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Nuclear Magnetic Resonance Spectroscopy and Multivariate Statistical Analysis: A Robust Approach for Avocado Oil Authentication

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01 Introduction

Avocado oil is a prominent edible oil in the global market, highly sought for its beneficial health attributes due to high monounsaturated fatty acid, carotenoids, and polyphenol contents. The surge in popularity has led to widespread adulteration with lower-grade oils like corn, safflower, sunflower, soybean, and others, primarily to address shortages in the global supply and reduce costs. Whilst chromatographic methods are conventionally employed in assessing the quality of oils, these are cumbersome, time consuming and laborious. Moreover, the fatty acid profile of avocado oil is heterogeneous as it is influenced by the processing and manufacturing conditions, therefore demanding robust analytical methods which can accurately capture and probe such large chemical variance. Nuclear Magnetic Resonance (NMR) spectroscopy and multivariate statistical analysis (MSA) were employed to address these analytical challenges. Authentic avocado oils and those blended with adulterants were analyzed using proton NMR spectroscopy and a spectral library of untargeted and semi-quantitative analytical capabilities was built. These samples were parallelly analyzed using gas chromatography (GC) as per the Association of Official Analytical Chemists (AOAC) guidelines.

02 Objectives

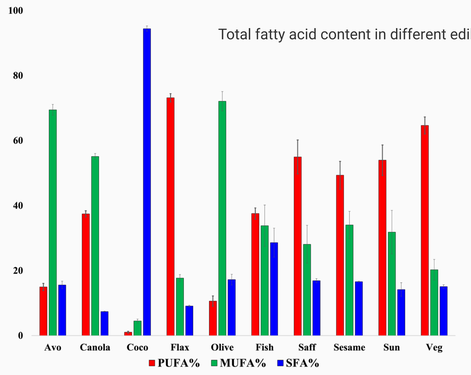
- To build a sophisticated spectral library for non-targeted differentiation of edible oils.
- To differentiate authentic vs. adulterated oils using NMR spectroscopic data and statistical models.
- To identify the adulterant.
- To assess the natural variabilities in the fatty acid profile among Avocado oil samples.

03 Materials and Methods

A sophisticated proton NMR spectral library comprising of several types of edible oils from different origins and extraction processes was built using proprietary Purity-IQ (PIQ) methods. Several Avocado oils were purchased by Catania oils from the market, some of them intentionally adulterated and submitted to Purity-IQ for NMR analysis as blind samples. These samples were independently analyzed by Catania oils using Gas Chromatography (GC) for cross-validation of NMR results.

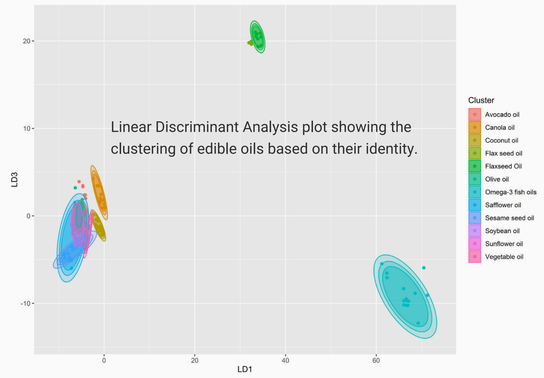
04 Fatty Acid Profiles in Different Edible Oils

The monounsaturated (MUFA), polyunsaturated (PUFA), and Saturated (SFA) fatty acid contents (in %) observed in different edible oils measured using NMR spectroscopy are plotted in the Figure below (Avo-Avocado; Coco-Coconut; Saff-Safflower; Sun-Sunflower; Veg-Vegetable). The overall fat profiles are unique to each type of oil and aids in authenticating edible oils in conjunction with non-targeted analytics employing multivariate statistical analysis.



05 Multivariate Statistical Analysis

- Due to distinct spectroscopic profiles of different edible oils, the samples group into respective clusters based on their identities using a non-targeted approach as seen in the figure below, obtained from Linear Discriminant Analysis of NMR spectral datasets.
- The three ellipsoids in each cluster mark different levels of consistency: innermost = >98%; middle = 95 – 98%; outermost = 90–95%.
- By applying test samples against this statistical model, both oil identity and consistency in terms of their fatty acid profiles can be assessed with ease.



06 Fatty Acid Profiles of Blind Samples Using Gas Chromatography

Sample #	Identity	Fatty Acid Composition as Determined by Gas Chromatography (Expressed as % of Total Fatty Acids)								
			C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C22:0
		Proposed Codex Standard for Avocado oil	11.0-26.0	4.0-17.1	0.1-1.3	42.0 - 75.0	7.8 - 19.0	0.5 -2.1	ND - 0.7	ND - 0.3	ND - 0.5
		Avocado oil Supplier Standard	5-25	2-12	3.0 Max	50-74	6-20	3.0 Max			
53	Avocado oil		11.00	2.09	2.28	69.28	13.20	0.61	0.33	0.27	0.28
215	Avocado oil		7.43	0.28	3.22	38.93	48.25	0.17	0.29	0.18	0.60
390	Avocado oil		10.63	0.64	3.11	59.81	23.60	0.47	0.44	0.25	0.30
558	Avocado oil		10.27	1.74	2.46	70.65	12.83	0.49	0.37	0.26	0.39
820	Avocado oil		7.46	1.26	2.38	72.81	14.00	0.26	0.30	0.29	0.62
313	50% Avo 50% Sun		5.68	0.70	2.71	78.75	10.13	0.20	0.28	0.26	0.80
278	25% Avo 75% Sun		4.81	0.45	2.86	81.48	8.24	0.15	0.26	0.26	0.84
843	100% Sun		3.93	0.14	3.03	84.56	6.17	0.13	0.29	0.26	0.99
415	75% Avo 25% Saff		6.80	0.99	2.25	73.92	14.08	0.25	0.30	0.27	0.53
124	50% Avo 50% Saff		6.17	0.70	2.15	74.94	14.11	0.28	0.33	0.20	0.48
869	25% Avo 75% Saff		5.44	0.41	2.02	79.99	14.29	0.31	0.35	0.21	0.40
941	100% Saff		4.83	0.11	1.92	77.11	14.33	0.15	0.36	0.28	0.33

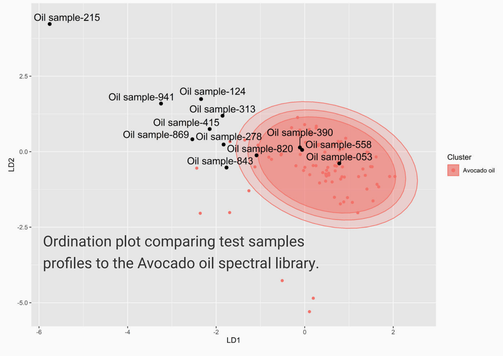
- Values in red did not meet Supplier Standard specifications for 100% Avocado oil.
- None of the samples met Codex Proposed Standard for 100% Avocado oil.
- All 100% Avocado oils tested were commercially obtained from various retail outlets.
- Samples 215 and 390 appeared to be adulterated and not 100% Avocado oil.
- Fatty Acid profile testing cannot identify the adulterating oil type or percentage of adulteration.

07 Identities of blind samples predicted using NMR Spectroscopy through a non-targeted lipidomic approach

Samples	Percent Probabilities (%)					Oil Identity by NMR	True Identity
	Avocado	Olive	Safflower	Soybean	Sunflower		
53	95.98	3.07	0.41	0.00	0.53	Avocado oil	Avocado oil*
215	0.00	0.00	0.00	100	0.00	Soybean oil	Avocado oil*
390	86.03	13.78	0.16	0.00	0.04	Avocado oil	Avocado oil*
558	88.31	7.17	3.52	0.00	1.01	Avocado oil	Avocado oil*
820	95.33	3.45	0.72	0.00	0.50	Avocado oil	Avocado oil*
313	39.31	1.45	40.50	0.00	18.74	Avo and Saff blend	50% Avo + 50% Sun
278	40.50	0.51	19.65	0.00	39.35	Avo and Sun blend	25% Avo + 75% Sun
843	21.60	0.26	28.17	0.00	49.97	100% Sun	100% Sun
415	67.20	4.10	22.00	0.00	6.70	Avo and Saff blend	75% Avo + 25% Saff
124	61.36	2.17	21.78	0.00	14.69	Avo and Saff blend	50% Avo + 50% Saff
869	42.93	3.15	45.83	0.00	8.10	Avo and Saff blend	25% Avo + 75% Saff
941	26.58	1.32	55.28	0.00	16.82	100% Saff	100% Saff

*Market-purchased and as claimed on the label

- Samples with prediction probabilities >85% for Avocado and <10% for other clusters are deemed authentic Avocado oils, in-line with their fatty acid profiles measured using GC.
- Samples landing in the innermost ellipsoid of the Avocado oil cluster ordination plot (figure below) built with non-targeted (full spectral profiling) are considered authentic, provided their predictions in non-Avocado cluster (table above) is <10%. Therefore, samples 53, 558, and 820 are consistent with the expected Avocado oil spectral profile and are concluded to be pure samples. Although sample 390 lands within the ellipsoid, its prediction for olive oil is >10% and hence does not pass the testing criteria for pure oil.
- Safflower and Sunflower oils are the common adulterants, and their prediction probabilities are significant for the blends.



- The statistical model can be used to assess the consistency of the samples with respect to the samples in the spectral library.
- Samples deemed to be authentic align well with the Avocado oil cluster and the adulterated oils land outside the cluster substantiating the deviation in their fatty acid profiles.
- This approach can be used to assess batch-to-batch consistencies of Avocado oils, compare samples from different processing conditions and extractions, and validate suppliers and vendors.

08 Conclusions

- The NMR-based spectroscopic data and statistical models clearly differentiated authentic vs. adulterated oils with high confidence as well as identified the source of adulteration, cross-validated by the corresponding GC data.
- NMR spectroscopy in conjunction with MSA proves to be a robust analytical method that can compete effectively with current analytical methods with additional benefits such as reliability, ease of use, high throughput sampling, minimal sample preparation steps, and easy data interpretation.
- The spectral library offers analytical capabilities to test batch-to-batch consistency, increasing the value of NMR spectroscopy in edible oil analysis.