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Analysis of the Lipid Component and the Sterol Ester Fraction for the Detection of Soft Wheat in Durum Wheat Flour and Pasta

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Abstract: Food adulteration consists of changing the original structure of a food, and so, by its definition, it is a process not admitted by law. Adulterations can not only have commercial consequences, but also hygienic and nutritional ones, and in some cases, they can cause a serious danger to public health. Therefore, it is of great interest to understand and identify the modifications that alter the original chemical composition of a food item (nutrition label). Among the food processing sectors, the pasta food chain is a fascinating production process, which finds its roots in a mixture of a few, simple ingredients; in particular, in its basic formulation to produce pasta, exclusively durum wheat mixed with water is used, while soft wheat and therefore the flour obtained from it are destined for bakeries and the confectionery industry. In this work, a procedure was developed that allows the detection of the possible presence of soft wheat in durum wheat-based products, both in the flour and after the pasta-making process. It is to point out that this adulteration is only commercial fraud and there are no consequences for people's health. In detail, the method was based on the isolation of the lipid fraction of flours and pastas and using the gas chromatographic analysis of the sterol ester fraction, which were not altered during the pasta-making phase, because they have very high boiling points. Based on the evaluation of some specific ratios between sterol esters, it was possible to trace the percentage of soft wheat present in a mixture with durum wheat, both in flour products and pastas.

Keywords: soft wheat; durum wheat; adulteration; sterol esters; flour products; pasta; gas chromatography; lipids

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1. Introduction

Wheat is a grass of the Triticum genus, of which numerous species are known [1]. The most common and important ones from a commodity point of view are Triticum aestivum or vulgare, commonly called soft wheat, and Triticum durum or durum wheat. Soft wheat lends itself to being cultivated at very different latitudes and climates, hot, temperate and cold, and by grinding it, soft wheat flour or simply flour is obtained, intended mainly for the production of bread and desserts [2]. Instead, durum wheat can only be grown in areas with a warm climate and, due to this limitation, its cost is higher than that of soft wheat [3]. From the milling of durum wheat, durum wheat flour is obtained, commonly known as semolina, which is particularly suitable to produce pasta [4]. Consequently, semolina is the granular product obtained from the grinding and subsequent sifting of

durum wheat. A recent review by Cecchini et al. provides an overview of the strengths and weaknesses of the rheological tests currently used to evaluate the quality of durum wheat semolina [5]. Sifting is the process of gradually separating ground wheat to obtain flours of different fineness. Instead, the product obtained from the grinding and subsequent sifting of durum wheat, after the extraction of the semolina, is called durum wheat granulated flour. Therefore, the granulated flour simply undergoes a different decortication process, which results in a higher ash content. This makes it less valuable than semolina and less brilliant. Whenever we talk about durum wheat semolina pasta, we instinctively associate the image of this product with Italy, where pasta is still prepared according to the traditional recipe, which involves the exclusive use of semolina [6]. On the other hand, Italy is the first producer of pasta in the world and in addition to being a leader in production, it is also the country where it is consumed in the greatest quantity (about 23 kg *pro capita* per year) [7–10]. However, pasta is a very widespread food product, even outside Italian borders. Further, 25% of the pasta consumed in the world and 75% consumed in Europe are produced by Italian pasta factories. More than half of the pasta produced in Italy are exported abroad; Germany, the United Kingdom, France, the United States and Japan are confirmed by themselves as the most receptive countries, purchasing a total of 58% of Italian pasta exports [11]. Compared to its basic formulation, despite being composed of only two ingredients (semolina and water), pasta owes its particular characteristics to two large variables, on which it is possible to act to modulate its properties. One of these two variables is the production process, which involves the transformation of the mixture of ingredients, appropriately dosed, into the finished product, through the kneading, shaping and drying phases (dry pasta). The other variable is the formulation; in fact, the basic formulation of pasta can be modified with the addition of other ingredients, to confer particular characteristics (special features), such as enrichment of nutrients to increase its nutritional value (enriched pastas) [12]. On the other hand, in recent years, pasta has evolved, consumer awareness of it has increased and it has been reinterpreted and adapted to new lifestyles. Regarding the horizons for the next few years, pasta will experience new types with alternative flours or ingredients, will be preserved in more ecological and biodegradable packaging and will see the addition of many new formats.

Based on all these characteristics, the pasta food chain is a fascinating production process, which finds its roots in a simple mixture of flour and water, durum wheat semolina and water. The pasta production process can be summarized as follows: the wheat is sieved, transformed into a dough, which is then drawn depending on the type of processing (dry or fresh) or shape, and subsequently dried. In particular, the raw materials used to produce semolina pasta are solely semolina and water. Their rigorous, constant and continuous dosage is an indispensable prerequisite to produce high quality pasta. Therefore, in order to protect the consumers, followed by that of safeguarding competition between operators in the food sector, the identification and repression of food fraud is necessary. The term food fraud indicates the production and distribution of food that does not comply with current regulations. The advantage of this scam for companies is to modify the product in order to lower production costs and consequently increase their profit (commercial or economic fraud). Food frauds are divided into two types: those of a health nature, which can affect people's health, and those of a commercial nature, which damage the contractual rights of another operator or the consumer and have no consequences on people's heath [13]. Recently, several instrumental analytical methods have been proposed, which, in combination with different chemometric approaches, can be used for the traceability and authenticity of different food products, including pasta derived from durum wheat [14–16].

Starting from these premises, the objective of the present work is to develop a rapid and reliable analytical method to detect the presence of soft wheat mixed with durum wheat, both in flours as such and in the pasta obtained from them after the production process [17]. This further potentiality introduced by this methodology consists of the analysis and detection of the fraud not only in the mixing of the soft wheat flour with semolina, but also in pasta (finite product). Instead, the previously used protein-based

method correctly worked only on the mixed flour with semolina and not on pasta and other bakery products, whose proteins are temperature sensitive [18]. Furthermore, it was proposed to evaluate whether this method could also be applied to trace the quantity of soft wheat present in samples of various dried pastas purchased in the market. On the other hand, conventional methods based on the analysis of protein components do not allow the recognition of soft wheat due to the denaturation of these compounds following the heat treatments to which pasta is subjected; this method can be only applied to mixtures of soft wheat in semolina flour. Therefore, in this analytical procedure, attention was paid to the analysis of the lipid component (triglycerides, sterols and sterol esters) using high resolution gas chromatography (HRGC) of flour products, which is notoriously stable at high temperatures, because it is made up of non-thermolabile substances. Furthermore, the identification of the sterol ester fraction was carried out after transesterification of the non-polar lipid fraction of the flour products using gas chromatography–flame ionization detector (GC-FID) and gas chromatography–mass spectrometry (GC-MS).

2. Materials and Methods

2.1. Chemicals, Reagents, Products and Instrumentation

All solvents and reagents were of analytical grade. Ethyl ether, n-hexane, anhydrous methyl alcohol and dichloromethane were purchased from Merck (Darmstadt, Germany); anhydrous sodium sulfate, sodium hydroxide and hydrochloric acid were purchased from Carlo Erba (Milan, Italy) and Sigma-Aldrich Co. (Buchs, Switzerland); cylinder nitrogen, helium, air and hydrogen were chromatographic grade and were purchased from Sol S.p.A. (Marcianise, Caserta, Italy). All pasta samples used in this study were purchased from a local market.

2.2. HRGC Analysis of Lipids Extracted from Flour Products as Is

The gas chromatograph was equipped with a programmable injector (Programmable Temperature Vaporization, PTV) and flame ionization detector (FID) (Autosystem XL Perkin Elmer, Norwalk, CT, USA), equipped with a fused silica capillary column l = 30 m, i.d. 0.25 mm, f.t. 0.25 μm with 65% phenyl 35% methylsilicone stationary phase, 65-TG-HT (Restek Corporation, Bellefonte, PA, USA). Gas chromatographic conditions: carrier gas: hydrogen, column flow rate of 2 mL/min; auxiliary gases: hydrogen and chromatographic air; detector temperature: 370 °C; injector programmed (PTV): 70 °C for 0.1 min, increase at 999 °C/min up to 370 °C and hold for 5 min; chamber programmed: 250 °C for 2 min, increase rate of 5 °C/min up to 360 °C and hold for 5 min. A total of 1 μL of the sample was injected and the injection mode was split 1:100.

2.3. HRGC Analysis of Flour Products after Transesterification

The gas chromatograph was equipped with a programmable injector (Programmable Temperature Vaporization, PTV) and flame ionization detector (FID) (DANI mod. 86.10 HT, Milan, Italy). The fused silica capillary column l = 60 m, i.d. 0.25 mm, f.t. 0.25 μm with 90% bis-cyanopropyl-10%-phenylsilicone stationary phase (Restek Corp., Bellefonte, PA, USA). The gas chromatographic conditions were: carrier gas: helium, column flow rate of 1.5 mL/min; auxiliary gases: hydrogen and chromatographic air; detector temperature: 250 °C; injector programmed: 60 °C for 0.1 min, increase at 999 °C/min up to 240 °C and hold for 3 min; chamber programmed: 150 °C for 2 min, increase at 8 °C/min up to 240 °C and held for 10 min. A total of 1 μL of the sample was injected and the injection mode was split 1:100.

2.4. GC-MS Analysis

The GC-17A gas chromatograph was equipped with a split-splitless injector and Shimadzu QP-5000 mass spectrometer as the detector connected with Class 5000 acquisition interface (Shimadzu Corp., Kyoto, Japan). Fused silica capillary column l = 30 m, i.d. 0.25 mm, f.t. 0.10 μm with 65% phenyl methylsilicone stationary phase (Restek Corp.,

Bellefonte, PA, USA). The gas chromatographic conditions were the following: carrier gas: helium, column flow rate of 2 mL/min; ionization energy: 70 eV; acquisition: 50–500 a.m.u.; injector temperature: 350 °C; chamber programmed: 250 °C for 2 min, increase at 5 °C/min up to 360 °C and hold for 5 min. A total of 1 µL of the sample was injected and the injection mode was split 1:100.

2.5. Extraction by Percolation of Fat from Flour Products

In total, 100 g of finely ground matrix was extracted with 100 mL of diethyl ether by percolation onto a cylindrical column, and then, the eluted enriched diethyl ether was passed onto an anhydrous sodium sulfate column to eliminate water traces. Then, the eluate was dried using a rotary evaporator at a temperature of 50 °C while the residual traces of diethyl ether were removed under a nitrogen flow. The lipid fraction obtained was kept at 4 °C in the freezer until the analyses. A sketch of the process is shown in Figure 1.

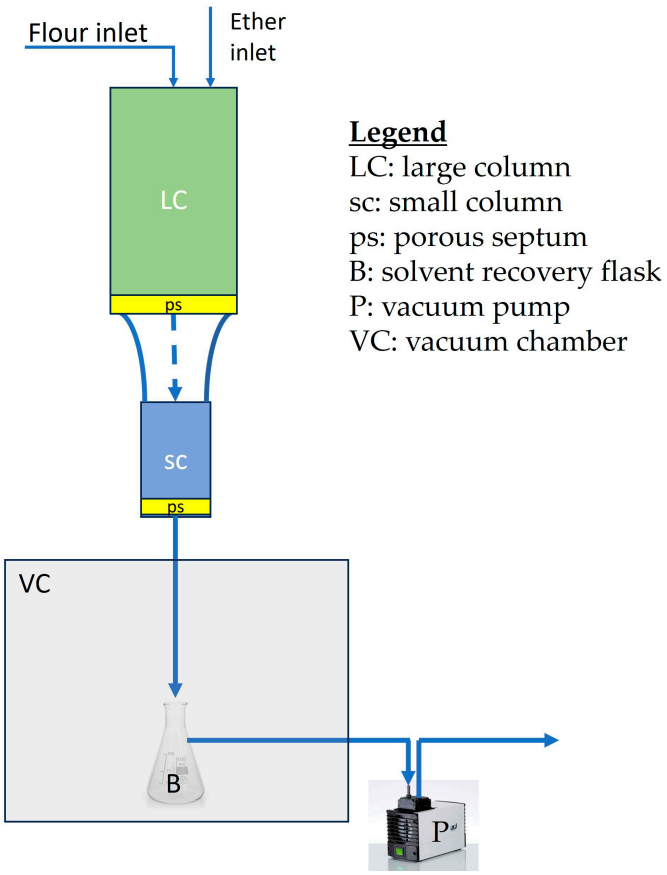

Figure 1. System for the rapid extraction of fat from flours.

2.6. Transesterification of the Extracted Fat

To 50 mg of the fat extracted by percolation, 1 mL of n-hexane was added. The sample was mixed vigorously on a mechanical stirrer until the lipids were completely dissolved. To prepare the transesterifying reagent (2M hydroxide potassium in methanol solution), 8 g of sodium hydroxide was weighed and then transferred in 100 mL of anhydrous methanol in a polyethylene container. The reagent solution was stirred until sodium hydroxide (2N methanolic soda) was completely dissolved. The 5% (*w*/*v*) fat solution in n-hexane was added with 400 µL of the transesterifying reagent, vortexed for 30 s and neutralized with the addition of 400 µL of 2N hydrochloric acid. The sample was centrifuged for 30 s at 4000 rpm and 1 mL of the supernatant organic phase was injected into GC.

2.7. Extraction of the Non-Polar Fraction of the Fat from Flour Products

To determine the composition of the non-polar fraction of the fat extracted from flour products, 50 mg of fat was weighed, to which 500 µL of dichloromethane was added. The sample was vortexed until the fat was dissolved and transferred onto an SPE column filled with SiOH (Silica gel) phase previously ambientized with two volumes of dichloromethane. The non-polar fraction of the fat was eluted with 30 mL of dichloromethane and the eluate was dried using a rotary evaporator; the residual traces of the solvent were eliminated under a flow of nitrogen. A 5% (*w*/*v*) n-hexane solution was prepared from the non-polar fraction obtained. The non-polar fraction recovered from the fat extracted from the flours was subjected to HRCG analysis, according to the conditions reported in the Materials and Methods section. In a similar way, gas chromatographic analysis was carried out on the non-polar fraction previously transesterified with 2N methanolic soda. Furthermore, to analyze the chemical composition of the sterol ester fraction, a gas chromatography analysis coupled with mass spectrometry (GC-MS) was carried out on the fat of the flour products after transesterification. In this way, GC-MS analysis provided the detailed molecular profiles of the organic compounds, which are identified by comparison with standard compounds or mass spectra libraries.

3. Results

3.1. Principle of the Method

The fat found in the finely ground flour or pasta was extracted by percolation on a column packed with an appropriate quantity of diethyl ether. The collected eluate was dried using a rotary evaporator and subsequently under nitrogen flow. A 5% hexane solution of the recovered fat was prepared, which, after transesterification with 2N sodium hydroxide in methanol, was analyzed using HRGC with a 65% phenyl methylsilicone polar column. The presence of soft wheat flour is highlighted by the examination of a particular configuration of four doublets of chromatographic peaks. Finally, from the comparison of the ratios between the most significant doublets and from the construction of an appropriate calibration line, it was possible to trace the quantity of soft wheat present in the mixture with the durum wheat.

3.2. Evaluation of the Rapid Extraction Method Used as an Alternative to Conventional Methods

The extraction of fats from soft wheat and semolina flours was carried out using a rapid procedure, which consisted of percolating the extracting solvent diethyl ether under pressure (under vacuum) over them [19]. The official extraction methods for soft wheat and durum wheat flours involve "batch" extraction or extraction using Soxhlet [20,21]. The first method is based on the contact of the solid matrix with the liquid for a minimum time of at least 24 h, while the second, which requires at least six hours, involves the extraction of the fat with a reflux solvent using the Soxhlet apparatus. Both methods are analytically valid but require long extraction times, and so, they are not suitable for the analyses of many samples. Therefore, in this work, the possibility of accelerating extraction times by introducing the percolation system of the extracting solvent was evaluated. In this case, the use of diethyl ether allowed the extraction of free lipids (non-polar lipids) from the solid matrix. The extraction process is based on the fact that as the liquid percolates on the packed column, the fat is removed from the flour and transported with the solvent front, since the stationary phase does not exert any interaction towards these compounds and the liquid that passes over the already extracted layers and helps to make the extraction of free lipids exhaustive.

First, the completeness of extraction was evaluated on the samples of soft wheat flour, semolina and finely ground pasta depending on the volume of diethyl ether used. Table 1 shows the results relating to fat recovery. As can be seen, the minimum volume of diethyl ether required to exhaust 100 g of soft wheat flour is 100 mL; in fact, the difference in recovery compared to that obtained using 120 mL is negligible. The same recovery trend was also found for semolina and pasta.

Table 1. Percentage recovery of free fat from 100 g of soft wheat flour, semolina and finely ground pasta. These experiments were performed at room temperature.

Diethyl Ether Volume, mL	Soft Wheat Flour, %	Semolina, %	Pasta, %
30.0 ± 0.2	18.7 ± 0.3	13.6 ± 0.2	11.5 ± 0.3
50.0 ± 0.5	35.8 ± 0.5	31.4 ± 0.4	30.7 ± 0.5
70.0 ± 0.7	68.9 ± 0.8	64.8 ± 0.5	62.6 ± 0.6
100 ± 0.3	99.4 ± 0.9	99.0 ± 0.7	99.2 ± 0.9
120 ± 0.3	99.9 ± 0.8	99.8 ± 0.9	99.7 ± 0.9

Table 2 shows the percentage comparison of fat extracted with the three methods used: batch, Soxhlet and the proposed method (hereinafter referred to as the rapid fat method). The reported results highlight that the three procedures lead to the same quantity of fat extracted for the three matrices, within the limits of experimental errors (the values, expressed in percent w/w, reported in Table 2 are the results of the average of three determinations). Therefore, the rapid fat method represents a valid alternative to traditional extraction methods, especially to minimize the extraction time.

Table 2. Amount of fat in percentage obtained with the 3 methods. The values, expressed in percent w/w, are the results of the average of three determinations. These experiments were performed at room temperature.

Type of Method	Soft Wheat Flour, %	Semolina, %	Pasta, %
Rapid method	1.04 ± 0.02	0.69 ± 0.03	0.15 ± 0.02
Soxhlet	1.08 ± 0.03	0.70 ± 0.02	0.16 ± 0.01
Batch	1.06 ± 0.02	0.73 ± 0.01	0.17 ± 0.02

Finally, the fats obtained from the partial extractions and those obtained from the total extraction for each matrix were qualitatively analyzed using GC. Figure 2 shows the gas chromatograms of flour fat recovered with partial extraction (18.7% recovery) versus exhaustive extraction. As can be observed, the chromatographic profiles are equivalent regarding the relative distribution of the peaks. This shows that, even if the free fat of the flours is not quantitatively extracted, the relative distribution of the lipid components (free hydrocarbons and fatty acids, free sterols, triglycerides, sterol esters and waxes) remains constant. Similar results were obtained by comparing the fats coming from partial and total extractions of semolina and pasta. This result highlights that the quantification of mixing of soft wheat in durum wheat is not affected by the partial extraction of lipidic fraction. Moreover, there is no other dependencies due to temperature variation, that is quantified in terms of 10 °C range with respect to room temperature (from +15 °C to +25 °C). However, these experiments were conducted at room temperature.

3.3. Effect of Temperature on the Stability of the Lipid Component

To evaluate the effect of temperature on the lipid component of flours, the fats extracted from 10 common soft wheat flours and 10 commercial semolina flours were kept at room temperature and then they were analyzed. The same flours were subjected to a temperature of 130 °C for 60 min and the extracted fat was analyzed using gas chromatographic analysis. The comparison of the percentage distribution of the glyceride families and sterol esters in the two cases does not show appreciable variations. This result is notable because the proposed method can be applied not only to a mix of flours, but also to pastas after the production process.

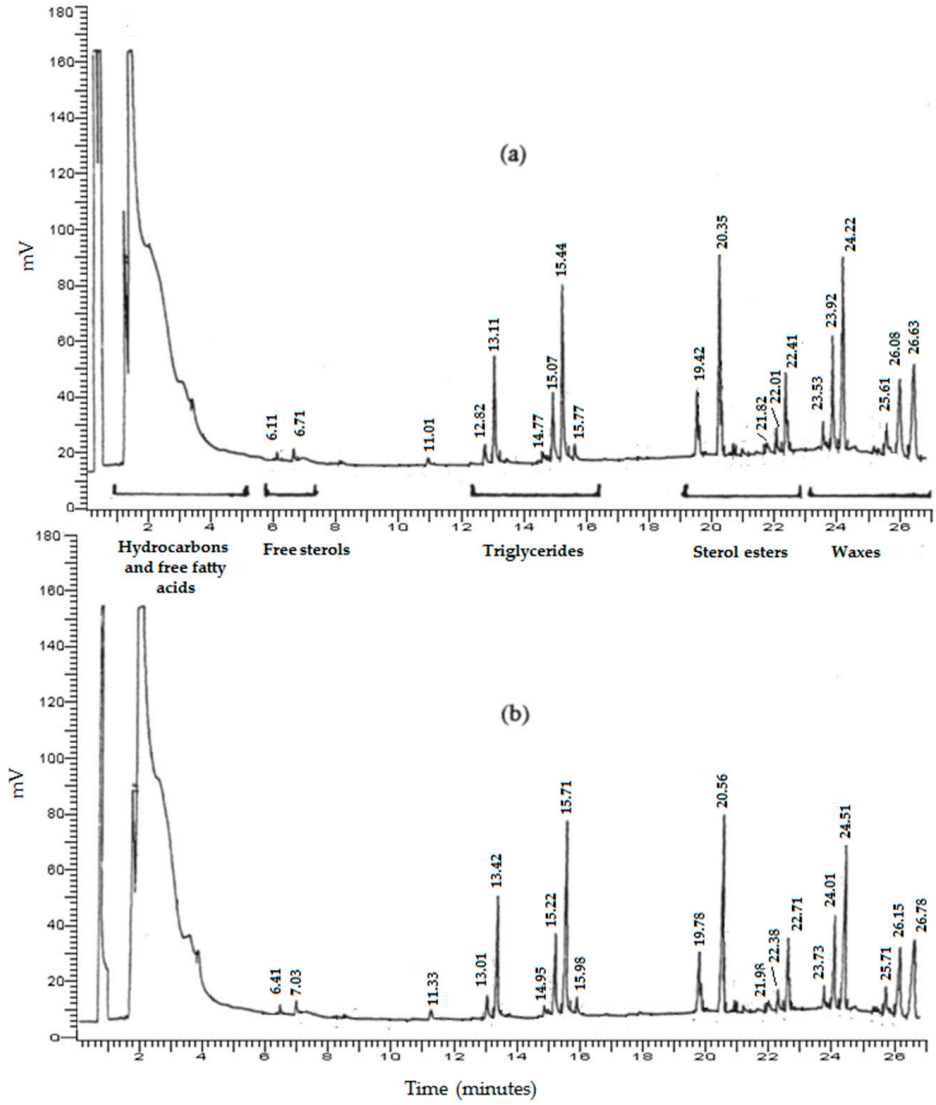

Figure 2. Comparison between the gas chromatograms of the fat as it is from a partially (about 50% of total lipids) extracted commercial flour (**a**) and of the same fat after total recovery of lipidic fraction (**b**). Panel a indicates the areas relating to the elution of the most representative lipid components in the fat of flour products.

Figure 3 shows the gas chromatograms obtained by analyzing two commercial samples: one of a soft wheat flour fat and one of semolina as is. The comparison of the lipid components highlights a difference in the areas of the peaks of sterol esters, between the retention times ranging between 19 and 21 min, due to the presence of two doublets of higher peaks in the flour and practically negligible in the semolina. Subsequently, the non-polar fractions were isolated from the fats according to the procedure reported in the Materials and Methods section. The comparison between the gas chromatograms, relating to the non-polar fractions of the flour and semolina, shown in Figure 4, does not highlight any further difference; the triglyceride area disappears due to the retention exerted by the silica gel, while the areas of the sterol esters and of the waxes remain practically unchanged when compared with the relative gas chromatograms of the fats analyzed as such (Figure 4).

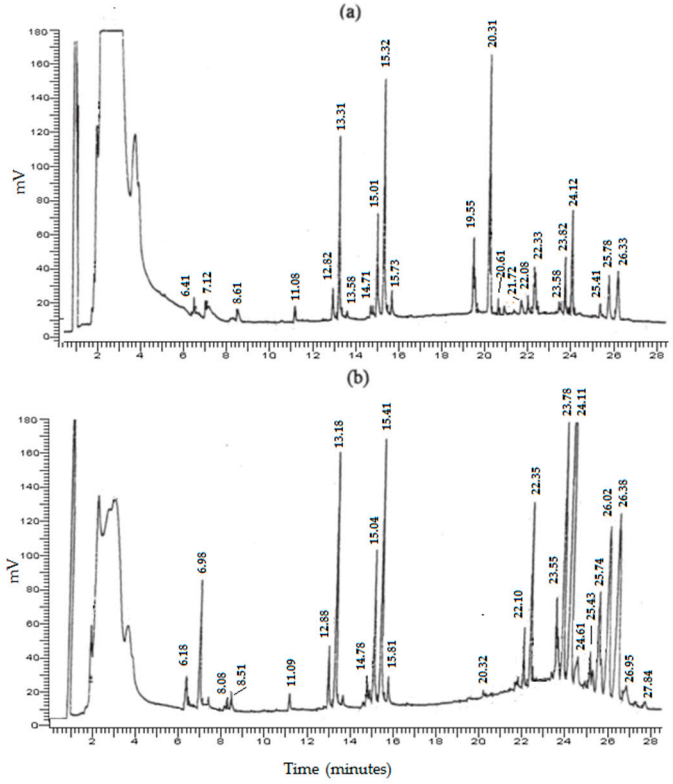

Figure 3. Comparison between the chromatographic analysis of fat from two commercial samples as is: (**a**) soft wheat flour and (**b**) semolina.

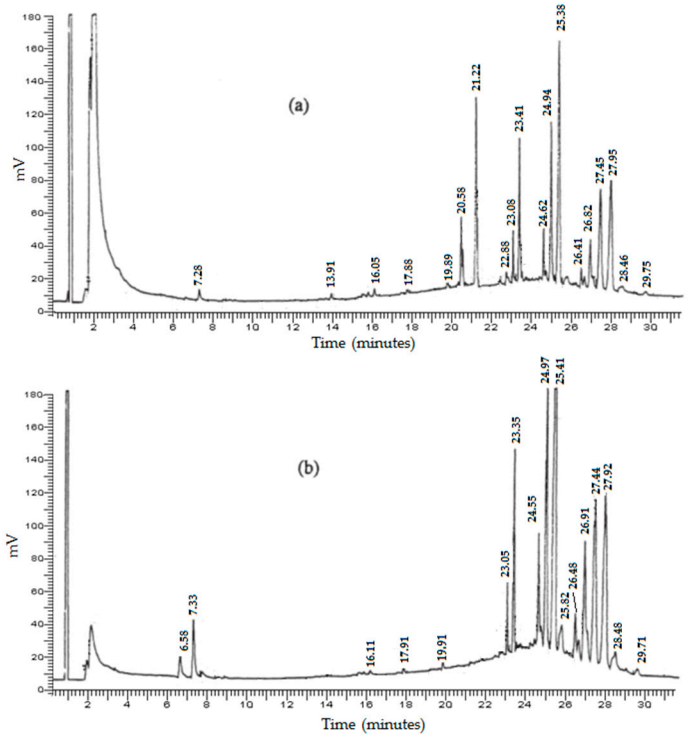

Figure 4. Comparison between the chromatographic analysis of the non-polar fat fraction of two commercial flour products: (**a**) soft wheat flour and (**b**) semolina.

3.4. GC-MS Analysis of Sterol Esters after Transesterification of the Non-Polar Lipid Fraction of Flour Products

The GC-MS analysis of the fats extracted from soft wheat flours and semolina allowed us to ascertain only the presence of campesterol palmitate and beta-sitosterol palmitate. On the contrary, it is possible to highlight the absence of campesterol oleate and beta-sitosterol oleate among the fraction of sterol esters, despite the significant presence of oleic acid, as reported in the literature [22]. According to this reference, Melis et al. used normal-phase HPLC-ELSD to compare the lipid profiles of different wheat flours [22]. Normal-phase high-performance liquid chromatography (HPLC) is widely used in combination with evaporative light scattering detection (ELSD) to separate and detect lipids in various food samples.

The possible cause of the absence of campesterol oleate and beta-sitosterol oleate could be due to the overlap of molecules of other nature present in greater quantities than these esters, which did not allow their identification. The mass spectra of these interfering compounds indicated the likely presence of long-chain esters, similar to waxes. On the other hand, a previous experiment made it possible to verify that the waxes and triglycerides in n-hexane were quantitatively and rapidly transesterified using 2N methanolic soda, while in the same experimental conditions, the esters of long-chain sterols did not react. For this reason, the fat from soft wheat and durum wheat flours was subjected to the transesterification reaction, and from the comparison of the gas chromatograms relating to the fat as is with the transesterified ones, it was highlighted that the profiles were different (Figures 3 and 5, respectively). The peaks relating to triglycerides and waxes were not present, while in the wax area, there are doublets of peaks identified by mass spectrometry as: campestanol oleate (peak 5) and campesterol oleate (peak 6); beta-sitostanol oleate (peak 7) and beta-sitosterol oleate (peak 8), whose numbering is indicated in Figure 6a; furthermore, even the first two doublets, reported in the literature [23,24] as single peaks of campestanol palmitates (peak 1) and beta-sitostanol (peak 3), were split into two different peaks due to an unsaturation in the perihydrophenanthrene cyclopentane ring of the sterol part of the esters and identified as campesterol palmitates (peak 2) and beta-sitosterol (peak 4) (Figure 6a); this better separation attributed to a higher polarity of the stationary phase used. The peak ratios related to sterol esters are typically different in the two types of fat. The qualitative–quantitative analysis method for the differentiation of soft wheat from durum wheat and the relative dosage of the mixtures was based on this configuration. In particular, Caboni et al. [23] showed that fatty acid steryl esters were useful for discriminating between hexaploid and tetraploid wheats, both qualitatively and quantitatively, using GC and HPLC chromatographic techniques. On the other hand, the analysis of cereal lipids is very challenging due to the complex lipidome comprising several hundred individual compounds present in a wide range of concentrations. A work by Hammann et al. reports a method for lipid profiling of seven cereals using high-temperature gas chromatography coupled with high-resolution mass spectrometry (GC/Q-TOF MS) [24].

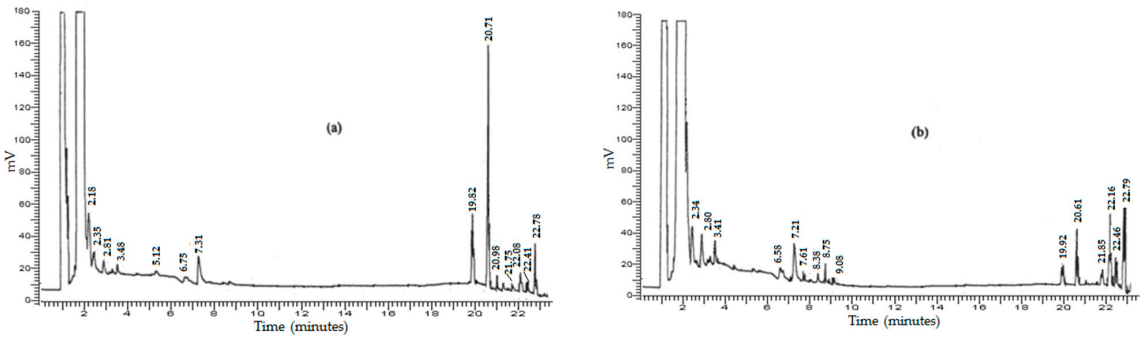

Figure 5. Comparison of the chromatographic analysis of fat from two commercial samples after transesterification with 2N methanolic soda: (**a**) soft wheat flour and (**b**) semolina.

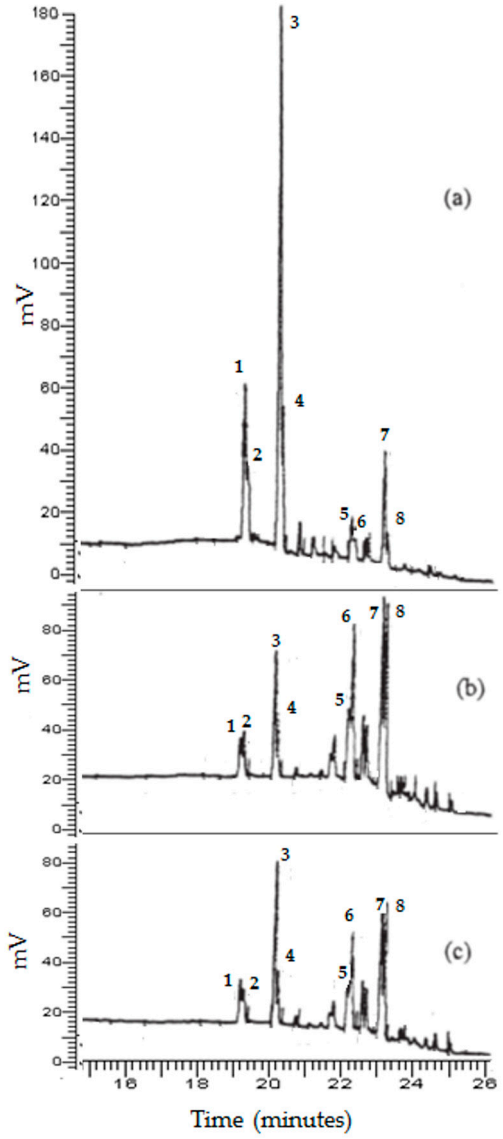

Figure 6. Gas chromatograms relating only to the sterol ester area of commercial flour products: (**a**) soft wheat flour; (**b**) semolina and (**c**) mixture of the two flours with 10% (*w*/*w*) soft wheat flour. Numbers reported on the diagrams are peak ratios, whose variation depends on the percentage of soft wheat flour to semolina.

Previously, Sarwar and Mc Donald had proposed a method in which these esters could be taken as indicators of the presence of soft wheat flour mixed with semolina [25]. The method involved the use of high-pressure liquid chromatography (HPLC) but had some limitations; the accuracy of the results depended, among other things, on the complete extraction of the fatty substance. This operation was obtained by carrying out a preliminary acid hydrolysis of the initial matrix, which, in addition to being long, did not always lead to accurate results. Therefore, the proposed protocol based on transesterification aimed to develop a faster and more accurate method that was independent of the total recovery of the fat and which consequently allowed the analysis to be freed from the addition of an internal standard with an undisputed advantage for the overall analytical procedure. Differences were looked for in the acidic composition of the two fats after transesterification, but, as shown in Figure 7, no differences that could be exploited for analytical purposes were highlighted in relation to their identification. The composition of fatty acids is quite similar in the two flours for palmitic acid (15%), stearic acid (4%), oleic (20%), linoleic (40%) and linolenic acid (10%).

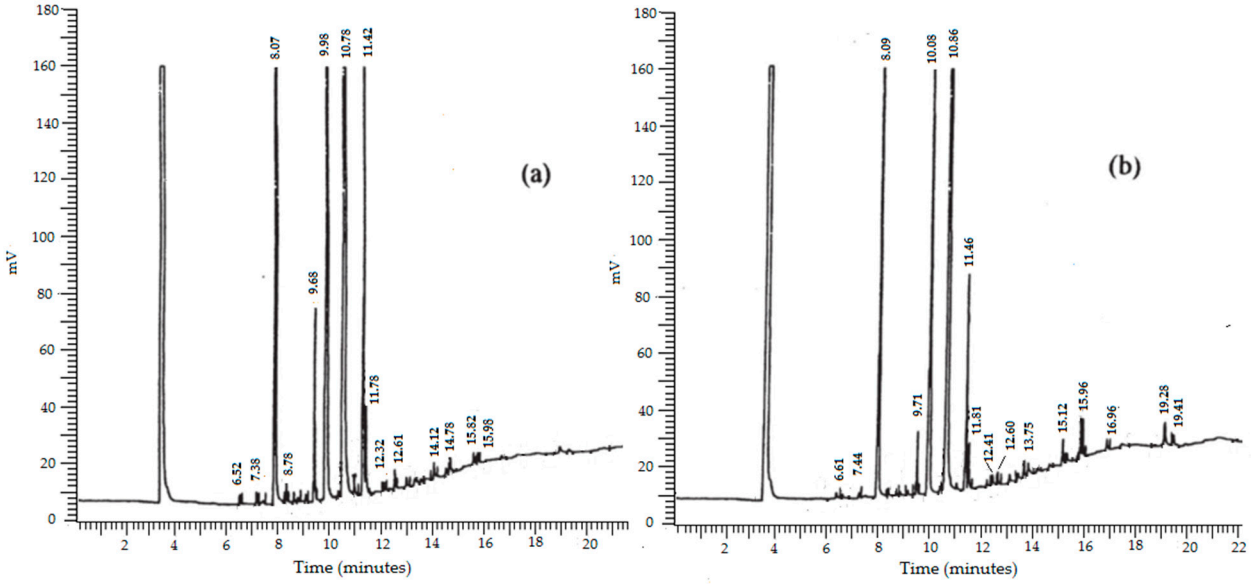

Figure 7. Comparison between the chromatographic analysis of the acid composition of two commercial flours: (**a**) semolina and (**b**) soft wheat flour obtained from the analysis of the fats extracted and then transesterified with 2N methanolic soda.

3.5. Characteristic Ratios of Sterol Esters in Flours and Semolina after Transesterification

To identify specific indices that allow the presence of soft wheat flours in the semolina to be highlighted and quantified, the fats extracted from 10 soft wheat flours and 10 commercial semolina flours were transesterified and then analyzed using gas chromatography. Table 3 shows the ratios between the heights of peaks 3 and 6 and the ratios between peaks 3 and 7. As can be seen, the values of the ratios indicated are included in satisfactorily narrow ranges that are different between the two matrices. The experimental determination of these ratios allows us to trace the degree of purity of each of the two matrices. By adding an appropriate internal standard (cholesteryl palmitate) to the fat before carrying out the transesterification reaction, it was possible to determine the quantity by weight of the sterol esters contained in the starting flours. The results indicated a presence of this fraction five-times greater in soft wheat flour than in semolina (Table 3). Therefore, these results highlight a great sensitivity of the detection method in soft wheat mixed with durum wheat.

Table 3. Characteristic ratios of representative peaks relating to 10 commercial samples of soft wheat flour (SWF) and 10 samples of semolina (S) and abundances of the related sterol fractions.

Samples SWF and S	Peak Ratio 3/6 SWF	Peak Ratio 3/6 S	Peak Ratio 3/7 SWF	Peak Ratio 3/7 S	SWF Sterol Esters mg/100 g	S Sterol Esters mg/100 g
1	4.3 ± 0.2	0.89 ± 0.03	1.5 ± 0.1	0.67 ± 0.03	0.015 ± 0.002	0.035 ± 0.002
2	8.0 ± 0.3	0.86 ± 0.03	2.3 ± 0.2	0.62 ± 0.04	0.017 ± 0.003	0.030 ± 0.003
3	5.0 ± 0.2	0.70 ± 0.02	1.3 ± 0.1	0.74 ± 0.05	0.013 ± 0.002	0.028 ± 0.002
4	7.6 ± 0.4	0.75 ± 0.03	2.3 ± 0.2	0.74 ± 0.04	0.018 ± 0.001	0.029 ± 0.001
5	12.7 ± 0.4	0.60 ± 0.02	2.9 ± 0.2	0.71 ± 0.03	0.019 ± 0.002	0.026 ± 0.003
6	13.7 ± 0.3	0.69 ± 0.04	3.2 ± 0.3	0.67 ± 0.05	0.020 ± 0.002	0.027 ± 0.002
7	13 ± 05	0.72 ± 0.05	4.3 ± 0.3	0.70 ± 0.06	0.021 ± 0.001	0.028 ± 0.002
8	5.0 ± 0.2	0.61 ± 0.03	1.3 ± 0.1	0.66 ± 0.05	0.020 ± 0.002	0.026 ± 0.003
9	9.6 ± 0.2	0.76 ± 0.04	2.5 ± 0.2	0.70 ± 0.03	0.013 ± 0.001	0.028 ± 0.002
10	10.7 ± 0.5	0.81 ± 0.05	2.5 ± 0.2	0.67 ± 0.04	0.018 ± 0.002	0.029 ± 0.002

3.6. Detection of the Presence of Soft Wheat in Pasta

In order to verify whether these same indices could be used to identify and quantify the presence of flour in pasta, doughs were prepared with only flour and semolina. Parts of the mixtures were dried at room temperature; two other aliquots were dried at 70 °C and 130 °C for one hour, respectively. The fats extracted by the described procedure were transesterified and analyzed using GC. Table 4 shows the characteristic ratios as well as the quantity of fat extracted per 100 g of flour. As can be seen, the quantity of fat extracted is approximately 15% of the quantity extracted from flour products not subjected to kneading, regardless of the drying temperature. Nonetheless, the characteristic ratios between the peaks taken as reference for both flours and semolina do not undergo appreciable variations. The reduction in the recovery of free lipids has already been highlighted in previous studies, in which it is reported that during the preparation of the dough, 90% of them bind to the three-dimensional protein structure that is generated due to contact with water [26].

Table 4. Trend of the characteristic ratios of soft wheat flour (SWF), semolina (S) and doughs of the related flours dried at different temperatures and percentage of fat extracted (DD stands for dough dried).

Samples	Characteristic Ratio 3/6	Characteristic Ratio 3/7	Percentage of Fat Extracted, %
SWF	13.0 ± 0.2	3.1 ± 0.3	1.04 ± 0.02
DD of SWF (25 °C)	12.8 ± 0.3	3.0 ± 0.2	0.18 ± 0.01
DD of SWF (25 °C)	13.3 ± 0.2	3.2 ± 0.3	0.15 ± 0.01
DD of SWF (25 °C)	12.7 ± 0.2	3.3 ± 0.2	0.16 ± 0.01
S	0.81 ± 0.1	0.67 ± 0.1	0.80 ± 0.03
DD of S (25 °C)	0.79 ± 0.1	0.66 ± 0.1	0.15 ± 0.01
DD of S (70 °C)	0.80 ± 0.2	0.65 ± 0.2	0.17 ± 0.02
DD of S (130 °C)	0.83 ± 0.1	0.68 ± 0.1	0.16 ± 0.01

3.7. Rapid Alternative Method to the Official Methods for Checking the Semolina Used for Pasta Making

The food industries of the pasta food chain routinely carry out checks on semolina to certify its genuineness, using the official analysis methods already mentioned. In this work, an alternative method to carry out this control was evaluated.

Starting from a semolina and a soft wheat flour analyzed as they were, mixtures containing 2, 5 and 10% respectively of soft wheat flour in the semolina were prepared. Figures 5 and 8 show the gas chromatograms relating to the transesterified fat of soft wheat flour and semolina separately and those relating to the fats extracted from the mixtures prepared as above and transesterified. As can be seen, the chromatogram relating to the 2% mixture of soft wheat flour presents a chromatographic profile that begins to differ from that of a semolina. The gas chromatogram relating to the 5% mixture leaves no doubt about the presence of soft wheat flour in the semolina. Furthermore, as the soft wheat flour content increases, a growth in the peaks that are initially low in the semolina is observed: peak 1 surpasses peak 2 and peak 3 exceeds peaks 6, 7 and 8. This is found already in the 5% mixture, distancing the similarity of the global profile from that typical of pure semolina. This effect is even more marked in the 10% mixture (Figure 8).

Table 5 shows the representative ratios of soft wheat flour and semolina used for the preparation of the mixtures. The theoretical calculation envisaged for the mixing, starting from the extreme values obtained for the soft wheat flour and the semolina, is in excellent agreement with the ratios obtained experimentally. The convergence of the values is possible if a factor of five is considered in the quantity of the sterol fraction contained in the flour compared to that of the semolina.

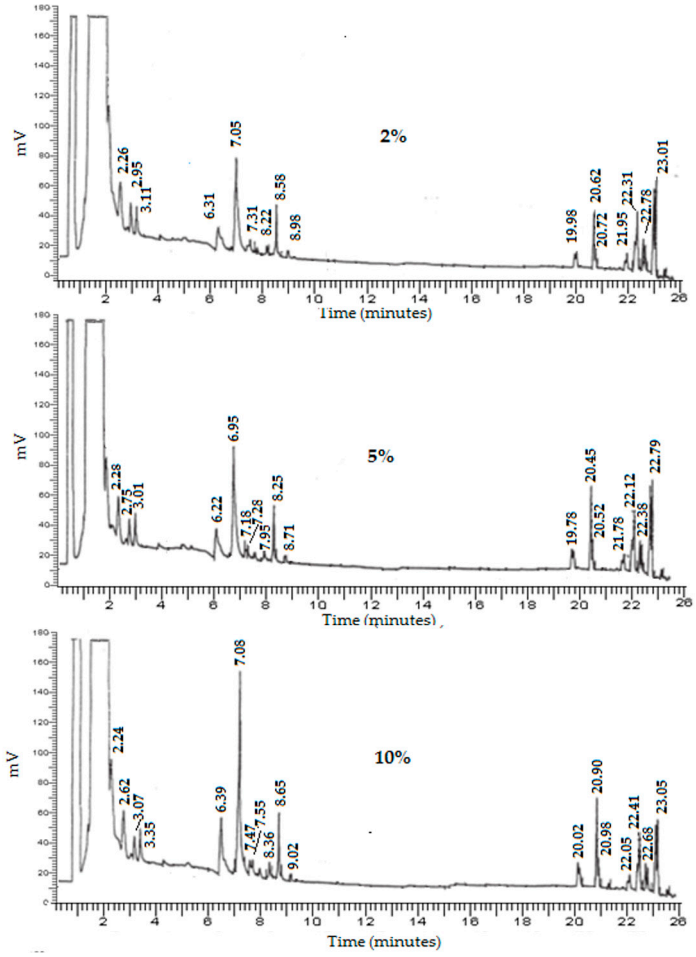

Figure 8. Gas chromatographic analysis of fats extracted from mixtures of commercial soft wheat flour at 2, 5 and 10% *w*/*w* in semolina after transesterification.

Table 5. Trend of the theoretical and experimental ratios characteristic of commercial samples of soft wheat flour (SWF), semolina (S) and their mixtures as such.

Samples	Peak Ratio 3/6 Theoretical	Peak Ratio 3/6 Experimental	Peak Ratio 3/7 Theoretical	Peak Ratio 3/7 Experimental
SWF	-	13.0 ± 0.2	-	3.1 ± 0.2
S	-	0.81 ± 0.04	-	0.67 ± 0.02
2% SWF mixture	1.07	1.04 ± 0.03	0.91	0.95 ± 0.04
5% SWF mixture	1.43	1.51 ± 0.02	1.20	1.24 ± 0.03
10% SWF mixture	2.13	2.00 ± 0.03	1.61	1.55 ± 0.02

3.8. Detection of the Presence of Soft Wheat in Pasta at Various Drying Times

The procedure previously described was used to verify its potential to identify and quantify the presence of soft wheat flour in pasta. Mixtures of commercial samples containing semolina and percentages of 2, 5 and 10% *w*/*w* of soft wheat flour respectively were prepared, from which three series of pastas subjected to different drying temperatures were produced. Part of the mixtures were dried at room temperature; two other aliquots were dried for 1 h at 70 °C and 130 °C, respectively. The fats extracted with the described procedure were transesterified and analyzed using GC. Moreover, the considerations made for flour mixtures can be also applied to pasta. In fact, as can be seen in Figure 9, where the gas chromatograms relating to the fats recovered from the pasta treated at 130 °C are shown, the trend of the profile of the sterol ester fraction is practically identical to that of the mixtures reported in the previous figure (Figure 8).

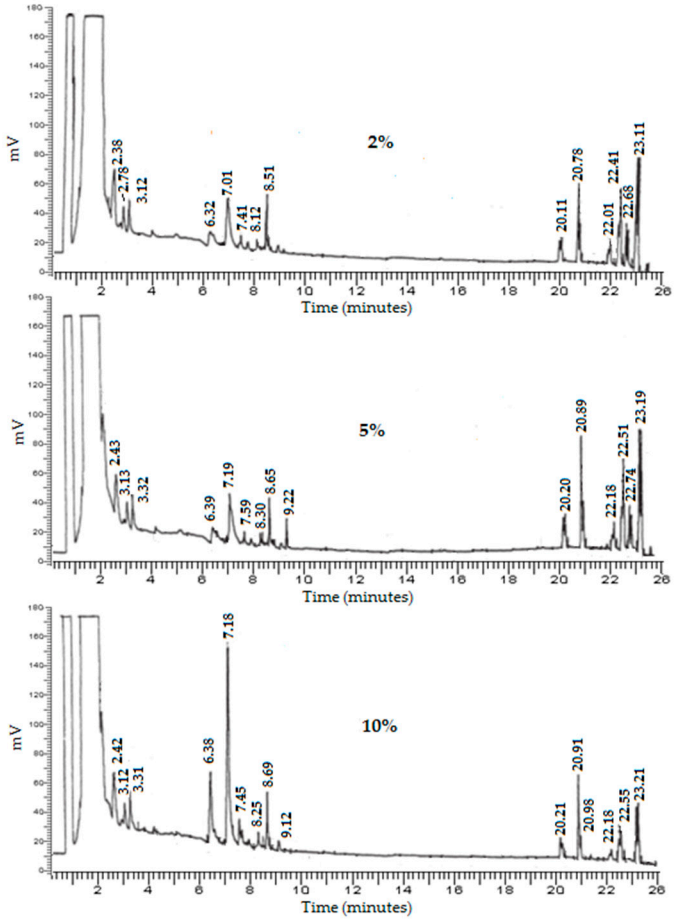

Figure 9. Gas chromatographic analysis of fats extracted from pasta obtained from mixtures of commercial soft wheat flour at 2, 5 and 10% in semolina and treated at 130 °C, after transesterification.

4. Conclusions

The study of the lipid component of soft wheat flours and semolina has allowed the identification of a class of compounds, sterol esters, which is contained in constant and greater quantities in soft wheat flours compared to semolina, where it is practically negligible [27–29]. We would like to highlight that this method represents an approach to the possibility of the application of a procedure to detect frauds based on mixing soft wheat with semolina worldwide, after defining a calibration procedure with local growing natural matrices.

The peaks identified in the literature as beta-sitosterol and campesterol palmitates were found to be well resolved using gas chromatography analysis on a 65% phenyl methylsilicone capillary column and to be split into two further peaks, which differ due to an unsaturation in the structure of the sterol part of the esters. The identification of these doublets, in addition to two other doublets of sterol esters identified as campesterol and campestanol oleates, the former, and beta-sitosterol and beta-sitostanol oleates, the latter, allowed the analysis to be separated from the extraction exhaustive of the fat in the flour products, providing the ability to monitor the characteristic ratios of the flours and semolina. The thermal stability of sterol esters has made it possible to develop a rapid analysis method, which is able to provide reliable results regarding the mixing of soft wheat with durum wheat not only in the initial matrices, but also in pasta, regardless of the drying temperature. Finally, compared to conventional methods, the rapid extraction of fat from flour products by percolation is advantageous, allowing the analysis of many samples in a short time with the aim of detecting fraud derived from the addition of soft wheat flour to semolina both in flour products and in pastas.

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