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Experimental evaluation of temperature, nutrients, and initial concentration on medium-chain carboxylic acids production from winery wastes

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ABSTRACT

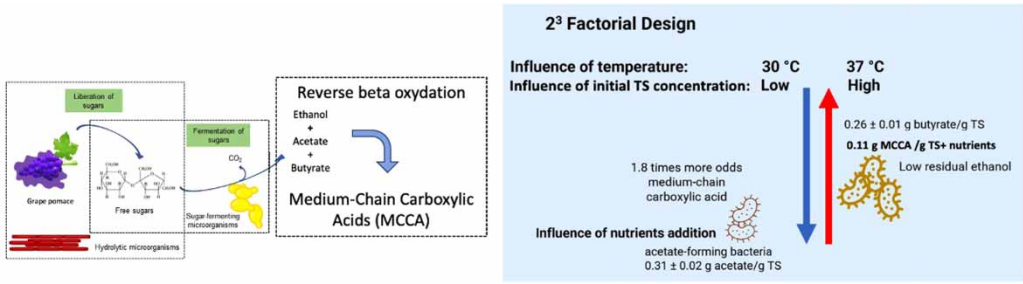
In the wine industry, grape processing is accompanied by waste generation, such as grape stalks, winery wastewater, and grape pomace (GP). GP can be used to produce value-added compounds such as medium-chain carboxylic acids (MCCA). This work aimed to determine the operational conditions (temperature, addition of nutrients, and initial waste concentration) to improve MCCA production using waste GP from the winery industry as a substrate. The electron donor (ethanol) and electron acceptor (acetate) were directly generated from the GP and consecutively used to produce MCCA. The treatment with high concentration, temperature, and nutrient addition promotes caproic acid's maximal yield and concentration (0.11 $\pm$ 0.02 g MCCA/g TS). Nutrients' presence and temperature significantly affected electron acceptor production. The addition of nutrients and 30 °C leads to elevated acetate production. However, at 37 °C, butyrate and MCCA were mainly produced without adding nutrients, and high ethanol consumption was observed. A higher metabolic diversification was observed at 37 °C than at 30 °C. Temperature and nutrient availability significantly affected the metabolic pathway and the type of carboxylic acid produced.

Key words: biorefinery, caproate, carboxylic acids, grape pomace, waste biomass

HIGHLIGHTS

- Factors affecting the MCCA production from grape pomace waste were studied.
- Ethanol, acetate, and butyrate were *in situ* generated and used for MCCA production.
- High temperature and nutrients maximize MCCA production.
- Low temperature and nutrient addition favored acetate production.
- 1.8 more odds MCCA were produced at low than high temperatures.

GRAPHICAL ABSTRACT



INTRODUCTION

In Mexico, the area under vines reached 36–39 thousand ha of vineyards, with an annual average growth of 6.5% (IOV 2022). Consequently, Mexico has doubled its wine production in 2 years from 39 million to 80.6 million liters (IOV 2022). In the

wine industry, grape processing is accompanied by waste generation, such as grape stalks, winery wastewater, and grape pomace (GP). GP is the remaining waste from pressing grapes to collect the juice, including grape skin, pulp, seeds, stems, and residual juice (Zheng *et al.* 2012). The composition of GP depends on the grape variety and the proportion of seeds, skin, and stem, among others (Corbin *et al.* 2015). It has been estimated that 1.8 kg of GP is produced for every 10 L of wine (de Campos *et al.* 2008). The world wine production was estimated at 257.7 million hL in 2019; therefore, 18 KT of GP are generated yearly (IOV 2022).

In white wine production, GP is not present in ethanolic fermentation; once the white grape is mechanically pressed to recover the juice, it is discarded. A high pulp and juice quantity remains in the white GP compared with the red GP processing, where the skin and pulp are part of the fermentation process to intensify the color and aroma properties of the red wine (Mendes *et al.* 2013; Corbin *et al.* 2015). The amount of grapes converted to wine is about 70–80%, while the rest, 20–30%, is disposable as fresh for GP or dried for red GP (Corbin *et al.* 2015).

White and red grape pomaces require environmental management, and their utilization to produce value-added compounds does not compete with food or land use. For instance, it has been reported that GP has been used to produce ethanol and other value-added compounds (Mendes *et al.* 2013). Although ethanol is attractive, higher energy density compounds, such as medium-chain carboxylic acids (MCCA), are wanted. The reasons are the higher market price, easy and cheap extraction, and purification, and because they are precursors to renewable chemical compounds (Wu *et al.* 2019). MCCA are produced through chain elongation, where an electron donor (ethanol) and an electron acceptor, such as the short-chain carboxylic acids (SSCA), are required (Villegas-Rodríguez & Buitrón 2021). Waste biomass can provide electron donor and acceptor components.

Wu *et al.* (2019) classified waste biomass according to the component characteristics of the electron donors. Type I contains electron donors and acceptors, such as wine lees (Kucek *et al.* 2016). In type II, the electron acceptor is produced from waste biomass, and the electron donor is added externally, such as the organic fraction of municipal solid waste plus exogenous ethanol (Grootscholten *et al.* 2014). Finally, the electron donor and electron acceptor are produced from waste biomass in type III biomass. This waste biomass can be first processed to produce the electron donor and acceptor through a hydrolytic-acidogenic process. For instance, Khor *et al.* (2017) reported a two-stage process for generating caproic acid from grass, with high productivity of 0.99 g/L/h. GP could be categorized as waste biomass type III. Thus, it is likely to find the optimal conditions to simultaneously produce and upgrade the electron donor (ethanol) and electron acceptor (acetate, butyrate) to MCCA in one stage from GP waste. GP waste does not require complex pretreatment to release fermentable sugars since alcoholic fermentation of residual carbohydrates occurs under anaerobic conditions. However, the valorization of GP to produce medium-chain carboxylic acid has yet to be systematically studied.

Commonly, ethanol is produced during fermentation. Still, butyrate fermentation is possible (Liu *et al.* 2022). Operational parameters such as temperature can improve the electron transfer efficiency from an electron donor to chain elongation mechanism reducing the electron transfer to competitive pathways such as excessive ethanol oxidation. The proposal is that the ethanol produced by the waste fermentation is then used to elongate SCCA to MCCA by β-oxidation reverse. However, care should be taken since excessive ethanol oxidation may occur through a competitive pathway where the energy is not used to produce MCCA (Wu *et al.* 2019). Thus, determining how temperature, nutrient availability, and initial concentration affect MCCA production when using waste from the effluent winery industry is of interest.

The main objective of this work was to evaluate the fermentation conditions of temperature, nutrients addition, and initial concentration of GP to produce and upgrade the electron donor (ethanol) and electron acceptor (short-chain carboxylic acid) to medium-chain carboxylic acid in one stage.

METHODS

Substrate and inoculum

Fresh white GP waste was collected from a local winery in Queretaro. For the experimental work, seeds and stems were removed, the remaining waste was pressed, and the solid part was referred to as GP waste. The characterization of GP waste is shown in Table 1. Fresh bovine rumen with a total solid content of 0.172 ± 0.01 g/g was used as a source of microorganisms, and it was collected from a municipal slaughterhouse at Queretaro, Mexico. The suitability of ruminal fluid as inoculum was determined in a previous study (Villegas-Rodríguez & Buitrón 2021); therefore, that inoculum was considered to use in this investigation.

Table 1 | Contents of soluble compounds, hemicellulose, cellulose, lignin, and ash in white GP

Parameters	Value
Soluble compounds (%)	50.1
Hemicellulose (%)	4.5
Cellulose (%)	12.7
Lignin (%)	32.8
Ash (%)	0.002
Total solids (g/g)	0.18 ± 0.00
Volatile solids (g/g)	0.14 ± 0.00
Soluble reducing carbohydrates (mg/g fresh grape pomace)	24.0 ± 1.5
Phenol compounds (mg gallic acid equivalents/g volatile solids of grape pomace)	4.6 ± 0.3

Experimental design

As a first attempt, a 2^3-full factorial design was performed where the addition of nutrients, temperature, and concentration of white GP waste was chosen as independent factors, and its influence on the MCCA concentration and yield and butyrate to acetate ratio was studied (Table 2). The total number of experiments was eight, each one with three replicates. The temperature factor was selected to determine its effect on microbial community development. It has been discussed that the optimum temperature range for MCCA-producing bacteria is between 30 and 37 °C (Wu *et al.* 2019). The GP waste can be limited in components to sustain the MCCA production; therefore, nutrient addition was evaluated. Finally, the initial GP waste concentration was evaluated due to the possibility of the accumulation or consumption of a precursor, such as ethanol or SCCA, that may have a critical effect on MCCA production.

Experiments were carried out in 150 mL serum bottles with a working volume of 100 mL. Each bottle was inoculated with 0.23 ± 0.002 g of total solids (TS)/L of bovine rumen content and fed with 11.07 ± 0.04 or 21.02 ± 0.04 g TS/L of GP as a substrate according to Table 2. The content in the serum bottles was resuspended with a mineral solution with or without nutrient addition. The initial pH of the solution was adjusted to 6.8 by using 5 M NaOH. This pH was selected accordingly to previous results obtained in our group (Villegas-Rodríguez & Buitrón 2021). The duration of the experiment was 23 days.

DSZM (German collection of microorganisms and cell cultures) medium (*Clostridium kluyveri* medium 52, DSZM) was used for the experiments (ethanol and acetate were omitted) where external nutrient addition was required (Table 2). Anaerobic conditions were established by flushing nitrogen gas. The bottles were kept in incubators at the required temperature (Table 2) and shaken at 150 rpm. Once a week, a sample from each bottle was taken and held at −20 °C until analysis was performed.

Aqueous soluble compounds, cellulose, hemicellulose, lignin, and ash were determined using the modified Van Soest method (ANKOM A200 Filter bag technique, Technology, USA; Van Soest *et al.* 1991). Peels and pulp were dried in an

Table 2 | Coded and actual factors and variable levels

	Coded factors and variable levels			Actual factors and variable levels		
Experiment number	X_1	X_2	X_3	Nutrients[a]	Temperature (°C)	Solids concentration (g TS/L)
1	−	−	−	N (−)	30	21.00 ± 0.09
2	−	−	+	N (−)	30	11.03 ± 0.00
3	−	+	−	N (−)	37	10.99 ± 0.09
4	−	+	+	N (−)	37	20.96 ± 0.01
5	+	−	−	N (+)	30	11.11 ± 0.00
6	+	−	+	N (+)	30	21.09 ± 0.09
7	+	+	−	N (+)	37	11.16 ± 0.04
8	+	+	+	N (+)	37	21.04 ± 0.04

[a]Nutrients is a categorical variable, and N (−) and N (+) means without and with nutrients addition, respectively; TS: total solids content.

oven at 105 °C until constant weight. Aqueous soluble compounds, hemicellulose, cellulose, lignin, and ash, were quantified from the difference between neutral detergent, acid detergent, and crude fiber analysis (Van Soest *et al.* 1991).

Analytical methods

Collected samples were centrifuged for 30 s at 3,600 rpm. Supernatants were diluted and filtered through a 0.22 μm pore-size membrane. The filtered sample was used to determine volatile fatty acids (VFA, such as acetate, propionate, isopropionate, butyrate, isobutyrate, valerate, and isovalerate), alcohols (ethanol and butanol), and MCCA (caproate, heptanoate, caprylate) by gas chromatograph (Agilent Technologies 7890B, USA) equipped with a flame ionization detector and a nitroterephthalic-acid-modified polyethylene glycol column (Agilent DB-FFAP column, 15 m × 530 μm × 1 μm) (Villegas-Rodríguez & Buitrón 2021). Reducing sugars were analyzed according to Miller (1959). In contrast, TS and volatile solids (VS) were determined based on Villegas-Rodríguez & Buitrón (2021).

Statistical analysis

Graphs were created using the Design Expert software (Stat-Ease Inc., USA). Data were analyzed using analysis of variance (ANOVA, supplementary information). A post hoc analysis was performed when significant differences were found. Three variables were studied to achieve a significant MCCA product selectivity from GP waste: temperature, nutrient addition, initial concentration of GP, and their interactions.

RESULTS AND DISCUSSION

GP as electron donor and acceptor producer

White GP waste was characterized after destemming. The soluble compounds present in the GP represented 50.1%. Zheng *et al.* (2012) reported up to 50% of the dry weight of soluble carbohydrates. In the present study, fermentable carbohydrates (24.0 ± 1.5 mg reducing sugars/g fresh GP) represent 13.4%. The lignin content was 32.8%, while cellulose and hemicellulose represented 12.7 and 4.5%, respectively. Cellulose and hemicellulose require pretreatment to release fermentable sugars and subsequent utilization for MCCA production (Filippi *et al.* 2021). The total phenolic content in the GP utilized was 4.6 ± 0.3 mg phenols per g VS (Table 1). It has been reported that the inhibitory phenol concentration is above 120 mg phenols/g VS (Hernandez & Edyvean 2008); hence, the amount present in the GP waste may not be inhibitory for microorganisms.

The high availability of sugars in GP waste suggests that a previous pretreatment is unnecessary. Although GP waste is a complex feedstock, with almost 50% of its content being lignocellulose, the recovery of the electron donor and electron acceptor for MCCA production is accessible in a practical way. In this sense, results indicate that sugars were entirely consumed by day 3 in all treatments and mainly used for ethanol fermentation (Figure 1). Similar effects were observed by Wang *et al.* (2021) using glucose and pit mud as inoculum for caproate production.

Ethanol is a crucial compound in the metabolism when MCCA are the target compound. In general, for all the conditions, after 3 days, the initial biomass (g VS) transformed to ethanol (g) was 18.2 ± 2.0% and 1.5 ± 0.2% to butyrate. Residual ethanol was found at day 23 only in the treatments with high TS.

The temperature influenced the consumption of ethanol. At 30 °C, the residual ethanol represented 15% (as mmol C), considering all the metabolites. In comparison, at 37 °C, residual ethanol was less than 2% (Figure 2). Therefore, a high temperature will be needed to transform efficiently high initial concentrations of GP waste.

It was observed that GP fermentation produced ethanol, acetate, and butyrate, allowing MCCA formation. Nutrient addition positively influences the MCCA generation. In this sense, the higher caproate concentrations were observed with nutrient addition (99.5–105.0 mmol C/L, 30°, and 37 °C, respectively). The initial solids concentration does not seem to influence the amount of MCCA produced, suggesting no inhibition by overloading occurred (Figure 2(a)).

Nutrient addition and temperature significantly interacted with acetate and butyrate production. At 30 °C, the higher acetate and butyrate were produced with nutrients; however, at 37 °C, higher acetate and butyrate concentrations were generated without nutrients. Butyrate was the primary metabolite in the tests without nutrients ($p = 0.026$). In contrast with nutrient addition, acetate was produced as the primary metabolite (Figure 2(b)).

Higher MCCA yields were obtained with nutrient addition. That is explained because the nutrient solution comprises essential substances that activate microorganisms producing the MCCA, such as yeast extract and vitamins. For instance, *C. kluyveri* utilized yeast extract for cellular synthesis. However, its lack leads *C. kluyveri* to use ethanol and acids for cellular synthesis, reducing MCCA production (San-Valero *et al.* 2020).

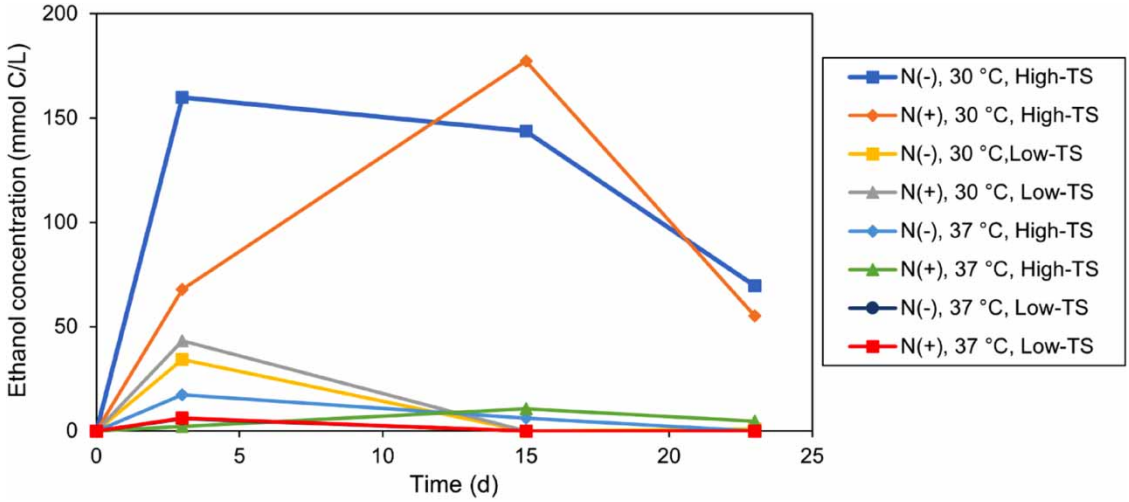


Figure 1 | Ethanol evolution in the batch culture. Treatments are coded by factor nutrient addition [without: N (−) and with: N (+)], temperature, and high or low GP waste concentration as TS.

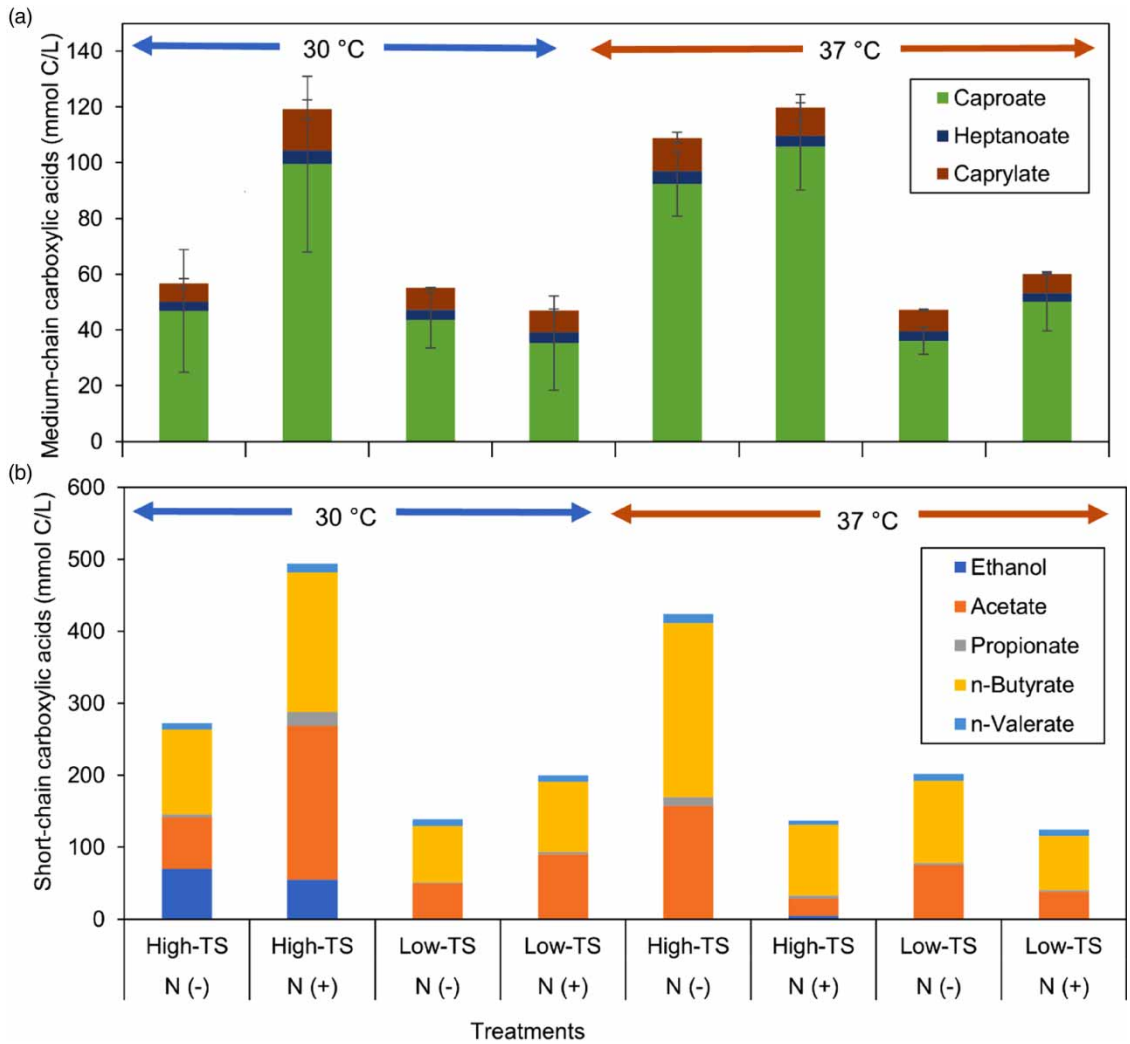


Figure 2 | The concentration of metabolic products after 23 days of fermentation as a function of the initial total solids concentration (TS), temperature and nutrient addition [without: N (-) and with: N (+)].

Temperature and nutrient presence influence the bacterial metabolism of acetate or butyrate production. The highest acetate yield (0.31 ± 0.02 g/g TS) was obtained with 30 °C, nutrient addition, and high initial GP waste concentration (Figure 3(a) and 3(b)). The temperature was a determinant factor ($p < 0.02$) because the same treatment, but at 37 °C, presented lower acetate yield (0.04 ± 0.005 g/g TS), indicating that acetate-forming bacteria could be more active at low temperatures. The acetate production was not significantly influenced by the initial concentration of GP ($p = 0.533$). Under the studied conditions, no optimal point for acetate production was found (see the supplementary information for ANOVA data).

Production of carboxylic acids

Production of caproate and caprylate was observed since day 3, even when acetate was not detected. However, MCCA could also be produced from ethanol and butyrate. Butyrate possibly was generated from the oxidation of the present carbohydrates (Nascimento *et al.* 2022). The inoculum from ruminal fluid showed an efficient conversion to carboxylic acids from GP waste

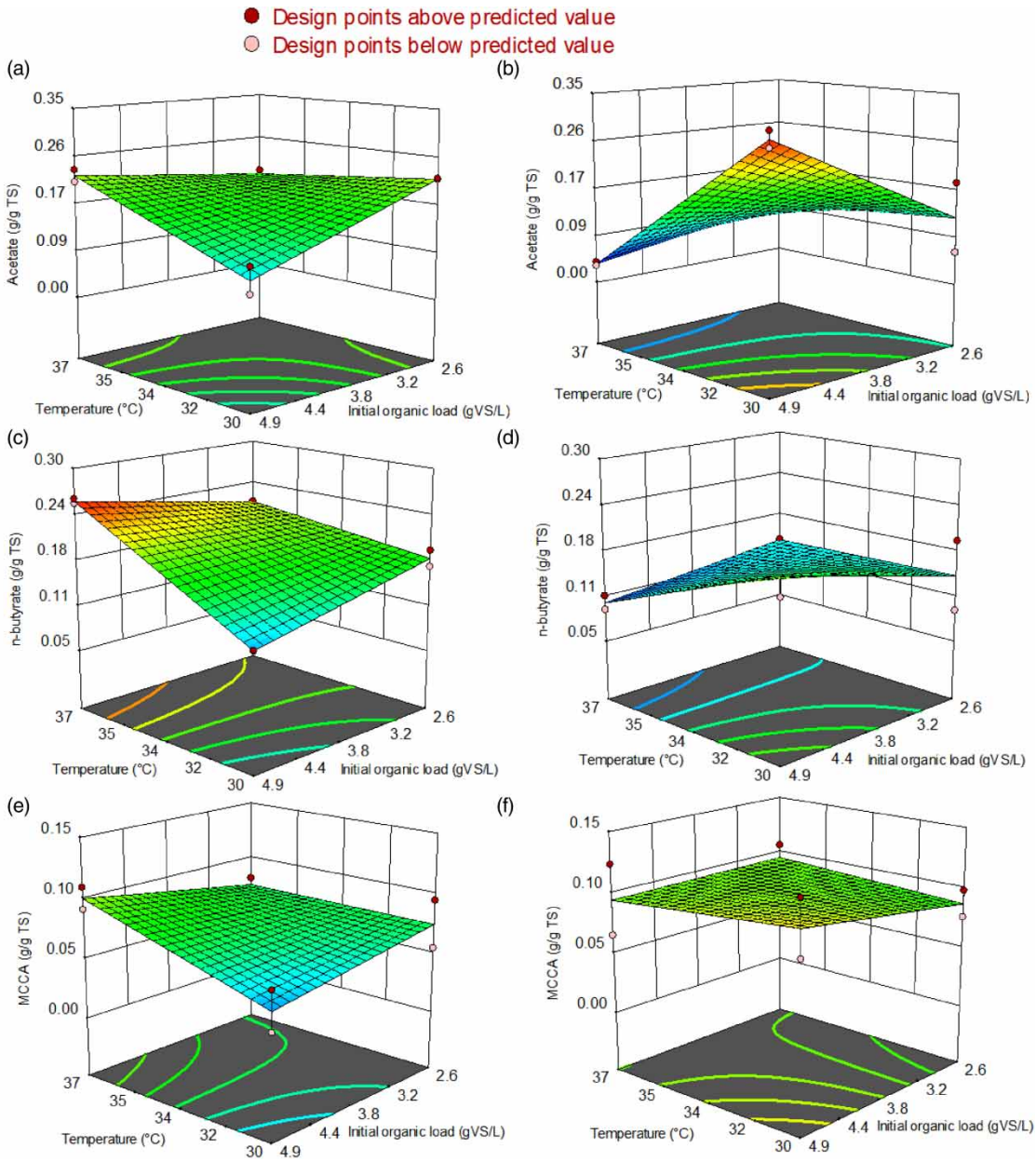


Figure 3 | The influence of the nutrient addition on acetate, n-butyrate, and medium-chain carboxylic acid yields as g of compound per g of TS grape pomace (after 23 fermentation days). Without nutrients addition: (a,c,e) and with nutrient addition: (b,d,f).

(Villegas-Rodríguez & Buitrón 2021). The ruminal fluid contains microorganisms that produce MCCA, such as *Clostridium kluyveri* (Jeon *et al.* 2017; San-Valero *et al.* 2020), *Megasphaera hexanoica*, and *Eubacterium pyruvativorans* (Wu *et al.* 2019). It has been discussed that the ruminal microorganisms' metabolism primarily produces acetate; however, when an electron donor is added, more reduced compounds, such as caproate, are produced (Bolaji & Dionisi 2017).

The high temperature combined with no nutrient addition produced 0.26 ± 0.005 g of butyrate/g TS, representing 28% more than the condition with nutrient addition (Figure 3(b) and 3(c)). The level of the studied factors does not lead to obtaining an optimal point in the butyrate response. The lower butyric acid production (0.09 g TS/g) was observed without nutrients and the lower temperature.

The maximum yield of MCCA was produced with nutrients and 37 °C (0.11 ± 0.02 g of MCCA/g TS) contrasting to the condition without nutrients and 30 °C (0.05 ± 0.02 g of MCCA/g TS) as shown in Figure 3(e) and 3(f). Carrillo-Reyes *et al.* (2019) reported that caproate was 1.6 times higher in treatments with nutrient addition than without from winery wastewater. A higher butyrate/acetate ratio resulted from the nutrient addition.

Figure 4 shows the abundance of metabolites in the products on day 23 of fermentation. The initial GP waste concentration and temperature factors had no interaction, except for heptanoate production ($p = 0.012$). However, odd carboxylic acids were found 1.8 times more in treatments under 30 °C than at 37 °C (Figure 4(a)). Odd carboxylic acids are typically found in fermentations with propionic acid supplied in addition to the electron donor (Ganigué *et al.* 2016). *Propionibacterium* showed a better growth performance and a higher propionic acid yield at the lower temperature (30 °C) (Candry *et al.* 2020). It has been found that temperature gradient affects the inoculum composition.

As discussed, higher acetate yields were observed at 30 °C while at 37 °C, butyrate and MCCA yields were higher. Lower temperatures were related to Bacteroidetes/Proteobacteria as the acetate-forming bacteria (*Acetobacter*); however, MCCA-producing microorganisms *(Clostridium kluyveri, Megasphaera elsdenii, M. indica, Rumminococcaceae bacterium* CPB6) grow better at temperatures close to 37 °C (Wu *et al.* 2019; Liu *et al.* 2022). The optimal temperature for butyric acid-producing bacteria, such as *Clostridium butyricum* and *C. thermobutyricum,* is typically 37 °C (Dudek *et al.* 2022). Results suggest that MCCA production improved at 37 °C, even for mixed culture.

The temperature effect is also related to the metabolites in the system and not only to the present microbiota. In most cases, butyric acid production represented more than 50% of carboxylic acid produced; the only exceptions were two treatments where the temperature was 30 °C (Figure 4(b)).

The treatments under low temperatures mainly produce acetate, and higher temperatures produce more reduced carboxylic acids. Low temperature and high initial concentration of GP waste decreased the ethanol consumption, causing an inhibition effect by the alcohol. Toxic ethanol concentration reported for MCCA production is between 200 and 400 mmol/L (Kucek *et al.* 2016). The opposite occurred at 37 °C, where ethanol assimilation was observed.

In GP fermentation, sugars are quickly fermented, reaching ethanol concentrations of 19–22 g/L (Mendes *et al.* 2013). In the present study, after 3 days, the highest ethanol concentration was 3.7 ± 0.46 g/L. Therefore, the initial GP concentration utilized did not cause ethanol accumulation or substrate inhibition in all cases. The mixed culture allowed ethanol generation and sequentially consumption for MCCA production. Higher GP concentration must be studied for optimal cell growth.

The average CO_2 composition in the biogas headspace was 94.0 ± 2.8% after 23 days of fermentation, which is typical for ethanol fermentation. H_2 was only present in treatments with a high initial concentration of GP waste and 30 °C (2.5 ± 0.8%). The average methane composition was lower than 4.5% in all treatments. Excessive ethanol oxidation could be avoided if the H_2 partial pressure is maintained above 3.04 kPa (Grootscholten *et al.* 2014; Wu *et al.* 2019).

Further assays staging the process are desirable. The first stage focused on producing the electron donor, such as ethanol, and the second focused on building the electron acceptor, such as butyrate. Thus, the electron donor can elongate butyrate to caproate and caprylate.

The results obtained in this study demonstrated the feasibility of using winery waste, mediated by the ruminal microbiota, to produce MCCA and butyrate, as well as the effect of temperature, nutrient addition, and initial concentration. The maximum MCCA yield was observed at 37 °C, nutrient presence, and high initial GP waste concentration.

CONCLUSIONS

The electron donor and acceptor required to produce MCCA were effectively generated from GP waste. Results indicated that temperature and nutrient availability affected the metabolic pathway. The presence of nutrients positively affects when

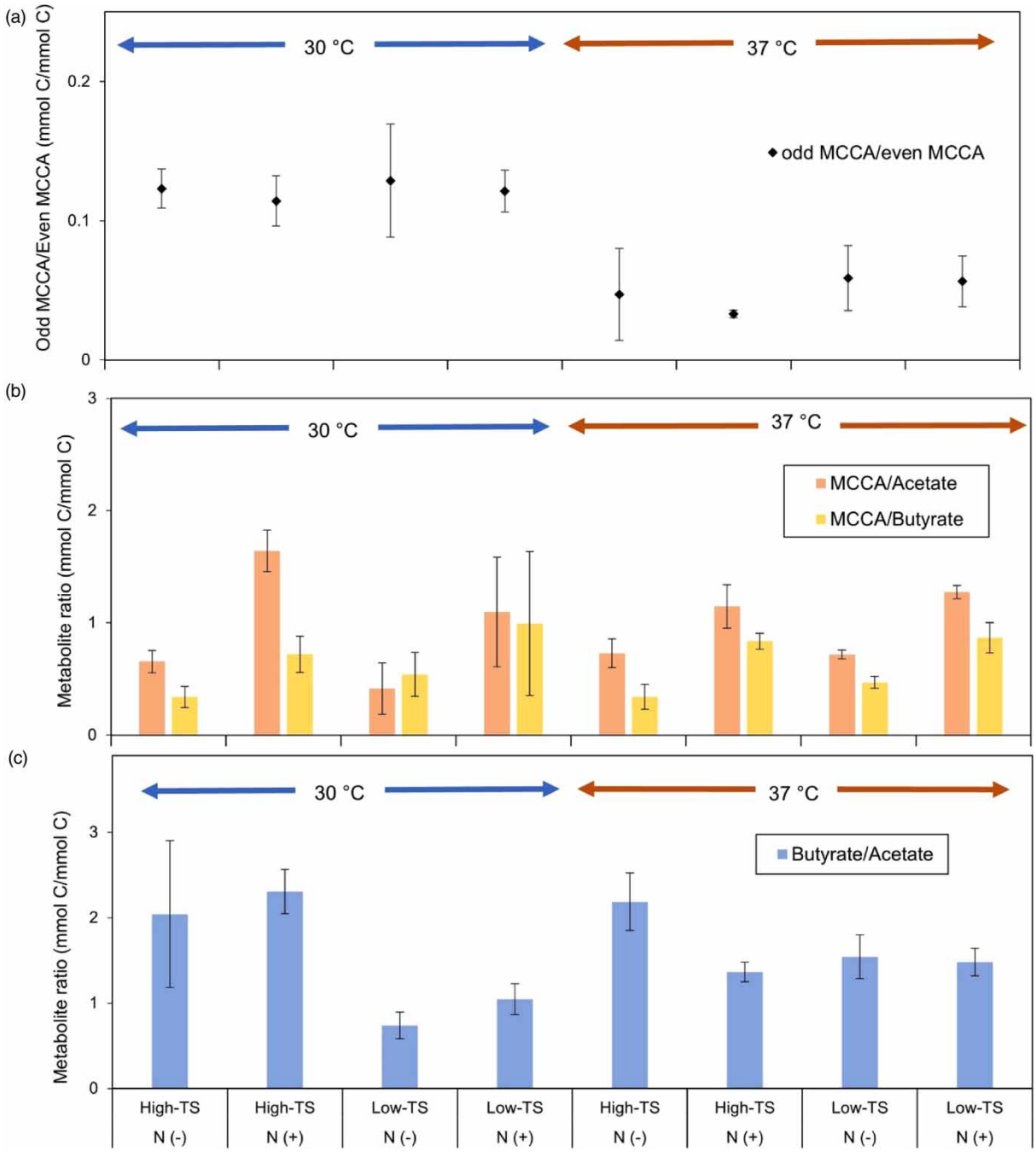


Figure 4 | Ratios for the different acids produced after 15 days of fermentation as a function of the initial TS concentration and nutrient addition [without: N (−) and with: N (+)]. (a) the odd/even carboxylic acids, (b) medium-chain carboxylic acid/butyrate and medium-chain carboxylic acid/acetate ratio, (c) butyrate/acetate ratio.

fermentation is conducted at 30 °C. Therefore, the carboxylic acid produced strongly depends on the chosen fermentation conditions. The treatment with high concentration, 37 °C, and nutrient addition promotes caproic acid's maximal concentration and yield. Nutrient addition and 30 °C lead the microorganisms' metabolism to acetate production, while 37 °C and absence of nutrients conducted to butyrate and MCCA production. A higher metabolic diversification was observed at 37 °C than at 30 °C.

AUTHOR CONTRIBUTIONS

E. Hernández-Correa and G. Buitrón helped with study conception and design. E. Hernández-Correa prepared the material, collected the data, and analyzed the data; E. Hernández-Correa wrote the original draft; G. Buitrón wrote the review and edited the article; G. Buitrón conducted funding acquisition.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

REFERENCES

Bolaji, I. O. & Dionisi, D. 2017 Acidogenic fermentation of vegetable and salad waste for chemicals production: effect of pH buffer and retention time. *J. Environ. Chem. Eng.* **5** (6), 5933–5943. https://doi.org/10.1016/j.jece.2017.11.001.

Candry, P., Ulcar, B., Petrognani, C., Rabaey, K. & Ganigué, R. 2020 Ethanol: propionate ratio drives product selectivity in odd-chain elongation with *Clostridium kluyveri* and mixed communities. *Bioresour. Technol.* **313**, 123651. https://doi.org/10.1016/j.biortech.2020.123651.

Carrillo-Reyes, J., Albarrán-Contreras, B. A. & Buitrón, G. 2019 Influence of added nutrients and substrate concentration in biohydrogen production from winery wastewaters coupled to methane production. *Appl. Biochem. Biotechnol.* **187** (1), 140–151. https://doi.org/10.1007/s12010-018-2812-5.

Corbin, K. R., Hsieh, Y. S., Betts, N. S., Byrt, C. S., Henderson, M., Stork, J., DeBolt, S., Fincher, G. B. & Burton, R. A. 2015 Grape marc as a source of carbohydrates for bioethanol: chemical composition, pretreatment and saccharification. *Bioresour. Technol.* **193**, 76–83. https://doi.org/10.1016/j.biortech.2015.06.030.

de Campos, L. M., Leimann, F. V., Pedrosa, R. C. & Ferreira, S. R. 2008 Free radical scavenging of grape pomace extracts from Cabernet sauvingnon (*Vitis vinifera*). *Bioresour. Technol.* **99** (17), 8413–8420. https://doi.org/10.1016/j.biortech.2008.02.058.

Dudek, K., Molina-Guerrero, C. E. & Valdez-Vazquez, I. 2022 Profitability of single and mixed culture fermentations for the butyric acid production from a lignocellulosic substrate. *Chem. Eng. Res. Des.* **182**, 558–570. https://doi.org/10.1016/j.cherd.2022.04.018.

Filippi, K., Georgaka, N., Alexandri, M., Papapostolou, H. & Koutinas, A. 2021 Valorisation of grape stalks and pomace for the production of bio-based succinic acid by *Actinobacillus succinogenes*. *Ind. Crops Prod.* **168**, 113578. https://doi.org/10.1016/j.indcrop.2021.113578.

Ganigué, R., Sánchez-Paredes, P., Baneras, L. & Colprim, J. 2016 Low fermentation pH is a trigger to alcohol production, but a killer to chain elongation. *Front. Microbiol.* **7**, 702. https://doi.org/10.3389/fmicb.2016.00702.

Grootscholten, T. I. M., Strik, D. P. B. T. B., Steinbusch, K. J. J., Buisman, C. J. N. & Hamelers, H. V. M. 2014 Two-stage medium chain fatty acid (MCFA) production from municipal solid waste and ethanol. *Appl. Energy* **116**, 223–229. https://doi.org/10.1016/j.apenergy.2013.11.061.

Hernandez, J. E. & Edyvean, R. G. J. 2008 Inhibition of biogas production and biodegradability by substituted phenolic compounds in anaerobic sludge. *J. Hazard. Mater.* **160** (1), 20–28. https://doi.org/10.1016/j.jhazmat.2008.02.075.

International Organisation of Vine and Wine. 2022 *The International Organisation of Vine and Wine*. Available from: https://www.oiv.int/what-we-do/data-discovery-report?oiv (accessed 8 November 2022).

Jeon, B. S., Kim, S. & Sang, B. I. 2017 *Megasphaera hexanoica* sp. nov., a medium-chain carboxylic acid-producing bacterium isolated from a cow rumen. *Int. J. Syst. Evol. Microbiol.* **67** (7), 2114–2120. https://doi.org/10.1099/ijsem.0.001888.

Khor, W. C., Andersen, S., Vervaeren, H. & Rabaey, K. 2017 Electricity-assisted production of caproic acid from grass. *Biotechnol. Biofuels* **10** (1), 1–11. https://doi.org/10.1186/s13068-017-0863-4.

Kucek, L. A., Xu, J., Nguyen, M. & Angenent, L. T. 2016 Waste conversion into n-caprylate and n-caproate: resource recovery from wine lees using anaerobic reactor microbiomes and in-line extraction. *Front. Microbiol.* **7**, 1892. https://doi.org/10.3389/fmicb.2016.01892.

Liu, J., Huang, J., Li, H., Shi, B., Xu, Y., Liu, J., Zhang, D., Tang, J. & Hou, P. 2022 Effect of temperature on fermentative VFAs production from waste sludge stimulated by riboflavin and the shifts of microbial community. *Water Sci Technol.* **85** (4), 1191–1201. https://doi.org/10.2166/wst.2022.020.

Mendes, J. A., Xavier, A. M., Evtuguin, D. V. & Lopes, L. P. 2013 Integrated utilization of grape skins from white grape pomaces. *Ind. Crops Prod.* **49**, 286–291. https://doi.org/10.1016/j.indcrop.2013.05.003.

Miller, G. L. 1959 Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.* **31**, 426–428. https://doi.org/10.1021/ac60147a030.

Nascimento, T. R., Cavalcante, W. A., de Oliveira, G. H. D., Zaiat, M. & Ribeiro, R. 2022 Modeling dark fermentation of cheese whey for H_2 and n-butyrate production considering the chain elongation perspective. *Bioresour. Technol. Rep.* **17**, 100940. https://doi.org/10.1016/j.biteb.2021.100940.

San-Valero, P., Abubackar, H. N., Veiga, M. C. & Kennes, C. 2020 Effect of pH, yeast extract and inorganic carbon on chain elongation for hexanoic acid production. *Bioresour. Technol.* **300**, 122659. https://doi.org/10.1016/j.biortech.2019.122659.

Van Soest, P. V., Robertson, J. B. & Lewis, B. A. 1991 Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* **74** (10), 3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2.

Villegas-Rodríguez, S. & Buitrón, G. 2021 Performance of native open cultures (winery effluents, ruminal fluid, anaerobic sludge and digestate) for medium-chain carboxylic acid production using ethanol and acetate. *J. Water Process Eng.* **40**, 101784. https://doi.org/10.1016/j.jwpe.2020.101784.

Wang, H., Gu, Y., Zhou, W., Zhao, D., Qiao, Z., Zheng, J., Gao, J., Chen, X., Ren, C. & Xu, Y. 2021 Adaptability of a caproate-producing bacterium contributes to its dominance in an anaerobic fermentation system. *Appl. Environ. Microbiol.* **87** (20), e01203–21. https://doi.org/10.1128/AEM.01203-21.

Wu, Q., Bao, X., Guo, W., Wang, B., Li, Y., Luo, H., Wang, H. & Ren, N. 2019 Medium chain carboxylic acids production from waste biomass: current advances and perspectives. *Biotechnol. Adv.* **37** (5), 599–615. https://doi.org/10.1016/j.biotechadv.2019.03.003.

Zheng, Y., Lee, C., Yu, C., Cheng, Y. S., Simmons, C. W., Zhang, R., Jenkins, B. M. & VanderGheynst, J. S. 2012 Ensilage and bioconversion of grape pomace into fuel ethanol. *J. Agric. Food Chem.* **60** (44), 11128–11134. https://doi.org/10.1021/jf303509v.

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