Polyphenol bioactivity evolution during the spontaneous fermentation of vegetal by-products

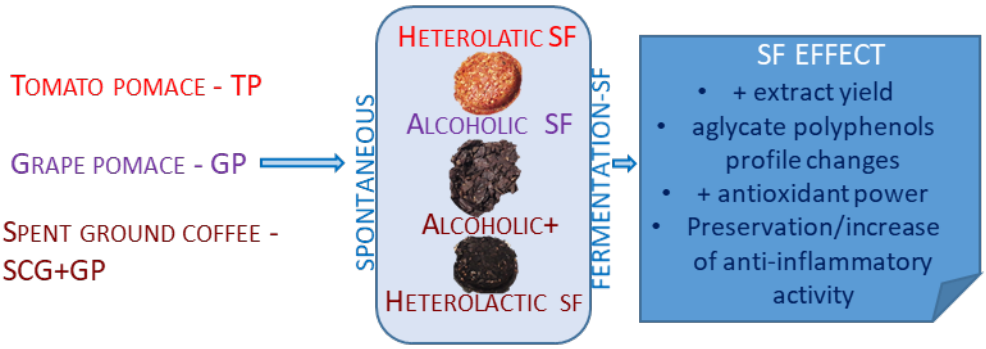
Parisa Abbasi-Parizad[a], Patrizia De Nisi[a], Tommy Pepè Sciarria[a], Alessio Scarafoni[b], Pietro Squillace[a], Fabrizio Adani[a], Barbara Scaglia[a, *]

[a]Ricicla Group Labs. - Department of Agricultural and Environmental Sciences (DISAA), University of Milan, via Giovanni Celoria 2, 20133 Milan, Italy.

[b]Department of Food, Environmental and Nutritional Sciences (DeFENS), University of Milan, via Giovanni Celoria 2, 20133 Milan, Italy.

*Corresponding author: Barbara Scaglia, Via Celoria 2, Milano, 20133- Italy, email: barbara.scaglia@unimi.it

VISUAL ABSTRACT



Abstract

Food industry by-products such as grape pomace (GP), tomato pomace (TP), and spent coffee grounds (SCG) are rich in polyphenols (PP) but are easily biodegradable. The aim of this study is to test Spontaneous Fermentation (SF) as treatment to modify PP profile and bioactivity. The results highlighted that the by-products' organic matter and the microbial populations drove the SF evolution; heterolactic, alcoholic, and their mixtures were the predominant metabolisms of TP, GP, and SCG+GP co-fermentation. Increases in the extractable amounts and antiradical activity occurred for all the biomasses. Regarding the aglycate-PPs (APP), i.e. the most bioreactive PPs, significant changes occurred for TP and GP but did not influence the anti-inflammatory bioactivity. The co-fermentation increased significantly chlorogenic acid and consumed most of the APPs, acting as a purification system to obtain a highly concentrated APP fraction, so that the extract might be employed for a specific purpose.

Abbreviations: Polyphenols: PP; Aglycone polyphenol fraction: APP; Total polyphenol content: TPC; 1,1-diphenyl-2-picrylhydrazyl: DPPH; Antiradical Activity: AA; Lactic Acid Bacteria: LAB; Yeast: Y; Interleukine-8: IL-8; Interleukine-1beta: IL-1β.

Keywords: Agro-industrial wastes, Spontaneous fermentation, Phenolic compounds, Co-Fermentation Metabolites, Anti-inflammatory property

1 Introduction

The market for polyphenols (PP) corresponded to USD 1.28 billion in 2018, with a perspective of increase expressed as Compounded Average Growth Rate (CAGR) of +7.2% from 2019 to 2025 (https://www.grandviewresearch.com/industry-analysis/polyphenols-market-analysis). Currently, the most common PP feedstocks are grape seed, apple, peach, olive, and citrus pomace (https://www.grandviewresearch.com/industry-analysis/polyphenols-market-analysis) and the PP extracts from these are destined for the cosmetic, nutraceutical, pharmaceutical and functional foods/beverages sectors. To enlarge the potential feedstocks of PP, other biomasses have been considered, with particular reference to the vegetal wastes of the food industry (i.e., peel, seeds, etc.).

Our recent results indicated that among tomato pomace (TP), grape pomace (GP), red corn cob (RCC), and spent coffee grounds (SCG), the latter has the best potential in term of bioactivity. In addition, SCG guarantees an almost constant supply throughout the year of large amounts of biomass (Abbasi-Parizad et al., 2021). On the other hand, the seasonality of the agri-food industry hinders the subsequent re-use in particular for biomasses that have high moisture and easily degradable organic fraction contents (i.e., TP and GP), which are the most susceptible to fast microbial degradation. To overcome this limitation, it is necessary to resort to effective and economical systems to preserve and possibly improve the active fractions. Fermentation is a technology, which is widely applied, since it allows the maintenance of the organic matter (OM) characteristics for a long time, thanks to the metabolic activity of yeast (Y) and/or lactic bacteria (LAB) that create an adverse environment for pathogen and degradative microorganisms. Controlled fermentation (CF), i.e. that based on the inoculum of selected strains, has been widely applied in the food sector because of its guaranteed constant metabolic pathways and the improved characteristics of the products, such as digestibility, sensory and nutritional properties of the foods

(Ng, Than, & Yong, 2021; Sabater et al., 2020). The modification of PP has been already described and depends on the metabolic activity of the micro-organisms present to break the bounds among PP and vegetal components (fiber, protein, and sugars) or to consume free PP (aglycate fraction, APP) and PP conjugated with monomers (i.e., sugars or N-compounds).

As an alternative to a controlled one, spontaneous fermentation (SF) may be carried out by LAB and Y endogenous microorganisms (Verni, Verardo, & Rizzello, 2019). The existence of an autochthonous community, yet selected based on the OM characteristics, was advantageous in terms of metabolic capability to act on PP. Recently, SF was tested on legumes and whole cereals chosen for their high content of bound PP (i.e., no extractable PP fraction), the activity of which was limited by physical inaccessibility and poor chemical reactivity. For both foods significant changes in PP occurred, thanks to the presence of several hydrolytic enzymes (i.e., tannase, cellulose, etc.) (Hur et al., 2014; Teles et al., 2019). The application of the same approach to vegetal by-products depends not only on the characteristics of the raw biomass but also on the industrial process already undertaken that affects the OM and microbial community composition. LAB have been described for both tomato and grape peels; however, after industrial treatment, the TP community was again rich in LAB whilst in GP, that came from alcoholic or malolactic fermentation, the community was made up by a mix of Y and LAB (Hur et al., 2014), while SCG was subjected to recolonization by molds and fungi after the roasting process sterilization (Anh et al., 2017).

Together with the chemical profile, bioactivity is a fundamental characteristic of PP. The fermentation increased the antiradical/antioxidant power as consequence of reducing metabolites production (Verni, Verardo, & Rizzello, 2019). However, the existence of a dose-effect between the APP versus anti-inflammation on Caco-2 cells was found for the TP, both raw and fermented (Abbasi-Parizad et al., 2020). SF has never been applied as a pre-treatment to modify PP to produce ingredients for nutraceutical and fortified foods, although developments in this sector are expected

to grow significantly to support the economic perspective of increase (CAGR 7.9%, reaching USD 275.77 billion by 2025) (https://www.grandviewresearch.com/industry-analysis/polyphenols-market-analysis).

In this work, SF was tested as a system to improve the deliverability of putrescible vegetal by-products for longer times in relation to the production season and, at the same time, as a treatment to modify PP profiles and the bioactivity of the extract.

2 Materials and Methods

2.1 Wastes collection and chemical characterisation

Two wastes from the food industry (tomato pomace (TP) and grape pomace (GP)) and one waste from the catering sector (spent coffee grounds (SCG)) were studied. The TP was sampled in a full-scale plant for tomato sauce production (OPOE Gruppo Cavicchi Scarl, Dodici Morelli, FE, Italy), the GP of red Merlot wine came from a wine–producer (Poncarale, Brescia, Italy), and the SCG, derived from different coffee varieties, was sampled in the canteen of the Agriculture Faculty of the University of Milan, Italy (Abbasi-Parizad et al., 2021). About 10 kg were sampled for each biomass and immediately stored at 4°C then the biomasses were chemically characterized for dry weight (DW), pH and ammonia (NH_3), according to the methods described previously (Abbasi-Parizad et al., 2020). The Volatile Solids (VS) i.e. the organic carbon based molecules of the by-product that volatilize at 550°C was employed as indicator of the organic matter evolution (Abbasi-Parizad et al., 2020).

2.2. Chemical characterisation of PP and antiradical test

The quali-quantitative characterization of PP was performed on the extracts, achieved by adding to the dry sample a hydro-ethanolic mix (70:30 v/v) in the ratio of 1:10 (dry weight/volume of

122 extraction). The quantity of PP content was determined by Folin-Ciocâlteu method and expressed
123 as total polyphenol content (TPC) (Abbasi-Parizad et al., 2021).
124 The antiradical activity (AA) of the extract was assessed with the DPPH radical scavenging method
125 and the results expressed as mmol Trolox equivalent (TE) g^{-1} DW (Abbasi-Parizad et al., 2021). A
126 solution (125 µM) of DPPH (Prot. N. D9132, Sigma Aldrich, Darmstadt, Germany) in methanol was
127 prepared and then added to extracts at different concentrations, then the decrease in absorbance
128 was recorded at 517 nm after 30 min by a spectrophotometer (Cary 60 UV-Vis, Agilent Technologies,
129 Santa Clara, CA, USA).
130 2.2.1. A*glycate polyphenol fraction chemical speciation*
131 The aglycate polyphenol fraction (APP) was quali-quantitatively characterized by using HPLC Agilent
132 1260 Infinity (Agilent Technologies, Santa Clara, California, United States). Briefly, 20 µL of the
133 extract was injected in HPLC equipped with a Kinetex® column (5 µm diameter, 250 x 4.6 mm) with
134 a flow rate of 0.5 mL min^{-1}. The mobile phase was made by CH_3CN and the solvent was composed
135 of distilled water acidified with acetic acid (98:2 v/v, pH 2.8). The detection of the molecules was
136 done at 260 nm for phenolic acids and stilbene and at 350 nm for flavonoids (Abbasi-Parizad et al.,
137 2020). The quantification of molecules was based on analysis of standards purchased from Sigma
138 Aldrich (Darmstadt, Germany).
139 *2.3. Storage trials and biomass chemical characterization*
140 About 500 g of fresh biomass was stored in airtight glass containers (Abbasi-Parizad et al., 2020).
141 The exit of air was enhanced by blowing through N_2 at the start of the process. The bottles were
142 stored at 20°C in dark conditions for up to 140 days. Basing on preliminary trials, SCG was not
143 adapted to be stored alone since fast contamination by moulds occurred, therefore co-fermentation
144 was attempted with GP, and the optimal proportion of 31.2:68.8 SCG:GP DW/DW was applied. The
145 fermentation evolution was monitored every 15 days through the determination of pH, DW, VS,

146 TPC, AA, NH_3 as reported before. The metabolites (lactate, formate, acetate, propionate, acetate,
147 ethanol) and simple sugars (galacturonic acid, glucose, and arabinose) were analyzed and quantified
148 by a Shimadzu HPLC (Shimadzu Corporation, Tokyo, Japan), equipped with a Hi-Plex H Agilent
149 column (300 × 7.7 mm, PL1170-6830) (Agilent Technologies, Santa Clara, CA, USA). Briefly, 5mL of
150 H_2SO_4 (0.05 M) were added on 5 grams of biomass and the final of 25 mL was reached by adding
151 distilled H_2O. The samples were stirred for a few seconds and then were left to rest for 30 minutes
152 and then successively centrifuged at 10,000 rpm for 15 min and filtered (0.45 µm Millipore Teflon
153 membrane). The injecting volume was 20 µL, using an isocratic 4 mmol L^{-1} sulfuric acid eluent (flow
154 rate of 0.4 mL min^{-1} and temperature was 50 °C for 40 min). Quantification was then made according
155 to the retention time of authentic standard curves using the Labsolution 5.90 software package
156 (Shimadzu Corporation, Tokyo, Japan) integrating the area under each compound detection peak
157 (Papa et al., 2020). The best AA for each biomass was applied as a screening criterion to select the
158 best time for storage and then the extract was characterized for its APP profile and anti-
159 inflammatory property test.

160 *2.4. Anti-inflammatory properties of the extracts*

161 Anti-inflammatory potential was assessed essentially as described previously (Abbasi-Parizad et al.,
162 2020). Two different concentrations of each extract from raw and fermented biomasses were
163 prepared and administered to Caco-2 cells in the presence of 20 ng mL^{-1} of IL-1β. Cells stimulated
164 with IL-1β alone were considered as the positive control, which allowed setting 100% of
165 inflammation induction in the experimental study. The expression of cytokine IL-8 was measured by
166 RT-qPCR, and GAPDH expression levels were used as a normalizer. Phenolic acids standards (ferulic
167 acid and chlorogenic acid) and flavonoid (naringenin) from Sigma Aldrich (Darmstadt, Germany)
168 were tested as references.

169 *2.5 Statistical analysis*

The results were analysed by the ANOVA bootstrap, Duncan test; for the anti-inflammatory test, the results are reported as averages of three biological replicates and the statistical difference is indicated by $p < 0.05$, Tukey's test.

Principal Component Analysis (PCA) was applied to correlate the bioactivities (AA and % inflammation status reduction i.e., IL-8 m-RNA expression reduction) to the SF's most important metabolites. The PCA was conducted using the raw data and the Principal Components (PCs) with eigenvalues >1 were retained; thus, among them were selected the two that have the best correlation relationships (r close to -1 or +1) with the highest number of starting parameters for graphical representation. All statistical analyses were carried out using SPSS statistical software SPSS 25 (IBM, New York, NY, USA).

3 Results and Discussion

3.1 Waste fermentation: metabolic trend

All wastes showed high moisture content that favored microorganisms' colonization (Table 1). TP had a significant concentration of lactic acid and smaller amounts of propionic and acetic acids and ethanol, all indicators of the existence of several metabolic pathways with a prevalence of LAB (Abbasi-Parizad et al., 2020). The LAB found was typical of tomato peel, however, their presence in the TP was attributable, above all, to the recolonization that occurred after industrial treatment enhanced by the suitable OM fraction. Regarding this topic, galacturonic acid confirmed the employment of pectin as a LAB substrate (Abbasi-Parizad et al., 2020). During the process, lactate and acetate (i.e., the most important indicators of hetero-lactic fermentation) had a progressive increase during the days of storage, reaching the highest concentration at 56 and 84 days respectively when the lactate: acetate proportion augmented the value of 2-3, typical of hetero-lactic fermentation, which was thus maintained until the end of the process (Table 2).

GP is derived from the previous alcoholic and malolactic fermentation driven by Y and LAB (Arcena et al., 2020). The presence of both communities was confirmed by their metabolites at the beginning of the fermentation process (t=0 d); however, after that, a reduction occurred, probably due to the difficulty of both communities to adapt to the new conditions or because of the existence of LAB-Y inhibition which may have been triggered by the respective metabolites' toxicity and/or biological competition (Table 1) (Mahboubi et al., 2018). From that situation, the glucose and ethanol increased at 42 d and 56 d respectively, when the Y overcame the metabolic impasse and become predominant with respect to the LAB.

The SCG+GP co-fermentation was largely influenced by the main component GP but with some differences. The ethanol peak at 14 d was an anticipation of the Y activity in comparison with that which occurred for the GP alone. The event was limited, and the ethanol trend was very similar to that of GP. Indeed, the sugar profile was changed: during the co-fermentation, the amount of glucose was different, since it now originated from both the GP and SCG cellulose fractions, moreover, arabinose was also present. In the SCG the arabinose was a component of hemicellulose and melanoidins, big molecules made by sugars, proteins, and chlorogenic acid generated during the green coffee roasting (Mussatto, Ballesteros, & Teixeira, 2011; Moreira, Nunes, & Coimbra, 2012; Burniol-figols, Cenian, & Gavala, 2016). To verify the effective contribution of SCG OM to sustain the microbial activity, the concentration of the main metabolites and sugars previously discussed $(SCG+GP)_M$ was now tentatively attributed to SCG (SCG_M) and GP (GP_M). The calculation of SCG_M was attempted for the lactate, acetate, propionate, glucose, arabinose, and ethanol as: $SCG_M=(SCG+GP)_M-(GP_{alone}*0.688)$ where GP_{alone} were the metabolites measured during the GP fermentation and the starting SCG:GP ratio (31.2%:68.8%) was considered constant since no VS changes occurred during the process. The results included both positive and negative data (SI Fig. S1), the first was an indicator of the use of SCG OM as the substrate of biological activity. This

218 approach allowed us to attribute the ethanol peak at 14 d to the consumption of SCG glucose and
219 to identify arabinose as the feedstock of the metabolism that produced propionate, lactate, and
220 acetate and that evolved in a stable hetero-lactic fermentation after 56 d of the process. The glucose
221 and ethanol showed negative values, interpretable as an increase of the OM GP-based metabolism
222 which occurred in comparison with that of GP_{alone}, stimulated indirectly by the different quali-
223 quantitative OM (Fig. S1). The result of the co-fermentation was thus the development of a different
224 community (LAB+Y as main groups) compared with that found for GP alone (Y as the main group)
225 and confirmed the possibility of carrying out the SF of non-adapted by-products and modulating the
226 final effect by using another biomass as a trigger.
227 The safety degree of the fermentation is a fundamental topic for the production of ingredients
228 destined for foods. The SF products based on LAB, Y and LAB+Y metabolism are widely applied in
229 the food sector since the development of LAB and Y based communities guarantee adequate safety
230 of the food. The sanitation effect was determined by the creation of unfavorable environmental
231 conditions for the growth of pathogen microorganisms. The hydrogen peroxides and bacteriocines
232 were recently identified as LAB bacteriostatic molecules however, the main sanitation effect
233 occurred in presence of LAB is due to the lactate production for its ability to pass through the
234 membrane and reduce the cytoplasm pH (Abbasi-Parizad et al., 2021; Voidarou et al., 2020).
235 Similarly, the Y and *Saccaromyces cerevisiae* in particular have been extensively studied about their
236 bacteriostatic effect associated to the high ethanol concentration, secretion of killer toxin and
237 competition for nutrients (Rima, Steve, & Ismail, 2012).
238
239 *3.2. Effect of fermentation on polyphenols content*
240 The TP was the only biomass for which higher TPC with respect to the raw one was reached during
241 the process (Table 1). Although the TPC is usually employed as a PP measurement, the reliability of

242 the correspondence was different for raw and fermented biomass in which a large portion of TPC
243 was made by molecules produced by microorganisms (Abbasi-Parizad et al., 2020). The same
244 metabolites as well as the smaller PP are itself subject to consumption, thus the intensity of
245 metabolic activity was the most probable cause of its reduction. At the start of the process the AA
246 of TP was lower than those of GP and SCG+GP which were very similar. Together with PP,
247 carotenoids are a class of antioxidants that can contributed to the AA. No carotenoids were reported
248 for SCG while the lutein plus beta-carotene and trans-lycopene were the principal molecules for GP
249 and TP respectively. Taking into consideration the experimental condition, only the hydrophilic
250 carotenoids i.e., beta-carotene was extractable, however, due to their low concentration with
251 respect to the PP content their contribution to the AA was very limited. For the TP, in this concept,
252 the contribution of trans-lycopene was excluded for its strong lipophilicity in spite of its high
253 concentration.
254 The AA increased for all treatment and biomass and the peaks which were found happened at a
255 different time in correspondence with, or very near to, the metabolite ones. Both enzymatic and
256 non-enzymatic activities were responsible for the increase of antioxidant properties during
257 fermentation (Hur et al., 2014). The enzymes determined the production of low-weight molecules
258 which have better antioxidant power than the original ones, and the nature of which depended on
259 the OM properties.
260 The presence of free antioxidant amino acids has been described from legumes' fermentation,
261 moreover the production of reducing sugars was expected from the fiber-rich biomasses used in
262 this work. The metabolic activity greatly affected the OM composition, and a direct effect was the
263 augmentation of the extractable fraction registered at metabolic peaks in comparison with the
264 starting values (+70%, +90%, +80% for TP, GP, and SCG+GP respectively). Nevertheless, no
265 significant correlation was found between extracts and AA, implying that a fraction of all molecules

266 had bioactivity. The expression of AA on extract content highlighted that the reduction of the AA
267 occurred for GP and SCG+GP (-74% and -80% respectively) while it remained constant for TP. This
268 result, together with the extract amount modification, suggested that the metabolic activity of GP
269 and SCG+GP did not have a significant effect on increasing AA, to the contrary of what happened
270 for TP.
271
272 *3.3 APP characterization*
273 The APPs of the raw biomasses were similar, although with different amounts and proportions
274 (Abbasi-Parizad et al., 2021). During the fermentations, the consumption as metabolic substrates
275 and non-biologically mediated degradation (Rondeau, Gambier, & Brosse, 2013) led to APP
276 decreases for all the biomasses (-51.2%, -77.7%, and -65% for TP, GP, and SCG+GP respectively)
277 (Table 3).
278 The TP hetero-lactic fermentation caused a general reduction of all phenolic acids, thus no
279 significant profile change occurred from a qualitative point of view (Table 3). However, phenolic
280 acids increased during GP (+83% phenolic acid of GPraw) with the augmentation of ellagic, ferulic,
281 and above all *p*-coumaric acids. Ellagic acid is a component of tannins, thus its increase was
282 attributable to the Y tannase activity, in agreement with the literature (Musingo et al., 2001; Pinelo,
283 Arnous, & Meyer, 2006); the non-core lignin monomers were the feedstock of ferulic and *p*-
284 coumaric acids from which aglycone forms were derived, thanks to Y cellulase action (Pinelo,
285 Arnous, & Meyer, 2006; Kyoung et al., 2009).
286 Although the GP is the main constituent of SCG+GP fermentation, the phenolic acids had a dissimilar
287 evolution determined by the different microbial communities (Y+LAB). A limited increase for ferulic
288 and *p*-coumaric acids occurred while the ellagic acid decreased. However, the great difference with
289 respect to the GP_{alone} process was for the aglycate chlorogenic acid augmentation (Table 3). Green

290 coffee is very rich in chlorogenic acid and is often employed as a feedstock to produce a chlorogenic-
291 acid-based extract. The melanoidins synthesis which occurs during roasting, however, reduces the
292 chlorogenic acid extractability since it is strongly linked by covalent bonds in the new molecules to
293 arabinose chains (Moreira et al., 2015). This new result, together with the arabinose presence as
294 the metabolite discussed before, suggests that the LAB used melanoidins as substrate and the
295 breakdown of bonds had a positive side effect which was the release of chlorogenic acid.
296 The high reactivity of the aglycate flavonoids explained the significant reduction that occurred for
297 all biomasses (-54.7%, -85%, -89% for TP, GP and SCG+GP respectively) (Table 3) (Kapcum &
298 Uriyapongson, 2018). The exception to the general behavior was quercetin, which increased tenfold
299 during TP fermentation (Table 3). This can probably be attributed to the existence of LAB enzymes
300 able to hydrolyze quercetin-glycosides (rutin) and quercetin-cellular component to improve the
301 aglycate quercetin content (Martins et al., 2016; Meinim, Cabezudo, & Romanini, 2019). During the
302 GP and SCG+GP SF, the naringenin chalcone augmentation could be attributable to the naringenin
303 conversion in the presence of a specific metabolic pattern typical of vegetal metabolism, but which
304 has recently been identified in microorganisms too (Moore et al., 2002).
305 Trans-resveratrol was the only component of the stilbene class and it showed an increase in
306 concentration for all biomasses but to different degrees. The effect was limited for TP (+33.2%)
307 whilst it was much greater for the other fermentations (Table 3). The increase of trans-resveratrol
308 was already described for GP, and several hydrolytic enzymes (i.e., tannase, pectinase, cellulase,
309 and β-glucosidase) showed the metabolic capability to transform resveratrol-glycosylated and/or
310 oligomeric forms (e.g., piceid) into the aglycate form (Kammerer, Claus, & Carle, 2005; Martins et
311 al., 2016). However, the relevant growth found for GP and SCG+GP may suggest microbial synthase
312 activity (Langcake & McCarthy, 1979; Martins et al., 2016).
313

314 *3.4 Anti-inflammatory activity performance of the extracts*

315 All extracts showed anti-inflammatory activity and their dependence on the APP dose was
316 statistically significant (SI, Table S1). For TP and GP, the raw and fermented samples had the same
317 dose-effect relationships, described by the linear regression equations (y = -5.08*(TP extract dose)
318 + 115, R^2=0.96, p<0.05; y = -6.17*(GP extract dose) + 138, R^2=0.99, p<0.05) (Abbasi-Parizad et al.,
319 2020) (Fig. 1). Accordingly, the minimum and maximum effective doses, i.e., the lowest and the
320 highest concentrations at which the bioactivity was null or maximum, allowed comparisons to be
321 made of the anti-inflammatory potential of TP and GP (Fig. 1). TP extracts showed significant
322 activities at a lower concentration than GP, while the total elimination of the induced inflammatory
323 status occurred at very similar doses. Although there was a general reduction in total APP content,
324 a balanced qualitative anti-inflammatory effect was observed. This may be due to the increase of
325 some molecules such as quercetin, t-resveratrol, *p*-coumaric, ellagic and ferulic acids, which are
326 known to have great bioactivity (Table 3) (Hur et al., 2014; Rodríguez-Morgado et al., 2015; Bucić-
327 Kojić et al., 2020). However, the evaluation of direct involvement of each molecule to give the
328 observed effects is difficult when complex matrices are tested in terms of synergism or antagonism
329 triggered by absorbability, competition for cell transportation and similar interactions (Yang et al.,
330 2014). A direct assessment was thus attempted to compare the measured bioactivities with respect
331 to standard molecules. TP and GP raw and fermented extracts (APP=15 µg L^{-1}) gave comparable anti-
332 inflammatory reduction when compared with ferulic acid, chlorogenic acid, and naringenin tested
333 at the same concentration. The best performance was that of SCG_{raw}, since a similar inflammatory
334 status reduction took place as for the reference (around 20%, that means a reduction of 80%), and
335 this was obtained with a dose tenfold lower (Fig. 1).

336 With respect to GP_{raw} and SCG_{raw}, their mixture did not give a synergistic anti-inflammatory effect,
337 thus less bioactivity occurred, probably due to the dilution of APP. In conflict with the trend found

for GP and TP, the fermentation of SCG+GP significantly improved the anti-inflammatory activity (Fig. 1). Again, considering as reference, an inflammation reduction of 80%, the SCG+GP fermented extract was effective at 14 μg mL^{-1} dose, which was 1.7-fold lower than that of $SCG+GP_{raw}$ necessary to obtain the same effect. This different behavior was explicable when taking into consideration that the APP composition changed completely; the APP of the mix was GP_based at the start (quercetin made by 70% APP of $SCG+GP_{raw}$) and SCG_based after fermentation (chlorogenic acid made by 60.7% APP of SCG+GPF). Taking into account the great increase in chlorogenic acid and the presence of other powerful molecules such as apigenin, *t*-resveratrol, and naringenin chalcone, a higher anti-inflammatory effect was expected: however, chlorogenic acid acts as a flavonoids antagonist when it has a higher concentration than other molecules, as in the case of the APP SCG+GPF (Hajimehdipoor, Shahrestani, & Shekarchi, 2014).

To valorize the great effects that occurred with co-fermentation (storage of biomass and APP evolution) and to avoid the negative interactions, the separation of GP and SCG was a possible post-treatment to obtain potentially two extracts from GP and SCG (López-Barrera, Vázquez-Sánchez, & Campos-Vega, 2016).

Thanks to the significant reduction that occurred for most of the APP, and the ratio between GP and SCG, the SCGF_APP is assumed to be composed almost totally of chlorogenic acid and apigenin with a concentration of 3-4-fold higher than that of the mixture. The second extract from GPF was expected to have *t*-resveratrol and naringenin chalcone as the main APPs at concentrations at least 1.5-fold higher than that of the mix. Similar concentrations and degree of purity are unusual for raw vegetal extracts and can be considered positive properties to address the subsequent employment of these extracts with specific functionalities.

3.5 SF metabolism effect on bioactivities

362 To better understand how the bioactivities evolution depended on the SF communities, PCA was
363 performed. The PCA results gave four significant PCs in which PC1 and PC2 explained 79.5 % of the
364 starting data.
365 The PC1 was well correlated with glucose (r=0.96), ethanol (r=0.98), TPC (r=0.88), AA (r=0.93)
366 acetate (r=-0.89), propionate (r= - 0.92) and galactose (r= - 0.83). Indeed, the PC2 was directly
367 correlated to the % inflammation reduction (r=0.82) and lactate content (r=0.82). Together PC1 and
368 PC2 represented 90% of the starting variables thus were employed for graphical representation of
369 the results (Fig. 2 a,b). Taking into consideration the indicators vs. PC relationships, the PC1 was
370 associated with the increase of the AA and to the presence of a Y-based community whilst the PC2
371 was related to the inflammation reduction and LAB-based community (Fig. 2a).
372 The plot of the extracts in the PC1-PC2 space (Fig. 2b) showed on the PC1 axis, two different groups
373 at negative (TP, TPF) and positive (GP+SCG, GP+SCGF, GP, GPF) values corresponded to lowest and
374 highest AA, respectively. However, no significant distance occurred among raw and fermented
375 samples for the same biomass suggesting that all SF metabolisms had a limited effect in the AA. A
376 similar situation was recorded on the PC2 for the GP and SCG+GP but not for the TP by-product TPF
377 positioned in higher well-separated position in relation to the TP (I and IV quadrants respectively)
378 suggesting that only the SF LAB based community positively affected the anti-inflammatory
379 bioactivity of the extract.
380

381 **4 Conclusion**

382 SF was effective to preserve for a long time the putrescible by-products of the food industry. The
383 organic matter composition and microbial populations were the factors that mainly drove the
384 subsequent metabolism and PP evolution. The storage conditions guaranteed the preservation of
385 anti-inflammatory activity and improved the AA power of the extract when the SF was carried out

386 for single biomasses. However, co-fermentation of grape and coffee wastes had a great effect in
387 terms of APP number reduction and can be considered a purification system to obtain extracts
388 destined for a pure molecule-like use. The LAB-based community of TP seemed to be a promising
389 starter to increase anti-inflammatory capability. Further development will be addressed to identify
390 and select specific microorganisms for anti-inflammatory extract production in sterilized conditions.

References

1. Abbasi-Parizad, P., De Nisi, P., Adani, F., Pepé Sciarria, T., Squillace, P., Scarafoni, A., Iametti, S., & Scaglia, B. (2020). Antioxidant and Anti-Inflammatory Activities of the Crude Extracts of Raw and Fermented Tomato Pomace and Their Correlations with Aglycate-Polyphenols. *Antioxidants*, 9(2), 179. https://doi.org/10.3390/antiox9020179.
2. Abbasi-Parizad, P., De Nisi, P., Scaglia, B., Scarafoni, A., Pilu, S., & Adani, F. (2021). Recovery of phenolic compounds from agro-industrial by-products: Evaluating antiradical activities and immunomodulatory properties. *Food and Bioproducts Processing*, 127, 338–348. https://doi.org/10.1016/j.fbp.2021.03.015.
3. Anh, Q., Cho, E., Thi, L., Trinh, P., Jeong, J., & Bae, H. (2017). Bioresource Technology Development of an integrated process to produce D -mannose and bioethanol from coffee residue waste. *Bioresource Technology*, 244, 1039–1048. https://doi.org/10.1016/j.biortech.2017.07.169.
4. Arcena, M. R., Leong, S. Y., Hochberg, M., Sack, M., Mueller, G., Sigler, J., Silcock, P., Kebede, B., & Oey, I. (2020). Evolution of volatile and phenolic compounds during bottle storage of merlot wines vinified using pulsed electric fields-treated grapes. *Foods,* 9(4), 1–21. https://doi.org/10.3390/foods9040443.
5. Bucić-Kojić, A., Fernandes, F., Silva, T., Planinić, M., Tišma, M., Šelo, G., Šibalić, D., Pereira, D. M., & Andrade, P. B. (2020). Enhancement of the anti-inflammatory properties of grape pomace treated by Trametes versicolor. *Food & Function*, 11(1), 680–688. https://doi.org/ 10.1039/c9fo02296a.
6. Burniol-figols, A., Cenian, K., Skiadas, I. V, & Gavala, H. N. (2016). Integration of chlorogenic acid recovery and bioethanol production from spent coffee grounds. *Biochemical Engineering Journal*, 116, 54–64. https://doi.org/10.1016/j.bej.2016.04.025.
7. Brejda, J. J., Karlen, D. L., Smith, J. L., & Allan, D. L. (2000). Identification of regional soil quality factors and indicators II. Northern Mississippi Loess Hills and Palouse Prairie. https://doi.org/10.2136/sssaj2000.6462125x
8. Global polyphenol market 2019, Retrived from: https://www.grandviewresearch.com/industry-analysis/polyphenols-market-analysis. Accessed March 2019; Report ID: 978-1-68038-127-6.
9. Hajimehdipoor, H., Shahrestani, R., & Shekarchi, M. (2014). Investigating the synergistic antioxidant effects of some flavonoid and phenolic compounds. *Research Journal of Pharmacognosy*, 1(3), 35–40. http://www.rjpharmacognosy.ir/article_5776.html.
10. Rima, H., Steve, L., & Ismail, F. (2012). Antimicrobial and probiotic properties of yeasts: from fundamental to novel applications. *Frontiers in microbiology*, 3, 421. https://doi.org/10.3389/fmicb.2012.00421.

11. Hur, S. J., Lee, S. Y., Kim, Y. C., Choi, I., & Kim, G. B. (2014). Effect of fermentation on the antioxidant activity in plant-based foods. In *Food Chemistry* (Vol. 160, pp. 346–356). Elsevier Ltd. https://doi.org/10.1016/j.foodchem.2014.03.112.
12. Kammerer, D., Claus, A., Schieber, A., & Carle, R. (2005). A novel process for the recovery of polyphenols from grape (Vitis vinifera L.) pomace. *Journal of Food Science*, 70(2). https://doi.org/10.1111/j.1365-2621.2005.tb07077.x.
13. Kapcum, C., & Uriyapongson, J. (2018). Effects of storage conditions on phytochemical and stability of purple corn cob extract powder. *Food Science and Technology*, 38, 301–305. https://doi.org/10.1590/1678-457x.23217.
14. Kyoung, H., Jung, E., Hee, M., Yook, Y., Sun, S., Hill, J., & Won, S. (2009). Characterization of increased phenolic compounds from fermented Bokbunja (Rubus coreanus Miq.) and related antioxidant activity. *Journal of Pharmaceutical and Biomedical Analysis*,49, 820–827. https://doi.org/10.1016/j.jpba.2008.12.024.
15. Langcake, P., & McCarthy, W. V. (1979). The relationship between resveratrol production to infection of grapevine leaves by Botrytis cinerea. Vitis, 18(3), 244–253.
16. López-Barrera, D. M., Vázquez-Sánchez, K., Loarca-Piña, M. G. F., & Campos-Vega, R. (2016). Spent coffee grounds, an innovative source of colonic fermentable compounds, inhibit inflammatory mediators in vitro. *Food Chemistry*, 212, 282–290. https://doi.org/10.1016/j.foodchem.2016.05.175.
17. Mahboubi, A., Cayli, B., Bulkan, G., Doyen, W., De Wever, H., & Taherzadeh, M. J. (2018). Removal of bacterial contamination from bioethanol fermentation system using membrane bioreactor. *Fermentation*, 4(4), 1–18. https://doi.org/10.3390/fermentation4040088.
18. Martins, I. M., Roberto, B. S., Blumberg, J. B., Chen, C. O., & Macedo, G. A. (2016). Enzymatic biotransformation of polyphenolics increases antioxidant activity of red and white grape pomace. *FRIN*, 89, 533–539. https://doi.org/10.1016/j.foodres.2016.09.009.
19. Meini, M. R., Cabezudo, I., Boschetti, C. E., & Romanini, D. (2019). Recovery of phenolic antioxidants from Syrah grape pomace through the optimization of an enzymatic extraction process. *Food Chemistry*, 283, 257–264. https://doi.org/10.1016/j.foodchem.2019.01.037.
20. Moore, B. S., Hertweck, C., Hopke, J. N., Izumikawa, M., Kalaitzis, J. A., Nilsen, G., O'Hare, T., Piel, J., Shipley, P. R., Xiang, L., Austin, M. B., & Noel, J. P. (2002). Plant-like biosynthetic pathways in bacteria: From benzoic acid to chalcone. *Journal of Natural Products*, 65(12), 1956–1962. https://doi.org/10.1021/np020230m.
21. Moreira, A. S. P., Coimbra, M. A., Nunes, F. M., Passos, C. P., Santos, S. A. O., Silvestre, A. J. D., Silva, A. M. N., Rangel, M., & Domingues, M. R. M. (2015). Chlorogenic acid-arabinose hybrid domains in coffee melanoidins: Evidences from a model system. *Food Chemistry*, 185, 135–144. https://doi.org/10.1016/j.foodchem.2015.03.086.

22. Moreira, A. S. P., Nunes, F. M., Domingues, M. R., & Coimbra, M. A. (2012). Coffee melanoidins: Structures, mechanisms of formation and potential health impacts. *Food and Function*, 3(9), 903–915. https://doi.org/10.1039/c2fo30048f.
23. Musingo, M. N., Sims, C. A., Bates, R. P., O'keefe, S. F., & Lamikanra, O. (2001). Changes in ellagic acid and other phenols in muscadine grape (Vitis rotundifolia) juices and wines during storage. American *Journal of Enology and Viticulture*, 52(2), 109–114.
24. Mussatto, S. I., Ballesteros, L. F., Martins, S., & Teixeira, J. A. (2011). Extraction of antioxidant phenolic compounds from spent coffee grounds. *Separation and Purification Technology*, 83(1), 173–179. https://doi.org/10.1016/j.seppur.2011.09.036.
25. Ng, Z. X., Than, M. J. Y., & Yong, P. H. (2021). Peperomia pellucida (L.) Kunth herbal tea: Effect of fermentation and drying methods on the consumer acceptance, antioxidant and anti-inflammatory activities. *Food Chemistry*, 344, 128738. https://doi.org/10.1016/j.foodchem.2020.128738.
26. Papa, G., Pepè Sciarria, T., Carrara, A., Scaglia, B., D'Imporzano, G., & Adani, F. (2020). Implementing polyhydroxyalkanoates production to anaerobic digestion of organic fraction of municipal solid waste to diversify products and increase total energy recovery. *Bioresource Technology*, 318. https://doi.org/10.1016/j.biortech.2020.124270.
27. Pinelo, M., Arnous, A., & Meyer, A. S. (2006). Upgrading of grape skins : Significance of structural components and extraction techniques for phenol release. *Trends in Food Science & Technology*, 17(11), 579-590. https://doi.org/10.1016/j.tifs.2006.05.003.
28. Rodríguez-Morgado, B., Candiracci, M., Santa-María, C., Revilla, E., Gordillo, B., Parrado, J., & Castaño, A. (2015). Obtaining from grape pomace an enzymatic extract with anti-inflammatory properties. *Plant Foods for Human Nutrition*, 70(1), 42–49. http://doi.org/10.1007/s11130-014-0459-0.
29. Rondeau, P., Gambier, F., Jolibert, F., & Brosse, N. (2013). Compositions and chemical variability of grape pomaces from French vineyard. *Industrial Crops and Products*, 43, 251–254. https://doi.org/10.1016/j.indcrop.2012.06.053.
30. Sabater, C., Ruiz, L., Delgado, S., Ruas-Madiedo, P., & Margolles, A. (2020). Valorization of Vegetable Food Waste and By-Products Through Fermentation Processes. *Frontiers in Microbiology*, 11, 1–11. https://doi.org/10.3389/fmicb.2020.581997.
31. Teles, A. S. C., Chávez, D. W. H., Oliveira, R. A., Bon, E. P. S., Terzi, S. C., Souza, E. F., Gottschalk, L. M. F., & Tonon, R. V. (2019). Use of grape pomace for the production of hydrolytic enzymes by solid-state fermentation and recovery of its bioactive compounds. *Food Research International*, 120, 441–448. https://doi.org/10.1016/j.foodres.2018.10.083.
32. Verni, M., Verardo, V., & Rizzello, C. G. (2019). How fermentation affects the antioxidant properties of cereals and legumes. *Foods,* 8(9), 362. https://doi.org/10.3390/foods8090362.

33. Voidarou C, Alexopoulos A, Tsinas A, Rozos G, Tzora A, Skoufos I, Varzakas T, Bezirtzoglou E. Effectiveness of Bacteriocin-Producing Lactic Acid Bacteria and Bifidobacterium Isolated from Honeycombs against Spoilage Microorganisms and Pathogens Isolated from Fruits and Vegetables. *Applied Sciences*. 2020; 10(20):7309. https://doi.org/10.3390/app10207309.
34. Yang, Y., Bai, L., Li, X., Xiong, J., Xu, P., Guo, C., & Xue, M. (2014). Transport of active flavonoids, based on cytotoxicity and lipophilicity: an evaluation using the blood–brain barrier cell and Caco-2 cell models. *Toxicology in Vitro*, 28(3), 388–396. https://doi.org/10.1016/j.tiv.2013.12.002.

TABLES

Table 1. Characterization of by-product during spontaneous fermentation.

By-products		Time (Days)						
		0	14	29	42	56	84	140
TP	DW (% w.w.)	27.6±0.3 a[*,#]	26.5±0.1 a	27.2±0.2 a	26.1±0.2 a	26.3±0.2 a	25.7±0.1 a	25.4±0.4 a
	VS (% DW)	94.4±0.6 a	95.1±0.3 a	96.7±0.2 b	98.4±0.1 b	97±0.1 b	97.3±0.2 b	97.5±0.3 b
	TPC (mg GAE g^{-1} DW)	4.6±0.3 bc	3.3±0.1 a	3.6±0.1 ab	4.1±0.1 ab	5.8±0.6 c	4.3±0.3 ab	4.8±0.5 bc
	AA (µM TE g^{-1} DW)	21.5±0.1 a	26.3±0.3 b	38.1±0.1 f	34.3±0.1 e	30.6±0.3 c	33±0.6 de	31.7±1.6 cd
	Extract (g kg^{-1} DW)	258±86 a	-	453±39 b	-	-	-	-
GP	DW (% w.w.)	36.01±2.9 a	34.6±0.1 a	37.02±1.7 a	34.8±0.8 a	33.4±1 a	34.4±0.2 a	33.4±0.3 a
	VS (% DW)	93±1.1 a	92±0.2 a	91.3±1.1 a	91±1.5 a	89.4±1 a	89.7±0.7 a	92.4±0.1 a
	TPC (mg GAE g^{-1} DW)	22.8±0.6 c	16.5±0.3 b	10.2±0.3 a	10.6±0.3 a	12.2±0.2 ab	11.1±0.3 a	14.7±0.8 b
	AA (µM TE g^{-1} DW)	87.8±0.9 d	68.1±0.1 b	79.7±0.2 c	78.9±0.1 c	93.5±1.4 e	87.5±0.4 d	53.1±0.9 a
	Extract (g kg^{-1} DW)	246±52 a				474±48 b		
SCG+GP	DW (% w.w.)	37.3±0.1 a (40±0.1)b **	36.3±0.1 a	38.6±1.1 a	39.2±0.2 a	36.5±0.5 a	34±0.2 a	34.7±0.4 a
	VS (% DW)	95.1±0.2 a (99.8±0.1)b	94.9±0.2 a	94.1±2.3 a	94.1±0.3 a	94.5±0.1 a	94.5±0.1 a	93.2±0.3 a
	TPC (mg GAE g^{-1} DW)	18.8±0.6 c (10.1±0.5) a	16.5±0.3 b	10.2±0.3 a	10.8±1.1 a	13.4±0.9 b	10.9±0.4 a	15.3±0.5 b
	AA (µM TE g^{-1} DW)	88.4±0.6 e	67.9±0.2 c	92.5±0.6 e	59.1±0.4 b	76.3±1.9 d	66±2.5 c	51.6±1.4 a

		(89.8±0.3) e	
	Extract (g kg^{-1} DW)	318±12 a (462±48 b)	581±103 c

* Data are the mean (n = 3) ± SD. Different letters in the same raw are statistically different (ANOVA, p <0.05, post-test Duncan).

TP: Tomato Pomace; GP: Grape Pomace; SCG: Spent coffee grounds; SCG+GP: Spent coffee grounds + Grape Pomace; DW: dry weight; VS: Volatile Solid; AA: Antiradical activity; TPC: Total polyphenol content. The data of raw biomasses came from Abbasi-Parizad et al., 2021.

**SCG characterization

Table 2. Changes in pH, ammonia, organic acids, sugars and ethanol during fermentation.

	Time (day)	pH	NH_3*	Formate	Acetate	Propionate	Lactate	Galacturonic acid	Glucose	Arabinose	Ethanol
						mg g^{-1} DW					
TP	0	3.8 ±0.03 a	0.3±0.01 a	-	4.4±0.2a	8.9±0.3a	10.8±1.1 a	1.7±0.4 a	0.6±0.01a	-	4.8±0.6a
	14	3.7±0.02 a[a]	0.4±0.02 a	-	8.8±0.6 b	9.3±0.4 a	38.7±2.6b	2±0.1 a	0.6±0.03 a	-	5.1±2.4 ab
	29	3.6±0.01 a	0.4±0.01 a	-	7.6±0.2 b	7.4±0.03 a	34.7±0.8 b	4.1±0.3 ab	0.9±0.13 b	-	5.1±0.3 ab
	42	3.6±0.08 a	0.6±0.08 b	-	8.7±0.9 b	8.8±0.9 a	43.9±3.6 c	5.2±2.5 b	1.07±0.01 bc	-	5.7±2.5 b
	56	3.5±0.05 a	0.8±0.03 c	0.2±0.3 a	8.7±0.2 b	9.6±1.3 a	53.6±3.6 d	11.5±0.1 c	1.2±0.08 cd	-	3.7±0.08 a
	84	3.5±0.04 a	0.6±0.02 b	2.1±0.03 c	13.4±0.3 c	8.5±2.9 a	49.8±2.2 cd	2.2±0.2 a	1.1±0.01 cd	-	6.5±0.3 ab
	140	3.5±0.06 a	0.7±0.01 c	1.4±0.1 b	13.9±0.4 c	7.9±0.5 a	55±2.8 d	8.8±1 c	1.3±0.01 d	-	7.2±0.2 ab
GP	0	3.47 ± 0.03 a	0.19±0.02 c	0.33±0.1 a	0.64±0.04 a	0.6±0.03 a	24.15±0.9 ab	-	46.97±1.8 a	-	140.2±1.7 b
	14	3.5±0.02 a	0.21±0.02 d	0.4±0.1 a	0.5±0.04 a	1.4±0.1 b	23.5±0.3 ab	-	53.06±0.3 ab	-	122±2.2 ab
	29	3.5±0.01 ab	0.15±0.01 ab	0.3±0 a	0.5±0.08 a	1.4±0.2 b	18.5±0.4 a	-	45.4±3.2 a	-	100±2.3 a
	42	3.5±0.01 ab	0.17±0.01 bc	0.4±0.02 a	0.6±0.2 a	1.5±0.1 b	23.02±2.2 ab	-	55.1±3.6 b	-	114.1±2.4 ab
	56	3.5±0.01 ab	0.2±0.01 cd	0.4±0.04 a	0.8±0.04 b	1.6±0.1 b	22.21±2.7 ab	-	53.7±2.08 ab	-	144±2.2 c
	84	3.5±0.01 b	0.12±0.02 a	0.4±0.04 a	1.1±0.02 b	1.7±0.2 b	26.53±0.1 b	-	52.21±4.5 ab	-	133±3.4 bc
	140	3.4±0.02 a	0.16±0 b	0.3±0.1 a	1.4±0.02 c	1±0.6 a	22.81±2.8 ab	-	54±1.1 ab	-	126±1.8 abc

SCG +GP	0	3.47±0.03 a 6.4±0.02 b	0.17±0.03 a (0±0)**	0±0 (0.44±0.02)	0.6±0.56 a (0.34±0.01)	1.9±0.55 b (0±0)	14.04±0.7 a (0.64±0.02)	-	28.22±1.4 b (0.4±0.02)	0±0 (0.05±0.001)	77.1±1.09 ab (0±0)
	14	3.6±0 a	0.18±0.02 a	0.3±0.2 ab	0.95±0.2 b	2.6±2.07 b	15.95±0.1 a	-	29.31±2.2 b	0.8±0.1 a	86.1±4.3 c
	29	3.7±0.007 a	0.19±0.02 a	0.34±0.04 ab	1.44±0.2 c	0.5±0.04 a	13.56±0.4 a	-	19.45±0.2 a	1.9±1.6 a	72.9±3.3 a
	42	3.6±0.007 a	0.17±0.01 a	0.2±0.04 ab	1.26±0.6 c	1.6±0.4 ab	18.15±1.7 a	-	36.54±1.06 c	0.8±0.2 a	68.88±2.9 a
	56	3.6±0.007 a	0.17±0 a	0.18±0.2 ab	2.51±0.8 d	1.9±0.14 b	16.89±2.3 a	-	32.5±2.6 bc	1.1±0.4 a	79±1.13 c
	84	3.6±0.01 a	0.15±0.03 a	0.43±0.06 b	2.58±0.3 d	0.7±0.3 a	19.67±3.7 a	-	27.39±2.5 b	0.9±0.1 a	84.2±2.05 c
	140	3.5±0.014 a	0.18±0.01 a	0.07±0.1 a	2.1±0.3 d	1.1±0.1 ab	18.14±3.2 a	-	20.69±1.44 a	2.02±0.9 a	80.23±3.9 c

Data are the mean (n = 3) ± SD. Different letters in the same column are statistically different (ANOVA, p <0.05, post-test Duncan).
TP: Tomato Pomace; GP: Grape Pomace; SCG: Spent coffee grounds; SCG+GP. DW: Dry weight.

**SCG characterization

Table 3. APP content of the raw and fermented extract.

extract	TP	TPF	GP	GPF	SCG+GP	SCG+GPF
APP	μg g^{-1} DW extract					
Gallic acid	1004±112 b*,#	832±30 a	1146±20 b	633±32a	900±32 b (588 ±7)**	251±21 a
Chlorogenic acid	756±15 b	461±10 a	0±0	27±6	597±14 a (1281±30)	9898±16 b
Syringic acid	50±6	0±0	67±8 b	22±6 a	36±6 (0±0)	0±0
Caffeic acid	205±2 b	36±6 a	451±16 b	259±19 a	303±39 b (122±11)	24±6 a
Ferulic acid	83±2 b	29±6 a	252±16 a	1270±39 b	219±32 a (166±14)	933±18 b
p-Coumaric acid	231±21 b	74±8 a	55±4 a	996±32 b	57±5 a (53±8)	157±19 b
Vanillic acid	122±24	0±0	70±6 a	68±13 a	38±3 (0±0)	0±0
Ellagic acid	0±0	0±0	1004±12 a	2426±25 b	548±42 b (0±0)	437±11 a

Cinnamic acid	387±27 b	188±9 a	67±6 b	13±2 a	37±10 b (0±0)	4±0 a
Sinapic acid	125±4 a	113±12 a	0±0	0±0	71±16 (152±13)	0±0
Sum of phenolic acids	2964±213 b	1734±81 a	3112±91 a	5715±175 b	2807±201 a (2362±83)	11705±92 b
Rutin	434±39 a	204±21 a	3764±45 b	1044±39 a	2419±71 b (781±18)	65±5 a
Quercetin	46±12 a	410±13 b	55333±496 b	4042±46 a	30226±742 b (33±6)	26±5 a
Catechin	0±0	0±0	0±0	0±0	287±32 (620±19)	0±0
Epicatechin	0±0	0±0	1215±57 b	35±4 a	2716±68 b (4409±34)	98±7 a
Apigenin	57±2 b	11±3 a	3858±36 b	576±16 a	3584±210 b (3180±22)	2086±11 a
Myricetin	380±19 b	46±8 a	537±20 b	193±11 a	293±48 b (0±0)	122±13 a
Kaempferol	806±50 b	344±6 a	1736±28 b	96±16 a	2651±90 b	16±3 a

Naringenin	2845±27 b	863±20 a	3419±24	0±0	(3665±19) 1864±51 (0±0)	0±0
Naringenin Chalcone	2728±31 b	697±36 a	0±0	3785±45	0±0 (0±0)	1453±15
Sum of all flavonoids	8123±238 b	3676±118 a	69941±716 b	10548±197 a	44084±1320 b (15049±118)	4602±84 a
trans-Resveratrol Sum of stilbenes	825±58 a	1099±11 b	79±8 a	776±20 b	43±7 a (0±0)	736±24 b
Sum of all APP	11087±451 b	5410±200 a	73053±806 b	16263±372 a	46892±1520 b (17381)	16308±175 a

TP: Tomato Pomace; TPF: Tomato Pomace Fermented; GP: Grape Pomace; GPF: Grape Pomace Fermented; SCG: Spent coffee grounds; SCG+GP: Spent coffee grounds + Grape Pomace; SCG+GPF: Spent coffee grounds + Grape Pomace Fermented; DW: Dry weight.

* Data are the mean (n = 3) ± SD. Different letters between the raw and fermented samples for the same biomass are statistically different (ANOVA, p <0.05, post-test Duncan). The data equal to 0±0 were excluded by the statistical analysis.

[#] Aglycate polyphenol faction (APP) and other data of raw biomasses came from Abbasi Parizad et al. (2021).

** SCG characterization

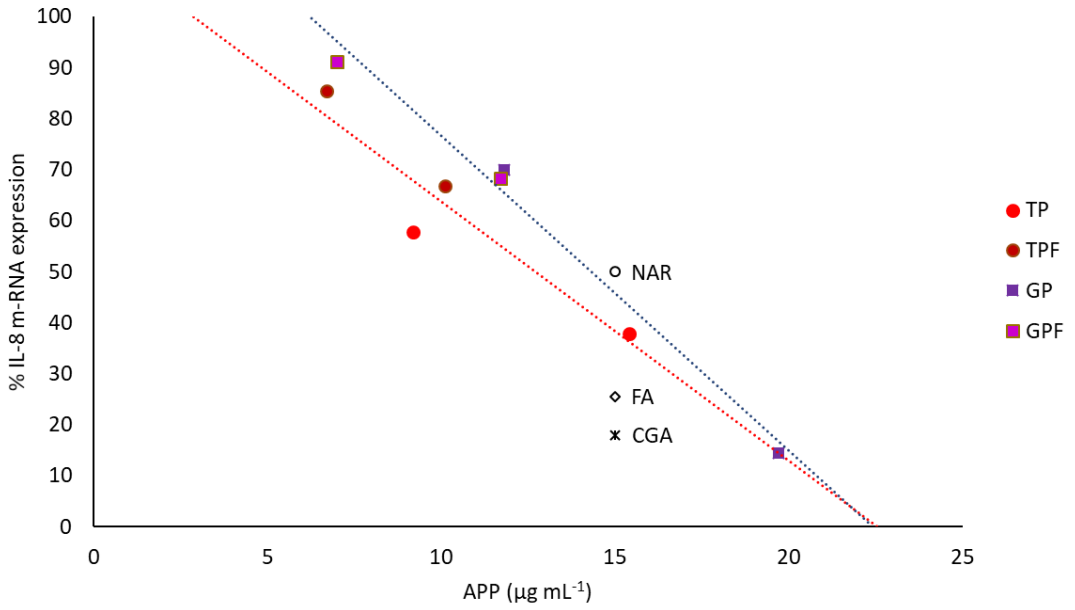
Caption figure

Figure 1. Anti-inflammatory effect of the extract of raw and fermented TP and GP (a) and SCG, SCG+GP raw and fermented (b) on cytokine IL-8 expression in Caco-2 cells. TP: Tomato Pomace; TPF: Tomato Pomace Fermented; GP: Grape Pomace; GPF: Grape Pomace Fermented; SCG: Spent coffee grounds; SCG+GP: Spent coffee grounds + Grape Pomace; SCG+GPF: Spent coffee grounds + Grape Pomace Fermented.

Figure 2. PCA plots of the influence of the metabolic evolution of SF versus bioactivities. Fig. 2a provides information on the metabolites that are significantly different during fermentation and bioactivities (AA and % IL-8 m-RNA expression reduction i.e. % inflammation status reduction) into PCA space indicating the load of each parameters in defining the PC1 (x axis) and the PC2 (y axis). Fig. 2b provides information on the extracts (TP: Tomato Pomace; TPF: Tomato Pomace Fermented; GP: Grape Pomace; GPF: Grape Pomace Fermented; SCG: Spent coffee grounds; SCG+GP: Spent coffee grounds + Grape Pomace; SCG+GPF: Spent coffee grounds + Grape Pomace Fermented.) similarity/dissimilarity (i.e. samples are close/far respectively) on the basis of communities and bioactivity evolution (Fig. 1a). Samples and parameters in the same position (Fig. 1a, b) indicate the influence of the SF metabolism (i.e LAB, Y and LAB+Y based) in the evolution of bioactivities.

Fig. 1

a


100
90
80
70
60
50
40
30
20
10
0
% IL-8 m-RNA expression
0
5
10
15
20
25
APP (µg mL-1)
NAR
FA
CGA
TP
TPF
GP
GPF

b

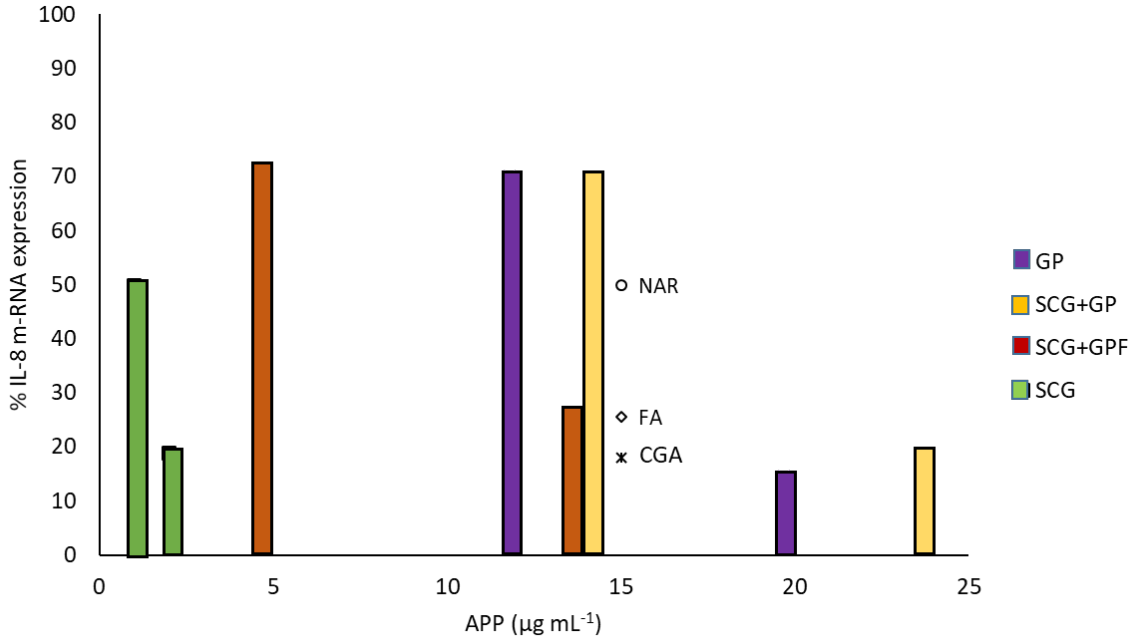
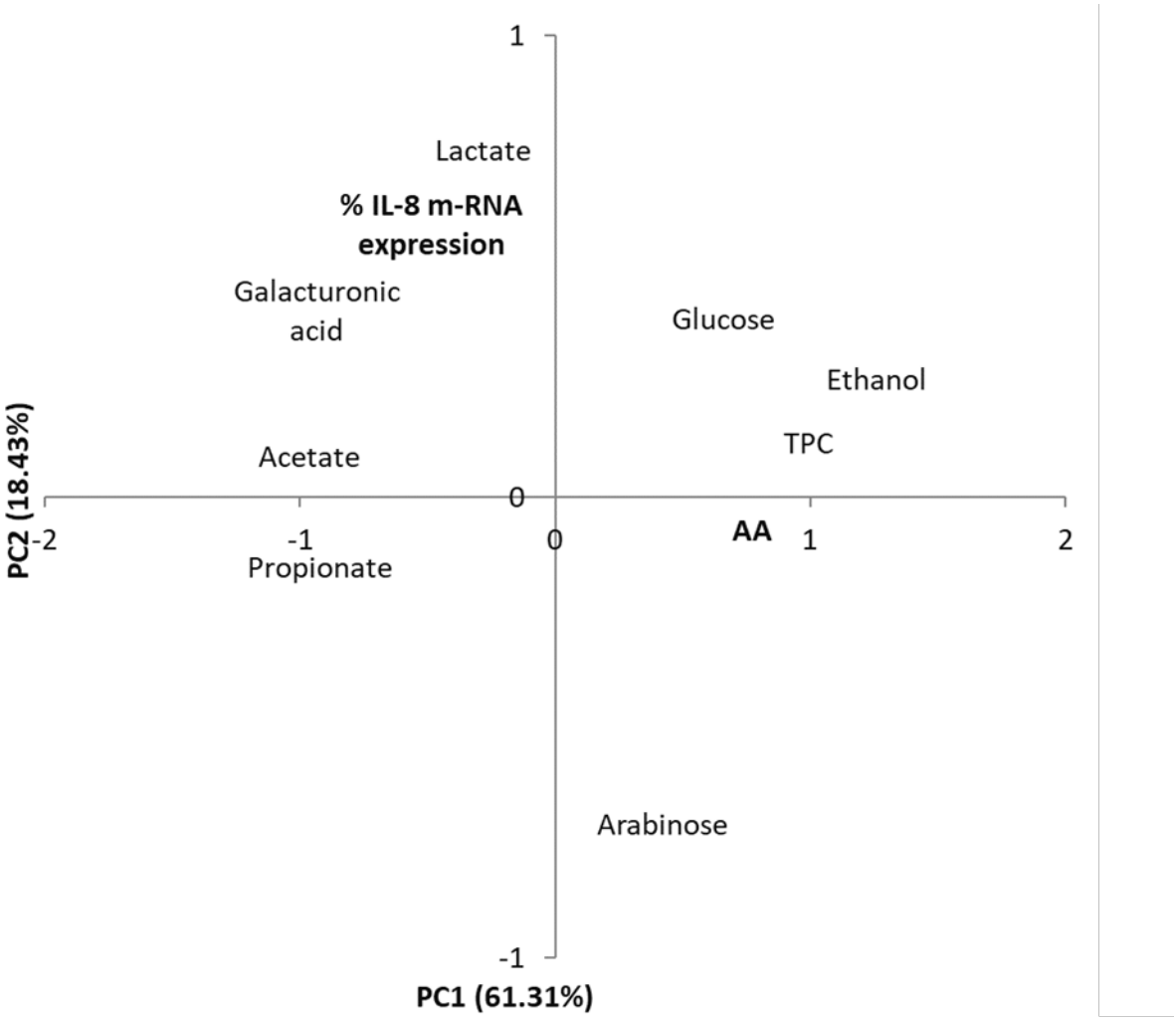
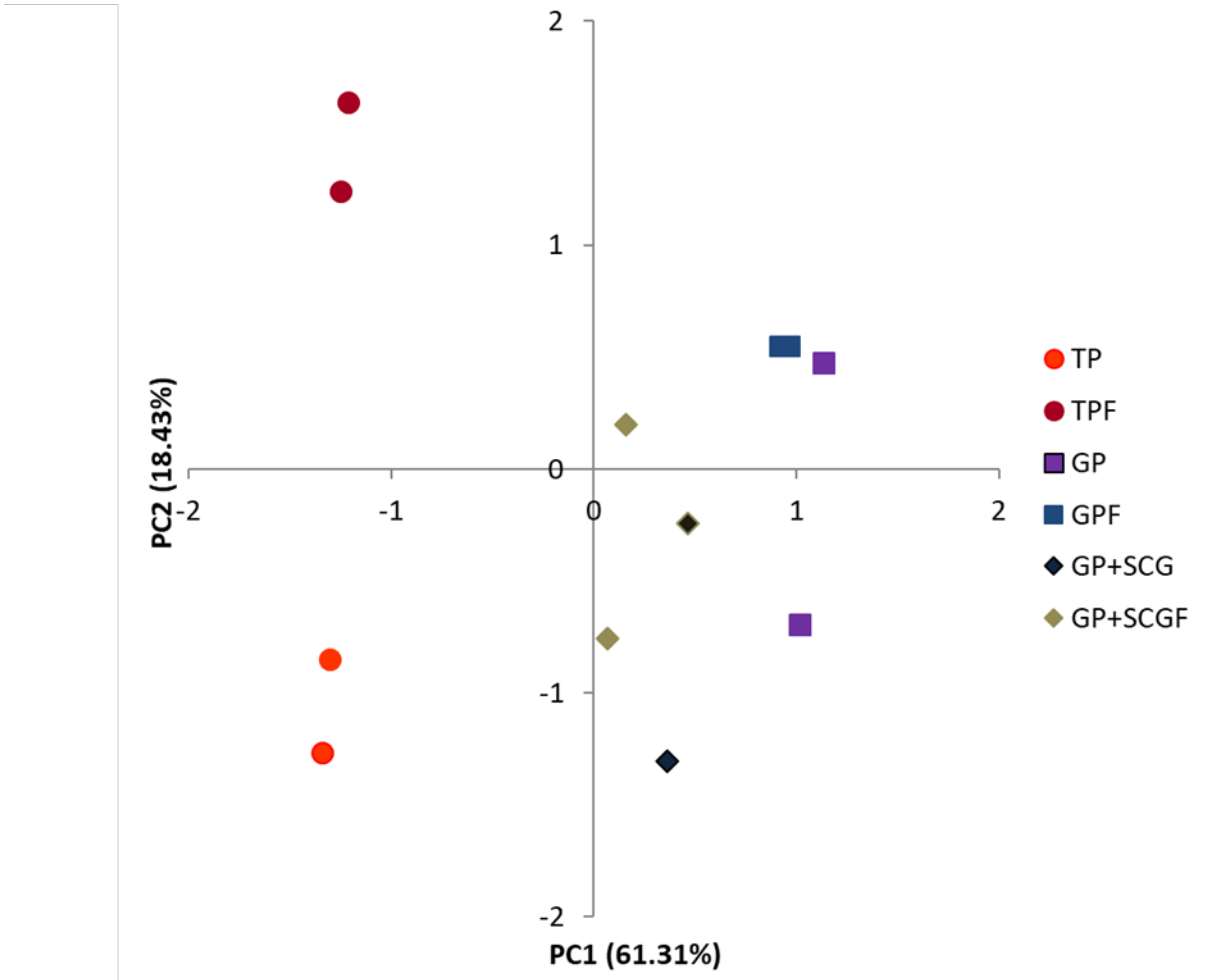

100
90
80
70
60
50
40
30
20
10
0
% IL-8 m-RNA expression
0
5
10
15
20
25
APP (µg mL-1)
NAR
FA
CGA
GP
SCG+GP
SCG+GPF
SCG

Fig.2


1
Lactate
% IL-8 m-RNA expression
Galacturonic acid
Glucose
Ethanol
TPC
Acetate
0
-2
-1
Propionate
0
AA
1
2
PC2 (18.43%)
-1
PC1 (61.31%)
Arabinose


2
1
0
-1
-2
-2
-1
0
1
2
PC2 (18.43%)
PC1 (61.31%)
TP
TPF
GP
GPF
GP+SCG
GP+SCGF

Supporting Information

Polyphenol bioactivity evolution during the spontaneous fermentation of vegetal by-products

Parisa Abbasi-Parizad[a], Patrizia De Nisi[a], Tommy Pepè Sciarria[a], Alessio Scarafoni[b], Pietro Squillace[a], Fabrizio Adani[a], Barbara Scaglia[a,*]

Fig. S1. Metabolite's trend attributable to the SCG fraction during SCG+GP co-fermentation.

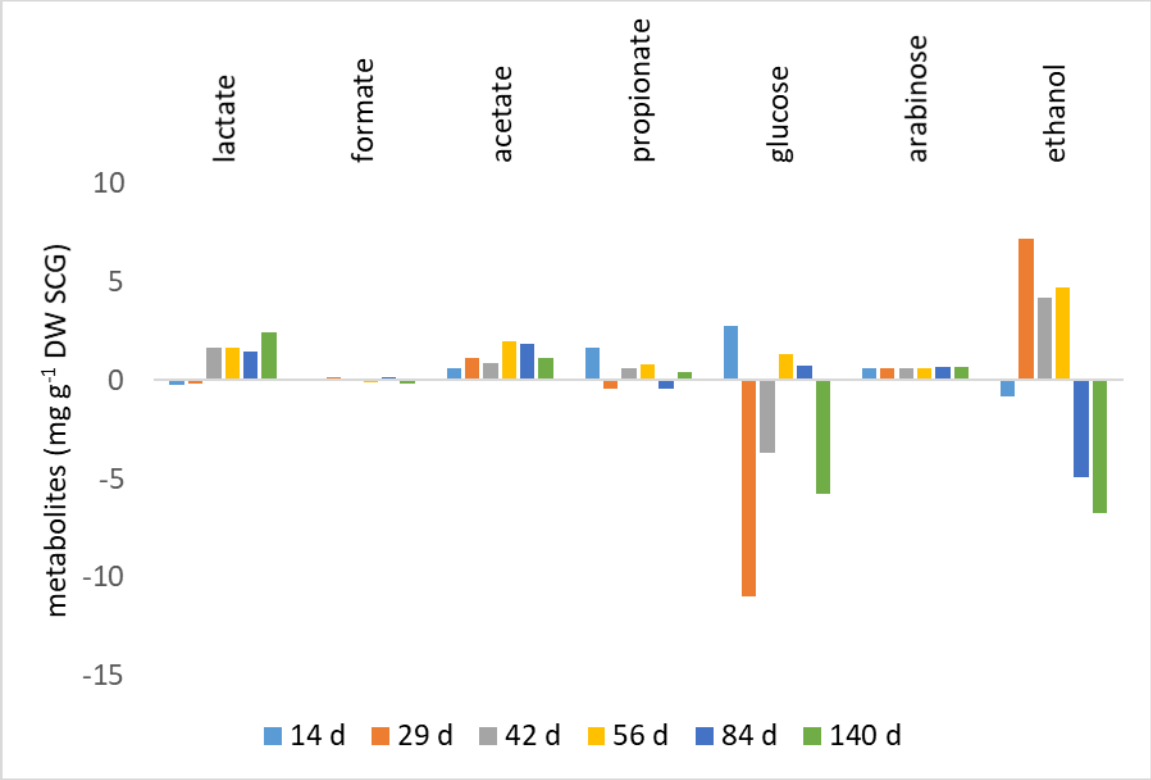


Table S1. Agro-industrial by-products PP-Dose Effect on cytokine IL-8 expression fold.

Biomass	Dose μg APP mL^{-1}	Inflammation status IL-8 m-RNA expression fold
TP	9	5.2 ± 0.2b*
	15	3.4 ± 0.3a
TP_F	7	7.7 ± 0.9b
	10	6 ± 0.2a
GP	12	6.3 ± 0.5b
	20	1.3 ± 0.2a
GP_F	7	8.2 ± 0.6b
	12	6.1 ± 0.7a
SCG	3	4.5 ± 0.5b
	7	1.7 ± 0.2a
$GP+SCG_F$	5	6.4 ± 0.3b
	14	5.7 ± 0.6a
Control (IL-1 β)	-	9 ± 0.3

*value followed by different letters for the same biomass are statistically different, $p<0.05$.

TP: Tomato Pomace; TP_F: Tomato Pomace Fermented; GP: Grape Pomace; GP_F: Grape Pomace Fermented; SCG: Spent coffee grounds; $SCG+GP_F$: Spent coffee grounds + Grape Pomace Fermented.