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A preliminary approach to predictive modelling of extra virgin olive oil stability†

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Abstract: The aim of this work was to set up a phenomenological model to predict the stability of extra virgin olive oil based on combined stability/instability indices. A screening of stability/instability indices was carried out by multivariate statistical analyses on company data for virgin olive oil. Screened indices were acidity, oleic acid content and bitter taste. The ability of these indices to predict stability was checked by measuring the following degradation parameters on 11 oil samples of different compositions planned by a fractional factorial design (FFD): peroxide value, UV spectroscopic indices, minor polar component content, oxidative status of fatty acids, antioxidant activity and sensory descriptors of aroma, taste and flavour. Experimental data were processed by multivariate statistical analyses. A combination of acidity, oleic acid content and bitter taste values was significantly able to predict oil stability expressed by the peroxide value, the K_{232} UV index and the oxidative status of fatty acids.

Keywords: extra virgin olive oil; stability indices; degradation parameters; predictive modelling

INTRODUCTION

Degradation occurs during the processing and shelf-life of extra virgin olive oil (EVOO), and success on the market may also depend on the stability of EVOO. Degradation may result in exceeding legal limits for eg acidity, peroxide and UV spectroscopic index values; it may result in variations in nutritional quality of the product, since antioxidant content decreases and free radical content increases; it may result in variations in sensory descriptors such that appreciation of the product decreases, since aroma, colour, taste and flavour attributes change and some unpleasant sensory attributes may occur.[1–8]

Degradation is mainly due to lipid oxidation[9], although enzymatic reactions also occur during processing. Some enzymatic reactions affect autoxidation: triglycerides are hydrolysed by lipase, while phenolic compounds are oxidised by both polyphenol oxidase and peroxidase.[10,11] Other enzymatic reactions are responsible for both pleasant oil flavours (eg the lipoxygenase pathway) and increasing antioxidant content (eg due to glycosidases).[12–14]

The extent and rate of degradation depend on the following operating conditions during the processing and shelf-life of EVOO: variety, growth conditions, pest control, harvesting, transportation and storage of olives; milling, malaxing, extraction and filtration of oils; packaging and commercial activities of oils.[15] Operating factors that promote degradation include long times, exposure to oxygen, presence of water, exposure to light and high temperatures and are associated with high levels of polyunsaturated fatty acids in the oil and enzymes in the olive paste and a low level of oil antioxidants.

The biggest companies produce EVOO by blending. For these companies, prediction of stability represents a useful tool to select the virgin oil purchased and to optimise the blending operation.

A large number of data on parameters and analytical methods for measuring EVOO degradation are available. Although some models are reported in the literature, either they describe degradation but do not predict it or they show limited predictability.[4,5,7,16–18]

The aim of this work was to set up a phenomenological model to predict the stability of EVOO based on combined composition indices. Effects of olive variety, crop season and processing on EVOO stability should be defined and explained by different values for composition indices.

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EXPERIMENTAL

EVOO stability was studied according to the following experimental approach in two steps:

- screening of stability/instability composition indices;
- checking for significant relationships between screened indices and EVOO degradation.

Screening of indices was carried out by multivariate analysis procedures on data for virgin oil purchased from Carapelli Firenze SpA (Florence, Italy). Data were derived from 63 chemical and 18 sensory parameters measured on oils purchased from four different Mediterranean areas during the 1999–2001 olive crop seasons.[9] Data processing showed that only a few composition indices may be related to degradation of EVOO (Table 1) in terms of peroxide value and UV spectroscopic parameters. Assuming a relationship between oleic acid content and the other fatty acids, these indices were further reduced to acidity, oleic acid content and bitter taste of EVOO.

This hypothetical relationship with stability was checked by measuring the extent of EVOO degradation in a large number of oil samples differing in screened indices. Eleven EVOO samples from the 2002 olive oil season were planned by a fractional factorial design (FFD) to optimise the analytical effort, using consistent minimum and maximum values for indices reflecting the company's historical data. Table 2 shows both the planned and the experimental composition of oil samples; a few differences were found between the ideal and actual levels, but these were acceptable to the FFD.

Table 1. Hypothetical relationship between EVOO stability and some composition indices as derived from company's data processing

Stability/instability index	Hypothetical relationship with oil stability
Acidity	Indirect
Palmitic acid content	Indirect
Stearic acid content	Direct
Oleic acid content	Direct
Linoleic acid content	Indirect
Bitter taste	Direct

Table 2. Comparison between planned and experimental composition of EVOO samples optimised by FFD

	Planned composition			Experimental composition		
Sample number	Acidity (% oleic acid)	Oleic acid content (%)	Bitter taste	Acidity (% oleic acid)	Oleic acid content (%)	Bitter taste
1	0.4	60	1	0.42	60.0	1.0
2	0.8	60	1	0.80	60.6	1.0
3	0.4	80	1	0.39	80.7	1.0
4	0.8	80	1	0.84	78.6	1.0
5	0.4	60	3	0.39	70.4	2.7
6	0.8	60	3	0.78	69.8	2.2
7	0.4	80	3	0.42	80.0	3.1
8	0.8	80	3	0.82	77.9	2.8
9	0.6	70	2	0.58	71.9	1.9
10	0.6	70	2	0.58	71.9	1.9
11	0.6	70	2	0.58	71.9	1.9

EVOO samples were either prepared by blending, assuming that the additive property of screened indices is applicable, or directly purchased from Mediterranean areas.

Measurement of EVOO stability/instability indices

Acidity (% oleic acid), fatty acid content (%) and bitter taste intensity were measured by the EU official methods.[20]

Measurement of EVOO degradation parameters

Peroxide value (meqO_2 kg^{-1}) was measured by the EU official method.[20]

Spectroscopic parameters, ie K_{232} and K_{270}, in the UV region were measured by the EU official method[20] on a 0.4% (w/v) solution of oil in isooctane.

Minor polar component content (ie oleuropein and ligstroside derivatives) was determined by high-performance liquid chromatography (HPLC) using a modification of the method of Cortesi *et al*.[21] Oil (2 g) was dissolved in 4 ml of methanol, and 1 ml of a methanolic solution (10 mg l^{-1}) of syringic acid was added as an internal standard. The mixture was agitated for 1 h, then centrifuged for 15 min at 4500 rpm; 5 ml of extract was obtained. The extract was filtered through a membrane (0.22 μm) before performing the HPLC analysis with a UV detector at 280 nm. Tyrosol content (mg kg^{-1}) was calculated using a standard curve for tyrosol (Merck, Milan, Italy) at concentrations of 2–20 mg kg^{-1} in methanol. Hydroxytyrosol content and the sum of decarboxymethyloleuropein and oleuropein aglycon contents (mg kg^{-1}) were calculated as the equivalent of tyrosol. Total minor polar component content was calculated as the sum of chromatographic peak areas.

Lipid oxidation status was evaluated according to Cortesi and Rovellini[22] and Rovellini *et al*.[23] The oxidative profile and chemical structure of oxidation products had already been characterised by HPLC/electrospray mass spectrometry (ES-MS) by Rovellini *et al*.[24] After a transesterification reaction with 1.0 M sodium benzyloxide in benzylic alcohol to form benzylester derivatives of fatty acids, dienoic and trienoic conjugated isomer contents (%) were measured by HPLC with a UV detector at 230 nm, while oxidised fatty acid contents (%) were measured by HPLC at 230 and 275 nm.

Antioxidant activity was evaluated by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging test according to Visioli *et al*[25] with some modifications. The polar fraction of oil (2 g) was extracted with methanol (5 ml). The mixture was agitated for 1 h and centrifuged for 15 min at 4500 rpm to separate the polar from the lipid fraction. Different solutions of methanolic extracts were added to a 25 mg l^{-1} methanolic solution of DPPH (Sigma, St

Table 3. Definition of sensory descriptors

Sensory descriptor	Definition
Green olive aroma	Typical aroma of green olive perceived by sense of smell
Astringency	Set of tactile sensations caused by dryness in oral mucosa
Pungency	Tactile itching sensation perceived in mouth during swallowing
Green olive flavour	Typical aroma of green olive involving taste and sense of smell during swallowing

Louis, MO, USA). The decrease in absorbance was measured at 515 nm up to 15 min (when a constant value was reached). I_{50}, defined as the amount of original oil sample (mg) required to lower the initial DPPH concentration by 50%, was extrapolated from a dose–response curve.

Sensory descriptors of EVOO were assessed by a trained panel of 25 judges according to the difference-from-control method.[26] An EVOO sample from Carapelli was used as reference. A common sensory vocabulary was used, including the following four descriptors: green olive aroma, astringency, pungency and green olive flavour. Table 3 defines the individual descriptors. Judges were requested to evaluate the intensity of descriptors as compared with the reference by assigning a score between +5 (more intense) and −5 (less intense).

Data processing

The fractional factorial design was carried out by the Modde 8.0 software package (Umetri, Umeå, Sweden).

Principal component analysis (PCA) and partial least squares (PLS) regression were used to classify samples by the Unscrambler 7.8 software package (Camo AS, Oslo, Norway).

RESULTS AND DISCUSSION

The screened stability/instability composition indices were in agreement with literature data.[11,27–29] Relationships between oleic acid content and the other screened fatty acids were also verified experimentally. The following linear relationships were obtained:

$$\mathrm{PAc} = q - m\mathrm{OAc} \quad (q = 37.09, m = 0.34; r = 0.99)$$

$$\mathrm{LAc} = q - m\mathrm{OAc} \quad (q = 53.75, m = 0.61; r = 0.99)$$

$$\mathrm{SAc} = q + m\mathrm{OAc} \quad (q = 0.26, m = 0.034; r = 0.67)$$

where PAc, LAc, SAc and OAc are palmitic acid, linoleic acid, stearic acid and oleic acid contents (%) respectively.

Other composition indices such as carotenoid, chlorophyll pigment and phenolic compound contents, which were not measured by the company, may be considered in agreement with literature data.[8,30] In this work a methodological approach was applied to simplify the experimental design. As a first hypothesis, indices which are usually and easily measured by companies were studied; therefore other stability/instability indices were not taken into account.

A different approach was applied to select degradation parameters. A large number of parameters were considered in order to enable us to determine their significance in measuring EVOO degradation.

All experimental data (Table 4) were processed by multivariate analysis procedures. A multidimensional map of all samples related to stability/instability indices and degradation parameters was obtained by PCA. The relevant loading plot is shown in Fig 1. The model explained more than 60% of data variability along the first (PC1) and second (PC2) principal components.

As compared with well-known degradation parameters, ie peroxide value and K_{232}, stability/instability indices were positioned according to our hypothesis: the more acidity the more degradation, the more oleic acid content the less degradation, and the more bitter the taste the less degradation was observed.

Bitter taste was relatively close to sensory descriptors astringency and pungency, sum of aglycons and minor polar component content. This showed a tendency to exhibit reciprocal relationships between the above-mentioned parameters, thus confirming some literature data.[12,13,30] The cluster around peroxide value also aroused attention, in agreement with Rovellini *et al*.[23]

PLS modelling with latent variables (Fig 2) was then applied to evidence a significant relationship between stability/instability indices (ie independent variables) and degradation parameters (ie dependent variables).

Coefficient values (Q_2) showed that some indices had good prediction capability ($Q_2 > 0.60$). Our hypothesis was then statistically confirmed, and peroxide value, K_{232}, oxidised fatty acid content at 230 nm and dienoic and trienoic conjugated fatty acid contents were shown to be significant parameters to measure EVOO degradation. Conversely, in our opinion, the high prediction coefficient of the astringency parameter depended on the relationship between astringency and bitter taste (see Fig 1) rather than on the significance of astringency to measure EVOO degradation.[30,31]

Antioxidant component content, measured both indirectly (ie antioxidant activity) and directly (ie minor polar component content), was not found to be a significant degradation parameter. This may depend on the fact that antioxidant components played opposite roles in EVOO degradation. On the one hand, their content decreased as a result of oil oxidation and could be used to measure degradation; on the other hand, they slowed down degradation because of their antioxidant activity, resulting in higher oil stability.

PLS data processing also allowed us to set up mathematical models which were able to predict EVOO degradation as a function of the combination of acidity, oleic acid content and bitter taste. The models

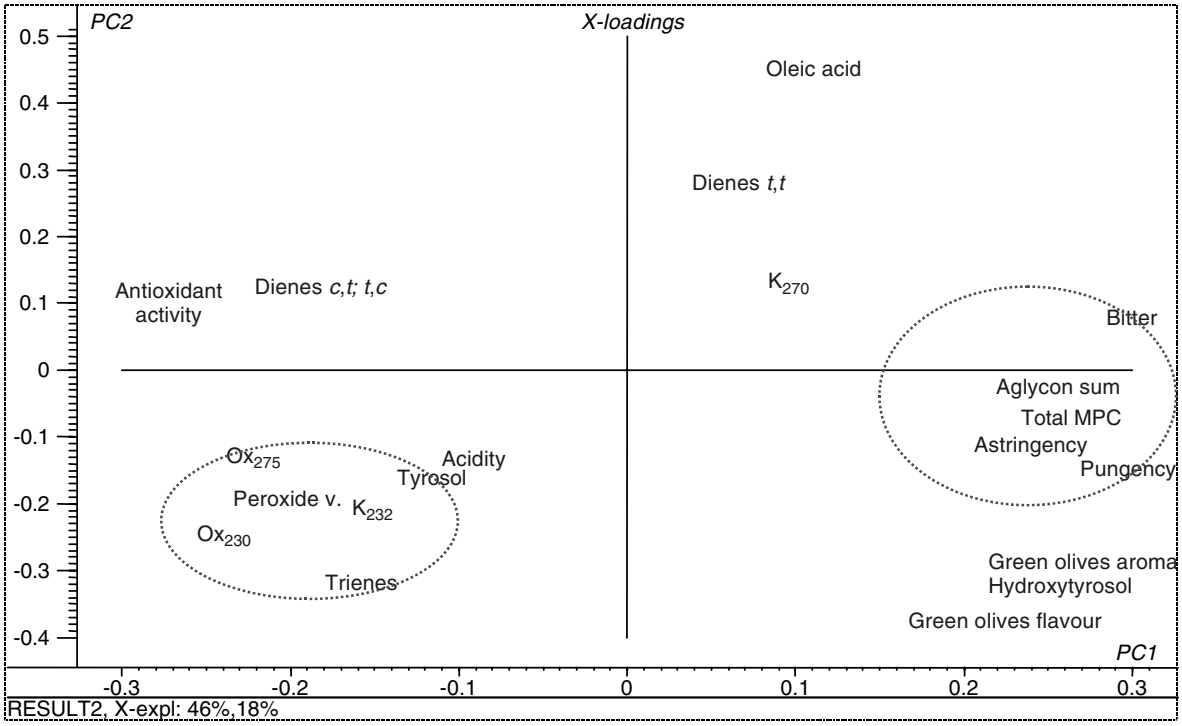

Figure 1. Sample loading plot by PCA method.

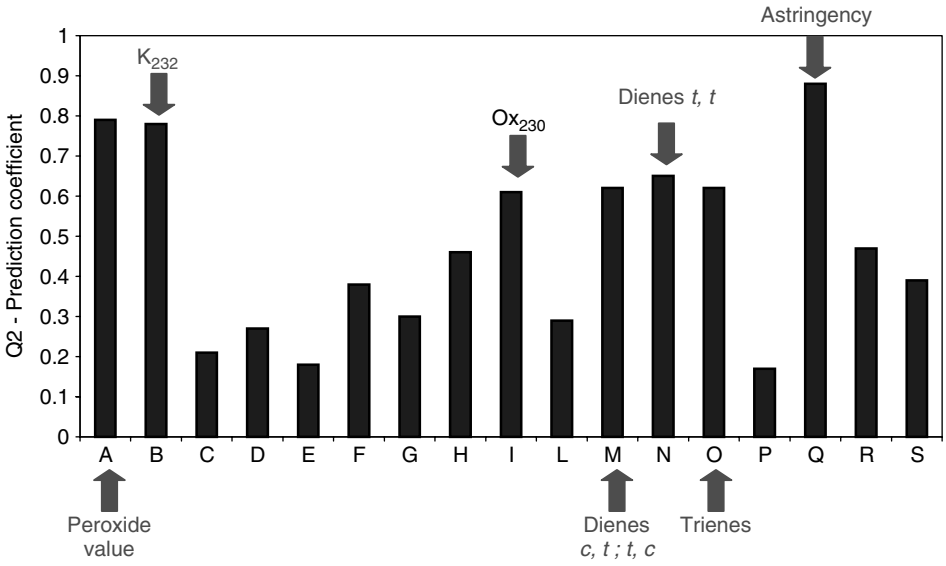

Figure 2. Goodness of fitting between variables by PLS method: A = peroxide value, B = K_{232}, C = K_{270}, D = antioxidant activity, E = hydroxytyrosol, F = tyrosol, G = aglycon sum, H = total MPC, I = Ox_{230}, L = Ox_{275}, M = dienes *cis–trans*, *trans–cis*, N = dienes *trans–trans*, O = trienes, P = green olive aroma, Q = astringency, R = pungency, S = green olive flavour.

were empirical polynomial models as follows:

$$Y = c + a_1X_1 + a_2X_2 + a_3X_3 + a_4X_1X_2 + a_5X_1X_3 + a_6X_2X_3 \quad (1)$$

where Y is the degradation parameter to be predicted, X_1 is the acidity, X_2 is the oleic acid content and X_3 is the bitter taste. Constant values for the selected degradation parameters are reported in Table 5.

Figure 3 shows an example of isoresponse curves for prediction of the peroxide value. They were obtained by varying acidity and oleic acid content while maintaining bitter taste constant. Therefore the curves represented regions of equal oil degradation, which were determined by different combinations of the three selected composition indices.

CONCLUSIONS

The experimental results allowed us to express the phenomenological model of extra virgin olive oil stability shown in Fig 4.

Oil stability may be a function of some indices which directly or indirectly reflect its chemical composition.

Table 4. Experimental data for degradation parameters of oil[a]

Sample number	Peroxide value ($meqO_2kg^{-1}$)	K_{232}	K_{270}	Antioxidant activity (I_{50}, mg)	Hydroxytyrosol ($mg\,kg^{-1}$)	Tyrosol ($mg\,kg^{-1}$)	Aglycon sum ($mg\,kg^{-1}$)	Total MPC	Ox_{230} (%)	Ox_{275} (%)	Dienes *c–t*, *t–c* (%)	Dienes *t–t* (%)	Trienes (%)	Green olive aroma	Astringency	Pungency	Green olive flavour
1	8.81	1.86	0.18	48.98	15.87	20.04	113.46	1 322 036	2.24	1.98	1.56	0.08	0.15	0.44	0.68	0.05	0.62
2	19.31	2.89	0.15	99.73	15.36	16.99	54.24	926 794	2.76	7.72	1.81	0.08	0.43	−0.13	0.19	−1.15	−0.37
3	13.02	2.19	0.23	102.94	5.62	18.05	37.20	727 869	2.00	4.60	1.82	0.10	0.14	−0.62	−0.09	−1.81	−1.24
4	12.65	2.18	0.13	115.90	6.64	17.71	30.76	713 694	2.24	4.54	2.26	0.11	0.20	−0.20	0.08	−2.07	−0.60
5	13.64	2.53	0.27	61.77	10.16	11.64	73.27	1 279 719	1.58	5.01	1.42	0.12	0.22	0.10	1.00	1.00	−0.35
6	15.23	2.61	0.25	80.82	9.94	14.58	66.00	1 201 702	2.12	4.95	1.69	0.09	0.28	−0.24	0.53	1.20	−0.45
7	8.22	1.94	0.19	29.28	16.81	12.56	193.69	1 692 181	1.00	2.21	1.46	0.10	0.13	0.11	0.94	1.69	−0.46
8	8.51	2.03	0.17	38.29	20.43	12.75	159.41	1 464 475	1.35	2.40	1.72	0.09	0.08	0.35	0.68	0.68	0.06
9	13.30	2.30	0.16	50.28	15.56	16.96	83.36	1 106 098	1.88	4.12	1.67	0.10	0.23	0.10	0.54	1.14	0.44
10	13.47	2.32	0.20	59.39	16.29	18.37	92.12	1 184 795	2.08	4.38	1.61	0.10	0.21	0.70	0.49	0.49	0.22
11	13.44	2.37	0.20	54.58	17.08	19.39	88.27	1 231 342	1.79	3.80	1.74	0.11	0.27	0.48	0.53	0.58	0.23

[a] Aglycon sum = sum of decarboxymethyloleuropein and oleuropein aglycon contents; total MPC = minor polar component content; Ox_{230} and Ox_{275} = oxidised fatty acid content at 230 and 275 nm respectively; dienes *c–t*, *t–c*, dienes *t–t* and trienes = dicnoic and trienoic conjugated fatty acid contents.

Figure 3. Isoresponse curves for peroxide value (bitter taste = 2).

Figure 4. Phenomenological model of extra virgin olive oil stability.

These indices, namely *stability/instability indices*, may cause sensitivity to degradation during oil shelf-life. Their value may result from both olive quality and process operating conditions. On the other hand, when the oil is extracted and filtered, their value may remain constant.

Based on our studies, the following indices were shown to be significant: acidity value was indirectly related to stability, while oleic acid content and bitter taste were directly related to stability.

Degradation of extra virgin olive oil can be monitored by measuring parameters, namely *degradation parameters*, which should be sensitive to small degrees of oil degradation. Their values and kinetic variations may depend on both stability/instability indices and operating conditions of oil packaging and commercial activities (ie time, exposure to light, exposure to oxygen, temperature).

Based on our studies, measurements of peroxide value, spectroscopic UV parameter K_{232} and lipid oxidation status (ie dienoic *trans–trans*, dienoic *cis–trans*, *trans–cis* and trienoic conjugated fatty acid contents, oxidised fatty acid content at 230 nm) were found to be significant.

The phenomenological model was applied to set up some predictive models of oil degradation extent. These models may also be useful for companies to

Table 5. Constant values for empirical polynomial models with respect to selected degradation parameters

Degradation parameter	c	a_1	a_2	a_3	a_4	a_5	a_6
Peroxide value ($meqO_2kg^{-1}$)	−77.921	115.143	1.148	28.910	−1.411	−3.679	−0.367
K_{232}	−6.364	10.911	0.106	2.981	−0.133	−0.328	−0.037
Ox_{230} (%)	−0.280	4.805	0.032	1.164	−0.054	−0.099	−0.020
Dienes *cis–trans*, *trans–cis* (%)	0.990	−0.510	0.006	0.054	0.023	−0.262	−0.002
Dienes *trans–trans* (%)	−0.051	−0.103	0.002	0.136	0.002	−0.040	−0.001
Trienes (%)	−1.562	2.960	0.020	0.563	−0.034	−0.183	−0.006

predict the rate of oil degradation, though in an indirect, semi-quantitative way. In order to have a stable oil, values for acidity, oleic acid content and bitter taste may be predicted which minimise degradation parameters. The above predictive models are naive because they depend on oil degradation history and hence are only able to record some of the situations of a company's operating reality. In clear terms, values for degradation parameters of extra virgin olive oil will be predicted by combined stability/instability indices. After storage, degradation parameters may change as a result of lipid oxidation, but models, being based on constant indices, are not able to predict a new degradation extent. However, by replicating the experimental design several times, models can be set up which better predict oil degradation extent, because they describe a company's common reality for purchase of virgin olive oil and blending operating conditions.

Research is currently being carried out to verify the application of our phenomenological model to directly predict the rate of oil degradation. In our opinion, under the same storage conditions the same combination of stability/instability indices may result in an equal constant rate of degradation parameter kinetics.

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