Food Chemistry 192 (2016) 965–971

Contents lists available at ScienceDirect

Food Chemistry

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

Lipid nutritional value of legumes: Evaluation of different extraction methods and determination of fatty acid composition

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ARTICLE INFO

Article history:
Received 10 April 2015
Received in revised form 20 July 2015
Accepted 21 July 2015
Available online 23 July 2015

Keywords:
Legumes
Lentils
Lipid extraction methods
Saturated fatty acids
Unsaturated fatty acids

ABSTRACT

This study sought to contribute to the assessment of the nutritional properties of legumes by determining the fatty acid (FA) composition of 29 legume samples after the evaluation of nine extraction methods. The Folch method and liquid–solid extraction with hexane/isopropanol or with hexane/acetone were investigated, as was the effect of previous hydration of samples. Soxhlet extractions were also evaluated with different solvent mixtures. Results on FA composition using the hexane/isopropanol extraction method were the same in terms of FA composition of the Folch method, but the extraction yield was only around 20–40% of that of the Folch method preceded by hydration. Some types of legumes showed particularly interesting values for the ratio of polyunsaturated fatty acids (PUFAs) *n*-6/*n*-3, such as lentils, with the value of 4.0, and Azuki beans, at 3.2. In lentils, the PUFAs% ranged from 42.0% to 57.4%, while in Azuki beans it was 57.5%.

1. Introduction

Legumes include lentils (*Lens culinaris* L.), beans (*Phaseolus vulgaris* L.), peas (*Pisum sativum* L.), chickpeas (*Cicer arietinum* L.), lupins (*Lupinus albus* spp.), fava beans (*Vicia faba* or *Faba vulgaris*), soy beans (*Glycine max*) and others. Cultivated for thousands of years (Messina, 1997), they have played an important role in the traditional diets of many regions throughout the world (Caprioli et al., 2010; Chung, Liu, Hoover, Warkentin, & Vandenberg, 2008). Among European countries, the highest legume consumption is observed around Mediterranean, with daily consumption between 8 and 23 g per person (Kalogeropoulos et al., 2010). It has been reported that inclusion of legumes in the daily diet has many beneficial effects in the control and prevention of chronic metabolic diseases such as diabetes mellitus and coronary heart disease (Sagratini et al., 2009). Moreover, cooked legumes are considered an excellent source of vegetable proteins, and are also rich in starch, dietary fiber, minerals, and vitamins (Ruiz et al., 1996). While many research groups have focused their studies on specific bioactive components such as phenolics (Duenas, Sun, Hernandez, Estrella, & Spranger, 2003) or cholesterol lowering soyasaponins (Sagratini et al., 2013; Vila-Donat et al., 2014), there are few studies concerning the lipophilic phytochemical contents or the fatty acid (FA) profile of legumes (Konopka, Czaplicki, & Rotkiewicz, 2006; Zhang et al., 2014). Zhang et al. (2014) analyzed the FA composition of 20 Canadian lentil cultivars after extraction of the lipid fraction with hexane/isopropanol (*i*-PrOH). Ryan, Galvin, O'Connor, Maguire, and O'Brien (2007) determined the FA profile of various types of foods, including such legumes as peas, beans and lentils, concluding that their FA profile could be favorable from a cardio-protective perspective. They also used hexane/*i*-PrOH as extraction solvents. In another study, Kalogeropoulos et al. (2010) used the Bligh and Dyer method (Bligh & Dyer, 1959) to extract lipids from several types of cooked legumes, rather than raw, in order to quantify FA content in the form in which the legumes are actually consumed in Mediterranean countries. The determination of the specific profile and content of FA in pulse foods is necessary to better understand the health benefits they provide. Thus, the aims of our work were (a) to compare different extraction methods for the analysis of FAs in legumes and (b) to apply the best extraction methodology for the evaluation of FA composition in 29 different pulse samples. Folch, Bligh and Dyer, and their modified procedures are the most appropriate methods for lipid extraction from most of the matrices (Bligh & Dyer, 1959; Dunstan, Volkman, & Barrett, 1993; Erickson, 1993; Ewald, Bremle, & Karlsson, 1998; Folch, Lees, & Stanley, 1957). However, the toxicity of chloroform ($CHCl_3$) and methanol (MeOH) has led researchers to evaluate possible substitutes, such

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http://dx.doi.org/10.1016/j.foodchem.2015.07.102
0308-8146/

as hexane and *i*-PrOH (Gunnlaugsdottir & Ackman, 1993; Hara & Radin, 1979; Molina Grima et al., 1994; Smedes, 1999; Undeland, Harrod, & Lingnert, 1998), especially when the extracts are intended for use as dietary supplements. Thus, we applied such conventional methods as those of Folch et al., and Soxhlet, but with some modifications, namely the previous hydration of the matrix and the combination of solvents, in order to improve the extraction yield and obtain a more reliable fatty acid profile. After the optimization, 29 legume samples were analyzed. To the best of our knowledge, to date, this is the first study presenting comprehensive FA composition data on so many types of legumes, determined after having evaluated the best extraction method.

2. Materials and methods

2.1. Chemicals and materials

HPLC grade MeOH, *i*-PrOH, dichloromethane (CH_2Cl_2), and $CHCl_3$ were purchased from Sigma–Aldrich (Milano, Italy), whereas HPLC grade acetic acid 99–100% was bought from J.T. Baker B.V. (Deventer, The Netherlands). Acetone and hexane solvents for residue analysis were supplied by Fluka-Riedel-deHaën (Milano, Italy). Sodium sulfate anhydrous, sodium chloride and sodium hydroxide were purchased from Panreac Quimica SA (Barcelona, Spain). Deionized water (>18 MΩ cm resistivity) was obtained from a Milli-Q SP Reagent Water System (Millipore, Bedford, MA, USA).

2.2. Legume samples

Twenty-nine raw legumes, including nine lentil samples (*L. culinaris*), nine bean samples (*P. vulgaris*), two pea samples (*P. sativum*), three chickpea samples (*C. arietinum*), two fava bean samples (broad bean, *V. faba*), one chickling sample (grass pea, *Lathyrus sativus*), one Azuki bean sample (*Vigna angularis*), one black eyed bean (*Vigna unguiculata*) and one soybean sample (*G. max*), were analyzed.

Variety and trade names of these legume materials investigated are listed in Table 1. The precise origin was known for most of the samples, whereas for some of them (samples 21 and 26), only the packing location was available. Legumes were bought from supermarkets in Camerino (Italy) or were kindly provided by a local seeds company (Fertitecnica Colfiorito) with the exception of samples 27–29, which were purchased from local grocery stores in Camerun.

2.3. Extraction procedures

Each extraction was performed in triplicate on a 5 g finely ground dry sample, obtained using a kitchen grinder (Jolly blender, Johnson, Elettrodomestici s.p.a., Italy). The extraction procedures were evaluated with the 'Lenticchia Colfiorito Alta Qualità' lentil sample.

2.3.1. Hexane/isopropanol extraction (Hexane/i-PrOH stirring)

The hexane/*i*-PrOH 3:2 (15 mL) solvent mixture was added to the sample and left under magnetic stirring at room temperature for 2 h (Ryan et al., 2007). The suspension was then filtered and the residue was washed twice with 10 mL of the solvent mixture. After the addition of 6.7% aqueous solution w/v of sodium sulfate anhydrous, the lipid extract was vigorously shaken for 30 s and centrifuged at 5000 rpm for 10 min. The upper liquid phase thus collected was evaporated with a rotary evaporator until dryness. The lipid extract was weighted and transmethylated.

2.3.2. Soxhlet extractions (Soxhlet hexane/acetone and Soxhlet hexane/CH_2Cl_2)

Extractions were performed using 150 mL of acetone/hexane (1:4) and CH_2Cl_2/hexane (1:4) as solvents (Manirakiza, Covaci, & Schepens, 2001). After 5 h, the extraction mixture was dried with a rotary evaporator, weighted and then transmethylated.

Table 1
Type, trade names, origin and botanical classification of the analyzed legumes.

Number	Type of legume	Trade Name	Origin	Botanical classification
1	Lentil	Lenticchie Fertitecnica Colfiorito Alta Qualità	Italy	*Lens culinaris*
2	Lentil	Lenticchie Terra e Sole	Italy	*Lens culinaris*
3	Lentil	Lenticchie Fertitecnica Colfiorito	Italy	*Lens culinaris*
4	Lentil	Lenticchie Azienda Agricola Monte Castello	Italy	*Lens culinaris*
5	Lentil	Lenticchie Casteluccio di Norcia	Italy	*Lens culinaris*
6	Lentil	Lenticchie degli Altipiani Umbri	Italy	*Lens culinaris*
7	Lentil	Lenticchie Colfiorito Qualità Oro	Italy	*Lens culinaris*
8	Lentil	Lenticchie nera di Sicilia Bio	Italy	*Lens culinaris*
9	Hulled red lentil	Lenticchie rosse decorticate Fertitecnica Colfiorito	Turkey	*Lens culinaris*
10	Chickling (grass pea)	Cicerchia Tenuta Mattioni	Italy	*Lathyrus sativus*
11	Pea bean	Piselli secchi Fertitecnica Colfiorito	Canada	*Pisum sativum*
12	Pea bean	Piselli verdi spezzati Bio	Italy	*Pisum sativum*
13	Chickpea	Ceci Terra e Sole	Italy	*Cicer arietinum*
14	Chickpea	Ceci Fertitecnica Colfiorito	Italy	*Cicer arietinum*
15	Chickpea	Ceci della Puglia Orti Italiani	Italy	*Cicer arietinum*
16	Soybean	Soia gialla Fertitecnica Colfiorito	Canada	*Glycine max*
17	Fava bean (broad bean)	Fave intere Simply	Italy	*Vicia faba*
18	Fava bean (broad bean)	Fave spezzate Fertitecnica Colfiorito	Egypt	*Vicia faba*
19	Navy bean	Fagioli tondini Fertitecnica Colfiorito	Canada	*Phaseolus vulgaris*
20	Cannellini bean	Fagioli cannellini Terra e Sole	Italy	*Phaseolus vulgaris*
21	Roman bean	Fagioli borlotti Simply	–	*Phaseolus vulgaris*
22	Butter bean	Fagioli corona Fertitecnica Colfiorito	Poland	*Phaseolus lunatus*
23	Black eyed bean	Fagioli occhio Fertitecnica Colfiorito	Peru	*Vigna unguiculata*
24	Black bean	Fagioli neri Fertitecnica Colfiorito	Mexico	*Phaseolus vulgaris*
25	Roman bean	Fagioli borlotti Terra e Sole	Italy	*Phaseolus vulgaris*
26	Azuki bean	Azuchi verdi Fertitecnica Colfiorito	–	*Vigna angularis*
27	Kidney bean	Fagioli rossi	Cameroon	*Phaseolus vulgaris*
28	Black bean	Fagioli neri	Cameroon	*Phaseolus vulgaris*
29	White bean	Fagioli bianchi	Cameroon	*Phaseolus vulgaris*