Yonekura *et al.*
Food Production, Processing and Nutrition (2024) 6:46
https://doi.org/10.1186/s43014-024-00227-7

Food Production, Processing and Nutrition

RESEARCH

Open Access

Human α-glucosidase inhibition and phytochemical profile of natural and shinzuke treated olives: implications from the processing method

Lina Yonekura[1*], Haruka Hisada[1] and Jittipon Intravichakul[1]

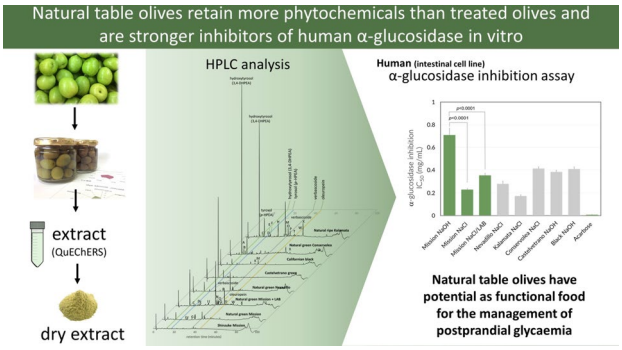
Abstract

Olive (*Olea europaea*) phytochemicals are associated with a reduced risk of type 2 diabetes. Table olives typically have higher phytochemical concentrations than olive oil, but their impact on human intestinal α-glucosidase is largely unknown. Shinzuke, the most common trade preparation in Japan, are alkali-treated non-fermented green olives. In contrast, natural olives are debittered by brining, which may enhance phytochemical retention. We evaluated the α-glucosidase inhibitory effect of Shinzuke and natural green olives produced in-house and compared them with commercial table olives. Eight types of table olives were tested against human intestinal α-glucosidase. Shinzuke Mission was the least effective (IC_{50} 0.710 ± 0.058 mg/mL), while Natural green olives showed double to triple activity, with a non-competitive mechanism. Natural table olives retained more phytochemicals than shinzuke and other treated olives, and are good inhibitors of human α-glucosidase in vitro, exhibiting potential as a functional food for the management of postprandial glycaemia.

Keywords Table olives, *Olea europaea*, α-Glucosidase inhibitor, Debittering, Secoiridoids, Type-2 Diabetes

*Correspondence:
Lina Yonekura
yonekura.lina@kagawa-u.ac.jp
Full list of author information is available at the end of the article

Graphical Abstract



Introduction

Type 2 diabetes (T2D) is a chronic and highly prevalent metabolic disorder characterized by hyperglycaemia and altered insulin secretion and function (World Health Organization 2020). Although it is usually diagnosed through fasting glucose tests, elevated postprandial glycaemia is one of the first signs of T2D. Prolonged hyperglycaemia may increase the risk of coronary heart disease, ischaemic stroke and other vascular disease-related deaths (Sarwar et al. 2010).

Starch digestion by salivary and pancreatic α-amylases produce disaccharides and oligosaccharides that need further digestion into glucose, prior to its uptake by the enterocytes and release in the bloodstream. The human intestine brush border harbours α-glucosidases that are responsible for the breakdown of disaccharides and oligosaccharides (Fatmawati et al. 2011). Oral hypoglycaemic drugs, such as acarbose and voglibose, work as α-glucosidase inhibitors, slowing down starch and sucrose breakdown and preventing the abrupt rise of postprandial glycaemic levels. However, acarbose is frequently associated with gastrointestinal side effects (such as diarrhoea and bloating) and abnormal liver function (Sea-tan 2017; Van De Laar et al. 2005). An effective management of T2D prioritize lifestyle and dietary adjustments, and ideally does not rely exclusively on medication. As hyperglycaemia triggers excessive generation of reactive oxygen species, the resulting oxidative stress gives rise to a number of complications (Sarwar et al. 2010), which emphasizes the importance of dietary antioxidants. Diets rich in plant foods contain a wide range of phytochemicals, many of which with α-glucosidase inhibitory activity (Nair et al. 2013) alongside antioxidant capacity. For example, strawberries, and other anthocyanin rich foods, onions and shallots, peppers, tomatoes and tea (Assefa et al. 2019; Giampieri et al. 2015), along with isolated flavonoids (Barber, Houghton, Williamson, Nilsson, & Hernandez-Hernandez 2021) have been reported to inhibit α-glucosidase. Therefore, the search for α-glucosidase inhibitory activity in foodstuff will provide crucial data for a more effective dietary management and symptom alleviation of T2D. Moreover, α-glucosidase inhibitors also modify insulin secretion and suppress appetite, thus helping in the management of obesity, another risk factor for T2D (Schnell et al. 2016).

To ensure relevance to humans, it is crucial to measure α-glucosidase inhibitory activity using enzymes of human origin. Mediterranean diet has been associated with a decrease of T2D risk, along with the improvement of several other cardiometabolic risk factors (Martín-Peláez et al. 2020). Olive oil, one of the pillars of the Mediterranean diet, has been linked to a reduced risk of T2D (Guasch-Ferré et al. 2015), with reports highlighting inhibitory effects of oleuropein, hydroxytyrosol, and luteolin found in olive oil and olive mill waste on α-glucosidase from baker's yeast (Saccharomyces cerevisiae) in vitro (Collado-González et al. 2017; Hadrich et al. 2015; Mwakalukwa et al. 2020). However, it is important to note that although baker's yeast and rat intestinal α-glucosidases have been widely used to assess potential anti-diabetic effect of phytochemicals, their amino acid sequence and crystal structure differ from that of human intestinal α-glucosidase (Ernits et al. 2021; Ong & Le 2015; Shen et al. 2015). Therefore, the inhibitory effects of phytochemicals may not be directly comparable across different enzyme sources (Pyner et al. 2017). To address this, our study specifically investigated the α-glucosidase inhibitory effects of olive extracts using human intestinal α-glucosidase from Caco-2 cells. Additional data was also obtained with the commonly used baker's yeast and rat intestinal α-glucosidases, to allow comparisons with literature data.

Table olives are processed into a variety of trade preparations in order to reduce the concentration of oleuropein and other compounds responsible for the bitterness and astringency of raw olive fruits (Colmagro, Collins, & Sedgley 2010). The most common trade preparation in Japan is the 'Shinzuke', which consists of a short (6 to 12 h) treatment of green olives (usually from Mission variety) with sodium hydroxide to decompose the bitter phytochemicals, followed by thorough rinsing, short brining and immediate heat treatment, without the traditional fermentation step typical of Spanish (or Sevilla) style olives (Okai 2014). However, the main drawbacks of the Shinzuke and Spanish style olives arise from the use of sodium hydroxide, which requires repeated rinsing cycles and generates large amounts of waste water with high alkalinity, organic matter, phenolic content and salinity (Rincón-Llorente, De la Lama-Calvente, Fernández-Rodríguez, & Borja-Padilla 2018). Apart from the obvious environmental burden, and the cost of treating the waste water before disposal, the rapid alkaline hydrolysis and the intense rinsing involved in the Shinzuke method gives way to a final product with very low levels of phytochemicals. Natural olives are processed by brining for several months until the olives reach desired organoleptic characteristics. During the brining period, enzymes in the olive flesh and/or from the natural flora in the olive surface slowly hydrolyse the bitter compounds, which are eluted due to osmotic pressure and concentration gradients between the fruit and the brine (Ramírez et al. 2016). In this method, a stepwise decomposition of oleuropein and ligstroside takes place, generating a range of compounds such as oleuropein and ligstroside aglycones, oleacein, oleocanthal, hydroxytyrosol and tyrosol (Ozturk 2014). The slow conversion and elution of phytochemicals in the debittering process of natural olives may give better control over the phytochemical profile of the final product, enabling the elaboration of table olives with higher levels of functional compounds, and hopefully higher α-glucosidase inhibitory activity.

In this study we screened the α-glucosidase inhibitory activity of three in-house preparations of natural green and Shinzuke olives and compared them with other commercial table olives. Assays were done with human intestinal cell-derived α-glucosidase, which enables a better assessment of the expected effect in humans. Comparative and mechanistic investigations were also performed with the widely used α-glucosidases from *Saccharomyces cerevisiae* and rat small intestine acetone powder.

Materials and methods

Table olive preparations

Olives of Mission and Nevadillo blanco cultivars were harvested at stage 3 (green ripe) at the Shozu Olive Research Institute—Kagawa Prefecture Agricultural Experimental Station in October, 2019. Mission olives were processed into the following types of table olives: shinzuke olives, natural green and natural green olives with the addition of a *Lactobacillus pentosus* starter (natural green Misson + LAB). For the shinzuke olives, 1.6 kg raw olives were soaked into 2 L of 1.8% NaOH (food grade) solution until 2/3 diffusion into the flesh, then washed 7 times (without exposing the olives to air) over two days until the NaOH was largely removed and water pH was lower than 10. Olives were then transferred to 5% brine, and subsequently to 7% brine, while adjusting to pH 5.0 with citric acid (food grade), and kept at 4 °C until NaCl concentration of brine and olives reached 6% and pH did not rise above 5.0 (2–3 days). Brine was then packed in 150 mL glass jars with fresh brine (to final NaCl concentration of 3%), and pasteurized (internal temperature 80 °C, 5 min). For the natural green Mission and Nevadillo blanco olives, 2 kg olives were slitted to the pit once and soaked in 6% brine for 5 days, transferred to 10% brine (to reach flesh salt concentration of 6%), then topped with a 1 cm layer of vegetable oil (Nissin salad oil, The Nisshin OilliO Group Ltd., Japan). Monitoring and adjustment of pH to 4.5–5.0 with citric acid (when required) were done very 2–3 days, while 90% of the brine was changed every week. Natural green Mission olives with LAB were prepared by soaking 1.8 kg olives in 3L of 6% brine, then adding 1.6×10^{11} cfu of a *Lactobacillus pentosus* starter (VegeStart 60, a gift from Chr. Hansen japan Co., Ltd), previously dissolved in 0.9% NaCl. Monitoring of pH and salt levels were done every 2–3 days but no adjustments were made. Processing times were 92 days for the natural green Mission, 76 days for the natural green Mission + LAB, and 35 days for the natural green Nevadillo table olives, at the end of which olives were packed with fresh brine and pasteurized, as described above. All olive preparations were stored at 4 °C until analyses.

Castelvetrano and Californian Black olives from Italy were purchased from Kobe Bussan Co., Ltd (Hyogo, Japan). Natural green Conservolea and Natural ripe Kalamata olives were from the Agriculture Cooperative of Rovies (Greece). A list of all table olive preparations used in this study, along with the main aspects of their processing methods are given on Table 1.

Chemicals

All chemicals, unless mentioned, were of reagent grade and obtained from FUJIFILM Wako (Japan), Nacalai Tesque (Kyoto, Japan), Kanto Chemical Co. (Kanagawa, Japan) or Sigma-Aldrich.

The Caco-2 cell line was obtained from RIKEN BioResource Research Center Cell Bank (Ibaraki, Japan).

Table 1 Description of table olive preparations used in this study

Table olives	Processing characteristics
Shinzuke Mission[a]	Debittering by 1.8% NaOH, repeated rinsing, short brining at 4 °C (no fermentation)
Natural green Mission[a]	Brining with natural fermentation, at 6% NaCl
Natural green Mission + LAB[a]	Brining with fermentation induced by addition of *L. pentosus* starter, at 6% NaCl
Natural green Nevadillo[a]	Brining with natural fermentation, at 6% NaCl
Castelvetrano green	Debittering by NaOH for 8 h, addition of salt and alkaline brining for 6–8 days, mild washing with water (Lanza 2012); prepared with Nocellara del Belice olives
Californian black	Debittering and oxidation by NaOH with aeration, rinsing and brining with ferrous gluconate or ferrous lactate (Kailis & Harris 2008); cultivar not specified
Natural ripe Kalamata	Debittering by multiple soakings in water or weak brine for approximately 1 wk, followed by brining with added wine vinegar (Kailis & Harris 2008)
Natural green Conservolea	Initial brining at 3.5% NaCl containing 0.15% lactic acid, followed by salt additions up to 8% and natural fermentation for 6–8 months (Agriculture Cooperative of Rovies 2017)

[a] Prepared in-house; detailed procedure described in the Materials and Methods section

Oleuropein was purchased from Panreac (Barcelona, Spain), and verbascoside from Funakoshi Co. (Tokyo, Japan). α-Glucosidase from *Saccharomyces cerevisiae* and acetone powder from rat small intestine were obtained from Sigma-Aldrich. Hydroxytyrosol (3,4-dihydroxyphenyl ethanol) and tyrosol (*p*-hydroxyphenyl ethanol) were from Tokyo Chemical Industry Co. (Tokyo, Japan). 3.4-DHPEA-EDA (oleacein) was isolated as previously reported (Sato et al. 2014). Ultra-pure water (resistivity > 18 MΩ.cm) was used in all experiments.

Extraction of phenolic compounds from table olives

Table olives were extracted using the QuEChERS method (Anastassiades et al. 2003), with modifications to allow fat removal from the acetonitrile phase. Five grams of olive flesh, 6.5 mL water and 10 mL acetonitrile were homogenized for 2–3 min using a Polytron® (Kinematica PT1035) probe homogenizer, in a 50 mL polypropylene tube. Then, 1 g NaCl, 1 g trisodium citrate, 0.5 g sodium dihydrogen citrate sesquihydrate, and 4 g $MgSO_4$ were added and the mixture was vigorously shaken for 1 min. To remove excess oil, 5 mL hexane was added, and the tube was further shaken for 1 min. After centrifugation (3000 rpm, 10 min), the hexane layer was removed, and two additional extractions were done with fresh hexane. The acetonitrile layer was stored at -20 °C until analyses. The extraction process was carried out in triplicate for each sample.

Analyses of total phenolics and individual compounds by UFLC in table olive extracts

Defatted QuEChERS extracts were dried under reduced pressure and dissolved in DMSO. Total phenolics were determined by the Folin-Ciocalteu method. UFLC analyses were performed using a Prominence UFLC system (Shimadzu) equipped with an InterSustain AQ-C18 column (5 µm particle size, 2.1 × 250 mm, GL Sciences) at 40 °C. The mobile phases A (water:acetonitrile 99:1, with 0.1% formic acid) and B (acetonitrile:water 75:25, with 0.1% formic acid) were set to a gradient program starting with a linear increase from 0 to 5% B in 5 min, with further increments to 35% B at 50 min, 50% B at 80 min, 100% B at 85 min, followed by isocratic elution at 100% B until 100 min. The injection volume was 5 µL and the flow rate was 0.3 mL/min. The compounds (hydroxytyrosol, oleuropein, 3.4-DHPEA-EDA, tyrosol, and verbascoside) were identified by retention time and comparison with spectra of standard compounds using PDA detector (SPD-M20A, Shimadzu) set at 279, 274 and 329 nm. Quantification was performed using a calibration curve made by reference standards.

Inhibitory activity on human intestinal α-glucosidase from Caco-2 cells

Caco-2 cells (RIKEN BRC Cell Bank, Ibaraki, Japan) were cultured in D-MEM medium supplemented with MEM non-essential amino acids, 40 U/mL Penicillin, 40 µg/mL Streptomycin, 0.1 µg/mL Amphotericin B and 15% inactivated foetal bovine serum. Cells were maintained at 37 °C with 5% CO_2. Medium was replaced every 2–3 days, and passages were made at 80–90% confluency. Caco-2 cells (passage number 57) cultured for 3 weeks were removed from the culture medium, washed 3 times with chilled PBS, and scraped off with 1 mL of 0.1 M phosphate buffer, pH 6.9, containing protease inhibitors (Protease Inhibitor Cocktail Set I, animal-derived-free, FUJIFILM Wako), and homogenized with a probe sonicator. The obtained cell homogenate was kept on ice and used within the same day. Growth medium and additives were from FUJIFILM Wako or Nacalai Tesque.

Defatted QuEChERS extracts from table olives were evaporated to dryness under reduced pressure, and

inhibitor solutions were prepared by dissolving the dry extract in 20% DMSO. Ten microliters of inhibitor solution (or 20% DMSO as the negative control) and 45 µL of Caco-2 homogenate (2 mU/mL) were combined and preincubated for 15 min. Subsequently, 45 µl of 10 mM maltose in 0.1 M phosphate buffer (pH 6.9) was added and further incubated in a water bath at 37 °C for 15 min. After enzyme inactivation in a water bath (95 °C, 10 min) and centrifugation (3000 rpm, 5 min), the concentration of glucose in the supernatant was quantified using the LabAssay™ Glucose kit (FUJIFILM Wako). Inhibitory activity was calculated as normalized response (percentage), relative to glucose concentration in the negative control wells, where 20% DMSO was added instead of the olive extract. IC_{50} values were determined using GraphPad Prism 8 (Dotmatics). This was done by fitting a dose–response curve to normalized response data at different inhibitor concentrations (ranging from 0 to 0.60 mg dry olive extract/mL), with a variable slope, based on the following equation: $\text{Normalized response (\%)} = 100/(1 + ([\text{inhibitor}]^{\text{HillSlope}})/(\text{IC50}^{\text{HillSlope}}))$ All assays were performed in triplicate and Acarbose was used as the positive control.

Inhibitory activity assay against yeast (Saccharomyces cerevisiae) and rat intestinal α-glucosidases

Table olive dry extracts were dissolved to required concentrations using 50% DMSO. Fifty microliters of the diluted olive extracts (or 50% DMSO as the negative control) and 50 µL of 1 U/mL *Saccharomyces cerevisiae* α-glucosidase (Type I, Sigma-Aldrich) in 0.1 M phosphate buffer (pH 6.9) were added to each well of a 96-well plate and preincubated for 15 min. Fifty microliters of 5 mM 4-nitrophenyl-α-D-glucopyranoside in 0.1 M phosphate buffer was added and incubated at room temperature for 5 min. The absorbance at 405 nm was measured using a microplate reader (MULTISKAN FC, Thermo Fisher Scientific). Inhibitory activity against rat intestinal α-glucosidase was assayed with the same method, substituting yeast α-glucosidase with rat intestinal acetone powder (Sigma-Aldrich) as the enzyme source, at 10 mU/mL. Assay replicates, positive control and IC_{50} calculations followed the same procedures described for inhibitory assays using human α-glucosidase.

For the inhibition kinetics, the above procedure was done with varying concentrations of substrate (0.17, 0.50, 1.0, 2.0 and 3.0 mM 4-nitrophenyl α-D-glucopyranoside) and inhibitors (0.5 to 3.0 mg/mL of table olive extracts; for details see Supplemental Table 1), with absorbance values recorded every 20 s for 20 min. Kinetic parameters and the mechanism of enzyme inhibition were determined using GraphPad Prism 8 (Dotmatics) by nonlinear fitting. Lineweaver–Burk plots were used only for the purpose of visualization and confirmation of the inhibitory mechanism.

Statistical analyses

Comparisons between α-glucosidase inhibitory activities were done by ANOVA, followed by the Tukey-Krammer test, using GraphPad Prism. Pearson correlation analysis was done to explore the relationship between UFLC peak areas and α-glucosidase inhibitory activity. Differences and correlation values were considered significant at $p < 0.05$. PCA analyses were performed using MetaboAnalyst 5.0 (https://www.metaboanalyst.ca) upon autoscaling of UFLC peak areas from table olive extracts and pIC_{50} values from human α-glucosidase inhibition assays.

Results and discussion

Processing methods and phytochemicals

Green Mission olives processed by the shinzuke method had remarkably low polyphenol content (26.0 ± 7.1 µg/g)

Table 2 Concentration of functional compounds and total polyphenols in various table olive preparations

	Hydroxytyrosol	Tyrosol	Verbascoside	Oleuropein	Total polyphenols[a]
	µg/g fresh weight				
Shinzuke Mission	nd	nd	nd	nd	26.0 ± 7.1
Natural green Mission	14.77 ± 1.68	2.26 ± 0.27	29.61 ± 3.20	18.1 ± 2.0	626.0 ± 50.1
Natural green Mission + LAB	86.60 ± 7.97	5.98 ± 0.60	190.87 ± 17.32	157 ± 12	1641.7 ± 120.1
Natural green Nevadillo	20.42 ± 0.37	6.78 ± 0.24	76.98 ± 0.04	nd	538.8 ± 39.5
Castelvetrano green	173.45 ± 25.18	26.53 ± 4.30	nd	nd	na
Californian black	13.32 ± 0.74	5.81 ± 0.54	nd	nd	na
Natural ripe Kalamata	264.17 ± 11.72	69.25 ± 3.83	161.85 ± 6.98	nd	805.3 ± 28.6
Natural green Conservolea	379.9 ± 5.0	69.10 ± 2.72	nd	nd	228.1 ± 3.6

Values are expressed as average ± standard deviation of three replicates; *nd* not detected, *na* not analysed. Oleacein was not detected in any of the table olive preparations

[a] Expressed as gallic acid equivalents

and no detectable amounts of hydroxytytosol, tyrosol, verbascoside and oleuropein (Table 2). The polyphenol concentration in Shinzuke olives is five to 20-times lower than those typically found in Spanish-style olives, which usually contain 130–510 μg/g of hytroxytyrosol alongside other polyphenols (Blekas et al. 2002; Johnson, Melliou, Zweigenbaum, & E. Mitchell 2018; Marsilio et al. 2005). The NaOH treatment in the Shinzuke method is not harsher than that of Spanish-style olives, but the repeated rinsing in the Shinzuke process was probably responsible for leaching out the hydrolysis products, leading to a product with very low polyphenol concentration. In contrast, green Mission olives from the same batch prepared by the natural method (with or without addition of LAB) had higher retention of hydroxytyrosol, verbascoside and oleuropein (Table 2), and higher α-glucosidase inhibitory activity (Table 3). Our results are in consonance with a previous study where natural olives had clearly higher polyphenol and secoiridoids contents, compared to treated olives produced with Ascolana tenera olives of the same ripening stage (Marsilio et al. 2005). Differences between cultivars were also evident, as natural green olives produced in-house with Nevadillo Blanco and Mission had distinct concentrations of verbascoside (76.98 ± 0.04 *vs* 29.61 ± 3.20 ug/g fresh weight) and oleuropein (negligible *vs* 18.1 ± 2.0 ug/g fresh weight). Kalamata and Conservolea, which are traditionally processed by the natural brining method, were characterized by high levels of hydroxytyrosol and tyrosol (Table 2) along with other phytochemicals (Table 2, Fig. 1). The natural method relies on internal enzymes (esterases), brining (compound diffusion to the brine) and fermentation by autochthonous yeasts and lactic acid bacteria to lower the concentration of secoiridoids in the olive flesh. In contrast to the shinzuke method, where secoiridoids are hydrolysed by NaOH and olives are produced in one-week time, the natural method required several months but offered better control over the concentration of the bioactive phytochemicals in the final product. The natural method has been traditionally used in Mediterranean countries, being the processing method of highly appreciated olives such as Kalamata (Greece), Aloreña de Málaga (Spain), and Olive di Gaeta (Italy). Both the cultivar and the processing method deeply influenced the concentration of phytochemicals in the final product and, as expected, natural brining methods led to table olives with a more complex profile and higher overall phytochemical concentration.

Within the treated olives, Californian black olives were devoid of oleuropein and verbascoside, and contained very low concentrations of hydroxytyrosol and tyrosol (13.3 ± 0.7 and 5.8 ± 0.5 μg/g, Table 2). This phytochemical profile is somewhat expected as the long lye treatment followed by extensive oxidation during the rinsing steps lead to oxidation and polymerisation of hydroxytyrosol, along with complex formation when ferrous gluconate is added (Marsilio et al. 2001). On the other hand, Castelvetrano green olives had very high hydroxytyrosol

Table 3 The inhibitory effect (IC_{50} values) of various table olive extracts and pure compounds against α-glucosidases from human intestinal (Caco-2) cells, yeast and rat intestines

	type of processing	α-glucosidase inhibition IC_{50}		
		Human (Caco-2)	Rat intestine	Yeast
			mg dry extract/mL	
Shinzuke Mission	NaOH	0.710 ± 0.058^{a}	6.92 ± 0.23^{a}	8.32 ± 0.42^{a}
Natural green Mission	NaCl	0.230 ± 0.011^{d}	4.86 ± 0.21^{c}	5.51 ± 0.39^{b}
Natural green Mission + LAB	NaCl	0.352 ± 0.020^{bc}	5.19 ± 0.21^{c}	6.46 ± 0.42^{ab}
Natural green Nevadillo	NaCl	0.281 ± 0.022^{cd}	4.77 ± 0.15^{c}	5.18 ± 0.14^{b}
Castelvetrano green	NaOH	0.384 ± 0.013^{bc}	6.56 ± 0.18^{ab}	7.96 ± 0.65^{a}
Californian black	NaOH	0.409 ± 0.017^{b}	5.41 ± 0.21^{bc}	6.77 ± 0.31^{ab}
Natural ripe Kalamata	NaCl	0.174 ± 0.010^{d}	na	na
Natural green Conservolea	NaCl	0.413 ± 0.014^{b}	na	na
			mg/mL	
Acarbose		0.0079 ± 0.0009^{e}	6.51 ± 0.47^{ab}	2.03 ± 0.07^{c}
Hydroxytyrosol		na	na	0.0296 ± 0.0004
Tyrosol		na	na	0.0390 ± 0.0011
Verbascoside		na	na	0.0791 ± 0.0018
Quercetin		na	na	0.0066 ± 0.0002

Values are expressed as best fit values ± standard error; *na* not analysed

Different superscript letters denote significant differences by one-way ANOVA, followed by multiple comparisons by the Tukey's test ($p < 0.05$)

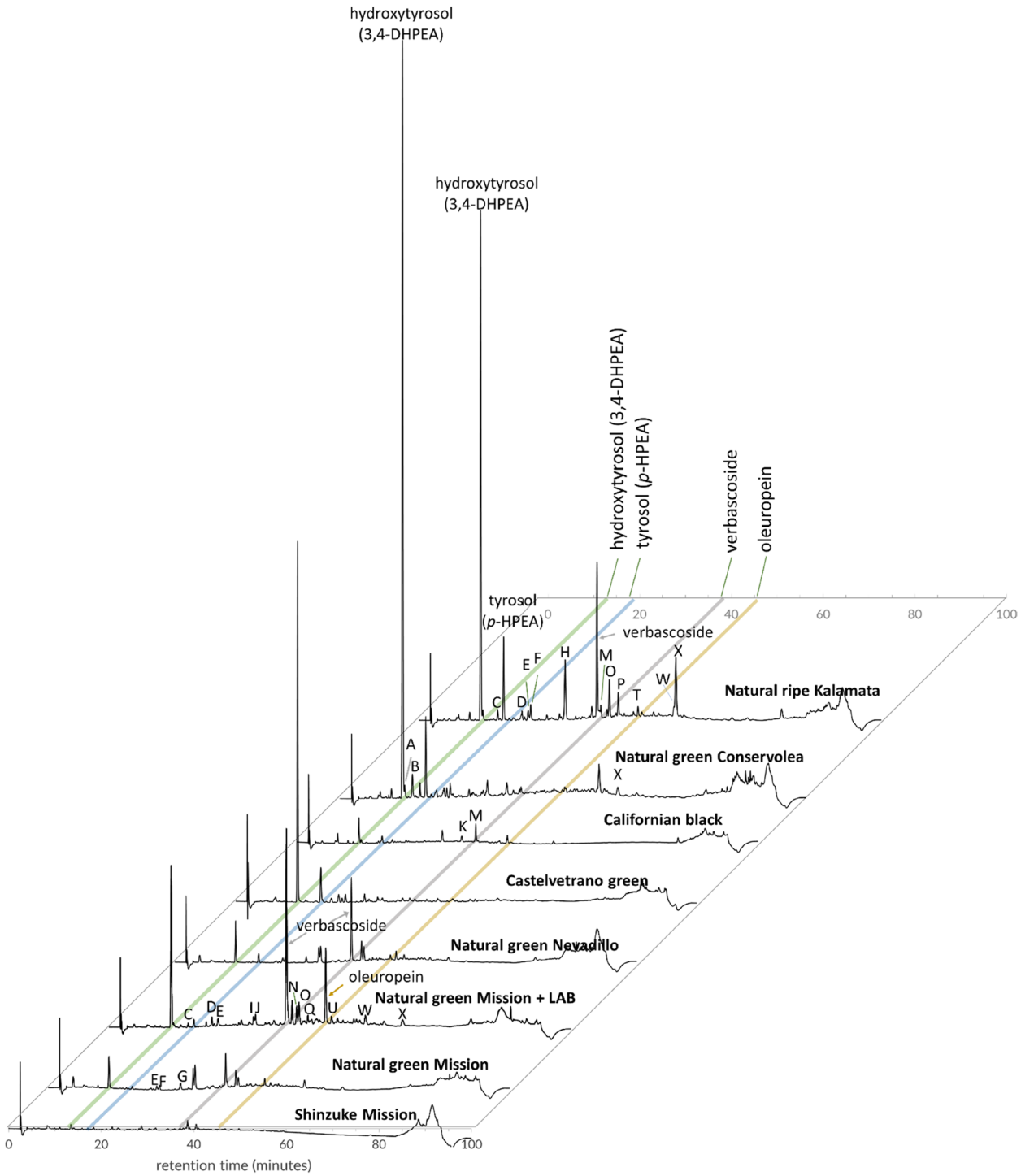


Fig. 1 UFLC chromatograms of table olive extracts, obtained at 278 nm. Hydroxytyrosol, tyrosol, verbascoside and oleuropein were quantified with pure standards. Letters denote unassigned peaks

and tyrosol (173 ± 25 and 26.53 ± 4.30 ug/g, Table 2) compared to other treated olives, which could a result of the characteristic Castelvetrano process where salt is added directly to the olives in alkaline solution (Lanza 2012), limiting the loss of secoiridoid hydrolytic products due to rinsing steps.

UFLC chromatograms of table olive extracts are shown in Fig. 1. Apart from the quantified compounds,

22 peaks (Fig. 1, peaks A to X) were also detected but remained unidentified due to the lack of authentic standards. The chromatograms also show a trend of fewer peaks in sodium hydroxide-treated olives, suggesting that treated olives are less appealing as functional foods. The maximum absorption wavelengths of peaks A to X are as follows: A, 258 nm and 293 nm; B, 274 nm; C, 274 nm; D, 264 nm and 265 nm; E, 259 and 292 nm; F, 320 nm; G, 277 nm and 308 nm; H, 307 nm; I and J, 328 nm; K, 278 nm; L, 327 nm; M, 344 nm; O, 325 nm; P and T, 311 nm; Q, 318 nm; S, 336 nm; R, 271 and 336 nm; U, 280 and308 nm; V, 367 nm; W, 347 nm, and X, 282 nm. UV–vis chromatograms of each peak are shown on Supplemental Figure 1.

Inhibitory activity on human α-glucosidase and its relationship with olive phytochemicals

The inhibitory effect of Shinzuke Mission on human intestinal α-glucosidase was the weakest among all tested table olives (Table 3, IC_{50} 0.710 ± 0.058 mg/mL), while Mission olives processed without NaOH treatment had double to triple inhibitory activity (Table 3, Natural green Mission + LAB IC_{50} 0.352 ± 0.20 and Natural green Mission IC_{50} 0.230 ± 0.011 mg/mL). Natural green olives prepared with Mission and Nevadillo varieties showed similar α-glucosidase inhibition (IC_{50} 0.281 and 0.352 mg/mL). Overall there was a tendency of stronger α-glucosidase inhibitory activity by natural table olives, in agreement with the higher concentration and number of phytochemicals, as shown in Table 2 and Fig. 1. However, the variety effect is also evident, thus both processing and the original phytochemical profile play a role in the biological activity of the final product. Nevertheless, most olives had comparable or stronger α-glucosidase inhibitory activity than that of green tea extract, a well-known natural α-glucosidase inhibitor with an IC_{50} value of 0.500 ± 0.003 mg/mL (Pyner et al. 2017).

Many polyphenols, especially flavonoids, have been associated with reduction of T2D risk in epidemiological studies, improvement of postprandial glycaemia in animal models, and inhibition of α-glucosidase activity in vitro (Barber et al. 2021; Kim, Keogh, & Clifton 2016), however the majority of data coming from studies using non-human α-glucosidases. Nevertheless, some studies using human α-glucosidase also suggested that baicalein (Nishioka et al. 2020), quercetin (Barber et al. 2021), tea flavonoids (Pyner et al. 2017) and other plant extracts are promising hypoglycaemic agents.

To elucidate which specific compounds were accountable for the α-glucosidase inhibition we performed a correlation analysis between the log transformed IC_{50} values (pIC_{50}) for human α-glucosidase and the peak areas of the UFLC chromatograms obtained from each olive preparation (Supplemental Figure 2). The α-glucosidase inhibitory activity was not correlated with hydroxytyrosol, tyrosol, oleuropein, and oleacein. On the other hand, peaks O (λ_{max} 325 nm) and F (λ_{max} 320 nm) were correlated ($p < 0.05$) with Caco-2 α-glucosidase inhibition. The inhibitory effect on human α-glucosidase seems to be result of a combination of compounds, and therefore could not be assigned to one or few specific peaks. Many flavonoids have been reported to inhibit α-glucosidase activity both in humans and in assays using yeast α-glucosidase, so a thorough study on each olive polyphenol would be needed to assess their contribution to the overall inhibitory activity observed in the present study.

The PCA analysis between UFLC peak areas and the inhibitory activity (pIC_{50}) on human α-glucosidase (Fig. 2) showed that 70.8% of the activity variance could be explained by the phytochemical profile (*i.e.* peak areas), distributed in PC1 (49.0%) and PC2 (21.8%). The score plot showed three clusters of high, medium, and low activity olives (Fig. 2). Natural Ripe Kalamata olives showed the highest activity, associated with F and C peaks and several other peaks unique to this olive preparation. Peak C is very likely a compound similar to tyrosol, based on its characteristic UV absorption spectrum (λ_{max} 274 nm) and retention time (Fig. 1). Natural green Mission + LAB, Natural green Mission and Natural green Nevadillo were moderately active against human α-glucosidase and associated with verbascoside, oleuropein and peaks G, J, I and N, which were only featured in the chromatograms of this cluster.

Natural olives retained more phytochemicals and usually showed stronger inhibition of α-glucosidase, when compared with treated olives. Extracts of natural table olives were comparable to green tea extract as human α-glucosidase inhibitor. Green tea has been shown to lower postprandial glucose in clinical trials (Miyoshi et al. 2015; Takahashi et al. 2014), suggesting that olives are also good candidates as functional food for management of postprandial glucose. However, the anti-glycaemic effect could not be explained by the concentrations of a single olive secoiridoid or any other phytochemical, suggesting that several compounds may be responsible for this function.

Inhibitory activity against yeast (Saccharomyces cerevisiae) and rat intestinal α-glucosidases

Shinzuke Mission and Castelvetrano were the olives with the weakest inhibitory activity on yeast α-glucosidase (Table 3, IC_{50} 8.32 ± 0.42 and 7.96 ± 0.65 mg dry extract/mL), and rat intestinal α-glucosidase (Table 3, IC_{50}

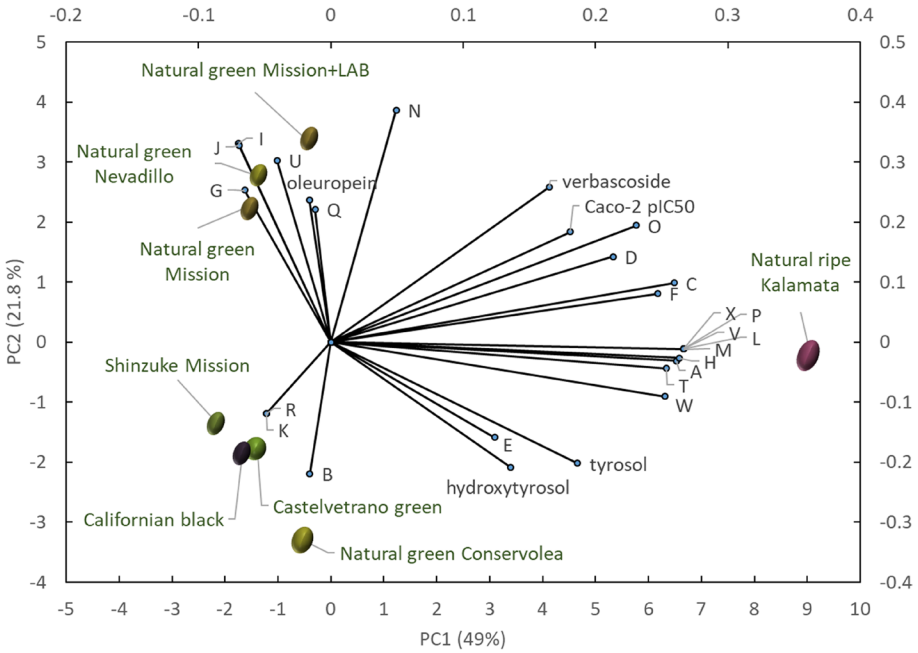


Fig. 2 PCA biplot between pIC50 (Caco-2 cell α-glucosidase) and peak area of various table olive extracts

6.92 ± 0.23 and 6.56 ± 0.18 mg dry extract/mL). In contrast, Natural green Mission and Nevadillo table olives showed the highest activities (IC_{50} 4.86 ± 0.21 and 4.77 ± 0.15 mg dry extract/mL, respectively), which were approximately half the activity of acarbose (Table 3, IC_{50} 2.03 ± 0.07 dry extract/mL). When tested against rat intestinal α-glucosidase, IC_{50} values of olive extracts were slightly lower than those obtained with yeast α-glucosidase. Olive extracts were equivalent or superior to acarbose (Table 3) as inhibitors of rat intestinal α-glucosidase, which conflicts with the stronger inhibitory effect of acarbose on human α-glucosidase.

Most published studies on bioactive compounds for α-glucosidase inhibition utilize in vitro assays based on yeast α-glucosidase, as it is widely available as purified enzyme. α-Glucosidases can be found in yeast, fungi, bacteria, plants, archaea and animals but, despite carrying the same name, those enzymes have a wide range of structures and substrate specificity (Ong & Le 2015). Therefore, it is predictable that inhibitors would also act differently to each enzyme, and that is why we focused on the results obtained with human intestinal α-glucosidase. Inhibitory activities of olive extracts (IC_{50} values) on human α-glucosidase were approximately ten-fold (lower IC_{50} values) of yeast α-glucosidase, but this relationship was not true for acarbose. Although acarbose shows very strong inhibitory activity on human α-glucosidase, it is far less efficient when tested on yeast and rat intestinal α-glucosidases (Table 3), thus leading to a false impression that olive extracts would be as efficient as a medicinal drug prescribed for the management of postprandial glucose in T2D patients. IC50 values obtained with human (Caco-2) intestinal α-glucosidase were correlated with those obtained with yeast α-glucosidase, however absolute values were much lower for the human enzyme (Supplemental Figure 3). In addition, no correlation was found between IC_{50} values obtained with human and rat enzymes. Therefore, caution should be taken when comparing with other in vitro studies using non-human α-glucosidases, as also emphasised by other authors (Barber et al. 2021; Kan et al. 2021).

Mechanism of inhibition of α-glucosidase

To gain some insight on the mechanistic aspects of inhibition, we performed kinetic experiments using yeast α-glucosidase, as isolation of human α-glucosidase could not be done in time for this work. Extracts from Natural green Mission, Natural green Nevadillo, Natural green Mission + LAB, Natural ripe Kalamata, Natural green Conservolea and Castelvetrano green olives inhibited yeast α-glucosidase through a non-competitive mechanism, as indicated by Alpha values around (0.743 to 1.391, Table 4) and typical Lineweaver–Burk plots with increasing inhibitor concentration leading to higher y-intercepts (Supplemental Figure 4), and K_i values between 0.292 and 2.76 mg/mL (Table 4). Shinzuke Mission and California

Table 4 Kinetic parameters of the inhibitory effect of table olive extracts and acarbose on yeast α-glucosidase

	Main mechanism of inhibition	V_{max}	Alpha	K_i	K_m
		nM/min		*mg/mL*	*nM*
Shinzuke Mission	Uncompetitive	11.38 (11.56 ~ 7.172)	0.3444	17.44	79.56 (88.42 ~ 54.14)
Natural green Mission	Non-competitive	10.62 (9.586 ~ 7.384)	0.9044	2.758	78.93 (75.74 ~ 90.88)
Natural green Mission + LAB	Non-competitive	11.99 (11.26 ~ 5.141)	1.391	1.204	75.51 (80.90 ~ 123.5)
Natural green Nevadillo	Non-competitive	10.44 (10.23 ~ 1.483)	0.9336	0.3303	99.36 (108.3 ~ 99.12)
Castelvetrano green	Non-competitive	11.85 (10.86 ~ 1.264)	1.064	0.9862	87.43 (89.78 ~ 68.03)
Californian black	Uncompetitive	11.36 (11.04 ~ 6.899)	0.04754	55.34	77.22 (78.40 ~ 50.22)
Natural ripe Kalamata	Non-competitive	10.28 (10.29 ~ 1.012)	0.7431	0.2920	80.51 (61.84 ~ 138.8)
Natural green Conservolea	Non-competitive	12.00 (12.04 ~ 0.447)	1.016	2.304	63.55 (56.77 ~ 80.98)
Acarbose	Competitive	13.85 (13.48 ~ 14.15)	$\sim 10^{115}$	0.2579	39.16 (35.17 ~ 326.3)

Kinetic parameters were calculated by fitting data into the mixed inhibition model using GraphPad Prism. V_{max} and K_m without inhibitor obtained with the mixed model; values between parentheses are the range of V_{max} and Km obtained by non-linear regression at each inhibitor concentration

Alpha = 1 denotes non-competitive mechanism, where inhibitor affinity is equal for both free enzyme and enzyme–substrate complex (non-competitive mechanism); Alpha > 1 indicate preferential binding to the free enzyme (deviation towards competitive mechanism), with very large Alpha values being a feature of pure competitive mechanism. Alpha < 1 indicates inhibitor binding favourably to the enzyme–substrate complex, approaching an uncompetitive model

Black olives fitted better within an uncompetitive model, as evidenced by the low binding affinity towards the free enzyme (Alpha values 0.3444 and 0.0475, Table 4), larger K_i values (55.34 and 17.44 mg/mL, Table 4) and Lineweaver–Burk plots displaying constant slopes (Supplemental Figure 4). It is clear that the α-glucosidase inhibition by treated olives containing low concentration of bioactives are mechanistically different from the natural olives, and that both cases divert from the typical competitive mechanism featured by acarbose, with a very high Alpha value (approx. 10^{115}). Acarbose is a widely used oral hypoglycaemic drug used for the management of type 2 diabetes, and has been previously shown high binding affinity to both human and yeast α-glucosidase, inhibiting α-glucosidase by a competitive mechanism (Barber et al. 2021; Rosak & Mertes 2012; Van De Laar et al. 2005).

Although some olives showed K_i similar to that of Acarbose, it is important to emphasize that we performed mechanistic studies using yeast α-glucosidase. More accurate mechanistic studies should be done with isolated olive biochemicals against human α-glucosidases.

Each table olive weighs approximately 4–10 g, which gives approximately 14–140 mg extract per olive. Considering the ingestion of a portion size of five olives, and the postprandial small bowel water content of approximately 40 to 120 mL (Marciani et al. 2010), we reached an estimate that olive extract concentrations could range from 0.6 to 18 mg/mL in the small bowel following a meal. The lowest concentration is already within the range of α-glucosidase IC_{50} values of the olives in this study, indicating that glycaemic lowering effect is attainable with olive intake at usual amounts.

Conclusion

Processing deeply influenced the profile and concentration of phytochemicals in table olives, overcoming cultivar variations. Natural table olives retain more phytochemicals than treated olives and are stronger inhibitors of human α-glucosidase in vitro, exhibiting potential as functional food for the management of postprandial glycaemia. Although some extracts from table olives showed strong α-glucosidase inhibitory activity, their inhibitory strength was not correlated with the main olive secoiridoids. Further studies are needed to identify the active compounds.

Abbreviation

T2D Type 2 diabetes

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43014-024-00227-7.

Additional file 1: Supplemental Table 1. The concentration of various table olive extracts in yeast α-glucosidase inhibition kinetics. **Supplemental Figure 1.**UV-vis spectra of each peak of UFLC chromatograms of table olive extracts, in retention time order. Hydroxytyrosol, peak A, peak B, Tyrosol, peaks C and D. UV-vis spectra of each peak of UFLC chromatograms of table olive extracts, in retention time order. Peaks E to J. UV-vis spectra of each peak of UFLC chromatograms of table olive extracts, in retention time order. Peaks K and L, Verbascoside, peaks M to O. UV-vis spectra of each peak of UFLC chromatograms of table olive extracts, in retention time order. Peaks P to U. UV-vis spectra of each peak of UFLC chromatograms of table olive extracts, in retention time order. Peaks V to X.**Supplemental Figure 2.**Pearson correlation coefficients between UFLC chromatogram peak areas and pIC_{50} values on human α-glucosidase. Correlation coefficients and significance levels (when applicable) are shown on the labels of each bar.**Supplemental Figure 3.**Correlation of inhibitory effects (IC_{50}) of olive extracts and acarbose on yeast and rat intestinal α-glucosidase.

Acknowledgements
We thank the Shozu Olive Research Institute for providing access to the olive orchards, and supplying raw olives. Special acknowledgement to Takeyasu Kubota and Hideaki Shibata for sharing their knowledge on olive processing in Japan.

Authors' contributions
LY: conceptualization, methodology, formal analysis, visualisation, writing—review & editing, supervision. HS: writing—original draft, formal analysis, data curation. JI: Investigation, writing—original draft.

Funding
This work has been partly supported by the 'Initiative for Realizing Diversity in the Research Environment' from MEXT (Ministry of Education, Culture, Sports, Science and Technology of Japan), through the host institution.

Availability of data and materials
All data generated or analysed during this study are included in this published article [and its Supplementary information files].

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Graduate School of Agricultural Sciences, Kagawa University, 2393 Ikenobe, Miki-Cho, Kita-Gun, Kagawa 761-0795, Japan.

Received: 1 September 2023 Accepted: 2 January 2024
Published online: 05 July 2024

References

Agriculture Cooperative of Rovies. (2017). Processing methods of olives. Retrieved February 24, 2022, from http://roviesolives.gr/en/video.html

Anastassiades, M., Lehotay, S. J., Štajnbaher, D., & Schenck, F. J. (2003). Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. *Journal of AOAC International, 86*(2), 412–431. https://doi.org/10.1371/journal.pone.0029268

Assefa, S. T., Yang, E.-Y., Chae, S.-Y., Song, M., Lee, J., Cho, M.-C., & Jang, S. (2019). Alpha glucosidase inhibitory activities of plants with focus on common vegetables. *Plants, 9*(1), 2. https://doi.org/10.3390/plants9010002

Barber, E., Houghton, M. J., Williamson, G., Nilsson, A., & Hernandez-Hernandez, O. (2021). Flavonoids as human intestinal α-glucosidase inhibitors. *Foods, 10*(8), 1939. https://doi.org/10.3390/FOODS10081939

Blekas, G., Vassilakis, C., Harizanis, C., Tsimidou, M., & Boskou, D. G. (2002). Biophenols in table olives. *Journal of Agricultural and Food Chemistry, 50*(13), 3688–3692. https://doi.org/10.1021/jf0115138

Collado-González, J., Grosso, C., Valentão, P., Andrade, P. B., Ferreres, F., Durand, T., … Gil-Izquierdo, Á. (2017). Inhibition of α-glucosidase and α-amylase by Spanish extra virgin olive oils: The involvement of bioactive compounds other than oleuropein and hydroxytyrosol. *Food Chemistry*, *235*, 298–307. https://doi.org/10.1016/J.FOODCHEM.2017.04.171

Colmagro, S., Collins, G., & Sedgley, M. (2010). Processing Technology of the Table Olive. In *Horticultural Reviews* (pp. 235–260). https://doi.org/10.1002/9780470650783.ch5

Ernits, K., Kjeldsen, C., Persson, K., Grigor, E., Alamäe, T., & Visnapuu, T. (2021). Structural insight into a yeast maltase-the BaAG2 from blastobotrys adeninivorans with transglycosylating activity. *J. Fungi, 7*, 816. https://doi.org/10.3390/jof7100816

Fatmawati, S., Shimizu, K., & Kondo, R. (2011). Ganoderol B: a potent α-glucosidase inhibitor isolated from the fruiting body of Ganoderma lucidum. *Phytomedicine, 18*(12), 1053–1055. https://doi.org/10.1016/J.PHYMED.2011.03.011

Giampieri, F., Forbes-Hernandez, T. Y., Gasparrini, M., Alvarez-Suarez, J. M., Afrin, S., Bompadre, S., … Hong, Y. (2015). Strawberry as a health promoter: an evidence based review. *Food Funct.*, *6*(5), 1386–1398. https://doi.org/10.1039/C5FO00147A

Guasch-Ferré, M., Hruby, A., Salas-Salvadó, J., Martínez-González, M. A., Sun, Q., Willett, W. C., & Hu, F. B. (2015). Olive oil consumption and risk of type 2 diabetes in US women. *The American Journal of Clinical Nutrition, 102*(2), 479–486. https://doi.org/10.3945/AJCN.115.112029

Hadrich, F., Bouallagui, Z., Junkyu, H., Isoda, H., & Sayadi, S. (2015). The α-Glucosidase and α-Amylase Enzyme Inhibitory of Hydroxytyrosol and Oleuropein. *Journal of Oleo Science, 64*(8), 835–843. https://doi.org/10.5650/JOS.ESS15026

Johnson, R., Melliou, E., Zweigenbaum, J., & Mitchell, A. E. (2018). Quantitation of oleuropein and related phenolics in cured Spanish-style green, California-style black ripe, and greek-style natural fermentation olives. *Journal of Agricultural and Food Chemistry, 66*(9), 2121–2128. https://doi.org/10.1021/acs.jafc.7b06025

Kailis, S. G., & Harris, D. (2008). *Producing Table Olives*. Landlinks Press.

Kan, L., Capuano, E., Fogliano, V., Verkerk, R., Mes, J. J., Tomassen, M. M. M., & Oliviero, T. (2021). Inhibition of α-glucosidases by tea polyphenols in rat intestinal extract and Caco-2 cells grown on Transwell. *Food Chemistry, 361*, 130047. https://doi.org/10.1016/j.foodchem.2021.130047

Kim, Y. A., Keogh, J. B., & Clifton, P. M. (2016). Polyphenols and glycemic control. *Nutrients*. Multidisciplinary Digital Publishing Institute. https://doi.org/10.3390/nu8010017

Lanza, B. (2012). Nutritional and Sensory Quality of Table Olives. In *Olive Germplasm - The Olive Cultivation, Table Olive and Olive Oil Industry in Italy* (pp. 343–372). https://doi.org/10.5772/51723

Marciani, L., Cox, E. F., Hoad, C. L., Pritchard, S., Totman, J. J., Foley, S., … Spiller, R. C. (2010). Postprandial Changes in Small Bowel Water Content in Healthy Subjects and Patients With Irritable Bowel Syndrome. *Gastroenterology*, *138*(2), 469–477.e1. https://doi.org/10.1053/J.GASTRO.2009.10.055

Marsilio, V., Campestre, C., & Lanza, B. (2001). Phenolic compounds change during California-style ripe olive processing. *Food Chemistry, 74*(1), 55–60. https://doi.org/10.1016/S0308-8146(00)00338-1

Marsilio, V., Seghetti, L., Iannucci, E., Russi, F., Lanza, B., & Felicioni, M. (2005). Use of a lactic acid bacteria starter culture during green olive (Olea europaea L cv Ascolana tenera) processing. *Journal of the Science of Food and Agriculture, 85*(7), 1084–1090. https://doi.org/10.1002/JSFA.2066

Martín-Peláez, S., Fito, M., & Castaner, O. (2020). Mediterranean diet effects on type 2 diabetes prevention, disease progression, and related mechanisms. A review. *Nutrients, 12*(8), 1–15. https://doi.org/10.3390/NU12082236

Miyoshi, N., Pervin, M., Suzuki, T., Unno, K., Isemura, M., & Nakamura, Y. (2015). Green tea catechins for well-being and therapy: prospects and opportunities. *Botanics: Targets and Therapy, 5*, 85–96. https://doi.org/10.2147/BTAT.S91784

Mwakalukwa, R., Amen, Y., Nagata, M., & Shimizu, K. (2020). Postprandial hyperglycemia lowering effect of the isolated compounds from olive mill wastes - An inhibitory activity and kinetics studies on α-glucosidase and α-amylase enzymes. *ACS Omega, 5*(32), 20070–20079. https://doi.org/10.1021/ACSOMEGA.0C01622/SUPPL_FILE/AO0C01622_SI_001.PDF

Nair, S. S., Kavrekar, V., & Mishra, A. (2013). In vitro studies on alpha amylase and alpha glucosidase inhibitory activities of selected plant extracts. *European Journal of Experimental Biology, 3*(1), 128–132.

Nishioka, H., Mizuno, T., Iwahashi, H., & Horie, M. (2020). Changes in lactic acid bacteria and components of Awa-bancha by anaerobic fermentation. *Bioscience, Biotechnology and Biochemistry, 84*(9), 1921–1935. https://doi.org/10.1080/09168451.2020.1771677

Okai, M. (2014). *Ketteiban Sodatetetanosimu Ori-bunohon (The Definitive Guide to Growing and Enjoying Olives)*. (T. Abe & Hachigatusha, Eds.). Shufunotomo Co.,LTD.

Ong, Q., & Le, L. (2015). Comparative study on human **A**-glucosidase. *IFMBE Proceedings, 46*, 351–354. https://doi.org/10.1007/978-3-319-11776-8_86

Ozturk, A. (2014). Understanding the characteristics of oleuropein for table olive processing. *Journal of Food Processing & Technology, 05*(05), 1–6. https://doi.org/10.4172/2157-7110.1000328

Pyner, A., Nyambe-Silavwe, H., & Williamson, G. (2017). Inhibition of human and rat sucrase and maltase activities to assess antiglycemic potential: Optimization of the assay using acarbose and polyphenols. *Journal of Agricultural and Food Chemistry, 65*(39), 8643–8651. https://doi.org/10.1021/ACS.JAFC.7B03678/SUPPL_FILE/JF7B03678_SI_001.PDF

Ramírez, E., Brenes, M., García, P., Medina, E., & Romero, C. (2016). Oleuropein hydrolysis in natural green olives: Importance of the endogenous enzymes1. Ramírez, E., Brenes, M., García, P., Medina, E. & Romero, C. Oleuropein hydrolysis in natural green olives: Importance of the endogenous enzymes. Food Chem. 206, 204–209. Food Chemistry, 206, 204–209. https://doi.org/10.1016/j.foodchem.2016.03.061

Rincón-Llorente, B., De la Lama-Calvente, D., Fernández-Rodríguez, M. J., & Borja-Padilla, R. (2018). Table olive wastewater: Problem, treatments and future strategy. A review. *Frontiers in Microbiology, 9*(JUL), 1641. https://doi.org/10.3389/FMICB.2018.01641/BIBTEX

Rosak, C., & Mertes, G. (2012). Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy Critical evaluation of the role of acarbose in the treatment of diabetes: patient considerations. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*, 5–357. https://doi.org/10.2147/DMSO.S28340

Sarwar, N., Gao, P., Kondapally Seshasai, S. R., Gobin, R., Kaptoge, S., Di Angelantonio, E., … Wormser, D. (2010). Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. *The Lancet*, *375*(9733), 2215–2222. https://doi.org/10.1016/S0140-6736(10)60484-9

Sato, A., Shinozaki, N., & Tamura, H. (2014). Secoiridoid type of antiallergic substances in olive waste materials of three japanese varieties of olea europaea. *Journal of Agricultural and Food Chemistry, 62*(31), 7787–7795. https://doi.org/10.1021/jf502151b

Schnell, O., Weng, J., Sheu, W. H. H., Watada, H., Kalra, S., Soegondo, S., … Grzeszczak, W. (2016). Acarbose reduces body weight irrespective of glycemic control in patients with diabetes: results of a worldwide, non-interventional, observational study data pool. *Journal of Diabetes and Its Complications*, *30*(4), 628–637. https://doi.org/10.1016/J.JDIACOMP.2016.01.023

Sea-tan, S. (2017). Water extract from leaf and stem of White Mugwort inhibits enzyme activity of D -amylase and D -glucosidase. *Journal of Nutrition Asscociation of Thailand, 52*(June 2018), 16–22.

Shen, X., Saburi, W., Gai, Z., Kato, K., Ojima-Kato, T., Yu, J., … Yao, M. (2015). Structural analysis of the α-glucosidase HaG provides new insights into substrate specificity and catalytic mechanism. *Acta Crystallographica Section D: Biological Crystallography*, *71*, 1382–1391. https://doi.org/10.1107/S139900471500721X

Takahashi, M., Miyashita, M., Suzuki, K., Bae, S. R., Kim, H. K., Wakisaka, T., … Yasunaga, K. (2014). Acute ingestion of catechin-rich green tea improves postprandial glucose status and increases serum thioredoxin concentrations in postmenopausal women. *British Journal of Nutrition*, *112*(9), 1542–1550. https://doi.org/10.1017/S0007114514002530

Van De Laar, F. A., Lucassen, P. L., Akkermans, R. P., Van De Lisdonk, E. H., Rutten, G. E., & Van Weel, C. (2005). α-Glucosidase inhibitors for patients with type 2 diabetes: Results from a Cochrane systematic review and meta-analysis. *Diabetes Care*. American Diabetes Association. https://doi.org/10.2337/diacare.28.1.154

World Health Organization. (2020). *Diagnosis and management of type 2 diabetes (HEARTS-D)*. Geneva.

Publisher's Note