil sample as reference. The identities of the triglycerides in olive oil have been confirmed by several workers (9). The results from the GC analyses were used to confirm the retention time assignments for the major components of each HPLC peak. Table 1 shows the GC results for a Turkish sample. The following abbreviations are used for fatty acids: L = 18:2; O = 18:1; S = 18:0; P = 16:0; Pa = 16:1; Ln = 18:3; and B = 20:1. It should be noted that the HPLC oil extracts contained all ether extractable materials, not just the triglycerides; thus, the presence of fatty acids other than linoleic in HPLC peak #1 is not unexpected. Table 2 shows the HPLC average triglyceride composition for oils from five known origins. CA and CA seconds are first and second quality grade nuts from California. As can be seen, the two Turkish types closely resemble each other and can easily be distinguished from both Iranian and California samples. However, the Iranian and California samples appear virtually identical. A close inspection of Table 2 reveals that the California types appear to have marginally higher percentages of palmitic-containing triglycerides than the Iranian. This difference can be clearly expressed by taking the ratios of certain palmitic triglycerides to their oleic analogs. These ratios, obtained from the HPLC data, are shown in Table 3.

Although there have been studies of vegetable oils by DSC in the past, such as those by Kawanura (10) and Dyszel (11), the application of DSC for determining the country of origin is unique. It requires an interpretation of the DSC curves to reveal the differences caused by origin. Figure 2 illustrates the melting curve obtained from a composite sample of first quality California pistachio nuts. The area designated A between the temperatures of 250 and 260K is clearly the major fraction of the total area under the curve. In contrast, Figure 3, the Turkish pistachio composite, demonstrated the reversal of area percents where A is clearly less than B. Figure 4, the Iranian pistachio sample, falls in between the profiles for the Californian and the Turkish. The thermal events being followed by the DSC are primar-

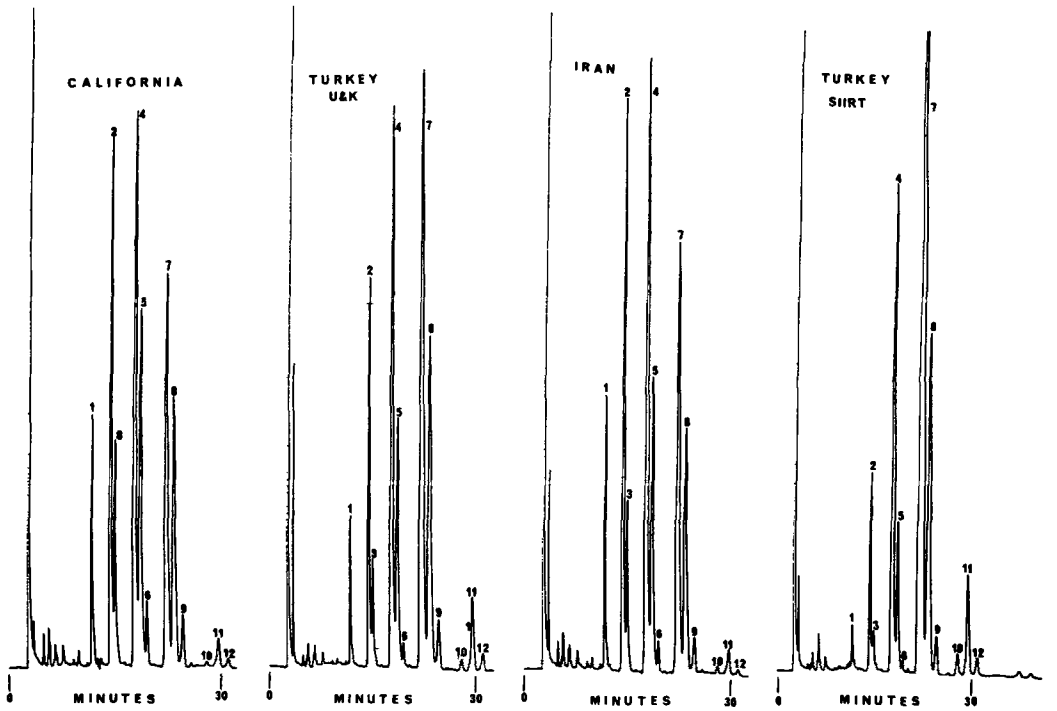

FIG. 1. HPLC curves for four pistachio oils: A, Turkey; B, Turkey-Siirt; C, California; and D, Iran. Triglycerides: 1, LLL, 2, LLO; 3, LLP; 4, LOO; 5, LOP; 6, LPP; 7, OOO; 8, POO; 9, POP; 10, OOB; 11, SOO; and 12, SOP.

TABLE 1

Fatty Acid Percentages of HPLC Peaks[a] by GC

	HLPC PEAK #									
	-1-	-2-	-3-	-4-	-5-	-6-	-7-	-8-	-9-	-11-
Fatty Acids										
16:0	4.1		35.2		26.1	68.4	1.0	36.2	68.8	1.5
16:1			1.6		0.7					
18:0					0.8			1.2		31.1
18:1	12.6	32.2	1.6	68.3	46.8		97.5	61.0	31.2	57.4
18:2	83.4	67.8	61.6	31.6	25.4	31.6	1.5	1.4		

[a]Major triglyceride found in HPLC Peak: LLL, LLO, LLP, OLO, OLP, PLP, OOO, OOP, POP, and SOO.

TABLE 2

Average Percentage of Triglyceride Composition by HPLC

Triglyceride	Iran	CA	CA Seconds	Turkey: Siirt	Turkey: U + K
LLL	4.8	4.6	6.5	1.0	2.1
LLO	16.1	14.5	18.1	4.7	8.9
LLP	4.5	5.9	6.9	1.3	2.4
LOO	25.4	22.2	23.2	16.9	22.2
LOP	11.7	14.4	14.1	5.2	8.5
LPP	1.0	2.0	1.7	<0.2	0.6
OOO	21.8	19.3	16.1	47.0	34.2
POO	12.1	13.6	10.8	17.7	16.2
POP	1.6	2.4	1.8	1.7	1.9
	<0.2	<0.2	<0.2	0.8	0.6
SOO	1.1	1.2	0.7	4.4	3.0
SOP	<0.2	<0.2	<0.2	1.0	0.6

TABLE 3

Ratios of Selected Palmitic Triglycerides to Their Oleic Analogs by HPLC

Ratio	Iran	CA	CA Seconds	Turkey: Siirt	Turkey: U + K
LLP/LLO	27.7	40.8	37.0	26.1	26.6
LOP/LOO	46.6	64.8	60.0	29.6	34.2
POO/OOO	55.6	70.9	66.7	36.8	42.9

ily the melting of the various triglycerides. This has been verified by the similarity in the thermal profiles of the ground nuts and the extracted oils.

Figure 5 illustrates the DSC curves for a ground pistachio nut sample and the corresponding extracted oil. It should be noted that for the same sample weight, the thermal peaks are larger and sharper for the oil than the nut sample, although the A/B ratio was the same for both. This diminution of the magnitude of thermal events is due to the presence of non-oil materials in the nut which contribute to the weight but not to the thermal transitions. It was observed that the DSC runs of the ground nut composite samples showed more variation from sample to sample than did the extracted oil samples and less variation than an equivalent

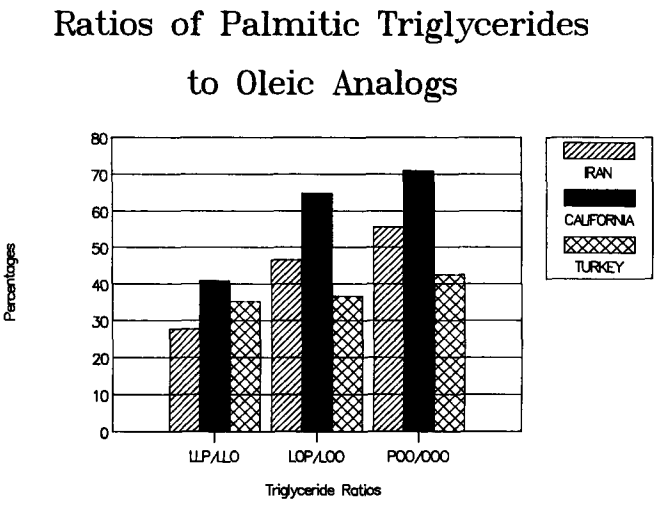

FIG. 2. DSC melting curve for pistacho nuts from California. A and B are designated measured partial areas.

number of samples taken from whole nuts. This was expected and reflects the sampling problem inherent in using milligram size samples to represent a less than perfectly homogeneous product.

In general, the lower C-number triglycerides have a lower melting temperature than do the higher C-number. The relative areas reflect the amount of energy it takes to melt that portion of the sample. Assuming that the molar heat of fusion for the triglycerides is equivalent, then the area under the curve represents the relative amount of the triglyceride fraction present. The ratio of area A divided by area B under the curve was tabulated. The A/B ratio for first quality Californian pistachio nut oil was greater than two, California seconds greater than three, the Iranian pistachios between one and two, and the Turkish less than one. Figure 6 displays in bar chart format the area percents of areas A and B for these same pistachio nut oils. Here the differences between origins become obvious. The observed differences were intrinsic to the nuts themselves and were unaffected by processing. Samples of roasted, salted or colored nuts displayed the same behavior as did their raw counterparts.

DSC has been shown to be a sensitive and useful technique for the assignment of country of origin for pistachio nuts. This method has been adopted by the U.S. Customs Service as the method of choice for screening pistachio nuts for country of origin. The drawback of small sampling sizes for nonhomogeneous natural products is overcome by taking an extract of the oil from a composite sample for analysis. The results have been shown to correlate with those obtained for the analysis of the pistachio nut oils by HPLC. The HPLC results paralleled the DSC curves as those samples having triglycerides of lower C-number and those with greater unsaturation displayed correspondingly lower melting temperatures, giving an increased area to segment A. The resulting DSC area ratio can be used directly to determine the country of origin of pistachio nuts.

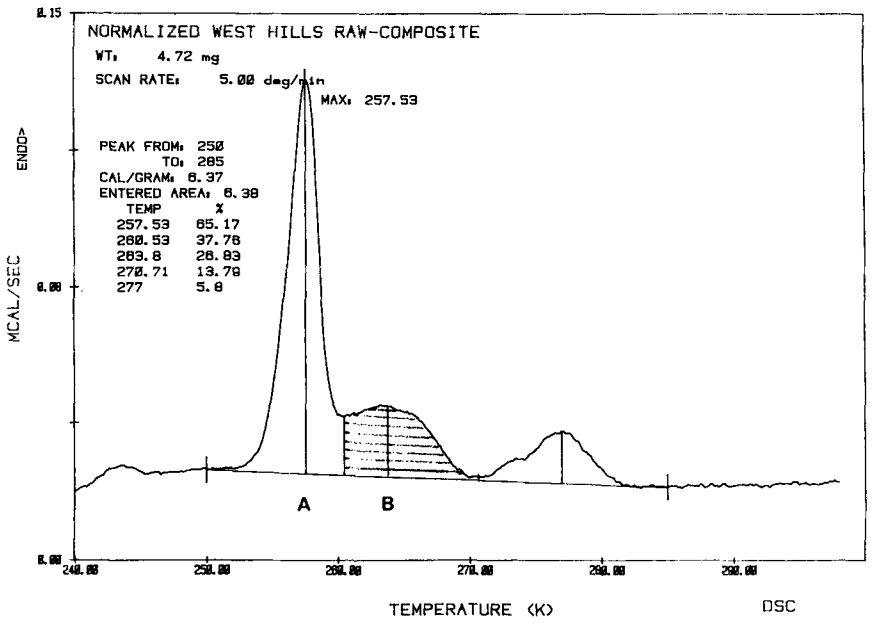

FIG. 3. DSC melting curve for pistachio nuts from Turkey. A and B are designated measured partial areas.

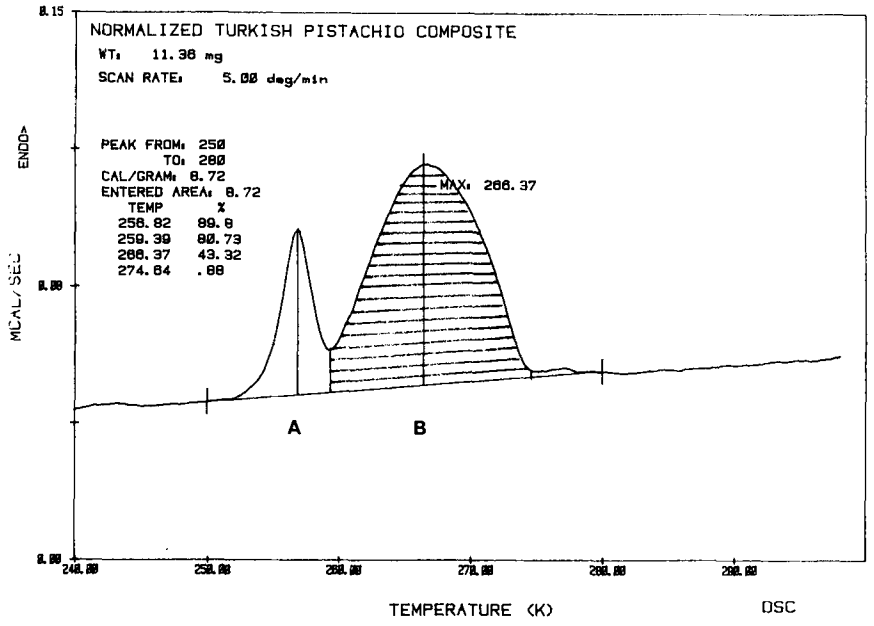

FIG. 4. DSC melting curve for pistachio nuts from Iran. A and B are designated measured partial areas.

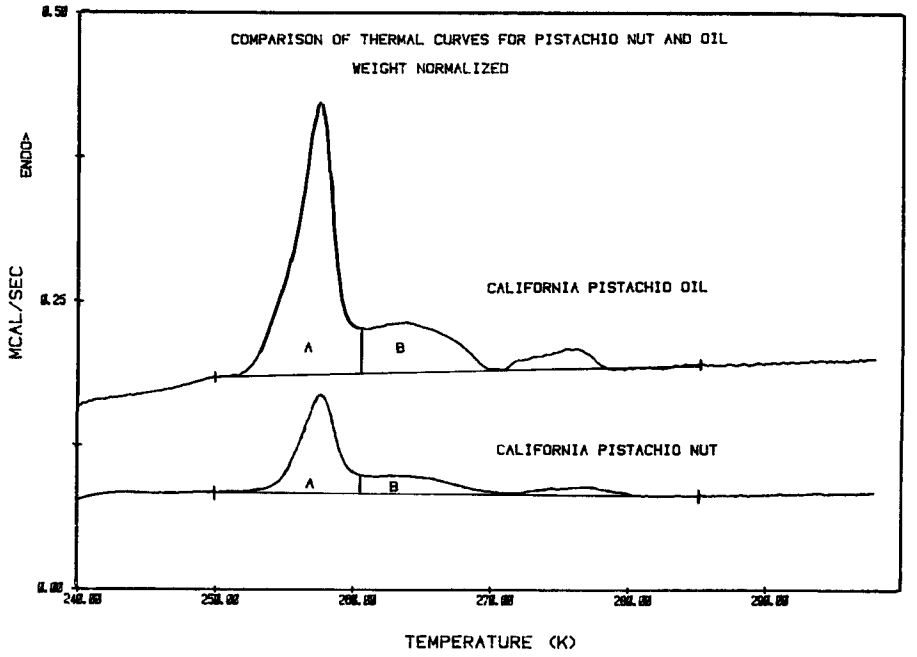

FIG. 5. Comparison of DSC curves for pistachio nuts and extracted oil on a weight normalized basis. The DSC curves are from the composite nut sample and the oil extracted from it. A and B are designated measured partial areas.

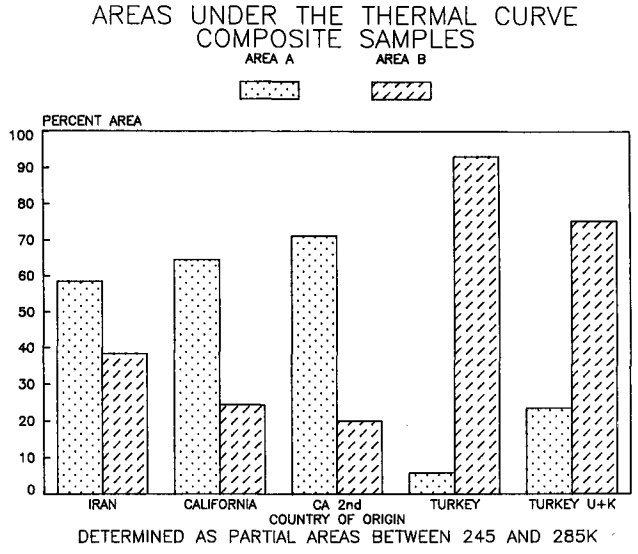

FIG. 6. Comparisons of partial areas under the DSC curves for pistachio nuts of known origin.

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