EXTRACTION OF NATURAL ANTIOXIDANTS (CAROTENOIDS AND TOCOPHEROLS) FROM By-PRODUCTS OF THE TOMATO PROCESSING INDUSTRY

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The supercritical fluid extraction of natural antioxidants present in by-products of the tomato industry was studied. The recovery of carotenoids (licopene and β-carotene) and tocopherols (α- and γ-tocopherol) was investigated as a function of the drying process of the raw material, of the extraction parameters, temperature (60 and 80°C) and pressure (30 and 50 MPa), and of the use of sunflower oil as a non conventional cosolvent.

INTRODUCTION

Carotenoids represent an important group of natural pigment that are essential for the human health. Their consumption is strongly recommended because, according to epidemiological studies, their intake can be associated with a reduced risk of developing heart diseases or certain types of cancers [1-3]. Among the several carotenoids, β-carotene and lycopene are of the utmost importance and recently lycopene has been affirmed as generally recognized as safe (GRAS) in the United States [4], so that now its use in food formulations has been made easier. The major sources of lycopene are tomatoes and tomato products and this pigment can represent more than 85% of all the carotenoids present in the fruits; its concentration can vary from 30 to 200 ppm in the fresh products or from 430 to 3000 ppm on a dry basis [5]. Tomato skins can be a viable source of lycopene, as they contain about five times more lycopene than the whole tomato pulp [6], and considering that more than one third of the tomatoes delivered to processing plants ends as processing wastes, mainly constituted by seeds and skins, the recovery of this carotenoid could represent an alternative for the valorization of the by-products of the tomato industry. Furthermore, tomatoes at the ripening stage contain α-tocopherol and γ-tocopherol at the average concentration of 3.5 and 1.2 μg/g, respectively [7].

The utilization of supercritical CO_2 (SC-CO_2) for the extraction of various carotenoids from a number of matrices, including tomatoes, has been studied by several authors [8-19]; but the available studies deal with the extraction of only very limited amounts of tomato products and with the use of different organic compounds as cosolvent. The aim of this research was to investigate the recovery of the antioxidant fraction contained in tomato by-products and represented by both carotenoids and tocopherols. Furthermore, the utilization of an unconventional cosolvent, sunflower oil, to enhance the recovery of the antioxidants was studied.

MATERIALS AND METHODS

Materials. Tomato seeds and skins were obtained from an industry producing concentrated

tomato sauce and located in the Campania region. The material was collected immediately prior to the disposal step and then stored at -20°C until utilized for the extraction tests. In order to avoid any channeling or packing of the bed during the extractions, the skins and seeds was dried up to a final moisture content of about 10%. Three different drying systems were tested: sun drying, oven drying at 105°C and drying with an industrial Sandvik apparatus. When using the Sandvik equipment, the drying process was conducted on batches of 2.5 kg of fresh product, with air at 60 °C flowing through the vegetable mass at the velocity of 2.5 m/s over an area of 0.17 m^2 for 100 minutes. Regardless of the drying procedure, the product was ground immediately before the extraction using a laboratory mill, and the measured average particle size was 0.38 mm.

Extractions. Supercritical fluid extractions were performed with a home built apparatus described elsewhere, using a 192 cm^3 extraction vessel (316 SS tube, 800 mm x 17.5 mm i.d.). [20]. The experiments were conducted on a constant quantity of sample (55 g), testing as extraction conditions the pressures of 30 and 50 MPa and the temperatures of 60 and 80°C. The CO_2 mass flow rate was set to 18 g/min, and the total amount of solvent that went through the bed of solids ranged from 15 to 29 g CO_2/g (skins+seeds), according to the operating conditions, until no significant amount of extracted material could be collected. The addition of sunflower oil as cosolvent was made taking into account its solubility in the supercritical fluid at the various extraction conditions. In order to obtain an oil laden stream of CO_2 flowing through the bed of solids, the oil was loaded onto the ground matrix at the inlet side of the extractor, and the added amounts were 5,25, 11,2 and 18 g for the extraction conducted at 30 MPa and 60 °C, 50 MPa and 60 °C, and 50 MPa and 80 °C, respectively.

Analyses. Moisture content of the vegetable matrix was determined according to standard methods [21] and the amount of total extractables was determined gravimetrically, after organic solvent extraction according to the procedure utilized by Ollanketo et al. [22]. Lycopene and β-carotene content in the matrix and in the extracts was assessed by RP-HPLC; the pigments were separated on a 5 μm Luna C_{18} column (250 x 4.6 mm) (Phenomenex, USA), equipped with a C_{18} pre-column, utilizing MeOH/H_2O (95:5) (solvent A) and CH_2Cl_2 (solvent B) as eluents at the flow rate of 1 mL/min. A linear gradient was used to go from 60% A/40% B to 90% A/10% B in 15 min. and the total analysis time was 18 minutes. Detection was performed at 450 nm and the peaks were tentatively identified by comparing their retention times to those of lycopene and β-carotene standards (Sigma, Milano, Italy); their quantification was then obtained using the external standard technique over a concentration range going from 2.5 to 250 ng/μL.

Tocopherol content in the extract was assessed by HPLC according to the method of Pocklington and Dieffenbacher [23]. The extracts (100 mg) were diluted in 10 mL of hexane and an aliquot of 20 μL was injected onto a 5 μm Adsorbosil silica column (250 x 4.6 mm) (Alltech, Milano, Italy) equipped with a pre-column (Adsorbosil 5 μm). Isocratic separation of the compounds of interest was achieved using n-hexane/2-propanol (99.3/0.7) as mobile phase at the flow rate of 1 mL/min. Solute detection was accomplished by using a fluorescent detector, with excitation and emission wavelengths set at 290 and 330 nm, respectively. Peaks were tentatively identified by comparing their retention times to those of α- and γ-tocopherol standards (Sigma, Milano, Italy); their quantification was then obtained using the external standard technique over a concentration range going from 0.7 to 66 ng/μL.

RESULTS AND DISCUSSION

Much of the scientific reports about SFE of carotenoids from tomato, deal with the

extraction of only limited amounts of vegetable material, usually between 0.3 and 2.5 g, and this is mainly due to the instrumentation utilized in the studies, that does not allow to use extraction vessels bigger than 10 cm^3 [8-12]. Only BAYSAL et al. [13] have worked with higher amounts of matrix, about 55 g, a quantity similar to that utilized in the present work. Furthermore, in the above mentioned studies the range of extraction temperatures and pressures is very wide, going from 35 to 110°C and from 8.6 to 40 MPa, and this makes very difficult to compare the various results, considering also that at the reported conditions the CO_2 density varies from 400 g/L to more than 800 g/L. Given that SPANOS et al. [14] have reported 27.6 MPa as the pressure value of the inversion point for the solubility of β-carotene in SC-CO_2, and in order to work at relatively high values of CO_2 density and Hildebrand solubility parameter (δ), in the present study the extractions were conducted at the pressures and temperatures of 30 and 50 MPa, and 60 and 80 °C, respectively (Table 1).

Pressure (MPa)	**Temperature (°C)**			
	60		80	
	Density (g/cm^3)	δ ($cal^{1/2}/cm^{3/2}$)	Density (g/cm^3)	δ ($cal^{1/2}/cm^{3/2}$)
30	0,829	7,131	0,747	6,364
50	0,934	8,019	0,876	7,485

Table 1. CO_2 density and Hildebrand solubility parameter values for the experimental conditions tested [24, 25].

	Fresh tomato skins	**Dried tomato skins**
Total lipid content (g/100 g dry matter)	52.6	37.2
Lycopene content (μg/g dry matter)	1574.0	189.0
b-**carotene content** (μg/g dry matter)	172.0	107.0
a-**tocopherol content** (μg/g dry matter)	92.7	28.6
g-**tocopherol content** (μg/g dry matter)	161.0	102.0

Table 2. Total lipid, carotenoids and tocopherols content in fresh and dried tomato skins.

Because of the high moisture content (> 90%), the tomato skins had to be dried up to a water content of no more than 10 %. The vegetable matrix was dried with different procedures, but sun drying and oven drying did not give good results, because of the development of undesirable processes, such as growth of molds and fermentations in the first

case, and browning of the matrix in the second. Better results were obtained using the Sandvik apparatus; three different drying temperature (40, 60 and 80 °C) were tested, and working at 60 °C allowed to dry the product up to the desired moisture level in a reasonable time, without any significant modification of the moieties of interest. However, it should be pointed out that the drying step caused a major reduction of the amount of components that could be recovered from the matrix, especially lycopene (Table 2). This reduction could be ascribed to physical modifications of the structure of the skins during the drying process, modifications that could hinder the mass transfer and diffusion phenomena throughout the

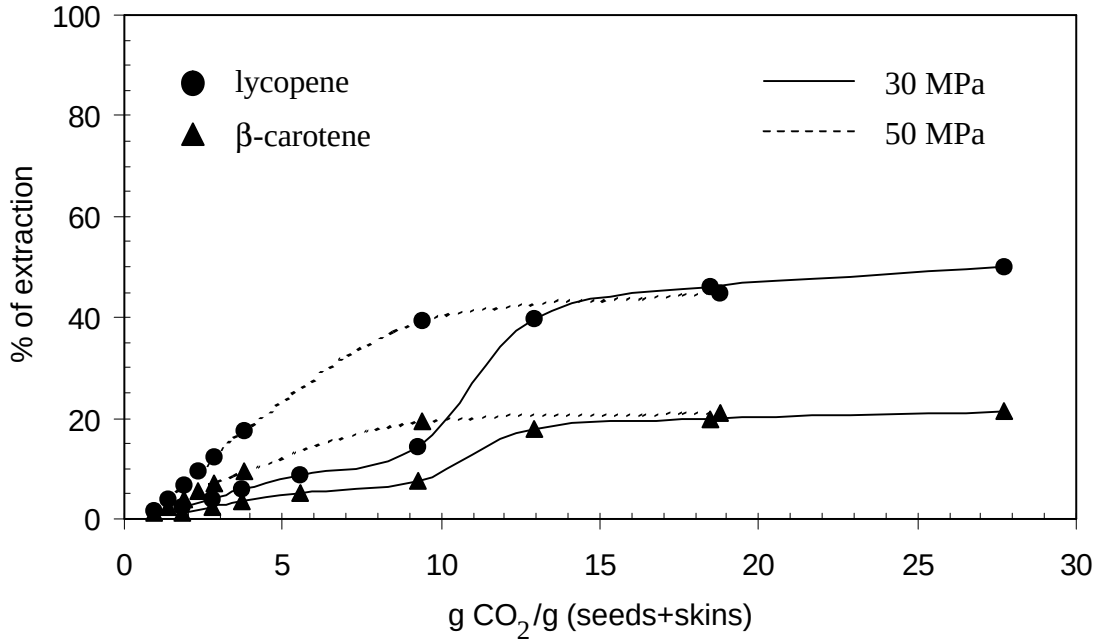

Figure 1. Percentages of lycopene and β-carotene recovery at 60°C and different extraction pressures.

vegetable matrix. The analytical data corroborated this hypothesis, because no presence of degradation products of the moieties of interest could be detected. Furthermore, Giovannelli et al. [26] reported that lycopene content of tomato halves at 10% final moisture content decreased to a maximum of 10% after drying at 110°C for 4 h and did not change during drying at 80°C for 7 h. Also Inakuma et al. [27] reported that air drying could cause a reduction of the amount of extractable and suggested the use of ethanol for drying the substrate. However, this method, even if appropriate for laboratory tests, can not be considered suitable at industrial scale, where tons of skins should be treated.

In Figure 1 the percentages of lycopene and β-carotene extracted at 60 °C and at 30 and 60 MPa are plotted as a function of the specific mass of solvent. The percentages were calculated with reference to the extractable amounts as determined on the dried material by organic solvent extraction. Increasing the solvent density from 0.83 to 0.93 did not cause a major increase of the carotenoids recovery; however, at 50 MPa the extraction process was quicker. Further reduction of the extraction time was observed operating at 50 MPa and 80 °C, when the percentage of lycopene recovered reached the level of about 60%, for a g CO_2/g (skins+seeds) ratio of 15. Conversely, β-carotene recovery showed only a minor increase, reaching the final value of 23%. Of interest is the shape of the extraction curves at 30 MPa. At this CO_2 compression level the amount of carotenoids extracted remained very low until the

specific mass of solvent reached the value of 9; afterwards a sharp increase of the carotenoids recovery was observed. This extraction profile could be explained considering that when the supercritical fluid has a somehow limited solvent power, there is a competition between the carotenoids and the oil dispersed in the matrix and deriving from the crushed tomato seeds

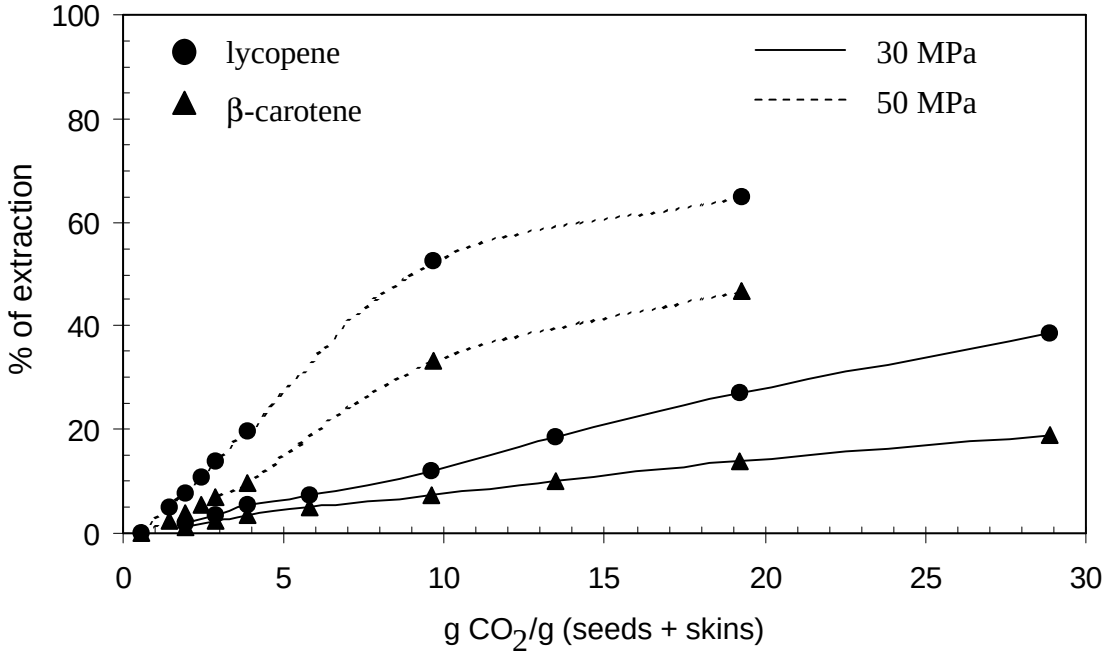

Figure 2 Percentages of lycopene and β-carotene recovery at 60°C and different extraction pressures, with the use of sunflower oil as cosolvent.

mixed with the skins. Because triglycerides are very soluble in SC-CO_2 at the tested conditions, they could almost completely saturate the CO_2 and a substantial extraction of the carotenoids could be observed only after that the major part of the oil was removed from the matrix. This hypothesis could be corroborated by the carotenoids recoveries observed when sunflower oil was added as cosolvent (Figure 2). In this case, while the final quantities of lycopene and β-carotene extracted were similar to those observed previously, the shape of the extraction curves was linear, without any sudden increase, because the amount of oil present in the matrix (natural + added) was not limited.

While several authors have studied the possibility of using various organic compounds (hexane, chloroform, methanol, ethanol, diethyl ether, acetone and water) as cosolvents for the enhancement of lycopene extraction with SC-CO_2, their results are somehow controversial, because using the same cosolvent different authors have reported very different results [9, 11, 13]. Furthermore, some of the proposed substances are not acceptable for the extraction of compounds for human consumption. Because carotenoids are lipophylic moieties and are usually utilized by the food industry as oil-based solutions, the use of sunflower oil as cosolvent was also investigated in order to improve the extraction recoveries. The choice of a vegetable oil was made also taking into account the fact that the bioavailability of lycopene is enhanced when tomato products are consumed in formulations containing triglycerides [28].

The percentages of recovery of tocopherols and carotenoids measured at various extraction conditions, with or without the use of sunflower oil, are reported in Table 2. For the extraction

conducted with the addition of the oil, the α-tocopherol recovery percentages were calculated excluding the contribution of the sunflower oil to the total α-tocopherol content. In the table the data are also tabulated with reference to the content of the moieties of interest determined on a dry (D) or wet (W) basis. Extraction yields higher than 100% indicate that for that particular combination of pressure and temperature conditions, the SFE process succeeded in extracting a quantity of antioxidants higher than that obtained through the use of traditional organic solvents on the dried vegetable matter.

At isothermal conditions (60 °C), increasing the extraction pressure from 30 to 50 MPa caused a 30% reduction of the tocopherol recovery, while lycopene and β-carotene were much less affected by the pressure enhancement. Increasing the extraction temperature at isobaric conditions (50 MPa) had a positive effect on the recovery of all the antioxidants of interest. The use of sunflower oil as cosolvent allowed always better recoveries for α-tocopherol, and at 50 MPa and 80°C this moiety was practically completely removed from the substrate. For γ- tocopherol the most interesting results were obtained at 30 MPa and 60°C without any cosolvent addition, when almost 90 % of the compound was recovered.

Extractions conditions (P, T)	**Cosolvent use**	**Percentage of recovery**							
		α-tocopherol		γ- tocopherol		lycopene		β–carotene	
		D	W	D	W	D	W	D	W
30 MPa 60 °C	No	256	78,9	136	86,2	49,9	6,0	21,5	13,4
	Yes	290	89,3	108	68,5	38,6	4,6	18,9	11,8
50 MPa 60 °C	No	92	28,3	97	61,4	45,7	4,9	21,2	12,6
	Yes	145	44,9	86	54,5	64,8	7,8	46,6	29,0
50 MPa 80 °C	No	206	63,6	122	77,3	58,5	7,0	23,2	14,4
	Yes	320	98,9	80	50,8	27,4	3,3	23,5	14,6

Table. 2. Percentages of recovery of tocopherols and carotenoids at different extraction conditions. Reported values are calculated with reference to the content determined by organic solvent extraction in the dried (D) or in the wet (W) tomato skins and seeds.

For lycopene and β-carotene the data show that when compared to the extractable amounts determined on the wet product, the recoveries were quite low; however, with respect to the extractable quantities determined on the dried material, recoveries of almost 65% and 47% of lycopene and β-carotene could be obtained at 50 MPa and 60°C when using the vegetable oil as cosolvent. As far as the evaluation of the extraction yields, and the identification of the best extraction conditions (50 MPa, 60°C and the use of sunflower oil as cosolvent) in comparison with analogous researches, it is not easy to compare these experimental data with those found in the literature. In particular Cadoni et al. [9] have reported recoveries of 87% for lycopene and 13% for β-carotene working with small amounts of fresh tomato skins (2.5 g). The authors performed a dual step extraction procedure, working initially at 40 °C and 27.2 MPa

and then rising isobarically the temperature to 80°C. In their work Baysal et al. [13] treated by-products of the tomato industry and reported that at the temperature of 65°C and the pressure of 30 MPa lycopene and β-carotene yields were 20 and 40% respectively. Conversely, for Ollanketo et al. [11] the optimum extraction conditions were represented by 40 MPa and 110 °C, with 100% recovery for lycopene. However, for their experiments the authors utilized minute amounts (0.3 g) of tomato skins, manually obtained starting from tomatoes bought in a local store. Finally Rozzi et al. [12] working on 2 g of real industry by-products having a low lycopene content (11.8 ppm) and a moisture level of about 48%, found that operating at 34.5 MPa and 86°C they could obtain lycopene recoveries of about 60%.

CONCLUSIONS

In this work real by-products of the tomato industry were utilized to study the possibility to recover the antioxidant moieties naturally present in the tomato skins and seeds. Working with a combination of temperatures (60 and 80 °C) and pressures (30 and 50 MPa) as well as with the utilization of sunflower oil as cosolvent, several data where generated regarding the best extraction conditions for recovering extracts enriched in individual components. With the aim of maximizing the yield of both the carotenoid and the tocopherol fraction, the most suitable SFE conditions were found to be the pressure of 50 MPa and the temperature of 60 °C, with the use of sunflower oil as a cosolvent. The recovery of the various compounds was, however, severely affected by the drying procedure, that represents a crucial step in treating with SC-CO_2 real industrial by-products.

REFERENCES

[1] ZIEGLER, R.G., J. Nutr., Vol. 119, **1989**, 116.

[2] SIEMENSMA, A.D., Natural mixed carotenoids and supplementation for foods. Quest International, Bussum, The Nederlands, **1996**.

[3] GIOVANNUCCI, E., CLINTON, S.K., Proc. Soc. Exp. Biol. Med., Vol. 218, **1998**, p. 129.

[4] CRANDALL, L., Inform, Vol. 14, **2003**, p. 62.

[5] LEONI, C., I derivati industriali del pomodoro, S.S.I.C.A., Parma, Monografie tecnologiche, n.7, **1993**.

[6] SHARMA, S.K., LE MANGUER, M., It. J. Food Sci., Vol. 8, **1996**, p. 107.

[7] ABUSHITA, A.A., HEBSHI, E.A., DAOOD, G.H., BIACS, P.A., Food Chemistry, Vol. 60, **1997**, p. 207.

[8] SNAGUN, M.K., BURFORD, M.D., BARTLE, K.D., CLIFFORD, A.A., Proceedings of the 3rd European Symposium on Analytical SFC and SFE. 6-8 September, Uppsala, Sweden, **1995**, p. 62.

[9] CADONI, E., DE GIORGI, M.R., MEDDA, E., POMA, G., Dyes and Pigments, Vol. 44, **2000**, p. 27.

[10] GÒMES-PRIETO, M.S., CAJA, M.M., SANTA-MARÌA, G.. J. Am. Oil Chem. Soc., Vol. 79, **2002**, p. 897.

[11] OLLANKETO M., HARTONEN K., RIEKKOLA M.L., HOLM Y., HILTUNEN R., Eur. Food Res. Technol., Vol. 212, **2001**, p. 561.

[12] ROZZI, N.L., SINGH, R.K., VIERLING, R.A., WATKINS, B.A., J. Agric. Food Chem., Vol. 50, **2002**, p. 2638.

[13] BAYSAL, T., ERSUS, S., STARMANS, D.A.J., J. Agric. Food Chem., Vol. 48, **2000**,

p. 5507.
[14] SPANOS, G.A., CHEN, H., SCHWARTZ, S.J., J. Food Sci., Vol. 58, **1993**, p. 817.
[15] FAVATI, F., KING, J.W., FRIEDRICH, J.P., ESKINS, K., J. Food. Sci., Vol. 53, **1988**, p. 1532.
[16] MARSILI, R., CALLAHAN, D., J. Chromatogr. Sci., Vol. 31, **1993**, p. 422.
[17] BARTH, M.M., ZHOU, C., KUTE, K.M., ROSENTHAL, G.A., J. Agric. Food Chem., Vol. 43, **1995**, p. 2876.
[18] BIRTIGH, A., JOHANNSEN, M., BRUNNER, G., J. Supercritic. Fluids, Vol. 8, **1995**, p. 46.
[19] SOVOVA, H., STATEVA, R.P., GALUSHKO, A.A., ROUMIANA, P., J. Supercritic. Fluids, Vol. 21, **2001**, p. 195.
[20] FAVATI, F., DI LAURO, R., PIETRAFESA A., GALGANO, F, LO VOI, A., Proceedings of the 6th International Symposium on Supercritical Fluids, 28-30 April, Versailles, France, **2003**.
[21] A.O.A.C. "Official Methods of Analysis" 15th Ed., Association of Official Analytical Chemists, Arlington, Virginia, USA, **1990**.
[22] OLLANKETO, M., HARTONEN, K., RIEKKOLA, M.L., HOLM, Y., HILTUNEN, R., Eur. Food Res. Technol., Vol. 212, **2001**, p. 561.
[23] POCKLINGTON, W.D., DIEFFENBACHER, A., Pure & Applied Chem., Vol. 60, **1988**, p. 877.
[24] ISCO Software SFSOLVER, **1991**. ISCO Inc., Lincoln, Nebraska, USA.
[25] KENNEDY, G.C., Am. J. Sci., Vol. 252, **1954**, p. 225.
[26] GIOVANNELLI, G., ZANONI, B., LAVELLI, V., NANI, R., J. Food Eng., Vol. 52, **2002**, p. 135.
[27] INAKUMA T., YASUMOTO M., KOGUCHI M., KOBAYASHI T., Nippon Shokuhin Kagaku Kogaku Kaishi, Vol. 45, **1998**, p. 740
[28] ANESE, M., CALLIGARIS, S., Industrie Alimentari, Vol. XL, **2001**, p. 1.