# Supplementary Material

# Green separation by using nanofiltration of *Tristerix tetrandus* fruits and identification of its bioactive molecules through MS/MS spectrometry

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## Membrane material specification

**Table S1.** Technical specifications for the NF membranes DL (Suez (GE)$^{TM}$), NFW (Synder$^{TM}$), and NDX (Synder$^{TM}$).

| **Series** | **DL** | **NFW** | **NDX** |
|---|---|---|---|
| **Feed** | Foods/Industrial | Foods/Industrial/Wastewater | Foods/Industrial/Wastewater |
| **Type** | Low Energy, Low Pressure | Softening | High rejection, Softening |
| **pH Range** | 2-10 | 4-10 | 3-10.5 |
| **Flux (GFD)/psi** | 28/220 | 50-55/110 | 35-45/110 |
| **$MgSO_4$ Rejection** | 98.0% | 97.0% | 95.0% |
| **Pore size/MWCO** | ~150-300 Da | ~300-500 Da | ~500-700 Da |
| **Polymer** | Polyamide-TFC | Polyamide-TFC | Polyamide-TFC |

* Information provided by Sterlitech Corp. (USA).

**Table S2.** List of the metabolites that were identified tentatively ([[M+H]$^-$, M+H]$^+$), and those quantified by UHPLC-ESI-MS-MS during the membrane treatments carried-out (DL, NFW, and NDX). The table also shows some particular molecular and chemical properties of the presented metabolites.

| Tentative Identification | Molecular weight (Da) | Theoretical Mass (*m/z*) | [M] | [M-H]$^-$ | [M+H]$^+$ | F-DL(t5) | P-DL(t180) | F-NFW(t5) | P-NFW(t180) | F-NDX(t5) | P-NDX(t180) | pKa | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | **Phenolics** | | | | | | |
| Quinic acid | 192.17 | 192.06 | $C_7H_{12}O_6$ | $C_7H_{11}O_6^-$ | $C_7H_{13}O_6^+$ | Yes | Yes | Yes | Yes | Yes | | 3.46 | Simirgiotis et al. 2016, Yang and Rainville, 2020 |
| 3-O-caffeoylquinic acid (3-CQA) | 354.31 | 354.10 | $C_{16}H_{18}O_9$ | $C_{16}H_{17}O_9^-$ | $C_{16}H_{19}O_9^+$ | Yes | Yes | Yes | Yes | Yes | Yes | 3.59, 8.59 | Simirgiotis et al. 2016, Fernández-Galleguillos et al. 2023 |
| p-coumaroyl malate | 280.23 | 280.06 | $C_{13}H_{12}O_7$ | $C_{13}H_{11}O_7^-$ | $C_{13}H_{13}O_7^+$ | Yes | | | | Yes | | 3.33 | Simirgiotis et al. 2016 |
| Isorhamnetin | 315.02 | 316.06 | $C_{16}H_{12}O_7$ | $C_{16}H_{11}O_7^-$ | $C_{16}H_{13}O_7^+$ | | | | | | Yes | 6.38 | Simirgiotis et al. 2016, Fernández-Galleguillos et al. 2023 |
| Apigenin | 269.05 | 270.05 | $C_{15}H_{10}O_5$ | $C_{15}H_9O_5^-$ | $C_{15}H_{11}O_5^+$ | | | | | Yes | | 6.57 | de Dicastillo et al. 2017, Simirgiotis et al. 2016 |
| Ferulic acid | 194.18 | 194.06 | $C_{10}H_{10}O_4$ | $C_{10}H_9O_4^-$ | $C_{10}H_{11}O_4^+$ | | | Yes | Yes | | Yes | 4.30, 8.81 | Li et al. 2018, Cabrera-Barjas et al. 2020 |
| Ellagic acid | 302.20 | 302.01 | $C_{14}H_6O_8$ | $C_{14}H_5O_8^-$ | $C_{14}H_7O_8^+$ | Yes | | Yes | | Yes | | 6.69, 7.45, 9.61, 11.50 | Jincy and Sunil, 2022, de Dicastillo et al. 2017 |
| 7-O-Methylisorhamnetin | 329.07 | 329.07 | $C_{17}H_{14}O_7$ | $C_{17}H_{13}O_7^-$ | $C_{17}H_{15}O_7^+$ | | | | | | Yes | | Simirgiotis et al. 2016S |
| **Compounds identified and quantified** | | | | | | | | **Phenolics** | | | | | |
| Gallic acid | 170.12 | 170.02 | $C_7H_6O_5$ | $C_7H_5O_5^-$ | $C_7H_7O_5^+$ | Yes | | Yes | | Yes | | 4.4, 8.5, 10.3, 13.0 | López et al. 2018, Junqueira-Gonçalves et al. 2015, Pérez-Almeida et a. 2022 |
| Cryptochlorogenic acid | 354.31 | 354.10 | $C_{16}H_{18}O_9$ | $C_{16}H_{17}O_9^-$ | $C_{16}H_{19}O_9^+$ | Yes | Yes | Yes | Yes | Yes | Yes | 3.44 | Simirgiotis et al. 2016 |
| Chlorogenic acid | 354.31 | 354.10 | $C_{16}H_{18}O_9$ | $C_{16}H_{17}O_9^-$ | $C_{16}H_{19}O_9^+$ | Yes | Yes | Yes | Yes | Yes | Yes | 3.59, 8.59 | Simirgiotis et al. 2016, Navarro-Orcajada et al. 2021, Fernández-Galleguillos et al. 2023 |
| Caffeic acid | 180.16 | 180.04 | $C_9H_8O_4$ | $C_9H_7O_4^-$ | $C_9H_9O_4^+$ | Yes | | Yes | | Yes | | 4.62, 9.07, 11.2 | López et al. 2018, Salehi et al. 2021, Navarro-Orcajada et al. 2021 |
| p-Coumaric acid | 164.16 | 164.05 | $C_9H_8O_3$ | $C_9H_7O_3^-$ | $C_9H_9O_3^+$ | Yes | Yes | Yes | Yes | Yes | Yes | 4.34, 8.83 | Junqueira-Gonçalves et al. 2015, Aguilar-Hernández et al. 2017 |
| Rutin | 610.52 | 610.15 | $C_{27}H_{30}O_{16}$ | $C_{27}H_{29}O_{16-}$ | $C_{27}H_{31}O_{16}^+$ | Yes | | Yes | | Yes | | 7.21, 7.52 | Xiao et al. 2021 |
| Quercetin | 302.23 | 302.40 | $C_{15}H_{10}O_7$ | $C_{15}H_9O_7^-$ | $C_{15}H_{11}O_7^+$ | Yes | | Yes | | Yes | | 7.17, 8.26, 10.13, 12.30, 13.11 | Junqueira-Gonçalves et al. 2015, Aguilar-Hernández et al. 2017, Xiao et al. 2021 |

| | | | | | | | **Amino acids** | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Tentative Identification** | **Molecular weight (Da)** | **Theoretical Mass (*m*/*z*)** | **[M]** | **$[M-H]^-$** | **$[M+H]^+$** | **F-DL(t5)** | **P-DL(t180)** | **F-NFW(t5)** | **P-NFW(t180)** | **F-NDX(t5)** | **P-NDX(t180)** | **pka1 (α-carboxyl group), pka2 (α-amino group)** | **References** |
| Aspartic acid | 133.10 | 133.04 | $C_4H_7NO_4$ | $C_4H_6O_4N^-$ | $C_4H_8O_4N^+$ | Yes | Yes | Yes | Yes | Yes | | 1.88, 3.65, 9.60 | Cabrera-Barjas et al. 2020, Idrees et al. 2020 |
| Proline | 115.13 | 115.06 | $C_5H_9NO_2$ | $C_5H_8O_2N^-$ | $C_5H_{10}O_2N^+$ | | Yes | | Yes | | | 1.99, 10.60 | Cabrera-Barjas et al. 2020, Idrees et al. 2021 |
| Valine | 117.15 | 117.08 | $C_5H_{11}NO_2$ | $C_5H_{10}O_2N^-$ | $C_5H_{12}O_2N^+$ | | | Yes | Yes | Yes | Yes | 2.32, 9.62 | Cabrera-Barjas et al. 2020, Idrees et al. 2022 |
| Tryptophan | 204.23 | 204.09 | $C_{11}H_{12}N_2O_2$ | $C_{11}H_{11}N_2O_2^-$ | $C_{11}H_{12}N_2O_2^+$ | | Yes | | | | | 2.83, 9.39 | Cabrera-Barjas et al. 2020, Idrees et al. 2023 |
| Leucine | 131.17 | 131.17 | $C_6H_{13}NO_2$ | $C_6H_{12}NO_2^-$ | $C_6H_{14}NO_2^+$ | | | | Yes | | | 2.36, 9.60 | Cabrera-Barjas et al. 2020, Idrees et al. 2024 |
| Isoleucine | 131.17 | 131.17 | $C_6H_{13}NO_2$ | $C_6H_{12}NO_2^-$ | $C_6H_{14}NO_2^+$ | | | | Yes | | | 2.36, 9.61 | Cabrera-Barjas et al. 2020, Idrees et al. 2025 |
| Methionine | 149.21 | 149.05 | $C_5H_{11}NO_2S$ | $C_5H_{10}NO_2S^-$ | $C_5H_{12}NO_2S^+$ | | | | | | Yes | 2.28, 9.21 | Cabrera-Barjas et al. 2020, Idrees et al. 2026 |
| Arginine | 174.20 | 172.22 | $C_6H_{14}NO_4S$ | $C_6H_{13}NO_4S^-$ | $C_6H_{15}NO_4S^+$ | | | | | | Yes | 2.17, 9.04, 12.48 | Cabrera-Barjas et al. 2020, Idrees et al. 2027 |

## Membrane permeate filtration

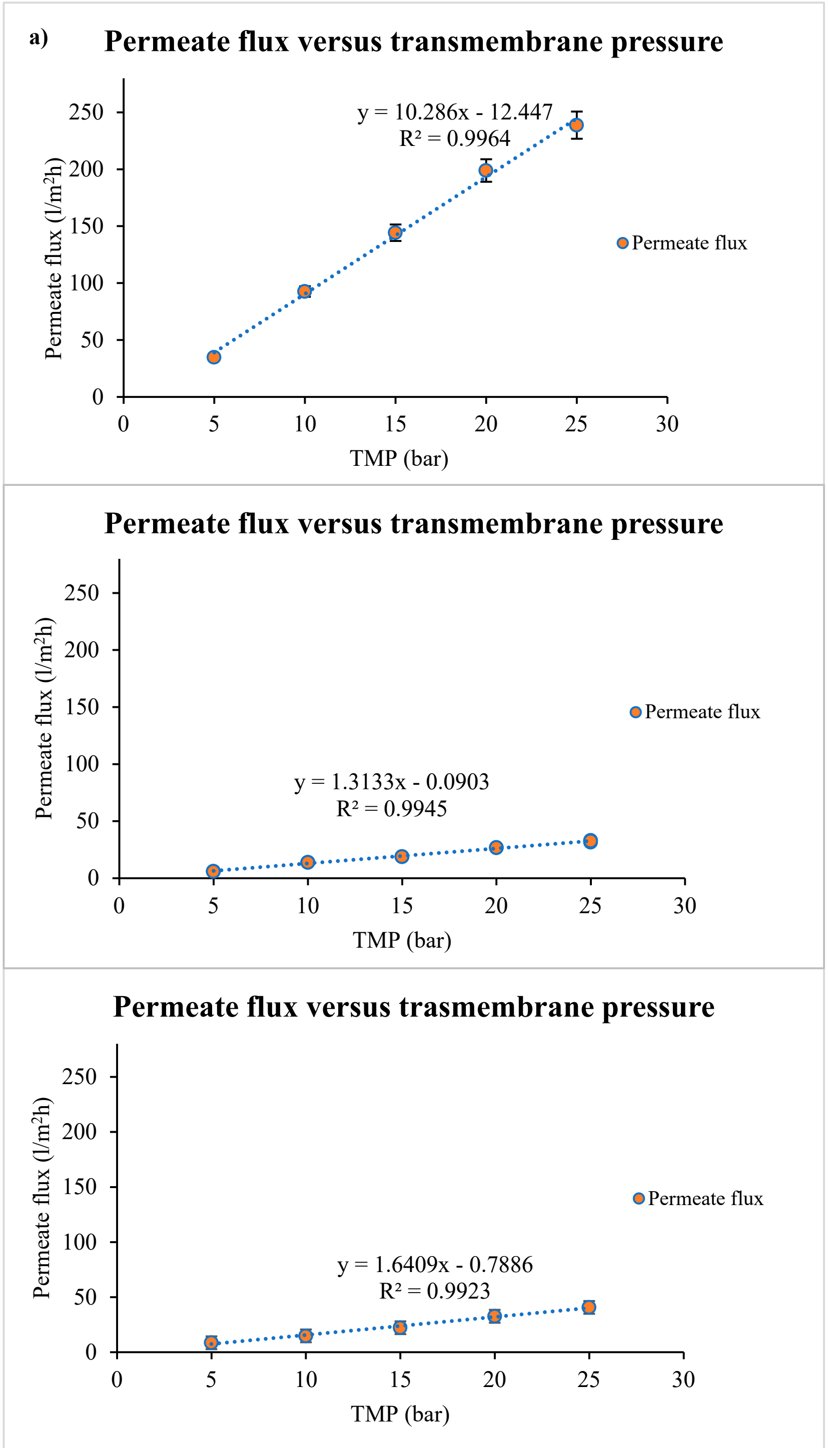
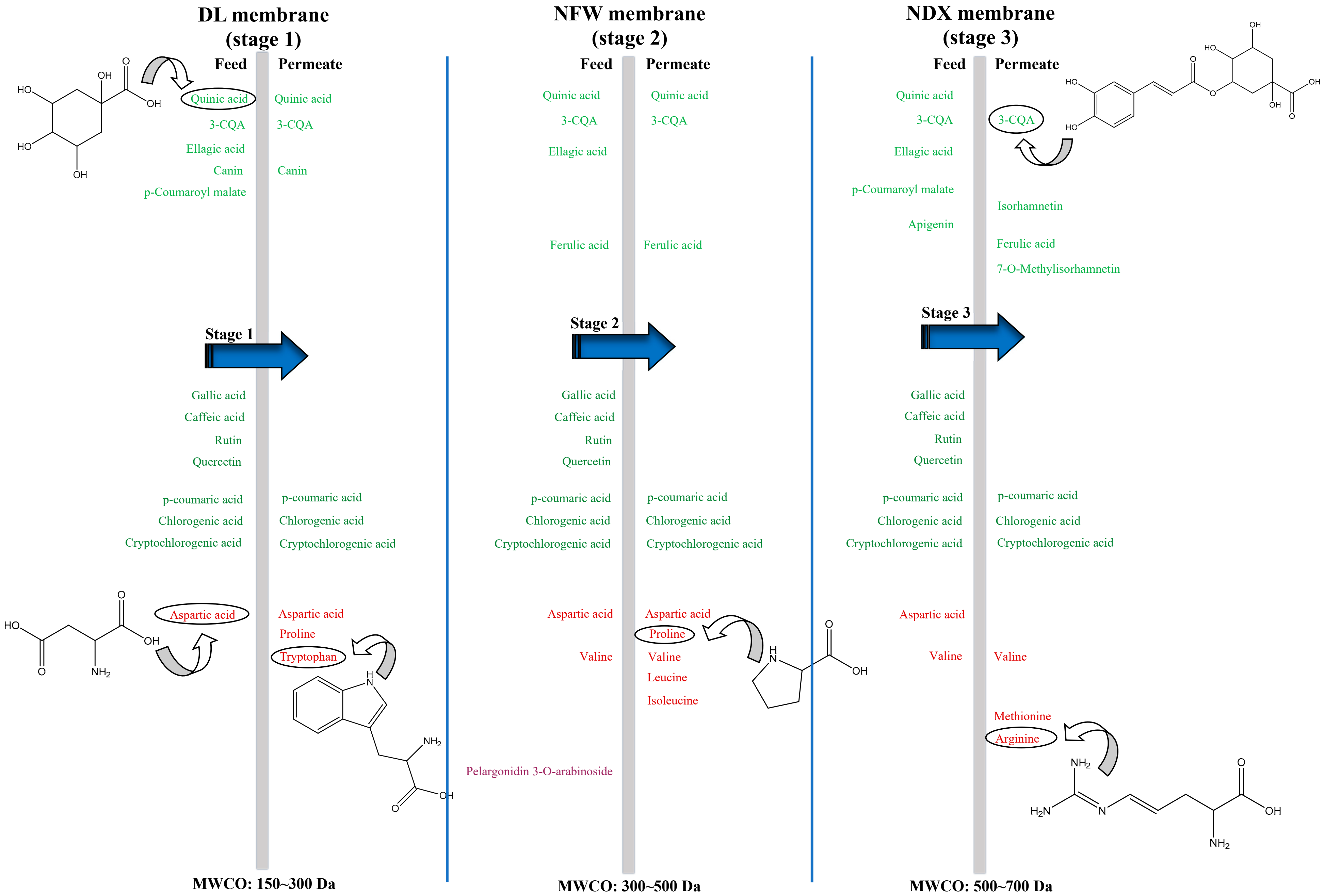


**Figure S1.** Curves of TMP (bar) values versus permeate-flux (l/m$^2$h) for the new membranes tested during the filtration of distilled water: a) DL membrane, b) NFW membrane, c) NDX membrane, (processing temperature: 20 °C).



DL membrane (stage 1)
Feed
Permeate
Quinic acid
3-CQA
Ellagic acid
Canin
p-Coumaroyl malate
Quinic acid
3-CQA
Canin
Stage 1
Gallic acid
Caffeic acid
Rutin
Quercetin
p-coumaric acid
Chlorogenic acid
Cryptochlorogenic acid
p-coumaric acid
Chlorogenic acid
Cryptochlorogenic acid
Aspartic acid
Aspartic acid
Proline
Tryptophan
MWCO: 150~300 Da
NFW membrane (stage 2)
Feed
Permeate
Quinic acid
3-CQA
Ellagic acid
Ferulic acid
Quinic acid
3-CQA
Ferulic acid
Stage 2
Gallic acid
Caffeic acid
Rutin
Quercetin
p-coumaric acid
Chlorogenic acid
Cryptochlorogenic acid
p-coumaric acid
Chlorogenic acid
Cryptochlorogenic acid
Aspartic acid
Valine
Pelargonidin 3-O-arabinoside
Aspartic acid
Proline
Valine
Leucine
Isoleucine
MWCO: 300~500 Da
NDX membrane (stage 3)
Feed
Permeate
Quinic acid
3-CQA
Ellagic acid
p-Coumaroyl malate
Apigenin
3-CQA
Isorhamnetin
Ferulic acid
7-O-Methylisorhamnetin
Stage 3
Gallic acid
Caffeic acid
Rutin
Quercetin
p-coumaric acid
Chlorogenic acid
Cryptochlorogenic acid
p-coumaric acid
Chlorogenic acid
Cryptochlorogenic acid
Aspartic acid
Valine
Valine
Methionine
Arginine
MWCO: 500~700 Da

**Figure S2.** Schematic membrane fractionation process and representation of the identified molecules in each stream (feed and permeate) during the treatments DL, NFW, and NDX, respectively. The mentioned membrane treatments were carried-out consecutively.

**Supporting references**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