Near Infrared Spectroscopic Determination of Olive Oil Adulteration with Sunflower and Corn Oil

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ABSTRACT

Determination of authenticity of extra virgin olive oils has become very important in recent years due to the increasing public concerns about possible adulterations with relatively cheap vegetable oils such as sunflower oil. This study was focused on the application of near infrared (NIR) spectroscopy in conjunction with multivariate calibration to identify the adulteration of olive oils. NIR transmittance measurements were made on pure olive oil and olive oil adulterated with varying concentrations (4-96%, v/v) of sunflower and corn oil in two sets of 26 binary and ternary mixtures. Multivariate calibration models were generated using genetic inverse least squares (GILS) method and used to predict the concentration of adulterants along with the concentration of olive oil in the samples. Over all, standard error of predictions ranged between 2.49 and 2.88% (v/v) for the binary mixtures of olive and sunflower oil and between 1.42 and 6.38% (v/v) for the ternary mixtures of olive, sunflower and corn oil.

Key words: near infrared spectroscopy, olive oil, adulteration, multivariate calibration, genetic algorithms

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INTRODUCTION

Olive oil is a valuable food product as compared with other vegetable oil products. As a result, the adulteration of olive oil with cheaper vegetable oil becomes a real concern. For this reason, the analysis of edible oils for possible adulterants is very important for food safety and protection of consumers. Based on the extraction method used, there are various types of olive oil on the market today. Extra virgin olive oil is obtained from the olive by purely mechanical means, and the lower grade oils are obtained by solvent extraction, heat treatment, esterification or refining. The composition of the oils is based on the fatty acids present and their locations on the glycerol backbone. This composition varies not only with the type of oil and extraction method but also with the geographical origin and meteorological effects during the growth and harvest of the olives[1]. This variation can be used for oil authentication and the identification of adulteration. Various physical and chemical tests have been used to establish the authenticity of olive oil and to detect the level of adulterants in it[2-4]. Studies related to olive oil adulteration were mostly carried out with chromatographic methods in recent years[5-7]. However, while chromatographic methods offer high sensitivity and accuracy, they are also time consuming and expensive. On the other hand, spectroscopic methods may offer faster and cheaper analysis alternatives[8-10].

Near infrared (NIR) spectroscopy[11] has become a popular method for simultaneous chemical analysis and is being studied extensively in a number of fields such as process monitoring[12], biotechnology[13], and pharmaceutical and food industry[14] because of the potential as an on-line, nondestructive and noninvasive analysis. The NIR spectrum covers the range from 780 nm to 2500 nm and most of the absorption bands observed in this region are due to overtones and combinations of the fundamental mid-IR molecular vibrational bands. Although all the fundamental vibrational modes can have overtones, the most commonly observed bands arise from the C–H, O–H or N–H bonds in the molecules.

Modern spectroscopic methods are so fast that they can generate hundreds of spectra in a few minutes for a sample containing multiple components. Unfortunately, univariate calibration methods are not suitable for this type of data, as they require an interference free system. Multivariate calibration deals with data of instrument responses measured on multiple wavelengths for a sample that usually contains more than one component. In recent years, advances in chemometrics and computers have led to the development of several multivariate calibration methods[15-18] for the analysis of complex chemical mixtures.

Inverse Least Squares (ILS) is based on the inverse of Beer's Law where the concentration of an analyte is modeled as a function of absorbance. Genetic Inverse Least Squares (GILS) is a modified version of original ILS method in which a small set of wavelengths are selected from a full spectral data matrix. It has evolved to an optimum solution using a genetic algorithm (GA) and has been applied to a number of wavelength selection problems[19-23]. GA's are non-local search and optimization methods that are based upon the principles of natural selection[24-26].

In this work, genetic algorithms based calibration

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method GILS was tested with the aim of establishing calibration models that have a high predicability for the NIR spectroscopic determination of olive oil adulteration with sunflower and corn oil.

I. *Genetic Inverse Least Squares*

The major drawback of the classical least squares (CLS) method is that all the interfering species must be known and their concentrations are included in the model. This need can be eliminated by using the ILS method. In the ILS method, the concentration of a component is modeled as a function of absorbance. Because modern spectroscopic instruments are stable and can provide excellent signal-to-noise (S/N) ratios, it is believed that the majority of errors lie in the reference values of the calibration sample, rather than the measurement of their spectra. In fact, in many cases the concentration data of calibration set is generated from another analytical technique that already has its inherent errors which might be higher than those of the spectrometer (for example, Kjeldahl protein analysis used to calibrate NIR spectra).

The ILS model for *m* calibration samples with n wavelengths for each spectrum is described by

$$C = AP - E_C \tag{1}$$

where C is the *m* x *l* matrix of the component concentrations, A is the *m* x *n* matrix of the calibration spectra, P is the *n* x *l* matrix of the unknown calibration coefficients relating *l* component concentrations to the spectral intensities and E_C is the *m* x *l* matrix of errors in the concentrations not fit the model. In the calibration step, ILS minimizes the squared sum of the residuals in the concentrations. The biggest advantage of ILS is that equation (1) can be reduced for the analysis of single component at a time since the analysis is based on an ILS model invariant with respect to the number of chemical components in the analysis. The reduced model is given as

$$c = Ap - e_c \tag{2}$$

where c is the *m* x *l* vector of concentrations for the component that is being analyzed, p is *n* x *l* vector of calibration coefficients and e_c is the *m* x *l* vector of concentration residuals unfit for the model. During the calibration step, the least-squares estimate of p is

$$\hat{p} = (A'A)^{-1}A' \cdot c \tag{3}$$

where $\hat{p}$ is the vector of estimated calibration coefficients. Once $\hat{p}$ is calculated, the concentration of the analyte of interest can be predicted with the equation below.

$$\hat{c} = a' \cdot \hat{p} \tag{4}$$

Where $\hat{c}$ is the scalar estimated concentration and a is the spectrum of the unknown sample. The ability to predict one component at a time without knowing the concentrations of interfering species has made ILS one of the most frequently used calibration methods.

The major disadvantage of ILS is that the number of wavelengths in the calibration spectra should not be more than the number of calibration samples. This is a big restriction since the number of wavelengths in a spectrum will generally be much more than the number of calibration samples and the selection of wavelengths that provide the best fit for the model is not a trivial process. Several wavelength selection strategies, such as stepwise wavelength selection and all possible combination searches, are available to build an ILS model which the data fit best.

GA are global search and optimization methods based upon the principles of natural evolution and selection as developed by Darwin. Computationally, the implementation of a typical GA is quite simple and consists of five basic steps including initialization of a gene population, evaluation of the population, selection of the parent genes for breeding and mating, crossover and mutation, and replacing parents with their offspring. These steps have taken their names from the biological foundation of the algorithm.

GILS is an implementation of a GA for selecting wavelengths to build multivariate calibration models with reduced data set. GILS follows the same basic initialize/breed/mutate/evaluate algorithm as other GA's to select a subset of wavelengths which is unique in the way it encodes genes. Detailed working principles of GILS were described elsewhere[19-21] but for completeness, a brief outline was given here. Following is a flow chart for the GILS algorithm used in this study.
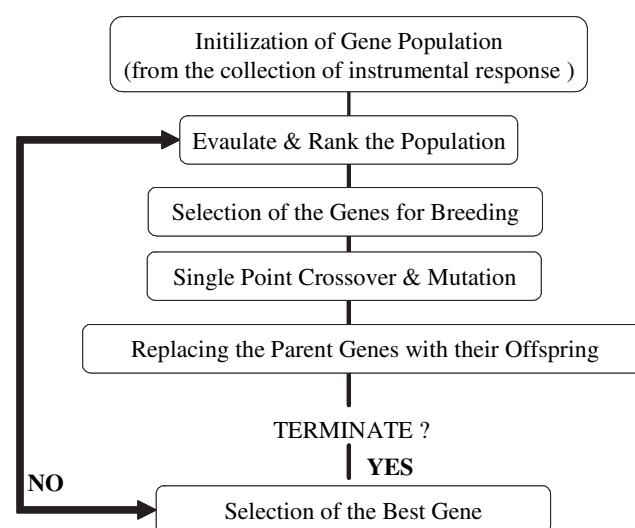
A gene is a potential solution to a given problem and the exact form may vary from application to application. Here, the term gene is used to describe the collection of instrumental response at the wavelength range given in the data set. The term 'population' is used to describe the

collection of individual genes in the current generation.

The first generation of genes is created randomly with a fixed population size. The size of the gene pool is a user defined even number in order to allow breeding of each gene in the population. It is important to note that the larger the population size, the longer the computation time. The number of instrumental responses in a gene is determined randomly between a fixed low limit and high limit. Once the initial gene population is created, the next step is to evaluate and rank the genes using a fitness function, which is the inverse of the standard error of calibration (SEC). The SEC is calculated as

$$SEC = \sqrt{\frac{\sum_{i=1}^{m}(c_i - \hat{c}_i)^2}{m-2}} \qquad (5)$$

where c_i is the actual and $\hat{c}_i$ is the predicted concentration of ith sample i^{th} for m number of samples.

The third step is where the basic principle of natural evolution is put to work for GILS. This step involves the selection of the parent genes from the current population for breeding using a roulette wheel selection method according to their fitness values. After the selection procedure is completed, the selected genes are allowed to mate top-down in pairs whereby the first gene mates with the second gene and the third one with the fourth one and so on as illustrated in the following example:

Parents

$$S_1 = (A_{347}, A_{251}, \# A_{379}, A_{218}) \qquad (6)$$

$$S_2 = (A_{225}, A_{478}, \# A_{343}, A_{250}, A_{451}, A_{358}, A_{231}, A_{458}) \qquad (7)$$

The points where the genes are cut for mating are indicated by #.

Offspring

$$S_3 = (A_{347}, A_{251}, A_{343}, A_{250}, A_{451}, A_{358}, A_{231}, A_{458}) \qquad (8)$$

$$S_4 = (A_{379}, A_{218}, A_{225}, A_{478}) \qquad (9)$$

where A_{347} represents the instrument response at the wavelength given in subscript, S_1 and S_2 represent the first and second parent genes and S_3 and S_4 are the corresponding genes for the offspring. Here the first part of S_1 is combined with the second part of the S_2 to give the S_3, likewise the second part of the S_1 is combined with the first part of the S_2 to give S_4. After crossover, the parent genes are replaced by their offspring and the offspring are evaluated. The ranking process is based on their fitness values following the evaluation step. Then the selection for breeding/mating starts all over again. This is repeated until a predefined number of iterations is reached.

The GILS method is an iterative algorithm and

therefore there is a high possibility that the method may easily overfit the calibration data so that the predictions for independent sets might be poor. To eliminate possible overfitting problems, cross validation is used in which one spectrum is left out of the calibration set and the model is constructed with *m-1* samples. Then this model is used to predict the concentration of the leftout sample. This process is continued until all samples are left out at least once in the iteration.

At the end, the gene with the lowest SEC (highest fitness) is selected for the model building. This model is used to predict the concentrations of components being analyzed in the prediction (test) sets. The success of the model in the prediction of the test sets is evaluated using standard error of prediction (SEP), given as

$$SEP = \sqrt{\frac{\sum_{i=1}^{m}(c_i - \hat{c}_i)^2}{m}} \qquad (10)$$

Because random processes are heavily involved in GILS as in all the GA's, the program has been set to run several times for each component in a given multi-component mixture in this study. The best run, (i.e. the one generating the lowest SEC for the calibration set and at the same time producing SEP's for prediction sets that are in the same range with the SEC) is subsequently selected for the evaluation and further analysis. The termination of the algorithm can be done in many ways. The easiest way is to set a predefined iteration number for the breeding/mating cycles.

GILS has some major advantages over the classical univariate and multivariate calibration methods. First of all, it is quite simple in terms of the mathematics involved in the model building and prediction steps, but at the same time it has the advantages of the multivariate calibration methods with a reduced data set since it uses the full spectrum to extract genes. By selecting a subset of instrument responses, it is able to eliminate nonlinearities that might be present in the full spectral region.

MATERIALS AND METHODS

Olive oil, sunflower oil and corn oil samples were purchased from a local grocery store. Two sets of binary and ternary mixtures with 26 samples in each were prepared. In the first set, 24 binary mixtures of olive oil and sunflower oil samples were prepared by mixing appropriate volumes of each. In addition, two pure component samples were also prepared and the sample set was split into two subsets: calibration set and prediction set as outlined in Table 1. The second set contained the ternary mixtures of olive oil, sunflower oil and corn oil as shown in Table 2. Spectra were collected using a Bio-Rad Excalibur FTS 3000 NX Fourier Transform Near Infrared spectrometer (Bio-Rad Laboratories Europe Ltd., UK) between

Table 1. Percent composition of calibration and prediction sets used in binary mixtures of olive and sunflower oil

	Calibration set			Prediction set	
Sample	Olive oil (v/v%)	Sunflower oil (v/v%)	Sample	Olive oil (v/v%)	Sunflower oil (v/v%)
1	36	64	1	96	4
2	92	8	2	8	92
3	52	48	3	24	76
4	28	72	4	48	52
5	12	88	5	44	56
6	56	44	6	68	32
7	72	28	7	88	12
8	60	40	8	80	20
9	100	0	9	64	36
10	16	84	10	4	96
11	84	16			
12	32	68			
13	76	24			
14	0	100			
15	20	80			
16	40	60			

Table 2. Percent composition of calibration and prediction sets used in ternary mixtures of olive, sunflower, and corn oil

	Calibration set				Prediction set		
Sample	Olive oil (v/v%)	Sunflower oil (v/v%)	Corn oil (v/v%)	Sample	Olive oil (v/v%)	Sunflower oil (v/v%)	Corn oil (v/v%)
1	48	48	4	1	46	4	50
2	92	6	2	2	64	8	28
3	34	30	36	3	26	66	8
4	64	26	10	4	38	34	28
5	28	50	22	5	30	48	22
6	48	4	48	6	80	10	10
7	70	30	0	7	24	54	22
8	0	66	34	8	18	68	14
9	20	24	56				
10	32	2	66				
11	30	36	34				
12	20	14	66				
13	96	0	4				
14	22	72	6				
15	40	44	16				
16	24	6	70				
17	8	84	8				
18	52	40	8				

1000 and 2500 nm wavelength range with a wavelength interval of 4 nm. This spectrometer was equipped with a 250 W tungsten-halogen source, a calcium fluoride beam splitter and a lead selenide detector. Samples were held in a 2 mm pathlength infrasil quartz sample holder from Starna (Atascadero, CA).

All spectra were then transferred to a computer where the data processing programs were installed. The GILS

method was written in MATLAB programming language using Matlab 5.3 (MathWorks Inc., Natick, MA).

RESULTS AND DISCUSSION

The binary system was selected to demonstrate the feasibility of NIR spectroscopy coupled with genetic multivariate calibration for the examination of olive oil adulteration with sunflower and corn oils. The spectra of pure olive, sunflower and corn oil and their ternary mixture between the 1000 nm and 2500 nm are shown in Figure 1. Because of structural similarities, the spectral features of these oils are very much alike and only minute differences exist in some parts of the whole spectra. To illustrate these minute differences, a small portion of the spectra are also shown on the same figure, which is an enlarged view of the region between 1900 and 2200 nm. As seen in this magnified view of the spectra, there are very small differences between the spectra of these oils. Throughout the multivariate calibration process, it is expected that these differences will reveal the information necessary to build successful calibration models otherwise almost impossible with univariate calibration methods.

In order to prepare calibration models, 16 of 26 samples of the first set were used to build calibration set and the remaining 10 sample were reserved for prediction set to test the performance of the models. Two spectra were collected from each sample yielding a total of 52 spectra. The calibration models for both olive oil and sunflower oil were prepared with 32 spectra and then these models were tested with 20 independent prediction spectra which were not used in the calibration step. Because of the random nature of the GILS method, the program was set to run 30 times with 20 genes and 50 iterations. Since the GILS program was iterated 50 times in each run, full cross validation was applied during the model building step to avoid possible overfitting problems. For the second data set, 18 samples of ternary mixtures of olive, sunflower and corn oil were selected as calibration set and remaining 8 samples were used as prediction set as shown in Table 2. The same data processing was applied with GILS as outlined above for the first data set.

The SEC and SEP results for calibration and prediction sets, respectively along with the average percent recoveries (APR) and associated standard deviation (SD) values for the first data set are shown in Table 3. The following equations are used to calculate percent recovery (PR), APR and SD.

$$PR_i = \frac{\hat{c}_i}{c_i} \times 100\% \tag{11}$$

$$APR = \frac{\sum\limits_{i=1}^{m}\left(\frac{\hat{c}_i}{c_i} \times 100\%\right)}{m} \tag{12}$$
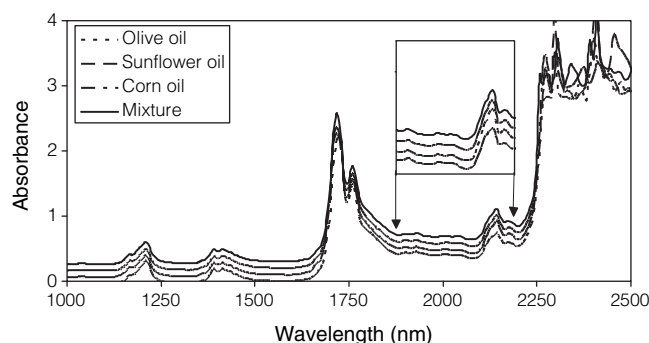

Figure 1. Near infrared spectra of olive, sunflower, corn oil and their ternary mixture between 1000 and 2500 nm. (Note that the sunflower, corn oil and the ternary mixture spectra have been ofsetted from the olive oil spectrum by a constant of 0.1, 0.2, and 0.3, respectively, to make it more clear).

Table 3. Standard error of calibration (SEC) and standard error of prediction (SEP) results for calibration and prediction sets, respectively along with average percent recoveries (APR) and associated standard deviation (SD) values for the binary mixtures of olive and sunflower oil

| Data sets	Parameters	Olive oil	Sunflower oil
Calibration set	SEC (v/v%)	1.68	2.01
	APR	100.31	99.32
	SD	5.48	5.96
Prediction set	SEP (v/v%)	2.49	2.88
	APR	99.78	100.74
	SD	4.52	5.64

$$SD = \sqrt{\frac{\sum\limits_{i=1}^{m}(PR_i - APR)^2}{m-1}} \tag{13}$$

where c_i is the actual and c_i is the predicted concentration of ith sample i^{th} for m number of samples as defined above.

As seen in the Table 3, the SEC and SEP values ranged between 1.68 and 2.88% by volume for both olive oil and sunflower oil in the binary mixtures. The APR values ranged between 99.32 and 100.74% with the SD around 5.00% for both olive oil and sunflower oil. Considering the fact that any possible olive oil adulteration attempt may include up to 30% or more vegetable oil by volume, these values seem to be a good prediction for a fast identification. Results of ternary data set are shown in Table 4. Here the SEC and SEP values of olive oil were 0.92 and 1.42% (v/v) respectively, showing a good agreement between binary and ternary data sets. The APR and the associated SD values were 100.09% and 5.66%, respectively, for the prediction set of olive oil. As the results of the binary data set, the GILS method is also successful in the prediction of olive oil content of the ternary samples. On the other hand, the SEC and SEP values of sunflower and corn oil in the second data set were somewhat higher than those obtained for olive oil. In fact, a close examina-

Table 4. Standard error of calibration (SEC) and standard error of prediction (SEP) results for calibration and prediction sets, respectively along with average percent recoveries (APR) and associated standard deviation (SD) values for the ternary mixtures of olive, sunflower, and corn oil

Data sets	Parameters	Olive oil	Sunflower oil	Corn oil
Calibration set	SEC (v/v%)	0.93	3.74	2.32
	APR	99.87	102.27	94.85
	SD	3.62	32.71	26.54
Prediction set	SEP (v/v%)	1.42	5.42	6.38
	APR	100.09	80.87	104.73
	SD	5.66	41.87	46.65
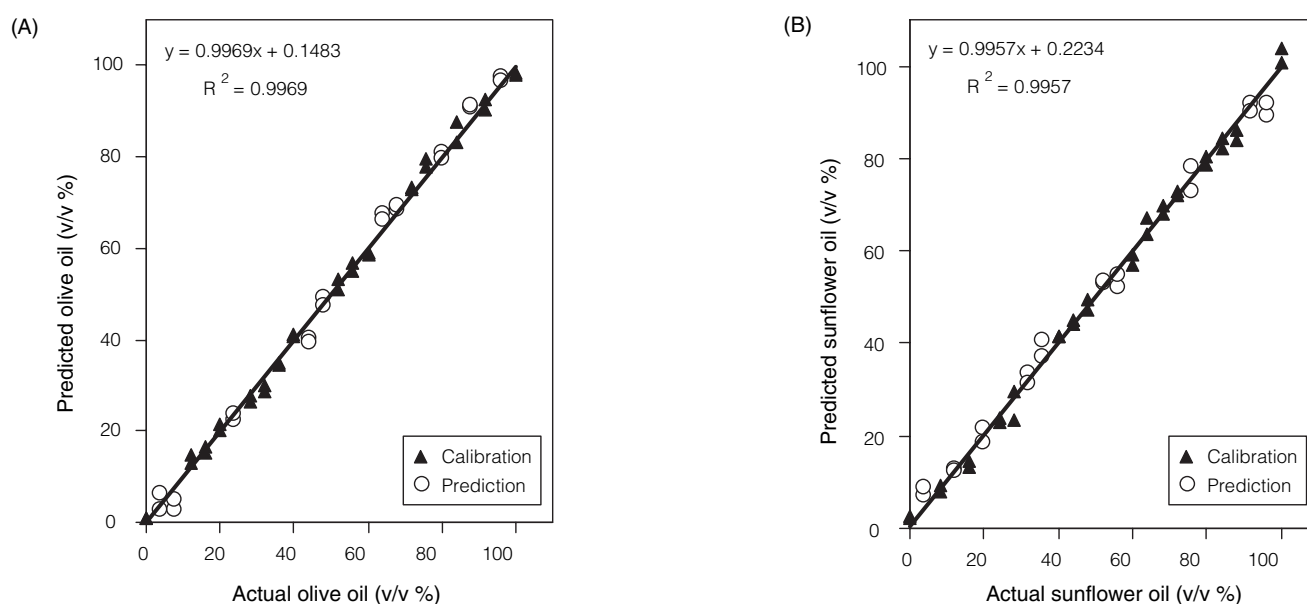
Figure 2. Actual versus NIR predicted concentrations of olive oil (A) and sunflower oil (B) for the binary set.

tion of the APR and associated SD values for prediction sets of sunflower and corn oil reveals that the models developed by GILS method were not as successful as in the case of binary data set. One possible reason for this could be the increased complexity of the ternary system compared to a binary system.

The plot of actual versus NIR predicted concentrations for both olive and sunflower oil is illustrated in Figure 2 for the first data set. It is evident that the proposed method is able to predict adulteration of olive oil in a wide dynamic range from 5% to 95% by volume. The actual versus NIR predicted concentrations of olive, sunflower and corn oil in the ternary system are shown in Figure 3. As can be seen, a very good prediction was observed for olive oil content of the ternary samples but it is not so true for the other two components. However, if the goal is to determine the purity of the olive oils which are suspected for the adulteration with cheaper vegetable oils, the GILS method is still able to determine the olive oil content of the ternary systems within its

error range. As a result, it is concluded that NIR spectroscopy in conjunction with multivariate calibration can be used for the fast identification of olive oil adulteration with cheaper substitutes.

Because GILS is a wavelength selection based method, it is interesting to observe the distribution of selected wavelengths in multiple runs over the entire full spectral region. The frequency distribution of selected wavelengths in 30 runs for olive, sunflower and corn oil is illustrated in Figure 4. As can be seen, the absorbance values in the region between 2200 and 2500 nm were over 3 and as a result strong nonlinearities arose. However, the GILS method is able to detect these nonlinearities as no wavelengths survived in final best genes higher than 2200 nm. The most frequently selected wavelengths correspond to the region between 1700 and 1900 nm where a large peak exists. This is a strong indication that the genetic algorithm incorporated GILS method is focusing on the regions where most concentration related information is contained.
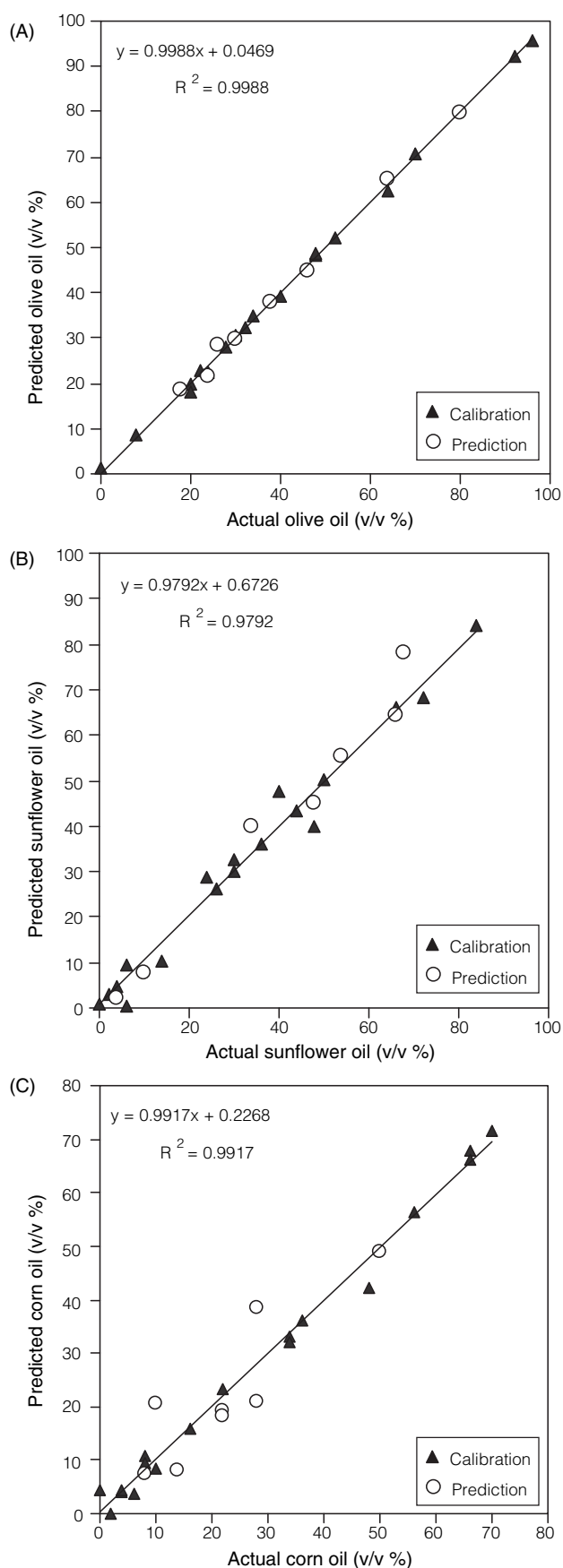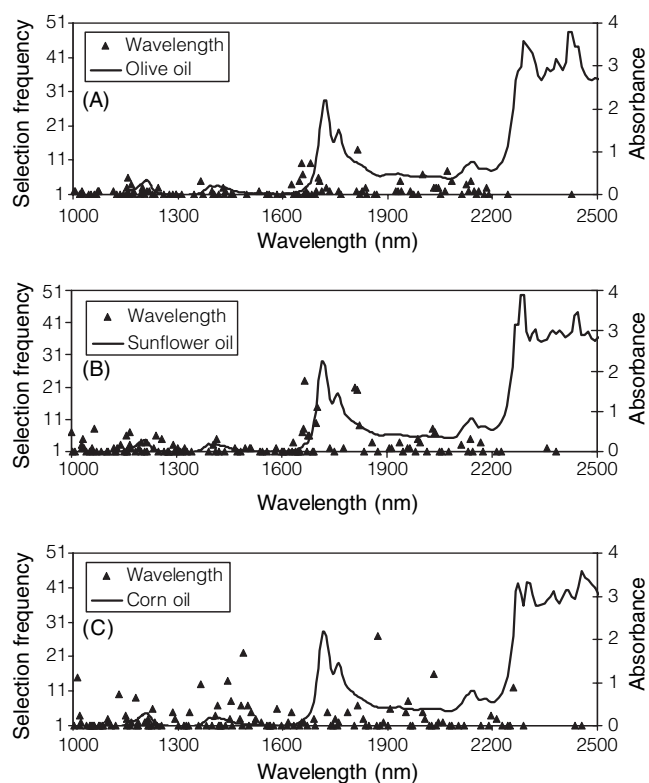
Figure 4. Distribution of the selected wavelengths by the GILS method for a total of 30 runs with 20 genes and 50 iterations in each run. (A) olive oil; (B) sunflower oil; (C) corn oil.

CONCLUSIONS

This study demonstrated the application of NIR spectroscopy with multivariate calibration to the determination of olive oil adulteration with sunflower and corn oil. The fact that the standard error of prediction values are below 3% (v/v) for the binary set and for olive oil of the ternary system, the NIR spectroscopy can be used as a fast screening method for possible olive oil adulteration. On the other hand, the genetic algorithm used in the GILS method is able to select and extract the most relevant information to build successful calibration models that has high predicability for the independent test samples.

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Figure 3. Actual versus NIR predicted concentrations of olive oil (A), sunflower oil (B), and corn oil (C) for the ternary set.

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