Supplementary Materials

Fermentation of Date Pulp Residues Using *Saccharomyces cerevisiae* and *Pichia kudriavzevii* - Insights into Biological Activities, Phenolic and Volatile Compounds, Untargeted Metabolomics, and Carbohydrate Analysis post in vitro Digestion

Nadia S. Alkalbani [1], Muneeba Zubair Alam [1], Anas Al-Nabulsi [2], Tareq M. Osaili [2,3], Amin Olaimat [4], Shao-Quan Liu [5], Afaf Kamal-Eldin [1] and Mutamed Ayyash [1,*]

[1] Department of Food Science, College of Agriculture and Veterinary Medicine, United Arab Emirates University (UAEU), Al Ain P.O. Box 15551, United Arab Emirates; 950223010@uaeu.ac.ae (N.S.A.); 201890043@uaeu.ac.ae (M.Z.A.); afaf.kamal@uaeu.ac.ae (A.K.-E.)
[2] Department of Nutrition and Food Technology, Jordan University of Science and Technology, Irbid 21121, Jordan; anas_nabulsi@just.edu.jo (A.A.-N.); tosaili@sharjah.ac.ae (T.M.O.)
[3] Department Clinical Nutrition and Dietetics, University of Sharjah, Sharjah P.O. Box 27272, United Arab Emirates
[4] Department of Clinical Nutrition and Dietetics, Faculty of Applied Medical Sciences, The Hashemite University, Zarqa 13133, Jordan; aminolaimat@hu.edu.jo
[5] Department of Food Science and Technology, Faculty of Science, National University of Singapore, Science Drive 2, Singapore 117542, Singapore; fstlsq@nus.edu.sg
* Correspondence: mutamed.ayyash@uaeu.ac.ae

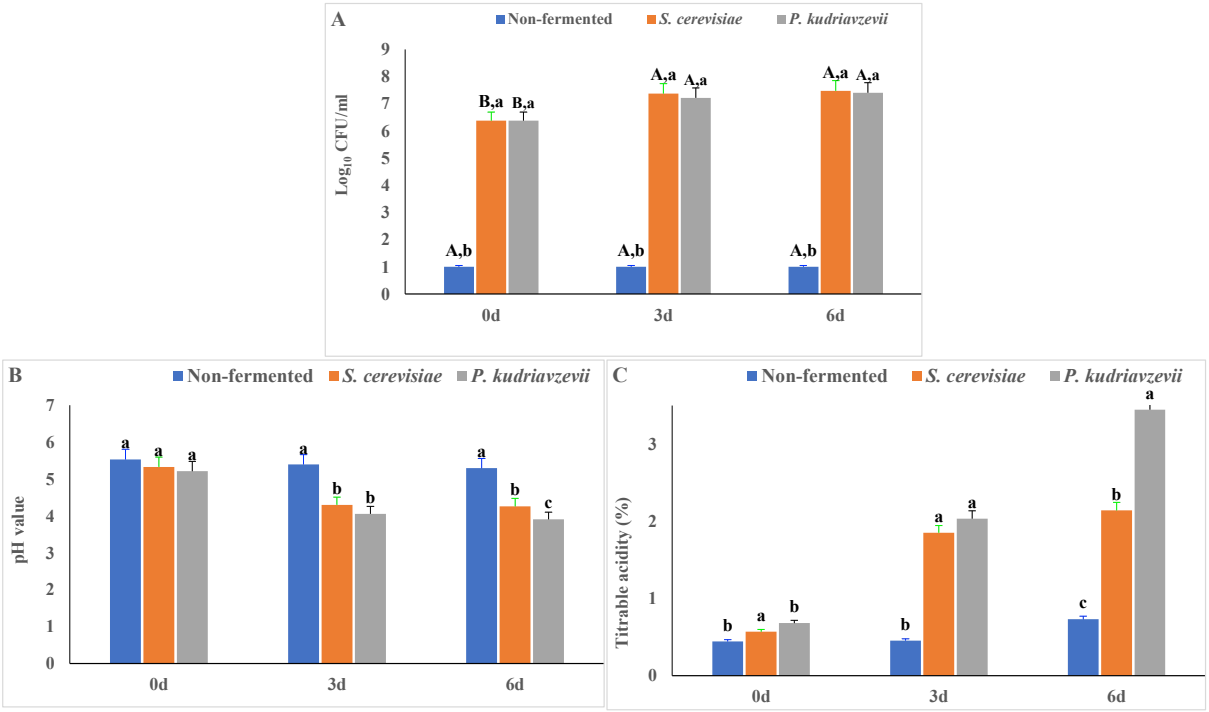

Figure S1. Yeast strains proliferation (A), pH (B) and titratable acidity (C) of the non-fermented and fermented date pomace by *S. cerevisiae* and *P. kudriavzevii* during at 25 °C for 6 days. Values are the mean values (n=3). Error bars express standard deviations.

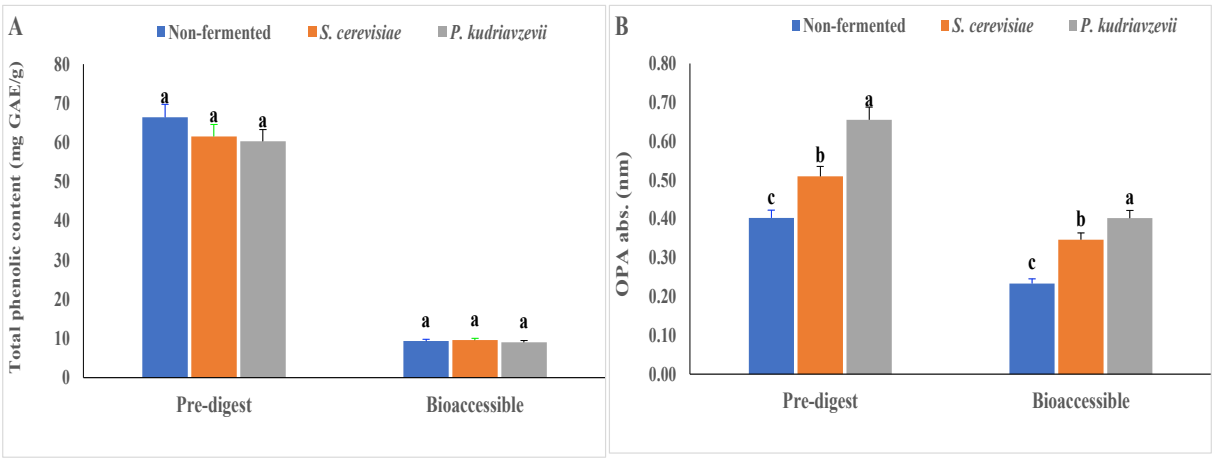

Figure S2. Total phenolic compounds (A) and OPA absorbances (B) of undigested samples and bioaccessible portion of non-fermented (control) and fermented date pomace by *S. cerevisiae* and *P. kudriavzevii*. Values are mean values (n=3). Values are the mean values (n=3). Error bars express standard deviations.

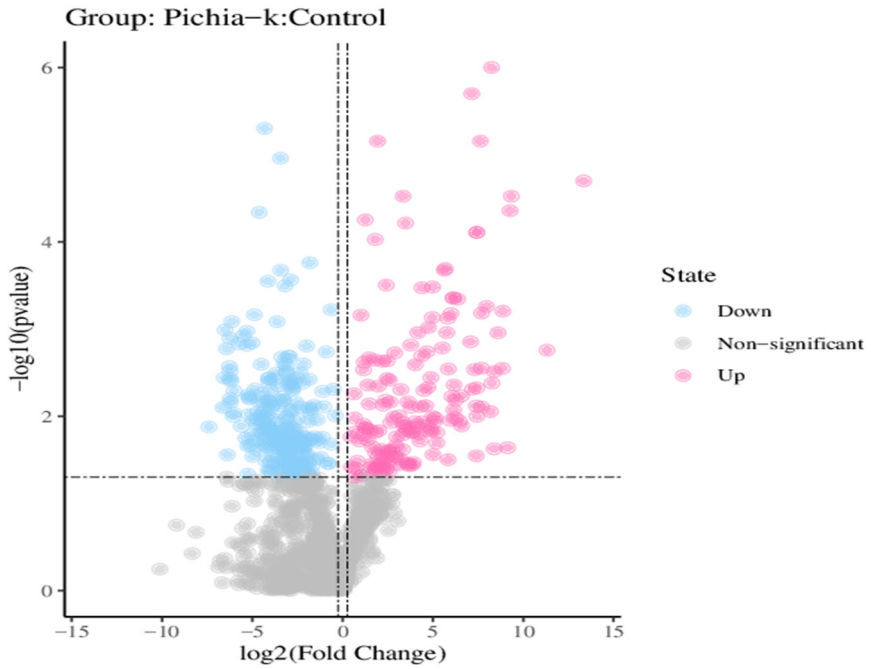

Figure S3. A volcano map displays the differential metabolites between non-fermented and fermented date palm pomace by *P. kudriavzevii.*

Table S1. Relative peak area (RPA%) and peak areas (x10^5) of main volatile compounds in non-fermented (control) and fermented date pomace by *S. cerevisiae* and *P. kudriavzevii*

		S. cerevisiae		***P. kudriavzevii***		**Control**	
Compounds	**RT**	**Peak Area**		**Peak Area**		**Peak Area**	
Acids							
4-Methyl-2-oxovaleric acid	19.67	0.15	0.05	0.00	0.00	0.00	0.00
Acetic acid	19.76	1.68	0.23	2.10	0.31	1.42	0.23
Isobutanoic acid	22.60	0.14	0.02	0.41	0.07	0.06	0.02
Butanoic acid	24.09	0.10	0.02	0.06	0.01	0.03	0.01
2-Methylbutanoic acid	25.03	0.10	0.02	0.59	0.10	0.12	0.02
Hexanoic acid	28.92	1.29	0.11	0.22	0.01	0.06	0.03
Heptanoic acid	31.13	0.13	0.00	0.10	0.02	0.12	0.02
Octanoic acid	33.24	1.17	0.17	0.26	0.09	0.12	0.01
Nonanoic acid	35.27	0.52	0.24	0.72	0.26	0.17	0.05
Benzoic acid	40.21	0.11	0.06	0.08	0.01	0.03	0.02
Subtotal		**5.40**		**4.55**		**2.14**	
RPA (%)		**1.26**		**1.60**		**1.92**	
Alcohols							
Isobutanol	9.63	1.69	0.24	2.53	0.29	0.00	0.00
Isoamyl alcohol	12.95	38.26	9.23	30.21	2.50	7.74	1.28
1-Hexanol	17.16	0.24	0.11	0.17	0.10	0.27	0.04
2-Ethyl-1-hexanol	21.02	1.29	0.39	0.79	0.10	0.94	0.46
2-Phenylethanol	30.95	134.23	15.35	46.97	4.58	2.85	1.59
2-Furanmethanol	25.18	0.10	0.01	0.07	0.01	0.13	0.03
Subtotal		**175.82**		**80.74**		**11.94**	
RPA (%)		**40.99**		**28.33**		**10.72**	
Aldehydes							
Benzaldehyde	21.97	2.06	0.79	5.95	1.78	0.14	0.05
Benzeneacetaldehyde	24.82	0.56	0.16	0.27	0.02	0.18	0.07
4-Methylbenzaldehyde	25.09	0.14	0.03	0.14	0.02	0.00	0.00
2,4-Dimethylbenzaldehyde	28.88	1.87	0.08	1.60	0.85	0.17	0.01
Subtotal		**4.64**		**7.96**		**0.49**	
RPA (%)		**1.08**		**2.79**		**0.44**	
Esters							
Ethyl acetate	5.33	74.65	14.03	92.28	27.27	36.19	14.57
Ethyl propanoate	6.33	1.76	0.16	3.28	0.26	1.50	0.16
Isoamyl acetate	9.97	0.61	0.26	1.17	0.20	0.00	0.00
tert-Butyl formate	15.96	0.03	0.01	0.03	0.01	0.04	0.00
Ethyl octanoate	19.07	0.13	0.01	0.00	0.00	0.00	0.00
Ethyl decanoate	24.26	0.07	0.01	0.00	0.00	0.00	0.00

Phenethyl acetate	28.53	0.52	0.13	0.52	0.19	0.22	0.03
Methyl salicylate	27.93	0.14	0.05	0.08	0.01	0.08	0.00
Subtotal		**77.90**		**97.36**		**38.02**	
RPA (%)		**18.16**		**34.16**		**34.14**	
Ketones							
Acetone	4.73	1.30	0.27	2.19	1.02	3.86	0.75
2-Methyl-3-thiolanone	21.86	0.03	0.00	0.00	0.00	0.00	0.00
Butyrolactone	24.71	0.03	0.01	0.03	0.00	0.00	0.00
Subtotal		**1.37**		**2.22**		**3.86**	
RPA (%)		**0.32**		**0.78**		**3.47**	
Phenols							
2,4-Di-*tert*-butylphenol	37.88	78.89	12.22	61.03	7.14	46.24	9.30
2-Acetyl-4-methylphenol	36.24	0.10	0.02	0.00	0.00	0.00	0.00
t-Butylhydroquinone	62.17	0.12	0.02	0.10	0.02	0.09	0.02
Guaiacol	29.59	0.02	0.00	0.01	0.00	0.01	0.01
Subtotal		**79.13**		**61.14**		**46.35**	
RPA (%)		**18.45**		**21.45**		**41.62**	
Others							
Dimethyl ether	5.960	84.23	41.32	30.60	12.57	8.05	3.15
2-Acetylfuran	21.469	0.11	0.03	0.11	0.00	0.13	0.01
1*H*-Indene, octahydro-2,2,4,4,7,7-hexamethyl-, *trans*-	26.735	0.03	0.003	0.16398	0.052	0.1772	0.044
Naphthalene	27.214	0.18	0.01	0.10	0.02	0.11	0.04
Isobutyric anhydride	29.685	0.05	0.01	0.02	0.01	0.02	0.01
1-Phenylpropane-1,2-diol	30.156	0.03	0.00	0.02	0.00	0.02	0.00
3-Acetyl-1*H*-pyrroline	31.938	0.05	0.01	0.04	0.01	0.05	0.00
Subtotal		**84.69**		**31.06**		**8.56**	
RPA (%)		**19.74**		**10.90**		**7.69**	
Total		**428.94**		**285.03**		**111.36**	

Value = mean±standard deviation (SD), n=3
RPA (%) = (Base peak area/total) × 100

Table S2. Top 10 enriched metabolic pathways with identified metabolites.

Pathway	**KEGG Names**
2-Oxocarboxylic acid metabolism	L-Glutamic acid Oxoglutaric acid L-Methionine L-Isoleucine L-Tryptophan 2-Isopropylmalic acid (-)-threo-isodihomocitric acid L-(+)-Valine (1*E*)-*N*-Hydroxy-4-(methylsulfanyl)-1-butanimine 4-Methylthiobutylthiohydroximate (*E*)-5-(methylsulfanyl)pentanal oxime
Biosynthesis of amino acids	L-Glutamic acid Oxoglutaric acid L-Arginine L-Methionine L-Isoleucine L-Tryptophan 2-Isopropylmalic acid Shikimate D-(-)-3-Phosphoglyceric acid L-(+)-Valine
ABC transporters	L-Glutamic acid L-Arginine L-Isoleucine a-Lactose Xylitol 1,4-D-xylobiose Glycerin L-(+)-Valine Phthalic acid
Aminoacyl-tRNA biosynthesis	L-Glutamic acid L-arginine L-Methionine L-Isoleucine L-Tryptophan L-(+)-Valine
Glucosinolate biosynthesis	L-Methionine L-Isoleucine L-Tryptophan L-(+)-Valine (1*E*)-*N*-Hydroxy-4-(methylsulfanyl)-1-butanimine 4-Methylthiobutylthiohydroximate (*E*)-5-(methylsulfanyl)pentanal oxime
Tryptophan metabolism	L-Tryptophan 4-Hydroxy-2-quinolinecarboxylic acid

	Indole-3-acetic acid Indole-3-ethanol Skatole Indole 5-(3'-carboxy-3'-oxopropyl) -4,6-dihydroxypicolinate
Butanoate metabolism	(*R*)-Malate L-Glutamic acid Oxoglutaric acid Diacetyl (+/-)-2-Hydroxyglutaric acid
Plant hormone signal transduction	N6-(*delta*-2-isopentenyl)-adenine Indole-3-acetic acid Salicylic acid
Pentose and glucuronate interconversions	Oxoglutaric acid L-arabitol Ribitol Xylitol Glycerin
C5-Branched dibasic acid metabolism	Itaconate L-Glutamic acid Oxoglutaric acid (+/-)-2-Hydroxyglutaric acid