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Chemometric study on the effect of cooking on bioactive compounds in tomato pomace enriched sauces

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Tomato pomace (TP) is an underutilized source of bioactive compounds with potential application in the food sector. A factorial experiment was designed to compare three culinary techniques, Thermomix®, Roner®, and traditional pan-frying, for the preparation of tomato sauces, enriched or not with TP, applying two temperatures and two cooking times. A multivariate analysis was performed on all the results obtained for the metabolites. The addition of TP significantly increased the content of bioactive compounds, especially phenolic compounds. OPLS-DA models were generated using cooking technique, temperature, and time as discriminant factors. The cooking technique had a greater effect on the phenolic content than cooking temperature or time. Thermomix® released bioactive compounds from the tomato into the sauce to a similar extent as pan-frying. Roner® proved to be effective in preserving the volatile fraction of the sauce. The Thermomix® significantly increased the amount of bioactive compounds, while the Roner® increased the volatile compounds.

Amidst growing consumer demand for functional foods, tomato by-products are generating considerable interest in the field of food science because of their high concentration of nutritive and functional components[1,2]. Moreover, there is an urgent need to tackle food waste due to its profound implications for both environmental sustainability and food security. The agri-food sector generates significant amounts of waste, including by-products from food processing. Tomato processing, in particular, yields significant quantities of by-products such as peels, seeds, and pomace. These wastes, if not properly managed, contribute to environmental pollution and constitute a loss of resources, considering they contain valuable bioactive compounds with potential health benefits[3]. Tomato pomace (TP), composed of approximately 60% seeds and 40% peel, is the major by-product of tomato processing and contains significant amounts of bioactive compounds as well as fiber and fatty acids[4].

Several studies have shown that tomato waste is a rich source of bioactive components[3,5–7]. Culinary home practices have a significant impact on the content of bioactive compounds in tomato sauces[8,9]. For example, during thermal processing, some nutrients may be affected by oxidation and degradation processes[10]. Given the widespread consumption of tomato sauces, it is of interest to enhance their antioxidant potential by enrichment with tomato by-products[11]. The practice of food enrichment is an innovative approach that can yield products of high nutritional value[12]. The Roner®, also known as a *sous vide* machine, consists of a temperature-controlled water bath and a vacuum sealing system, which minimizes food exposure to oxygen during cooking, thereby reducing the loss of volatile organic compounds (VOCs). A precise and consistent temperature can be maintained throughout the cooking process, typically lower than in traditional methods[13]. The Thermomix® is a versatile appliance that combines several cooking functions (chopping, blending, cooking, and mixing). It features a built-in heating element and a stainless-steel bowl with integrated blades. In tomato sauce preparation, this machine chops and mixes the ingredients

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and heats them at a controlled temperature, ensuring a gentle cooking process that preserves flavor[14].

So, it is necessary to ascertain how these cooking techniques affect bioactive compound levels and VOCs. In the present study, the effect of different cooking techniques on the bioactive compound profile of TP-enriched tomato sauces was investigated for the first time, comparing traditional pan-frying with more innovative systems using Thermomix® and Roner® kitchen appliances, and testing a range of variables such as temperature, cooking time, the form of heat application, and oxygen availability. In this work, a factorial design was used to investigate the differences in bioactive compounds of TP-enriched tomato sauces prepared using three different cooking techniques (Thermomix®, Roner®, and pan-frying), with two different temperatures (70 and 90 °C) and times (15 and 30 min). The same preparations without TP enrichment were used as a control, using a multivariable approach.

Results

Effects on bioactive compounds

Phenolic compounds, carotenoids, vitamin E, and VOCs of tomato sauces were analyzed to investigate how processing and TP enrichment affected their content. A total of 97 minor compounds were identified and quantified, including 54 phenolic compounds, 15 carotenoids, three forms of vitamin E, and 25 VOCs. Detailed information on the identified bioactive compounds is provided in the Supplementary Information (Table S1).

Among the total identified phenolic compounds, 70% were classified as phenolic acids, with the remaining 30% allocated to flavonoids. The most abundant phenolic acid in the TP-enriched sauces was caffeoyl-hexose II followed by *p*-hydroxybenzaldheyde. The third most abundant compound was 3-caffeoylquinic acid (chlorogenic acid). Finally, homovanillic acid hexose III, 2,5-dihydroxybenzoic acid, dicaffeoylquinic acid III, 3,4-dihydroxyhydrocinnamic acid and gentisic acid were the lowest quantified compounds in all samples with values below 0.5 µg/g. Among flavonoids, rutin was by far the most abundant flavonoid detected in tomato sauce, being twice as abundant in sauces enriched with TP. Other flavonoids such as eriodictyol-*O*-hexoside, quercetin-3-glucoside, and hesperetin were only found in the TP-enriched sauces. We detected trace amounts of kaempferol and luteolin in both TP-enriched and non-enriched tomato sauces, and kaempferol-*O*-rutinoside in TP-enriched sauces.

The most abundant carotenoid was lycopene, and its concentration was not affected by TP-enriched tomato sauces. Lycopene isomers such as 5-*Z*-lycopene were also more abundant than other identified carotenoids, followed by phytoene and other lycopene isomers. Regarding vitamin E, alpha-tocopherol was the predominant isoform that we found in tomato sauce, with a slightly lower concentration in sauces enriched with TP. Alpha-tocotrienol was the second most abundant, and its concentration was slightly higher in the TP-enriched tomato sauces.

The most abundant VOCs in the prepared tomato sauces were 6-methyl-5-heptene-2-one, 4-methyl-2-pentanol, and 2,2,4,6,6-pentamethylheptane. 6-Methyl-5-heptene-2-one was the only one that increased in the TP-enriched sauces, the other two VOCs having practically the same concentration in the TP-enriched and non-enriched tomato sauces.

Changes in composition in enriched sauces

A multivariate statistical analysis was performed to evaluate the effect of TP enrichment on the concentration of phenolic compounds, carotenoids, vitamin E, and VOCs in tomato sauces. The color-coded PCA score plot for the TP-enrichment factor (Fig. 1) clustered the data on bioactive components of non-enriched and TP-enriched sauces. In this model, the PC1 accounted for 44.5% of the variance, indicating that TP-enrichment had a high impact on the composition of bioactive and minor compounds in the tomato sauces prepared in this work.

In addition, to determine the differences in concentration of bioactive compounds between TP-enriched and non-enriched sauces, a PLS-DA model was built using TP-enrichment as a factor. The results of the validation model are provided in the Supplementary Information (Table S2). Plots of the validation model are provided in the Supplementary Information (Fig. S1). Figure 2 shows how the components are clearly separated when this factor is considered.

Table 1 shows the marker compounds of TP enrichment, together with the VIP score, the *p*-value of the *t*-test, and the concentration of the bioactive compounds. The components most affected by the enrichment were phenolic compounds, their content in the tomato sauce more than doubling. Regarding phenolic acids, some compounds such as caffeoyl-hexose and its derivatives, 4-hydroxybenzoic acid, 2,6-dihydroxybenzoic acid, 2,5-dihydroxybenzoic acid, and *p*-coumaric acid were not identified in the non-enriched sauces but were found at high levels after the enrichment. The flavonoid content also increased in the enriched tomato sauces, especially rutin and naringenin chalcone.

Although TP is known to be rich in carotenoids, but in this study did not find any significant increase in carotenoid levels in the TP-enriched tomato sauces. Similarly, the concentration of vitamin E (tocopherols and tocotrienols) and VOCs seemed unaffected.

Effect of culinary techniques and conditions

To evaluate the effects of culinary techniques and conditions, OPLS-DA models were generated, using cooking technique, temperature, and time as discriminant factors. As shown in Fig. 3, all models clearly separated the experimental variables according to the bioactive compound content. The parameters used to validate the models are listed in Table S2 in the Supplementary Information. In addition, all plots of the models shown in Figs. S2–S4 in the Supplementary Information.

As expected, the processing conditions had a significant influence on the content of bioactive compounds in tomato sauces. Table 2 shows the bioactive and minor compounds selected as markers to evaluate the effects of the culinary techniques and conditions.

The marker compounds of the cooking technique were mainly VOCs, in addition to some phenolic compounds, carotenoids, and vitamin E. The VOCs hexanal, 2-hexenal, 3-hexen-1-ol, and 1-hexanol increased with Roner® processing.

Certain phenolic compounds exhibited divergent responses according to the technique, decreasing in concentration when subjected to Roner® processing, while increasing in sauces prepared in the Thermomix® and/or a frying pan. The contents of gentisic acid and naringenin dihexose II were higher when using pan-frying and the Thermomix®, respectively, whereas the levels of sinapic acid-*O*-hexoside, 3-(2-hydroxyphenyl) propanoic acid, and phloretin-*C*-dihexoside were similar between the two techniques.

The lowest levels of carotenoids were also found in tomato sauces cooked with the Roner®. The highest levels of lycopene (175.12 ± 38.63 µg/g FW) and 13-*Z*-lycopene (2.09 ± 0.47 µg/g FW) were found in those prepared by pan-frying. The highest levels of both forms of vitamin E were found in sauces prepared in the Thermomix®: α-tocopherol (16.38 ± 12.21 µg/g FW) and α-tocotrienol (6.13 ± 2.66 µg/g FW).

Regarding the culinary conditions, the marker compounds, including phenolic acids, carotenoids, and VOCs, experienced a slight increase at the higher temperature of 90 °C (Table 2). Although without significant differences, the shorter cooking time of 15 minutes resulted in a slightly higher content of bioactive compounds compared to 30 minutes.

Discussion

This study evaluated the potential of TP for use in the food sector to improve the nutritional properties of tomato sauce. We prepared tomato sauces enriched and non-enriched with TP, using different cooking techniques and conditions to evaluate their bioactive compounds. We studied phenolic compounds, carotenoids, vitamin E, and VOCs.

TP is known to be rich in bioactive compounds. In our study, we have observed that the tomato sauces studied generally had a higher content of phenolic acids compared to flavonoids, and in most cases, we have seen that several compounds were not identified in non-enriched tomato sauces, which makes us see that TP was rich in these compounds. This finding agrees with previous studies that also report phenolic acids as the

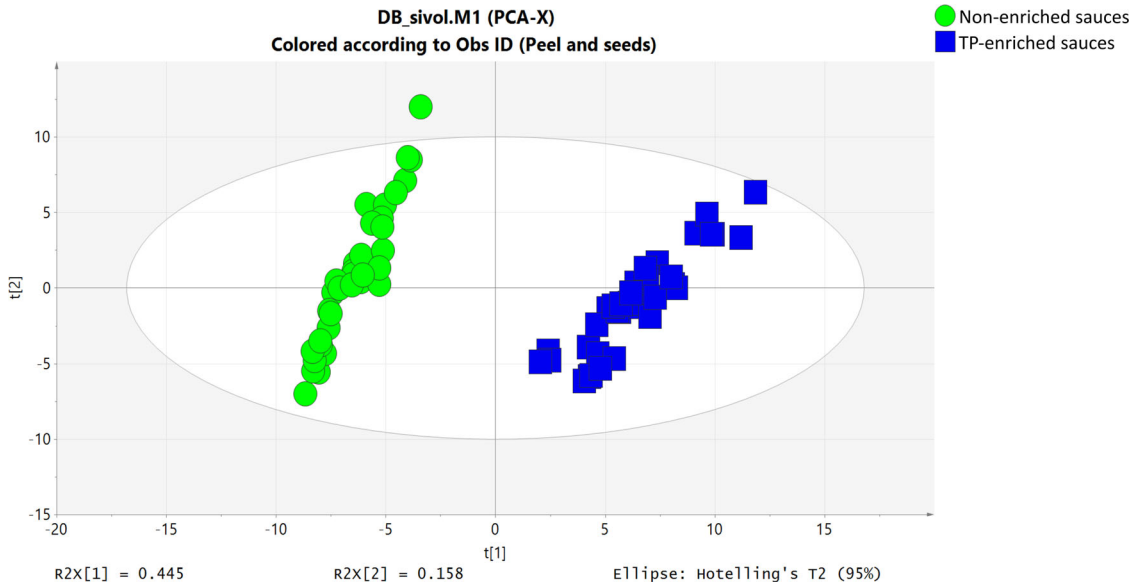

Fig. 1 | PCA score plot of the PC1, colored and shaped according to the factor of TP-enrichment.

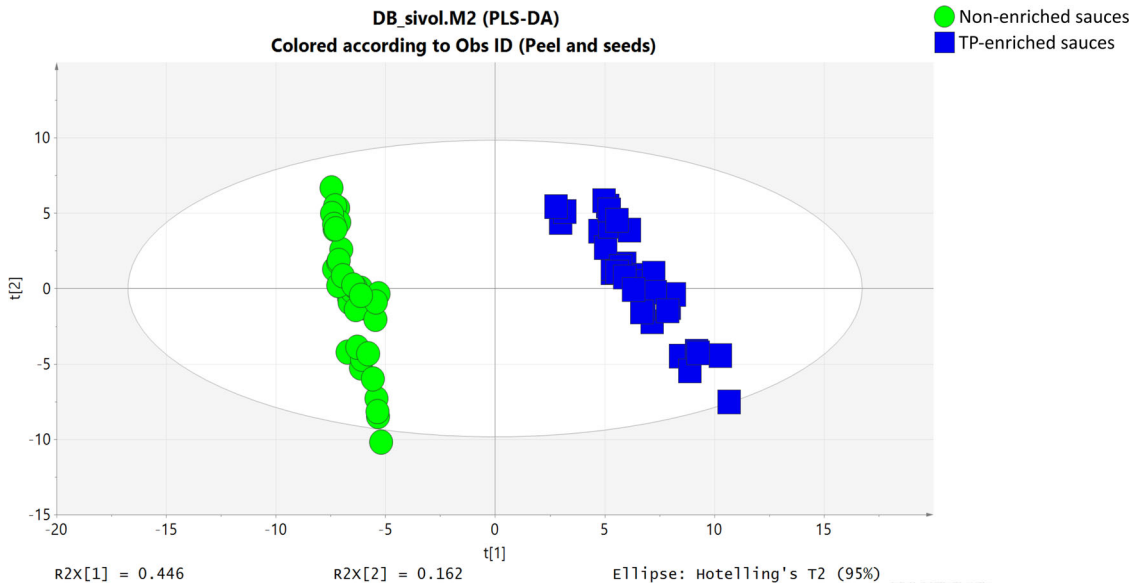

Fig. 2 | PLS-DA score plot for tomato pomace (TP)-enriched tomato sauces.

predominant phenolic compounds in TP[15,16], although others have found flavonoids to be the most abundant[5,17,18]. The relative concentrations of flavonoids and phenolic acids in TP can vary depending on factors such as tomato variety, ripeness, processing methods, and environmental conditions.

Previous studies of phenolic acids in TP have determined *p*-coumaric acid[17–19] and tentatively identified caffeic acid hexoses[20]. However, other phenolic acids have not been reported in tomato residues until now, despite being present in fresh tomato[21], perhaps due to insufficiently in-depth analysis. In agreement with the present findings, earlier studies of tomato sauces have identified *p*-coumaric acid[8,22], caffeoylquinic acids and dicaffeoylquinic acids[22], and caffeoyl-hexose (or caffeic acid hexoses)[23]. In contrast, previously identified compounds such as *o*-coumaric acid[22,23] and ferulic hexose[24] were not detected in the tomato sauces analyzed in the present study.

Among flavonoids, naringenin derivatives, and rutin have been detected in wastes from different tomato varieties[17,25]. In the present work, rutin was twice as abundant in sauces enriched with TP. Quercetin and derivatives have also been identified in tomato by-products in previous studies[4,18]. Other flavonoids reported in TP include kaempferol, luteolin, chrysin, catechin, and epicatechin[25].

In this study, the most abundant carotenoids in the tomato sauces were lycopene, in agreement with previous reports[26,27]. Lycopene is a highly reactive carotenoid that readily undergoes oxidation and/or isomerization during processing[28]. The processing of tomato sauces produces lycopene *Z*-isomers, which are the most available forms for the human body[29]. Vallverdú-Queralt et al.[27] reported that short-term, high-quality processing of tomato sauces results in a higher concentration of bioactive molecules with benefits for human health[27].

Alpha-tocopherol is the predominant isoform of vitamin E found in fresh tomatoes and tomato sauce, as corroborated in this study, with higher levels observed in enriched tomato sauces. This isoform is a potent antioxidant and the most biologically active form of vitamin E, playing a crucial role in protecting cells from oxidative damage[30].

The most abundant VOCs were 6-methyl-5-heptene-2-one, 4-methyl-2-pentanol and 2,2,4,6,6-pentamethylheptane, which are part of a complex

Table 1 | Bioactive compound markers of enriched tomato sauces

Factor	Compound	VIP-value	*p*-value	Non-enriched tomato sauce	TP-enriched tomato sauce
Tomato pomace enrichment	Caffeoyl-hexose IV	1.60559	2.4241E−049	0.00 ± 0.00	22.23 ± 2.69
	Caffeoyl-hexose II	1.59776	3.7337E−045	0.00 ± 0.00	64.97 ± 9.26
	p-Hydroxybenzaldehyde	1.59100	2.9396E−048	1.99 ± 1.02	38.70 ± 5.41
	Phenolic acids	1.58622	3.032E−046	115.03 ± 12.96	311.09 ± 28.94
	Caffeoyl-hexose I	1.58534	7.4434E−042	0.00 ± 0.00	8.33 ± 1.37
	Total phenolics	1.58503	3.9603E−046	170.70 ± 19.82	439.99 ± 39.19
	4-Hydroxybenzoic acid	1.58306	7.4434E−042	0.00 ± 0.00	1.89 ± 0.32
	2,6-Dihydroxybenzoic acid	1.57182	2.7722E−038	0.00 ± 0.00	0.87 ± 0.17
	Homoeriodictyol	1.56913	1.8372E−041	0.02 ± 0.02	0.88 ± 0.17
	Eriodictyol	1.56700	5.144E−041	0.04 ± 0.02	0.93 ± 0.17
	Eriodictyol-*O*-hexoside	1.56435	2.3377E−036	0.00 ± 0.00	0.42 ± 0.09
	2,5-dihydroxybenzoic acid	1.56400	2.4143E−036	0.00 ± 0.00	0.38 ± 0.08
	Caffeoyl-hexose III	1.56234	1.393E−034	0.00 ± 0.00	20.57 ± 4.41
	p-Coumaric acid	1.56214	2.4143E−036	0.00 ± 0.00	0.95 ± 0.20
	Quercetin *O*-hexoside	1.56087	1.7152E−035	0.00 ± 0.00	0.69 ± 0.15
	Quercetin-*O*-rutinoside-*O*-hexoside	1.55962	3.1271E−039	0.26 ± 0.06	1.29 ± 0.21
	Flavonoids	1.55554	2.344E−038	55.67 ± 7.84	128.91 ± 13.88
	Rutin	1.55089	1.5793E−037	30.33 ± 4.18	63.29 ± 6.12
	Naringenin chalcone	1.5487	1.1175E−036	1.96 ± 1.89	13.56 ± 1.92
	Caffeoylmalic acid	1.53642	9.222E−034	3.94 ± 0.38	7.46 ± 0.83
	Naringenin 7-glucoside	1.52345	1.9228E−032	0.04 ± 0.04	1.27 ± 0.33
	Homovanillic acid hexose II	1.52226	1.664E−032	2.03 ± 0.34	4.15 ± 0.47
	Hesperetin	1.51394	3.7008E−029	0.00 ± 0.00	0.06 ± 0.02

mixture of VOCs that give tomato sauce its characteristic taste and flavor. 6-Methyl-5-heptene-2-one, ubiquitous in fruits and vegetables, has a strong fruity and slightly floral aroma, contributing to the fruity and aromatic notes of tomato sauce. 4-Methyl-2-pentanol, commonly found in a variety of foods, is an alcohol with a slightly fruity, floral, and alcoholic aroma, which may contribute to the overall complexity of tomato sauce flavor. Commonly found in citrus fruits, the cyclic terpene 2,2,4,6,6-pentamethylheptene contributes to the citrus notes and aromatic profile of tomato sauce[31,32].

A clear difference was observed between the enriched and non-enriched tomato sauces in terms of bioactive compound content. In the principal component analysis, the TP-enriched and non-enriched samples were separated by the PC1, which was responsible for 44.5% of variance, indicating that the TP-enrichment factor had a strong influence on the bioactive compound composition in the tomato sauces prepared in this work. The components most affected by the enrichment were phenolic compounds, their content in the tomato sauce more than doubling. As mentioned above, TP is rich in phenolic compounds[3]. The addition of TP to tomato sauces directly increases the content of these compounds in the sauces, possibly due to the transfer of these compounds from TP to the sauce. Other phenolic compounds, not present in the not-enriched sauces, were quantified in TP-enriched sauces. These polyphenols detected in the TP-enriched sauces may be bound compounds released from the TP matrix in the sauce[33]. Although TP is known to be rich in carotenoids, mainly lycopene, β-carotene, and lutein[7,34], this study did not find any significant increase in carotenoid levels in the TP-enriched tomato sauces.

In general, bioactive compounds are more concentrated in the pomace (peel and seeds) than in the whole raw fruit from which it derives, regardless of the factors that may influence the content of bioactive compounds, such as tomato variety, agronomic conditions[35], processing[22], food matrix[24] and others.

Hence, incorporating this by-product into tomato sauces can potentially provide a functional food with significantly enhanced nutritional value and health-promoting properties[36]. The bioactive compounds found in pomace have antioxidant, anti-inflammatory, anti-cancer, and cardioprotective affects[37,38]. In addition, the reuse of processing by-products in food production contributes to sustainability by reducing food waste and the environmental impact of its disposal, an approach aligned with the principles of circular economy and sustainable development[3].

When assessing the impact of cooking techniques, it was observed that Thermomix® processing was comparable with the traditional pan-frying method in effectively releasing bioactive compounds from tomato into the sauce. This result is of particular interest for consumers seeking to minimize kitchen time and effort without compromising the quality of meals. Compared to the Thermomix®, cooking with a Roner® was better at preserving flavor and aroma but less efficient at releasing bioactive compounds. The marker compounds of the cooking technique were mainly VOCs, in addition to some phenolic compounds, carotenoids, and vitamin E. The VOCs hexanal, 2-hexenal, 3-hexen-1-ol, and 1-hexanol increased with Roner® processing. These compounds, which are important for food aroma and flavor, have been previously described in fresh tomatoes and tomatoes sauces[32,39]. The Roner® cooker is equipped with a thermostat, enabling precise temperature control (maintained between 5 and 100 °C) during water bath cooking with continuous water circulation. As the Roner® cooking technique involves minimal evaporation, resulting in a higher ratio of volatile compounds production than evaporation, it is effective in preserving VOCs. Consequently, this method facilitates the creation of sauces with enhanced taste and aroma[32].

In contrast, the tomato sauces prepared with the Roner® exhibited the lowest concentrations of phenolic and carotenoid compounds in the experiment. This outcome could be attributed to the absence of stirring during the cooking process, in contrast with traditional methods, where a spatula is conventionally employed. Stirring is known to play a crucial role in the release of bioactive compounds from foods during cooking. Additionally, the lower heat transfer of the Roner® method might hinder certain

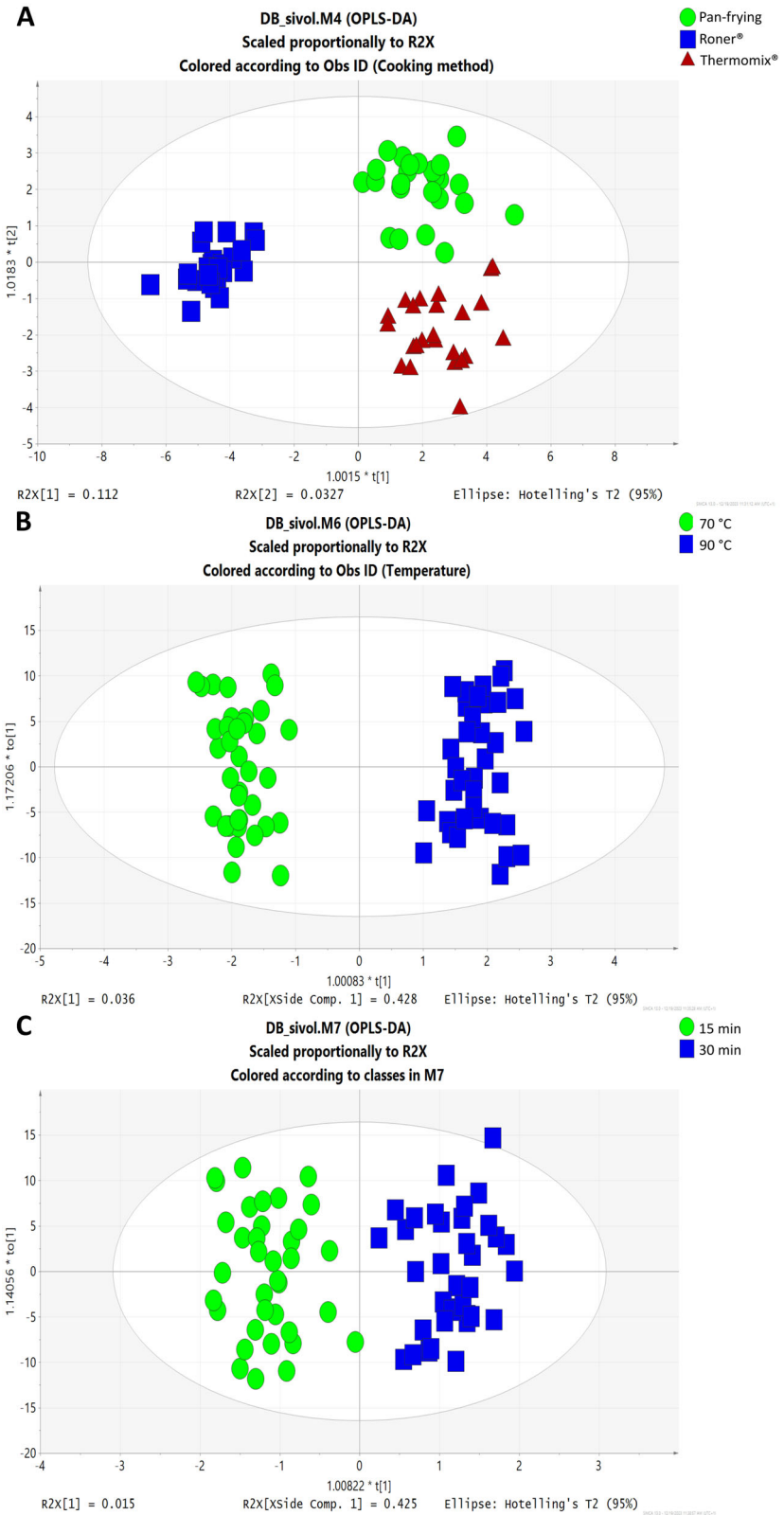

Fig. 3 | OPLS-DA-score plot. A Cooking technique. **B** Temperature (°C). **C** Time (minutes).

chemical transformations essential for optimal compound extraction. Notably, as few published studies have utilized the Roner®, there are no results available in the literature for comparison.

Certain phenolic compounds exhibited divergent responses according to the technique, decreasing in concentration when subjected to Roner® processing, while increasing in sauces prepared in the Thermomix® and/or a frying pan. This may be because Thermomix® cooking allows precise control of temperature and cooking time, which can optimize the release of phenolic compounds without causing significant degradation[14]. Cooking in a pan is less controllable and direct exposure to oxygen can cause degradation of phenolic compounds; however, in this study, both types of cooking allowed ~~for~~ a

Table 2 | Markers of bioactive and other minor compounds in tomato sauces considering cooking factors

Factor	Compound	VIP-value	*p*-value	Thermomix®	Roner®	Pan-frying
Cooking technique	2,2,4,4-Tetramethyloctane	2.25289	0.00017263	0.12 ± 0.07	0.19 ± 0.04	0.20 ± 0.06
	Hexanal	1.9846	8.136E−12	0.52 ± 0.42	1.53 ± 0.32	0.82 ± 0.35
	Octanal	1.86985	0.0061007	0.05 ± 0.06	0.02 ± 0.05	0.00 ± 0.00
	2-Hexenal	1.71266	0.0026653	0.59 ± 1.15	1.56 ± 0.99	0.70 ± 0.39
	Gentisic acid	1.70411	0.002729	0.01 ± 0.01	0.02 ± 0.02	0.06 ± 0.07
	Sinapic acid-O-hexosid	1.701	2.133E−08	2.99 ± 0.36	2.29 ± 0.22	2.99 ± 0.36
	Heptane, 3-[(1,1-dimethylethoxy) methyl]	1.67544	0.0048567	0.01 ± 0.02	0.00 ± 0.00	0.00 ± 0.00
	2,5-Dimethyl-2-undecene	1.65884	0.0058979	0.02 ± 0.03	0.00 ± 0.00	0.00 ± 0.00
	3-(2-Hydroxyphenyl) propanoic acid	1.65167	3.156E−08	7.49 ± 0.99	5.72 ± 0.75	7.46 ± 1.02
	Naringenin dihexose II	1.62105	0.002169	0.41 ± 0.17	0.21 ± 0.17	0.25 ± 0.21
	Phloretin-C-dihexoside	1.6151	4.946E−06	3.54 ± 0.62	2.77 ± 0.51	3.79 ± 0.65
	13-Z-Lycopene	1.5173	0.00046939	1.81 ± 0.62	1.43 ± 0.35	2.09 ± 0.47
	3-Hexen-1-ol	1.51693	1.250E−06	0.08 ± .08	0.28 ± 0.186	0.08 ± 0.04
	Lycopene	1.51361	2.746E−05	158.15 ± 62.54	100.50 ± 38.33	175.12 ± 38.63
	1-Hexanol	1.50404	3.061E−06	0.00 ± 0.00	0.17 ± 0.19	0.00 ± 0.00
	α-Tocotrienol	1.50067	0.00093551	6.13 ± 2.66	3.88 ± 1.03	4.79 ± 1.21
Factor	**Compound**	**VIP-value**	***p*-value**	**70 °C**	**90 °C**	
Temperature	Protocatechuic acid	4.21403	7.017E−11	0.20 ± 0.08	0.42 ± 0.13	
	9-*Z*-Lycopene	2.90013	0.0015525	3.54 ± 1.61	5.98 ± 2.96	
	2,2,4,6,6-Pentamethylheptane	2.72295	0.0015408	1.31 ± 0.34	0.96 ± 0.33	
	Acetone	2.56237	0.0024903	0.17 ± 0.05	0.24 ± 0.08	
	7-*Z*-Lycopene	2.13035	0.029557	2.01 ± 0.51	2.44 ± 0.58	
	Dicaffeoylquinic acid III	2.03975	0.040198	0.37 ± 0.07	0.49 ± 0.21	

greater release of phenolic compounds compared to cooking with the Roner®.

The effects of cooking techniques on bioactive compounds are known to depend on several factors, principally the food matrix, but also the cooking time and temperature, and the surface exposed to water and oxygen[40]. Cattivelli et al.[41] observed that frying yielded a higher phenolic content in cooked onion compared to baking, boiling, and grilling[41]. Similarly, fried vegetables (potato, tomato, and pumpkin) were found to have a higher phenolic content than those cooked by sautéing and boiling[42]. Steaming is another technique that effectively preserves phenolic content as it minimizes leaching of water-soluble compounds and reduces oxidative degradation[43]. Martini et al.[44] observed that different cooking techniques had different effects on the release of specific phenolic compounds; baking and grilling resulted in a higher release of bioavailable caffeoylquinic acids, whereas frying resulted in higher levels of di-caffeoylquinic acids and hydroxycinnamic acid amides[44]. Ilyasoglu and Burnaz (2015) found that steaming was the most effective method for preserving antioxidant molecules in fresh and frozen kale, followed by microwaving and boiling as the next best options for home cooking[45].

Variable effects of processing on tomato phenolic content have been reported. A reduction in content has been attributed to the type of compound, the cooking technique, the processes of leaching and complexation with other compounds[46–48], and the release of oxidative and hydrolytic enzymes that were not completely deactivated[46]. On the other hand, an increase in total phenolic content[46,49]may be due to the release of compounds from the matrix[37], alterations of plant cell structure because of stress factors, or inactivation of oxidative enzymes[50].

The study highlights that the bioactive composition of tomato sauces is less influenced by cooking temperature and time than the cooking technique and enrichment with pomace, both of which had a significant impact. Heat treatment disrupts cellular structures and releases phenolic compounds from their biological matrices, making them more accessible. This process can also modify the chemical structure of bioactive compounds, transforming insoluble forms into more soluble ones. In addition, cooking can lead to the hydrolysis of various components, releasing bioactive compounds and increasing their extractability[40,51]. In terms of cooking time, phenolic compounds are susceptible to oxidation; longer cooking times increase the exposure of phenolic compounds to oxidative processes[8].

In summary, tomato sauces enriched with TP had higher levels of bioactive compounds; this could be due to the fact that the different cooking techniques improved their increase due to their different mechanisms of action, such as cell disruption, chemical transformation, enzyme inactivation, hydrolysis and improvement of solubility. Although temperature and time had a less effect, a higher temperature for a shorter time allowed to obtain higher concentrations of bioactive compounds in the enriched sauces. The results suggest that tomato pomace, a tomato processing by-product rich in antioxidants, has potential as an ingredient in foods with enhanced functionality. As well as benefiting human health, such an application would promote sustainability by reducing tomato waste.

Methods

Chemicals

Standards used for compound identification and quantification were sourced from various suppliers as follows: homovanillic acid, apigenin, quercetin, naringenin, rutin, quercetin dihydrate, quercetin-3-glucoside taxifolin, *o*-coumaric acid, *m*-coumaric acid, 3-(4-hydroxyphenyl)propionic acid, 3,4-dihydroxyhydrocinnamic acid, sinapic acid, 3-(2,4-dihydroxyphenyl)propionic acid, cinnamic acid, chlorogenic acid, caffeic acid, verbascoside, benzoic acid, neochlorogenic acid, ellagic acid, vanillic acid, 4-hydroxybenzoic acid, 2,5-dihydroxybenzoic acid, gallic acid, 2,6-dihydroxybenzoic acid, vanillic acid, 3,5-dihydroxybenzoic acid, p-hydroxybenzoic acid, phenylacetic acid, hesperidin, luteolin and eriodictyol were obtained from Sigma-Aldrich; naringenin-7-*O*-glucoside, epicatechin gallate, ethylgallate, and kaempferol from Extrasynthese; epicatechin, ferulic acid, *p*-coumaric acid, syringic acid,

Table 3 | Experimental level of the factors used in the full factorial design

Treatment	Control			Treatment	Enriched with TP		
	Cooking Method	Temperature (°C)	Time (min)		Cooking method	Temperature (°C)	Time (min)
C1	Thermomix®	70	15	T1	Thermomix®	70	15
C2	Thermomix®	70	30	T2	Thermomix®	70	30
C3	Thermomix®	90	15	T3	Thermomix®	90	15
C4	Thermomix®	90	30	T4	Thermomix®	90	30
C5	Roner®	70	15	T5	Roner®	70	15
C6	Roner®	70	30	T6	Roner®	70	30
C7	Roner®	90	15	T7	Roner®	90	15
C8	Roner®	90	30	T8	Roner®	90	30
C9	Pan-frying	70	15	T9	Pan-frying	70	15
C10	Pan-frying	70	30	T10	Pan-frying	70	30
C11	Pan-frying	90	15	T11	Pan-frying	90	15
C12	Pan-frying	90	30	T12	Pan-frying	90	30

3-hydroxybenzoic acid and myricetin from Fluka; quercitrin and methyl gallate from Phytolab; and naringenin chalcone; all-*E*-α-carotene, all-*E*-β-carotene, all-*E*-lycopene, phytoene, α-tocopherol and 4-methyl-2-pentanol from Chromadex. Methanol (LC-MS grade) was supplied by Merck (Darmstadt, Germany) as well as acetonitrile, ethanol, hexane, tert-butyl methyl ether (TBME).

Tomato sauce preparation

The tomato sauces were prepared following a conventional recipe in an industrial kitchen at Torribera Campus, University of Barcelona (Santa Coloma de Gramenet, Spain). Tomatoes of the traditional variety *Lycopersicon esculentum* Mill, *c. v.* Pera were bought in Barcelona markets, washed, crushed with a mixer (model R5 Plus, Robot Coupe®), and weighed according to the factorial design.

To determine the best cooking technique for maximizing the bioactive compound content of the final product, tomato sauces were prepared using a 2 × 3 × 2 × 2 factorial design. Thus, using tomato without peel and seeds as a control, three techniques were compared, two using novel appliances (Thermomix® and Roner®) and the other traditional pan-frying, with the application of two cooking times (15 and 30 min), and two temperatures (70 and 90 °C). This resulted in a total of 24 series for each culinary technique, as shown in Table 3. Each tomato sauce was prepared with 200 g of tomatoes and 20 mL of refined olive oil, and in no case were tomato peel and seeds removed. To verify that the refined olive oil was free of phenolic compounds was analyzed by LC-ESI-LTQ-Orbitrap-MS. To supplement the sauces with extra peel and seeds, 12 g of crushed TP (Conesa group, Spain) was added to the tomato paste before cooking. The processing of each sauce was repeated three times.

Thermomix® apparatus. A Thermomix® apparatus (model TM6-1, Vorwerk, Germany) was programmed with the selected temperatures and cooking times, and the tomato sauce was cooked with continuous stirring. All samples were vacuum packed and kept frozen (−20 °C) until analysis.

Roner® apparatus. In a Roner® apparatus (model 9999951, J.P. Selecta S.A., Abrera, Spain), a plastic vacuum bag containing tomato sauce was placed in the water bath heated to the desired temperature, and cooked for 15 or 30 min. All samples were kept frozen (−20 °C) until analysis.

Pan-frying. The tomato sauce was cooked directly in a frying-pan once the specified temperatures had been reached (measured by a thermometer) for the indicated times with continuous stirring. All samples were vacuum-packed and kept frozen (−20 °C) until analysis.

Phenolic profile

The phenolic compounds were extracted as in a previous study[52]. Tomato sauce (0.5 g) was mixed with 5 mL of a solution composed of methanol and milli-Q water (8:2 v/v) and homogenized for 30 seconds. The samples were sonicated for 10 min in an ice-bath to prevent compound oxidation, and then centrifuged at 4000 rpm for 10 min at 4 °C. The resulting supernatant was transferred to a glass tube. The solid residue underwent a second extraction under the same conditions as detailed above. Both supernatants were pooled and evaporated with a vacuum evaporator (miVac DNA concentrator, Genevac LTD, Warminster, England). Finally, all samples were reconstituted with 2 mL of milli-Q water containing 0.1% formic acid, filtered through a 0.22 µm polytetrafluoroethylene filter, transferred to a 2 mL amber vial, and stored at −80 °C until analysis. Phenolic compound extraction was carried out in triplicate for each sample.

The phenolic compounds in tomato sauce were identified and quantified using liquid chromatography coupled to high-resolution mass spectrometry as in a previous study[53], with some modifications. An Accela chromatograph (Thermo Scientific, Hemel Hempstead, UK) equipped with a photodiode array detector, a quaternary pump, and a thermostated autosampler was employed. A BEH C18 column (50 mm × 2.1 mm) i.d., 1.7 µm (Milford, MA, United States) maintained at 30 °C was used for the chromatographic separation. The injection volume was 5 µL, and the samples were maintained at 4 °C. The mobile phase consisted of an A phase of water (0.1% formic acid) and a B phase of acetonitrile (0.1% formic acid). The gradient conditions applied were as follows: 0–2 min, 0–5% B; 2–15 min, increase of phase B up to 18%; 15–26 min, increase of phase B up to 100%, maintaining these conditions for one min; and 27–28 min, decrease of phase B until 5%. Finally, the column was equilibrated for 2 min after returning to the initial conditions. The flow rate applied was 400 µL/min.

For the mass spectrometry (MS) analysis, an LTQ-Orbitrap Velos mass spectrometer (Thermo Scientific, Hemel Hempstead, UK) equipped with an electrospray ionization source was operated in negative mode. The specific parameters were as follows: source voltage, 3 kV; sheath gas, 50 a.u. (arbitrary units); auxiliary gas, 20 a.u.; sweep gas, 2 a.u.; and capillary temperature, 375 °C. Tomato sauce extracts were analyzed in Fourier transform mass spectrometry (FTMS) mode at a resolving power of 30,000 at m/z 900, and data-dependent MS/MS events were collected at a resolving power of 15.000 at m/z 900. The most intense ions detected in the FTMS spectrum were selected for the data-dependent scan. Parent ions were fragmented by high-energy collisional dissociation with normalized collision energy of 35% and an activation time of 10 min. The data analyses and instrument control were performed with Xcalibur 3.0 software (Thermo Fisher Scientific).

Table 4 | LOD and LOQ of standards constituents of phenolic compounds

Phenolic compounds	LOD (µg/Kg)	LOQ (µg/Kg)
4-hydroxybenzoic acid	16.84	56.12
2,5-dihydroxybenzoic acid	5.40	18.01
2,6-dihydroxybenzoic acid	7.06	23.55
3,5-dihydroxybenzoic acid	9.89	32.98
Gentisic acid	1.61	5.38
p-Coumaric acid	4.62	15.40
Ferulic acid	10.97	36.58
Naringenin	0.14	0.45
Naringenin chalcone	0.77	2.56
Naringenin 7-glucoside	1.07	3.58
Hesperetin	0.08	0.27
Kaempferol	0.07	0.25
Taxifolin	0.90	3.01
Quercetin	0.13	0.43
Rutin	1.90	6.33

Phenolic compounds were identified using commercial standards. Where no reference standard was available, the identification was based on chemical composition and MS/MS fragmentation patterns[53].

Phenolic compounds were quantified using pure standards when available. Otherwise, the compound was tentatively quantified with its aglycone or using a compound with a similar chemical structure. Calibration curves were constructed using standard solutions at concentrations ranging from 0.1 to 5.0 µg/mL and were linear with correlation coefficients above 0.98. The validation parameters for the methods are presented in Table 4.

Carotenoid profile and vitamin E

The extraction of carotenoids and vitamin E from tomato sauces was carried out following a previously described method[54], with some modifications. Tomato sauce was weighed (0.5 g) and homogenized with 5 mL of ethanol and *n*-hexane (4:3 v/v), mixed for 20 seconds, sonicated in an ice bath for 10 min, and centrifuged at 4000 rpm for 20 min at 4 °C. The apolar phase was separated into a flask. The extraction was performed twice, with 500 µL of milli-Q water added to the second extraction to improve phase separation. The two supernatants were mixed and evaporated to dryness with a stream of nitrogen. Subsequently, the samples were reconstituted in 1 mL of TBME, filtered through a 0.2 µm PTFE filter, and stored in a 2 mL amber vial at −80 °C. Each sample was replicated twice.

Identification and quantification were performed according to a previously described protocol (Rinaldi), with some modifications. First, a QTRAP4000 triple quadrupole mass spectrometer (Sciex, Foster City, CA, USA) equipped with an APCI ionization source operating in positive-ion and multiple reaction monitoring (MRM) mode was used to identify carotenoids in tomato sauces.

A UPLC system coupled to a diode array detector (DAD) was used to quantify the carotenoids and vitamin E. The separation was performed on an Acquity TM UPLC (Waters, Milford, MA., USA), with a YMC C_{30} column (250 × 4.6 mm, 5 µm) (Waters Co., Milford, MA, USA), using a flow rate of 0.6 mL/min at 25 °C. The injection volume was 10 µL. The mobile phase consisted of methanol 90% (A), TBME and methanol (8:2 v/v) (B), and water (C). A gradient was used to separate the carotenoid compound under the following conditions: 0 min, 90% A; 10 min, 75% A; 20 min, 50% A; 25 min, 30% A; 35 min, 10% A; 43 min, 6% A; 48 min, 6% A; 50 min, 90% A; and 57 min, 90% A. The DAD detector was used in the range of 220 to 700 nm and the chromatograms were recorded at a 450, 350, and 295 nm.

Table 5 | LOD and LOQ of standards carotenoids and vitamin E

Compound	LOD (mg/kg)	LOQ (mg/kg)
Lycopene	0.07	0.25
β-carotene	0.06	0.18
Lutein	0.12	0.40
Phytoene	0.11	0.36
α-tocopherol	0.22	0.74

Carotenoid compounds were identified based on the M1/M3 masses from previously reported MRM experiments, retention times and absorption spectra[54]. The carotenoids were quantified using external calibration curves. The standards used were lycopene for lycopene derivatives, lutein for cryptoxanthin and fucoxanthin, α-carotene for violaxanthin, β-carotene for β-carotene derivatives, phytoene, and α-tocopherol. The results were expressed as mg/kg FW. The calibration curves for the carotenoid and vitamin E standards were linear with correlation coefficients exceeding 0.99. The validation parameters for the methods are presented in Table 5.

Volatile organic compound analysis

The VOCs were analyzed as previously described[55]. An internal standard (IS), obtained by dissolving 0.1 g of 4-methyl-2-pentanol in 20 g of refined olive oil, was added to the samples to give an approximate concentration of 10,000 mg/kg. The tomato sample (2 g) was placed in a 20 mL glass vial, sealed with a polytetrafluoroethylene septum, and allowed to equilibrate for 10 min at 40 °C with shaking. Subsequently, the sample was subjected to solid phase microextraction by exposing the fiber to the headspace at 40 °C for 40 min. The volatile fraction was analyzed by gas chromatography-mass spectrometry (GC-MS) (QP2010 Ultra, Shimadzu, Kyoto, Japan) using an autosampler (AOC-5000 plus, Shimadzu, Kyoto, Japan) and a polar phase capillary column (TG-WAXMS: length 60 m, internal diameter 0.25 mm and coating 0.50 µm; Thermo Fisher Scientific, Waltham, MA, USA). The VOCs were identified by comparing their mass spectra with those reported in the reference library of the instrumental software; the retention times of the compounds were compared with those of pure standards, when available, to confirm the identification. VOCs were quantified using the equation: (Aa / Ais)* Cis, where Aa is the area of the analyte, Ais is the area of the IS, and Cis is the exact concentration of the IS. The results are expressed as mg/kg FW.

In this work, the same method was applied in terms of chromatographic conditions and sample preparation as reported in other studies[56,57] but using a different quantification method. The calibration curve for the IS was built in the range 0.05–10.00 mg/kg and the regression coefficient (R^2: 0.99) was determined. Some of the volatile compounds detected in this research work are in common with those generally present in virgin olive oils (octane, hexanal, 3-methyl-1-butanol, (E)-2-hexenal, (E)-2-heptenal, 6-methyl-5-hepten-2-one, 1-hexanol, nonanal, acetic acid) and, only for these molecules, the method performance parameters (linearity, repeatability, reproducibility, recovery, limit of detection (LOD) and limit of determination or quantification (LOQ)) have been presented in the two above-mentioned publications.

Statistical analysis

A multivariate analysis was performed on all the results obtained for the studied metabolites. All statistical analyses were conducted using SIMCA software v13.0.3.0 (Umetrics, Sweden) and Metaboanalyst 6.0 (https://www.metaboanalyst.ca). First, a principal component analysis (PCA) was carried out to visualize the natural distribution and clustering of the samples. Supervised models were used to identify the marker compounds associated with the different levels in the factorial design. A partial least squares discriminant analysis (PLS-DA) was performed, with TP-enrichment selected as a factor, followed by an orthogonal projection to latent structures discriminant analysis (OPLS-DA) to determine the effect of cooking technique,

temperature, and time on the content of phenolic compounds, carotenoids, vitamin E and VOCs in the tomato sauce. The OPLS-DA model was employed to analyze these three factors (cooking technique, temperature, and time) as it provides better separation than the PLS-DA model (Lozano-castellon 2022), which failed to separate between groups. For these models, the data were logarithmically transformed using the auto-transform option in the software. Variables of importance in the projection (VIP) score were calculated to select the most significant variables for each factor, i.e., those with a VIP score higher than 1.5. Goodness of fit (R^2Y) and goodness of prediction (Q^2Y) were used to validate the models. Outliers were detected using Hotelling's T2 (95% and 99% confidence). In addition, the model was validated using an ANOVA of the cross-validated residuals with an accepted p-value < 0.01. Finally, a permutation test with 200 permutations was performed to rule out overfitting.

In addition, t-student (TP-enrichment, temperature, and time) or one-way ANOVA (cooking technique) tests were used to determine the statistical significance of the data obtained. All data underwent logarithmic transformation, and the false discovery rate parameter (<0.05) was applied.

Data availability

Supplementary Information is available for this publication. Tables with the characteristics of bioactive compounds detected in tomato sauce, model validation parameters, Plots of Hotelling's and residuals of each model used to analyze the data, are included.

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Author contributions

R.M.L.R., A.V.Q., J.R. and M.P. acquired the funding. M.P., R.M.L.R. and A.V.Q designed this study. J.G.C., C.J.R., M.I. and X.T. made the tomato sauces. J.G.C., C.J.R., D.P. and A.L.Y. carried out the extraction, determination and quantification of phenolic compounds. C.M.P. and J.L.C. extracted, determined and quantified the carotenoids compounds. C.M.P., E.C., E.V. and A.B. performed the determination and quantification of volatile organic compounds. J.G.C., J.L.C. and A.L.Y. performed the acquisition of data. J.G.C. and J.L.C. contributed to data analysis and visualization. J.G.C. and A.L.Y. wrote the original draft. J.C.G., J.L.C, E.C., E.V., A.L.Y., J.R., A.V.Q., A.B., R.M.L.R. and M.P. reviewed and edited the manuscript. All the authors have read and agreed to the version of the manuscript to be published.

Competing interests
The authors declare no competing interests.

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