Eur Food Res Technol (2004) 219:251–253
DOI 10.1007/s00217-004-0945-y

ORIGINAL PAPER

José Luis Peñalvo · M. Conçeicão Castilho · M. Irene N. Silveira · M. Cruz Matallana · M. Esperanza Torija

Fatty acid profile of traditional soymilk

Received: 22 March 2004 / Revised: 28 April 2004 / Published online: 1 July 2004

Abstract Soymilk is a water extract of soybeans, closely resembling dairy milk in physical appearance and composition. Most fatty acids in soybean and its derivates are unsaturated, and therefore susceptible to oxidation. The aim of this study was to evaluate the possible effect of the thermal conditions during the elaboration process of soymilk on its fatty acid profile. For this, the fatty acid composition of soymilk, okara (soymilk residue), and soybean were studied by gas chromatography with flame ionization detection (GC-FID). No major differences in the fatty acid patterns were found.

Keywords Fatty acid profile · Thermal treatment · Okara · Soybean · Soymilk

Introduction

Soybeans contain approximately 18–24% of total lipids, including 90% of neutral lipids, 7% phospholipids, and 3% glycolipids. Fatty acids (FA) are distributed between 15% saturated and 80% unsaturated. The most abundant FA in soybeans is linoleic acid (C18:2 *n*-6) representing approximately half of the total content, although it also contains considerable levels of the other essential FA, i.e., linolenic acid (C18:3 *n*-3) [1, 2]. Humans are capable of converting linoleic acid to arachidonic acid (C20:4 *n*-6), and to a lesser extent, linolenic acid to eicosapentanoic (C20:5 *n*–3) and docosahexanoic (C22:6 *n*-3) acids. These highly unsaturated FA exert a number of physiological actions in humans, and their intake has been related to a lower incidence of certain chronic diseases such coronary heart disease [3, 4]

Traditional soymilk is a water extract of whole soybeans. Because of its optimal nutritional profile and resemblance to dairy milk, it can be used as an animal milk complement or substitute, in cases of lactose intolerance. Soybean FA distribution has been studied to some extent [2] but no investigations have been carried out on the effect that the elaboration conditions may exert on the final FA profile of soymilk. Traditional soymilk extraction implies a soaking step followed by a thermal treatment for 1.5 h [5]. These conditions may promote lipid auto-oxidation [6], and therefore the FA distribution may be altered from the original pattern in soybeans. Our work is aimed at obtaining the FA profile of soymilk and okara (solid residue after soymilk extraction) in order to observe the possible compositional changes and the PUFA/SFA ratio evolution during soymilk extraction.

J. L. Peñalvo (✉)
Institute for Preventive Medicine, Nutrition and Cancer,
Folkhälsan Research Center, Division of Clinical Chemistry,
University of Helsinki,
Biomedicum C315a, PO Box 63, 00014 Helsinki, Finland
e-mail: jose.penalvo@helsinki.fi
Tel.: +358-9-19125454
Fax: +358-9-19125452

M. C. Matallana · M. E. Torija
Dpto Nutrición y Bromatología II: Bromatología,
Facultad de Farmacia,
Universidad Complutense de Madrid,
Madrid, Spain

M. C. Castilho · M. I. N. Silveira
Laboratório de Bromatologia e Hidrologia,
Faculdade de Farmácia,
Universidade de Coimbra,
Coimbra, Portugal

Materials and Methods

Soybeans (*Glycine max* L. var. Tokyo) were kindly provided by the manufacturer. Impurities and foreign material were removed by hand, and the cleaned seeds were preserved at constant temperature and humidity until their use. The elaboration process of traditional soymilk was as follows: 20 g of soybeans were thoroughly washed with tap water, drained, and finally soaked overnight at room temperature. After removal of the water, the wet beans were crushed, and the resultant paste was submerged in water to be boiled at ~90 °C for 1.5 h. The boiled product was then cooled at room temperature and filtered by 0.45 mm mesh, separating the soymilk and the residue i.e. okara.

For lipid extraction, approximately 5 g of ground soybean or okara, or 20 mL of soymilk were extracted on a mechanical shaker at 420 min^{-1} for 30 min with 20 mL of chloroform/methanol (2:1)

mixture acidified with 1 mL of glacial acetic acid. After centrifugation (380×*g* for 15 min), the supernatant was separated, and the residue was extracted twice with 20 mL of the same extraction mixture. The combined supernatants were treated with 60 mL of 3% potassium chloride and gently shaken. After separation of the phases, the organic layer was collected in a clean, dry, and preweighed round bottom vacuum flask, and evaporated to dryness in rotavapor. Fatty acid methyl esters (FAMEs) were prepared from the lipid extract according to official methods [7]: 1 g of fat was dissolved in 2 mL of *n*-heptane in a test tube with 1 mL of 2 M sodium hydroxide in dry methanol and the mixture shaken vigorously in vortex. After 5 min at room temperature the mixture was centrifuged at 380x*g* for 2 min. The supernatant layer was decanted and an aliquot (0.3 μL) was directly injected into the gas-chromatograph. All reagents used in this sample pre-treatment were pro-analysis (Carlo Erba, Milano, Italy; Merck, Darmstadt, Germany). Analyses were carried out in a Carlo Erba GC 6000 Vega Serie II, equipped with a split/splitless injector and a EL-580 flame ionization detector. A fused-silica capillary column SP 2380 (60m×0.25 mm×0.20 μm film thickness) with 90% biscyanopropyl/10% cyanopropylphenyl stationary phase (Supelco, Bellefonte, US) was used. Injector and detector temperatures were 240 °C and 250 °C, respectively. Oven program temperature was 175 °C for 20 min, raised to 220 °C at a rate of 5 °C/min and held at 220 °C for 10 min. FAMEs were identified by comparing their retention time with those of reference standards (Sigma, St. Louis, US). The FA profile of the samples was calculated as the area percentage of the major FA.

Results and discussion

Total lipid content was 23% for soybeans, 4.4% for okara, and 1.4% for soymilk, for corresponding moisture values of 7.3%, 75%, and 95%, respectively (results not shown in table). The FA profiles of soybean, soymilk, and okara are presented in Table 1. In soybean the major FA is linoleic acid (58%), followed by oleic acid (21%), palmitic (9.6%), α-linolenic (7.8%), and steraric acid (3.4%). This pattern was similarly repeated both in soymilk and okara. Other FA, like myristic, palmitoleic, margaric, arachidic, behenic, and lignoceric acids appeared at much lower levels in all three different matrices. The ratio *n*-6/*n*-3 is normally used to assess the balance between essential FA in the diet [3]. This value is, in our case, approximately 7.2 in the three matrices, which led us to conclude that the essential fatty acids remain unmodified during the thermal treatment. Furthermore, the ratio agrees with the recommended limits proposed by the FAO [8]. In relation to the PUFA/SFA ratio, soybeans are characterized by a high content (65%) of PUFA and a lower content of saturated fatty acids (SFA) (14%). Both soymilk and okara maintain the proportions of the initial soybean with 63% and 62% of PUFA, respectively. The mean PUFA/SFA ratio for soybeans was 4.69 and for soymilk 3.97, indicating that %SFA is slightly increased during the elaboration process, mainly due to higher percentages of palmitic and stearic acids. *Trans*-FA were found to represent 0.09% of the total FA composition in soybeans. This value is the same for okara but lower percentages were found in soymilk, suggesting that the thermal treatment during the elaboration of soymilk does not promote *trans*-FA formation. The equivalence between the FA profile of soymilk and okara, and the initial profile of the soybean imply that this fraction is not altered during the different steps that the elaboration of traditional soymilk includes. Only *cis*-vaccenic acid (C18:1 *n*–7), a positional isomer of oleic acid (C18:1 *n*–9), appears in soymilk and okara at low percentage (1.5%). It should be notice that *cis*-vaccenic acid was not present in the original soybean, and therefore its appearance during the elaboration of soymilk may be due to the thermal treatment. It has been reported that thermal treatment of fats may promote the formation of *trans*-isomers of essential fatty acids [9], and that the intake of those can be positively correlated with higher levels of cholesterol and cardiovascular disease [10]. In our study, *trans*- isomer content was very low in all cases and the levels found do not represent any significant health risk [11].

Table 1 Fatty acid profile of soybean, okara, and soymilk (% of total fatty acids)[a]

Fatty acid	Soybean	Okara	Soymilk
C14:0	0.06	0.08	0.07
C15:0	0.01	nd	0.02
C15:1	0.01	nd	nd
C16:0	9.64	10.7	10.7
C16:1 (*n*–7)	0.07	0.07	0.05
C17:0	0.08	0.09	0.07
C17:1	0.04	0.06	0.04
C18:0	3.46	3.56	3.79
C18:1 t	0.02	nd	nd
C18:1 *(n–9)*	21.1	19.6	20.4
C18:1 *(n–7)*	nd	1.49	1.56
C18:2 t	0.04	0.07	0.03
C18:2 (*n*–6)	56.7	55.1	54.8
C20:0	0.28	0.32	0.28
C18:3 t	0.01	nd	nd
C18:3 (*n*–3)	7.80	7.67	7.53
C22:0	0.33	0.36	0.39
C24:0	0.09	0.10	0.09
% SFA	13.8	15.2	15.3
% MUFA	21.2	21.2	22.1
% PUFA	64.6	62.8	62.4
PUFA/SFA	4.66	4.13	4.06
n-6/*n*-3	7.27	7.18	7.28
% Trans	0.07	0.07	0.03

[a] Mean % abundance (CV<1 in all cases) of triplicate analyses
nd, not detected

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