Talanta 97 (2012) 420–424

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Separation of olive proteins by capillary gel electrophoresis

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ARTICLE INFO

Article history:
Received 18 January 2012
Received in revised form
23 April 2012
Accepted 29 April 2012
Available online 7 May 2012

Keywords:
Capillary gel electrophoresis
Molecular mass
Olive
Protein profile

ABSTRACT

Olive proteins are not well known and there are still a lot of unknown information requiring further studies focused on the determination and characterization of these proteins. Despite the widely use of gel electrophoresis, this is the first time that capillary gel electrophoresis (CGE) is applied to separate proteins extracted from olive fruits. Seven common peaks were identified in the twenty olive varieties studied in this work. According to their migration times, these seven peaks could correspond to molecules with molecular masses of 11.0 ± 0.4, 13.9 ± 0.5, 16.3 ± 0.8, 22.1 ± 0.6, 30 ± 1, 48 ± 1, and 53 ± 2 kDa. All of the determined molecular masses could be attributed to proteins and four of them have been previously observed by SDS-PAGE. The electrophoretic profiles were also evaluated for their capability to differentiate olive varieties according to their presumed geographical origin. Results demonstrated that this method could successfully classify the studied olive varieties by its combination with multivariate chemometrics tools.

1. Introduction

Olives are composed of water (50%), oil (22%), carbohydrates (19.1%), cellulose (5.8%), proteins (1.6%), and minerals (ash) (1.5%) [1]. As it is known so far, olive proteins are distributed between the olive mesocarp and the olive stone (constituted by the seed and the endocarp), being mainly present in the olive stone [2,3]. Olive proteins present in the olive stone, most of them contained in the olive seed, belong to two main families: seed storage proteins (SSPs) and oleosins. Storage proteins are formed during seed development [4]. According to Alché [4], the most abundant storage proteins in the mature olive seed belong to the 11 S protein family, accounting for approximately 70% of the total seed proteins. A sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis showed that storage proteins in olive seed mainly consisted of two hydrogen-bonded subunits of 41 and 47.5 kDa [4–6]. Moreover, the precursor of 41 kDa generated three polypeptides of 21.5, 25.5, and 27.5 kDa when reduced, while the other precursor yielded two polypeptides of 20.5 and 30.0 kDa [4]. In addition to these storage proteins, proteins associated with fatty bodies called oleosins are hydrophobic proteins from oleaginous seeds stabilizing oil bodies. The oil bodies are composed of a core of triacylglycerols surrounded by a monolayer of phospholipids in which different proteins are inserted [7]. Oleosins are insoluble in water, but could be easily dissolved using a surfactant such as SDS, according to Huang et al. [8]. Ross et al. [9] found two different oleosins of 22 and 50 kDa in olive seeds. The 22 kDa protein was assigned to an oleosin located on the surface of the oil bodies. These authors [9] also analyzed the oil bodies from the olive mesocarp tissue not detecting any oleosin. However, the mesocarp contains proteins (1.3–1.8% of the dry weight of the olive fruit) that are still not well-known [1]. Indeed, Hidalgo et al. [10] reported that some proteins present in the oil bodies of the olive fruit mesocarp could pass to the oil during their extraction. Moreover, Zamora [1] found a polypeptide of 4.6 kDa by SDS-PAGE analysis regardless of the variety of the olive fruit or the maturity stage.

Up to date, SDS-PAGE is the most used technique to separate olive proteins and to determine their molecular masses [1,4,5,9–13]. Further refinement of the technique has been recently observed by the off-line analysis of the SDS-PAGE bands using MALDI-TOF MS and/or nanoLC-MS/MS [14,15]. Capillary electrophoresis (CE) presents a high potential for the separation, detection, and determination of biomolecules such as proteins. In this context, capillary gel electrophoresis (CGE) is an electrophoretic mode which uses a sieving matrix as buffer for the separation of high molecular weights compounds [16]. CGE presents the advantages of automation, on-column detection, and higher resolution than the traditional SDS-PAGE. Thus, the aim of this work was to use CGE as an alternative to SDS-PAGE for

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http://dx.doi.org/10.1016/j.talanta.2012.04.055

the separation of olive proteins and to study the potential of obtained protein profiles to differentiate olive varieties according to their presumed geographical origin.

2. Experimental section

2.1. Reagents and Materials

HPLC grade acetonitrile (ACN) and 2-mercaptoethanol were obtained from Scharlau Chemie (Barcelona, Spain). Hydrochloric acid, sodium hydroxide pellets, tris(hydroxymethyl)aminomethane (Tris), SDS, methanol, chloroform, and acetone were obtained from Merck (Darmstadt, Germany). All solutions were prepared with ultrapure water from a Milli-Q system (Millipore, Bredford, MA). ProteomeLab^{TM} SDS-MW Analysis Kit was purchased from Beckman (Beckman Coulter, Inc., Fullerton, CA). Olive samples from twenty different varieties were supplied by the Olive World Germplasm Bank of IFAPA (Junta de Andalucía, Córdoba, Spain). To avoid possible pedoclimatic influences, all varieties were ground in the same geographical zone, under the same climatic factors. Moreover, fruit samples were harvested at the same time and at the same stage of maturity, being the own variety the only difference among them. Table 1 shows the varieties, their origin and geographical extension and different denominations found in Spain, according to Rallo et al. [17].

2.2. Apparatus

Electropherograms were obtained using a HP^{3D}CE instrument (Agilent Technologies, Palo Alto, CA) equipped with an on-column DAD for UV detection and spectra collection. The experiments were performed in fused-silica capillaries (Polymicro Technologies Phoenix, Arizona, USA) of 50 μm id and an effective length of 23 cm (31.5 cm of total length). A replaceable gel from ProteomeLab^{TM} SDS-MW Analysis Kit from Beckman (Beckman Coulter, Inc., Fullerton, CA) designed for the separation of protein-SDS complexes was used to fill the capillary. A capillary conditioning was performed every six injections consisting of a 0.1 M sodium hydroxide rinse for 15 min, an acidic rinse (4 min) with 0.1 M HCl followed by Milli-Q water for 4 min, and a gel rinse for 15 min. The CGE selected conditions were: capillary temperature 25 °C, applied voltage −20 kV, and UV detection at 210, 254, and 280 nm with a bandwidth of 5 nm in all cases. Sample injection was performed electrokinetically at −5 kV for 20 s.

2.3. Sample preparation

Olive samples were prepared according to a previous procedure developed in our research group [18]. Briefly, the stone and pulp were homogenized separately in a domestic miller (Kenwood Ibérica, Barcelona, Spain). 2 g of the stone and pulp mixture was added to 20 mL of chloroform/methanol (2:1, v/v) and it was vortexed vigorously. A centrifugation (Heraeus Instrument, Hanau, Germany) at 1500 *g* for 15 min was carried out twice. After centrifugation, the pellets were removed and the proteins in the liquid phase were precipitated with 40 mL of cold acetone at −20 °C for 1 h. Precipitated proteins were separated by centrifugation (Multifuge 3 LR Heraeus, Buckinghamshire, England) at 10,000 *g* for 5 min. Proteins were solubilized in 0.5 mL of 100 mM Tris–HCl buffer (pH 9.0) containing 1% (m/v) SDS and filtered through 0.45 μm Titan filters (Rockwood, Tennessee) prior to their injection in the CE system.

2.4. Molecular masses determination

In order to determine the molecular masses corresponding to the electrophoretic peaks, a calibration curve was obtained using seven molecular size standards from Beckman (Beckman Coulter, Inc., Fullerton, CA): 10, 20, 35, 50, 100, 150, and 225 kDa. Within this size range, the logarithm of the protein molecular mass is

Table 1
Olive varieties studied indicating their country of origin, the most common zones for their cultivation in Spain and different Spanish denominations found (Rallo et al., 2005; http://www.oliveoilsource.com/).

Category[a]	Variety origin	Most common zones for olive variety cultivation in Spain	Other Spanish denominations
Mean Varieties	*'Arbequina' ESP*	Cataluña, Aragón, and less common in Andalucía	*Blanca*
	'Empeltre' ESP	Aragón and Baleares principally, Castellón, Tarragona, and Navarra	*Aragonesa, Común, de Aceite, Fina, Injerto, Macho, Mallorquina, Navarro, Negral, Payesa, Salseña, Terra Alta,* and *Zaragona*
	'Lechín Sevilla' ESP	Sevilla, Córdoba, Cádiz, and Málaga	*Lechín, Ecijano, Zorzaleño, Alameño, Cordobés, Lechino,* and *Manzanilla Serrana*
	'Morrut' ESP	Castellón and Tarragona	*Morruda, Montserratina, Regués, Rocha,* and *Roig*
	'Villalonga' ESP	Province of Valencia and north zone of Alicante	*Forna, Manzanet, Manzanilla, Sevillano*, and *Valenciana*
Secondary Varieties	*'Lucio' ESP*	Granada	*Lucio Gordo* and *Plateado*
Spread Varieties	*'Alameño de Cabra' ESP*	Córdoba, Granada, and Sevilla	*Alameño (H)*[b]
	'Azul' ESP	Alhama de Granada (Granada) and Huelma (Jaén)	–
	'Negrillo de Estepa' ESP	Estepa (Sevilla) and Campiña-Penibética (Córdoba)	*Negrillo*
Local Varieties	*'Bolvino' ESP*	Belchite (Zaragoza)	–
	'Caballo' ESP	Huelma (Jaén)	–
	'Carrasquillo' ESP	Montefrío (Granada)	*Negrete*
	'Negro de el Carpio' ESP	El Carpio (Córdoba)	*Negro*
	'Nevado Basto' ESP	Baena and Cabra (Córdoba)	–
	'Nevado Rizado' ESP	Cabra (Córdoba)	–
	'Torcío de Cabra' ESP	Cabra (Córdoba)	*Torcío*
Other Varieties	*'Belluti' TUR*	Turkey	–
	'Samsun Tuzlamalik' TUR	Turkey	–
	'Rosciola' ITA	Italy	–
	'Barnea' ISR	Israel	–

[a] Category established according to the cultivar expansion.
[b] H (homonymia): different varieties with the same name.