橄榄油的聚集荧光猝灭及吸收光谱特性的研究

王泓鹏，万 雄*

中国科学院上海技术物理研究所空间主动光电技术重点实验室，上海 200083

摘 要 素有“液体黄金”之称的橄榄油已成为健康食用油的代名词，不仅身价陡增，而且在非产地市场也已成为一种畅销油。在橄榄油检测技术中光谱法与其他技术相比具有快速、无损、无样品处理等优势而备受关注，而不同的光谱检测方法在检测的物质成分上各有侧重，例如红外光谱法侧重于脂肪酸含量的检测、拉曼光谱法侧重于分子的检测、荧光光谱法侧重于光敏物质的检测以及吸收光谱法侧重于光敏物质和不饱和脂肪酸的检测等。荧光及吸收光谱对光敏物质反应极其灵敏，而橄榄油富含叶绿素等光敏物质，因此荧光及吸收光谱成为一种鉴别橄榄油的有效技术手段。叶绿素是一种含有环卟啉结构的有机分子，该类分子结构具有吸光特性，且不同的叶绿素吸收光谱各异，其中绿色植物的叶绿素a含量最多。为深入研究叶绿素的吸收光谱及荧光特性在橄榄油鉴别中的应用，将特级初榨橄榄油中掺入不同比例的玉米油，已达到间接调控橄榄油中叶绿素含量的目的，测量不同掺伪比例橄榄油的荧光及吸收光谱并研究与叶绿素浓度的相关性，以此来研究叶绿素浓度与掺伪量对橄榄油吸收光谱及荧光特性的影响。取10份同批次的特级初榨橄榄油，将其中9份按照等比例稀释，并对10份样品按照掺伪量依次排序；依次采集这10份样品的荧光及吸收光谱，比较叶绿素浓度与掺伪量的相关性及对这两种光谱技术在橄榄油鉴别中的影响。随着叶绿素浓度的上升，荧光强度由弱变强，并在某一时刻后会出现荧光强度急剧减弱的现象，即聚集荧光猝灭。这种现象主要是由于叶绿素的环卟啉分子结构引起的分子间π—π作用，使未被激发的低能分子与高能分子堆叠在一起，能量的辐射跃迁(荧光)也转变为分子间的能量转移(热能交换)。对于吸收光谱，随着叶绿素浓度的上升，吸收光谱的强度也逐渐增强。橄榄油中叶绿素吸收的能量主要去向包括镁电子辐射跃迁产生荧光以及分子间热能交换两部分，而橄榄油的吸收光谱并未出现类似于聚集荧光猝灭的现象，且吸收光谱强度与掺伪浓度间存在近似线性相关的关系。结果表明：当聚集荧光猝灭出现时，叶绿素吸收的能量仍然与浓度呈线性相关，此时高、低能分子堆叠引起的热能交换效率提高。

关键词 叶绿素；荧光；吸收光谱；特级初榨橄榄油；检测

中图分类号：O675.3 **文献标识码**：A **DOI**：10.3964/j.issn.1000-0593(2019)08-2421-04

引 言

国内已成为全球粮油最大的消费市场，橄榄油的消耗量也连年攀升。然而除国内少数地区(如甘肃陇南)可产橄榄油并满足部分自给外，国内橄榄油的消费绝大部分只能靠进口，而国内消费者对橄榄油的等级、品质等认识有限，以及不法商贩在利益的驱动下，勾兑假冒橄榄油甚至贩卖工业级橄榄油并以次充好。橄榄油掺伪的现象在国际市场上也屡见不鲜，国内外的研究机构对橄榄油的鉴别开展了深入的研究，例如常规理化检测法[1-2]、色谱法[3-5]、光谱法[6-10]、核磁共振法[11]、电子鼻[12]等。以油橄榄鲜果为原料，采用机械冷榨、过滤等物理工艺提取的酸度值小于0.8%的特级初榨橄榄油是一种等级最高、品质最好并可直接饮用的橄榄油。特级初榨橄榄油富含多酚化合物、游离脂肪酸及光敏物质等具有保健功效的天然物质成分，这类特殊成分造就了其高昂的“身价”。橄榄油成分的特殊性是各类检测技术手段的关键，就光谱法而言，有侧重于脂肪酸成分的红外光谱法、侧重于分子振动的拉曼光谱法以及侧重于光敏物质的荧光光谱法等。针对光敏物质(叶绿素)对橄榄油检测的影响，开展橄榄

收稿日期：2018-06-04，**修订日期**：2018-10-11

基金项目：中国科学院上海技术物理研究所创新专项(CX110)，民用航天技术预先研究项目(D030207)，载人航天领域预先研究项目(0209)和国家高技术研究发展计划(863)项目(2015AA021103)资助

作者简介：王泓鹏，1989年生，中国科学院上海技术物理研究所空间主动光电技术重点实验室助理工程师
e-mail：wanghongpeng813@163.com *通讯联系人 e-mail：wanxiong@mail.sitp.ac.cn

油中不同浓度的叶绿素对吸收光谱及荧光特性影响的研究。

本研究的目的在于分析荧光及吸收光谱在橄榄油鉴别中的应用及叶绿素浓度对光谱特性的影响。图 1 所示，几种常见植物油的紫外可见吸收光谱，图中特级初榨橄榄油的吸收光谱与其他植物油种类具有显著差异，6 个较为明显的吸收峰主要由叶绿素 a、叶绿素 b 以及类胡萝卜素等光敏物质引起。图 1(a)及(b)表明特级初榨橄榄油富含光敏物质且含量以叶绿素 a 为主，此外光敏物质的含量受橄榄油的产地、气候、品种种类、加工工艺及货期等因素的影响。

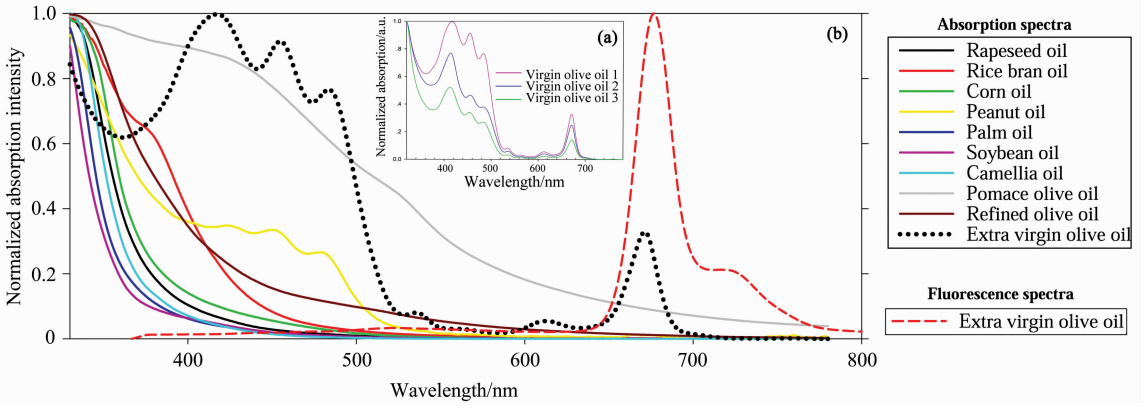

图 1 (a) 不同品牌的橄榄油可见吸收光谱图；(b) 不同植物油的可见吸收光谱图

Fig. 1 (a) The visible absorption spectrum of extra virgin olive oil of different brands; (b) The visible absorption spectrum of different vegetable oils

1 实验部分

1.1 仪器与样本

仪器：岛津公司 SolidSpec-3700/3700DUV 分光光度计，光谱范围 185～3 300 nm。其可见光区的探测器为光电倍增管(PMT)，波长采样间隔 1 nm，波长扫描速度 5 000 nm·min^{-1}，波长重复性 0.08 nm，分辨率 0.1 nm，基线平整度 0.002 Abs(320～1 600 nm 的可见光区)。532 nm 连续激光器，海洋 USB4000 光谱仪，荧光探头。比色皿采用石英比色皿(厚度 1 cm)。

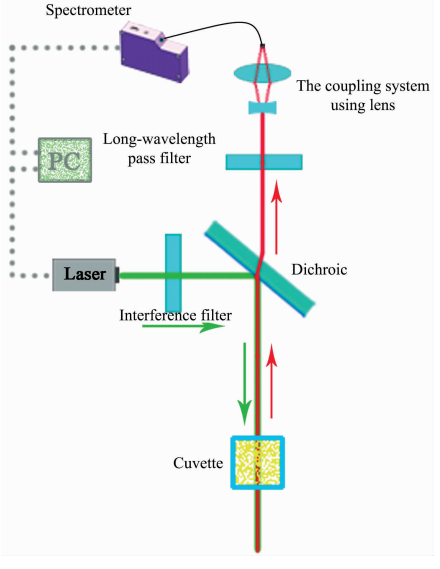

图 2 实验装置原理图

Fig. 2 Schematic diagram of experimental setup

样本：准备相同批次的特级初榨橄榄油 10 份(每份 10 mL)并编号 10 组，将玉米油(精炼玉米油中叶绿素含量极低)按比例 1∶2∶3∶4∶5∶6∶7∶8∶9 依次加入后 9 组样品中。

1.2 方法

荧光特性：荧光实验装置如图 2 所示，使用移液枪将样本注入石英比色皿中(约 2/3 的含量)。分别采集这 10 组样本的荧光发射光谱，如图 3 所示。

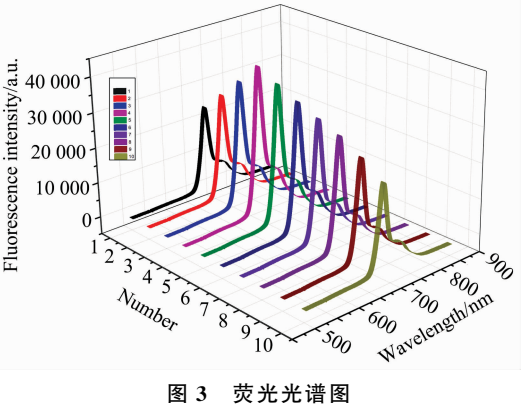

图 3 荧光光谱图

Fig. 3 The fluorescence spectrum

吸收光谱：首先确保分光光度计控制室温恒定，以确保采集时油温度恒为 25 ℃。然后调试检测仪，选定测量光谱的波长范围、光谱带宽等，并使用移液枪将样本注入石英比色皿中(约 2/3 的含量)。最后分别采集这 10 组样本的可见吸收光谱如图 4 所示。

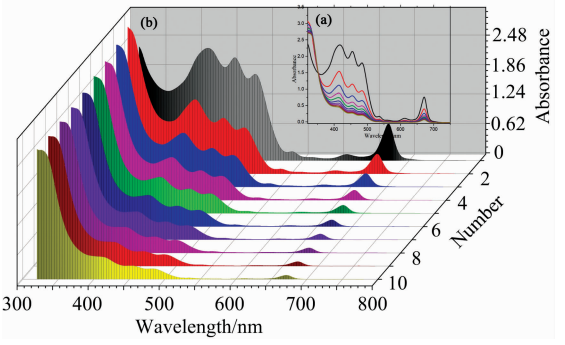

图 4　可见吸收光谱图

Fig. 4　The visible absorption spectrum

2　结果与讨论

2.1　叶绿素浓度变化对荧光光谱特性的影响

对比含有不同叶绿素浓度的特级初榨橄榄油的荧光光谱数据发现：在某一叶绿素浓度范围内，特级初榨橄榄油的荧光强度随着叶绿素浓度的增加而增强；当叶绿素浓度超过某一值后，荧光强度会随着叶绿素浓度的增加而降低。

叶绿素的聚集荧光猝灭是造成这一现象的原因。叶绿素是一类含有环卟啉分子结构的天然有机化合物，其中绿色植物中以叶绿素 a 的含量为主，叶绿素 a 的吸收波长在 430 nm 附近，反射波长在 500～550 nm 范围内(绿色植物呈现出颜色根本原因)。叶绿素主要是以 Mg 为中心的环卟啉分子结构，这种分子结构密布大量的电子云，使叶绿素吸收光能并进入激发态，从而发出低能量的荧光。一般情况下，荧光物质的浓度越高，荧光强度就越强，因此可以用荧光强度来表征荧光物质的浓度。但叶绿素的分子结构决定了其在定量分析上存在不可避免的缺陷。平面结构的叶绿素分子导致其分子运动较简单，当叶绿素较为分散时，分子与分子间几乎无能量交换，外界吸收的光能只能以能级跃迁的方式消耗，从而激发较强的荧光；但当叶绿素浓度达到一定值时，分子间产生堆叠效应，分子运动更加单一，此时当从外界吸收光能后，能量更容易在高、低能分子间转移消耗，因此荧光会减弱甚至消失。

实验中荧光猝灭的现象只是一批橄榄油样品中的一个特例，并不是所有的特级初榨橄榄油都会出现聚集荧光猝灭的现象，原因是橄榄油中叶绿素的浓度必须达到发生荧光猝灭的阈值。然而，橄榄油中光敏物质的浓度受产地环境、气候、加工工艺、存储条件以及货架期等因素的影响。因此，同产地不同批次甚至是同批次不同存储方式的橄榄油中光敏物质的含量及成分比也存在差异，从而增加了发生荧光猝灭现象的不确定性。

2.2　叶绿素浓度变化对可见吸收光谱特性的影响

在对比含有不同叶绿素浓度的特级初榨橄榄油的可见吸收光谱数据发现：叶绿素浓度与可见吸收光谱的吸收峰表现出正相关性，即随着叶绿素浓度的增加，可见吸收光谱的吸收峰强度也一直呈现正反馈效应，并未出现类似于荧光的聚集荧光猝灭现象。

橄榄油中叶绿素吸收的光能主要以荧光及热能两种形式消耗。当叶绿素浓度增加引起聚集荧光猝灭时，荧光辐射消耗的能量虽然减弱，但减少的能量却以分子间热量交换的形式消耗，因此聚集荧光猝灭效应并未对吸收光谱造成负反馈的影响。

3　结　论

吸收光谱及荧光特性对于橄榄油的鉴定具有重要意义。荧光可应用于橄榄油的快速定性分析，适用于短周期、大批量检测要求的领域(进出口海关等)，然而叶绿素的聚集荧光猝灭效应使其在定量分析上存在严重缺陷(即在某一浓度范围内荧光强度可能难以准确反映叶绿素浓度信息)。可见吸收光谱可用于橄榄油的定性及定量分析，但与荧光相比，可见吸收光谱法难以满足短周期、大批量检测的要求。

References

[1] Internet: Codex Standard for Table Olives. (Codex Stan. 66-1981). Downloaded from http://www.codexalimentarius.org/input/download/standards/243/CXS_066e.pdf. on 25/9/2013.
[2] GB 23347—2009. National Standards of the People's Republic of China(中华人民共和国国家标准). Olive Oil and Olive-Pomace Oil(橄榄油、油橄榄果渣油).
[3] Carranco N, Farrés-Cebrián M, Saurina J, et al. Foods, 2018, 7(4): 44.
[4] Jiang Z, Wang Y, Zheng Y, et al. Journal of Separation Science, 2016, 39(15): 2928.
[5] Sales C, Cervera M I, Gil R, et al. Food Chemistry, 2017, 216: 365.
[6] Mora-Ruiz M E, Reboredo-Rodríguez P, Salvador M D, et al. European Journal of Lipid Science and Technology, 2017, 119(1): 1600099.
[7] Georgouli K, Del Rincon J M, Koidis A. Food Chemistry, 2017, 217: 735.
[8] Ferreiro-González M, Barbero G F, álvarez J A, et al. Food Chemistry, 2017, 220: 331.
[9] Aparicio-Ruiz R, Tena N, Romero I, et al. Grasasy Aceites, 2017, 68(4): 219.
[10] Merás I D, Manzano J D, Rodríguez D A, et al. Talanta, 2018, 178: 751.
[11] Zhu W, Wang X, Chen L. Food Chemistry, 2017, 216: 268.
[12] Rodrigues N, Dias L G, Veloso A C A, et al. European Food Research & Technology, 2017, 243(4): 597.

Study on Fluorescence Quenching and Absorption Spectra of Olive Oil

WANG Hong-peng, WAN Xiong*

Key Laboratory of Space Active Opto-Electronics Technology, Shanghai Institute of Technical Physics of the Chinese Academy of Sciences, Shanghai 200083, China

Abstract The olive oil, known as "liquid gold", has become a synonym for healthy edible oil. It not only has a steep increase in its price, but also has become a best-selling oil in the non producing market. Spectral method has many advantages compared with other technologies, such as fast, nondestructive and non sample processing. Different spectral detection methods have a particular emphasis on the material components, for example, infrared spectroscopy focuses on the detection of fatty acid content, Raman spectroscopy focuses on the detection of molecules, fluorescence spectroscopy focuses on the detection of photosensitive substances, and absorption spectroscopy focuses on the detection of unsaturated fatty acids. Fluorescence and absorption spectra are very sensitive to photosensitive substances, and olive oil is rich in chlorophyll and other photosensitive substances. Therefore, fluorescence and absorption spectra have become an effective technique for identifying olive oil. Chlorophyll is an organic molecule containing the structure of cycloporphyrin. The molecular structure of this kind of molecular structure has the characteristics of absorption of light, and the absorption spectra of different kinds of chlorophyll are unique, among which the content of chlorophyll a in green plants is the most. In order to study the application of the absorption spectrum of chlorophyll and the fluorescence characteristics of the extra virgin olive oil, the content of chlorophyll in olive oil was indirectly regulated by mixing different proportion of corn oil in the special primary olive oil. The fluorescence and absorption spectra of different adulterated olive oil were measured and the phase of chlorophyll concentration was studied. The effecus of chlorophyll concentration and adulteration amount on the absorption spectra and fluorescence characteristics of olive oil were studied. 10 samples of the same batch of extra virgin olive oil were taken, 9 of them were diluted in equal proportion and 10 samples were sequentially ordered according to adulteration. The fluorescence and absorption spectra of the 10 samples were collected in turn, and the correlation between the concentration of chlorophyll and adulteration were compared and the effects of the two spectral techniques on the identification of olive oil were compared. With the increase of chlorophyll concentration, the fluorescence intensity becomes stronger and weakens sharply after a certain time, that is, the aggregation fluorescence quenching. This phenomenon is mainly due to the intermolecular π—π action caused by the molecular structure of the phyphyrphyrin, which makes the non excited low energy molecules and high energy molecules stacked together. The radiation transition of energy (Fluorescence) is also transformed into the energy transfer (heat exchange) between the molecules. As for the absorption spectrum, the intensity of absorption spectrum increases with the increase of chlorophyll concentration. The main energy of the absorption of chlorophyll in olive oil consists of two parts, including the emission of magnesium electron emission and the intermolecular heat exchange, while the absorption spectrum of olive oil does not appear like aggregation fluorescence quenching, and there is an approximate linear correlation between the absorption spectrum intensity and the adulteration concentration. The results show that when the fluorescence quenching occurs, the energy of the absorption of chlorophyll is still linearly related to the concentration, and the efficiency of heat exchange caused by the stacking of high and low energy molecules increases.

Keywords Chlorophyll; Fluorescence; Absorption spectrum; Extra virgin olive oil; Detection

(Received Jun. 4, 2018; accepted Oct. 11, 2018)

* Corresponding author