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The impact of industrial processing on health-beneficial tomato microconstituents

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ABSTRACT

The effect of industrial processing was investigated on the stability of tomato carotenoids, phenolic compounds and ascorbic acid. A deep insight in the processed products allowed the quantification of caffeic acid hexosides, which are far more important contributors than the well-known chlorogenic acid, dicaffeoylquinic acids and quercetin oligosaccharides (new feruloyl, sinapoyl and syringoyl derivatives of quercetin apiosylrhamnosylglucoside). (*E*)-β-Carotene and (*E*)-lycopene were also quantified along with different mono- and di-(*Z*)-isomers of lycopene which were tentatively assigned. Processing of fresh tomato into paste had an overall positive effect on the contents in phenolic compounds, no effect on lycopene and a slight and high detrimental effect on β-carotene and ascorbic acid, respectively. The balance between the increase in tomato matrix extractability and microconstituent catabolism was further observed in two contrasted transformations of paste into sauce. Overall, the nutritional quality of tomato-processed products, except for ascorbic acid, is mainly preserved through manufacture.

1. Introduction

Tomato is a fruit widely consumed either fresh or processed possessing recognized nutritional qualities. As a daily source of carotenoids (lycopene, β-carotene), ascorbic acid and a range of phenolic compounds, its regular consumption could reduce risks of developing cardiovascular diseases and cancers, especially prostate cancer (Campbell et al., 2004; Sesso, Liu, Gaziano, & Buring, 2003).

However, tomato is a fruit with a limited shelf life and a short natural period of production. Processing could respond to the need for a year-round consumption of this healthy fruit. Basic tomato paste is actually produced by a multiple step transformation of tomato including washing, breaking, skin and seed removal by sieving, concentration, canning, pasteurization and finally storage. Several mild or severe heat treatment steps are required to inactivate microorganisms, soften the tissue to separate pulp from epicarp and decrease the water content. In hot break processing, temperatures up to 90 °C lead to the inactivation of pectinolytic enzymes (pectin methylesterase and endopolygalacturonase) known to lower viscosity as well as lipoxygenases involved in aroma production. By contrast, temperatures lower than 70 °C, as used in cold break processing, maintain enzyme activity leading to less viscous and more aromatic products, such as tomato juice. Another economically valuable product, tomato sauce, is produced by homogenizing tomato paste, at high temperature, with water, starch and vegetable oil as main ingredients. During thermal treatments, various (photo) chemical reactions could take place leading to the degradation of the antioxidant microconstituents and finally to an altered nutritional quality of the final product. Besides, the presence of vegetable oil as in tomato sauce may lead to lipid oxidation, contributing to degradation reactions.

In processes involving tomato slicing and thermal treatments, ascorbic acid, because of its low redox potential, appears to be rapidly oxidized in dehydroascorbic acid before further irreversible degradation (Capanoglu, Beekwilder, Boyacioglu, Hall, & De Vos, 2008; Dewanto, Wu, Adom, & Liu, 2002; Gahler, Otto, & Böhm, 2003). Factors such as pH, temperature and time processing impact its stability although dissolved oxygen and transition metal ions from stainless steel equipments could be of greater influence. Among carotenoids, (*E*)-lycopene, the red tomato pigment, is present in a 10- to 20-fold level compared to (*E*)-β-carotene, a provitamin A carotenoid. In the course of processing, they may first readily isomerize from all-*E* to *Z*-conformation, although a decrease in total carotenoid content may indicate further degradation pathways such as oxidative cleavage (Capanoglu et al., 2008; Seybold, Fröhlich, Bitsch, Otto, & Böhm, 2004; Shi & Le Maguer, 2000). By contrast, an increase in total carotenoid concentration was reported in the dry matter of tomato paste by Re, Bramley, and Rice-Evans (2002) and Abushita, Daood, and Biacs (2000) while

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the latter also found a higher isomerization rate for (*E*)-β-carotene compared to (*E*)-lycopene. These differences may be first ascribed to carotenoid extractability from protein complexes and crystalline structures upon cell lysis. Indeed, carotenoid extraction appears to be largely improved when thermal treatment precedes mechanical destructuration (Dewanto et al., 2002). Data dealing with the evolution of tomato phenolics are scarcer. The total polyphenol content was found stable in the heat treatment of crushed tomatoes by Dewanto et al. (2002), while decreased in puree manufacture (George et al., 2011). Only two literature studies report specific evaluations for individual phenolic compounds. According to Re et al. (2002), the processing of fresh tomato into tomato paste increases the contents in chlorogenic acid and glycosides of hydroxycinnamic acids, whereas rutin is not affected and naringenin largely degraded. By contrast, Capanoglu et al. (2008) observed no overall change for chlorogenic acid and quercetin glycosides in tomato paste manufacture, while naringenin, resulting from the cyclization of its isomeric form naringenin chalcone, disappeared to a large extent. Overall, the evolution of health-beneficial tomato microconstituents during tomato processing remains unclear.

The aim of the present work is to investigate the individual stability of tomato carotenoids, phenolic compounds and ascorbic acid along the industrial transformation of fresh tomato into tomato paste then tomato paste into tomato sauce. Extraction and analysis methods specifically developed for each tomato-based product allowed the identification and quantification of a diversity of undescribed carotenoid isomers, new hydroxycinnamic acid derivatives (caffeic acid-4-*O*-glucoside, *p*-coumaric acid-4-*O*-glucoside, dicaffeoylquinic acids, feruloyl, sinapoyl and syringoyl derivatives of quercetin apiosylrhamnosylglucoside) and several naringenin glycosides.

2. Materials and methods

2.1. Solvents and chemicals

Petroleum ether, ethanol, methanol and methyl-*tert*-butyl ether were from Fisher scientific (Illkirch, France), acetone and orthophosphoric acid from Prolabo (Leuven, Belgium), formic acid from Merck (Darmstadt, Germany) and anhydrous sodium sulfate from Fluka (Saint-Quentin Fallavier, France). HPLC grade water was prepared using a Milli-Q system (Millipore, Bedford, MA).

Naringenin, chlorogenic acid, rutin and ascorbic acid were purchased from Sigma–Aldrich (Saint-Quentin Fallavier, France), prunin, eriodictyol and eriodictyolchalcone from Extrasynthese (Genay, France) and (*E*)-β-carotene from Fluka (Saint-Quentin Fallavier, France). Dihydrokaempferol, 3,4- and 4,5-dicaffeoylquinic acids were obtained from Phytolab (Vestenbergsgreuth, Germany) and 3,5-dicaffeoylquinic acid from Dr. J.L. Poëssel (INRA, Avignon, France). Standards of (*E*)-lycopene, caffeic acid-4-*O*-glucoside and *p*-coumaric acid-4-*O*-glucoside were synthesized in the laboratory according to Ernst, 2002; Galland, Mora, Abert-Vian, Rakotomanomana, & Dangles, 2007, respectively. Standards of (13*Z*)- and (5*Z*)-lycopene were a gift from Dr. V. Böhm (Friedrich Schiller University, Jena, Germany). A standard of phytoene was supplied by DSM Nutritional Products (Kaiseraugst, Switzerland).

2.2. Sampling and processing conditions

Fresh tomatoes (FTa) and the corresponding tomato paste (Pa) were supplied by a local tomato processing factory in August 2008 (*Process A*). Tomato fruits of Nautico, Montégo and Elégy varieties were harvested in the Provence region, analyzed and processed within the week. Processing included washing, crushing, cold-breaking at 70 °C, sieving, water evaporation until 30% dry matter, can filling (70 g net weight) and sterilization at 95 °C for 40 min. The cans were next cooled under a water spray to reach 30 °C.

Tomato sauce was produced using two markedly different processes, as in used in local factories.

Process B: A tomato paste (Pb, 36% dm), a tomato pulp (Pub) and the sauce (Sb) prepared from these two ingredients were supplied by a tomato-based product factory in February 2008. A first set of ingredients including olive oil (1.5% final), onions, corn starch (1.5% final), 25% w/w tomato pulp (6.5% dm), sugar and salt (both 1.5% final) were mixed and brought to 60, 80 and 120 °C in 2 min using a scraped surface heat exchanger. This set is added in a 40:60 weight ratio to tomato paste (Pb) diluted from 36% to 14% dm in water and preheated at 95 °C on a plate heat exchanger. The resulting tomato sauce product (Sb) was stirred in a static mixer at 95 °C for 15 min before being canned (140 g net weight). Cans were immediately cooled under water flow (Fig. 1A).

Process C: Tomato paste (Pc) and the corresponding tomato sauce (Sc) were supplied by a local factory in April 2009. Water (69%) was mixed with a 30% dm tomato paste (Pc, 25.5%, prepared the same day), saccharose (2%), corn starch (1%), fresh onions (1%), rapeseed oil (0.5%) and salt (1%) in a stainless steel tank and heated to boiling. The resulting sauce (Sc) was then canned (180 g net weight) and pasteurized for 35 min at 96 °C before water cooling (Fig. 1B).

2.3. Extraction and analysis of ascorbic acid

Ascorbic acid was analyzed immediately after slicing and crushing fresh tomatoes in a blender or opening cans of pulp, paste or sauce. Aliquots of all tomato products were frozen at −20 °C until analyses for polyphenols and carotenoids.

2.3.1. Extraction

Two grams of fresh tomato, pulp and sauce and 1 g of paste, were diluted in 2 and 4 ml of 2% orthophosphoric acid, respectively. Mixing was achieved with a rotor stator homogenizer

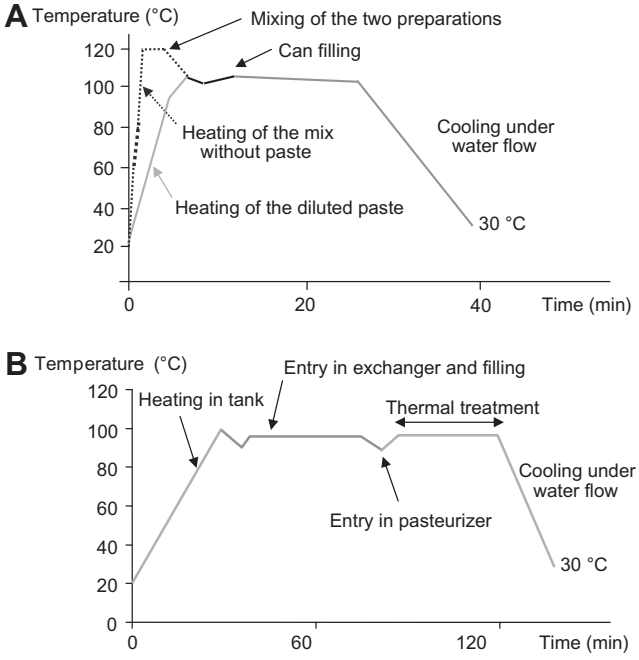

Fig. 1. Thermal backgrounds for processes B (A) and C (B).

(Ultra-Turax T25, IKA) for 1 min. The homogenizer tip was rinsed with 2 ml of diluted orthophosphoric acid. After centrifugation (10,000 rpm, 5 min, 4 °C), the supernatant was filtered with a 0.45 μm filter and immediately injected in HPLC–DAD.

2.3.2. Analysis

HPLC–DAD analyses were carried out on a Hewlett–Packard 1100 apparatus coupled to a UV–visible diode-array detector. Separation was obtained at 24 °C using an Alltima C18 column (5 μm, 150 mm × 4.6 mm) and a guard column (7.5 mm × 4.6 mm). The mobile phase was a solution of 0.05% aqueous formic acid with a flow rate of 0.5 ml min^{-1}. Ascorbic acid was detected at 254 nm.

2.4. Extraction and analysis of carotenoids

2.4.1. Extraction

Four grams of frozen tomato, pulp or sauce were homogenized (9500 rpm, 1 min) with 15 ml of acetone/petroleum ether (50/50, v/v). For paste, 1 g is homogenized with 2 ml of water and 13 ml of acetone/petroleum ether (50/50, v/v). The homogenizer tip was rinsed with 5 ml of solvent. After glass wool filtration, the residue is extracted twice more under magnetic stirring (5 min) using 20 ml of the same solvent mixture. The upper layer of the biphasic extract is transferred to a 50 ml volumetric flask and brought to volume with petroleum ether. After drying over anhydrous sodium sulfate, the carotenoid containing solution is concentrated in vacuo before solubilization in a small volume of dichloromethane/methanol (50/50, v/v) and injection in HPLC–DAD–MS.

2.4.2. Analysis

HPLC–DAD–MS analyses were carried out on a Hewlett–Packard 1050 apparatus coupled to a UV–visible diode-array detector and a Micromass platform LCZ 4000 mass spectrometer. Mass analyses were performed in the positive electrospray ionization mode with cone voltages of 17 and 32 V and a desolvation temperature of 250 °C. Separation was obtained at 30 °C using an YMC C30 column (3 μm, 250 mm × 4.6 mm) and a guard column (7.5 mm × 4.6 mm).

The mobile phase consisted of water (A), methanol (B) and methyl-*tert*-butyl ether (C). The gradient used at a flow rate of 1 ml min^{-1} was 40% A, 60% B at initial time, 30% A, 70% B at 10 min, 25% A, 70% B, 5% C at 15 min, 20% A, 70% B, 10% C at 20 min and 4% A, 11% B, 85% C at 60 min. Carotenoids were detected at 452 and 472 nm. Authentic standards were used for calibration curve.

2.5. Extraction and analysis of phenolic compounds

2.5.1. Extraction

Aliquots of 16 g for frozen tomato, pulp or sauce and 4 g for paste were extracted three times with, respectively, 30 and 5 ml of ethanol using a rotor stator homogenizer (9500 rpm, 1 min) and separated by centrifugation (10,000 rpm, 5 min, 4 °C). The homogenizer tip was rinsed with 2 ml of solvent. The combined extracts were concentrated in vacuo and the residue taken up in 10 ml (tomato, pulp and sauce) or 5 ml (paste) of methanol before injection on HPLC–DAD and fast HPLC–DAD–MS–MS.

2.5.2. Analysis

For identification of phenolic compounds, fast HPLC–DAD–MS–MS analyses were carried out on a Waters Acquity Ultra Performance LC apparatus coupled to a UV–visible diode-array detector and a Bruker Daltonics HCT Ultra mass spectrometer. The conditions for the MS operating in the negative electrospray ionization mode were as follows: capillary voltage, 2 kV; nitrogen flow rate, 12 l min^{-1}; nebulization pressure, 60 psi; desolvation temperature, 365 °C. The ion trap was operated in the Ultrascan mode from *m/z* 100–1300. Separation was obtained at 30 °C using a Waters HSST3 C18 column (1.8 μm, 50 mm × 2.1 mm) and a guard column. The mobile phase consisted of 0.05% aqueous formic acid (A) and methanol (B). The flow rate was 0.4 ml min^{-1} and the gradient as follows: 15% B at initial time, 21% B at 2.27 min, 23% B at 4.76 min, 60% B at 12.2 min and 100% B at 15 min. For quantitative determination, HPLC–DAD analyses were carried out on a Hewlett–Packard 1100 apparatus coupled to a UV–visible diode-array detector. Separation was obtained at 30 °C using an Alltima C18 column (5 μm, 150 mm × 4.6 mm) and a guard column (7.5 mm × 4.6 mm). The solvent system was 0.05% aqueous formic acid (A) and methanol (B) at a flow rate of 0.5 ml min^{-1} (0–46 min) and 0.7 ml min^{-1} (46–118 min). The gradient was as follows: 15% B ($t = 0$), 21% B at 20 min, 23% B at 42–52 min, 60% B at 107 min and 100% B at 112 min. Phenolic compounds were quantified at 280 nm except naringenin chalcone at 365 nm. Authentic standards were used for calibration curves.

2.6. Statistical analyses

Analyses of variance were performed using XLStat 2008.3.02 (Addinsoft SARL, France). Results are expressed as means ± SD. ANOVA was performed to identify differences among groups.

3. Results and discussion

3.1. Identification

Analysis of the various industrial tomato products permitted an exhaustive identification of phenolic compounds, alkaloids and carotenoids. Available standards were injected in parallel runs and compared for retention time, mass fragmentation and UV–visible spectra with compounds detected in the tomato-based products. Other molecules were tentatively assigned by comparison with literature data.

3.1.1. Identification of phenolic compounds and alkaloids

In fresh tomato, pulp, paste and sauce, 57 different phenolic compounds were identified as presented in Table 1. Hydroxycinnamic acids were largely represented, either in the form of acylated quinic acid derivatives, or as hexoside derivatives. Caffeic acid, the main aglycone, was present in 3-*O*-, 4-*O*- and 5-*O*-caffeoylquinic acid as well as in 3,4-, 3,5-, and 4,5-dicaffeoylquinic acids. The latter compound was minor compared to the other isomers and was not detected in paste Pb. Additionally, 3,4,5-tricaffeoylquinic acid and its never described hexoside derivative (t_R 8.5 min, *m/z* 839) were detected in all tomato products. *p*-Coumaric acid was detected once as *p*-coumaroylquinic acid and twice as hexoside derivatives. Five different hexosides of caffeic acid were detected in our study while a metabolome database from 96 cultivars (Moco et al., 2006) revealed up to six isomeric caffeic acid hexosides. Indeed, structural variations can arise from linkage of the hexose moiety to the 3-hydroxy, 4-hydroxy and carboxylic acid positions, whereas glucose, galactose or mannose may be common plant hexoses. Caffeic acid-4-*O*-glucoside and *p*-coumaric acid-4-*O*-glucoside display maximal absorption wavelengths at, respectively, 290 and 294 nm which are characteristic of the electronic density modification induced by the glycosylation of the hydroxyl group at carbon-4. Ferulic acid and sinapic acid as well as a commonly abundant plant phenolic acid, syringic acid, were also found as hexosides. New or recently described phenolics were detected in the possible forms of hexosides of dihydroxyphenylacetic acid, hydroxyphenylpropionic acid, dihydroxyphenylpropionic acid and its O-methylated derivative in all tomato-based products.

Table 1
Phenolic compounds and alkaloids identified in tomato products.

t_R (min)	λ_{max} (nm)	$[M-H]^-$ (*m/z*)	Fragment ions (*m/z*)	Identification	Origin
1.7	292sh, *324*	353	191 > 179, 135	3-*O*-Caffeoylquinic acid[a,d,e]	FTa, Pa, Pb, Pub, Sb, Pc, Sc
1.7		341	323, 233, 203, 179, 101	Caffeic acid-hexose (I)[a,d,e]	FTa, Pa, Pb, Pub, Sb, Pc, Sc
1.9		503	413, 341, 179	Caffeic acid-dihexose	FTa, Pa, Pub
2.0		341	281, 251, 233, 203, 179, 161, 135	Caffeic acid-hexose (II)[a,d,e]	FTa, Pa, Pb, Pub, Sb, Pc, Sc
2.2	222, *294*	325	651, 325, 163, 119	*p*-Coumaric acid-4-*O*-β-D-glucoside[c]	FTa, Pa, Pb, Pub, Sb, Pc, Sc
2.2	236sh, *290*, 316sh	341	179, 135	Caffeic acid-4-*O*-β-D-glucoside[c]	FTa, Pa, Pb, Pub, Sb, Pc, Sc
2.5	*288*	329	311, 209, 167	Dihydroxyphenylacetic acid-hexose	FTa, Pa, Pb, Pub, Sb, Pc, Sc
2.5		327	165, 121	Hydroxyphenylpropionic acid-hexose[e]	FTa, Pa, Pb, Pub, Sb, Pc, Sc
2.5	236, 298sh, *325*	341	323, 281, 251, 221, 179, 135	Caffeic acid-hexose (III)[a,d,e]	FTa, Pa, Pb, Pub, Sb, Pc, Sc
2.6	216, *279*	343	298, 223, 181, 137	Dihydroxyphenylpropionic acid-hexose	FTa, Pa, Pb, Pub, Sb, Pc, Sc
2.9	216, *279*	343	298, 223, 181, 137	Dihydroxyphenylpropionic acid-hexose	FTa, Pa, Pb, Pub, Sb, Pc, Sc
3.1		325	265, 187, 163, 145, 119	*p*-Coumaric acid-hexose [a,,d,e]	FTa, Pa, Pb, Sb, Pub, Pc, Sc
3.1	298sh, *323*	341	323, 281, 221, 179, 135	Caffeic acid-hexose (IV)[a,,e]	FTa, Pa, Pb, Sb, Pub, Pc, Sc
3.1	298sh, *326*	353	191	5-*O*-Caffeoylquinic-acid[a,c,d,e]	FTa, Pa, Pb, Sb, Pub, Pc, Sc
3.4	223sh, *280*, 300sh	357	195, 136	Hydroxymethoxyphenylpropionic acid-hexose[e]	FTa, Pa, Pb, Pub, Sb, Pc, Sc
3.5	219, 234, 298sh, *326*	353	191 < 179 < 173, 135	4-*O*-Caffeoylquinic acid[a,d,e]	FTa, Pa, Pb, Pub, Sb, Pc, Sc
3.5	236, 295sh, *318*	355	295, 217, 193, 175, 134	Ferulic acid-hexose[a,d,e]	FTa, Pa, Pub
3.6		385	223, 208, 179, 164	Sinapic acid-hexose[e]	FTa, Pa, Pub, Sb, Pc, Sc
4.0		903	741, 609, 300	Quercetin–glucose–rhamnose–apiose-hexose[d]	FTa, Pa, Pb, Pub, Sb, Pc, Sc
4.4	*289*, 326sh	595	577, 475, 415, 385, 355, 313	Naringenin dihexose[a,d]	FTa, Pa, Pb, Pub, Sb, Pc, Sc
4.5	310	353	191	5-*O*-Caffeoylquinic acid (*cis* form)[b]	FTa, Pa
4.8	237, 299sh, *310*	337	191, 163	*p*-Coumaroylquinic acid[a,e]	FTa, Pa, Pb, Pub, Sb, Pc, Sc
5.4	*254*, 265sh, *351*	771	609, 301	Rutin-hexose[a,d]	FTa, Pa, Pb, Pub, Sb, Pc, Sc
5.7	248sh, *277*, 325sh	557	359, 299, 255, 197, 153	Syringic acid-hexose derivative	FTa, Pa, Pb, Pub, Sb, Pc
6.1	*284*, 310sh	631	433, 271	Naringenin-hexose derivative	FTa, Pa, Pb, Pub, Sb, Pc, Sc
6.1		611	491, 449, 287, 151	Dihydrokaempferol-dihexose	FTa, Pa, Pc, Sc
7.3	248, *291*, 324sh	433	387, 343, 313	Naringenin-hexose (I)[d]	FTa, Pa, Pb, Pub, Sb, Pc, Sc
7.9	250sh, *282*	449	287, 151, 135	Eriodictyol-hexose[dd]	FTa, Pa, Pb, Pub, Sb, Pc, Sc
8.0		625	463, 301	Quercetin-dihexose	Pub, Sb
8.1		359	197, 153	Syringic acid-hexose	FTa, Pa, Pb, Pub, Sb, Pc, Sc
8.1	*284*, 330sh	433	313, 271, 151	Naringenin-hexose (II)	FTa, Pa, Pb, Pub, Sb, Pc, Sc
8.5	248, 290sh, *324*	839	677, 515, 353	Tricaffeoylquinic acid-hexose	FTa, Pa, Pb, Pub, Sb, Pc, Sc
9.0	*256*, 268sh, 293sh, *355*	741	609, 547, 475, 343, 300	Quercetin 3-*O*-(2″-*O*-β-apiofuranosyl-6″-*O*-α-rhamnopyranosyl-β-glucopyranoside)[a,b,d]	FTa, Pa, Pb, Pub, Sb, Pc, Sc
9.2	*282*, 336sh	433	271	Naringenin-7-*O*-glucoside[c] (prunin)	FTa, Pa, Pb, Pub, Sb, Pc, Sc
9.2	242, 300sh, *324*	515	353, 335, 299, 203, 191 < 179 < 173	3,4-Dicaffeoylquinic acid[a,c,d,e]	FTa, Pa, Pb, Pub, Sb, Pc, Sc
9.2		1227	1095, 1065, 933, 771	Esculeoside B (I)[a,d,e]	FTa, Pa, Pb, Pub, Sb, Pc, Sc
9.3	242, 300sh, *328*	515	353, 191 > 179 > 173	3,5-Dicaffeoylquinic acid[a,c,d,e]	FTa, Pa, Pb, Pub, Sb, Pc, Sc
9.3		433	271, 151	Naringenin-hexose (III)	FTa, Pa, Pb, Pub, Sb, Pc, Sc
9.4	*285*, 330sh	597	477, 417, 387, 357, 315	Phloretin–di-hexose[b]	FTa, Pa, Pb, Pub, Sb, Pc, Sc
9.7	*256*, 293sh, *354*	609	301	Rutin[a,c,d,e]	FTa, Pa, Pb, Pub, Sb, Pc, Sc
10.1		1227	1095, 1065, 933, 771	Esculeoside B (II)[a,e]	FTa, Pa, Pb, Pub, Sb, Pc, Sc
10.1	242, 300sh, *328*	515	353, 299, 255, 191 < 179 < 173	4,5-Dicaffeoylquinic acid[a,c,d,e]	FTa, Pa, Pub, Sb, Pc, Sc
10.3		433	313, 271, 151	Naringenin-hexose (IV)	FTa, Pa, Pc, Sc
10.3		947	741, 723, 591, 300, 271	Quercetin–glucose–rhamnose–apiose-sinapic acid	FTa, Pa, Pb, Pub, Sb
10.5	250, 268, *292*, 321sh	287	151, 135, 107	Eriodictyol[c]	FTa, Pa, Pub, Sb, Pc, Sc
10.5	230sh, *268*, 290, *350*	593	345, 285	Kaempferol-3-*O*-rutinose[a,d]	FTa, Pa, Pb, Pub, Sb, Pc, Sc
10.5	250, 268, *292*, 321	917	741, 723, 609, 591, 300, 271	Quercetin–glucose–rhamnose–apiose-ferulic acid	FTa, Pa, Pb, Pub, Sb, Pc, Sc
10.5		1269	1137, 1107, 1065, 1047, 975, 933, 867, 813, 753	Lycoperoside F, G ou esculeoside A (I)[a,d,e]	FTa, Pa, Pub, Pb, Sb
10.7		1269	1209, 1137, 1107, 1095, 1047, 975, 933, 867, 813, 753	Lycoperoside F, G or esculeoside A (II)[a,d,e]	FTa, Pa, Pub, Pb, Sb
10.7		1049		Lycoperoside H (I)[a,d]	FTa, Pa, Pb, Pub, Sb, Pc, Sc
10.7		887	741, 723, 609, 591, 300, 271	Quercetin–glucose–rhamnose–apiose-*p*-coumaric acid[a]	FTa, Pa, Pb, Sb, Pub, Pc, Sc

(*continued on next page*)

Table 1 (*continued*)

t_R (min)	λ_{max} (nm)	$[M-H]^-$ (*m/z*)	Fragment ions (*m/z*)	Identification	Origin
10.3		433	271, 253	Naringenin-hexose (V)	FTa, Pa, Pb, Pub, Sb, Pc, Sc
11.0		1049		Lycoperoside H (II)[a,d]	FTa, Pa, Pb, Pub, Sb, Pc, Sc
11.4		921	877, 741, 723, 609, 300	Quercetin–glucose–rhamnose–apiose-syringic acid	FTa, Pa, Pb, Pub, Sb, Pc, Sc
11.4	*290*, 330sh	271	177, 151, 119, 107	Naringenin[a,c,d,e]	FTa, Pa, Pb, Sb, Pub, Pc, Sc
11.4	251, 293, *328*	677	515, 353, 335, 191 < 179 = 173	Tricaffeoylquinic acid[a,d,e]	FTa, Pa, Pb, Sb, Pub, Pc, Sc
11.9	243, 341ep, *368*	271	177, 151, 107	Naringenin chalcone[a,d,e]	FTa

FTa: fresh tomato; Pa: tomato paste prepared with FTa; Pb: tomato paste; Pub: tomato pulp; Sb: tomato sauce prepared with Pb and Pub; Pc: tomato paste; Sc: tomato sauce prepared with Pc. Wavelength of maximal UV–visible absorption italicised.

[a] According to Moco et al. (2006, 2007).
[b] According to Slimestad et al. (2008).
[c] Standard eluted in the same chromatographic conditions.
[d] According to Mintz-Oron et al. (2008).
[e] According to Gomez-Romero et al. (2010).

The dihydroxyphenylpropionic acid aglycone (t_R 2.6 and 2.9 min, *m/z* 343) is favoured over homologous homovanillic acid (Gomez-Romero, Segura-Carretero, & Fernandez-Gutierrez, 2010) owing to the retention time, the absence of even fragment ions typical for methoxylated phenolic compounds and the presence of an O-methylated derivative. Additionally, the presence of 2-(2,5- and 2-(2,3-dihydroxyphenyl)acetic acid, 3-(2- and 3-(4-hydroxyphenyl)propionic acid has been observed in a few plant species (Kindl, 1969; Smolarz & Nowak, 1998).

Flavonoids are highly represented in tomato, although their occurence has been almost exclusively restricted to tomato epidermis. Flavonol hexosides such as quercetin dihexose–deoxyhexose–pentose (t_R 4.0 min, *m/z* 903), quercetin dihexose–deoxyhexose (t_R 5.4 min, *m/z* 771), quercetin hexose–deoxyhexose–pentose (t_R 9.0 min, *m/z* 741) and quercetin hexose–deoxyhexose (t_R 9.7 min, *m/z* 609) were detected in fresh tomato, as well as in all the processed products (Moco et al., 2007). Quercetin dihexoside (t_R 8.0 min, *m/z* 625) was only found in pulp Pub and the corresponding sauce Sb. The identity of the quercetin hexose–deoxyhexose–pentose (t_R 9.0 min, *m/z* 741) was recently assigned by NMR as quercetin 3-*O*-(2″-*O*-β-apiofuranosyl-6″-*O*-α-rhamnopyranosyl-β-glucopyranoside) (Slimestad, Fossen, & Verheul, 2008). Acylation of quercetin-hexose–deoxyhexose–pentose with *p*-coumaric acid (t_R 10.7 min, *m/z* 887) was observed in all the studied tomato products as reported recently (Mintz-Oron et al., 2008; Moco et al., 2007). Since the quercetin glycoside part was structurally assigned and this quercetin trisaccharide detected in all the studied products, we propose that the newly reported derivatives bearing ferulic acid (t_R 10.5 min, *m/z* 917), sinapic acid (t_R 10.3 min, *m/z* 947) and syringic acid (t_R 11.4 min, *m/z* 921) could arise from the specific acylation of quercetin 3-*O*-(2″-*O*-β-apiofuranosyl-6″-*O*-α-rhamnopyranosyl-β-glucopyranoside). Interestingly, these diversely acylated compounds were detected in fresh tomato fruits and in the various processed products, except for the sinapoyl derivative which was not detected in paste Pc and sauce Sc. Last, kaempferol-3-rutinose was the only other flavonol detected in fresh and processed tomato products (Mintz-Oron et al., 2008; Moco et al., 2007).

Flavanones are ubiquitary secondary metabolites in tomato. The uncyclized form, naringenin chalcone (λ_{max} 368 nm) exerts protective effects against light-induced oxidative stress and it is thus mainly located in tomato epidermis. This highly acid-sensitive molecule was only detected in fresh tomato fruit (FTa). Indeed, tissue destructuration induced by processing or homogenization for sample extraction is known to displace the equilibrium towards cyclized naringenin. In this work, no acidification was performed in order to prevent chalcone cyclization during polyphenol extraction. Naringenin was however detected in fresh and processed tomato along with a naringenin dihexoside and five isomers of naringenin hexoside. Owing to the lack of absorption spectra, naringenin and its chalcone form could not always be distinguished. However, the presence of these hexosides in processed products is in favour of the cyclized form. Moreover, a structurally related compound (t_R 6.1, *m/z* 631) could not be unambiguously assigned although it displayed the *m/z* 433 and 271 fragments typical of naringenin hexosides.

The compound eluting at t_R 10.5 min displays a parent ion at *m/z* 287 and a fragment ion at *m/z* 151 corresponding to the cyclized carboxydihydroxyphenyl moiety typical for flavonoids with a 5,7-dihydroxylation pattern for the A nucleus. LC–MS analysis of authentic eriodictyol and dihydrokaempferol, the two isomeric compounds matching these mass data, revealed the presence of eriodictyol similarly to recent findings by Mintz-Oron et al. (2008). Furthermore, we also report the presence of an eriodictyol hexoside (t_R 7.9 min, *m/z* 449) in fresh tomato as in all the processed products along with an eriodictyol dihexoside (t_R 6.1 min, *m/z* 611) in FTa, Pa, Pc and Sc.

The last phenolic compound to be detected appeared to be phloretin-3′,5′-di-*C*-glucoside at t_R 9.4 min. The UV spectrum exhibited absorption maximum at 285 nm with a shoulder at 330 nm as found by Slimestad et al. (2008). A parent ion at *m/z* 597 and fragments at *m/z* 477, 417, 387 and 357 revealing the loss of two hexose units confirmed the assignment of this structure.

Steroidal alkaloids were also detected in fresh and processed tomato after mass search of the parent ion as found in databases (Gomez-Romero et al., 2010; Mintz-Oron et al., 2008; Moco et al., 2006). Two isomeric esculeosides B (t_R 9.2 and 10.1 min, *m/z* 1227), displaying fragmentation patterns similar to those in Moco et al. (2006), were identified in all the studied products. The parent ion *m/z* 1269 corresponding to lycoperoside F or lycoperoside G or esculeoside A was detected twice at t_R 10.5 and 10.7 min in agreement with reported findings. Besides, fragment ions *m/z* 1107, 975, 813 revealed the loss of terminal xylose and glucose units from these pentasaccharides. Moreover, all the tomato products studied contained trace amounts of two lycoperoside H isomers (t_R 10.7 and 11.0 min, *m/z* 1049), although no fragmentation could be detected. Finally, it is noteworthy that all the phenolic and alkaloid compounds detected in the initial products (FTa, Pb, Pub, Pc) were recovered in the processed products (Pa, Sb, Sc), outlining their overall stability.

3.1.2. Identification of carotenoids

Up to 28 different carotenoids were identified or tentatively identified in tomato, pastes, pulp and sauces (Table 2). As expected, (*E*)-lycopene was the main carotenoid in fresh tomato and in all tomato products analyzed (Tables 3–5). Seventeen lycopene *Z*-isomers were also detected in the various tomato products. Two of them were identified as (5*Z*) and (13*Z*) after the injection of the

purified standards. Other lycopene *Z*-isomers were tentatively identified by comparing their UV–visible spectra with (*E*)-lycopene and by comparison with literature data (Breitenbach, Braun, Steiger, & Sandmann, 2001; Hengartner, Bernhard, Meyer, Englert, & Glinz, 1992). In reference to an (*E*)-carotenoid, the UV–visible spectra of the mono *Z*-isomers usually present a small hypsochromic shift (2–6 nm) and a new absorption band, called the *cis*-peak, at around 142 nm below the longest wavelength of maximum absorption (Britton, 1995). The intensity of the *cis*-peak is higher as the *Z*-double bond is nearer to the centre of the molecule (15,15′ position). The relative intensity of a *cis*-peak is expressed as % A_B/A_{II}, with A_B: absorbance of the *cis*-peak and A_{II}: absorbance of the middle main absorption band. Using these considerations and literature data, four other mono *Z*-isomers, namely (15*Z*), (11*Z*), (9*Z*) and (7*Z*), could be tentatively identified. Among those, (11*Z*) is not described in the literature and its structure was assigned based on its intermediate value of % A_B/A_{II} (0.36), between those of (9*Z*) and (13*Z*) lycopene isomers (0.16 and 0.58, respectively). Eleven di-(*Z*)-lycopenes were tentatively identified considering their λ_{max} and the presence and intensity of the *cis*-peak. Three di-*Z*-lycopenes were already described in the literature: (5*Z*, 9′*Z*) and (5*Z*, 13′*Z*) by Breitenbach et al. (2001) and (5*Z*, 5′*Z*) by Hengartner et al. (1992). Two others could be tentatively attributed to (5*Z*, 13*Z*)- and (5*Z*, 15*Z*)-lycopenes due to their λ_{max} and the high intensity of their *cis*-peak (respectively 0.53 and 0.64).

Four (*Z*)-lycopenes were detected in fresh tomatoes (FTa), namely (5*Z*)-, (9*Z*)- and (13*Z*)-lycopenes as well as a non-identified di-(*Z*)-isomer, which were still present in the corresponding tomato paste (Pa). However, processed products contain a wider variety of mono- and di-(*Z*)-lycopene isomers, which is in accordance with their possible formation from (*E*)-lycopene by heat treatment. Indeed, (7*Z*)- and (5*Z*, 9*Z*)-lycopenes were newly tentatively identified in the corresponding tomato paste (Pa). Additional lycopene isomers were detected in Pb, Pub and Sb although (15*Z*) and (5*Z*, 5′*Z*) tentatively identified in Pb and Pub, respectively, were not detected in the tomato sauce Sb. Paste Pc contained three mono-(*Z*)-lycopenes, which were still found in the sauce Sc along with the newly identified (11*Z*). Three of the four di-(*Z*)-isomers detected in Pc could be found in Sc along with four new di-*Z*-isomers.

Another lycopene-derived molecule, namely 1,2-epoxy-lycopene, was tentatively identified in fresh tomatoes and in all the processed products. 1,2-Epoxy-lycopene has already been found as a minor carotenoid in red tomato fruits (Ben-Aziz, Britton, & Goodwin, 1973).

Other carotenoids were also detected in fresh tomato and processed tomato products like (*E*)-β-carotene and phytoene which assignments were confirmed by the analysis of standards. Phytofluene, another biosynthetic precursor of lycopene, was also tentatively identified in all tomato products. β-Carotene is the second main carotenoid in tomato, confering to this fruit a provitaminic A value. One of its isomer tentatively identified as (13*Z*) due to its UV–visible spectra characteristics comparable with literature data (Böhm, Puspitasari-Nienaber, Ferruzzi, & Schwartz, 2002) was detected in Pa, Pb, Pub and Sb as well as in Pc and Sc. 1,2-Epoxy-phytoene was possibly present in all processed products, but not in fresh tomatoes. Although found in very small amounts in red tomato fruits (Britton & Goodwin, 1969), 1,2-epoxy-phytoene may result from oxidation of phytoene during processing in our study.

Finally, the first peak to elute at 41.18 min could correspond to a lutein mono-ester since it has an UV–visible spectra identical to that of lutein but a retention time higher than free lutein. Unfortunately, mass data were not available and the structure of the fatty acid could not be determined. Although free lutein is already known as a minor carotenoid of tomato, it was the first time that a lutein ester was detected in both fresh tomato and tomato processed products.

3.2. Industrial processing of fresh tomato into tomato paste

The impact of industrial processing on the stability of tomato phenolic compounds, carotenoids and ascorbic acid was assessed for the transformation of fresh tomato into tomato paste (Table 3). Hydroxycinnamic acids were not found in their free forms neither in fresh nor in processed tomato. Glycosylated derivatives were largely present with hexosides of caffeic acid and coumaric acid accounting, respectively for 23.0 mg and 13.2 mg/100 g dw in fresh tomato. This is in agreement with contents reported in the literature of, respectively, 27.5 and 15.7 mg/100 g dw for the sum of both glucosides and glucose esters of caffeic acid and coumaric acid in red cherry tomato pulp (Fleuriet & Macheix, 1985). Caffeoyl derivatives of quinic acid appeared as the second largest hydroxycinnamic acid group. The contents in monocaffeoylquinic acids and less described dicaffeoylquinic acids were unexpectedly close in this mixture of three tomato cultivars. In fact, chlorogenic acid may be underestimated owing to coelution with a caffeic acid hexoside isomer. Processing was shown to increase significantly by a ca. 1.5-fold factor the levels in hydroxycinnamic acid hexosides and dicaffeoylquinic acids, while this factor may be greater for monocaffeoylquinic acids. An improved extraction of these compounds could result from both tissue destructuration and thermal step. Re et al. (2002) studied a similar process although sampling after breaking and before the concentration step. After hot break, chlorogenic acid content increased by a factor 3 whereas it was not affected after cold break suggesting that heat is required for a better release of microconstituents from tomato cells. For hydroxycinnamic acid glycosides, they reported a slight increase in contents whatever the breaking step temperature as observed for cold break in our study. Additionally, we report for the first time the contents in two specific glycosides, namely caffeic acid-4-*O*- and *p*-coumaric acid-4-*O*-glucosides. They both appear as important contributors to the pool of hydroxycinnamic acid glycosides and their levels doubled upon processing.

Flavonols are highly represented with a major contribution of quercetin oligosaccharides at the level of 46.5 mg/100 g dw in fresh tomato. Rutin constitutes the two thirds of this pool, while its kaempferol analog is present in a lesser amount. Processing was apparently not found to affect flavonol contents, as observed by Capanoglu et al. (2008) for rutin and its apioside derivative. In fact, these authors found a 2-fold increase after the cold break step, then a reduction upon sieving while no impact of the evaporation and pasteurization steps. Additionally, Re et al. (2002) observed a larger decrease in rutin after sieving for the cold compared to the hot break step. These results clearly suggest that flavonol levels in paste are positively controlled by heat in the break step and negatively influenced by sieving, which is a critical operation for all microconstituents specifically located in tomato exocarp. Oxidizable quercetin oligosaccharides are probably fairly stable at the high temperatures required for the evaporation and pasteurization steps owing to low dissolved oxygen levels.

The flavanone stability was evaluated through the titration of naringenin chalcone, naringenin and two isomeric naringenin hexosides. Naringenin chalcone proved unstable and disappeared totally upon processing. The low amount of naringenin in the fresh fruits was increased by an 18-fold factor in the paste supporting the cyclization of only 22% of naringenin chalcone. The dramatic loss in naringenin chalcone has been classically observed for cold and hot break processes (Capanoglu et al., 2008; Re et al., 2002). The lipophilic chalcone, poorly extracted from the epicarp cells whatever the mechanical/thermal treatments used, is mainly eliminated upon sieving (Capanoglu et al., 2008). Chemical degradation

Table 2
Carotenoids and carotenoid-derived products identified in tomato products.

t_R (min)	λ_{max} (nm)	Intensity of *cis*-peak (% A_B/A_{II})	$[M+H]^+$ *m/z*	Identification	Origin
41.18	416, *446*, 474	–	–	Lutein mono-ester	FTa, Pa, Pb, Pub, Sb, Pc, Sc
42.09	276, *286*, 297	–	561	1,2-Epoxy-phytoene	Pa, Pb, Pub, Sb, Pc, Sc
50.01	275, *286*, 297	–	n.d.	Phytoene[a]	FTa, Pa, Pb, Pub, Sb, Pc, Sc
50.75	332, 348, 368	–	543	Phytofluene	FTa, Pa, Pb, Pub, Sb, Pc, Sc
51.71	332, *348*, 368	–	543	Isomer of phytofluene	FTa, Pa, Pb, Pub, Sb, Pc, Sc
51.95	290, 380, 400, *424*	–	n.d.	Isomer of ζ-carotene	FTa, Pa
51.97	338, 420, *446*, 468	0.44	537	(13*Z*)-β-Carotene[b]	Pa, Pb, Pub, Sb, Pc, Sc
52.92	426, *452*, 478	–	537	(*E*)-β-Carotene[a]	FTa, Pa, Pb, Pub, Sb, Pc, Sc
53.77	382, 402, *426*	–	n.d.	ζ-Carotene	FTa, Pa, Pb, Pub, Sb, Pc, Sc
57.16	362, 428, *456*, 488	0.27	537	Di-(*Z*)-lycopene	Sc
57.58	360, 430, *458*, 486	0.32	537	Di-(*Z*)-lycopene	Sb, Pb
57.65	430, *460*, 492	–	537	Di-(*Z*)-lycopene	Sc
57.76	360, 440, *468*, 498	0.73	537	(15*Z*)-Lycopene[c]	Pb
57.91	432, *462*, 490	–	537	Di-(*Z*)-lycopene	FTa, Pa, Pb, Sb, Pc, Sc
58.10	438, *458*, 490	–	537	Di-(*Z*)-lycopene	Sc
58.15	362, 440, *468*, 494	0.53	537	(5*Z*,13*Z*)-Lycopene	Pc
58.34	362, 436, *462*, 492	0.13	537	Di-(*Z*)-lycopene	Pb, Sb
58.58	362, 440, *466*, 494	0.64	537	(5*Z*,15*Z*)-Lycopene	Sc
58.70	362, 440, *464*, 494	0.36	537	(11*Z*)-Lycopene	Sc
59.18	362, 440, *466*, 496	0.58	537	(13*Z*)-Lycopene[a]	FTa, Pa, Pb, Pub, Sb, Pc, Sc
59.61	360, 432, *458*, 488	0.29	537	(5*Z*,13′*Z*)-Lycopene[d]	Sb, Pc, Sc
60.98	446, *472*, 502	–	553	1,2-Epoxy-lycopene	FTa, Pa, Pb, Pub, Sb, Pc, Sc
61.28	362, 440, *466*, 496	0.16	537	(9*Z*)-Lycopene[d]	FTa, Pa, Pb, Pub, Sb, Pc, Sc
61.38	362, 440, *466*, 498	0.12	537	(5*Z*,9′*Z*)-Lycopene[d]	Pa, Pb, Pub, Sb, Pc, Sc
61.67	446, *470*, 504	–	537	(7*Z*)-Lycopene[d]	Pa
61.87	450, *474*, 506	–	537	(5*Z*,5′*Z*)-Lycopene[c]	Pub
63.76	446, *472*, 502	–	537	(*E*)-Lycopene[a]	FTa, Pa, Pb, Pub, Sb, Pc, Sc
64.11	446, *472*, 502	–	537	(5*Z*)-Lycopene[a]	FTa, Pa, Pb, Pub, Sb, Pc, Sc

FTa, Pa, Pb, Pub, Sb, Pc and Sc as for Table 1. Wavelength of maximal visible absorption italicised.
[a] Standard eluted in the same chromatographic conditions.
[b] According to Böhm et al. (2002).
[c] According to Hengartner et al. (1992).
[d] According to Breitenbach et al. (2001).

of naringenin is doubtful because of its robust cyclic structure encompassing a monophenolic B ring.

(*E*)-Lycopene seemed to be rather stable during the preparation of paste Pa (Table 3). Data in the literature are controversial, some authors describing a loss in (*E*)-lycopene content by 32% (Capanoglu et al., 2008) others an increase by 36% (Abushita et al., 2000) and 260% (Re et al., 2002). Qualitatively, mono- and di-*Z*-isomers of lycopene originally present in FTa were conserved during paste preparation (Table 2), except (7*Z*)- and (5*Z*, 9′*Z*)-lycopenes which were newly tentatively identified. Lycopene *Z*-isomers decreased by ca. a 2-fold factor in tomato paste. Partial retro-isomerization of *Z*-isomers of lycopene into the most thermodynamically stable (*E*)-lycopene could have occured albeit with a low impact on (*E*)-lycopene content. Oxidative degradation of lycopene *Z*-isomers is the most likely pathway providing a diversity of yet undetected molecules. For (*E*)-β-carotene, a significant loss was observed in the processing of fresh tomato into tomato paste (Table 3), similarly to Abushita et al. (2000) and Capanoglu et al. (2008). This loss could be at least partly explained by the formation of (13*Z*)-β-carotene which was more easily detected in Pa than in FTa (Table 2).

Finally, half of the ascorbic acid present in the original fruit was lost along processing resulting in 185 mg/100 g dw in the final paste. Capanoglu et al. (2008) observed a similar loss although they showed a continuous degradation of this vitamin all over the process.

Heat treatment during breaking increases microconstituent extractability through cell wall and cell membrane disruption. Indeed, Lopez-Sanchez et al. (2011) showed a particle size reduction for tomatoes that were heat treated before blending compared to those treated in the reverse order. On the other hand, sieving affects negatively the levels in phenolics and carotenoids more specifically located in the epidermis, while heat-induced isomerization decreases (*E*)-carotenoid contents (Shi, Dai, Kakuda, Mittal, & Xue, 2008). On a nutritional point of view, process A, with a cold break step, was found very respectful of the microconstituents studied. The used evaporation and long pasteurization steps may not be as deleterious as expected for oxidizable ascorbic acid and phenolics because of low oxygen levels at elevated temperatures.

3.3. Industrial processing of tomato paste into tomato sauce

Two highly different industrial preparation of tomato sauces were evaluated for stability of phenolic compounds, carotenoids and ascorbic acid (Fig. 1). Manufacture of a tomato sauce (Sc) required tomato paste (Pc) as starting material, whereas a more complex process made use of both tomato paste (Pb) and pulp (Pub) for preparation of tomato sauce (Sb).

Levels in phenolic compounds for tomato paste Pb were very close to those found in tomato paste Pa except for rutin and naringenin (Table 4). By contrast, tomato paste Pc presented nearly twice as much phenolics compared to Pa and Pb in particular for caffeic acid hexosides, flavonols and flavanones (Table 5). The latter two classes being specifically located in the epidermis, these differences could suggest either a response to abiotic factors or a genotype influence or a lower loss upon sieving. In the three pastes, it is noteworthy that caffeic acid-4-*O*-glucoside contributes for half to caffeic acid hexosides. Moreover, this is a far more important contributor compared to the well-known chlorogenic acid. Processing of paste Pc into sauce Sc did not affect significantly the levels in the specific molecules or groups under evaluation. This indicates an apparent high thermal stability of the phenolic microconstituents during heating to the boiling and pasteurization. The oxygen concentration, which is ca. 258 μM (25 °C) after ingredient mixing in the open tank unit, decreases nearly 10 times upon heating to 96 °C to reach 27 μM concentrations in cans. Physicochemical conditions are thus minimally

Table 3
Evolution of microconstituents during the processing of fresh tomatoes in tomato paste (process A).

Compound	In fresh tomatoes (FTa) (mg/100 g of dry matter)	In tomato paste (Pa) (mg/100 g of dry matter)
Caffeic acid-hexosides including	23.0 ± 2.0[a]	29.3 ± 1.6[b]
Caffeic acid-4-*O*-glucoside	6.9 ± 1.1[a]	12.3 ± 1.4[b]
p-Coumaric acid-hexosides including	13.2 ± 1.5[a]	18.6 ± 0.8[b]
p-Coumaric acid-4-*O*-glucoside	7.5 ± 0.8[a]	17.0 ± 0.6[b]
Caffeoylquinic acids including	3.2 ± 1.1[a]	12.9 ± 1.9[b]
5-*O*-Caffeoylquinic acid	0.97 ± 0.42[a]	8.1 ± 1.6[b]
Dicaffeoylquinic acids including	2.35 ± 0.08[a]	3.25 ± 0.37[b]
3,5-Dicaffeoylquinic acid	0.71 ± 0.10[a]	1.04 ± 0.16[b]
3,4-Dicaffeoylquinic acid	1.31 ± 0.19[a]	2.20 ± 0.48[b]
4,5-Dicaffeoylquinic acid	0.33 ± 0.05[a]	nq[b]
Tricaffeoylquinic acid	1.1 ± 0.7[a]	nq[b]
Quercetin-oligosaccharides including	47 ± 9	47.4 ± 1.7
Rutin	30 ± 8	31.1 ± 0.9
Kaempferol-3-rutinose	3.9 ± 1.8	2.8 ± 0.9
Naringenin-hexosides including	2.50 ± 0.10[a]	3.71 ± 0.14[b]
Naringenin-7-*O*-glucoside	1.82 ± 0.06	1.72 ± 0.08
Naringenin	0.21 ± 0.04[a]	3.70 ± 0.06[b]
Naringenin chalcone	16 ± 10[a]	n.d.[b]
(*E*)-β-Carotene	4.3 ± 0.6[a]	3.19 ± 0.18[b]
(*E*)-Lycopene	150 ± 23	144 ± 7
(*Z*)-Lycopene	15.2 ± 2.4[a]	8.67 ± 0.28[b]
Ascorbic acid	380 ± 58[a]	185 ± 25[b]

Values with different letters are significantly different ($n = 3$, mean ± SD, Tukey's test, $p < 0.05$). nq: Not quantified (between detection and quantification limits); n.d.: not detected. Contents of four major caffeic acid-hexosides and two *p*-coumaric acid-hexosides are expressed in, respectively, caffeic acid-4-*O*-glucoside and *p*-coumaric acid-4-*O*-glucoside equivalents. Contents of caffeoylquinic acids and tricaffeoylquinic acid are expressed in 3,5-di-*O*-caffeoylquinic acid equivalent. Contents of quercetin oligosaccharides (rutin, rutin hexose, querc.-Glc-Rha-Api and querc.-Glc-Rha-Api-*p*-coumaric acid) and kaempferol-3-rutinose are expressed in rutin equivalent. Contents of major four naringenin hexosides are expressed in naringenin-7-*O*-glucoside equivalent. Content of naringenin chalcone is expressed in eriodictyol chalcone equivalent. Content in (*Z*)-lycopene corresponds to the sum of *Z*-isomers and is expressed in (*E*)-lycopene equivalent.

Table 4
Evolution of microconstituents during the processing of tomato paste and tomato pulp in tomato sauce (process B).

Compound	In tomato paste (Pb) (mg/100 g of dry matter)[a]	In tomato pulp (Pub) (mg/100 g of dry matter)[a]	In Pb + Pub (mg/100 g of dry matter)[b]	In tomato sauce (Sb) (mg/100 g of dry matter)[a]
Caffeic acid-hexosides including	26.7 ± 3.3	28.5 ± 2.0	26.8	38 ± 8
Caffeic acid-4-*O*-glucoside	10.4 ± 0.7	13.2 ± 0.7	10.6	22.3 ± 2.5
p-Coumaric acid-4-*O*-glucoside	14.1 ± 0.1	14.0 ± 0.8	14.1	26.5 ± 2.6
Caffeoylquinic acids including	13.9 ± 0.2	11.2 ± 0.7	13.7	19.9 ± 1.4
5-*O*-Caffeoylquinic acid	6.19 ± 0.17	6.9 ± 0.7	6.24	7.07 ± 0.48
Dicaffeoylquinic acids including	4.95 ± 0.16	9.9 ± 0.8	5.3	5.6 ± 1.5
3,5-Dicaffeoylquinic acid	3.79 ± 0.13	2.77 ± 0.26	3.7	2.4 ± 0.6
3,4-Dicaffeoylquinic acid	1.16 ± 0.08	7.2 ± 0.5	1.6	3.2 ± 0.9
4,5-Dicaffeoylquinic acid	n.d.	nq	–	nq
Tricaffeoylquinic acid	0.29 ± 0.01[a]	nq	0.27	nq
Quercetin oligosaccharides including	21.0 ± 0.2	50.9 ± 3.5	23.1	30.2 ± 3.3
Rutin	4.23 ± 0.14	40.1 ± 2.6	6.8	19.1 ± 1.5
Kaempferol-3-rutinose	3.70 ± 0.29	3.6 ± 0.6	3.7	3.8 ± 1.0
Naringenin-hexosides including	2.33 ± 0.05	2.32 ± 0.22	2.33	2.32 ± 0.14
Naringenin-7-*O*-glucoside	0.50 ± 0.03	0.47 ± 0.13	0.49	0.32 ± 0.08
Naringenin	1.00 ± 0.01	19.7 ± 2.8	2.34	4.24 ± 0.32
(*E*)-β-Carotene	4.1 ± 0.8	5.15 ± 0.22	4.2	4.2 ± 0.7
(13*Z*)-β-Carotene	1.13 ± 0.17	–	1.05	1.05 ± 0.03
(*E*)-Lycopene	196 ± 34	171 ± 7	194	209 ± 10
(*Z*)-Lycopene	45 ± 7	7.3 ± 0.9	42.2	23.8 ± 3.2
Ascorbic acid	90 ± 13	422 ± 112	113	17.0 ± 4.5

Legend as in Table 3 except for contents in quercetin oligosaccharides (rutin, querc.-Glc-Rha-Api and querc.-Glc-Rha-Api-ferulic acid) which are expressed in rutin equivalent. Contents of two major naringenin hexoses are expressed in naringenin-7-*O*-glucoside equivalent.

[a] Contents evaluated by analysis (mean ± SD, $n = 3$ for paste, pulp and sauce).

[b] Content estimation based on both dilution and the Pb/Pub ratio used in process B.

Table 5
Evolution of microconstituents during the processing of tomato paste in tomato sauce (process C).

Compound	In tomato paste (Pc) (mg/100 g of dry matter)	In tomato sauce (Sc) (mg/100 g of dry matter)
Caffeic acid-hexosides including	50.5 ± 4.4	47.3 ± 4.8
Caffeic acid-4-*O*-glucoside	27.7 ± 2.6	26.2 ± 2.4
p-Coumaric acid-4-*O*-glucoside	17.6 ± 1.9	15.5 ± 1.4
Caffeoylquinic acids including	16.5 ± 1.6	16.1 ± 2.3
5-*O*-Caffeoylquinic acid	9.9 ± 1.0	8.9 ± 1.1
Dicaffeoylquinic acids including	16.8 ± 1.9	15.1 ± 2.0
3,5-Dicaffeoylquinic acid	9.3 ± 1.4	8.5 ± 0.9
3,4-Dicaffeoylquinic acid	5.36 ± 0.19	4.8 ± 0.7
4,5-Dicaffeoylquinic acid	2.17 ± 0.29	1.8 ± 0.5
Tricaffeoylquinic acid	6.4 ± 1.9	4.8 ± 1.9
Quercetin oligosaccharides including	94 ± 9	84 ± 10
Rutin	65 ± 7	55 ± 7
Kaempferol-3-rutinose	1.8 ± 0.5	3.5 ± 1.1
Naringenin-hexosides including	12.1 ± 3.2	11.0 ± 1.0
Naringenin-7-*O*-glucoside	2.65 ± 0.02	2.27 ± 0.25
Naringenin	11.2 ± 1.0	8.4 ± 1.2
(*E*)-β-Carotene	3.0 ± 0.5[a]	2.35 ± 0.14[b]
(13*Z*)-β-Carotene	n.d.[a]	1.06 ± 0.07[b]
(*E*)-Lycopene	197 ± 40[a]	123 ± 10[b]
(*Z*)-Lycopene	17.7 ± 2.5[a]	27.0 ± 1.0[b]
Ascorbic acid	107 ± 8[a]	55 ± 5[b]

Legend as in Table 3 except for contents of five major naringenin hexosides, which are expressed in naringenin-7-*O*-glucoside equivalent. Values with different letters are significantly different (n = 3, mean ± SD, Tukey's test, $p < 0.05$).

oxidizing during pasteurization and polyphenols do not appear to be degraded by autoxidation reactions usually catalyzed by transition metal ions. Autoxidation of unsaturated lipids from rapeseed oil may be competing for available oxygen although lipid-derived oxygenated radicals potentially produced had apparently no influence on the stability of phenolic compounds.

In process B, starting tomato paste Pb and pulp Pub exhibited rather different rutin and naringenin compositions although similar levels in hydroxycinnamic acid derivatives. The dry matters of pulp Pub and paste Pb contributed, respectively for 7% and 93% to the final tomato sauce Sb. Predicted levels in microconstituents can thus be calculated and compared to levels effectively measured in sauce Sb. Hydroxycinnamic acid hexosides, monocaffeoylquinic acids, naringenin and quercetin oligosaccharides increased by a factor ca. 1.5–2 when the other phenolics were quite stable. No overall detrimental effect was thus outlined and the mixing/heating steps have resulted in an increase in extractability from the vegetable matrix.

Contents in (*E*)-β-carotene and (*E*)-lycopene were rather similar for the three pastes. (*E*)-Lycopene was significantly decreased by the processing of Pc into sauce Sc and the loss was only partially counterbalanced by the formation of *Z*-isomers. Degradation of (*E*)-lycopene might have occured during the different thermal treatments (heating in an open tank, waiting times in exchanger and pasteurization at 96 °C, Fig. 1B) either by autoxidation processes and/or by cooxidation by lipid peroxides arising from rapeseed oil. By contrast, the total concentration in β-carotene was stable, the loss of (*E*)-β-carotene being compensated by the formation of (13*Z*)-β-carotene.

Tomato paste Pb and tomato pulp Pub presented similar total concentrations in β-carotene whereas Pb was richer in total lycopene. The higher proportion of *Z*-isomers of carotenoids in Pb (about 25%) may be the result of more drastic thermal treatments used to produce the paste compared to the pulp. An overall stability was evidenced for carotenoids in process B albeit (*Z*)-lycopene isomers may have slightly evolved either by further oxidative degradation or by partial retro-isomerization.

Ascorbic acid proved to be a lot more sensitive than phenolic compounds and carotenoids in tomato sauce manufacture. Indeed, ca. half of the initial ascorbic acid in tomato paste Pc was degraded during the preparation of the sauce Sc. For the more complex process B, the degradation rate is even higher (85%). It is noteworthy that pulp Pub, a mildly processed tomato-based product, brought similar quantities of ascorbic acid as fresh fruits. This important loss may be related to the harsh process conditions used. Indeed, pulp and recipe-specific ingredients were brought to 60, 80 and finally 120 °C using a scraped surface heat exchanger. Diluted tomato paste was heated to 95 °C using a plate heat exchanger, blended with the previous mix before canning and cooling (Fig. 1A). Thus, the use of heat exchangers may be largely detrimental to ascorbic acid.

Interestingly, the two studied processes led to opposite evolutions of phenolic compounds and carotenoids. Process C with apparently less harsh conditions (heating to the boiling and pasteurization) but 3- to 4-fold longer processing times, did not increase the extraction rates of phenolics and carotenoids. Thus, the degradation of carotenoids was not counterbalanced by an additional release from tomato matrix as in process B.

4. Conclusion

This work gives a deep insight into the impact of processing on nutritionally relevant tomato microconstituents. Several hexosides of caffeic and *p*-coumaric acids were found as major contributors to the phenolic pool. In particular, contents in caffeic acid-4-*O*-glucoside and *p*-coumaric acid-4-*O*-glucoside in tomato fruits, pastes and sauces were higher than those in chlorogenic acid, the hydroxycinnamic acid derivative classically reported. For carotenoids, (*E*)-β-carotene and (*E*)-lycopene were quantified in tomato fruits, pastes and sauces along with a high number of different mono- and di-(*Z*)-isomers of lycopene which were tentatively assigned.

The industrial transformation of fresh tomato into paste had an overall positive effect on the contents in phenolic compounds, no effect on lycopene and a slight detrimental effect on β-carotene, while it promoted a drastic loss in ascorbic acid. Albeit markedly different industrial processes were used, the nutritional quality of the two resulting tomato sauces was mainly preserved, except for ascorbic acid. An increase in tomato matrix extractability may be counterbalanced by microconstituent catabolism as evidenced for carotenoids and phenolics. Carotenoids can be isomerized, but this effect is not detrimental to the nutritional value of tomato since *Z*-isomers of lycopene are more bioavailable than the (*E*)

form (Unlu et al., 2007). The influence of the main parameters, which are light, dissolved oxygen, transition metal ions and temperature, should be determined for each operation unit. Identification of kinetic parameters such as rate constants or activation energies is the first step toward strategies aiming at the preservation of the nutritional properties in processed tomato products.

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