Production of flavonoid and lignan aglycones from flaxseed and soy extracts by Bifidobacterium strains

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Complete List of Authors:	Peiroten, Angela; Instituto Nacional de Investigacion y Tecnologia Agraria y Alimentaria, Food Technology Álvarez, Inmaculada; ICTAN, USTA Landete, José Maria; Instituto Nacional de Investigacion y Tecnologia Agraria y Alimentaria, Food Technology
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1 **Production of flavonoid and lignan aglycones from flaxseed and soy extracts by**
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6 *Bifidobacterium* strains

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11 4 Ángela Peirotén¹, Inmaculada Álvarez², José M^a Landete^{1*}

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16 6 ¹Departamento de Tecnología de Alimentos, Instituto Nacional de Investigación y
17 7 Tecnología Agraria y Alimentaria (INIA). Carretera de La Coruña Km 7.5, 28040.
18 8 Madrid (Spain)
19
20 9 ²Unidad de Servicio de Técnicas Analíticas, Instrumentales y Microbiología (USTA),
21 10 Instituto de Ciencia y Tecnología de los Alimentos y Nutrición (ICTAN-CSIC), José
22 11 Antonio Novais, 10, 28040 Madrid, (Spain)

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29 14 *Address correspondence to José M^a Landete, landete.josem@inia.es

Abstract

Flaxseed and soybean have are an important source of phytochemical compounds, mainly, lignans and isoflavones, but also of flavones, flavanones and flavonols. These compounds appear mainly glycosylated in plant food, and must be converted into aglycones to increase their bioavailability. In this work we analyse the ability of *Bifidobacterium* strains (n = 25) to produce lignan and flavonoids aglycones from flaxseed and soybean extracts. Most of the *Bifidobacterium* strains increased the concentrations of secoisolariciresinol, daidzein, genistein, naringenin, eriodictiol, luteolin and apigenin. Moreover, *Bifidobacterium pseudocatenulatum* and *Bifidobacterium breve* strains showed high production of herbacetin, increased the kaempferol concentration and produced quercetin and quercetagetin. *B. pseudocatenulatum* INIA P815 and *B. breve* INIA P367 produced 32.37 ± 2.81 and 28.64 ± 3.36 mg respectively of herbacetin/g of lignan extract. *Bifidobacterium* strains transformed the glycosides of a wide range of flavonoids into their aglycones, increasing the antioxidant activity and improving their bioavailability.

Keywords: herbacetin; quercetagetin; quercetin; naringenin; kaempferol; *Bifidobacterium*; soy; flaxseed.

Introduction

Flavonoids and lignans are phenolic compounds present in many plant foods. Flavonoids are a diverse group of phytonutrients that encompasses several groups: anthocyanidins, flavanols, flavones, flavonols, flavanones and isoflavones. Although all these compounds are minor components of food, each type of flavonoids and lignans carries its own distinct set of actions and benefits for human health. Many studies have suggested the protective role of flavonoids in cardiovascular disease due to their antioxidant properties, which could also have influence in other diseases as cancer and neurodegenerative diseases (Airoldi, La Ferla, Orazio, Ciaramelli, & Palmioli, 2018; Zamora-Ros, Touillaud, Rothwell, Romieu, & Scalbert, 2014). Moreover, isoflavones and lignans are classified as phytoestrogens, compounds with structural similarity to estrogen and a weak affinity for the estrogen receptor (Vaya & Tamir, 2004). Phytoestrogen intake has been associated with the amelioration of menopausal symptoms and with decreased risks of breast and prostate cancer, cardiovascular disease and diabetes (Rietjens, Louisse, & Beekmann, 2017). Flavonoids and lignans appear in plant foods in the form of O- or C-glycosides that are not absorbed by the intestinal epithelium and must be firstly transformed into aglycones (Landete, Arques, Medina, Gaya, de Las Rivas, & Muñoz, 2016). The deglycosylation is carried out in our organism by the intestinal microbiota, which produce the aglycones and can further transform them into other bioactive compounds. The aglycones show higher bioavailability and antioxidant and estrogenic/antiestrogenic activities and thus it has been suggested that the beneficial effects of polyphenols could be exerted by their aglycones (Peirotén, Bravo, & Landete, 2019a).

61 Among the food products rich on phenolic compounds, flaxseed and soybean have been
62 studied for being particularly rich in lignans and isoflavones respectively. Flaxseed
63 (*Linum usitatissimum* L.), one of the oldest cultivated crops, continues to be widely
64 grown for oil, fibre and food (Oomah, 2001). Flax or flaxseed oil are used as ingredients
65 in baked foods, dairy products, milk, juices, dry pasta and meat products (Goyal,
66 Sharma, Upadhyay, Gill, & Sihag, 2014). There is an increasing interest in the health
67 effect of flaxseed and flax oil consumption due to its high content in biologically active
68 components, such as alpha-linolenic acid and lignans. Soybean (*Glycine max*) is a
69 legume widely cultivated for its multiple uses in food, feed and other industries
70 (McClure, Cocuron, Osmark, McHale, & Alonso, 2017). In addition, soy is of great
71 interest for human health due to its high content in isoflavones. Both soybean and
72 flaxseed contain also other groups of phenolic compounds, including several types of
73 flavonoids (Herchi, Arraez-Roman, Trabelsi, Bouali, Boukhchina, Kallel, *et al.*, 2014;
74 Landete, Hernandez, Robredo, Duenas, de las Rivas, Estrella, *et al.*, 2015).

75 In previous works we described how most *Bifidobacterium* strains were able to produce
76 the lignan aglycone secoisolariciresinol (SECO) from secoisolariciresinol diglucoside
77 (SDG) (Peirotén, Gaya, Álvarez, Bravo, & Landete, 2019b), which is the main lignan in
78 flaxseed (Toure & Xu, 2010). *Bifidobacterium* strains are also able to produce the
79 isoflavone aglycones daidzein and genistein (Gaya, Peirotén, Medina, & Landete,
80 2016b). The ability of bifidobacteria to deglycosilate a wide range of substrates seem to
81 be linked to a variety of β -glycosidases encoded in their genome (Guadamuro, Flórez,
82 Alegría, Vázquez, & Mayo, 2017). In this work, we explored if the ability of
83 *Bifidobacterium* strains to deglycosilate lignans and isoflavones also applies to other
84 flavonoids, such as flavanones, flavones, flavanols and flavonols. We chose a broth
85 enriched with flaxseed and soy extracts as a model of a food rich in a diversity of

phenolic compounds. A selection of 25 *Bifidobacterium* strains belonging to ten different species was chosen for the fermentations, after which the content in specific aglycones, the antioxidant activity and the total phenolic compounds were determined.

Material and methods

Bacterial growth conditions

The 25 *Bifidobacterium* strains tested in this study (Table 1) were selected from the INIA culture collection based on their biotechnological and probiotic properties. The *Bifidobacterium* were cultivated under strict anaerobic conditions at 37 °C in RCM broth (BD, Le Pont de Claix, France) and subcultured twice before using them in the fermentation experiments.

Fermentation of flaxseed and soy extracts by *Bifidobacterium* strains

Flaxseed extract (LinumLife™ EXTRA) and soy extract (SoyLifes EXTRA) were purchased from Frutarom Netherlands BV (Veenendaal, The Netherlands) and initially dissolve in DMSO (Sigma Aldrich, Poole, UK). BHI medium (Biolife, Milan, Italy) containing 0.5 g/L of L-cysteine was supplemented with flax seed extracts (2 g/L) and soy extracts (4 g/L) and the pH was adjusted to 7.4 with NaOH. Ten mL of these

enriched media was inoculated with the *Bifidobacterium* strains. The residual amount of DMSO present in the media was checked to not interfere with bacterial growth. The inoculated broths were incubated in sealed jars under anaerobic conditions using AnaeroGen sachets (Oxoid Ltd., Basingstoke, United Kingdom) at 37 °C for 5 days. Non-inoculated broths containing flaxseed and soy extracts were used as controls. The experiments were carried out in triplicate.

Extraction of flavonoids and lignans

After the incubation of the broths containing soy and flaxseed extracts, the extraction of flavonoids and lignans was performed twice with 2 mL of diethyl ether, and twice with 2 mL of ethyl acetate according to Gaya, Arqués, Medina, Álvarez, and Landete (2016a). The solvents were evaporated at room temperature under an N₂ stream, and the residue was dissolved in 300mL methanol/water (50:50, v/v). The extracts were filtered through a 0.22 mm cellulose acetate filter (Millipore, Madrid, Spain) before transferring them to HPLC vials and storing them at -20°C.

Flavonoids and lignans analysis by HPLC-ESI/MS and HPLC-ESI/MS/MS.

Flavonoids and lignans were analysed by HPLC-ESI/MS and HPLC-ESI/MS/MS as previously described (Gaya, *et al.*, 2016a; Gaya, Peirotén, Álvarez, Medina, & Landete, 2018). Briefly, separation of phenolic compounds was achieved on a reverse phase Nova-Pak C18 column (300 x 3.9 mm, 4 µm) (Waters, Barcelona, Spain). A volume of

25 μ L was injected and elution was performed with a gradient consisting of solvent A (water/acetic acid, 98:2 v/v), solvent B (water/acetonitrile/acetic acid, 78:20:2 v/v/v) and solvent C (acetonitrile) at a flow rate of 1 mL/min. Analyses were carried out using an Agilent 1200 series LC, comprised of quaternary pump with integrated degasser, thermostated autosampler, thermostated column compartment and diode array detector, coupled with an Agilent 6530 Accurate-Mass Quadrupole Time of Flight (Q-TOF) LC/MS with ESI-Jet Stream Technology (Agilent Technologies, Waldbroon, Germany). The Q-TOF acquisition method was 4GHz, mass range low 1700 m/z, negative polarity, drying gas 10 l/min and 350°C, sheath gas 11 l/min and 350°C, nebulizer 45 psi, capillary voltage 3500V, nozzle voltage 0V and fragmentor voltage 120V. For the identification and characterization of flavonoids MS/MS experiments were performed with 20V collision energy. Data acquisition (version B.05.01) and Qualitative Analysis (version B.07.00) of MassHunter Workstation Software were used (Agilent Technologies).

Quantification was done by means of external standard calibration curves, and the data expressed as mg of compound per litre of fermentation media. The different concentration of standard compounds were added to BHI medium and subsequently extracted and analysed as above mentioned. SECO, naringenin, eriodyctiol, hesperetin, luteolin, apigenin, vitexin, catechin, epicatechin, kaempferol, kaempferol-3-O-rutinoside, quercetin, isoquercetin, rutin, taxifolin and mirecetin, were purchased from Sigma-Aldrich, whereas herbacetin and quercetagetin were purchased from Extrasynthese (Genay, France) and daidzein, daidzin, genistein and genistin were purchased from LC Laboratories (Woburn, MA).

Determination of antioxidant activity and phenolic compounds

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Antioxidant activity was determined in triplicate by the ferric reducing antioxidant power (FRAP) method according to del Olmo, Picón, and Núñez (2018). An aliquot of 20 µl of supernatants were incubated at 37 ° C for 60 min with 100 µl of Milli-Q water and 900 µl of the FRAP reagent containing 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ, Sigma-Aldrich) and FeCl₃ 6 H₂O. The absorbance was read at 595 nm to monitor Fe³⁺ reduction and the formation of a colored TPTZ-Fe²⁺ complex. A standard curve was obtained using Trolox (Sigma-Aldrich) to allow quantification.

Phenolic compounds were determined in triplicate by the Folin-Ciocalteu colorimetric method adapted from Ferraces-Casais, Lage-Yusty, Rodríguez-Bernaldo de Quirós, and López-Hernández (2012). The fermented broths (100 µL) were mixed with a 6% Na₂CO₃ aqueous solution (1 mL) and the Folin-Ciocalteu reagent (100 µL). The mixture was incubated for 1 h at room temperature and the absorbance was read at 740 nm. Quantification was carried out using a gallic acid (Sigma Aldrich) standard curve.

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172 Results

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174 SECO production by *Bifidobacterium* strains

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SECO was detected in all the fermented broths, in levels higher than the control for the majority of the *Bifidobacterium* strains. The three *Bifidobacterium pseudocatenulatum* strains and two of the *Bifidobacterium breve* strains (INIA P367, INIA P776) produced the highest SECO concentrations (Table 2). These five strains produced concentrations of SECO higher than 100 mg/L, equivalent to 50 mg per gram of flaxseed extracts.

Isoflavone aglycones production by *Bifidobacterium* strains

The majority of *Bifidobacterium* strains analysed were able to convert the daidzin and genistin to their aglycones daidzein and genistein (Table 2). However, large differences in aglycone production were observed between strains. The higher levels of combined daidzein and genistein production were found in the three *B. breve* strains. *B. pseudocatenulatum* strains also showed high concentrations of the aglycones, and *B. pseudocatenulatum* INIA P846, and *B. pseudocatenulatum* INIA P946 also showed the complete deglycosylation of genistin (data not shown). Moreover, the two *Bifidobacterium dentium* strains, *Bifidobacterium animalis* INIA P913 and *Bifidobacterium bifidum* INIA P466 also produced high levels of daidzein and genistein. Daidzein concentrations higher than 150 mg /L, equivalent to 37.5 mg per gram of soy extract, were produced by the three *B. breve* strains, *B. pseudocatenulatum* INIA P846 and *B. pseudocatenulatum* INIA P946. While genistein concentrations higher than 200 mg/L, equivalent to 50 mg per gram of soy extract, were produced by the three *B. breve* strains and *Bifidobacterium catenulatum* INIA P825 (Table 2).

Flavanone aglycones produced by *Bifidobacterium* strains

An increase in the naringenin concentration was observed as a result of the metabolism of most of the *Bifidobacterium* strains (Table 4) (Figure S1). The *B. breve*, *B. pseudocatenulatum* and *B. catenulatum* strains, together with *B. dentium* INIA P886 and

205 *B. animalis* INIA P913, showed the highest levels, with a 5 fold increase over the
206 control.

207 Eriodictyol showed a slight increase compared to control as a result of the microbial
208 metabolism of most of the *Bifidobacterium* (Figure S1). On its part, hesperetin was not
209 detected either in the controls or as a product of the microbial metabolism (Table 4).

211 **Flavone aglycones produced by *Bifidobacterium* strains**

213 Luteolin was found in the all samples analysed (Figure S1), showing some
214 bifidobacterial strains small increases compared to control (Table 3). Interestingly,
215 *Bifidobacterium infantis* INIA P728 degraded most of the luteolin present in the broth.
216 Apigenin, not detected in the control broth, appeared as a consequence of the
217 metabolism of most of the *Bifidobacterium* strains (Table 3) (Figure S1). *B. breve* INIA
218 P12, *B. breve* INIA P776, *B. longum* INIA P750, *B. pseudocatenulatum* INIA P846 and
219 *B. pseudocatenulatum* INIA P946 were the strains with the highest apigenin production.
220 Vitexin, an apigenin flavone glucoside was detected in all the samples (Table 4), with
221 no apparent variation due to the microbial metabolism (data not shown).

223 **Flavanol aglycones**

225 Catechin and epicatechin were not detected either in the control or as a result of the
226 fermentation by the *Bifidobacterium* strains (Table 4).

228 **Flavonol aglycones produced by *Bifidobacterium* strains**

230 The flavonol aglycone found in higher concentrations was herbacetin (Table 3). It was
231 not present in the control and was detected in 15 of the *Bifidobacterium* strains. The
232 highest levels of herbacetin were produced by the three *B. breve* strains, *B.*
233 *pseudocatenulatum* INIA P815 and *B. pseudocatenulatum* INIA P946. Two compounds
234 present in the control sample were tentatively identified as glycosides of herbacetin
235 (Table 4). Herbacetin diglucoside concentrations decreased in those fermented broths in
236 which herbacetin was detected, and was totally consumed by *B. pseudocatenulatum*
237 INIA P815, the strain showing the highest herbacetin concentration.

238 A compound with equal molecular mass to that of herbacetin was detected and
239 identified as quercetin. Herbacetin and quercetin were identified by means of
240 comparison with the standard compounds (Figure 1A) and were confirmed by MS/ MS
241 (Figure 1B).

242 Quercetin was detected in low levels in four of the broths fermented by bifidobacteria,
243 corresponding to *B. breve* INIA P367, *B. breve* INIA P776, *B. pseudocatenulatum* INIA
244 P815 and *B. pseudocatenulatum* INIA P946 (Table 3). Of the two quercetin glycosides
245 studies, rutin was not detected in any sample while isoquercetin, was identified in
246 almost all of them. Isoquercetin was completely metabolized by *B. pseudocatenulatum*
247 INIA P815, and its levels decreased when *B. animalis* INIA P490, the two *B. dentium*,
248 the three *B. breve* and the remaining *B. pseudocatenulatum* strains fermented the broths.
249 Nevertheless, quercetin, the expected result of isoquercetin deglycosilation, was
250 detected only in some of those strains.

251 Similarly to quercetin, the aglycone quercetagenin (Figure 2) was not found in the
252 control and only in a few of the bifidobacteria-fermented broths, namely in *B.*
253 *pseudocatenulatum* INIA P946, *B. pseudocatenulatum* INIA P815, the three *B. breve*

strains and *B. dentium* INIA P886 (Table 3). Finally, the other flavonol aglycones tested, mirecitin and taxifolin, were not detected in any sample. Kaempferol was found in low concentrations in control samples and not all the *Bifidobacterium* strains were able to increase its levels (Table 3) (Figure 1S). The three *B. breve* strains, *B. pseudocatenulatum* INIA P815, *B. pseudocatenulatum* INIA P946 and *B. dentium* INIA P886 were the strains that produced the highest concentration of kaempferol, with a tenfold increase over the control. The glycoside kaempferol-3-O-rutinoside was not found in any of the samples.

Antioxidant activity and phenolic compounds

A small increase in antioxidant activity was observed for all the *Bifidobacterium* strains compared with the control broth with flaxseed and soy extracts. The highest increases were found in the broths fermented by *B. breve* INIA P367, *B. breve* INIA P776, *B. dentium* INIA P886 and *B. pseudocatenulatum* INIA P946 (Table 5).

The determination of phenolic compounds also showed moderate increments in the broths fermented with *Bifidobacterium* strains, although not all of them were superior to control. *B. breve* INIA P367 and *B. catenulatum* INIA P825 resulted in the highest reads for phenolic compounds.

Discussion

For years, soy and flaxseed have received attention because of their phytoestrogen content of isoflavones and lignans respectively. As seen in this and other works, both foods contain also other flavonoids (Correa, Li, Aldini, Carini, Oliver Chen, Chun, *et al.*, 2010; Herchi, *et al.*, 2014; Landete, *et al.*, 2015). All these compounds are present in plant food as glycosides and must be metabolized into aglycones to increase their bioavailability. Given their estrogenic nature, many studies have addressed the effect of lignan and isoflavones, such as SECO, daidzein and genistein, in menopausal symptoms and the prevention of breast cancer. Moreover, their effect in the lowering of risk of cardiovascular disease, metabolic syndrome and other cancers has been also described (Peirotén, *et al.*, 2019a; Rietjens, *et al.*, 2017; Sirotkin & Harrath, 2014). The effect of other flavonoids, such as flavanones, flavones and flavonols has been less studied. Quercetin, kaempferol, naringenin and eriodictyol exhibit antioxidant and anti-inflammatory effects, which confers them potential beneficial effects in preventing different inflammation-related diseases (Devi, Malar, Nabavi, Sureda, Xiao, Nabavi, *et al.*, 2015; Egert, Bosy-Westphal, Seiberl, Kürbitz, Settler, Plachta-Danielzik, *et al.*, 2009; Lou, Jing, Ren, Wei, & Zhang, 2012; Narváez-Mastache, Novillo, & Delgado, 2008; Venkateswara, Kiran, Rohini, & Bhagyasree, 2017). Herbacetin has been described to have anti-hyperglycaemic and anti-hyperlipidemic properties (Veeramani, Alsaif, & Al-Numair, 2018). Quercetagenin exhibited strong in vitro antioxidant, anti-diabetic and antilipidemic activities, having potential application for the treatment of obesity and diabetes (Wang, Xu, Chen, Tai, Liu, & Gao, 2016).

In previous works, *Bifidobacterium* strains have shown the ability to deglycoside SGD, the main lignan in flaxseed, into SECO (Peirotén, *et al.*, 2019b), and to transform the isoflavones daidzin and genistin into their aglycones daidzein and genistein (Gaya, *et al.*, 2016b). In agreement with that, the fermentation of a mixture of flaxseed and soy

extracts by the 25 *Bifidobacterium* strains tested resulted in the production of the aglycones SECO, daidzein and genistein.

Alongside isoflavones and lignans, the flaxseed and soy extracts contain a series of other flavonoid glycosides, which can be deglycosilated by the appropriate bacterial strains. Herbacetin diglucoside is part of the lignan macromolecule found in flaxseed (Struijs, Vincken, Verhoef, van Oostveen-van Casteren, Voragen, & Gruppen, 2007). Accordingly, this compound was found in the flaxseed and soy mixture. Several *B. breve* and *B. pseudocatenulatum* strains were able to produce herbacetin as a result of the deglycosilation of herbacetin diglucoside, in a range of concentrations between 21 and 33 mg of herbacetin per gram of flaxseed extract. Moreover, the flavonol aglycones quercetin and quercetagenin were produced mainly by *B. breve* and *B. pseudocatenulatum* strains. A possible explanation could be a common enzyme responsible for the deglycosilation of the glycosides of the three flavonols. This is, to our knowledge, the first time that the bacterial production of herbacetin and quercetagenin is described.

The production of quercetin by bacteria commonly found in food, as lactic acid bacteria and bifidobacteria, has been described in two *Enterococcus* strains (Schneider, Schwietz, Collins, & Blaut, 1999; Shin, Moon, Shin, Li, Lee, Kim, *et al.*, 2016). In our work, just two *B. breve* and two *B. pseudocatenulatum* were able to produce quercetin, although a decrease on the glycoside isoquercetin was observed also for other *Bifidobacterium* strains. Interestingly, a collection of *Bifidobacterium* strains tested for rutin deglycosilation showed that any of the strains was able to produce quercetin, not even the *B. pseudocatenulatum* strains that were able to deglycosilate hesperidin into hesperetin (Amaretti, Raimondi, Leonardi, Quartieri, & Rossi, 2015).

The simultaneous production of quercetin and kaempferol was carried out by *Lactobacillus plantarum* SDL 1413 from flavonoid glycosides (Lee, Oh, & Jeong,

2015). Moreover, *Bifidobacterium* strains have been described as capable of transforming kaempferol glycoside into its aglycone form (Marotti, Bonetti, Biavati, Catizone, & Dinelli, 2007). Accordingly, we found increases on kaempferol concentration in around half of the broths enriched with flaxseed and soy extracts and fermented by *Bifidobacterium* strains. Again, the highest concentrations were achieved by *B. breve* and *B. pseudocatenulatum* strains, together with *B. dentium* INIA P886.

Naringenin was produce by the majority of the *Bifidobacterium* strains, with higher figures in *B. breve*, *B. pseudocatenulatum*, *B. catenulatum* and *B. dentium* strains. Regarding the bacterial formation of this aglycone, it has been described in soybean flour fermented with *Lb. plantarum* (Landete, *et al.*, 2015).

Not all the aglycones studied were detected in the fermented broths. The lack of detection of hesperetin, catechin, epicatechin, mirecitin and taxifolin may be due to the absence of glycosidated precursors susceptible of being transformed by the glycosidases of the *Bifidobacterium* strains tested.

In spite of the difference in the profile of aglycones produced, the differences in antioxidant activity and phenolic compounds were not as high as it could have been expected. Nevertheless, the higher antioxidant activity was measured in some of the bifidobacterial strains showing the higher production of flavonoid aglycones, i.e. *B. breve* INIA P367, *B. breve* INIA P776 and *B. dentium* INIA P883, although the high aglycone content of other bifidobacteria-fermented samples did not have a reflection in the antioxidant figures. Something similar happened with the determination of phenolic compounds, which did not correlate with the quantified aglycones, although higher levels were achieved again by *B. breve* INIA P367, as well as by *B. catenulatum* INIA P825. The lack of correlation between this two measurements and the profile of compounds detected by HPLC-ESI/MS could be due the inability to identify and

quantify all the phenolic compounds present in the samples. The composition in glycosides of flaxseed and soy is complex, with presence of many different O- and C-glycosides. Glycosylation attenuated the antioxidant activity and therefore the deglycosylation reactions produce an increase in this activity (Cavia-Saiz, Busto, Pilar-Izquierdo, Ortega, Pérez-Mateos, & Muniz, 2010; Lee, *et al.*, 2015). Nevertheless, it has been described that the antioxidant activity of C-glucosides must be taken into account, as it could be higher than that of the O-glycosides (Xiao, 2017). Those C-glycosides are also less easily transformed into aglycones by the action of bacteria (Gaya, Peirotén, & Landete, 2017).

The previously described ability of *Bifidobacterium* strains to deglycosilate lignans and isoflavones from flaxseed and soy extracts has been confirmed in this work in a mixture of both extracts. Moreover, the production of other flavonoid aglycones has been observed, with an important role of *B. breve* and *B. pseudocatenulatum* strains. One of them, *B. pseudocatenulatum* INIA P815, which showed good levels of almost of the aglycones studied, had shown previously the ability to produce urolithins A and B that are phytoestrogens of interest in human health (Gaya, Peirotén, Medina, Álvarez, & Landete, 2018).

Conclusion

Besides isoflavones, lignans, flavonoids such as herbacetin, naringenin and kaempferol are also present in flaxseed and soy. The lignan and flavonoid aglycones have a high nutritional and health interest due to their increased bioactivity compared with the glycosides. *Bifidobacterium* is a genus with high glycosidase activity, in this work we

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376 show how that activity extents to several flavonoid compounds producing a wide
377 variety of flavonoid aglycones, some of them never described before as bacterial
378 products (herbacetin and quercetagenin). The *B. breve* and *B. pseudocatenulatum* strains
379 tested in this study stood out, showing high levels of SDG and isoflavones
380 deglycosilation alongside with a wide range of other flavonoids deglycosilated. Thus,
381 the use of these bacteria as probiotics, or in the fermentation of foods rich in flavonoids
382 and lignans, has a nutritional and health interest.

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For Peer Review

Compliance with Ethical Standards

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Compliance with ethical standards. This article does not contain any studies with human participants or animals performed by any of the authors.

Conflicts of Interest: Authors declare no conflict of interest.

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522 **Table 1.** *Bifidobacterium* strains tested in this work.

Strain	Origin	Reference
<i>B. adolescentis</i> INIA P549	breast-fed infant faeces	Rodríguez et al. 2012
<i>B. adolescentis</i> INIA P659	breast-fed infant faeces	Rodríguez et al. 2012
<i>B. animalis</i> INIA P490	breast-fed infant faeces	Rodríguez et al. 2012
<i>B. animalis</i> INIA P913	adult faeces	INIA culture collection
<i>B. bifidum</i> INIA P466	breast-fed infant faeces	Rodríguez et al. 2012
<i>B. bifidum</i> INIA P475	breast-fed infant faeces	Rodríguez et al. 2012
<i>B. breve</i> INIA 367	breast-fed infant faeces	Rodríguez et al. 2012
<i>B. breve</i> INIA P12	breast-fed infant faeces	Rodríguez et al. 2012
<i>B. breve</i> INIA P776	child faeces	Peirotén et al. 2018
<i>B. catenulatum</i> INIA P732	breast-fed infant faeces	Peirotén et al. 2018
<i>B. catenulatum</i> INIA P765	adult faeces	Peirotén et al. 2018
<i>B. catenulatum</i> INIA P825	child faeces	Peirotén et al. 2018
<i>B. dentium</i> INIA P883	adult faeces	INIA culture collection
<i>B. dentium</i> INIA P886	adult faeces	INIA culture collection
<i>B. infantis</i> INIA P593	breast-fed infant faeces	Rodríguez et al. 2012
<i>B. infantis</i> INIA P722	breast-fed infant faeces	Peirotén et al. 2018
<i>B. infantis</i> INIA P728	breast-fed infant faeces	Peirotén et al. 2018
<i>B. longum</i> INIA P750	child faeces	Peirotén et al. 2018
<i>B. longum</i> INIA P9	breast-fed infant faeces	Rodríguez et al. 2012

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5	<i>B. pseudocatenulatum</i> INIA P815	adult faeces	INIA culture collection
6	<i>B. pseudocatenulatum</i> INIA P846	child faeces	Peirotén et al. 2018
7	<i>B. pseudocatenulatum</i> INIA P946	breast-fed infant faeces	INIA culture collection
8	<i>B. pseudolongum</i> INIA P2	breast-fed infant faeces	Rodríguez et al. 2012
9	<i>B. pseudolongum</i> INIA P120	breast-fed infant faeces	Rodríguez et al. 2012
10	<i>B. pseudolongum</i> INIA P977	goat milk	INIA culture collection
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Table 2. Secoisolariciresinol (SECO) and isoflavone aglycones (mg per liter of fermentation media) produced from flaxseed and soy extracts after incubation with a collection of *Bifidobacterium* strains.

	Lignans	Isoflavones	
	SECO	Daidzein	Genistein
Control	3.9 ± 0.4	27.9 ± 2.3	38.6 ± 12.6
<i>B. adolescentis</i> INIA P549	3.8 ± 0.5	58.2 ± 7.7	62.5 ± 21.3
<i>B. adolescentis</i> INIA P659	5.8 ± 2.1	28.9 ± 5.6	57.2 ± 12.2
<i>B. animalis</i> INIA P490	74.6 ± 13.3	82.3 ± 9.7	101.2 ± 7.8
<i>B. animalis</i> INIA P913	33.5 ± 17.6	99.2 ± 24.5	156.2 ± 15.8
<i>B. bifidum</i> INIA P466	34.2 ± 1.3	131.6 ± 3.3	166.5 ± 21.3
<i>B. bifidum</i> INIA P475	9.4 ± 2.3	49.2 ± 14.2	72.4 ± 8.7
<i>B. breve</i> INIA 367	116.4 ± 13.7	187.3 ± 10.7	221.8 ± 9.7
<i>B. breve</i> INIA P12	81.3 ± 4.5	172.3 ± 31.2	210.2 ± 23.1
<i>B. breve</i> INIA P776	148.9 ± 16.8	181.8 ± 17.1	205.6 ± 21.2
<i>B. catenulatum</i> INIA P732	41.3 ± 12.6	147.3 ± 27.2	103.4 ± 33.5
<i>B. catenulatum</i> INIA P765	2.9 ± 2.1	34.7 ± 7.6	61.2 ± 8.1
<i>B. catenulatum</i> INIA P825	42.3 ± 3.6	67.2 ± 21.8	206.5 ± 8.9
<i>B. dentium</i> INIA P883	54.6 ± 6.5	141.2 ± 5.3	166.4 ± 22.4
<i>B. dentium</i> INIA P886	18.9 ± 8.5	107.6 ± 20.0	142.3 ± 9.3
<i>B. infantis</i> INIA P593	3.3 ± 0.8	51.6 ± 12.5	59.1 ± 9.1

<i>B. infantis</i> INIA P722	4.3 ± 1.1	23.3 ± 1.9	43.3 ± 2.9
<i>B. infantis</i> INIA P728	7.7 ± 1.5	57.3 ± 13.2	112.4 ± 4.6
<i>B. longum</i> INIA P750	12.0 ± 0.9	47.8 ± 14.5	98.7 ± 18.5
<i>B. longum</i> INIA P9	5.8 ± 0.7	40.3 ± 6.7	91.2 ± 8.2
<i>B. pseudocatenulatum</i> INIA P815	102.6 ± 57.6	139.4 ± 32.4	141.5 ± 21.7
<i>B. pseudocatenulatum</i> INIA P846	152.8 ± 22.1	158.4 ± 12.0	168.2 ± 18.8
<i>B. pseudocatenulatum</i> INIA P946	204.2 ± 35.3	154.8 ± 6.4	195.7 ± 11.1
<i>B. pseudolongum</i> INIA P2	13.6 ± 4.4	65.4 ± 2.3	73.2 ± 15.2
<i>B. pseudolongum</i> INIA P120	16.8 ± 7.5	46.7 ± 2.6	69.5 ± 11.7
<i>B. pseudolongum</i> INIA P977	15.8 ± 4.4	62.1 ± 16.9	101.6 ± 8.0

Table 3. Flavanones, flavones and flavonols (mg per liter of fermentation media) produced from flaxseed and soy extracts after incubation with a collection of *Bifidobacterium* strains.

	Flavanones		Flavones		Flavonols			
	Naringenin	Eriodictiol	Luteolin	Apigenin	Herbacetin	Kaempferol	Quercetin	Quercetagetin
Control	0.87 ± 0.06	0.29 ± 0.03	0.76 ± 0.06	n.d.	n.d.	0.19 ± 0.04	n.d.	n.d.
<i>B. adolescentis</i> INIA P549	0.31 ± 0.11	0.09 ± 0.04	0.81 ± 0.21	n.d.	n.d.	0.08 ± 0.04	n.d.	n.d.
<i>B. adolescentis</i> INIA P659	2.62 ± 0.17	0.39 ± 0.05	0.79 ± 0.41	0.22 ± 0.16	0.04 ± 0.02	0.19 ± 0.11	n.d.	n.d.
<i>B. animalis</i> INIA P490	2.70 ± 0.71	0.41 ± 0.03	1.16 ± 0.32	0.33 ± 0.09	0.57 ± 0.07	0.71 ± 0.09	n.d.	n.d.
<i>B. animalis</i> INIA P913	4.09 ± 0.71	0.45 ± 0.21	0.89 ± 0.13	0.40 ± 0.06	1.87 ± 0.43	0.87 ± 0.32	n.d.	n.d.
<i>B. bifidum</i> INIA P466	1.90 ± 0.08	0.31 ± 0.06	1.04 ± 0.34	0.19 ± 0.01	n.d.	0.28 ± 0.03	n.d.	n.d.
<i>B. bifidum</i> INIA P475	0.78 ± 0.23	0.34 ± 0.11	0.90 ± 0.06	0.07 ± 0.02	n.d.	0.12 ± 0.01	n.d.	n.d.
<i>B. breve</i> INIA 367	4.13 ± 0.56	0.52 ± 0.02	0.98 ± 0.18	0.27 ± 0.03	57.37 ± 6.73	2.00 ± 0.56	0.90 ± 0.08	0.93 ± 0.08
<i>B. breve</i> INIA P12	4.33 ± 1.01	0.50 ± 0.13	1.15 ± 0.07	0.55 ± 0.21	42.67 ± 8.09	2.29 ± 0.34	n.d.	0.63 ± 0.17
<i>B. breve</i> INIA P776	4.11 ± 0.38	0.56 ± 0.13	0.96 ± 0.10	0.46 ± 0.02	54.30 ± 13.81	2.30 ± 0.08	0.95 ± 0.19	0.53 ± 0.05
<i>B. catenulatum</i> INIA P732	3.97 ± 0.70	0.50 ± 0.11	1.06 ± 0.11	0.30 ± 0.03	0.02 ± 0.01	0.47 ± 0.08	n.d.	n.d.
<i>B. catenulatum</i> INIA P765	4.41 ± 0.30	0.58 ± 0.02	0.93 ± 0.36	0.36 ± 0.12	n.d.	0.41 ± 0.11	n.d.	n.d.
<i>B. catenulatum</i> INIA P825	4.35 ± 0.22	0.61 ± 0.15	0.77 ± 0.02	0.29 ± 0.15	0.04 ± 0.03	0.45 ± 0.01	n.d.	n.d.
<i>B. dentium</i> INIA P883	3.56 ± 0.15	0.46 ± 0.08	0.72 ± 0.32	n.d.	n.d.	0.28 ± 0.05	n.d.	n.d.
<i>B. dentium</i> INIA P886	4.02 ± 0.63	0.50 ± 0.16	1.09 ± 0.28	0.34 ± 0.012	16.22 ± 2.16	2.53 ± 0.17	n.d.	0.17 ± 0.02
<i>B. infantis</i> INIA P593	2.57 ± 0.33	0.38 ± 0.07	0.72 ± 0.17	0.18 ± 0.011	0.03 ± 0.01	0.21 ± 0.05	n.d.	n.d.
<i>B. infantis</i> INIA P722	3.02 ± 0.09	0.46 ± 0.13	0.74 ± 0.05	0.17 ± 0.03	n.d.	0.19 ± 0.15	n.d.	n.d.
<i>B. infantis</i> INIA P728	3.24 ± 1.41	0.50 ± 0.21	0.14 ± 0.02	0.13 ± 0.02	n.d.	0.13 ± 0.10	n.d.	n.d.
<i>B. longum</i> INIA P750	2.86 ± 0.43	0.45 ± 0.08	0.94 ± 0.08	0.49 ± 0.16	0.02 ± 0.01	0.21 ± 0.02	n.d.	n.d.
<i>B. longum</i> INIA P9	0.88 ± 0.09	0.35 ± 0.08	0.95 ± 0.11	n.d.	n.d.	0.08 ± 0.03	n.d.	n.d.
<i>B. pseudocatenulatum</i> INIA P815	4.21 ± 0.08	0.62 ± 0.05	1.15 ± 0.07	0.33 ± 0.09	64.74 ± 5.62	2.36 ± 0.41	0.98 ± 0.07	1.12 ± 0.32
<i>B. pseudocatenulatum</i> INIA P846	4.41 ± 0.65	0.60 ± 0.01	0.97 ± 0.19	0.50 ± 0.04	0.78 ± 0.19	0.88 ± 0.18	n.d.	n.d.
<i>B. pseudocatenulatum</i> INIA P946	4.38 ± 0.21	0.70 ± 0.43	0.86 ± 0.07	0.48 ± 0.010	56.90 ± 7.81	1.77 ± 0.05	0.97 ± 0.31	1.44 ± 0.16

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<i>B. pseudolongum</i> INIA P120	3.01 ± 0.17	0.49 ± 0.06	0.90 ± 0.21	0.40 ± 0.08	n.d.	0.26 ± 0.21	n.d.	n.d.
<i>B. pseudolongum</i> INIA P2	2.09 ± 0.21	0.41 ± 0.11	0.84 ± 0.16	n.d.	0.03 ± 0.01	0.17 ± 0.05	n.d.	n.d.
<i>B. pseudolongum</i> INIA P977	0.94 ± 0.16	0.48 ± 0.17	0.80 ± 0.05	n.d.	n.d.	0.14 ± 0.08	n.d.	n.d.
n.d. (not detected)								

For Peer Review

535 **Figure legends**

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537 **Figure 1.** HPLC-ESI (A) and HPLC-ESI-MS/MS (B, C) analyses to identify herbacetin and quercetin produced by *Bifidobacterium*.

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539 **Figure 2.** HPLC-ESI (A) and HPLC-ESI-MS/MS (B) analyses to identify quercetagenin produced by *Bifidobacterium*.

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Figure 1

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Figure 2

549 Graphical abstract

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Figure 1. HPLC-ESI chromatograms of flavonoid aglycones produced by *Bifidobacterium* strains.