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Metabolomics and Food Processing: From Semolina to Pasta

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Supporting Information

ABSTRACT: The objective of this study was to investigate the metabolite variations during industrial pasta processing (from semolina to dried pasta) for five different commercial products. Up to 76 metabolites were detected. Significant differences were observed between wholemeal and refined pasta samples, with the wholemeal pasta richer in many classes of compounds such as phytosterols, policosanols, unsaturated fatty acids, amino acids, carotenoids, minerals, and so on. Significant differences were also observed between samples of refined pasta apparently similar for the actual parameters used for the assessment of pasta quality. The results indicated that a number of metabolites undergo a transformation during the pasta-making process depending on the processing conditions adopted. The approach used in this work shows the high potential of metabolite profiling for food investigations with regard to process-related transformation, safety, and nutrition.

KEYWORDS: metabolomics, metabolic profiling, nutrition quality, pasta processing, PCA

INTRODUCTION

Dried pasta represents a traditional Italian food that is becoming widespread in many other countries.[1] The concept of pasta quality, at least for Italian consumers, is derived from traditional habits and history of production, and it is strictly dependent on the use of durum wheat semolina as the exclusive raw material.[2] The preparation of pasta at the industrial scale entails several steps from semolina to dough formation, dough extrusion, and, finally, drying of the pasta products. During the whole process the constitutive components of semolina are subjected to a number of modifications that affect, or define, the taste and nutritional quality of the final product.[3−5] More recently, considerable progress has been made to optimize pasta processing with specific attention to the drying process, with the result that most manufactures employ high-temperature drying technologies to generate high-quality pasta in a short time with the consequential increase in productivity.[3,4,6]

A significant level of attention has been paid to the enhancement of pasta nutritional quality, either by using flour from other cereals such as barley or semolina from genotypes with enhanced levels of nutritionally valuable compounds or by direct enrichment (fortification) with specific nutrients.[7,8] Indeed, the U.S. Food and Drug Administration (FDA) and the World Health Organization (WHO) consider pasta a good vehicle for the addition of nutrients, and pasta was among the first foods to be authorized by the FDA for fortification with vitamins and iron.[2] As an alternative to enrichment of the refined foods, the use of products derived from whole wheat generally contain comparatively more vitamins, minerals, antioxidants, and dietary fiber than those derived from refined semolina. Furthermore, the regular consumption of whole wheat and whole grain products is implicated in reduced incidences of chronic diseases (e.g., cardiovascular disease, cancer, and diabetes) and the control of obesity by lowering glycemic responses.[9,10]

The metabolome can be defined as the complete set of metabolites (small molecules) of a given sample and defines the biochemical phenotype of a cell or tissue. Metabolomics is then the comprehensive analysis in which all of the metabolites of a sample are identified and quantified at a specific time point.[11]

Although relatively young, and still very much in development, plant metabolomics is now being widely applied, and a key area of application is plant-derived human nutrition and health benefits. Over the past few decades there has been a significant increase in interest in the nutritional quality of foods, the link between quality/nutritional value, the origin of the raw materials, and the effects that different food production chain processes bring to bear on quality and nutritional properties. Insight into all of these areas can be gained by applying metabolomics. Advances in metabolomics technologies (liquid and gas chromatography−mass

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Table 1. Mean Value ± SD of Metabolites and Minerals Identified in Different Dried Pasta Samples[a]

			μg/g dw				
	IP	component	DW (shells)	D13 (rigatoni)	D13 V (rigatoni)	D12 (fusilli)	D12 V (rigatoni)
1	GC-MS	alanine	106.50 ± 4.50 a	nd	23.00 ± 1.00 c	29.33 ± 0.58 b	nd
2	GC-MS	valine	29.33 ± 3.06 a	18.67 ± 0.58 b	17.67 ± 2.31 b	20.67 ± 0.58 b	nd
3	GC-MS	leucine	24.33 ± 2.52 a	15.00 ± 1.00 bc	13.67 ± 1.53 c	18.33 ± 1.53 b	16.67 ± 0.58 bc
4	GC-MS	proline	nd	nd	nd	nd	nd
5	GC-MS	isoleucine	nd	nd	nd	nd	nd
6	GC-MS	glycine	29.00 ± 3.46 a	22.00 ± 1.00 b	24.33 ± 0.58 ab	22.00 ± 1.73 b	20.67 ± 1.15 b
7	GC-MS	serine	23.33 ± 2.89 a	16.00 ± 1.00 b	19.67 ± 1.53 ab	22.67 ± 2.31 a	19.00 ± 2.00 ab
8	GC-MS	threonine	18.33 ± 1.15 a	11.00 ± 1.00 b	11.67 ± 0.58 b	13.00 ± 2.00 b	12.00 ± 1.00 b
9	GC-MS	asparagine	242.00 ± 6.56 a	182.00 ± 7.21 b	203.67 ± 9.71 b	139.33 ± 7.77 c	150.33 ± 10.79 c
10	LC-MS	lysine	25.07 ± 0.10 a	24.45 ± 0.06 c	24.39 ± 0.01 c	24.65 ± 0.01 b	24.52 ± 0.01 bc
11	LC-MS	histidine	70.96 ± 4.11 a	47.71 ± 5.07 c	49.19 ± 0.53 c	58.70 ± 0.18 b	49.73 ± 1.88 c
12	LC-MS	arginine	973.13 ± 128.93 a	687.34 ± 57.72 b	675.99 ± 0.04 b	717.01 ± 23.23 b	655.12 ± 18.42 b
13	LC-MS	glutamine	323.23 ± 189.68 a	227.95 ± 24.94 a	281.20 ± 3.07 a	258.17 ± 10.25 a	217.05 ± 2.38 a
14	LC-MS	methionine	0.45 ± 0.05 a	0.41 ± 0.05 a	0.41 ± 0.00 a	0.46 ± 0.04 a	0.40 ± 0.05 a
15	LC-MS	tyrosine	13.95 ± 1.13 a	7.97 ± 0.87 c	8.44 ± 0.57 bc	10.42 ± 0.65 b	9.06 ± 0.58 bc
16	LC-MS	tryptophan	243.42 ± 26.32 a	152.77 ± 19.98 b	144.79 ± 5.24 b	178.70 ± 17.27 b	160.06 ± 8.76 b
17	GC-MS	aspartic acid	151.33 ± 7.64 b	153.67 ± 7.23 b	175.67 ± 8.62 a	146.33 ± 4.51 b	159.67 ± 4.73 ab
18	GC-MS	glutamic acid	229.00 ± 12.12 a	151.67 ± 7.23 b	174.67 ± 6.35 b	154.00 ± 7.00 b	162.67 ± 12.50 b
19	GC-MS	GABA	90.00 ± 6.93 a	63.00 ± 1.00 bc	67.67 ± 3.79 b	53.33 ± 6.11 c	nd
20	GC-MS	malic acid	1748.00 ± 180.71 a	1742.33 ± 77.47 a	1849.33 ± 84.33 a	1596.33 ± 163.18 a	1748.67 ± 42.22 a
21	GC-MS	citric acid	471.33 ± 18.72 a	207.33 ± 12.74 b	200.00 ± 5.29 b	186.67 ± 4.51 b	189.67 ± 3.79 b
22	GC-MS	gluconic acid	113.67 ± 13.05 a	124.67 ± 2.08 a	116.67 ± 7.09 a	70.67 ± 8.62 b	67.67 ± 3.06 b
23	GC-MS	arabinose	44.33 ± 4.51 a	15.00 ± 1.00 c	nd	28.00 ± 2.65 b	26.33 ± 2.08 b
24	GC-MS	sucrose	18790.67 ± 1833.29 a	9346.00 ± 250.14 b	9674.33 ± 359.37 b	9913.33 ± 1009.59 b	10499.00 ± 232.99 b
25	GC-MS	fructose	2057.33 ± 207.08 a	1054.00 ± 23.52 c	1051.33 ± 47.27 c	1417.33 ± 157.82 b	1290.67 ± 38.08 bc
26	GC-MS	glucose	2857.00 ± 314.72 a	1376.33 ± 32.72 b	3038.00 ± 128.31 a	1626.67 ± 167.83 b	3289.00 ± 123.76 a
27	GC-MS	maltose	60566.67 ± 6878.08 c	72624.33 ± 1662.97bc	70216.33 ± 2701.40bc	80094.33 ± 7522.26ab	86370.67 ± 3533.57 a
28	GC-MS	raffinose	8754.67 ± 1147.18 a	3760.33 ± 156.13 b	3766.00 ± 302.04 b	3480.67 ± 420.33 b	3748.33 ± 65358 b
29	GC-MS	sorbitol/galactitol	45.33 ± 1.53 b	55.67 ± 2.08 a	49.67 ± 3.21 ab	29.33 ± 2.08 c	27.33 ± 2.08 c
30	GC-MS	*myo*-inositol	145.67 ± 5.13 a	87.67 ± 1.53 bc	76.67 ± 2.52 c	91.67 ± 8.74 b	78.00 ± 1.73 c
31	GC-MS	tetradecanoic acid	5.10 ± 0.46 a	3.98 ± 0.49 b	3.19 ± 0.01 b	3.55 ± 0.29 b	3.70 ± 0.46 b
32	GC-MS	pentadecanoic acid	1.98 ± 0.25 a	1.25 ± 0.11 b	1.16 ± 0.09 b	1.45 ± 0.11 b	1.38 ± 0.15 b
33	GC-MS	hexadecanoic acid	156.05 ± 1.02 a	75.35 ± 2.25 cd	69.90 ± 0.53 d	89.95 ± 4.35 b	78.45 ± 3.79 c
34	GC-MS	heptadecanoic acid	0.90 ± 0.07 a	0.41 ± 0.04 c	nd	nd	0.55 ± 0.02 b
35	GC-MS	octadecanoic acid	10.13 ± 1.04 a	7.80 ± 0.64 b	7.16 ± 0.19 b	7.75 ± 0.82 b	7.28 ± 0.14 b
36	GC-MS	eicosanoic acid	1.03 ± 0.07 a	0.68 ± 0.00 bc	nd	0.83 ± 0.08 b	0.53 ± 0.08 c
37	GC-MS	docosanoic acid	2.00 ± 0.04 a	0.75 ± 0.00 b	0.64 ± 0.04 b	0.75 ± 0.00 b	0.70 ± 0.09 b
38	GC-MS	tetracosanoic acid	3.50 ± 0.04 a	1.30 ± 0.19 b	1.20 ± 0.05 b	1.43 ± 0.07 b	1.18 ± 0.09 b
39	GC-MS	hexacosanoic acid	nd	0.79 ± 0.04 b	nd	nd	0.90 ± 0.08 a
40	GC-MS	octacosanoic acid	2.40 ± 0.13 a	nd	nd	nd	nd
41	GC-MS	hexadecenoic acid	0.73 ± 0.03 b	nd	nd	nd	1.05 ± 0.10 a
42	GC-MS	γ-linolenic acid	nd	nd	nd	nd	nd
43	GC-MS	octadecadienoic acid	460.40 ± 5.57 a	166.40 ± 7.25 c	156.34 ± 0.64 c	197.40 ± 6.15 b	170.35 ± 6.45 c
44	GC-MS	octadecenoic acid	25.60 ± 1.26 a	15.33 ± 0.89 c	12.90 ± 0.08 c	19.23 ± 2.35 b	18.93 ± 0.66 b
45	GC-MS	α-linolenic acid	22.68 ± 0.98 a	6.78 ± 0.36 bc	6.00 ± 0.18 c	9.25 ± 1.99 b	9.18 ± 0.41 b
46	GC-MS	eicosenoic acid	2.35 ± 0.30 a	nd	nd	nd	0.90 ± 0.07 b
47	GC-MS	1-hexadecanol	0.80 ± 0.07 a	0.55 ± 0.02 b	nd	0.68 ± 0.09 ab	0.65 ± 0.07 ab
48	GC-MS	1-octadecanol	0.50 ± 0.04 a	0.26 ± 0.02 c	nd	0.34 ± 0.04 b	0.30 ± 0.00 bc
49	GC-MS	1-heneicosanol	0.55 ± 0.09 a	0.35 ± 0.04 bc	0.26 ± 0.01 c	0.40 ± 0.04 b	0.30 ± 0.00 bc
50	GC-MS	1-triacosanol	0.68 ± 0.00 a	0.23 ± 0.00 c	0.23 ± 0.00 c	0.23 ± 0.00 c	0.23 ± 0.00 c
51	GC-MS	1-tetracosanol	0.30 ± 0.01 a	nd	nd	nd	nd
52	GC-MS	1-hexacosanol	1.65 ± 0.00 a	nd	nd	nd	nd

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Table 1. Continued

			μg/g dw				
	IP	component	DW (shells)	D13 (rigatoni)	D13 V (rigatoni)	D12 (fusilli)	D12 V (rigatoni)
53	GC-MS	1-heptacosanol	0.98 ± 0.08 a	0.30 ± 0.02 b	0.23 ± 0.02 b	0.33 ± 0.04 b	0.25 ± 0.01 b
54	GC-MS	α-tocopherol	1.70 ± 0.11 a	0.25 ± 0.01 b	nd	0.30 ± 0.00 b	0.23 ± 0.01 b
55	GC-MS	β-tocopherol	0.80 ± 0.04 a	0.18 ± 0.01 b	nd	0.18 ± 0.04 b	0.15 ± 0.01 b
56	GC-MS	γ-tocopherol	0.83 ± 0.07 a	0.18 ± 0.01 b	nd	0.15 ± 0.00 b	0.15 ± 0.01 b
57	GC-MS	campsterol	41.63 ± 1.06 a	21.80 ± 1.23 c	20.29 ± 1.01 c	25.68 ± 1.97 b	23.55 ± 0.78 bc
58	GC-MS	stigmasterol	5.38 ± 0.67 a	nd	2.55 ± 0.15 b	nd	nd
59	GC-MS	β-sitosterol	83.10 ± 0.69 a	50.63 ± 0.65 cd	48.90 ± 1.43 d	55.95 ± 3.15 b	53.75 ± 1.02 bc
60	GC-MS	stigmastanol	16.83 ± 0.38 a	11.80 ± 0.41 bc	11.06 ± 0.41 c	12.75 ± 0.79 b	12.25 ± 0.38 bc
61	HPLC	lutein	0.52 ± 0.00 a	0.30 ± 0.03 c	0.19 ± 0.00 d	0.39 ± 0.01 b	0.39 ± 0.03 b
62	HPLC	zeaxanthin	0.05 ± 0.00 a	nd	nd	0.02 ± 0.00 b	0.01 ± 0.01 bc
63	HPLC	canthaxanthin	0.06 ± 0.00 a	0.03 ± 0.00 c	0.02 ± 0.00 d	0.04 ± 0.00 b	0.04 ± 0.00 b
64	HPLC	β-carotene	nd	nd	nd	nd	nd
65	LC-MS	niacin (vit B3)	10.95 ± 0.94 bc	7.62 ± 1.04 d	13.01 ± 0.35 ab	9.23 ± 1.00 cd	13.88 ± 0.79 a
66	LC-MS	panthotenic acid (vit B5)	5.94 ± 0.56 a	4.39 ± 0.40 b	4.37 ± 0.13 b	4.42 ± 0.42 b	4.20 ± 0.38 b
67	LC-MS	riboflavin (vit B2)	0.52 ± 0.18 b	0.14 ± 0.00 b	4.24 ± 0.02 a	0.31 ± 0.00 b	4.02 ± 0.35 a
68	LC-MS	thiamin (vit B1)	2.63 ± 0.22 c	1.28 ± 0.11 d	8.08 ± 0.30 b	1.72 ± 0.12 d	9.00 ± 0.62 a
69	LC-MS	chlorogenic acid	0.15 ± 0.08 a	0.06 ± 0.02 ab	0.04 ± 0.00 b	0.05 ± 0.01 ab	0.05 ± 0.01 ab
70	ICP-OES	Mg	966.00 ± 91.10 a	524.63 ± 3.39 b	528.89 ± 4.04 b	485.44 ± 18.80 c	506.01 ± 1.81 bc
71	ICP-OES	Ca	382.32 ± 47.81 a	228.50 ± 3.24 c	292.59 ± 3.95 b	235.62 ± 5.91 bc	235.90 ± 0.91 bc
72	ICP-OES	P	3126.35 ± 139.00 a	2078.53 ± 60.33 b	2067.15 ± 113.38 b	2011.03 ± 72.68 b	2120.98 ± 102.55 b
73	ICP-OES	Na	31.13 ± 3.05 a	8.59 ± 0.98 c	15.80 ± 0.90 b	11.15 ± 0.09 c	10.53 ± 1.00 c
74	ICP-OES	Fe	36.30 ± 1.28 d	26.93 ± 1.79 d	84.91 ± 2.90 a	42.99 ± 1.47 c	56.60 ± 6.77 b
75	ICP-OES	Mn	27.10 ± 0.41 a	8.97 ± 0.02 c	9.60 ± 0.18 b	8.33 ± 0.23 d	8.63 ± 0.06 cd
76	ICP-OES	Zn	21.93 ± 0.31 a	10.90 ± 0.09 c	11.27 ± 0.04 c	11.12 ± 0.49 c	13.06 ± 0.15 b
77	ICP-OES	Cu	3.66 ± 0.36 a	3.99 ± 0.09 a	3.74 ± 0.18 a	3.48 ± 0.16 a	3.96 ± 0.09 a
78	ICP-OES	Mo	0.50 ± 0.38 c	1.71 ± 0.24 bc	2.47 ± 0.36 b	3.02 ± 0.44 b	10.37 ± 1.07 a

[a] IP, instrumental platform used for quantification. DW, dried pasta wholemeal; D13, dried pasta with 13% protein content; D13 V, dried pasta with 13% protein content + vitamins; D12, dried pasta with 12% protein content; D12 V, dried pasta with 12% protein content + vitamins. Different letters in the same row indicate significant differences among pasta samples at $P \leq 0.05$ following the Tukey test. nd, not detected.

spectrometry (LC-MS, GC-MS), nuclear magnetic resonance spectroscopy (NMR), etc.) can significantly increase our knowledge of small molecules, allowing the food processor to design, modify, and refine processes in food production according to the desired quality and nutritional properties of the end products.[12,13]

To date, very few studies have used metabolomics to investigate the metabolite composition of wheat-derived products. Metabolomics has been employed to study changes in the metabolome of bread wheat associated with specific GM events,[14] different agricultural practices,[15] and aspects of climate change.[16] Much less is known about durum wheat and the modifications it undergoes during processes required to transform semolina into pasta. An exception is a previous study in which correlations between semolina composition and cooked pasta volatile components were described, highlighting the influence of different wheat cultivars on pasta flavor.[17]

The aim of this study was to use a validated GC- and LC-based metabolomics approach, along with mineral analysis, to describe the modifications induced during the industrial processes of semolina into pasta to obtain a detailed picture of the modifications associated with production of different kinds of pastas. A panel of different commercially available types of pasta was utilized to explore differences and changes in the content of the main nutritional and health beneficial metabolites.

MATERIALS AND METHODS

Materials. Five different sample sets were subjected to metabolomics analyses encompassing the common processes used to produce commercially available pasta from semolina by Tamma Industrie Alimentari (Foggia, Italy). Different batches of semolina were used, all of them from the same harvesting season (2008) and from the same growing region (Apulian Tavoliere, Italy). Each set was organized to sample three steps of pasta processing: semolina (S), extruded pasta (E), and dried pasta (D). The five types of pasta considered included "shells" from wholemeal semolina (W), "rigatoni" with 13% and "fusilli" with 12% protein content (P13 and P12, respectively), and "rigatoni" with 13 and 12% protein content fortified with 1‰ of a mixture containing iron lactate, thiamin mononitrate (vit B1), riboflavin (vit B2), and niacin (vit B3) dispersed in glucose (P13 V and P12 V, respectively). Depending on the pasta shape, the temperature and duration of the drying process varied: drying temperature was 83 °C for fusilli, 91 °C for rigatoni, and 86 °C for shells for 4 h and 10 min, 3 h and 56 min, and 3 h and 45 min, respectively.

The different combinations of samples and processing steps were codified as follows: SW, wholemeal semolina; S13, semolina with 13%

protein content; S13 V, semolina with 13% protein plus vitamins; S12, semolina with 12% protein content; S12 V, semolina with 12% protein content plus vitamins; EW, extruded pasta wholemeal; E13, extruded pasta with 13% protein; E13 V, extruded pasta with 13% protein content plus vitamins; E12, extruded pasta with 12% protein; E12 V, extruded pasta with 12% protein plus vitamins; DW, dried pasta wholemeal; D13, dried pasta with 13% protein content; D13 V, dried pasta with 13% protein content plus vitamins; D12, dried pasta with 12% protein content; D12 V, dried pasta with 12% protein content plus vitamins. After collection, the samples were freeze-dried, milled using a laboratory mill (Udy-Cyclone 1093 Foss Tecator), passed through a 0.5 mm sieve, and stored at −25 °C until the analysis. None of the samples were refrozen once opened, and all samples were analyzed within 3 months after freeze-drying.

GC-MS Profiling. The samples (100 mg dw) were weighed into a glass tube (10 mL) with a Teflon cap, and 100% methanol (1 mL), ultrapure water (1 mL), and $CHCl_3$ (3 mL) were added sequentially. Between and after each addition, the samples were vigorously vortexed (3 × 3 s). The samples were stored at 4 °C for 30 min and then centrifuged at 4000*g* for 10 min. Aliquots (50 μL) of polar and $CHCl_3$ phases (1 mL) were pipetted into tubes (2 mL). Ribitol (15 μL of a 0.20 mg/mL solution) and nonadecanoic acid methyl ester (20 μL of a 0.21 mg/mL solution) were added as internal standards, and the samples were dried in a Speed-vac RC 1022 (Thermo Electron Corp., France) for further analysis.

The polar residues were redissolved and derivatized for 90 min at 37 °C in methoxyamine hydrochloride in pyridine (70 μL, 20 μg/mL) followed by incubation with *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA, 120 μL) at 37 °C for 30 min. For derivatization of the nonpolar fraction, the residues were redissolved and derivatized for 30 min at 37 °C in MSTFA (70 μL). Derivatized samples were then transferred to GC vials for analysis.

Samples (1 μL) were injected onto the GC column in splitless mode. Polar metabolites were analyzed using a GC (Agilent 6890N, Agilent Technologies, USA) linked to a quadrupole MS (Agilent 5973, Agilent Technologies, USA). GC separation was achieved on a HP-5 ms capillary column (30 m, 0.25 mm i.d., 0.25 μm film thickness). The injection temperature was 280 °C, the transfer line and the ion source were set at 280 °C, and the quadrupole was adjusted to 180 °C. Helium was used as carrier gas at a constant flow rate of 1 mL/min. The oven was kept at a temperature of 70 °C for 1 min, then increased at a rate of 5 °C/min to 310 °C, and held for 15 min. Subsequently, the temperature was increased to 340 °C and held for 1 min. The system was equilibrated for 1 min at 70 °C before sample injection. The spectrometer was operated in electron-impact (EI) mode, the ionization voltage was 70 eV, and the detector was tuned according to the manufacturer's recommendations using tris(perfluorobutyl)amine (PFTBA). The scan range was from 30 to 700 amu, and mass spectra were recorded at 2.21 scan/s.

The chromatograms and mass spectra were evaluated using the AMDIS program. The absolute concentration of the metabolites was determined by comparison with standard calibration curves obtained for all metabolites reported in Table 1 in the range of 0.04−2.00 ng (using 5 point calibration) with r^2 values between 0.989 and 0.999.

Nonpolar metabolites were analyzed as above with a few modifications: the injection temperature and the transfer line were set at 250 °C; the oven was kept at a temperature of 70 °C for 5 min, then increased at a rate of 5 °C/min to 310 °C, and held for 1 min. Subsequently, the system was equilibrated for 6 min at 70 °C before sample injection. The mass spectra were recorded at 2.28 scans/s with an m/z 50−700 mass range. The standard calibration curves were obtained for all metabolites reported in Table 1 over a range from 0.05 to 3.00 ng (using 5 point calibration) with r^2 values between 0.992 and 0.999. For both polar and nonpolar profiling, the batches of analysis include 17 runs with two calibration standard mixes at the beginning and end of each batch.

The GC-MS quantifications were performed by a Chemstation program, whereas the internal standards used were chosen because they were absent in wheat samples and added after the extraction to control the instrumental performances. Standards and all chemicals used (HPLC grade) were purchased from Sigma-Aldrich Chemical Co. (Deisenhofen, Germany), and MSTFA was purchased from Fluka.

LC-MS Profiling. The samples (100 mg dw) were weighed into a 7 mL glass vial, and water/methanol (3 mL, 50:50 v/v) containing 1% acetic acid and 2 μM reserpine as internal standard was added to the vial and shaken for 60 min on an orbital shaker. The contents were allowed to settle, and then an aliquot (1000 μL) was placed into an autosampler vial containing an integral 0.2 μm filter (Whatman Mini-UniPrep). Electrospray ionization LC-MS analyses were performed on an LTQ Orbitrap XL (Thermo Scientific, Hemel Hempstead, U.K.) ion trap mass spectrometer coupled to a Surveyor HPLC System (Thermo Scientific). Metabolites were separated within 35 min on a C_{18} Synergi Hydro RP column (2 mm × 150 mm, 4 μm particle size; Phenomenex, Macclesfield, U.K.), fitted with a Security Guard column (Aqua 10 μm C_{18} cartridge, 2 mm i.d. × 4 mm lengthl Phenomenex) using a linear gradient from 0.2% formic acid in deionized water to 0.2% formic acid in 90% acetonitrile. Between analyses, the column was re-equilibrated for 10 min. The LC-MS quantification was performed by an Xcalibur program, and the internal standard was used for laboratory retention index calculation to assist compound tracking characterization and quantification by plotting the ratio of metabolite signal to the internal standard signal as a function of the metabolite concentration in the standards. Reserpine was chosen as the internal standard because it is not naturally present in pasta/wheat; it elutes approximately mid-run and does not coelute with any metabolite, therefore eliminating interferences and ion suppression of metabolites/internal standard. Furthermore, reserpine is stable in the matrix over the period the samples are on the autosampler. Examples of LOD, LOQ, and linearity have been reported in Table ST1 of the Supporting Information .

The mass spectrometer was set to scan from m/z 80 to 2000 at a rate of 250 ms. The instrument was tuned against two internal standards, reserpine for positive mode analysis and m-fluoro-DL-tyrosine for negative mode. Tuning is carried out using the instrument manufacturer's suggested method. Mobile phase flow rates and gas flows are set to give a stable signal. Solvent percentages are set to mimic those reached at 50% during the run time (linear gradient). Reserpine was infused through a T connection, allowing mixture with the mobile phase. The instrument was then set to run in autotune mode.

All chemicals (HPLC grade) were purchased from VWR International LTD (Lutterworth, U.K.), and the standards were purchased from Sigma-Aldrich Ltd. (Poole, U.K.).

Carotenoid Profiling. Carotenoids were extracted using a protocol similar to that reported by Digesù et al.,[18] with minor volume changes. Samples (2.0 g dw) were suspended in extraction buffer [hexane/acetone (8:2 v/v), 8 mL] containing 0.1% butylated hydroxytoluene (BHT). The mixtures were streamed under nitrogen for 10 s, vortexed for 30 s, and left in the dark at room temperature overnight. The samples were vortexed and centrifuged at 4000*g* for 15 min. The pellet was washed with hexane (4 mL) and centrifuged as above. The supernatants collected were filtered using GHP membrane syringe filters (0.45 μm, 25 mm), and the filtrate was dried under a gentle stream of nitrogen and reconstituted in methanol/dichloromethane (45:54 v/v, 400 μL). Final extracts were transferred into amber HPLC vials.

HPLC analyses were carried out using a Hewlett-Packard 1100 system (Hewlett-Packard, USA) with a diode array detector set for monitoring the wavelength at 450 nm. Carotenoids were separated using a YMC C_{30} (250 mm × 2.0 mm, 5 μm particle size) column coupled with a 20 mm × 2.0 mm, 5 μm guard column (YMC Inc., Waters, RSA). The column temperature was set at 25 °C. The mobile phases employed were (A) 83% methanol containing 15% *tert*-butyl methyl ether (TBME)

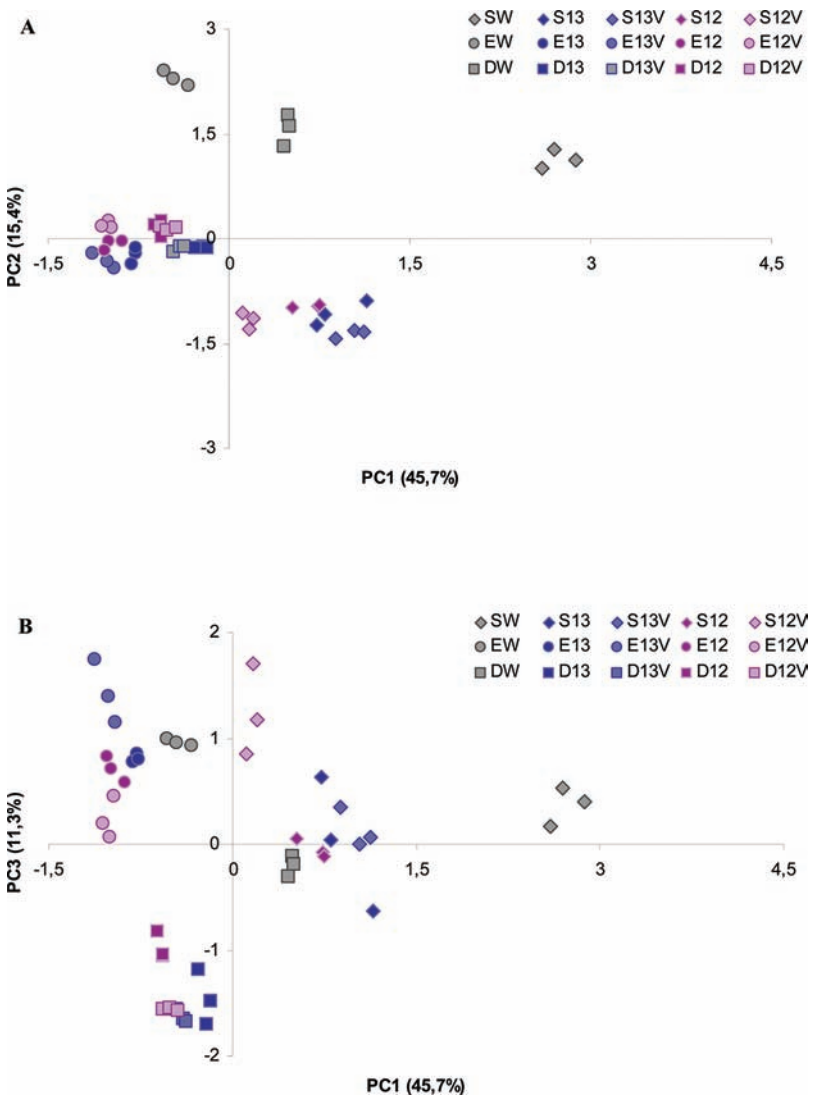

Figure 1. Principal component analysis (PCA) score plot for (A) principal components 1 and 2 and (B) principal components 1 and 3 of metabolites and minerals concentrations following pasta production process of different products: S, semolina; E, extruded pasta; D, dried pasta; 12 and 13 indicate the protein content; V, vitamin supplement. Three analytical replicates are presented for each sample.

and (B) 8% methanol in 90% TBME at a flow rate at 0.4 mL min^{-1}. The gradient elution program used was as follows: 0 min, 100% mobile phase A; 8 min, 70% mobile phase A and 30% mobile phase B; 22 min, 45% mobile phase A and 55% mobile phase B; 24 min, 5% mobile phase A and 95% mobile phase B; and 27 min, 100% mobile phase B. The total separation time was 50 min.

Carotenoids were identified by comparison to the retention times and absorption spectra of standards. Quantification was determined using linear standard curves (5 point calibration) in the range of 0.01−10.00 μg/mL with r^2 values of <0.999. Random standards were also injected during each sample run to monitor the performance of the instrument and evaluate intra- and interday variations. The limit of detection (LOD) for each carotenoid was as follows: lutein, 0.01 μg/mL; zeaxanthin, 0.02 μg/mL; β-carotene, 0.01 μg/mL; and canthaxanthin, 0.02 μg/mL. To measure the recovery rate, lutein and β-carotene standards were added to three replicates of the dry, powdered samples followed by carotenoids extraction and analysis using the methods above. An average recovery above 87% was calculated for both standards.

All chemicals (HPLC grade) were purchased from Sigma (St. Louis, MO). Lutein, zeaxanthin, canthaxanthin, and β-carotene standards were up to 99% pure and acquired from Industrial Analytical (Kyalami, RSA).

Mineral Profiling. Samples (300 mg dw) were digested in an ultrapure HNO_3−H_2O_2 mixture and digested in a microwave autoclave. Mineral concentrations were determined using ICP-OES (ARCOS, SPECTRO Analytical Instruments GmbH, Kleve, Germany) with the following operating conditions: power, 1.2 kW; plasma gas flow, 15 L/min; auxiliary gas flow, 1.5 L/min; nebulizer type, VGrove; pump speed, 15 rotations/min; sample delay time, 30 s; and stabilization time, 15 s.

Each element was measured at different wavelengths as reported in Table ST2 of the Supporting Information, and the results were the average of three replicates. The reference material RM 8436 (durum wheat flour), from the National Institute of Standards and Technology (NIST) (dried for 24 h at 25 °C), was used as quality control and digested and analyzed using the same method as applied for the pasta samples.

Statistical Analysis. All determinations were conducted in triplicate (one sample, from one batch of production, was three times

extracted and subjected to the different analyses in a randomized order). Analysis of variance (ANOVA) was carried out with respect to each metabolite/mineral and class of compound detected in semolina and extruded and dried pasta samples, and mean discrimination was performed by applying Tukey's test; statistically significant differences were determined at the probability level $P \leq 0.05$. To obtain a general and comprehensive characterization of the samples, the detected metabolites were subjected to principal component analysis (PCA), based on correlation, followed by factor analysis (FA). The PCA and FA procedures allowed us to synthesize the data set, avoid redundancy, and identify a limited number of new, uncorrelated variables named "factors" (linear combinations of the original ones) able to account for a significant portion of the total variance. Having defined the proper number of factors useful to summarize each data set, a procedure called "varimax rotation"[19] allowed the optimization of the factor loadings on each factor. PCA, FA, and ANOVA were performed using the software JMP (SAS Institute Inc., version 8).

RESULTS AND DISCUSSION

Principal Component Analysis of Analytes and Sample Discrimination. To follow the repertoire of metabolic changes that occur during the pasta-making process, an extensive metabolomics approach was undertaken covering primary and secondary metabolites. Aligned mineral analysis was also undertaken to fully describe the industrial transformations accompanying the production of five types of semolina into their corresponding pasta products.

In total, 78 chemical entities (metabolites and minerals) were detected, 51 of which were present in all samples. Considering each step of the pasta production process, in semolina 76 components were detected (65 common to all semolina samples); in extruded pasta 75 were detected (59 present in all extruded samples), and in dried pasta 74 were detected (55 common to all types of pasta) (see Table ST3 of the Supporting Information).

The data set obtained from the analysis was subjected to PCA to obtain a first representation of the metabolic and mineral differences in toto between the samples. The number of factors that can properly describe the data was determined on the basis of the eigenvalues and consequently on the percentage of the total variance explained by each factor, as shown in Figure SF1 of the Supporting Information, where the eigenvalues and the cumulative variance are plotted. Three main factors accounting for 72.4% of the total variances were selected: PC1−PC3 explained 45.7, 15.4, and 11.3% of total variance, respectively.

On basis of the factors selected by the varimax rotation method,[19] 37 metabolites had a positive loading on PC1 including tocopherols, sterols (with the exception of stigmastanol), β-carotene, zeaxanthin, and linoleic acid as well as most of the hydroxy fatty acids, saturated fatty acids, and sugars with the exception of fructose and arabinose. Maltose and Nniacin (vit B3) were the only metabolites negatively loaded to PC1.

Twenty-four metabolites and minerals were represented by PC2; compounds positively loaded to this factor included GABA, citric acid, fructose, and arabinose, some amino acids (including the essentials ones leucine and threonine but not in the dried pasta), most unsaturated fatty acids, and minerals with the exception of iron and molybdenum. The only metabolites negatively loaded to the second factor were riboflavin (vit B2) and the amino acid proline (except for proline in dried pasta).

Finally, 14 compounds were represented by PC3, with the exceptions of iron and molybdenum, all of them had a positive loading onto this factor. Among these metabolites there were amino acids (including lysine, methionine, and tryptophane), stigmastanol, pantothenic acid, and two carotenoids (lutein and cantaxanthin).

PCA score plots (Figure 1A) show that all wholemeal samples (semolina and extruded and dried pasta) were positively loaded onto PC2 and, whereas semolina and dried pasta were also positively loaded to PC1, the extruded samples of wholemeal were correlated to the first factor in negative mode. All of the refined semolina samples, regardless of their protein content, were positively and negatively correlated to PC1 and PC2, respectively, whereas the extruded and dried pasta samples were negatively correlated to PC1 and not particularly influenced by a second factor. Figure 1B shows the score plots of PC1/PC3, whereas those of PC2/PC3 are reported in Figure SF2 of the Supporting Information.

The largest differences in PC3 were observed between dried and extruded pasta samples (before and after the drying process). Also, the points for each sample/treatment were generally tightly grouped, highlighting their similarity within group for the components represented in these scores.

It is clear from the PCA score plots (Figure 1 and Figure SF2 of the Supporting Information) that pasta manufacturing processes have a great impact on the segregation of the samples in multivariate space and hence the global composition of metabolites, nutrients, and minerals. Furthermore, and perhaps as expected, the wholemeal products, which contain husk-derived components, are significantly different from the other types of pasta. In each food processing step, wholemeal samples had the highest content of many of the analytes reported and correlated to the three main factors in positive mode. Nevertheless, metabolic differences were also detected among samples made with refined semolina. At the retail level, these products are considered to be essentially the same apart from the inherent difference of protein content, added vitamins, and shape. However, this study shows that there are more significant differences in the metabolite, mineral, and nutrient levels that affect organoleptic and nutrient contents.

Table 1 displays the analytical data and the results of the ANOVA for all metabolites and minerals detected in dried pasta samples. The wholemeal pasta showed the highest content of tocopherols, carotenoids, unsaturated fatty acids (particularly oleic and linoleic acid), phytosterols (about twice in comparison to refined samples), and minerals with the exception of copper, which was about the same in all types of pasta. Notably, 1-tetracosanol and 1-hexacosanol were detected only in wholemeal pasta (Table 1). These metabolites are components of policosanol, which is a group of long-chain aliphatic ($C_{20}-C_{36}$) primary alcohols concentrated in the bran fraction of cereal grain.[20]

Significant differences were observed between the pastas containing 13 and 12% protein contents. The latter exhibited greater levels of reducing sugars (fructose and arabinose) and amino acids (serine, lysine, hystidine, and alanine, with alanine not detectable in D13). Additionally, unsaturated fatty acids (oleic, linoleic, and α-linolenic), phytosterols (campsterol, β-sitosterol, and stigmastanol), and carotenoids (lutein, zeaxanthin, and canthaxantin) were found more abundant in D12 than in D13.

Comparison between D13 V and D12 V showed significant differences: as for the samples without the fortification, the higher contents of unsaturated fatty acids (UFAs) were observed

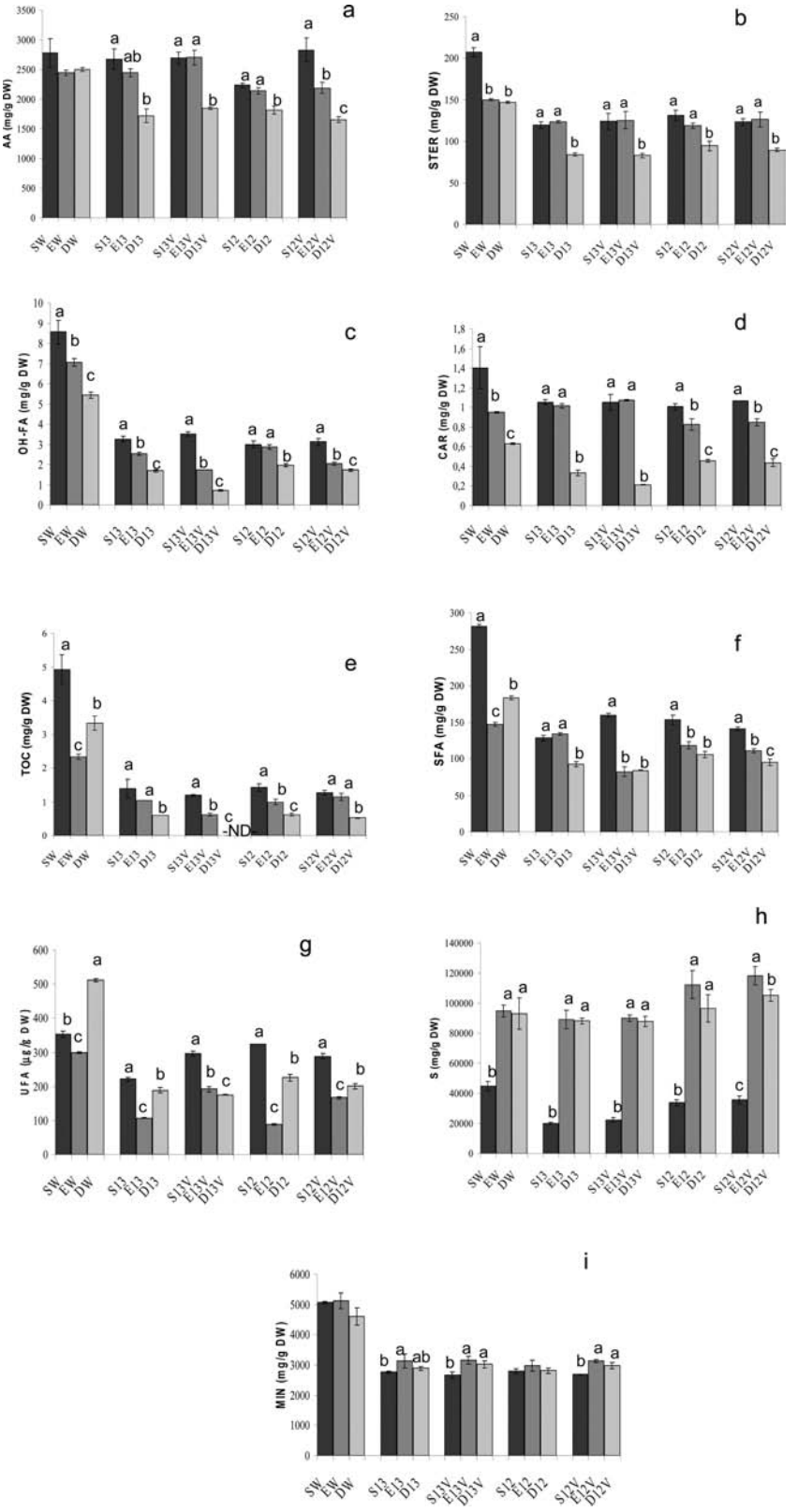

Figure 2. Evolution of selected compounds during pasta making of wholemeal, P12, P12 V, P13, and P13 V semolina. Error bars represent standard deviations. Different letters indicate significant differences among processing steps for each semolina at $P \leq 0.05$ following the Tukey test; ND, not detectable. AA, amino acids; STER, sterols; OH-FA, hydroxy fatty acids; CAR, carotenoids; TOC, tocopherols; SFA, saturated fatty acids; UFA, unsaturated fatty acids; S, sugars; MIN, minerals.

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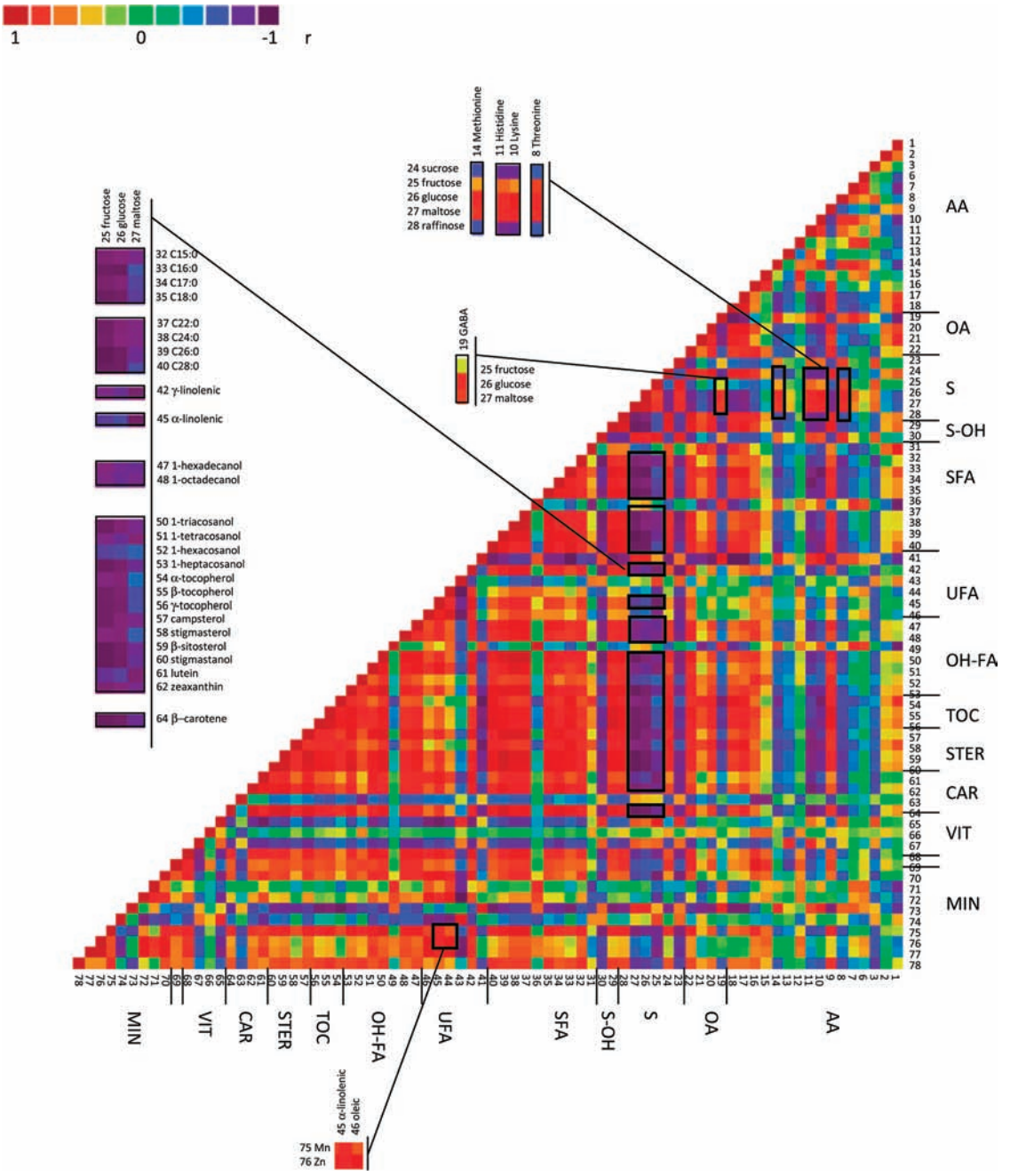

Figure 3. Visualization of metabolite and mineral cross correlations during wholemeal pasta making. Metabolites were grouped by compound class, and each square represents the correlation between the metabolite (or mineral) heading the column and the metabolite heading (or mineral) the row. Each square indicates a given *r* value resulting from a Pearson correlation analysis in a false color scale (see color key at top left). The metabolite and mineral notation is outlined in Table 1.

in pasta D12 V, whereas tocopherols, zeaxanthin, and most of the hydroxy fatty acids and alcohols were not detected in D13 V. In contrast, D13 V exhibited the greater content of asparagine, whereas alanine, valine, and GABA were totally absent in D12 V. For minerals, higher values were observed in D13 V with the exceptions of zinc and molybdenum, which were more abundant in D12 V. Finally, and not surprisingly, higher contents of thiamin, riboflavin, niacin, and iron were observed in the two pasta samples fortified with iron and vitamins (see Materials and Methods).

Evolution of Metabolites during Pasta Processing. The two samples of pasta characterized by 13% of protein content were derived from the same industrial process and produced to give the same shape (rigatoni), whereas pasta samples with 12% protein content were derived from two slightly different processes resulting in rigatoni and fusilli (with and without vitamins, respectively). The wholemeal pasta was produced as shells. The main differences among the three industrial processes concern a small but significant variation in temperature and the duration of the drying cycle. No significant differences were observed among

the processing steps for organic acid (OA) and polyalcohols (OH-S) (data not shown), whereas a significant decrease in free amino acids (AA), sterols (STER), hydroxy fatty acids (OH-FA), and carotenoids (CAR) was observed from semolina to dried pasta (Figure 2a−d, respectively). Phytosterol reductions were similar in all type of pasta (29.1, 29.6, 33.2, 28.2, and 27.3% for W, P13, P13 V, P12, and P12 V, respectively). In contrast, the reduction in the level of hydroxy fatty acids was dependent on the processing employed: the decrease from semolina to dried pasta was around 35% for shells (W sample) and fusilli (P12 sample), two industrial protocols characterized by a lower drying temperature than the process leading to rigatoni, for which the reduction was around 50% (P13, P13 V, and P12 V samples), suggesting that the degradation of these compounds has a distinct temperature dependency.

A significant total carotenoid loss was observed during processing. In wholemeal, P12, and P12 V the degradation after extrusion was 32, 17.8, and 20.4%, respectively, whereas in P13 and P13 V the loss was below 4%. After the drying step, further significant losses were observed for all kinds of pasta investigated, probably due to the high temperature employed in the processes. Overall losses of carotenoids from semolina to pasta observed in this study were between 54.7 and 80%. This broad variation is mainly due to the different kneading/extrusion times and drying temperature regimen in different pasta-making processes; in general, the higher the temperature, the greater the carotenoid loss. These findings are in complete agreement with Hidalgo et al.,[21] who reported an average carotenoid loss of 57% from semolina to pasta, whereas Fares et al.[22] reported a more intense degradation (77%) for pasta made with semolina from emmer wheat.

For tocopherols (TOC) and saturated fatty acids (SFA) the refined samples showed a progressive decrease during the pasta generation steps, whereas for wholemeal, after a significant decrease during the extrusion step (−52 and −47.8% for TOC and SFA, respectively), the total content of TOC and SFA increased significantly in dried pasta (Figure 2e,f).

The UFAs showed a significant decrease following extrusion, possibly due to the hydroperoxidation reaction catalyzed by lipoxygenase (LOX) enzymes (Figure 2g). The increase in UFA content in dried pasta samples compared to the extruded samples was probably due to the inactivation of LOX enzyme and the thermal hydrolysis of triacylglycerol, thereby releasing fatty acids during drying.

The total sugar content (S) significantly increased from semolina to extruded and dried pasta (Figure 2h), possibly highlighting thermally driven hydrolysis of polysaccharide material such as starch. The total mineral content (Figure 2i) showed a slight increase during the extrusion for all pasta processing, likely due to the mineral content of the tap water employed for the dough preparation followed by a nonsignificant decrease after the drying step regardless of the conditions adopted. Similar observations were reported by Cubadda et al.[23]

Correlation of Combined Metabolite Data Sets for Wholemeal Pasta. Correlation analysis was performed on the data set collected from the wholemeal pasta processing, the sample set showing the highest significant correlations in comparison to the other data sets (not shown). The analysis was based on Pearson correlation between all metabolites and minerals (Figure 3).

Metabolite−metabolite associations were positive in the majority of combinations, especially for nonpolar metabolites. Exceptions were the reducing sugars, which displayed significant negative correlation with most SFAs, UFAs (α- and γ-linolenic acid), most hydroxy fatty acids, tocopherols, sterols, and carotenoids (see enlarged section in the heat map of Figure 3).

The negative association between the increase in sugar content and the concomitant decrease of the most nonpolar compounds, especially in the extruded samples, can be viewed as a secondary, or indirect, association as it reflects the consequence of the high enzymatic activities of amylase, lypoxygenase, and peroxidases in the dough.[24,25]

The positive correlation between the reducing sugars fructose, glucose, and maltose and the free amino acids lysine, histidine, threonine, and methionine (see enlarged section in the heat map of Figure 3) may be explained by their interaction during the Maillard reaction. The reducing sugars (maltose, glucose, and fructose) were also positively correlated with the nonprotein amino acid γ-aminobutyric acid (GABA). The amino acids lysine, histidine, threonine, and methionine as well as GABA showed, in contrast to reducing sugars, a negative correlation with the nonreducing sugars raffinose and sucrose.

The strong positive correlation of manganese and zinc with oleic and α-linolenic acids probably reflects the common colocalization of these analytes in the germ of durum wheat kernel. Conversely, the negative correlation between UFA and iron reflects the different allocations of this component in the germ and bran fractions, respectively.

Use of Matabolomics in Food Investigations. Besides the major components starch, protein, and fats, foods contain a multitude of less abundant metabolites, some of which have relevant organoleptic and nutritional features. The application of metabolomics to the crop/food sector is increasing and being exploited to gain greater detailed insight into variation in food composition and the implications for organoleptic, nutritive, and health beneficial content.[12,13,26]

Real advances in the applications of metabolomics technologies have been highlighted by many research studies on staple crops such as tomato,[27−29] potato,[30,31] fruits,[32−34] and cereals, in particular, corn[35] and rice.[36,37] Also, for bread wheat several metabolomics studies have been undertaken,[14−16] but much less information is available for durum wheat and pasta.[17]

Many kinds of pasta are commercially available to satisfy the different demands and needs of the consumer, for example, wholemeal pasta, pasta with high and low protein contents, and pasta fortified with other nutrients. In general, the labels identify the constituents demanded by legislation and statutory requirements, and these are generally limited to energy value and the content of the main classes of compounds (sugars, proteins, fats, and, in the case of the wholemeal product, fiber). However, many more metabolites are present in the food, and the consumer is becoming more sophisticated and discerning, with a consequential desire for a more in-depth description of food and the potential nutritive value. The combined metabolic mineral analysis approach described here fulfills this requirement much more rapidly and economically than a corresponding series of targeted analyses would.

Common refined semolina, the staple ingredient in the majority of pasta, lacks the nutrient-rich bran and aleurone layers present in wholemeal semolina, in which the starchy endosperm, germ, and bran are present in the same relative proportions as in the intact caryopsis.[38] Consequently, metabolomics analysis of wholemeal pasta (Table 1) has highlighted the presence of policosanol, particularly tetracosanol (C_{24}) and hexacosanol (C_{26}),[20] and elevated phytosterols.[39] Several studies indicate

that the consumption of these compounds is effective in lowering LDL cholesterol and increasing HDL cholesterol levels.[40,41] Indeed, phytosterols have an approved health claim with regard to reducing the risk of cardiovascular disease,[42] but the clinical evidence for a positive effect of policosanols is in dispute.[43]

A deeper analysis of the metabolomics data showed differences between samples apparently similar besides the differences reported on the pasta labels. For example, pastas D13 and D12 are labeled differently only with regard to protein content, but metabolomics showed significant differences in phytosterols and UFA levels (Table 1), which are considered beneficial with regard to reducing the risk of coronary heart disease, hypertension, and type 2 diabetes.[41,42,44] These differences are likely to reflect a variation between stocks of durum wheat semolina traditionally classified (and paid for) on the basis of protein content only and a variation due to the different process conditions employed for pasta making.

If the dietary dose is correct, selected minerals can decrease the incidence of diseases of many degenerative diseases such as osteoporosis, arthritis, diabetes, and asthma. A balanced mineral intake is viewed as a way to achieve and/or prolong good health and metabolism. Current approaches to relieve micronutrient deficiencies include the promotion of balanced diets, supplementation, and food fortification.[45,46] Accordingly, in many countries pasta is fortified with vitamins and minerals to both enhance its nutritional value and create marketplace distinction. Metabolomics analysis of the fortified pasta confirmed the presence of elevated levels of iron and riboflavin (vit B2) in D13 V and D12 V samples, as declared by the manufacturer, but also identified additional differences in selected metabolite contents. For example, alanine, thiamin, aspartic acid, glucose, and some saturated fatty acids for P12 V and, for P13 V, also tocopherols, stigmasterols, hexadecanol, octadecanol, calcium, sodium, and manganese varied between the pasta samples with and without enrichment, suggesting that stocks of durum wheat semolina, although classified as the same, are different. This is probably a result of different factory intake times and subsequent batch-to-batch variation, and it could also affect organoleptic acceptance. A previous study highlighted that the flavor of cooked pasta differed significantly depending on the metabolite composition of volatile aldehydes, furans, and alcohols derived from fatty acids in the durum wheat cultivar.[17]

Metabolite Changes during Pasta Processing. The results of this work show that metabolite levels not only change during the pasta-making process but also depend on the processing conditions (Figure 3).

Oxidation is one of the main degradative reactions of phytosterols and is influenced by a number of factors, including temperature and reaction time.[47] Because thermal degradation is favored during the drying step, modifications of these conditions may help maintain the low content of phytosterols detected in wholemeal and refined pasta. Similar degradation trends were observed for hydroxy fatty acids, tocopherols, and carotenoids, suggesting that temperature adjustments could also help maintain the contents of these compounds in pasta. These trends in compound degradation suggest that the nutritional characteristics of pasta could be preserved at lower drying temperatures.

Although some of the carotenoids contribute to health benefits, some of the individual components are responsible for the bright yellow color of pasta that is considered to be desirable to the consumer. As part of pasta production, kneading leads to the incorporation of water and oxygen in the dough, thereby fostering the lipoxygenase (LOX)-mediated oxidation of polyunsaturated fatty acids, which in turn promote the oxidation of carotenoids.[48] As reported by Borrelli et al.[24] LOX activity is high during kneading, probably related to a greater availability of oxygen, water, and mixing-promoted substrate−enzyme−O_2 contact. Thus, the different carotenoid degradation levels observed among the samples are probably attributable to the degradation occurring during the kneading as a consequence of different characteristics of the semolina samples employed[49] and enzymatic activity.[24,50]

As reported by De Noni and Pagani[25] during the mixing of semolina with water, an extensive α-amylase activity takes place, consequentially increasing the content of reducing sugars. In fact, during kneading and extrusion, dough temperature (48 °C for all kind of process) and slightly acidic pH (6.1−6.2) conditions are optimal for amylase activity to increase the content of reducing sugars from semolina to dough extrusion. The subsequent high pressure employed inactivates amylase. After the drying phase, a decrease in total sugar content was evident, most likely driven by the Maillard reaction (MR). In fact, nonenzymatic browning related to the MR occurs easily during drying, and the protein−carbohydrate reactions lead to a change in the native structure of proteins and a degradation of amino acids with both free amino acids and small peptides in the matrix reported to be involved in the MR.[3,51] Acquistucci[6] showed that amino acids were differently involved in the MR and total essential amino acids decreased after extrusion in comparison to the unprocessed semolina.

In model studies with sugars and amino acids, Kwak and Lim[52] found that reducing sugars contribute to the browning in the following order: xylose > arabinose > glucose > maltose > fructose. With respect to pentose > hexose, this is also true for a wealth of other studies.[53] Furthermore, lysine was highlighted as one of the most reactive amino acids regarding Maillard formation,[52,53] and it exhibited the highest reactivity with each of these sugars during the MR. Our findings are in agreement with these results because there are positive correlations observed between reducing sugar and amino acids. As for amino acids, there are also reports of the loss of GABA in hydrothermally treated foods and cereal-based products,[54,55] suggesting that it behaves like most α-amino acids in the MR with reducing sugars.

The high-throughput analytical platforms used in this study have shown how a rapid and multicomponent view can be obtained of the chemical modifications taking place during the industrial production of different kinds of pastas. Although it is accepted that the use of raw material with good characteristics is essential to achieve a high-quality product, it is clear from this study (and many others) that the processing operations used can greatly affect product end quality. Even in cases when no additional nutritional enhancement had taken place, the pasta formation processes had significant impacts on components with known, or disputed, organoleptic, nutritional, and health beneficial parameters. Furthermore, this study has shown that pasta products, which are quantitatively similar according to food labels, exhibit many distinct metabolic differences. This study also demonstrates that metabolomics has a definite place in food quality, nutritional value, and safety issues; this specific role is often described with the new term "foodomics".[56,57] With the increasing power to resolve and correlate even more components, metabolomics has a chance to become an established part of the food analytical toolbox with a fundamental place in food

feedstock-to-product transition monitoring and process control. For this, further studies are required to show that metabolomics is not only suitable to monitor batch-to-batch variability but also a robust aid to improve product quality and associated nutritional value.

■ ASSOCIATED CONTENT

Supporting Information. Figures and tables giving additional test data, methodological information, and composition of samples. This material is available free of charge via the Internet at http://pubs.acs.org

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