TASC 2011 PROJECT REPORT:

COMPOSITE CHEMICAL PICTURE OF U.S. OLIVE OIL: REMOVAL OF POTENTIAL TRADE BARRIERS –YEAR 2

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Project Designed and Report Prepared by:
Selina Wang PhD, Research Director, *UC Davis Olive Center*

Under the Direction of:
California Olive Oil Council (COOC)
Patricia Darragh, Executive Director
Bruce Golino, Chair, Committee on Standards

The goals of this study were to (1) establish a database consisting of chemical/sensory profiles for US olive oils and (2) evaluate the 2010 US Standards for Olive Oil and Olive-Pomace Oil. As the US olive oil industry grows, it is vital to obtain information on chemical profiles of US olive oils and characterize the relationship of chemical profile to cultivar and geographical location. With such knowledge available, one can evaluate and further modify the current standards for US olive oils.

Introduction

Many studies have suggested that parts of the chemical profile (such as sterol and fatty acid profile) may vary based on olive cultivars, geographical locations and climate [1-5]. However, such studies have not been undertaken in the US.

The International Olive Council (IOC) is the international intergovernmental organization for olive oil and table olives and it has been at the forefront of developing chemical and sensory testing methods, and setting standards for different grades of olive oils. IOC standards for olive oils are based on chemical data gathered from North African countries and a small area of central and southern Europe (Spain, France, Italy and Greece), which represent the majority of the olive oil producing nations of the Mediterranean basin. IOC standards for olive oil may not be appropriate for US-produced olive oil, because of the differences in olive cultivars, geography and climate.

This 2010 TASC project builds on the work and results from the 2009 TASC project which provided significant data regarding Unites States olive oils that failed to meet the limits of some chemical compounds as set in various standards used to classify olive oil according to grade. Chief among the important results was the finding that a large percentage of oils containing the "Arbequina" variety have higher levels of Campesterol than is permitted by the IOC standard for an oil to be classified as extra virgin. "Arbequina" is the largest planted variety in the United States and represents in excess of 50 % of current production. In addition a significant number of oils had levels of linolenic acid that exceed the IOC limits.

In this study, sixty mono-varietal extra virgin olive oils produced in the US from the 2010 harvest were collected and analyzed using chemical and sensory parameters from the USDA (United States Department of Agriculture) standards for grades of olive oils.[i] Two additional chemical tests in the current Australian standards,[ii] DAGs and PPP, were also included. Both chemical and sensory tests were performed independently by three laboratories and three sensory panels, respectively. The laboratories are USDA-Blakely laboratory, Australian Oil Research Laboratory (AORL), and UC Davis Olive Center. The sensory panels are from Australian Oil Research Laboratory (AORL), UC Davis Olive Center, and California Olive Oil Council (COOC).

Method

Sixty domestic mono-varietal extra virgin olive oils were selected for this study; some were certified by COOC and some were not. All samples were from the 2010 harvest and were collected from February to April, 2011 by UC Davis Olive Center and COOC. Many attempts were made to include oils from outside of California, however, we were unable to find mono-varietal oils from other regions except for three samples from Texas. **Figure 1a and Figure 1b** show the producing regions of all sixty oil samples collected.

The sixty samples collected contained oil from fourteen different olive varietals. The breakdown of samples is shown in **Table 1**. Because "Arbequina" is the most common varietal for super-high density production in the US, 20% of our samples were oils made from "Arbequina" in various regions.

Sample were packaged and sent to the USDA-Blakely laboratory, AORL, UC Davis Olive Center and COOC for chemical and sensory analyses. All the chemical laboratories and sensory panels follow the same official procedures shown in **Table 2**. Testing results were then submitted by each testing facility to Dr. Selina Wang, research director at UC Davis Olive Center, where the data was complied and analyzed.[iii]

Figure 1a. Producing regions providing olives for the oil collected in this study

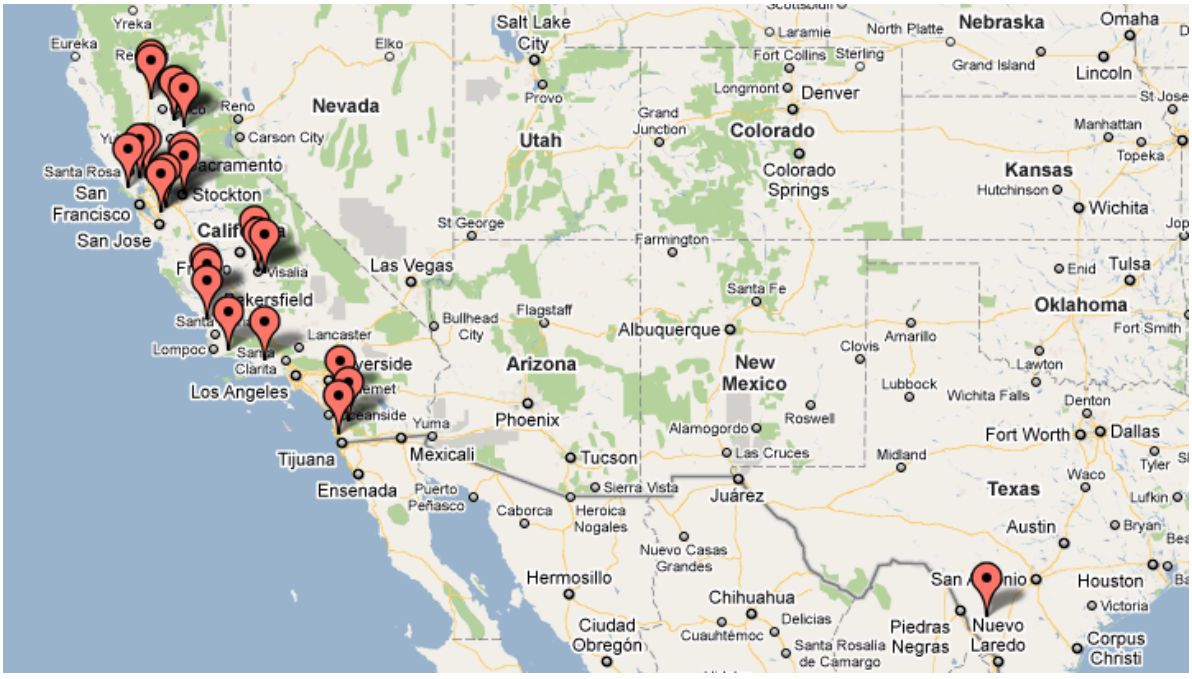


Figure 1b. Zoom-in picture of Figure 1a to show producing regions in California

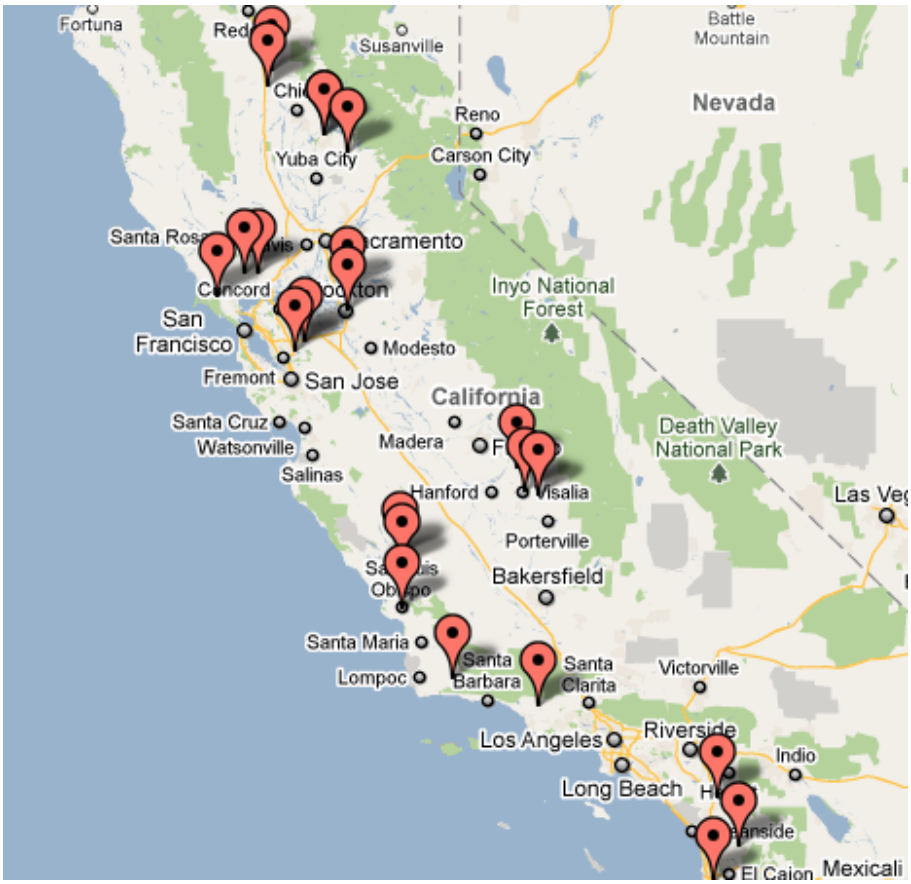


Table 1. Number of samples collected in this study containing each cultivar

Cultivar	Number of Samples
Arbequina	12
Arbosana	3
Ascolano	5
Barouni	1
Coratina	2
Frantoio	5
Kalamata	1
Koroneiki	6
Leccino	4
Manzanillo	4
Maurino	1
Mission	11
Sevillano	3
Taggiasca	2
Total	**60**

Table 2. Tests performed at AORL, USDA and UC Davis

Test	Method
Peroxide Value (PV)	ISO 3960:2007
Free Fatty Acids (FFA)	AOCS Ca 5a-40
UV Absorbency	IOC COI/T.20/Doc No. 19-2008
Stigmastadiene	IOC COI/T.20/Doc No. 11-2001
Fatty Acids Profile (FAP)	IOC COI/T.20/Doc No. 24-2001
Sterols	IOC COI/T.20/Doc No. 10-2001
Wax	IOC COI/T.20/Doc No. 18-2001
Equivalent Carbon Number (ECN 42)	IOC COI/T.20/Doc No. 20-2001
Pyropheophytin (PPP)	ISO 29841:2009
1,2-Diacylglycerols (DAGs)	ISO 29822:2009
Total Polyphenol Content	Modified Gutfinger method

ISO: International Organization for Standardization; AOCS: American Oil Chemists' Society; IOC: International Olive Council

Results and Discussion

Free Fatty Acids (FFA):
Free fatty acids are formed due to breakdown of the triacylglycerides in olive oils; fatty acids are "free" when they are not bound to other molecules. Many factors can affect free fatty acid content in an oil sample, such as quality and health of fruit, time between harvesting and extraction, time and temperature of oil extraction. All the samples passed the USDA limit (0.8), with the average value of the sixty samples was less than 25% of the below 0.2 (**Table 3**).

Peroxide Value (PV):
Peroxides are primary oxidation products that are typically formed during oil extraction, processing and storage. The average value of the sixty samples was 8, significantly lower than the USDA limit of 20. Four samples were found to have PV greater than 15, these samples also failed sensory and at least one other chemical test (**Table 3**).

UV Absorbency (K_{232}, K_{268}/K_{270} and ΔK):
Several factors may effect the UV spectrophotometric determination of K_{232}, $K_{268/270}$ (depending on the solvent used) and ΔK: age of the oil, time elapsed between harvesting and extraction, time and temperature of oil extraction and storage of oil. There were four samples that exceeded the limited for K232, all of which also failed sensory (**Table 3**).

Table 3. Summary of results for quality parameter: free fatty acids, peroxide values, and UV absorbance for 60 samples

	Free Fatty Acids (% oleic acid)	Peroxide Value (mEq O_2/kg)	K232 ($K^{1\%}_{1cm}$)	K268 ($K^{1\%}_{1cm}$)	ΔK ($K^{1\%}_{1cm}$)
USDA Limit	≤ 0.80	≤ 20	≤ 2.50	≤ 0.22	≤ 0.01
Average Value	0.18	8	1.89	0.12	<0.003
Highest Value	0.54	30	3.65	0.21	<0.003
Lowest Value	0.10	3	1.35	0.07	<0.003
Number of Samples Failed USDA Limit	0	2	4	0	0

Pyropheophytins (PPP):
Upon thermal degradation of olive oil, chlorophyll pigments break down to pheophytins and further to pyropheophytins. The ratio of pyropheophytins to the total pheophytins is considered to be a useful indicator to distinguish fresh oils from deodorized or aged oils. Since PPP is not part of USDA standards for olive oils, the limit adapted by Australian Olive Association (AOA) was used in this study. Laboratory results on all of the samples were well below the limit, which is consistent with the fact that they were produced less than six to nine months ago (**Table 4**).

1,2-Diacylglycerol Content (DAGs):
During the breakdown of triacylglycerides (glycerol moiety bound to three fatty acids), diacylglycerols (glycerol moiety bound to two fatty acids and one free fatty acid) are formed. Fresh extra virgin olive oil contains higher proportion of 1,2-diacylglycerols where olive oil from unsound fruits and refined olive oil have a higher proportion of 1,3-diacylglycerols. The high ratio of 1,2- and 1,3-diacylglycerols is thus believed to be a useful marker for sound fruits and fresh oils. Same as PPP, DAGs is not part of USDA standards and the AOA limit was again used in this study. Laboratory results on all the samples were significantly higher than the limit, again suggesting these samples were fresh and produced recently (**Table 4**).

Total Polyphenol Content:
Polyphenols are important antioxidants and have effects on the shelf life of olive oils. The level of polyphenols decreases as the fruit matures and is, therefore, an indicator for the ripeness of the harvested fruits. However, it is worth noting that polyphenol contents are highly affected by weather conditions, irrigation regime, genetics of the cultivar and processing practices. A large range of values was observed for each varietal, suggesting the polyphenol content was dependent on the practices of the growers (irrigation, time of harvesting (early or late)) and producers (processing method) in this study (**Table 4**).

Table 4. Summary of results for pyropheophytin *a* (PPP), 1,2-diacylglycerols (DAGs), and total polyphenol content for 60 samples

	Pyropheophytin *a* (% of total pheophytins)	1,2-Diacylglycerols (% of total 1,2 & 1,3 diacylglycerols)	Total Polyphenol (mg caffeic acid/kg)
USDA Limit	N/A AOA: ≤ 15	N/A AOA: ≥ 40	N/A
Average Value	2.4	81.0	250
Highest Value	14.1	94.1	670
Lowest Value	0.7	42.8	64
Number of Samples Failed AOA Limit	0	0	N/A

Fatty Acid Profile:

Fatty acids are the principle components of fats and may be saturated or unsaturated, based on their chemical structures. It has been well documented that fatty acid profiles are much influenced by the olive cultivars and environmental factors[iv] and hence USDA standards have allowed higher levels of linolenic acid than the IOC standard. We found that the increased linolenic acid (C18:3) in the USDA standard was appropriate for the various cultivars in this study, however, nine samples (0.4%) exceeded the USDA limit (0.3%) for heptadecenoic acid (C17:1). Interestingly, more than one sample of each of "Ascolano", "Kalamata", "Manzanillo", and "Sevillano" cultivars were amongst those nine samples, suggesting their correlation with higher levels of heptadecenoic acid (**Table 5**). This is consistent with our previous study, in which we found that both "Ascolano" and "Sevillano" exhibited a higher level of heptadecenoic acid. We suggest further research on the possibility of increasing the limit of heptadecenioc acid from 0.3% to 0.4% in the USDA standard.

Table 5. Summary of selected fatty acids results for 60 samples

	C16:0 (Palmitic acid %)	**C17:1** (Heptadecenioc acid %)	**C18:1** (Oleic acid %)	**C18:3** (Linolenic acid %)	**C20:1** (Gadoleic acid %)
USDA Limit	7.5 - 20.0	≤ 0.3	55.0 - 83.0	≤ 1.5	≤ 0.4
Average Value	12.4	0.2	74.0	0.9	0.3
Highest Value	21.2	0.4	80.7	1.3	0.4
Lowest Value	8.4	0.1	52.5	0.6	0.2
Number of Samples Failed USDA Limit	2	9	1	0	1

Sterols:
Sterols are minor constituents of oils and are characteristic markers for different seed and nut oils. Sterols are generally related to cultivar, although geographical locations can also affect the sterol profiles in olive oils.[v] Even though it has been documented that the "Arbequina" cultivar ("Arbosana" and "Koroneiki" cultivars as well) tends to have a higher level of campesterol than other cultivars, only one out of the sixty samples was found to exceed (4.6%) the USDA limit of 4.5% (**Table 6**). This particular sample was made from the "Koroneiki" cultivar. We believe the campesterol limit of 4.5% in the 2010 US Standards for Olive Oil and Olive-Pomace Oil is more suitable for US extra virgin olive oils than the 4.0% limit in the IOC standards, as there were several oils that fell between 4.0-4.5%. We also found three samples that had less total sterols than the limit (1000 mg/kg), and all of these were made from the "Koroneiki" cultivar; this suggests a possible correlation that is worth examining in future studies.

Table 6. Summary of selected sterols results for 60 samples

	Cholesterol (%)	Brassicasterol (%)	Campesterol (%)	Apparent B-Sitosterol (%)	Total Sterols (mg/kg)
USDA Limit	≤ 0.5	≤ 0.1	≤ 4.5	≥ 93.0	≥ 1000
Average Value	0.1	0.1	3.4	94.7	1527
Highest Value	0.3	0.1	4.6	96.3	2661
Lowest Value	0.1	0.1	2.3	92.4	914
Number of Samples Failed USDA Limit	0	0	1	1	3

Equivalent Carbon Number (ECN) 42:
This method compares the experimentally determined concentration in percentage of natural triacylglycerol types (of ECN 42), with a calculated concentration of all the theoretically possible triacylglycerols on the basis of the major fatty acid composition of the oil. Most of the oil samples were well below the limit of 0.2, according to AORL and USDA laboratories (**Table 7**).

Wax:
This method enables the separation of individual waxes for distinguishing between olive oil obtained by proper extraction and that obtained from olive pomace. One sample was found to exceed to limit (**Table 7**), and coincidentally, this sample also failed PV, K232, and sensory.

Table 7. Summary results of total sterols, equivalent carbon number (ECN) 42 and Wax for 60 samples

	ECN 42	Wax (mg/kg)
USDA Limit	≤ 0.2	≤ 250
Average Value	0.08	83
Highest Value	0.47	275
Lowest Value	0.01	33
Number of Samples Failed USDA Limit	2	1

Sensory:

As shown in **Table 8**, seven samples were found defective by more than one of the three sensory panels. **Table 9** shows the high, low and average scores of positive attributes (fruitiness, bitterness, and pungency) from the three panels. We found the average scores of fruitiness, bitterness and pungency are reasonably close between the three panels.

Table 8. Number of the 60 samples that had sensory defects from more than one panels

	Sensory Defects > 0
Number of Samples	7

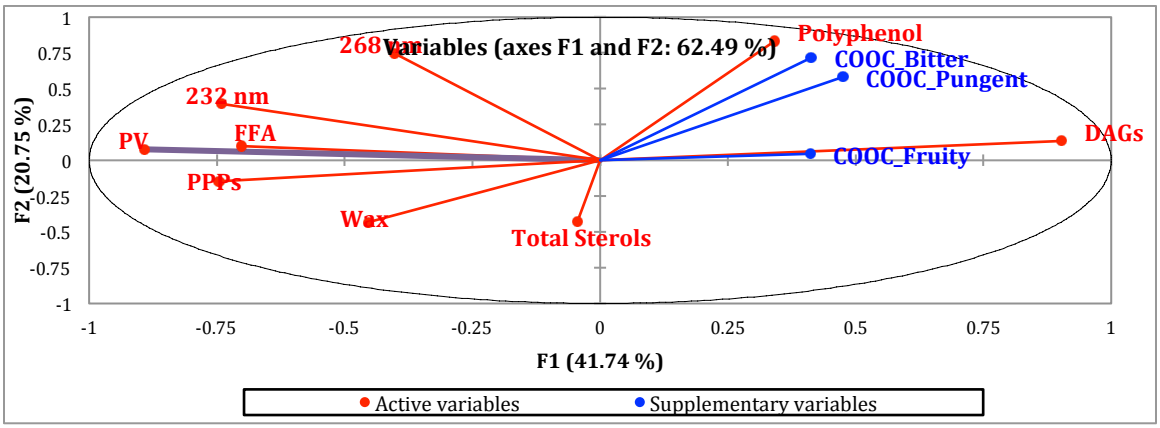
Table 9. Positive sensory attributes for 60 samples from the three sensory panels

	Fruit	Bitter	Pungency
Average of Samples (AORL)	4.22	3.30	4.00
Average of Samples (COOC)	4.30	3.30	3.70
Average of Samples (UC Davis)	4.60	3.60	3.90
Highest Value (AORL)	5.40	7.15	6.90
Highest Value (COOC)	5.85	5.40	5.80
Highest Value (UC Davis)	6.60	7.50	6.50
Lowest Value (AORL)	2.00	0.55	0.55
Lowest Value (COOC)	0.00	0.85	0.45
Lowest Value (UC Davis)	2.30	1.10	1.00

Principle Component Analysis (PCA):

Principal Component Analysis (PCA) was applied to graphically summarize the chemical and sensory data. PCA is known to be a useful tool for revealing the underlying structure of the sample space by reducing the dimensionality of a data set by finding a new set of variables, called principal components (PCs), that captures the greatest variability.[vi] In **Figure 2**, chemical variables were used as "active variables" whereas the positive sensory attributes were used as "supplementary variables" (COOC data is used here for illustration). The first two PCs accounted for 63% of the total variation of the data (PC1=42% and PC2=21%). Bitterness and pungency are found to be highly positively correlated with polyphenol, positively correlated with DAGs and negatively correlated with PPP, PV, FFA, K232. Fruitiness shows less strong correlations with chemical variables, but is slightly positively correlated with DAGs and negatively correlated with PPP, PV, FFA, K232.

Figure 2. PCA biplot for PC1 and 2 showing chemical and sensory variables



Pearson's correlation coefficient table, **Table 10**, shows the correlations between chemical variables and sensory attributes from the three sensory panels (AORL, UC Davis and COOC). Again, polyphenol correlates well (positively) with bitterness and pungency for results from all three panels, and slightly so with fruitiness for results from the AORL panel. PV correlates well (negatively) with fruitiness for results from all the panels, and well (positively) for results from the UC Davis panel. DAGs correlates more with bitterness and pungency than fruitiness for results from all three panels.

Table 10. Pearson's correlation coefficient between sensory attributes and chemical variables from three panels

Correlation matrix (Pearson (n-1)):

Variables	FFA	PV	232 nm	268 nm	DAGs	PPPs	Wax	Total Sterols	Polyphenol
AORL Fruit	**-0.332**	**-0.530**	-0.209	0.022	**0.362**	-0.109	-0.250	-0.106	**0.428**
AORL Bitter	**-0.379**	**-0.449**	-0.084	**0.325**	**0.473**	**-0.284**	**-0.455**	-0.154	**0.758**
AORL Pungent	**-0.374**	**-0.439**	-0.024	**0.257**	**0.429**	-0.256	**-0.377**	-0.213	**0.738**
UC Davis Fruit	**-0.404**	**-0.549**	**-0.319**	-0.219	**0.369**	-0.104	**-0.382**	-0.119	**0.260**
UC Davis Bitter	**-0.320**	**-0.436**	-0.067	**0.373**	**0.473**	**-0.326**	**-0.407**	**-0.260**	**0.897**
UC Davis Pungent	**-0.321**	**-0.445**	-0.057	**0.297**	**0.437**	**-0.296**	**-0.365**	**-0.312**	**0.798**
UC Davis Fusty/ muddy sediment	**0.539**	0.031	-0.203	-0.156	-0.157	-0.029	0.011	0.144	-0.206
UC Davis Musty-humid-earthy	0.099	0.092	-0.116	-0.060	0.001	0.039	0.009	0.111	-0.113
UC Davis Rancid	**0.539**	**0.807**	**0.351**	**0.331**	**-0.532**	**0.316**	**0.322**	0.095	**-0.301**
UC Davis Ripe fruit	*-0.056*	*-0.118*	*-0.201*	***-0.611***	*-0.085*	*0.231*	*0.039*	*0.131*	***-0.497***
UC Davis Green fruit	***-0.446***	***-0.546***	*-0.167*	*0.200*	***0.501***	*-0.249*	***-0.366***	*-0.208*	***0.731***
COOC Fruity	**-0.356**	**-0.489**	-0.204	-0.176	**0.364**	-0.129	**-0.268**	-0.079	0.178
COOC Bitter	**-0.263**	**-0.429**	-0.045	**0.363**	**0.384**	**-0.281**	**-0.450**	-0.250	**0.870**
COOC Pungent	**-0.377**	**-0.522**	-0.077	0.251	**0.409**	**-0.348**	**-0.376**	-0.197	**0.760**
COOC Winey	0.099	-0.089	-0.064	-0.080	-0.017	0.002	-0.065	0.118	0.105
COOC Fusty	0.099	0.092	-0.116	-0.060	0.001	0.039	0.009	0.111	-0.113
COOC Winey	0.099	-0.089	-0.064	-0.080	-0.017	0.002	-0.065	0.118	0.105
COOC Rancid	-0.118	0.107	0.021	-0.089	0.012	-0.033	-0.061	-0.070	-0.146

Values in bold are different from 0 with a significance level alpha=0.05

Conclusion

With careful planning and sample collection, we were able to accomplish our goals in full: providing an initial database of US olive oils and important information on how they were evaluated under the current USDA standards. We found that the increased linolenic acid (C18:3) and campesterol limit in the 2010 US Standards for Olive Oil and Olive-Pomace Oil is able to accommodate the various cultivars in this study. However, it may be worthwhile investigating if increasing the limit of heptadecenioc acid (C17:1) from 0.3%, which is what in the current standards, to 0.4% would be more suitable for the natural chemistry of domestic olive oils. In addition, our data does show that chemical tests such as PV and FFA have very high upper limit, which may allow poor quality oils to pass the standards.

PPP and DAGs are useful methods and important markers for quality of oils, and have been adopted by Australia during the course of this study. We believe it is important for the US to consider adopting these two methods as part of US standards.

The inclusion of sensory, a standard testing method from IOC/USDA as a quality parameter for olive oils, provided additional valuable information. Statistical analysis allowed us to correlate the chemical variables with sensory attributes, and we were able to conclude that polyphenol correlates well with bitterness and

pungency (all three panels), and slightly so with fruitiness (AORL panel only). PV correlates well with fruitiness (all three panels). DAGs correlates more with bitterness and pungency than fruitiness (all three panels).

In **Table 11**, a summary table, from AORL, shows that 4 samples failed at least one of the chemical tests for quality, 16 samples failed at least one of the chemical tests for purity (9 were from FAP) in the current USDA Standards, none of the samples failed the non-standards PPP or DAGs, and 10 samples failed sensory test. This result may lead to further investigation on where the limits should be set for PPP and DAGs if they are considered for US olive oil quality standards.

Table 11. Number of sample that failed at least one of the standard chemical tests for quality (FFA, PV, or UV), for purity (FAP, sterols, Wax, or ECN 42), non-standard tests (PPP or DAGs), sensory test, from AORL

	Chemical and Sensory Tests in USDA Standards			
	FFA, PV, or UV	**FAP, Sterols, Wax, or ECN 42**	**Sensory**	**PPP or DAGs**
Number of Samples that Failed	4	16	10	0

While the sample size was limited to 60 and additional data collection would be necessary in order to establish a reasonably complete database of extra virgin olive oils produced in the US, we firmly believe this study provided important fundamental information for the US olive oil industry

Future Work

To help the US olive oil industry to continue to thrive, we believe more studies are required that focus on the quality parameters of the current olive oil standards. While 2010 US Standards for Olive Oil and Olive-Pomace Oil covers purity parameters such as FAP, wax, sterols, and ECN 42, it lacks a reliable means of quantifying the "freshness" quality of the oils. Currently, it is not required for the manufactures to include a "best by" date or "best before" date on olive oil containers, and in our previous studies, even when a "best by" date is included, there is little correlation with olive oil quality. We would like to streamline the chemical methods focusing on quality parameters, such as PPP, DAGs, UV and to understand their impact on the initial quality parameters such as FFA. We would like to research the effect of storage time on these quality parameters as well as sensory quality. With statistical analysis, we anticipate the research will allow us to reasonably evaluate the "freshness" quality of the oil and predict its "best by" date. This research would estimate the "life" of olive oil and provide tools to the supply chain to provide better information to the consumer and professional buyer.

References

[i] United States Department of Agriculture (accessed 2010) United States standards for grades of olive oil and olive-pomace. http://www.usda.gov

[ii] Australian standard AS5264-2011 Olive oils and olive pomace oils, Standards Australia, Sydney, Australia

[iii] For the sake of simplifying this discussion, only the data from the IOC-certified AORL (Australian Olive Research Laboratory) is presented here. Laboratory data from USDA and UC Davis were found to be comparable.

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