



November 14, 2007

Mr. Andrew Stephens
Director for Bilateral Agricultural Affairs
Office of the U.S. Trade Representative
Winder Building
600 17th Street, N.W.
Washington, D.C. 20506

Re: United States-Israel ATAP Written Comments

Dear Mr. Stephens,

Pursuant to the Federal Register Notice of October 16, 2007 by the Office of the U.S. Trade Representative ("USTR")¹, Paramount Farms ("Paramount") of Los Angeles, California submits these comments on the review and renegotiation of the United States-Israel Agreement on Trade in Agricultural Products ("ATAP"). These comments refer specifically to the export to Israel of U.S. pistachios (HTSUS No. 0802.50). Paramount is the largest U.S. producer of pistachios and exports its products all over the world.

As these comments will elaborate, despite duty free treatment for U.S. pistachios, the U.S. pistachio industry continues to experience restricted access to the Israeli market due to the illegal export to Israel of Iranian pistachios primarily through the Republic of Turkey. This trade barrier violates both Israeli law and the Israel-Turkey Free Trade Agreement ("FTA"), distorts Israel's pistachio market, and contravenes the intent of the ATAP. As a result, the U.S. pistachio industry requests that a key negotiating objective of the USTR be to seek the removal of this trade barrier during the upcoming review and negotiation of the ATAP.

BACKGROUND

California represents approximately 98 percent of U.S. pistachio production. The industry produces an average of 300 million pounds of pistachios a year and employs thousands of Californians. The industry has grown more than three times in value in the past ten years to over US\$500 million in annual sales today. Industry sales are expected to grow to over US\$1 billion in the coming years. Exports are fundamental to this growth.

¹ Notice of Request for Public Comments on the Review and Renegotiation of the United States-Israel Agreement on Trade in Agricultural Products, 72 Federal Register 58,688 (2007).

Iran is the world's largest producer of pistachios and is the U.S. pistachio industry's largest competitor. Iranian pistachios are frequently contaminated with high levels of aflatoxin – a naturally occurring carcinogen – and often exceed Minimal Risk Levels (“MRLs”) for aflatoxin established by various countries. Led by Paramount, the U.S. pistachio industry has made significant investments to incorporate aflatoxin preventative measures into its farming and processing operations. The results of these safeguards are considerable: shipments of Iranian pistachios to the European Union (“EU”) have a 53-fold higher incidence of being rejected by the EU due to high aflatoxin levels than do U.S. pistachios.

Where there is a level playing field, the differentiators between U.S. and Iranian pistachios become the product itself. U.S. pistachios are higher quality and healthier than pistachios from Iran, and in markets where free trade is permitted, U.S. pistachio producers out-compete Iranian producers.

The Israeli pistachio market is worth an estimated US\$20 million per year for the U.S. pistachio industry. Unfortunately, this level of trade has not been achieved. According to trade figures from the Israeli Ministry of Agriculture, Turkey holds an estimated 83 percent share of Israel's pistachio market.² The U.S. by comparison holds a 5 percent share.³

TREATMENT OF PISTACHIOS UNDER ISRAELI LAW, THE ISRAEL-TURKEY FTA, AND THE ATAP

Israeli Law

Israeli national law prohibits the importation of goods and services – including pistachios – from Iran. Under Israel's Trading with the Enemy Act of 1939, any form of trading, direct or indirect, with Iran is prohibited. If Israeli customs authorities believe that goods are imported from Iran, they may block the import(s), and the importer(s) may be subject to certain penalties.

The Israel-Turkey FTA

Israel and Turkey signed an FTA in 1996. That agreement entered into force in 1997. Under the terms of this FTA, Israel agreed to reduce or eliminate import duties on select goods originating in Turkey, including agricultural products such as pistachios. Turkey may export to Israel up to 100 tons per year of pistachios duty free.⁴ The duty-free treatment of the first 100

² See, “Pistachio Import Data into Israel – 2004 to 2006”, Israeli Ministry of Agriculture (attached). To date, our research on pistachio exports to Israel has yielded considerably different figures. Given this discrepancy, we continue to work through contacts in the U.S., Turkey and Israel to gather accurate trade data. We will forward this to USTR once we obtain it.

³ See Id.

⁴ See Annex I to Protocol A Concerning Arrangements Applicable to the Importation into Israel of Agricultural and Pro-Agricultural Products Originating in Turkey, Israel-Turkey FTA, available at: <http://www.tamas.gov.il/NR/exeres/6E2AF981-E1A7-4081-AF71-398AC2547980.htm>. Additional information on this tariff rate quota (“TRQ”) is not readily available. Indeed, other credible sources indicate that Israel's Most Favored Nation (“MFN”) duty rate on pistachios may actually be zero, thus rendering a TRQ meaningless from a

tons of pistachios is contingent on compliance with the Rules of Origin set forth in the Agreement.⁵

The Israel-Turkey FTA defines products as originating in Turkey if they are either (1) wholly obtained in Turkey, or (2) are obtained in Turkey and incorporate materials not wholly obtained there but that have undergone sufficient working or processing in Turkey (i.e., “substantial transformation”).⁶ Vegetable products harvested in Turkey are considered wholly obtained there and should be eligible for duty-free treatment in Israel. Pistachio nuts harvested beyond Turkey’s borders do not meet any of the criteria for being wholly obtained in Turkey.⁷

It is highly unlikely that pistachios originating in Iran could be deemed “sufficiently worked or processed” in Turkey as to qualify as being of Turkish origin. The FTA details that the following are insufficient for establishing Turkish origination: preserving, washing or cleaning, partial or total bleaching, shelling of nuts, sifting, sorting, grading, placing in containers or packaging, affixing marks or logos, or mixing.⁸ As we understand it, there is little other working or processing of raw pistachio nuts that could conceivably enable pistachios from Iran to be considered of Turkish origin under the Agreement.

To avoid extending preferential treatment to products not of Turkish origin, the Israel-Turkey FTA requires that proof of origin accompany the imported product from Turkey and be submitted to Israeli customs authorities. Those products which on importation would benefit from the provisions of the Agreement must have either one of three forms: (1) a movement certificate EUR.1, (2) a movement certificate EUR-MED, or (3) in the case of an approved exporter, an invoice declaration.⁹ For import into Israel, these first two forms must be issued by the Turkish customs authority on application of the exporter. The exporter must be prepared to

commercial perspective. Regardless, we continue to seek more detailed information concerning the TRQ and Israel’s MFN duty rate and we share this information with USTR once we have it.

⁵ See Protocol A, Article III, Israel-Turkey FTA, available at: <http://www.tamas.gov.il/NR/exeres/8E7D35CF-D6A4-4150-9F0A-501F5BF7E9F6.htm>.

⁶ See Protocol B, Title II, Article 2 Concerning General Requirements of the Definition of the Concept of ‘Originating Products’, Israel-Turkey FTA, available at: <http://www.tamas.gov.il/NR/rdonlyres/B40B6802-1E7A-423F-9FBF-39BBF7DC1772/0/protokolBfinal.doc>.

⁷ See Id. at Article 2 Concerning Wholly Obtained Products, Israel-Turkey FTA, available at: <http://www.tamas.gov.il/NR/rdonlyres/B40B6802-1E7A-423F-9FBF-39BBF7DC1772/0/protokolBfinal.doc>.

⁸ See Id. at Article 7 Concerning Insufficient Working or Processing, Israel-Turkey FTA, available at: <http://www.tamas.gov.il/NR/rdonlyres/B40B6802-1E7A-423F-9FBF-39BBF7DC1772/0/protokolBfinal.doc>.

⁹ See Protocol B, Title V, Article 16 Concerning General Requirements of Proof of Origin, Israel-Turkey FTA, available at: <http://www.tamas.gov.il/NR/rdonlyres/B40B6802-1E7A-423F-9FBF-39BBF7DC1772/0/protokolBfinal.doc>.

provide documentation proving the originating status to the Turkish customs authorities.¹⁰ Approved exporters must also be prepared to submit such information.

Verification of proofs of origin “shall be carried out at random or whenever the customs authorities of the importing country have reasonable doubts as to the authenticity of such documents, the originating status of the products concerned, or the fulfillment of the other requirements....”¹¹ The subsequent verification shall be carried out by the Turkish authorities, and Israel may suspend the preferential trade treatment to the products concerned pending the results. In sum, the Israel-Turkey FTA provides for strict requirements that allow preferential treatment only to products originating in the two countries.

The ATAP

In 1985, the U.S. and Israel agreed to establish a Free Trade Area Agreement (“FTA”) between the two countries.¹² The purpose of the FTA is to “eliminate the duties and other restrictive regulations of commerce on trade between the two nations . . .”¹³ Because both governments held different interpretations of certain provisions of the FTA related to agricultural products, they signed an adjunct agreement to the FTA – the ATAP – in 1996.¹⁴ U.S. pistachio exports to Israel enjoy duty free treatment under the ATAP.¹⁵

Article 19 of the FTA permits either Party to invoke dispute settlement mechanisms if (1) a dispute arises, (2) a Party considers that the other Party has failed to carry out its obligations under the FTA, or (3) “one Party considers that measures taken by the other Party . . . severely distort the balance of trade benefits accorded by this Agreement or substantially undermine fundamental objectives of this Agreement”.¹⁶ Such dispute settlement mechanisms include consultations between both Parties.

¹⁰ See Id. at. Article 17 Concerning Procedure for the Issue of a Movement Certificate EUR.1 or EUR-MED, Israel-Turkey FTA, available at: <http://www.tamas.gov.il/NR/rdonlyres/B40B6802-1E7A-423F-9FBF-39BBF7DC1772/0/protokolBfinal.doc>.

¹¹ See Protocol B, Title VI, Article 33 Concerning Verification of Proofs of Origin, Israel-Turkey FTA, available at: <http://www.tamas.gov.il/NR/rdonlyres/B40B6802-1E7A-423F-9FBF-39BBF7DC1772/0/protokolBfinal.doc>.

¹² See United States-Israel Free Trade Area Agreement, Aug. 19, 1985, available at: http://tcc.export.gov/Trade_Agreements/All_Trade_Agreements/exp_005439.asp.

¹³ Id. at Article 1.

¹⁴ See United States-Israel Agreement on Trade in Agricultural Products, 1996 (renewed in 2004), available at: http://www.ustr.gov/assets/Trade_Agreements/Bilateral/Israel/asset_upload_file899_7902.pdf.

¹⁵ Id. at Annex A: Free Imports into Israel from USA, Pistachios (2004 Israeli Customs Tariff Number 0802.500).

¹⁶ Article 19(1)(a), U.S.-Israel FTA, available at: http://tcc.export.gov/Trade_Agreements/All_Trade_Agreements/exp_005439.asp

In confirming the U.S. understanding of the ATAP, then-USTR Robert Zoellick noted the following in a letter to then-Vice Prime Minister and Minister of Industry, Trade, Labor and Communications, Ehud Olmert:

To facilitate a better understanding of each party's agricultural economy in the course of future discussions on improving agricultural market access, either party may present information on factors of importance to their respective agricultural sectors. Such factors may include, but are not limited to: annual overall trade in a product, the scope of U.S.-Israeli trade in that product and the interests which each party has in expanding access to the other's market; trends in domestic production, prices and profitability of a product and the capacity for further market access; economic circumstances of producer, such as changes in rural income; the benefits of liberalization of agricultural trade, including benefits to consumers, trade and food processors; and additional agricultural policy considerations related to issues such as national and regional development priorities, government assistance, food security, special security conditions, and multilateral agricultural policy objectives. The aforementioned factors may be referenced by the parties and provide guidance in future discussions, including in the U.S.-Israel Joint Committee, on further improvements in agricultural market access.¹⁷

This understanding was confirmed by the Israeli Government in a subsequent letter from Mr. Olmert to Mr. Zoellick dated July 27, 2004.¹⁸

ISSUE

Evidence suggests that many, if not most, of Turkey's pistachio exports to Israel in fact consist of smuggled Iranian pistachios falsely identified as products of Turkey. Moreover, a considerable amount of the pistachios exported to Israel from the EU are in fact Iranian pistachios that have been re-exported after being rejected by the EU for having exceeded aflatoxin limits. This product is entering Israel undetected due to insufficient inspection procedures in Israel. These procedures fail to differentiate between Turkish and Iranian pistachios. Most importantly, this trade violates Israel's Trading with the Enemy Act, violates the Rule of Origin provision of the Israel-Turkey FTA, significantly distorts the Israeli pistachio market, and contravenes the intent of the ATAP by severely restricting U.S. access to Israel's market.

In June 2007, Paramount representatives traveled to Israel where they conducted informal interviews with sellers in the Tel Aviv nut market. These sellers confirmed that a majority of the

¹⁷ Letter to Ehud Olmert from Robert Zoellick, July 27, 2004, available at: http://www.ustr.gov/assets/Trade_Agreements/Bilateral/Israel/asset_upload_file899_7902.pdf.

¹⁸ Letter to Robert Zoellick from Ehud Olmert, July 27, 2004, available at: http://www.ustr.gov/assets/Trade_Agreements/Bilateral/Israel/asset_upload_file899_7902.pdf.

pistachios exported to and sold in Israel come from Turkey, but are in fact of Iranian origin. Moreover, Turkish exporters reported to Paramount that they are readily able to export Iranian pistachios to Israel.

Unfortunately, this is not a new issue. The U.S. pistachio industry has pressed the U.S. Government for many years to address Iran's infiltration of Israel's pistachio markets. Progress was made in the mid-late 1990s after Israel increased inspections and adopted methods to test pistachio imports to determine their country of origin. However, we have been informed by the USTR that during the October 2007 U.S.-Israel Joint Committee meeting, the Israeli Government noted that these new inspection and testing procedures had been successfully challenged in Israel's courts, ultimately forcing Israeli customs authorities to discontinue the reformed procedures. As a result, the Israeli import inspection system remains ineffective in prohibiting the entry of Iranian pistachios into the country.

This illicit trade carries with it a negative economic impact for Israel. Importation of Iranian pistachios into Israel under the guise of Turkish origin deprives Israel of tariff duties owed. According to the Israel-Turkey FTA, Israel has the right to request further documentation establishing the origin of the pistachios and to suspend the applicable preferential duties in the interim.

While these illicit imports violate Israeli law, they also negatively affect the U.S. pistachio industry's share of the Israeli market. Indeed, permitting Iranian pistachios into Israel undermines the fundamental objectives of the ATAP, and distorts the balance of trade benefits accorded by the agreement. Paramount estimates that, but for this trade barrier, U.S. pistachio producers could increase exports to Israel by approximately US\$20 million per year.

PROPOSED REMEDIES

Article 19 of the FTA permits the U.S. to seek resolution of a trade dispute, in part, through consultations with the Israeli Government. As noted herein, with particular respect to agricultural products, such consultations can include discussions of the capacity for further market access; benefits of liberalization of agricultural trade, including benefits to consumers, trade, and food processors; and agricultural policy considerations related to food security, among other matters.

The situation surrounding U.S. pistachio exports to Israel directly involves several of the issues above. To wit, the U.S. pistachio industry has a well documented desire to increase its access to the Israeli market. There are considerable benefits for Israeli consumers and food processors to increased imports of U.S. pistachios. As noted U.S. pistachios are a far healthier and safer food than Iranian pistachios. One need only look at the amount of Iranian pistachios that are regularly rejected from the EU due to excessive levels of aflatoxin, to understand that the safety of Israel's pistachio market would be greatly enhanced by increasing the amount of U.S. pistachios imports.

On behalf of the U.S. pistachio industry, Paramount requests that the trade-restrictive barrier discussed herein be addressed during the review and renegotiation of the ATAP. In

particular, we recommend that the USTR strongly encourage the Israeli government to enforce its own laws and the Rules of Origin provision in the Israel-Turkey FTA, to prohibit the importation into Israel of Iranian pistachios. By failing to do so, Israel has effectively – and significantly – limited U.S. access to Israel’s pistachio market.

Specific Recommendations

The U.S. pistachio industry recommends that four key remedies be discussed with the Israeli Government to help resolve the Iranian import issue. First, Israeli customs officials should require sworn affidavits from any exporter of pistachios into Israel confirming the country of origin of those pistachios. Second, Israeli customs officials should request verification from any exporter of pistachios into Israel claiming a preference under an FTA.

Third, Israeli customs officials should be trained to identify Iranian pistachios. With regard to this point, there are two primary methods by which to differentiate pistachios obtained from different geographical locations including California, Turkey and Iran. A physical/visual test is the most expedient method to employ in order to expedite product through customs. The commonly exported Turkish pistachio is the Antep variety. As evidenced by the attached Power Point presentation, this type of pistachio is easily distinguishable from the Iranian (Fandoghi) variety available in Israel.

The second type of test is a High Performance Liquid Chromatography (“HPLC”) analysis. Pistachios from different geographic locations generally contain different concentrations of various chemicals. HPLC testing measures the profile of trace element concentrations found in pistachios. From this, researchers are able to identify what geographic location a particular pistachio is from. Attached is a study that employed the HPLC test to determine the geographic location of various pistachio samples.¹⁹ The study concluded, in part, that a pistachio sample obtained in the Tel Aviv nut market was in fact the Iranian Fandoghi variety. While more accurate, the HPLC test is expensive and requires sophisticated equipment. The U.S. pistachio industry recognizes the potential inefficiency of employing such testing on a wide scale. We therefore recommend that HPLC testing be limited to periodic spot checks. It also should be used to test product deemed suspect via the physical/visual test.

Fourth, and finally, the U.S. pistachio industry recommends that the Israeli Government implement procedures by which to test pistachio imports for aflatoxin. As noted, we believe that a considerable portion of the exports to Israel of pistachios from the EU are in fact Iranian pistachios that were rejected by the EU for exceeding aflatoxin levels. Testing imports of pistachios in Israel for aflatoxin would help identify likely Iranian product, thereby protecting Israeli consumers from unhealthy Iranian pistachios.

¹⁹ See “Use of Anthocyanin Phytochemical Fingerprinting (Cyanidin-3-Glucosides and Cyanidin-3-Galactosides Content) as a Tool to Differentiate Pistachio Samples from Different Geographical Locations”, Memorandum from Dr. Navindra Seeram, Mark Hughes Cellular and Molecular Nutrition Laboratory, UCLA and Dr. Yanjun Zhang, Mark Hughes Cellular and Molecular Nutrition Laboratory, UCLA to Mark Masten, Paramount Farms, Inc., Aug. 31, 2007 (attached).

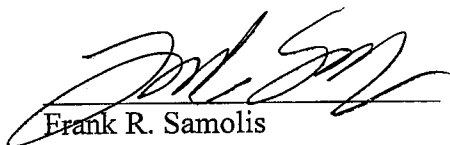
CONCLUSION

On behalf of the U.S. pistachio industry, I thank you for considering these comments. The U.S. pistachio industry stands ready to provide you with any assistance you require to successfully resolve this matter.

Sincerely,



Mark L. Masten
Vice President, Global Sales
Paramount Farms



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Counsel for Paramount Farms

Pistachio Import Data into Israel - 2004 to 2006

| Country | 2004 | | 2005 | | | | 2006 | | |
|----------------------|-----------|------------------|---------------------|------------|------------------|------------------|------------|----------------------|------------|
| | Volume | (KG's) | (lbs) | % of Total | (KG's) | (lbs) | % of Total | Value (\$'s) | % of Total |
| USA | | 144,891 | 318,760 | 4% | 407,879 | 897,334 | 12% | \$ 797,000 | 5% |
| Germany | | 360,181 | 792,398 | 9% | 365,100 | 803,220 | 11% | \$ - | 0% |
| England | | 537,550 | 1,182,610 | 14% | 151,500 | 333,300 | 4% | \$ 1,600,000 | 11% |
| Turkey | | 2,674,337 | 5,883,541 | 68% | 2,366,689 | 5,206,716 | 70% | \$ 12,100,000 | 83% |
| Jordan | | 103,000 | 226,600 | 3% | - | - | 0% | - | 0% |
| Luxemburg | | 97,700 | 214,940 | 2% | - | - | 0% | - | 0% |
| France | | 7,350 | 16,170 | 0% | - | - | 0% | - | 0% |
| Greece | | - | - | 0% | 22,000 | 48,400 | 1% | - | 0% |
| Croatia | | - | - | 0% | 45,000 | 99,000 | 1% | \$ 85,000 | 1% |
| Italy | | - | - | 0% | 520 | 1,144 | 0% | \$ 5,000 | 0% |
| Belguim | | - | - | 0% | 25,000 | 55,000 | 1% | - | 0% |
| Value | \$ | 3,925,009 | \$ 8,635,020 | | 3,383,688 | 7,444,114 | | \$ 14,587,000 | |
| | | | | | | | | | |
| Value (\$/lb) | \$ | | \$ 1.78 | | | \$ 2.63 | | | |

Source: Israeli Agricultural Ministry



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The Israeli Market

Confidential – Paramount Farms

Israel Pistachio Market: Import Volumes

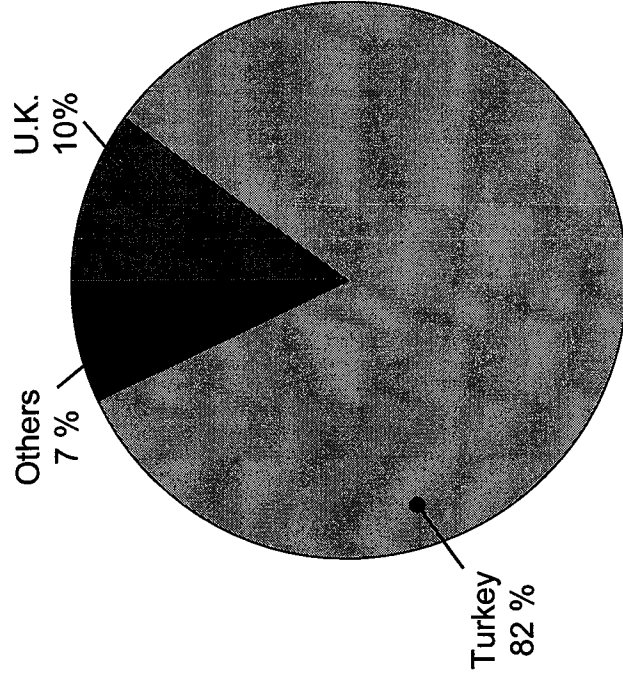
2



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Israel reports largest pistachio imports from Turkey.

➤ Israel Pistachio Import Volumes (in \$)



* 2006

Chart Key

- + 14,6 Million \$ imported by Israel
- + Turkey exported 12 million \$
- + U.K. exported 1,6 million \$

Path of Iranian Product



Iranian Product **→** **E.U.** **→** **Israel**

Pistachios are rejected by the E.U. for high aflatoxin levels and are then exported to Israel.

Iranian Product **→** **Turkey** **→** **Israel**

Israel reports largest pistachio imports from Turkey

Why Turkey?



- In 1996, a Free Trade Agreement was established between Israel and Turkey.
Source: Turkey-Israel Free Trade Agreement
- Although Turkey is 3rd largest producer of pistachios in the world, approximately 11% of its total production is exported.
Source: GAIN Report - TU4026
- Turkey imported Iranian pistachios ranging between 4 – 22 million pounds per year in 1999-2003.
Source: United Trade Atlas
- Several Turkish exporters reported that they were readily able to provide Iranian pistachios to be exported to Israel.

Ref. Turkish Exporters

Sultan Demir
BAHA ROĞLU TARI M
ÜRÜN.SA N.VE Tİ C.A .Ş.

Mehmet Kucukakin
KAHRAMA N TARIM ÜRÜNLERİ A .Ş.



Pistachio Product Overview vs. Israeli Pistachio Sample

| Varieties | Turkish | |
|-----------------|---|--|
| | Antep, Uzun, Kirmizi Halebi, Siirt | |
| | Red-Purple Crust Ivory Shells | |
| | 16 - 22 | |
| | 10 - 12 | |
| Characteristics | Visual | |
| | Length (mm) Range | |
| | Width (mm) Range | |
| | Diameter (mm) Range | |
| Scientific | Ratio Range | |
| | Cyanidin-3-Glucosides & Cyanidin-3-Galactosides Ratio | |
| | 68-70 | |

| Iranian | |
|---|--|
| Fandoghi Akbari, Ahmad Badami, Kaleghochi | |
| Brown-Red Crust Tan Shells | |
| 18 - 20 | |
| 12 - 14 | |
| 13 - 15 | |
| Ratio Range | |
| 0.7 - 54.94 | |

| Israel Sample | |
|----------------------------|--|
| Fandoghi | |
| Brown-Red Crust Tan Shells | |
| 18 - 20 | |
| 12 - 15 | |
| 13 - 16 | |
| Ratio | |
| 35.20 | |

Israeli Market: Proof of Iranian Product

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The pistachio sample from Israel was submitted to the University of Los Angeles to determine the country of origin using an HPLC - UV method.

Dr. Navindra Seeram concluded that the pistachio sample from Israel was in fact the Fandoghi variety from Iran.

Sources



- (1) Seeram, N.P.; Zhang, Y.; Heber, D.; Use of Anthocyanin Phytochemical Fingerprinting *Unpublished Paper (Personal Communication)*. Center for Human Nutrition, University of California L.A. 2007.
- (2) Hazekamp, Tom. Descriptors for Pistachio (Pistachia Vera L.). International Plant Genetic Resources Institute.
- (3) Central Bureau of Statistics
- (4) Turkey - Israel Free Trade Agreement. URL <www.dtm.gov.tr/dtmadmin/upload/ABISerbesTicare/bDb/israel.doc>
- (5) USDA Foreign Agricultural Service. GAIN Report TU 4026.
- (6) United Trade Atlas
- (7) World Horticulture Trade & U.S. Export Opportunities.

TO: Mark Masten,
Paramount Farms, Inc
CC: Dr. David Heber, Center of Human Nutrition, UCLA
FROM: Dr. Navindra Seeram, Mark Hughes Cellular and Molecular Nutrition
Laboratory, UCLA
Dr. Yanjun Zhang, Mark Hughes Cellular and Molecular Nutrition
Laboratory, UCLA

DATE: August 31, 2007

PROJECT: Use of Anthocyanin Phytochemical Fingerprinting (Cyanidin-3-Glucosides
and Cyanidin-3-Galactosides Content) as a Tool to Differentiate Pistachio
Samples from Different Geographical Locations

1. OBJECTIVE

To establish a method to differentiate pistachios obtained from different geographical locations by analyzing anthocyanin content (cyanidin-3-glucosides and cyanidin-3-galactosides)

2. SUMMARY

Thirteen pistachios samples were analyzed to determine the contents of cyanidin-3-glucosides and cyanidin-3-galactosides. The samples were extracted with methanol and the resulting extract was subjected to HPLC-UV analysis. The result showed that samples contained different levels of cyanidin-3-glucosides and cyanidin-3-galactosides.

3. SAMPLES

- 3.1 YZ/39/90A Sunkist California (nuts, purchased from a food store in LA)
- 3.2 YZ/39/90B Gold Series California (nuts, purchased from internet)
- 3.3 YZ/39/90C Gold Series Turkish (nuts, purchased from internet)
- 3.4 YZ/39/90D Westwood Iranian (nuts, purchased from a food store in LA)
- 3.5 YZ/39/90E SGPID, Inc Round (nuts, provided by Paramount Farms Inc.)
- 3.6 YZ/39/90F Fandoghi Variety Round (nuts, provided by Paramount Farms Inc.)
- 3.7 YZ/39/90G Ahmad Aghie Long Pistachio (nuts, provided by Paramount Farms Inc.)
- 3.8 YZ/39/90H Kalagouchi Variety Jumbo (nuts, provided by Paramount Farms Inc.)
- 3.9 YZ/39/90I Akbari Long (nuts, provided by Paramount Farms Inc.)
- 3.10 YZ/39/90J SGPID, Inc Long (nuts, provided by Paramount Farms Inc.)
- 3.11 YZ/39/90K Israel (nuts, provided by Paramount Farms Inc.)
- 3.12 YZ/39/90L Erdal Bezaroglu (kernel only, provided by Paramount Farms Inc.)
- 3.13 YZ/39/90M American (provided by Paramount Farms Inc.)

4. STANDARDS

- 4.1 Kuromanin chloride, ChromaDex, Lot#03-11605-221
- 4.2 Cyanidin-3-galactoside, purified at UCLA Center for Human Nutrition.

5. REAGENT

- 5.1 Water – Millipore Water in-house
- 5.2 Methanol – Solvent, Fisher Scientific
- 5.3 12M HCl – Solvent, Fisher Scientific
- 5.4 Acetonitrile – HPLC Grade, Fisher Scientific
- 5.5 H₃PO₄ – HPLC Grade, Fisher Scientific

6. EQUIPMENTS:

- 6.1 METTLER Toledo AT 261 Analytical balance
- 6.2 Beckman CS-6R Centrifuge
- 6.3 Assorted glassware
- 6.4 Waters HPLC system
- 6.5 Agilent Zorbax SB-C18, 4.6 x 250 mm column
- 6.6 Mr. Coffee Coffee Grinder IDS77

7. EXPERIMENTAL METHODS

7.1. Preparation of samples:

Weigh 50 pieces of pistachios. The kernels were separated from the shells and weighed. The kernels were ground in a coffee grinder for 15 seconds. About 1 g of ground kernels was dissolved in 5 mL of methanol and 300 µL of 12M hydrochloric acid. After sonicating for 20 minutes at room temperature, the solution was centrifuged at 10000 rpm for 2 minutes. The supernatant was loaded onto HPLC.

7.2 Preparation of Stock Standard solutions

1.0 mg of Kuromanin chloride (cyanidin-3-glucoside) was dissolved in 1.0 mL of methanol to make a 1000 µg/mL standard stock solution and kept at -20 °C before analysis.

7.3 Linearity dilution for Standard Curves

Linearity Standard #1

100 µL of the stock standard solution was pipetted into a 1.0-mL volumetric tube and diluted to volume with water/methanol (1:1 v/v), generating a solution at concentration of 100 µg/mL (S1).

Linearity Standard #2

50 µL of the stock standard solution was pipetted into a 1.0-mL volumetric tube and diluted to volume with water/methanol (1:1 v/v), generating a solution at concentration of 50 µg/mL (S2).

Linearity Standard #3

25 µL of the stock standard solution was pipetted into a 1.0-mL volumetric tube and diluted to volume with water/methanol (1:1 v/v), generating a solution at concentration of 25 µg/mL (S3).

7.4 HPLC Conditions for Cyanidin-3-Glucoside

Column: Zorbax SB C₁₈, 5µm 4.6x250 mm

Guard column: C₁₈, 5µm, 3.9x20 mm

Flow Rate: 0.75 mL/min

Mobile Phase: A: acetonitrile,

B: 0.4% H₃PO₄ in water

Pump Program: (Linear gradient)

| Time (min) | Flow (mL/min) | A (%) | B (%) |
|------------|---------------|-------|-------|
| - | 0.75 | 5 | 95 |
| 10 | 0.75 | 15 | 85 |
| 30 | 0.75 | 25 | 75 |
| 40 | 0.75 | 35 | 65 |
| 41 | 0.75 | 5 | 95 |
| 50 | 0.75 | 5 | 95 |

Injection Volume: 25 µL

Detection: 520 nm

8. RESULTS:

Calibration

Each linearity standard solution was injected in duplicate prior to the sample injections.

Sample

The ratio of kernels to the total weight of 50 pieces of pistachios is presented in **Table 1**. Double injections were made. Cyanidin-3-glucoside and Cyanidin-3-galactoside contents are presented in **Table 2**.

Chromatograms of the standards are present in **Figure 1** and **Figure 2** and the pistachio samples are presented in **Figure 3-15**. Standard curve of the kuromanin chloride is presented in **Figure 16**.

9. CONCLUSIONS & DISCUSSION

Cyanidin-3-glucoside and Cyanidin-3-galactoside in the pistachio samples were identified by comparison with the corresponding standards.

There is not a significant difference between the kernel to nut ratios of pistachios from different locations. It is difficult to get a conclusive jurisdiction of the locations by comparing only the kernel to nut ratio.

However, when calculating the ratio of typical anthocyanin contents, cyanidin-3-galactoside to cyanidin-3-glucoside, the pistachios from different locations have different ratios of the two-anthocyanin contents. The California pistachios have a cyanidin-3-galactoside to cyanidin-3-glucoside ratio of less than 10, while the Iranian pistachios have a very wide range, between 0.7 to 54.94 (perhaps because of the different varieties), whereas, the Turkish pistachios have the highest ratio at around 70.

We have concluded that the two samples in question (provided by Paramount Farms International):

- 1) Sample YZ/39/90L (Erdal Bezaroglu) is from Turkey by comparison with the Turkish sample purchased by UCLA i.e. Gold Series Turkish (YZ/39/90C) with a similar ratio ranging from ~68-70 (See Table 2).
- 2) YZ/39/90K is from Iran by comparison to Iranian samples provided by Paramount Farms (YZ/39/90F Fandoghi Variety Round) with a similar ratio of ~35 (See Table 2).

The result presented here is only a preliminary investigation based on a small number of samples. For a more conclusive investigation, a well-designed study that includes more samples should be conducted.

APPENDIX 1: TABLE

Table 1. Amount of Kernels in Pistachio Samples

| Number | Sample Name | UCLA ID | Weight of Kernels (g) | Weight of 50 pieces Pistachios (g) | Ratio of Kernels (%) |
|--------|--------------------------|-----------|-----------------------|------------------------------------|----------------------|
| 1 | Israel | YZ/39/90K | 37.8 | 68.1 | 55.5 |
| 2 | Gold Series Turkish | YZ/39/90C | 21.6 | 41 | 52.7 |
| 3 | Erdal Bezaroglu | YZ/39/90L | 18.5 | NA | NA |
| 4 | Ahmad Aghie Long | YZ/39/90G | 36.3 | 64.6 | 56.2 |
| 5 | Akbari Long | YZ/39/90I | 33.5 | 62.8 | 53.3 |
| 6 | SGPID, Inc Long | YZ/39/90J | 29.1 | 57.3 | 50.8 |
| 7 | Kalagouchi Variety Jumbo | YZ/39/90H | 35.1 | 69.1 | 50.8 |
| 8 | Fandoghi Variety | YZ/39/90F | 26.9 | 47.5 | 56.6 |
| 9 | SGPID, Inc Round | YZ/39/90E | 25.0 | 47.4 | 52.7 |
| 10 | Westwood Iranian | YZ/39/90D | 37.0 | 66.7 | 55.5 |
| 11 | American | YZ/39/90M | 34.9 | 66.6 | 52.4 |
| 12 | Sunkist California | YZ/39/90A | 31.5 | 58.5 | 53.8 |
| 13 | Gold Series California | YZ/39/90B | 37.0 | 69.1 | 53.5 |

Table 2. Cyanidin-3-glucosides and Cyanidin-3-galactosides contents in Pistachios

| Number | Sample Name | UCLA ID | Sample Weight (mg) | Cyanidin-3-glucoside (µg/g) | Cyanidin-3-galactoside (µg/g) | Ratio of Cyanidin-3-galactoside to Cyanidin-3-glucoside |
|--------|--------------------------|-----------|--------------------|-----------------------------|-------------------------------|---|
| 1 | | | | | | |
| 2 | Erdal Bezaroglu | YZ/39/90L | 1023 | 1.42 | 97.67 | 68.78 |
| 3 | Gold Series Turkish | YZ/39/90C | 1079 | 1.21 | 84.77 | 70.06 |
| 4 | Ahmad Aghie Long | YZ/39/90G | 1085 | 12.9 | 79.19 | 6.14 |
| 5 | Akbari Long | YZ/39/90I | 1044 | 6.08 | 8.71 | 1.43 |
| 6 | SGPID, Inc Long | YZ/39/90J | 1038 | 5.31 | 4.20 | 0.79 |
| 7 | Kalagouchi Variety Jumbo | YZ/39/90H | 1038 | 0.17 | 9.34 | 54.94 |
| 8 | | | | | | |
| 9 | SGPID, Inc Round | YZ/39/90E | 1091 | 1.46 | 68.05 | 46.6 |
| 10 | Westwood Iranian | YZ/39/90D | 1086 | 1.28 | 22.17 | 17.32 |
| 11 | American | YZ/39/90M | 1018 | 21.38 | 68.45 | 3.20 |
| 12 | Gold Series California | YZ/39/90B | 1086 | 11.32 | 42.61 | 3.76 |
| 13 | Sunkist California | YZ/39/90A | 1083 | 8.35 | 41.15 | 4.93 |

Note: The calculation of cyanidin-3-galactosides content was based on the standards of cyanidin-3-glucosides because cyanidin-3-galactosides standard was not available commercially.

APPENDIX 2: FIGURES

Figure 1. Standard Chromatogram of Kuromanin chloride

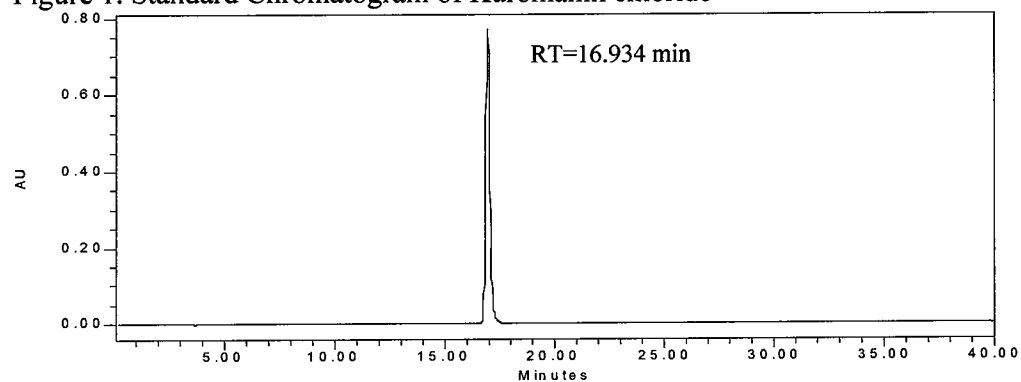


Figure 2. Standard Chromatogram of Cyanidin-3-galactoside

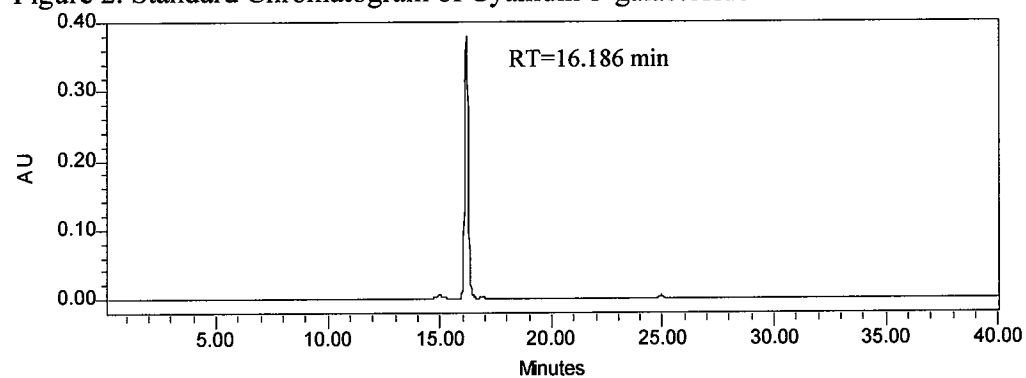


Figure 3. YZ/39/90K Israel

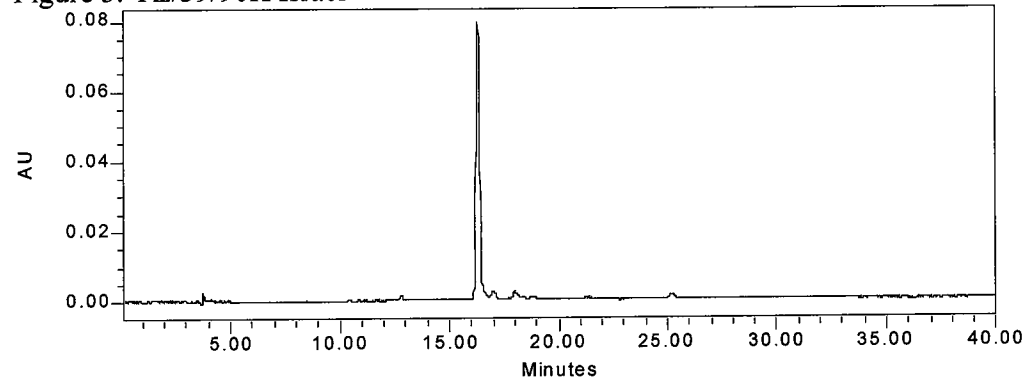


Figure 4. YZ/39/90L Erdal Bezaroglu

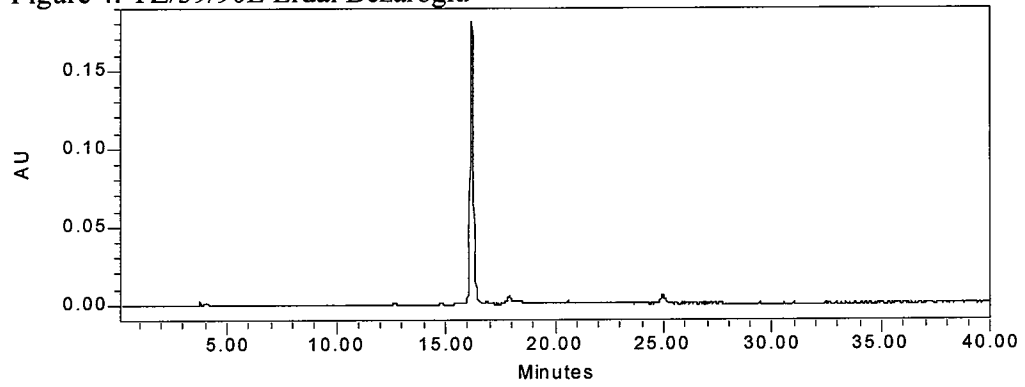


Figure 5. YZ/39/90C Gold Series Turkish

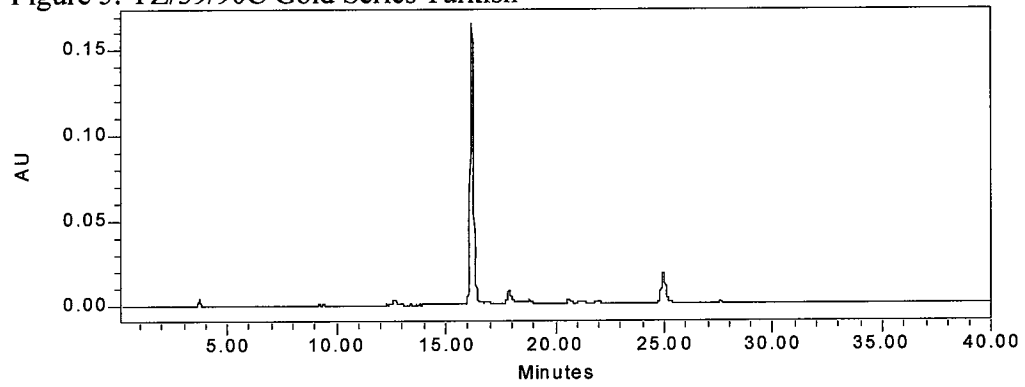


Figure 6. YZ/39/90D Westwood Iranian

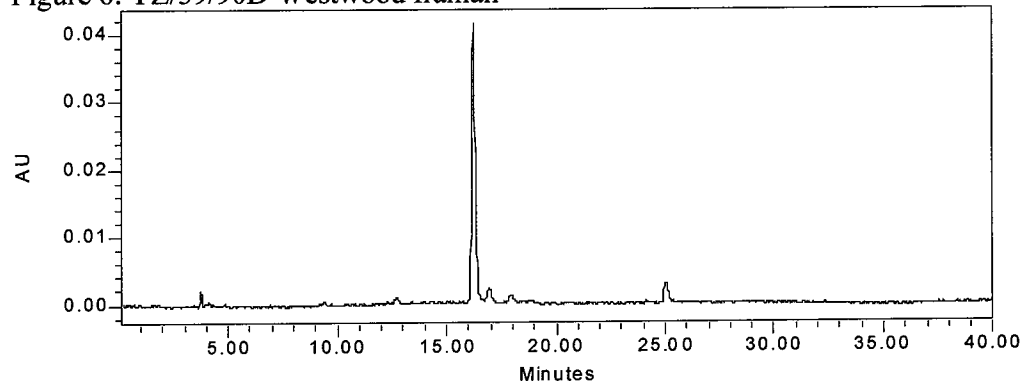


Figure 7. YZ/39/90F Fandoghi Variety

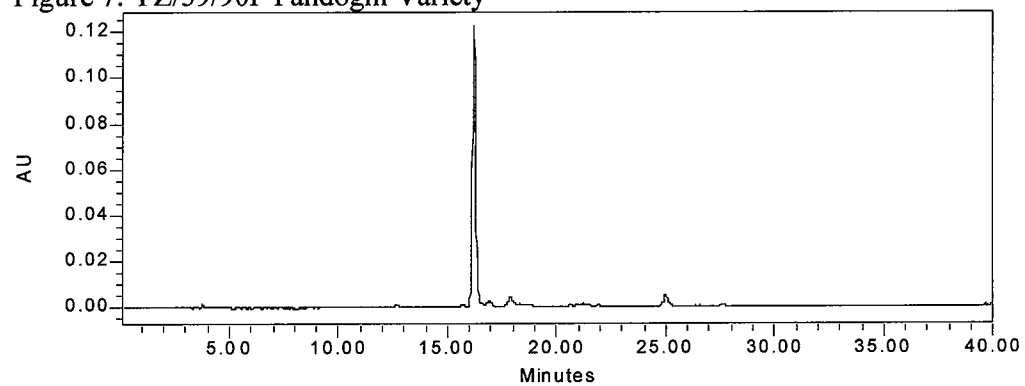


Figure 8. YZ/39/90H Kalagouchi Variety Jumbo

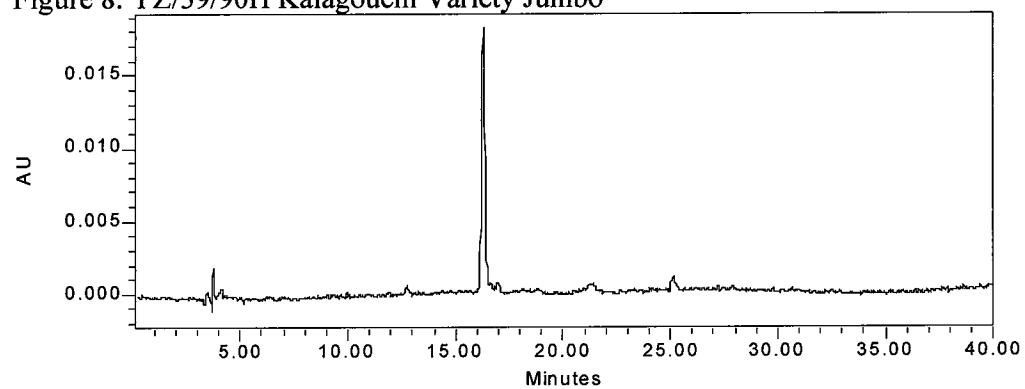


Figure 9. YZ/39/90I Akbari Long

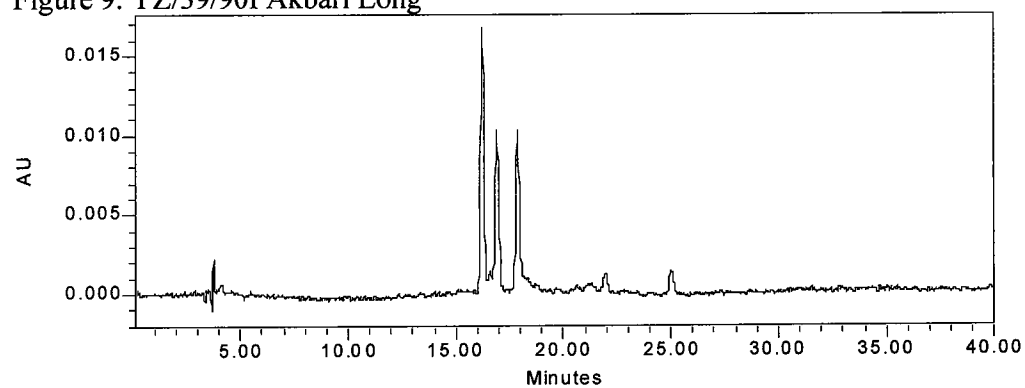


Figure 10. YZ/39/90J SGPID, Inc Long

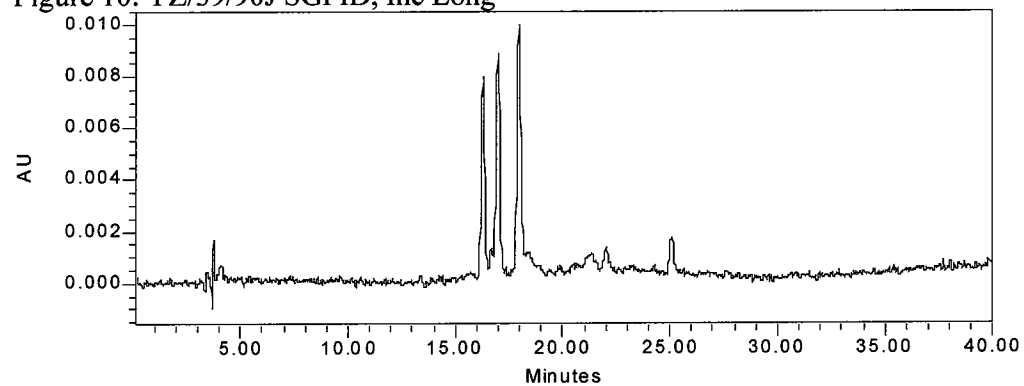


Figure 11. YZ/39/90G Ahmad Aghie Long

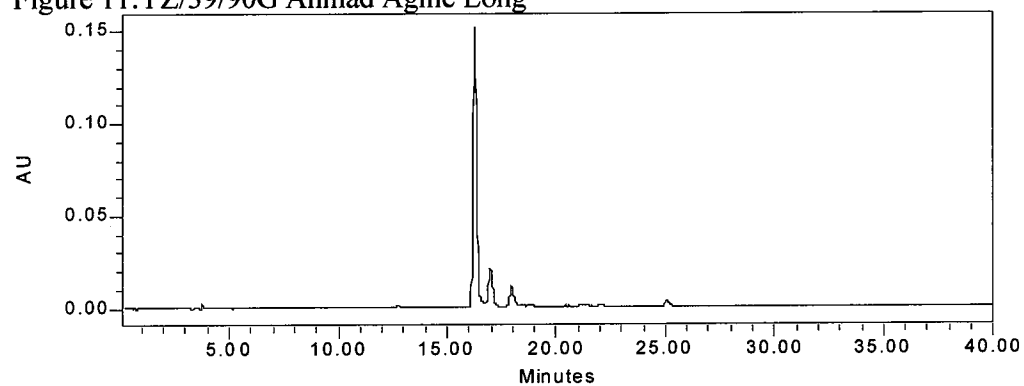


Figure 12. YZ/39/90E SGPID, Inc Round

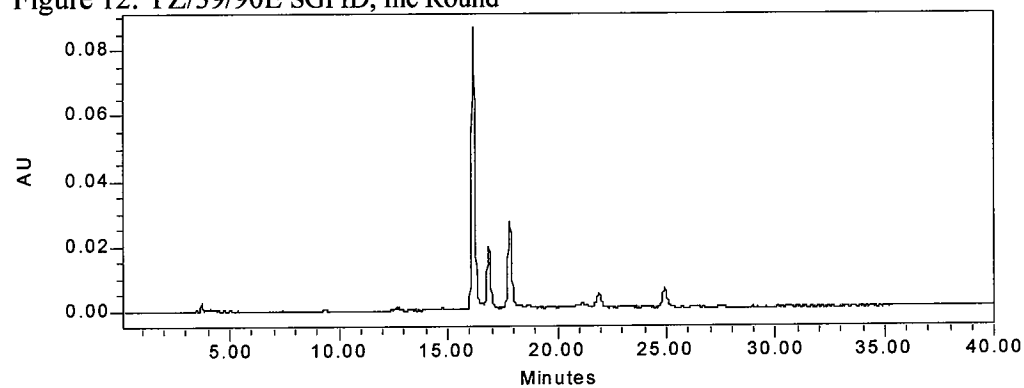


Figure 13. YZ/39/90A Sunkist California

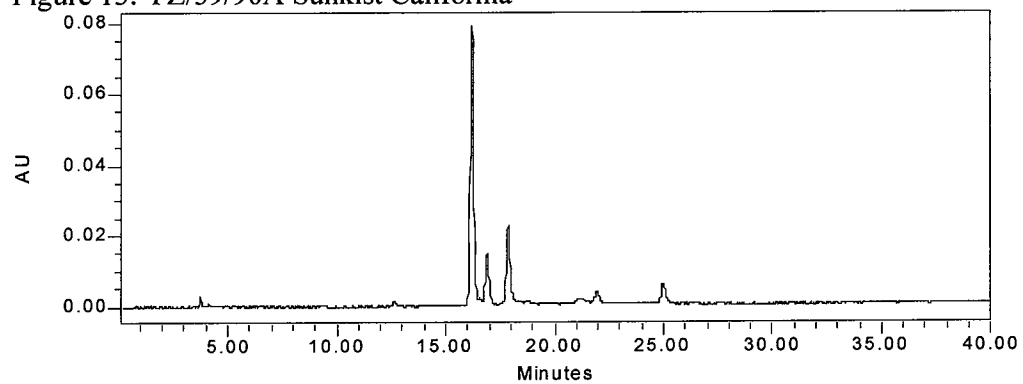


Figure 14. YZ/39/90B Gold Series California

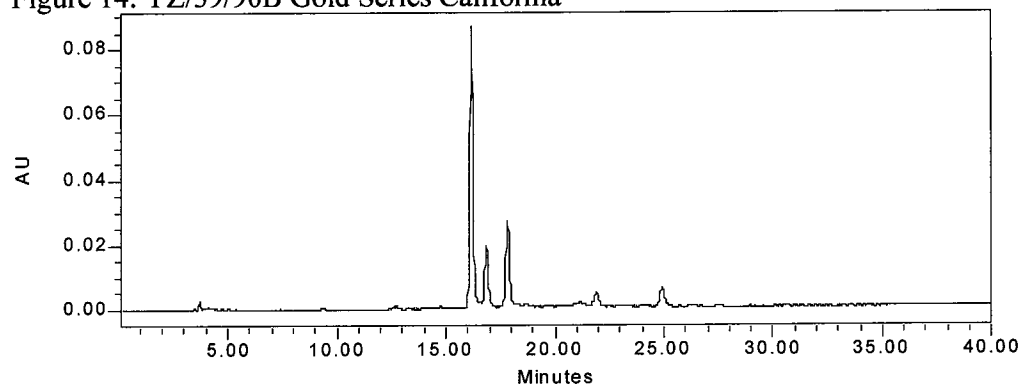
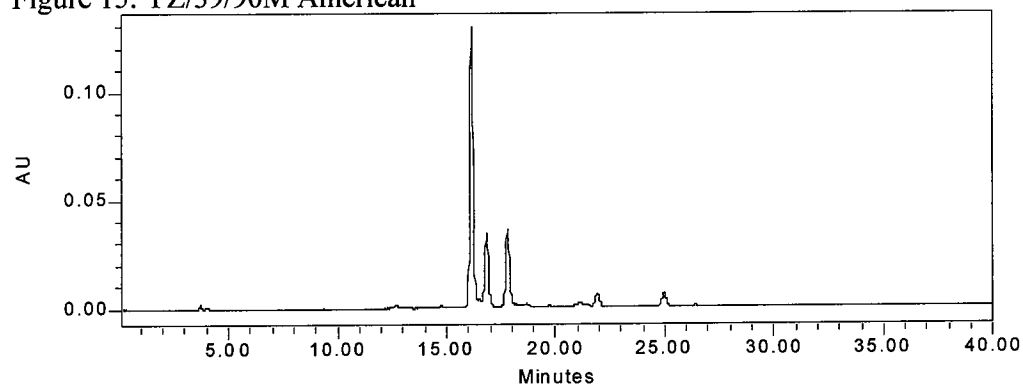


Figure 15. YZ/39/90M American



APPENDIX 3: Standard curve

Figure 16. Standard curve for Kuromanin Chloride

