

# Characterization of Almond Cultivars by the Use of Thermal Analysis Techniques. Application to Cultivar Authenticity

A. Beltrán Sanahuja · N. Grané Teruel ·  
M. L. Martín Carratalá · M. C. Garrigós Selva

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**Abstract** Almonds are subjected to thermal processes in the production of processed food and this can affect their thermal stability and lead to oxidation processes. In this work, almond samples from three different cultivars (Spanish Guara and Marcona, and American Butte) were characterized by using Differential Scanning Calorimetry (DSC) and Thermogravimetric Analysis (TGA) at different heating rates. Crystallization and melting parameters were determined by DSC; whereas thermal stability was studied by TGA, showing no apparent degradation for all samples up to around 290 °C. Butte samples showed the lowest DSC values and TGA initial degradation temperature. These results were linked with differences in fatty acid profiles between Butte and Spanish almond cultivars, Butte presenting higher linoleic acid content. Successful discrimination was obtained for samples analyzed at 2 and 10 °C min<sup>-1</sup> heating rates for DSC and TGA, respectively, by applying multivariate stepwise linear discriminant analysis (LDA). The results obtained proved the suitability of thermal analysis techniques combined with LDA for an easy and fast discrimination among different almond cultivars to control eventual adulteration in food processing.

**Keywords** Almond oil · *Prunus dulcis* · Differential scanning calorimetry · Thermogravimetric analysis · Multivariate data analysis · Classification · Authenticity

## Introduction

Almonds are nuts with low water and high fat and protein contents with many health advantages obtained from their regular consumption. The protein content of the kernels represents 16–31% of the total composition [1]. In relation to the fat fraction (53–63% referring to the dry weight of the almond), the high content of monounsaturated and polyunsaturated fatty acids (mainly oleic acid) gives almonds protective qualities against heart disease [2]. Almonds are also characterized by their content of minor compounds such as tocopherols and phyosterols which are associated with antioxidant defense [3]. The carbohydrate fraction represents only 3–8% of the overall composition of the kernel but it is sufficient to provide a sweet taste. Finally, almonds are rich in insoluble fiber which is associated with a reduction in the development of diseases like diabetes [1].

Almonds are typically used as snack foods and as essential ingredients in a variety of processed foods, especially in bakery and confectionery products, some of them original from the Valencia Community (Spain) as nougats [4]. These products are traditionally manufactured by using toasted almonds, sugar and honey; and they are protected by the Regulation Council of the Protected Designations of Origin Jijona and Alicante Nougat; with requirements on almonds belonging to Marcona, Valenciana, Mollar or Planet cultivars, raised and harvested in the municipality of Jijona (Spain) [5]. Marcona cultivar is one of the most commonly used for its recognized organoleptic quality. However, it is an early flowering variety with production often damaged by frost. For these reasons, the California Butte cultivar is sometimes used as a substitute for the Marcona variety, due to its lower cost compared with Spanish cultivars. The use of this late flowering

A. Beltrán Sanahuja (✉) · N. Grané Teruel ·  
M. L. Martín Carratalá · M. C. Garrigós Selva  
Analytical Chemistry, Nutrition and Food Sciences Department,  
University of Alicante, 03080 Alicante, Spain  
e-mail: ana.beltran@ua.es

cultivar constitutes the main reason for fraudulent practices in nougat production.

Some studies have been reported on the characterization of different almond cultivars and criteria for almond quality evaluation, to avoid adulteration practices. Fatty acid profiles [2], triglycerides and tocopherol composition [3] as well as the volatile compounds profile [6] have been proposed as discriminant parameters to differentiate almond cultivars. However, these methods are quite tedious, raising the need for fast and reliable methods. Thermal analysis techniques give adequate tools for such an objective, since they are easy-to-use and quite sensitive in the calculation of thermal profiles in almonds.

Thermal properties have been evaluated on different nuts by using thermal analysis techniques, such as Differential Scanning Calorimetry (DSC) and Thermogravimetric Analysis (TGA) [7]. DSC was used for the characterization of different seed oils by obtaining their thermal profiles [8, 9]. The quality of oils was dependent on their composition and this may be influenced by agronomy factors, such as cultivar and industrial factors derived from the oil processing. These modifications can be determined from DSC parameters such as melting and crystallization temperatures and enthalpies [10]. It has been demonstrated that the thermal profile is strongly dependent on the temperature-scanning rate and the thermal history of samples [11, 12]. Oxidation kinetic profiles of different edible oils using DSC have also been reported [13, 14]. TGA has been also used to study food composition under different thermal treatments [15, 16] such as the effect of antioxidants on oxidative stability of edible fats and oils [17].

Only a few studies on the thermal behavior of almonds have been reported in the literature [18]. Regarding the thermal stability of almond oils, previous studies confirmed that they were stable up to around 285 °C, with further mass losses at higher temperatures. Three stages corresponding to the degradation of polyunsaturated, monounsaturated and saturated fatty acids were reported by Differential Thermogravimetric Analysis (DTGA) [16]. On the other hand, DSC applied to the study of almond oils showed the existence of two exothermic events, due to oxidation reactions and oil decomposition, respectively. However, there is no evidence in the literature for studies on the thermal stability of different almond cultivars to obtain discriminant parameters.

The aim of the present work was the characterization, thermal analysis, and further classification of three different almond cultivars (Guara, Marcona and Butte) based on the use of thermal techniques (DSC and TGA) in order to obtain discriminant parameters to differentiate between Spanish and American almond cultivars; getting new concepts for the development of fast and reliable methods to help detect adulteration practices in food products.

## Materials and Methods

### Almond Samples

Twenty-four samples of almond oils from three different almond cultivars were selected: eight Marcona (M), eight Guara (Gu) and eight Butte (Bu). Marcona is a Spanish cultivar of recognized organoleptic quality but low resistance to hard climatic conditions. The Spanish Guara cultivar shows important morphological similarities to Marcona and higher resistance leading to the replacement of this latter cultivar in the production of Spanish nougat. Finally, the Butte cultivar is obtained from USA and it is sometimes included as an ingredient in the nougat manufacturing since it is one of the most widely grown cultivars in the world [19], easier to obtain, more resistant, and cheaper than both Spanish cultivars.

The main differences between almond cultivars can be obtained from their fatty acid composition. In this sense, some differences in terms of chemical composition found in the literature may result in different decomposition profiles. In this way, the Butte cultivar shows low concentrations of oleic and stearic acids and high linoleic acid content, while the Guara cultivar shows high stearic acid and low palmitoleic acid contents [2]. Some differences have been also reported in terms of volatile compounds, since octanal and nonanal are oleic acid derivatives and consequently their contents are expected to be lower in samples from the Butte cultivar compared with the Spanish almond cultivars [6].

All samples were acquired unshelled from cultivars grown in the same crop year. Marcona and Guara cultivars were collected from different Spanish locations and samples were kindly supplied by “Colefruse S.A” (San Juan, Alicante, Spain). Butte samples were grown in California and were obtained from a Spanish importer (Almendras Llopis, San Vicente Del Raspeig, Alicante, Spain). Almond shells were immediately removed by using a hammer and the seeds obtained were then stored at  $7 \pm 1$  °C to keep them fresh until oil extraction.

### Almond Oil Extraction

Almond seeds were ground in an electric grinder just before oil extraction. Seed fragments were sieved to get a sample size lower than 1.5 mm and stored in a desiccator. The extraction was carried out by applying an analytical method previously developed and reported elsewhere [2]. Five grams of each sample was extracted by using a commercial fat extractor (Selecta, Barcelona, Spain) with 40 mL of petroleum ether (analytical grade; Panreac, Barcelona, Spain) for 90 min. The temperature of the heating module was set at  $135.0 \pm 0.1$  °C, i.e. roughly two

times the boiling point of the extraction solvent. However, the temperature of the extraction process was fixed at 60 °C because of the boiling point of the petroleum ether, avoiding the generation of oxidation products.

The oil obtained for analysis was a mixture of twelve independent fat extractions and it was dried under nitrogen and kept sealed in an amber vial at  $-21 \pm 1$  °C in a freezer until required for analysis.

#### DSC Conditions

Calorimetric tests were performed with a TA Instruments DSC Q2000 V23.12 Build 103 (New Castle, DE, USA). Nitrogen was used as an inert atmosphere at a flow rate of 50 mL min<sup>-1</sup>. The DSC instrument was calibrated using indium (melting point 156.6 °C; melting enthalpy 28.45 J/g), according to standard procedure described in the user's manual issued by the manufacturer.

Then, 6–7 mg of oil was weighed to the nearest 0.1 mg into hermetically sealable aluminum pans (40 µL) and placed in the sample chamber. An empty sealed pan was used as a reference. The following thermal program was used: (1) sample loading at 50 °C (5 min hold); (2) cooling to -80 °C (5 min hold); and (3) heating to 50 °C. Analyses were performed in duplicate obtaining a good reproducibility (Relative standard deviation between 1 and 5%). Due to the strong dependence of results on the scanning rate, calorimetric tests were performed at 2, 5 and 10 °C min<sup>-1</sup>. Details of methods and criteria for selecting the time–temperature program are reported elsewhere [8].

#### TGA Conditions

Dynamic TGA tests were carried out by using a TGA/SDTA 851 Mettler Toledo (Schwarzenbach, Switzerland) thermobalance. 6–7 mg of oil samples was placed in alumina pans (70 µL) and heated from 30 to 700 °C at 2, 5 and 10 °C min<sup>-1</sup> under a nitrogen atmosphere (50 mL min<sup>-1</sup>).

#### Statistical Analysis

DSC and TGA experimental data were processed with the aid of the “SPSS statistical package Version 15.0”. The presence of differences between groups of samples using a set of variables was investigated by using stepwise linear discriminant analysis (LDA). This is a supervised pattern recognition technique used not only to obtain different classes in a set of data but also to get classification functions (i.e. vectors) allowing one to predict the group a sample belongs to by using the appropriate latent variables [20]. These latent variables are linear combinations of the initial selected variables that maximize the resolution between groups. The main criteria used for variable

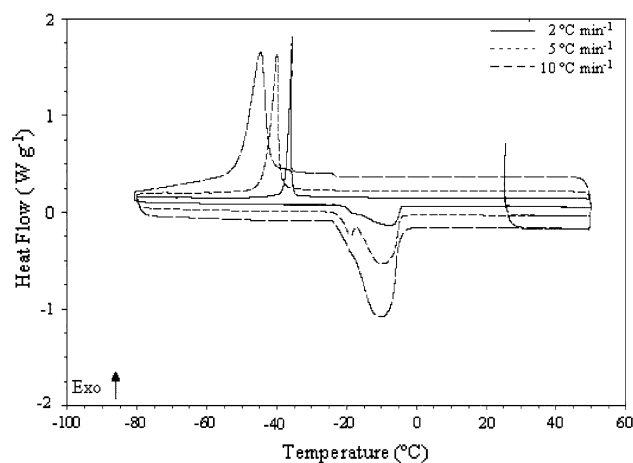
selection was to get the minimum Wilks' lambda ( $\lambda_w$ ). This parameter is calculated as the sum of the squares of the distances between points belonging to the same category divided by the total sum of their squares. In order to construct LDA vectors, the average values obtained for samples replicates were included, reducing the internal dispersion of categories. In order to validate the classification rule developed for this study, a test set was constructed on the basis of the leave-one-out algorithm. The validation of the methodology was carried out by leaving randomly 1/3 of our data “out”, using the remaining 2/3 to build an LDA model and testing the “stability” of our model with the 1/3 of the samples left out as unknowns.

## Results and Discussion

### DSC Analysis of Almond Oil Samples

The main difficulty in thermal characterization of vegetable oils comes from the great complexity of their thermal profiles, essentially due to the variation in their fatty acid composition as principal constituents of these samples. Therefore, it has been proposed that some edible oils do not show specific crystallization and melting temperatures since they crystallize and melt over a wide temperature range [12].

DSC curves obtained for Guara Pinoso almond oil at different cooling and heating rates are shown in Fig. 1. As expected, the DSC profile of this oil showed an exothermic peak at temperatures between -50 and -30 °C (depending on the program rate) corresponding to the oil crystallization and an endothermic peak at around -10 °C related to the melting of the previously formed crystals.



**Fig. 1** DSC curves obtained at different program rates (2, 5 and 10 °C min<sup>-1</sup>) for Guara Pinoso oil sample in nitrogen atmosphere (50 mL min<sup>-1</sup>)

It was also observed that as the cooling rate increased, the crystallization temperature shifted to lower values. This result was expected since high cooling rates lead to crystallization of a certain amount of the fat in a less stable form at a lower temperature [11]. The use of low cooling rates gave more time to allow interactions between triacylglycerols, leading to complete crystallization at narrow temperature ranges. For the purpose of this study, the determination of crystallization temperatures at different cooling rates can be useful since there is a close link between triacylglycerols composition and crystallization temperatures [21]. On the other hand, as the heating rate increased, some displacement of peaks to higher temperatures took place, but it was not significant for this study.

Crystallization and melting parameters (temperatures and enthalpies) were calculated from DSC curves for all almond oils (Table 1). It was concluded that values obtained for the repeatability of the process were dependent on the selected parameter and on the cooling/heating rate, with relative standard deviations between 1 and 5% in all cases.

The well-known effect of the poor thermal conductivity of oils was also observed in DSC results since the heating rate also influenced the sharpness of the endothermic peaks [22]. In this sense, the endothermic melting peaks were more pronounced in curves with high heating rates, as reported elsewhere [12].

A comparison of DSC results obtained for oil samples belonging to the three different almond cultivars was carried out (Table 1). Significant differences at the 5% level were observed, in particular when tests were carried out at  $2\text{ }^{\circ}\text{C min}^{-1}$  regarding to melting and crystallization parameters. It is important to note that Butte samples showed lower values for all parameters, i.e. crystallization temperature ( $T_c$ ), crystallization enthalpy ( $\Delta H_c$ ), melting temperature ( $T_m$ ) and melting enthalpy ( $\Delta H_m$ ), at all testing rates. These results can be attributed to the different fatty acid profiles between Butte and the Spanish almond cultivars. In this sense, it was observed that the Butte cultivar

shows a higher linoleic acid content [2] making it more prone to lipid oxidation. This fact clearly influences the thermal behavior of this American cultivar due to its high content in polyunsaturated fatty acids which resulted in lower decomposition enthalpies. On the other hand, all Spanish almonds showed similar DSC patterns and no conclusions for tendencies in their thermal parameters could be obtained.

A deeper study for classification of almond cultivars from DSC results can be carried out by using a stepwise LDA with the Wilk's lambda statistics for variable selection. In this case, the application of this statistical analysis led to two discriminant functions where 100% of the total variance was retained. The melting temperature was the only variable not included in the analysis since differences in results for different oil samples were not high enough to give clear results. Figure 2 shows the mean scores obtained for DSC results at  $2\text{ }^{\circ}\text{C min}^{-1}$  projected on the reduced space of the two discriminant functions. Similar results were obtained for each testing rate in all DSC runs. In conclusion, samples were correctly classified in 100% of the cases by using the calculated discriminant functions obtained from DSC results.

#### TGA Analysis of Almond Oil Samples

The thermal stability behaviour of all almond oils used in this work was studied by TGA. Figure 3 shows an example of TGA curves obtained for Butte oil at  $10\text{ }^{\circ}\text{C min}^{-1}$ . In this case, the almond oil was stable up to a temperature of  $289\text{ }^{\circ}\text{C}$  where the mass loss reached 5%.

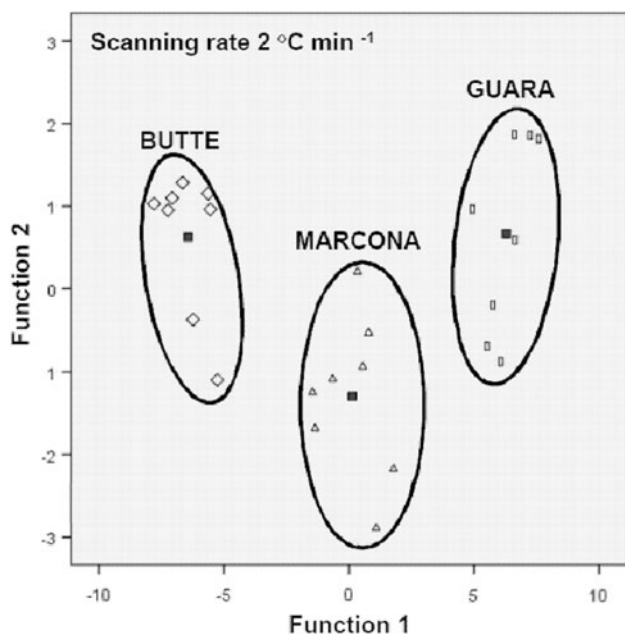
The relatively high stability of almond oil when compared to other vegetable oils [23] could be attributed to the high amount of saturated fatty acids in its composition (ranging from 7 to 9%) [2] and to the presence of  $\alpha$ -tocopherol as a major component [3]. It is well-known that  $\alpha$ -tocopherol is an efficient antioxidant and can be used as an alternative to thermal stabilizers in polymer formulations [24]. Therefore, the relatively high concentration of

**Table 1** Crystallization and melting parameters obtained from different almond oils at three program rates (2, 5 and  $10\text{ }^{\circ}\text{C min}^{-1}$ )

$T_c$  ( $^{\circ}\text{C}$ ), crystallization temperature;  $\Delta H_c$  ( $\text{J g}^{-1}$ ) crystallization enthalpy;  $T_m$  ( $^{\circ}\text{C}$ ), melting temperature;  $\Delta H_m$  ( $\text{J g}^{-1}$ ), melting enthalpy

<sup>a</sup> Mean value ( $n = 8$ )

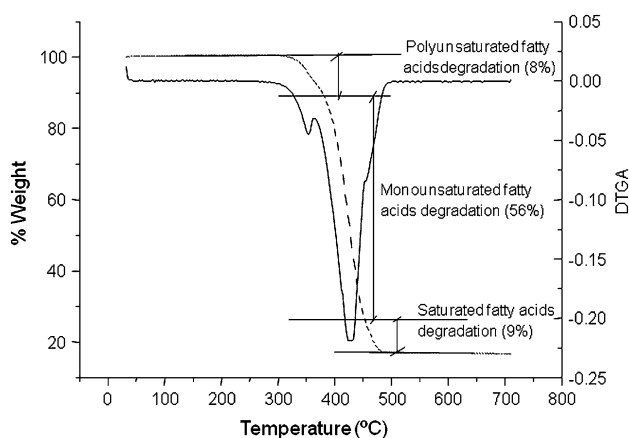
Almond cultivar	Analysis rate ( $^{\circ}\text{C min}^{-1}$ )	DSC parameters <sup>a</sup>			
		$T_c$ ( $^{\circ}\text{C}$ )	$\Delta H_c$ ( $\text{J/g}$ )	$T_m$ ( $^{\circ}\text{C}$ )	$\Delta H_m$ ( $\text{J/g}$ )
Marcona	10	-46.9	57.1	-11.6	65.5
Butte	10	-50.7	52.6	-12.6	61.2
Guara	10	-45.1	56.7	-10.6	64.6
Marcona	5	-42.3	58.0	-10.9	65.3
Butte	5	-45.7	52.1	-12.6	59.5
Guara	5	-40.7	56.5	-9.9	63.7
Marcona	2	-37.7	59.8	-8.9	59.0
Butte	2	-40.8	58.9	-11.5	58.3
Guara	2	-35.9	64.0	-7.8	62.3



**Fig. 2** DSC mean values for the two discriminant functions obtained from the analysis of oil samples

this compound in almond oil would lead to some thermal stabilization and finally to an increase in the initial decomposition temperature ( $T_i$ ) in these samples.

The TGA curve shown in Fig. 3 is a clear example of the thermal behavior obtained for all almond oils. A typical profile of sample weight loss with temperature occurring at different stages was observed. The first step, starting at 289 °C and reaching its maximum degradation rate at 352 °C, represents the initial phase of triglycerides degradation where the oxidation of the polyunsaturated fatty acids takes place. The second and third stages, i.e. the main degradation with a maximum rate at around 400 °C and a



**Fig. 3** Thermogravimetric profile (dashed line) and first derivative curve (continuous line) of Butte-1 almond oil at 10 °C min<sup>-1</sup> in a nitrogen atmosphere (50 mL min<sup>-1</sup>)

small shoulder observed in the DTGA curve at around 470 °C, represent the decomposition of monounsaturated and saturated fatty acids, respectively.

When comparing the TGA results obtained after the analysis of the three almond cultivars (Table 2) it is important to note that Butte samples showed lower values for  $T_i$  (about 289 °C) than those obtained for the Spanish varieties (around 296 °C). This result could be attributed to their above-indicated higher amounts of linoleic acid in its composition. In this regard, the Butte cultivar showed slightly lower thermal stability while Guara and Marcona samples were more resistant to high processing temperatures.

Regarding to the main degradation peak related to the decomposition of monounsaturated fatty acids, the Guara cultivar showed higher values than those obtained for the Butte and Marcona samples which were very similar. Finally, the weight loss resulting from the degradation of saturated fatty acids was clearly higher in Marcona samples supporting the idea of the higher saturated fatty acid content in this cultivar. As in the case of DSC results, the TGA values obtained for the repeatability of the process depended on the selected parameter and the cooling/heating rate, being between 1 and 5% (relative standard deviation) in all cases.

Significant differences at the 5% level were observed in TGA results for almond oil samples from different cultivars, this being more important when the analyses were carried out at the highest heating rate (10 °C min<sup>-1</sup>). In this case, linear statistical analysis led to two discriminant functions. The percentage of polyunsaturated, monounsaturated and saturated fatty acids obtained from the relative weight loss in TGA runs and  $T_i$  for the polyunsaturated fatty acids peak were included in this analysis. Figure 4 shows the mean scores for all almond cultivars, projected

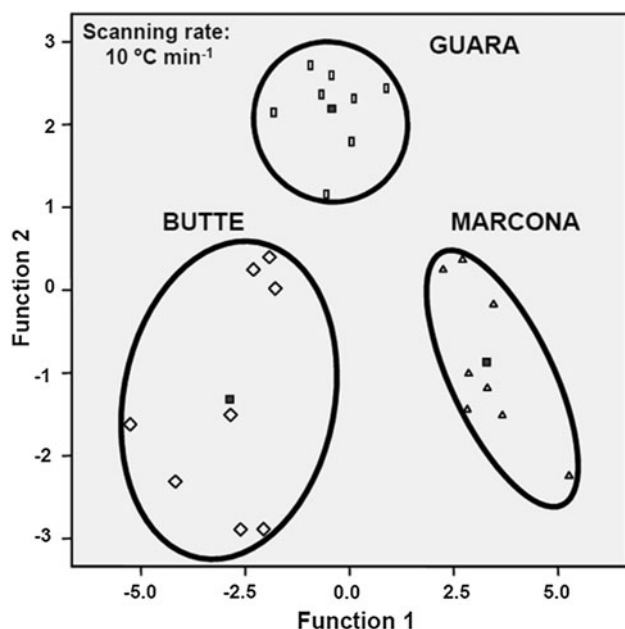
**Table 2** Thermal parameters obtained from different almond oils at 10 °C min<sup>-1</sup>

TGA parameters	Almond cultivar		
	Butte	Marcona	Guara
Weight loss (%) PFA	6.3	8.7	8.3
Weight loss (%) MFA	70.6	68.5	84.0
Weight loss (%) SFA	8.0	20.1	5.8
$T_i$ (°C) PFA degradation	289	297	296
$T_f$ (°C) PFA degradation	372	360	367
$T_i$ (°C) MFA degradation	372	360	367
$T_f$ (°C) MFA degradation	458	439	461
$T_i$ (°C) SFA degradation	458	439	461
$T_f$ (°C) SFA degradation	503	503	504

Mean value ( $n = 8$ )

PFA polyunsaturated fatty acids, MFA monounsaturated fatty acids, SFA saturated fatty acids,  $T_i$  initial temperature,  $T_f$  final temperature





**Fig. 4** TGA mean values for the two discriminant functions obtained from the analysis of oil samples

on the reduced space of the two discriminant functions. 100% of the total variance was retained and the samples were correctly classified in 91.7% of the cases.

## Conclusions

DSC and TGA have proved to be valuable and reliable techniques for the characterization of different almond oils. Both thermal techniques have shown their inherent advantages, including small sample size, minimal sample preparation, short experimental times and no use of chemical reagents.

Significant differences were observed in crystallization and melting parameters for oil samples extracted from different almond cultivars, in particular for DSC tests carried out at low rates ( $2^{\circ}\text{C min}^{-1}$ ). Thermal degradation profiles were obtained, with higher differences in TGA curves among almond cultivars observed at a  $10^{\circ}\text{C min}^{-1}$  heating rate. The percentage of polyunsaturated, monounsaturated and saturated fatty acids and initial degradation temperature of polyunsaturated fatty acids can be considered as the main parameters for TGA characterization of these samples.

Finally, the use of multivariate LDA applied to DSC and TGA results has been successful for the classification and discrimination of different almond cultivars. These results constitute a useful basis for the control of adulteration in food processing, particularly for the Protected Designation of Origin products.

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