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## **Release of phenolic acids from sunflower and rapeseed meals using different carboxylic esters hydrolases from *Aspergillus niger***

Oscar Laguna, Elise Odinet, Alexandra Bisotto, Bruno Barea, Pierre Villeneuve, Jean-Claude Sigoillot, Eric Record, Craig Faulds, Frédéric Fine, Laurence Lesage-Meessen, et al.

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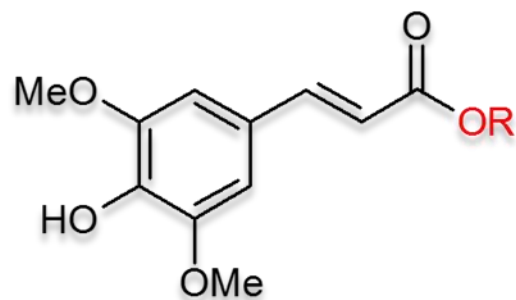
Abstract: Sunflower and rapeseed meals are agro-industrial coproducts that contain high amount of phenolics (1-4 % dry matter), mostly as esters of caffeic acid (CA) and sinapic acid (SA), respectively. The enzymatic hydrolysis of the ester bonds enables to recover the corresponding free phenolic acids that are bioactive compounds and platform molecules for various applications in green chemistry. Here we aimed to find the best route for producing free CA and SA by applying various fungal carboxylic ester hydrolases from recombinant *Aspergillus niger* strains either directly on crude meal or on their phenolic extracts obtained by methanolic extraction. Two types of meals were studied: (i) industrial (commercial) meals (I-meals), produced by a process that includes cooking at 95-100°C and steam desolventizing at 105-107°C, and (ii) non-industrial meals (NI-meals) obtained at pilot-scale with much milder heat treatment, that offer a higher total phenolic content. CA release through hydrolysis of sunflower meal (SFM) was successfully achieved with *A. niger* type-B feruloyl esterase (AnFaeB) and chlorogenic acid esterase (ChlE). Maximal amount of free CA released was of  $54.0 \pm 1.1$  to  $59.8 \pm 2.1$   $\mu\text{mol/g}$  defatted dry matter (DDM) from I-SFM (94-100% hydrolysis yield) against  $42.0 \pm 1.1$  to  $52.3 \pm 0.2$   $\mu\text{mol/g}$  DDM (59-73% hydrolysis yield) from NI-SFM in which CA release was hampered by a phenolic oxidation side-reaction, seemingly due to meal endogenous polyphenol oxidase activities. AnFaeB and ChlE hydrolysis of phenolic extracts from NI-SFM increased the CA amount obtained to 55.0-68.1  $\mu\text{mol/g}$  DDM (77-95% hydrolysis yield). In all cases, AnFaeB showed broader specificity towards SFM caffeoyl quinic acid isomers than ChlE. In particular, ChlE did not hydrolyze 3-O-caffeoylquinic acid. The maximal amount of free SA released by AnFaeA hydrolysis was  $41.3 \pm 0.3$   $\mu\text{mol/g}$  DDM from NI-SFM (50% hydrolysis yield) and  $32.3 \pm 0.4$   $\mu\text{mol/g}$  DDM from the phenolic extract (64% hydrolysis yield), with AnFaeA also having sinapine

transesterification activity that led to the synthesis of 1,2-di-O-sinapoyl- $\beta$ -D-glucose. Finally, of all the substrates tested for enzymatic hydrolysis in our conditions, I-RSM and NI-SFM extract showed the best compromise between initial total phenolic content, hydrolysis yields and amounts of CA/SA released.

## Rapeseed meal



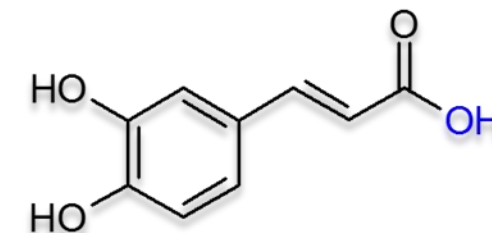
Phenolics  
≈ 2% DDM



Sinapic acid esters ≈ 98 %

*In situ*  
enzymatic hydrolysis

AnFaeA

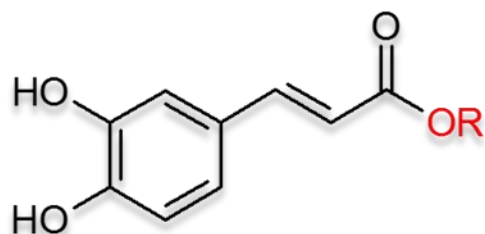


Free sinapic acid

## Sunflower meal



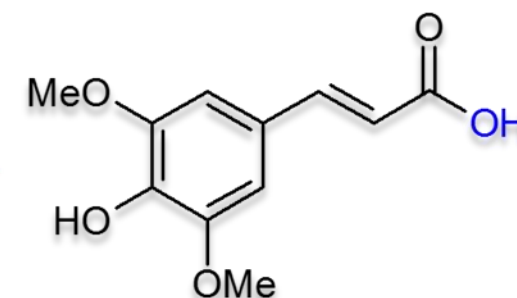
Phenolics  
≈ 3% DDM



Caffeic acid esters ≈ 95 %

*In situ*  
enzymatic hydrolysis

AnFaeB or ChIE



Free caffeic acid

- *Aspergillus niger* AnFaeA efficiently released free sinapic acid from rapeseed meal
- *A. niger* AnFaeB and ChIE efficiently released free caffeic acid from sunflower meal
- Enzymatic hydrolysis performed on methanolic extracts avoided phenolics degradation
- AnFaeA acyl transferase activity on sinapine led to 1,2-di-*O*-sinapoyl- $\beta$ -D-glucose
- Non-thermally-treated meals had the advantage of high initial phenolic contents

1 **Release of phenolic acids from sunflower and rapeseed meals using different**  
2 **carboxylic esters hydrolases from *Aspergillus niger***

3

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5 Jean-Claude Sigoillot<sup>b</sup>, Eric Record<sup>b</sup>, Craig B. Faulds<sup>b</sup>, Frédéric Fine<sup>c</sup>, Laurence  
6 Lesage-Meessen<sup>b</sup>, Anne Lomascolo<sup>b</sup> and Jérôme Lecomte<sup>a,d\*</sup>

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13

14 **Abstract**

15 Sunflower and rapeseed meals are agro-industrial coproducts that contain high  
16 amount of phenolics (1-4 % dry matter), mostly as esters of caffeic acid (CA) and  
17 sinapic acid (SA), respectively. The enzymatic hydrolysis of the ester bonds enables to  
18 recover the corresponding free phenolic acids that are bioactive compounds and  
19 platform molecules for various applications in green chemistry. Here we aimed to find  
20 the best route for producing free CA and SA by applying various fungal carboxylic ester  
21 hydrolases from recombinant *Aspergillus niger* strains either directly on crude meal or  
22 on their phenolic extracts obtained by methanolic extraction. Two types of meals were  
23 studied: (i) industrial (commercial) meals (I-meals), produced by a process that includes  
24 cooking at 95-100°C and steam desolventizing at 105-107°C, and (ii) non-industrial

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25 meals (NI-meals) obtained at pilot-scale with much milder heat treatment, that offer a  
26 higher total phenolic content. CA release through hydrolysis of sunflower meal (SFM)  
27 was successfully achieved with *A. niger* type-B feruloyl esterase (AnFaeB) and  
28 chlorogenic acid esterase (ChIE). Maximal amount of free CA released was of  $54.0 \pm$   
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42 compromise between initial total phenolic content, hydrolysis yields and amounts of  
43 CA/SA released.

44

45 **Keywords:** rapeseed and sunflower meals, *Aspergillus niger*, chlorogenate esterase,  
46 feruloyl esterase, sinapic acid, caffeic acid

47

## 48 Abbreviations

49 1,2-diSG: 1,2-di-*O*-sinapoyl- $\beta$ -D-glucose

50 3-CQA: 3-*O*-Caffeoylquinic acid

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- 51 3,4-diCQA: 3,4-di-*O*-caffeoylquinic acid
- 52 3,5-diCQA: 3,5-di-*O*-caffeoylquinic acid
- 53 4-CQA: 4-*O*-Caffeoylquinic acid
- 54 4,5-diCQA: 4,5-di-*O*-caffeoylquinic acid
- 55 5-CQA: 5-*O*-Caffeoylquinic acid
- 56 *A. niger*: *Aspergillus niger*
- 57 ACE: Angiotensin-I Converting Enzyme
- 58 AnFaeA: *A. niger* type-A feruloyl esterase
- 59 AnFaeB: *A. niger* type-B feruloyl esterase
- 60 CA: Caffeic acid
- 61 CAE: Caffeic acid equivalents
- 62 CAEsters: Caffeic acid esters
- 63 ChIE: Chlorogenic acid esterase
- 64 DDM: Defatted dry matter
- 65 DM: Dry matter
- 66 HPLC: High performance liquid chromatograph
- 67 I: Industrial
- 68 I-meals: Industrial meals
- 69 I-RSM: Industrial rapeseed meal
- 70 I-SFM: Industrial sunflower meal
- 71 MBTH: 3-methyl-2-benzothiazolinonehydrazone
- 72 MOPS: 3-(*N*-morpholino)propanesulfonic acid
- 73 nkat: nanokatal
- 74 NI: Non-industrial
- 75 NI-meals: Non-industrial meals
- 76 NI-RSM: Non-industrial rapeseed meal

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77 NI-SFM: Non-industrial sunflower meal

78 PPO: Polyphenol oxidase

79 RSM: Rapeseed meal

80 SA: Sinapic acid

81 SAE: Sinapic acid equivalents

82 SAEsters: Sinapic acid esters

83 SFM: Sunflower meal

84 SNP: Sinapine

85 TCAD: Total caffeic acid derivatives

86 TPC: Total phenolic compounds

87 TSAD: Total sinapic acid derivatives

88

## 89 1. Introduction

90 Rapeseed (*Brassica napus L.*) and sunflower (*Helianthus annuus L.*) are two of  
91 the most important oilseed crops in the world (Lomascolo *et al.*, 2012) and their oils are  
92 mainly used for human consumption or for biofuel production (Borredon *et al.*, 2011;  
93 Fine *et al.*, 2015). Rapeseed (RSM) and sunflower (SFM) meals are the main co-  
94 products of the seed pressing and de-oiling processes. In 2018, worldwide production of  
95 RSM and SFM was estimated at 39.2 Mt and 21.0 Mt respectively  
96 (<http://www.indexmundi.com/agriculture/>). They are mostly dedicated to animal feed  
97 because of their high protein content with about 38 g/100 g dry matter (DM) and 28  
98 g/100 g DM for RSM and SFM respectively (González-Pérez and Vereijken, 2007;  
99 Ivanova *et al.*, 2016; Ugolini *et al.*, 2015). In addition, studies have shown that these  
100 raw materials can also serve as a source of protein isolates with bioactive properties,  
101 such as Angiotensin-I Converting Enzyme (ACE) inhibitory activities, antioxidant or  
102 antithrombotic activities, and bile acid-binding capacities (Aider and Barbana, 2011;

103 Campbell *et al.*, 2016; Ugolini *et al.*, 2015). These protein isolates also possess  
104 emulsification and foaming properties and are considered as promising ingredients for  
105 industrial non-food applications such as adhesives and detergents, among others (Fetzer  
106 *et al.*, 2018, 2019). Moreover, RSM and SFM can also be used as substrates in  
107 microbial fermentative processes like saccharification and production of enzymes,  
108 antibiotics and other bioproducts (for a review, see Lomascolo *et al.*, 2012). Also, as  
109 outlined in the work of Licata *et al.* (2019), these meals represent a potential source of  
110 energy production through combustion processes or biogas production and could be  
111 considered as economically convenient in a short agro-energy chain.

112 RSM and SFM also have relatively high total phenolic compound (TPC) contents,  
113 ranging from 1-2 g/100 g DM for RSM and 2-4 g/100 g DM for SFM. These phenolics  
114 are almost exclusively sinapic acid esters (SAEsters) in RSM, with sinapine as the main  
115 derivative (80 % of the TPC content) and the remaining 20 % composed of mono-, di-  
116 and tri-sinapoyl esters of sugars and/or kaempferol (Baumert *et al.*, 2005; Milkowski  
117 and Strack, 2010; Siger *et al.*, 2013). In SFM, phenolics are mostly found as caffeic acid  
118 esters (CAEsters). 5-Caffeoylquinic acid (chlorogenic acid) is the major phenolic  
119 compound accounting for 70 % of the TPC content, while the remaining 30 % are other  
120 mono- and di-caffeoylquinic acids, as well as coumaroyl- and feruloylquinic acids. Free  
121 caffeic (CA) acid is also found in SFM but accounts for just 0.5 % of the TPC content  
122 (Guo *et al.*, 2017; Szydłowska-Czerniak *et al.*, 2011; Weisz *et al.*, 2009).

123 Phenolic acids, particularly hydroxycinnamic acids, are bioactive molecules with  
124 interesting properties that include antioxidant (Gullón *et al.*, 2018; Shahidi and  
125 Ambigaipalan, 2015), anti-inflammatory (Oueslati *et al.*, 2012) and antimicrobial  
126 (Ouerghemmi *et al.*, 2017) activities among others. These bioactive properties can be  
127 enhanced by modifying the hydrophilic/hydrophobic balance of the molecules  
128 (Laguerre *et al.*, 2013; Lecomte *et al.*, 2010; Szydłowska-Czerniak *et al.*, 2018). For

129 example, esterification with aliphatic alcohols of adequate chain-length improved their  
130 antioxidant activity in liposome suspensions, oil-in-water emulsions (Laguerre *et al.*,  
131 2010; Sørensen *et al.*, 2015) and even in cultured cells, as well as their bactericidal  
132 activity on selected bacteria (Suriyarak *et al.*, 2013). This phenomenon, related to the  
133 so-called “cut-off effect”, was attributed to a better location and concentration of esters  
134 at the interfacial membrane where lipid oxidation occurs (Laguerre *et al.*, 2011;  
135 McClements and Decker, 2018), and to their ability to cross or interact with membranes  
136 of living cells and microorganisms. Moreover, owing to their particular structure and  
137 reactivity, phenolic acids (i.e. hydroxybenzoic and hydroxycinnamic acids), as their  
138 corresponding vinyl phenols, have gained much importance during the last decade for  
139 the synthesis of new sustainable polymers and pre-polymers (Aouf *et al.*, 2012; Diot-  
140 Néant *et al.*, 2017; Pion *et al.*, 2014; Reano *et al.*, 2015; Wang *et al.*, 2011; Zago *et al.*,  
141 2015a). So any raw material and process enabling large-scale production and recovery  
142 of phenolic acids would positively facilitate the development of these sustainable active  
143 platform molecules and polymers. In this context, SFM and RSM, which are cheap,  
144 abundant, and rich in phenolics, make perfectly suitable candidates. It is worth noting  
145 that only few studies have reported the use of these two raw materials for the production  
146 of phenolic acids and derivatives like vinyl phenols (Kreps *et al.*, 2014; Odinet *et al.*,  
147 2017).

148         Microbial cinnamoyl and feruloyl esterases are carboxylic ester hydrolase  
149 enzymes (EC 3.1.1) that are able to hydrolyze ester linkages between hydroxycinnamic  
150 acids and sugars present in plant cell-walls (Faulds, 2010) with various *in vivo*  
151 specificities according to type of enzyme and origin of microorganism. For instance, the  
152 type-A feruloyl esterase from the filamentous fungus *Aspergillus niger* (called AnFaeA)  
153 is highly active on sinapic and ferulic acid methyl esters and on some 1,5-esterified  
154 feruloylated oligomers from cereals. Type-B feruloyl esterase from *A. niger* (so called

155 AnFaeB) is rather active on caffeic and *p*-coumaric acid methyl esters and on some 1,2-  
156 and 1,6-esterified feruloylated oligomers from sugar-beet pulp (Brézillon *et al.*, 1996;  
157 Faulds and Williamson, 1994). Another type of *A. niger* carboxylic ester hydrolase,  
158 called chlorogenic acid hydrolase (or chlorogenate esterase, ChIE) was primarily found  
159 to be highly specific to chlorogenic acid (5-*O*-caffeoylquinic acid) hydrolysis (Benoit *et*  
160 *al.*, 2007). Historically, pure AnFaes were shown to be essentially inactive when used  
161 directly on natural raw substrates. However, we recently studied the enzymatic  
162 hydrolysis of an industrial rapeseed meal (I-RSM) using a recombinant AnFaeA to  
163 produce sinapic acid (Odinot *et al.*, 2017) and demonstrated that this enzyme was highly  
164 effective on the raw RSM. In the best conditions, AnFaeA hydrolyzed most of the  
165 SAEsters present in the RSM, with an overall yield of 68-76 % (100% hydrolysis of  
166 sinapine).

167         Taking this previous work forward, here we aimed to find the best route and  
168 operating conditions for producing free CA and SA by applying various fungal  
169 carboxylic ester hydrolases from recombinant *Aspergillus niger* strains either directly on  
170 crude meal or on their phenolic extracts obtained by methanolic extraction. Two types  
171 of meals were studied: (i) I-meals (industrial meals), produced by a process that  
172 includes cooking at 95-100°C and steam desolventizing at 105-107°C, and (ii) NI-meals  
173 (custom-made non-industrial meals), obtained at pilot-scale with much milder heat  
174 treatment. In a general way, the NI-meals have a net higher phenolic content than the  
175 corresponding I-meals (Zago *et al.*, 2015b). We first studied the hydrolysis of sunflower  
176 meals (NI-SFM and I-SFM) with two types of fungal ester hydrolases and determined  
177 the best conditions for caffeic acid (CA) production. Exploiting their hydrolytic activity  
178 on CAEsters (Faulds, 2010; Gopalan *et al.*, 2015; Ramos-de-la-Peña and Contreras-  
179 Esquivel, 2016), we tested an *A. niger* type-B recombinant feruloyl esterase (AnFaeB)  
180 and a recombinant *A. niger* chlorogenate esterase (ChIE) on the SFMs. To the best of

181 our knowledge, these two enzymes have never been used on oilseed meals. Enzymatic  
182 hydrolysis was also performed on methanolic extracts from NI-SFM which are mainly  
183 composed of phenolics, minerals, and carbohydrates (Sripad and Narasinga Rao, 1987)  
184 but are devoid of the proteins and other meal constituents. Then, the same approach was  
185 applied to a non-industrial rapeseed meal (NI-RSM) and the corresponding phenolic  
186 extract, by using AnFaeA to release free SA. The results (hydrolysis yield and highest  
187 released SA amount) were compared with our previous data obtained on an I-RSM  
188 (Odinot *et al.*, 2017). All cases were compared and discussed based on the following  
189 parameters: hydrolysis yields, qualitative and quantitative composition of hydrolyzed  
190 hydroxycinnamoyl esters, and free CA or SA amount released per gram of substrate.

## 191 **2. Materials and Methods**

### 192 **2.1. Chemicals**

193 Sinapine thiocyanate (99.0 % HPLC) was isolated from rapeseed meal according  
194 to the method outlined by Mailer (2008). 3,4-di-*O*-caffeoylquinic, 3,5-di-*O*-  
195 caffeoylquinic and 4,5-di-*O*-caffeoylquinic acids (>99.0 % HPLC) were isolated from  
196 yerba mate leaves, following the procedure previously described in Tong *et al.* (2015).  
197 Sinapic acid (98 %) was from AlfaAesar (Karlsruhe, Germany). 4- and 5-caffeoylquinic  
198 (chlorogenic) acids (95 %), caffeic acid (>98 %), methanol and water (both HPLC  
199 grade, >99.9 %), NaOH (> 97%) and MOPS (3-(N-morpholino)propanesulfonic acid)  
200 buffer were purchased from Sigma–Aldrich (Saint-Quentin-Fallavier, France). ACS  
201 grade ethyl acetate (>99,5%) was purchased from Honeywell Riedel-de-Haën (Seelze,  
202 Germany)

### 203 **2.2. Plant materials**

#### 204 **2.2.1. Preparation of the non-industrial sunflower and rapeseed meals (NI-SFM 205 and NI-RSM)**

206 NI-SFM and NI-RSM were prepared at OLEAD oilseed–protein crops  
207 technology platform’s pilot oilseed plant (Pessac, France), according to the following  
208 methods. Whole sunflower (*Helianthus annuus L.*) seeds (75 kg) were first cold flaked  
209 in a flaker (Damman-Croes N.V., Roeselare, Belgium) equipped with two smooth 500  
210 mm-diameter contra-rotating cylinders spaced at 0.3 mm (capacity: 200 kg.h<sup>-1</sup>). Whole  
211 rapeseed (*Brassica napus L.*) seeds (120 kg) were first cold pressed on a MBU20 screw  
212 press (OLEXA®, Feuchy, France), fed at 75 kg.h<sup>-1</sup> of seeds, to remove 75% of the  
213 initial oil amount. The residual oil from the sunflower or rapeseed press cakes was  
214 further extracted by steeping in hexane at 50°C, in a 480 L-capacity Guedu Pilot  
215 Agitated Filter Dryer (De Dietrich Process Systems, Semur, France). Extraction was  
216 performed on 73 kg of each press cake by immersion in hexane for 15 min followed by  
217 filtration. Five or six successive immersion-filtration steps were required to remove the  
218 oil from the SFM and RSM, respectively. The defatted meals were then desolventized in  
219 the same device, under vacuum at 60°C and without steam injection. Then, the meals  
220 obtained were finely ground by a high-shear impact mill (Hosokawa-alpine, type  
221 UPZ100, Augsburg, Germany) using a 0.1-mm (for NI-RSM) or a 0.5-mm (for NI-  
222 SFM) grid operated at room temperature at a speed of 18,000 rpm, and a feeder speed of  
223 4 kg.h<sup>-1</sup>.

#### 224 **2.2.2. Industrial sunflower and rapeseed meals (I-SFM and I-RSM)**

225 I-SFM and I-RSM were provided by the Technical Centre for Oilseed Crops,  
226 Grain Legumes and Industrial Hemp (Terres Inovia, Pessac, France). I-SFM and I-RSM  
227 were obtained from seed pressing and hexane solvent oil extraction. Further industrial  
228 processing steps included: preconditioning at about 45°C, cooking at 95-100°C for 60  
229 min, then steam desolventizing at 107 ± 2°C for 80 ± 5 min in the case of I-RSM or 105  
230 ± 2°C for 50 ± 5 min in the case of I-SFM.

### 231 **2.3. Enzyme production**

232 The extracellular recombinant feruloyl esterase A (AnFaeA), feruloyl esterase B  
233 (AnFaeB) and chlorogenate esterase (ChIE) were produced, respectively, by the  
234 recombinant strains *A. niger* BRFM451, *A. niger* BRFM766 and *A. niger* BRFM891,  
235 formerly obtained in our Laboratory of Fungal Biodiversity and Biotechnology  
236 (UMR1163 BBF, Marseille, France), by genetic engineering of the host strain *A. niger*  
237 D15#26, as previously described (Benoit *et al.*, 2007; Levasseur *et al.*, 2004; Record *et*  
238 *al.*, 2003). Briefly, in this study, these strains were used to produce batches of AnFaeA,  
239 AnFaeB and ChIE, respectively, in a culture medium containing (per liter contents):  
240 glucose 50 g, NaNO<sub>3</sub> 5.95 g, KCl 0.52 g, MgSO<sub>4</sub>·7H<sub>2</sub>O 0.49 g, and 1 mL of trace  
241 elements (1000× stock, per liter: ZnSO<sub>4</sub>·7H<sub>2</sub>O 21.85 g, H<sub>3</sub>BO<sub>3</sub> 11 g, MnCl<sub>2</sub>·4H<sub>2</sub>O 4.95  
242 g, FeSO<sub>4</sub>·7H<sub>2</sub>O 5 g, CoCl<sub>2</sub>·6H<sub>2</sub>O 1.69 g, CuSO<sub>4</sub>·5H<sub>2</sub>O 1.6 g, Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O 1.5 g,  
243 EDTA-Na<sub>2</sub>·2H<sub>2</sub>O 64.76 g) in a 0.1 M citrate-sodium phosphate buffer at pH5  
244 (Levasseur *et al.*, 2004).

### 245 **2.4. Accession number of protein sequences**

246 The protein sequences of AnFaeA, AnFaeB and ChIE were registered in the  
247 NCBI database under accession numbers CAA70510, AJ309807, and ABK62698,  
248 respectively.

### 249 **2.5. Enzyme activity assay**

250 Esterase activity was assayed spectrophotometrically at either 37°C and 55°C for  
251 AnFaeA or 37°C and 50°C for AnFaeB and ChIE, as previously described (Odinot *et*  
252 *al.*, 2017), by monitoring the A<sub>335</sub> with respect to the rate of hydrolysis of 0.032 mM of  
253 the enzyme substrate in 88 mM of a 3-(*N*-morpholino)propanesulfonic acid (MOPS)  
254 buffer (pH 5.5 for AnFaeA and pH6 for AnFaeB and ChIE). The synthetic substrates  
255 used were methyl sinapate for AnFaeA, methyl caffeate and methyl *p*-coumarate for  
256 AnFaeB, and chlorogenic acid for ChIE. The extinction coefficients at 335 nm were (in

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257 L.mol<sup>-1</sup>.cm<sup>-1</sup>): 13,318 for methyl sinapate, 5,500 for sinapic acid, 19,524 for methyl  
258 ferulate, 4,409 for ferulic acid, 12,560 for methyl caffeate, 6,060 for caffeic acid, 821  
259 for methyl *p*-coumarate, 673 for *p*-coumaric acid and 15,423 for chlorogenic acid.  
260 Endogenous polyphenol oxidase (monophenol, *o*-diphenol:oxygen oxidoreductase, EC  
261 1.14.18.1) activity was determined in SFM as per a procedure adapted from Halaouli *et*  
262 *al.* (2005). Five grams of meal were homogenized in 50 mL sodium phosphate buffer  
263 (100 mM, pH 6.8) at 37°C for 3h under stirring at 200 rpm. After centrifugation at 9500  
264 × *g* for 40 min, the supernatant was used to assay monophenol oxidase (or cresolase)  
265 and diphenol oxidase (or catecholase) activity (Halaouli *et al.*, 2005). Briefly,  
266 monophenolase activity was determined in meal extract by monitoring the A<sub>484</sub>  
267 (extinction coefficient 22,300 L.mol<sup>-1</sup>.cm<sup>-1</sup>) with respect to rate of oxidation of 1.25 mM  
268 L-tyrosine in sodium phosphate buffer (50 mM, pH6.8) at 30°C, in the presence of 3-  
269 methyl-2-benzothiazolinonehydrazone (MBTH, 5 mM) and L-DOPA using a molar L-  
270 DOPA-to-L-tyrosine ratio of 0.057. Diphenolase activity was determined by monitoring  
271 A<sub>484</sub> (extinction coefficient 22,300 L.mol<sup>-1</sup>.cm<sup>-1</sup>) with respect to rate of oxidation of 4.2  
272 mM L-DOPA in sodium phosphate buffer (50 mM, pH6.8) at 30°C, in the presence of 5  
273 mM MBTH.

274 All enzymatic activities were expressed in nanokatal. One nanokatal of activity  
275 is defined as the quantity of enzyme that hydrolyzed 1 nmol of substrate per second.  
276 Experiments were performed in triplicate, and the standard deviation was lower than 1%  
277 of the mean.

## 278 **2.6. Determination of meal dry matter and lipid contents**

279 Dry matter content of the meals was determined as follow: around two grams  
280 (exactly weighed at +/- 0.01 mg) of meal were placed into an aluminium capsule  
281 (previously weighed dry with the same precision scale) and the whole was dried at 105  
282 °C until constant weight (difference between two weighings < 1%). Then, capsule and

283 meal were cooled in a desiccator at room temperature for 30 min and finally weighed.

284 The dried matter content was calculated from the difference between the weight of the  
285 sample (meal and capsule) before and after drying to constant weight.

286 Lipid content of the meals was determined by the method previously outlined in  
287 the work of Laguna *et al.* (2018) : Around two grams (exactly weighed at +/- 0.01 mg)  
288 of each fraction were placed into a filter paper envelope previously dried and weighed  
289 and the whole was dried at 105 °C until constant weight. Then, envelopes were  
290 extracted with n-hexane for 5 h using a Soxhlet apparatus. After extraction, envelopes  
291 were desolventized at room temperature for 12 hours, dried at 105 °C, then cooled in a  
292 desiccator at room temperature for 30 min and finally weighed. The oil content was  
293 calculated from the difference between the dry weight of the sample (meal and  
294 envelope) before and after defatting. Values were given as percentage of oil per gram of  
295 dry matter.

296 These two values were taken into account to express other results per gram of  
297 dry defatted matter. All experiments were done in triplicate.

## 298 **2.7. Methanolic extraction of phenolic compounds from meals**

299 The extraction of TPC from meal samples was carried out in closed tubes at  
300 75°C for 20 min using a meal-to-methanol ratio of 1:100 (w/v) following the procedure  
301 previously described in Laguna *et al.* (2018). After drying under a nitrogen stream,  
302 extracts were kept at -20°C until being processed. All experiments were done in  
303 triplicate.

## 304 **2.8. Determination of the potentially releasable phenolic acid content in meals** 305 **by alkaline hydrolysis**

306 Fifty milligrams of meal were mixed with 1.5 mL of methanol. Then, 6 mL of 2  
307 M NaOH were added to the mixture and incubated at 30°C for different times (15, 30,  
308 60 and 120 min). The mixture was then acidified to pH 2.0 using 4 M HCl with addition

309 of 300 mg NaCl and further extracted three times with ethyl acetate (2 mL) by stirring  
310 for 2 min at room temperature. After each extraction, samples were centrifugated (3000  
311  $\times g$ , 5 min) and supernatants were collected and pooled. The organic phase was dried  
312 under nitrogen stream and the residue was kept at  $-20^{\circ}\text{C}$  until HPLC analysis. All  
313 experiments were performed in triplicate. The amount of free phenolic acid released  
314 from meals after alkaline hydrolysis was used, when possible, as the 100% baseline  
315 reference for the calculations of enzymatic hydrolysis yields.

### 316 **2.9. Enzymatic hydrolysis of caffeic acid derivatives in sunflower meals and in** 317 **the corresponding phenolic extracts with AnFaeB and ChIE**

318 Fifty milligrams of the meal were weighed into a brown flask with a screw cap,  
319 and 5 mL of 100 mM MOPS buffer at pH 6 were added. The closed flasks were  
320 incubated in an orbital shaker (350 rpm) at  $50^{\circ}\text{C}$  run for 30 min for homogenization.  
321 After this pre-incubation step, two samples (mixture of meal and MOPS buffer) were  
322 cooled to  $-80^{\circ}\text{C}$  and freeze-dried. Phenolics were then extracted as described in section  
323 2.7, and their content determined and set as  $t = 0$ . In parallel, different amounts of ChIE  
324 and AnFaeB, from 0 to 30 nkat enzyme/g meal, were added to other pre-incubated  
325 samples. The hydrolysis assay was run for 10 to 240 min, at  $50^{\circ}\text{C}$  and 350 rpm. An  
326 enzyme-free control was also performed in the same conditions. After the desired  
327 treatment duration, the reaction mixture was cooled to  $-80^{\circ}\text{C}$  and freeze-dried.  
328 Phenolics were then extracted as described in section 2.7. After drying under a nitrogen  
329 stream, extracts were kept at  $-20^{\circ}\text{C}$  until HPLC analysis. Two independent trials were  
330 performed per experiment.

331 The enzymatic treatment was also applied to a dry methanolic extract obtained  
332 from 50 mg of NI-SFM as described in section 2.7. Hydrolysis was performed at  $50^{\circ}\text{C}$ ,  
333 as described above, on the dry phenolic extract solubilized in 5 mL MOPS buffer  
334 containing 30 nkat/g meal for AnFaeB or 10 nkat/g meal for ChIE, respectively. After

335 treatment, the reaction medium was cooled to  $-80^{\circ}\text{C}$ , then freeze-dried and finally  
336 extracted as described in section 2.7. After drying under a nitrogen stream, the dry  
337 residue was kept at  $-20^{\circ}\text{C}$  until HPLC analysis. Two independent trials were performed  
338 per experiment.

## 339 **2.10. Enzymatic hydrolysis of sinapic acid derivatives in rapeseed meal and in the** 340 **corresponding phenolic extracts with AnFaeA**

341 The enzymatic hydrolysis of NI-RSM with AnFaeA was performed in the  
342 optimized conditions previously determined on I-RSM by Odinot *et al.* (2017), with  
343 some modifications. Briefly, 50 mg of the meal was weighed into a brown flask with a  
344 screw cap and 5 mL of 100 mM MOPS buffer at pH 5.5 was added. The closed flasks  
345 were stirred in an orbital shaker (350 rpm) at  $55^{\circ}\text{C}$  run for 30 min for homogenization.  
346 After this pre-incubation step, two samples (mixture of meal and MOPS buffer) were  
347 cooled to  $-80^{\circ}\text{C}$  and freeze-dried. Phenolics were then extracted as described in section  
348 2.7 and their content determined and set as  $t = 0$ . In parallel, 39 nkat AnFaeA/g meal  
349 was added to other pre-incubated samples. The enzymatic hydrolysis assay was run at  
350  $55^{\circ}\text{C}$ , 350 rpm for 10 to 240 min. An enzyme-free control was also performed in the  
351 same conditions. After treatment, the reaction mixture was cooled to  $-80^{\circ}\text{C}$  and the  
352 mixture was freeze-dried. The methanolic extraction of phenolics was done as described  
353 in section 2.7. After drying under a nitrogen stream, extracts were kept at  $-20^{\circ}\text{C}$  until  
354 HPLC analysis. Two independent trials were performed per experiment. Enzymatic  
355 hydrolysis was also applied to a dry NI-RSM methanolic extract obtained from 50 mg  
356 of meal as described in section 2.7. Hydrolysis was performed at  $55^{\circ}\text{C}$ , as described  
357 above, on this dry phenolic extract solubilized in 5 mL MOPS buffer containing 39 nkat  
358 AnFaeA/g meal. After treatment, the reaction medium was cooled to  $-80^{\circ}\text{C}$ , then freeze-  
359 dried and finally extracted as described in section 2.7. After drying under a nitrogen

360 stream, the dry residue was kept at  $-20^{\circ}\text{C}$  until HPLC analysis. Two independent trials  
361 were performed per experiment.

## 362 **2.11. High Performance Liquid Chromatography (HPLC) analysis of phenolics** 363 **from hydrolyzed meals**

364 The dry phenolic extracts, obtained as described in the previous sections, were  
365 solubilized in 5 mL methanol/water (2/1, v/v) and filtered (0.45- $\mu\text{m}$  nylon filter) before  
366 HPLC analysis. Phenolic compounds were quantified with an XR UFLC Shimadzu  
367 liquid-phase chromatograph equipped with an SPD-M20A diode array detector (Kyoto,  
368 Japan). Separation was carried out with an ACE C18 reversed-phase column (5  $\mu\text{m}$ , 250  
369 mm  $\times$  4.6 mm, Phenomenex, Le Pecq, France). Injection volume, flow rate, and oven  
370 temperature were set at 20  $\mu\text{L}$ , 1 mL $\cdot\text{min}^{-1}$  and  $30^{\circ}\text{C}$  respectively. The gradient elution  
371 was performed using HPLC-grade water with 0.1% (v/v) acetic acid (A) and methanol  
372 with 0.1% (v/v) acetic acid (B) in the following conditions: 15% B (0–5 min), 15–80%  
373 B (5–30 min), 80–100% B (30–31 min), 100% B (31–35 min), 100-15% B (35–40 min).

### 374 **2.11.1. Sunflower samples**

375 Sunflower phenolics were identified according to retention times and quantified  
376 at their maximum detection wavelength, as previously described in Laguna *et al.*  
377 (2018). Total caffeic acid derivative (TCAD) content was calculated by summing peak  
378 area of all caffeoyl-containing molecules after normalization (i.e. each peak area was  
379 divided by the number of caffeoyl moiety contained in the corresponding molecule) and  
380 expressed in  $\mu\text{mol}$  of caffeic acid equivalent (CAE)/g DDM. CA was selected as  
381 external standard and the calibration curve was obtained from series of standard  
382 methanolic solutions from 12.5 to 800  $\mu\text{M}$  ( $R^2 = 0.9991$ ).

### 383 **2.11.2. Rapeseed samples**

384 SA and sinapine thiocyanate were chosen as external standards for the  
385 calibration curves. Their maximum detection wavelength and retention time were 323

386 nm–17.8 min and 328 nm–11.5 min, respectively. Total sinapic acid derivative (TSAD)  
387 content was calculated by summing peak area of all sinapoyl-containing molecules after  
388 normalization (i.e. each peak area was divided by the number of sinapoyl moiety in the  
389 corresponding molecule) and expressed in  $\mu\text{mol}$  of sinapic acid equivalent (SAE)/g  
390 DDM. Calibration curves were obtained from series of standard methanolic solutions  
391 from 12.5 to 200  $\mu\text{M}$  with  $R^2 = 0.9983$  for sinapic acid and  $R^2 = 0.9987$  for sinapine.

## 392 **2.12. Statistical analyses**

393 Means and standard deviations (SD) were calculated using Minitab Statistical  
394 Software v.18. The means were then compared using one-way ANOVA analysis, with  
395 level of significance set to  $\alpha = 0.05$ .

## 396 **3. Results and discussion**

### 397 **3.1. Characterization of raw materials**

#### 398 **3.1.1. Moisture, lipid and total phenolic compound contents of the meals**

399 The moisture and lipid contents of the meals used in this study are shown in  
400 Table 1, with values of 7-13% DM and 1.4-1.9% DM, respectively. These data were  
401 consistent with the values available in the literature (Lomascolo *et al.*, 2012).

402

403 Table 1 (suggested location)

404

405 The TPC initially present in SFM and RSM samples, as determined after methanolic  
406 extraction, are shown in Table 2 and Table 3 respectively. The TPC content of SFM  
407 samples, which was expressed as TCAD content as 95 % of SFM phenolics were found  
408 as caffeic acid esters (Karamać *et al.*, 2012; Weisz *et al.*, 2009), was  $71.7 \pm 0.5 \mu\text{mol}$   
409 TCAD/g DDM for NI-SFM and  $57.4 \pm 1.5 \mu\text{mol}$  TCAD/g DDM for I-SFM. These data  
410 were consistent with the values available in the literature (Baumert *et al.*, 2005; Cai and  
411 Arntfield, 2001; Weisz *et al.*, 2009). The TPC content of RSM samples, which was

412 expressed as TSAD content as 98 % of their phenolics were shown to be sinapic acid  
413 esters (Khattab *et al.*, 2010; Siger *et al.*, 2013), was  $64.2 \pm 1.2$   $\mu\text{mol}$  TSAD/g DDM for  
414 NI-RSM and  $44.8 \pm 0.2$   $\mu\text{mol}$  TSAD/g DDM for I-RSM. Thus, TPC content was 1.2- to  
415 1.4-fold higher in NI-meals than I-meals, in our conditions.

416 A mild alkaline treatment (NaOH 2 M, 30°C, 30 min) was applied to NI-SFM and I-  
417 SFM samples in order to determine their respective total releasable CA content  
418 (CAEsters in soluble form and potentially bound to the lignocellulosic matrix (Shahidi  
419 and Yeo, 2016)), to be taken as reference. Unfortunately, no CA was recovered (Table  
420 2) from either NI-SFM or I-SFM despite the mild alkaline treatment used. Indeed, the  
421 high chemical reactivity of CA make it prone to autoxidation at high pH due to its  
422 catechol function that rapidly reacts with NaOH and oxidizes in the presence of reactive  
423 oxygen species (Wildermuth *et al.*, 2016). Thus, as alkaline hydrolysis was not suitable  
424 for assessing the total potentially releasable CA from SFM, all calculations and  
425 comparisons between various enzymatic hydrolysis conditions were made on the basis  
426 of TCAD content after methanolic extraction, expressed as  $\mu\text{mol}$  CAE/g DDM, and  
427 determined from the values of  $71.7 \pm 0.5$   $\mu\text{mol}$  CAE/g DDM for NI-SFM and  $57.4 \pm 1.5$   
428  $\mu\text{mol}$  CAE/g DDM for I-SFM (Table 2). These values were taken as 100% reference for  
429 the calculations of enzymatic hydrolysis yields.

430

431 Table 2 (suggested location)

432

433 As done for SFM samples, the total amount of SA initially present in rapeseed meals as  
434 soluble conjugates and potentially releasable forms was determined after alkaline  
435 hydrolysis. The result was  $62.5 \pm 1.8$   $\mu\text{mol/g}$  DDM for NI-RSM and  $46.8 \pm 3.0$   $\mu\text{mol/g}$   
436 DDM for I-RSM (Table 3). These values were not significantly different from those of  
437 the initial TSAD content of each meal ( $64.2 \pm 1.2$   $\mu\text{mol}$  SAE/g DDM for NI-RSM and

438 44.8 ± 0.2 μmol SAE/g DDM for I-RSM), indicating that all the sinapic esters from the  
439 meal were hydrolyzed and recovered after our alkaline hydrolysis protocol. The latter  
440 values, which represented the total amount of potentially releasable SA per gram of  
441 DDM, were set as the 100% reference and used for the calculations of enzymatic  
442 hydrolysis yields. Unlike the significant loss of phenolics observed by Vuorela *et al.*  
443 after alkaline hydrolysis of RSM with a 4 M NaOH solution at 37°C for 4 h (Vuorela *et*  
444 *al.*, 2003), the milder conditions (NaOH 2 M, 30°C, 30 min) implemented here  
445 managed to not degrade the SA and its derivatives.

446

447 Table 3 (suggested location)

448

### 449 3.1.2. Characterization of meal phenolic composition

450 This study identified the phenolic compounds present in the I-SFM by HPLC-  
451 DAD. As shown by HPLC analysis of I-SFM methanolic extract (Fig. 1), the main  
452 phenolic compounds were CA esters such as mono- and di-caffeoylquinic acids, the  
453 most abundant being 4- *O*-caffeoylquinic acid (4-CQA) and 5-*O*-caffeoylquinic acid (5-  
454 CQA). These two compounds co-eluted and were named chlorogenic acid. Other minor  
455 phenolics were 3-*O*-caffeoylquinic acid (3-CQA), two di-caffeoylquinic acid (di-CQA)  
456 isomers (3,5- and 4,5-di-*O*-caffeoylquinic acids), 5-*O*-*p*-coumaroylquinic acid, 5-*O*-  
457 feruloylquinic acid, and CA. These results were in good agreement with other studies  
458 (Karamać *et al.*, 2012; Weisz *et al.*, 2009), and were comparable to the NI-SFM  
459 composition previously determined by Laguna *et al.* (2018).

460

461 Figure 1 (suggested location)

462

463 Two of our previous studies characterizing the phenolic composition of NI-RSM and I-  
464 RSM (Laguna *et al.*, 2018; Odinet *et al.*, 2017) showed that RSM phenolics comprised:  
465 sinapine, sinapoyl glucopyranoside, sinapic acid, di- and tri-sinapoyl gentiobiosides, di-  
466 sinapoyl glucopyranoside isomer, and sinapoyl keampferol derivatives.

### 467 **3.2. Release of free caffeic acid from sunflower meals by *A. niger* AnFaeB and** 468 **ChIE**

#### 469 **3.2.1. Enzymatic hydrolysis of I-SFM**

470 This study selected AnFaeB and ChIE for the hydrolysis of phenolic esters  
471 present in SFMs. Numerous studies have already shown that these enzymes can  
472 hydrolyze CAEsters in other raw materials such as coffee pulp, apple marc, autoclaved  
473 maize bran and sugar-beet pulp (Benoit *et al.*, 2006; Benoit *et al.*, 2007; Faulds, 2010;  
474 Gopalan *et al.*, 2015; Ramos-de-la-Peña and Contreras-Esquivel, 2016), but SFM has  
475 never yet been investigated. The optimum pH and temperature of these enzymes  
476 established in previous work were set to 6.0 and 50°C for synthetic substrates (e.g.  
477 methyl sinapate and 5-CQA) (Asther *et al.*, 2005; Benoit *et al.*, 2006).  
478 Given the drastic heat treatment undergone during the production process, it is  
479 reasonable to posit that I-SFM was somewhat recalcitrant to enzymatic degradation. We  
480 therefore traced the kinetics of hydrolysis of the major phenolic esters in I-SFM and the  
481 release of free CA as a function of enzyme amount (Fig. 2). In the presence of 20 to 30  
482 nkat AnFaeB/g meal, all the CAEsters, initially present in I-SFM, were completely  
483 hydrolyzed after 1 h of incubation (Fig.2, Supplementary data Fig. S1). An amount of 5  
484 nkat AnFaeB/g meal failed to completely hydrolyze the phenolics. An amount of 10  
485 nkat AnFaeB/g meal was able to hydrolyze all CAEsters except 3-CQA (Fig. 2). A  
486 minimum concentration of 20 nkat AnFaeB/g meal was thus required for complete  
487 hydrolysis of all phenolics, and the highest free CA content was obtained with 30 nkat  
488 AnFaeB/g meal after a 3 h incubation ( $59.8 \pm 2.1 \mu\text{mol/g DDM}$ ), which corresponded to

489 a 100% hydrolysis yield. AnFaeB thus showed high catalytic efficiency on all the  
490 mono- and dicaffeoylquinic acid isomers present in I-SFM (Fig. 2), together with the *p*-  
491 coumaroyl and feruloyl derivatives (Supplementary data, Fig. S1).

492

493 Figure 2 (suggested location)

494

495 In the presence of ChIE, the hydrolysis of 3,5-diCQA and 4,5-diCQA was complete  
496 after 3 h of incubation whatever the amount of enzyme. Conversely, 3-CQA was not  
497 hydrolyzed at all in any condition, even though 4-CQA and 5-CQA were 90%  
498 hydrolyzed after 2 h of incubation with ChIE (Fig. 2). The highest free CA content of  
499 obtained from I-SFM with ChIE was  $54.0 \pm 1.1$   $\mu\text{mol/g}$  DDM after 2–4 h incubation  
500 with 5–30 nkat enzyme/g meal. Compared to AnFaeB, this slightly lower value of free  
501 CA released was mainly due to the inability of ChIE to hydrolyze 3-CQA, which was  
502 still found at  $6.1 \pm 0.3$   $\mu\text{mol CAE/g}$  DDM whatever the enzyme concentration (Fig. 2,  
503 supplementary data Fig. S1).

504 AnFaeB has already been shown to have a broader specificity than ChIE for  
505 hydrolyzing chlorogenic acids from agricultural by-products such as maize bran and  
506 sugar-beet pulp (Benoit *et al.*, 2006). For instance, unlike ChIE, AnFaeB was found to  
507 hydrolyze 5-*O-p*-coumaroylquinic acid and feruloylated oligomers (Benoit *et al.*, 2007).  
508 This confirmed the decisive role of the moieties adjacent to the ester bond for correct  
509 alignment of the substrate in the active site of Faes. To the best of our knowledge, there  
510 is no literature data on the relative activity of AnFaeB and ChIE against the different  
511 mono- and di-caffeoylquinic acids. Here, we demonstrated that ChIE showed selectivity  
512 for different caffeoylquinic acid isomers in the SFM compared to AnFaeB. The  
513 concentrations of 30 nkat AnFaeB/g meal and 10 nkat ChIE/g meal were then chosen to  
514 be further applied on NI-SFM

**515 3.2.2. Enzymatic hydrolysis of NI-SFM**

516 Fig. 3A and Fig. 3B shows the results of enzymatic hydrolysis performed on NI-  
517 SFM with AnFaeB and ChIE, respectively. When AnFaeB was used, all the chlorogenic  
518 acid (4- and 5-CQA) was hydrolyzed after 10 min. When ChIE was used, 4- and 5-CQA  
519 were 95% hydrolyzed in our conditions. The highest concentration of free CA released  
520 in the reaction medium was observed after 30 min with AnFaeB ( $52.3 \pm 0.2 \mu\text{mol/g}$   
521 DDM) and ChIE ( $42.0 \pm 1.1 \mu\text{mol/g DDM}$ ). However, these values remained lower than  
522 the initial concentration of chlorogenic acid of  $55.8 \pm 0.9 \mu\text{mol CAE/g DDM}$  in the  
523 meal (Table 2). It is worth noting that there was a strong decrease in TCAD content  
524 over time (Fig. 3), with or without enzyme (control), resulting in a loss of TCAD  
525 compared to the initial content in the meal (Table 2). While the effect of other factors  
526 (including pH and temperature) cannot be totally ruled out, the most likely explanation  
527 was the presence of endogenous enzymes in the NI-SFM that degraded CA and its  
528 derivatives during the pre-incubation and incubation steps. Studies have already  
529 demonstrated the presence of polyphenol oxidase (PPO) in sunflower meal and its high  
530 activity on chlorogenic acid (Wildermuth *et al.*, 2016). Therefore, although our  
531 hydrolysis conditions (pH 6.0, 50°C) did not exactly correspond to PPO optimum pH  
532 and temperature (7.5 and 45°C respectively; Wildermuth *et al.*, 2016), we found it  
533 reasonable to posit that the residual PPO activity was sufficient to continuously oxidize  
534 chlorogenic acid and related compounds from the pre-incubation step. Here we  
535 measured PPO activity in endogenous SFM meal extracts and found  $1.02 (\pm 0.08)$   
536 nkat/mL diphenol oxidase activity and  $0.43 (\pm 0.02)$  nkat/mL monophenol oxidase  
537 activity, which are characteristic of a tyrosinase-type activity (Halaouli *et al.*, 2005). In  
538 addition, it is noteworthy that 15 putative PPO-encoding gene sequences were predicted  
539 in the publicly-available *Helianthus annuus* genome from the NCBI Database

540 (<https://www.ncbi.nlm.nih.gov/search/all/?term=polyphenol+oxidase+helianthus+annuus>)  
541 s).

542 Figure 3 (suggested location)

543

544 Owing to this degradation phenomenon, and regardless of enzyme (AnFaeB or  
545 ChIE), the maximal amount of CA released from NI-SFM remained lower than that  
546 released from I-SFM ( $52.3 \pm 0.2 \mu\text{mol/g DDM}$  versus  $59.8 \pm 2.1 \mu\text{mol/g DDM}$  for  
547 AnFaeB, and  $42.0 \pm 1.1 \mu\text{mol/g DDM}$  versus for  $54.0 \pm 1.1 \mu\text{mol/g DDM}$  for ChIE).  
548 Thus, it seemed that the heat treatment (cooking at 95–100°C followed by steam  
549 desolventizing at about 105°C) applied to I-SFM, but not NI-SFM, led to inactivation of  
550 endogenous enzymes in the SFM even if this heat treatment was also responsible for a  
551 significant decrease in TPC (Table 2). This constituted a major drawback that greatly  
552 limited the value of non-thermally-treated NI-SFM as a substrate for *in situ* enzymatic  
553 production of CA.

### 554 **3.3. AnFaeB and ChIE hydrolysis of NI-SFM methanolic extract**

555 Enzymatic hydrolysis was also performed on phenolic extracts from NI-SFM  
556 after a sequence of methanolic extraction, drying, and solubilization in reaction buffer.  
557 Methanol mainly extracted phenolic compounds, minerals and carbohydrates from the  
558 meal (Sripad and Narasinga Rao, 1987), but not the proteins, including the possible  
559 endogenous enzymes responsible for the degradation of phenolics. Fig. 4A shows the  
560 fast hydrolysis of CA esters by AnFaeB in the methanolic extract from NI-SFM at 50°C.  
561 After only 10 min, all the phenolic esters were hydrolyzed into free CA, which gave rise  
562 to a concentration of  $68.1 \pm 1.7 \mu\text{mol/g DDM}$  with 95 % hydrolysis yield (Table 4).

563

564 Figure 4 (suggested location)

565 Table 4 (suggested location)

566

567           When hydrolysis was carried out with ChIE at the same temperature (50°C),  
568 chlorogenic acid was hydrolyzed more slowly (30 min instead of 10 min) and was not  
569 completely converted even after 240 min (Fig. 4-B), and only a small fraction of 3-CQA  
570 got hydrolyzed (Table 4, Supplementary data Fig. S2). This typical feature of ChIE,  
571 already observed during I-SFM hydrolysis (see section 3.2.1), could explain why the  
572 highest concentration of CA was only  $55.4 \pm 0.3 \mu\text{mol/g DDM}$  after 60 min incubation,  
573 which corresponded to a hydrolysis yield of only 77 % (Table 4). It is worth noting that,  
574 in both cases (AnFaeB and ChIE), no decrease in the TCAD content was observed over  
575 enzymatic incubation time on methanolic extracts (Fig. 4), conversely to the crude NI  
576 meal (Fig. 3). This might indirectly confirm the presence of phenolic-degrading  
577 enzymes (i.e PPO for instance) in the meal that were removed after phenolic extraction  
578 with methanol.

579           On NI-SFM, the best CA yields were achieved through enzymatic hydrolysis of  
580 methanolic extract, particularly with AnFaeB that allowed fast, selective, and  
581 quantitative production of CA ( $68.1 \pm 1.7 \mu\text{mol/DDM}$  from methanolic extract  
582 hydrolysis against  $52.3 \pm 0.2 \mu\text{mol/g DDM}$  from crude NI-meal hydrolysis).

### 583 **3.4. Enzymatic hydrolysis of sinapic acid esters in NI-RSM and in the** 584 **corresponding methanolic extract with AnFaeA**

585           Hydrolysis conditions (pH, temperature and enzyme concentration) for the  
586 enzymatic hydrolysis of sinapic esters present in an I-RSM (a commercial RSM  
587 thermally treated by cooking at 95-100°C followed by steam desolventizing at about  
588 107°C) was previously established by Odinot *et al.* (2017). These conditions were  
589 implemented on NI-RSM and on the corresponding methanolic extract. NI-RSM was  
590 chosen for its higher total sinapic acid derivatives (TSAD) content compared to the I-  
591 RSM (Table 1).

**592 3.4.1. AnFaeA hydrolysis of NI-RSM**

593 The evolution of the concentration of sinapine (SNP), sinapic acid (SA) and  
594 TSAD during enzymatic hydrolysis of NI-RSM with AnFaeA (39 nkat/g meal, 55°C,  
595 pH 5.5) is charted in Fig. 5A. The maximum amount of SA released was  $41.3 \pm 0.3$   
596  $\mu\text{mol/g DDM}$  after 240 min of incubation with AnFaeA. Almost all the SNP initially  
597 present in the meal ( $31.7 \pm 0.6 \mu\text{mol SAE/g DDM}$ ) (Fig. 5A) and the mono- and di-  
598 sinapoyl glucose isomers (Supplementary data Fig. S3A) were hydrolyzed at the end of  
599 incubation.

600 However, kaempferol-based sinapic acid derivatives, as well as di-sinapoyl  
601 gentiobiosides and some tri-sinapoyl gentiobiosides were not (or partially) hydrolyzed  
602 by the enzyme (supplementary data, Fig.S3A). These results were in agreement with our  
603 previous work on I-RSM (Odinot *et al.*, 2017). TSAD content measured after the 30-  
604 min pre-incubation step (Fig. 5A) was decreased by about 16% compared to the initial  
605 content in the unprocessed meal ( $64.2 \pm 1.2 \mu\text{mol SAE/g DDM}$ ), possibly due to the  
606 activity of endogenous enzymes (possibly PPO) present in the crude meal. In the case of  
607 NI-RSM, no PPO-type activity was detectable in the meal in our assay conditions.  
608 However, it is noteworthy that a laccase-encoding gene (*AtTT10* gene) was identified in  
609 the genome of *Brassica napus* (Zhang *et al.*, 2013). This PPO was involved in  
610 proanthocyanidin polymerization and lignin biosynthesis as well as seed coat  
611 pigmentation. Conversely, this decrease was not observed in enzymatic hydrolysis  
612 applied to an I-RSM (Odinot *et al.*, 2017). Like for I-SFM, but in contrast to NI-RSM,  
613 the thermal treatments (95–107°C) applied to I-RSM seemed to inactivate endogenous  
614 phenolic-degrading enzymes.

615 Figure 5 (suggested location)

616

**617 3.4.2. AnFaeA hydrolysis of the NI-RSM methanolic extract**

618 Like for NI-SFM, enzymatic hydrolysis was also performed on phenolic extracts  
619 from NI-RSM obtained after a sequence of methanolic extraction, drying, then  
620 solubilization in reaction buffer. The time- concentrations of SNP, SA and TSAD  
621 during enzymatic hydrolysis of NI-RSM dry methanolic extract with AnFaeA (39  
622 nkat/g meal, 55°C, pH 5.5) is shown in Fig. 5B. Unexpectedly, the maximum amount of  
623 SA released ( $32.3 \pm 0.4 \mu\text{mol/g DDM}$ ) was lower than the amount obtained from NI-  
624 RSM ( $41.3 \pm 0.3 \mu\text{mol/g DDM}$ ) (Table 5), concomitantly with the appearance of a new  
625 phenolic compound in the reaction medium (Fig. 5B, green bar) that corresponded to a  
626 peak with a retention time of 22.25 min in our HPLC conditions (Supplementary data  
627 Fig. S3B).

Table 5 (suggested location)

628  
629  
630 Based on its retention time in the HPLC chromatogram (Compound **9**,  
631 Supplementary data Fig. S3B), its maximum wavelength absorption of 326 nm, and its  
632 mass and NMR ( $^1\text{H}$ ,  $^{13}\text{C}$ ) spectra (Supplementary data Fig. S4 and Fig. S5), the new  
633 compound was 1,2-di-*O*-sinapoyl- $\beta$ -D-glucose. Therefore, given the presence of  
634 significant amounts of glucose (Naczek & Shahidi, 1990), as well as mono- and di-  
635 sinapoyl glucose isomers in the NI-RSM extract (Laguna *et al.*, 2018), it can be  
636 reasonably assumed that this new compound resulted from transesterification of  
637 sinapine with the aforementioned molecules extracted from the meal. If so, these results  
638 would confirm the well-known acyl transferase activity of AnFaeA (Hatzakis and  
639 Smonou, 2005; Hüttner *et al.*, 2017; Vafiadi *et al.*, 2009). However, the reason why  
640 such a reaction only occurred in the NI-RSM methanolic extract remains to elucidate.  
641 TSAD content measured during enzymatic hydrolysis of the methanolic extract ( $59.2 \pm$   
642  $2.3 \mu\text{mol SAE/DDM}$ ) remained higher than TSAD content measured during meal

643 hydrolysis ( $53.1 \pm 1.0 \mu\text{mol SAE/ DDM}$ ). This might corroborate the presence of  
644 phenolic-degrading endogenous enzymes in the crude meal.

645 Hydrolysis of the NI-RSM methanolic extract was also performed at  $37^\circ\text{C}$  in order  
646 to assess the possible influence of temperature on yield of 1,2-di-*O*-sinapoyl- $\beta$ -D-  
647 glucose synthesis (Fig. 5C). At  $37^\circ\text{C}$ , TSAD concentration ( $65.6 \pm 2.3 \mu\text{mol/ DDM}$ )  
648 measured during hydrolysis remained stable throughout the incubation time at an  
649 equivalent level to the initial content in the unprocessed meal ( $64.2 \pm 1.2 \mu\text{mol SAE/g}$   
650 DDM). The decrease over time of SNP was slightly slower at  $37^\circ\text{C}$  than at  $55^\circ\text{C}$  (Fig.  
651 5C): hydrolysis was complete in less than 120 min at  $55^\circ\text{C}$  whereas 3% of SNP  
652 remained in the medium even after 240 min at  $37^\circ\text{C}$ . Production of 1,2-di-*O*-sinapoyl- $\beta$ -  
653 D-glucose was higher overall (e.g.  $8.0 \pm 0.8 \mu\text{mol SAE/g DDM}$  at  $37^\circ\text{C}$  against  $5.7 \pm$   
654  $0.3 \mu\text{mol SAE/g DDM}$  at  $55^\circ\text{C}$  after 240 min incubation), which may suggest that lower  
655 temperature facilitated acyl transfer more than sinapine hydrolysis, which occurs in both  
656 conditions.

657 Taken together, the results presented in section 3.4 converged to show the best  
658 yield of SA came from hydrolysis performed directly in the crude NI-RSM with  
659 AnFaeA compared to hydrolysis of the corresponding methanolic extract. The  
660 enzymatic treatment of the NI-RSM methanolic extract seemed to be characterized by  
661 acyl transfer reactions that reduce the overall yield of free SA release.

#### 662 4. Conclusion

663 It is possible to obtain free CA from sunflower meal and free SA from rapeseed  
664 meal by alkaline hydrolysis and solvent extraction (Vuorela *et al.*, 2003, Zago *et al.*,  
665 2015b), but in high temperature, strong base and toxic solvent conditions, which runs  
666 counter to a 'green' process. Here we showed that *A. niger* AnFaeB, ChIE and AnFaeA  
667 were very efficient catalysts for the enzymatic release of CA and SA from both  
668 industrial and non-industrial meals. To the best of our knowledge, this is the first time

669 that AnFaeB and ChIE were applied on sunflower meals as raw natural substrates.  
670 AnFaeB was able to hydrolyze all the mono- and di-caffeoylquinic acid isomers,  
671 together with the coumaroylquinic and feruloylquinic acid isomers identified in SFMs,  
672 whereas ChIE was unable to hydrolyze 3-CQA. The non-industrial meals processed  
673 under mild thermal conditions presented the advantage of higher initial phenolics  
674 contents. However, phenolic acid release was hampered by phenolic oxidation side-  
675 reactions, seemingly due to meal endogenous PPO-type activities, which is a major  
676 drawback for industrial applications. Here we found evidence that using an intermediary  
677 step of phenolic extraction with methanol, before enzymatic hydrolysis, could overcome  
678 this drawback for NI-SFM but not NI-RSM due to AnFaeA transesterification side-  
679 reactions. Nevertheless, this acyl transfer activity of AnFaeA could prove beneficial for  
680 the enzymatic synthesis of novel sinapic and ferulic acid esters with sugars or polyols.  
681 Of all the substrates tested for enzymatic hydrolysis, industrial rapeseed meal and non-  
682 industrial sunflower meal extract showed the best compromise between the three  
683 following parameters: initial TPC, hydrolysis yields, and amounts of CA and SA  
684 released. The proof of concept of such an enzymatic hydrolysis process was already  
685 successfully scaled-up in a 10-L bioreactor in the case of FaeA and raw industrial RSM  
686 (Odinot *et al.*, 2017). In addition, regarding enzymatic hydrolysis applied to meal  
687 methanolic extract, the extraction of phenolics by ethanol (less toxic and biosourced  
688 solvent, almost as efficient as methanol) followed by concentration/desolventization  
689 steps could be easily implemented at industrial scale as it is already done with hexane  
690 for instance. In the case of sunflower meal extract, this process may be advantageous  
691 since phenolic concentration as well as enzyme load would be better controlled, limiting  
692 matrix effect in the same time. This work opens new perspectives on the  
693 biotechnological applications of fungal Faes and ChIEs to other hydroxycinnamic acid-  
694 containing raw materials, including green coffee bean wastes, co-products of rapeseed

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695 and sunflower crops (e.g. stems, straws, seed shells, sunflower capitula) as well as other  
696 oilseed plants (mustard, flax).

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## 706 Author Contributions

707 Jérôme Lecomte, Oscar Laguna and Anne Lomascolo conceived, designed, supervised  
708 the experiments, and wrote the paper. Oscar Laguna, Elise Odinet, Alexandra Bisotto  
709 and Bruno Baréa performed the experiments. Frédéric Fine, Jean-Claude Sigoillot and  
710 Pierre Villeneuve contributed to the conception of the work and help to write the final  
711 draft of the paper. Craig B. Faulds and Laurence Lesage-Meessen brought expertise on  
712 feruloyl esterases. Eric Record is at the origin of *Aspergillus niger* recombinant strains.  
713 All the authors contributed to finalize the manuscript.

714

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716

## 717 References

718 Aider, M., Barbana, C., 2011. Canola proteins: composition, extraction, functional  
719 properties, bioactivity, applications as a food ingredient and allergenicity – A

- 720 practical and critical review. Trends Food Sci. Technol. 22, 21–39.  
721 <https://doi.org/10.1016/j.tifs.2010.11.002>
- 722 Aouf, C., Lecomte, J., Villeneuve, P., Dubreucq, E., Fulcrand, H., 2012. Chemo-  
723 enzymatic functionalization of gallic and vanillic acids: synthesis of bio-based  
724 epoxy resins prepolymers. Green Chem. 14, 2328-2336.  
725 <https://doi.org/10.1039/c2gc35558b>
- 726 Asther, Mi., Estrada Alvarado, M.I., Haon, M., Navarro, D., Asther, Ma., Lesage-  
727 Meessen, L., Record, E., 2005. Purification and characterization of a chlorogenic  
728 acid hydrolase from *Aspergillus niger* catalysing the hydrolysis of chlorogenic  
729 acid. J. Biotechnol. 115, 47–56. <https://doi.org/10.1016/j.jbiotec.2004.07.009>
- 730 Baumert, A., Milkowski, C., Schmidt, J., Nimtz, M., Wray, V., Strack, D., 2005.  
731 Formation of a complex pattern of sinapate esters in *Brassica napus* seeds,  
732 catalyzed by enzymes of a serine carboxypeptidase-like acyltransferase family?  
733 Phytochem 66, 1334–1345. <https://doi.org/10.1016/j.phytochem.2005.02.031>
- 734 Benoit, I., Asther, Mi., Bourne, Y., Navarro, D., Canaan, S., Lesage-Meessen, L.,  
735 Herweijer, M., Coutinho, P.M., Asther, Ma., Record, E., 2007. Gene  
736 overexpression and biochemical characterization of the biotechnologically  
737 relevant chlorogenic acid hydrolase from *Aspergillus niger*. Appl. Environ.  
738 Microbiol. 73, 5624–5632. <https://doi.org/10.1128/AEM.00374-07>
- 739 Benoit, I., Navarro, D., Marnet, N., Rakotomanomana, N., Lesage-Meessen, L.,  
740 Sigoillot, J.-C., Asther, Ma., Asther, Mi., 2006. Feruloyl esterases as a tool for the  
741 release of phenolic compounds from agro-industrial by-products. Carbohydr. Res.  
742 341, 1820–1827. <https://doi.org/10.1016/j.carres.2006.04.020>
- 743 Borredon, M.-E., Berger, M., Dauguet, S., Labalette, F., Merrien, A., Mouloungui, Z.,  
744 Raoul, Y., 2011. Débouchés actuels et futures du tournesol produit en France–  
745 Critères de qualité. Innov. Agron. 14, 19–38.

- 746 Brézillon, C., Kroon, P.A., Faulds, C.B., Brett, G.M., Williamson, G., 1996. Novel  
747 ferulic acid esterases are induced by growth of *Aspergillus niger* on sugar-beet  
748 pulp. *Appl. Microbiol. Biotechnol.* 45, 371–376.  
749 <https://doi.org/10.1007/s002530050698>
- 750 Cai, R., Arntfield, S.D., 2001. A rapid high-performance liquid chromatographic  
751 method for the determination of sinapine and sinapic acid in canola seed and  
752 meal. *J. Am. Oil Chem. Soc.* 78, 903–910. [https://doi.org/10.1007/s11746-001-](https://doi.org/10.1007/s11746-001-0362-4)  
753 [0362-4](https://doi.org/10.1007/s11746-001-0362-4)
- 754 Campbell, L., Rempel, C., Wanasundara, J., 2016. Canola/rapeseed protein: future  
755 opportunities and directions—workshop proceedings of IRC 2015. *Plants* 5, 17.  
756 <https://doi.org/10.3390/plants5020017>
- 757 Diot-Néant, F., Migeot, L., Hollande, L., Reano, F.A., Domenek, S., Allais, F., 2017.  
758 Biocatalytic synthesis and polymerization via ROMP of new biobased phenolic  
759 monomers: a greener process toward sustainable antioxidant polymers. *Front.*  
760 *Chem.* 5:126. <https://doi.org/10.3389/fchem.2017.00126>
- 761 Faulds, C.B., 2010. What can feruloyl esterases do for us? *Phytochem. Rev.* 9, 121–132.  
762 <https://doi.org/10.1007/s11101-009-9156-2>
- 763 Faulds, C.B., Williamson, G., 1994. Purification and characterization of a ferulic acid  
764 esterase (FAE-III) from *Aspergillus niger*: specificity for the phenolic moiety and  
765 binding to microcrystalline cellulose *Microbiol.* 140, 779-787.  
766 <https://doi.org/10.1099/00221287-140-4-779>
- 767 Fetzer, A., Herfellner, T., Eisner, P., 2019. Rapeseed protein concentrates for non-food  
768 applications prepared from pre-pressed and cold-pressed press cake via acidic  
769 precipitation and ultrafiltration. *Ind. Crops Prod.* 132, 396-406.  
770 <https://doi.org/10.1016/j.indcrop.2019.02.039>

**Manuscript**

- 771 Fetzer, A., Herfellner, T., Stäbler, A., Menner, M., Eisner, P., 2018. Influence of  
772 process conditions during aqueous protein extraction upon yield from pre-pressed  
773 and cold-pressed rapeseed press cake. *Ind. Crops Prod.* 112, 236-246.  
774 <https://doi.org/10.1016/j.indcrop.2017.12.011>
- 775 Fine, F., Lucas, J.-L., Chardigny, J.-M., Redlingshöfer, B., Renard, M., 2015. Food  
776 losses and waste in the French oilcrops sector. *OCL* 22, A302.  
777 <https://doi.org/10.1051/ocl/2015012>
- 778 González-Pérez, S., Vereijken, J.M., 2007. Sunflower proteins: overview of their  
779 physicochemical, structural and functional properties. *J. Sci. Food Agric.* 87,  
780 2173–2191. <https://doi.org/10.1002/jsfa.2971>
- 781 Gopalan, N., Rodríguez-Duran, L.V., Saucedo-Castaneda, G., Nampoothiri, K.M.,  
782 2015. Review on technological and scientific aspects of feruloyl esterases: A  
783 versatile enzyme for biorefining of biomass. *Bioresour. Technol.* 193, 534–544.  
784 <https://doi.org/10.1016/j.biortech.2015.06.117>
- 785 Gullón, B., Eibes, G., Moreira, M.T., Herrera, R., Labidi, J., Gullón, P., 2018. Yerba  
786 mate waste: a sustainable resource of antioxidant compounds. *Ind. Crops Prod.*  
787 113, 398–405. <https://doi.org/10.1016/j.indcrop.2018.01.064>
- 788 Guo, S., Ge, Y., Na Jom, K., 2017. A review of phytochemistry, metabolite changes,  
789 and medicinal uses of the common sunflower seed and sprouts (*Helianthus*  
790 *annuus* L.). *Chem. Cent. J.* 11:95. <https://doi.org/10.1186/s13065-017-0328-7>
- 791 Halaouli, S., Asther, Mi., Kruus, K., Guo, L., Hamdi, M., Sigoillot, J.-C., Asther, Ma.,  
792 Lomascolo, A., 2005. Characterization of a new tyrosinase from *Pycnopus*  
793 species with high potential for food technological applications. *J Appl Microbiol.*  
794 98, 332-343. <https://doi:10.1111/j.1365-2672.2004.02481.x>

**Manuscript**

- 795 Hatzakis, N.S., Smonou, I., 2005. Asymmetric transesterification of secondary alcohols  
796 catalyzed by feruloyl esterase from *Humicola insolens*. *Bioorganic Chem.* 33,  
797 325–337. <https://doi.org/10.1016/j.bioorg.2005.05.002>
- 798 Hüttner, S., Zezzi Do Valle Gomes, M., Iancu, L., Palmqvist, A., Olsson, L., 2017.  
799 Immobilisation on mesoporous silica and solvent rinsing improve the  
800 transesterification abilities of feruloyl esterases from *Myceliophthora*  
801 *thermophila*. *Bioresour. Technol.* 239, 57–65.  
802 <https://doi.org/10.1016/j.biortech.2017.04.106>
- 803 Ivanova, P., Chalova, V., Uzunova, G., Koleva, L., Manolov, I., 2016. Biochemical  
804 characterization of industrially produced rapeseed meal as a protein source in food  
805 industry. *Agric. Agric. Sci. Procedia* 10, 55–62.  
806 <https://doi.org/10.1016/j.aaspro.2016.09.009>
- 807 Karamać, M., Kosińska, A., Estrella, I., Hernández, T., Dueñas, M., 2012. Antioxidant  
808 activity of phenolic compounds identified in sunflower seeds. *Eur. Food Res.*  
809 *Technol.* 235, 221–230. <https://doi.org/10.1007/s00217-012-1751-6>
- 810 Khattab, R., Eskin, M., Aliani, M., Thiyam, U., 2010. Determination of sinapic acid  
811 derivatives in canola extracts using high-performance liquid chromatography. *J.*  
812 *Am. Oil Chem. Soc.* 87, 147–155. <https://doi.org/10.1007/s11746-009-1486-0>
- 813 Kreps, F., Vrbiková, L., Schmidt, Š., 2014. Industrial rapeseed and sunflower meal as  
814 source of antioxidants. *Int. Journal of Engineering Research and Applications* 4,  
815 45-54.
- 816 Laguerre, M., Bayrasy, C., Lecomte, J., Chabi, B., Decker, E.A., Wrutniak-Cabello, C.,  
817 Cabello, G., Villeneuve, P., 2013. How to boost antioxidants by lipophilization?  
818 *Biochimie* 95, 20–26. <https://doi.org/10.1016/j.biochi.2012.07.018>
- 819 Laguerre, M., López Giraldo, L.J., Lecomte, J., Figueroa-Espinoza, M.-C., Baréa, B.,  
820 Weiss, J., Decker, E.A., Villeneuve, P., 2010. Relationship between

- 821 hydrophobicity and antioxidant ability of “phenolipids” in emulsion: a parabolic  
822 effect of the chain length of rosmarinic acid esters. *J. Agric. Food Chem.* 58, 2869–  
823 2876. <https://doi.org/10.1021/jf904119v>
- 824 Laguerre, M., Wrutniak-Cabello, C., Chabi, B., López Giraldo, L.J., Lecomte, J.,  
825 Villeneuve, P., Cabello, G., 2011. Does hydrophobicity always enhance  
826 antioxidant activity? A cut-off effect of the chain length of functionalized  
827 chlorogenic acid esters on ROS-overexpressing fibroblasts: cut-off effect of  
828 phenolipid antioxidants. *J. Pharm. Pharmacol.* 63, 531–540.  
829 <https://doi.org/10.1111/j.2042-7158.2010.01216.x>
- 830 Laguna, O., Barakat, A., Alhamada, H., Durand, E., Baréa, B., Fine, F., Villeneuve, P.,  
831 Citeau, M., Dauguet, S., Lecomte, J., 2018. Production of proteins and phenolic  
832 compounds enriched fractions from rapeseed and sunflower meals by dry  
833 fractionation processes. *Ind. Crops Prod.* 118, 160–172.  
834 <https://doi.org/10.1016/j.indcrop.2018.03.045>
- 835 Lecomte, J., Giraldo, L.J.L., Laguerre, M., Baréa, B., Villeneuve, P., 2010. Synthesis,  
836 characterization and free radical scavenging properties of rosmarinic acid fatty  
837 esters. *J. Am. Oil Chem. Soc.* 87, 615–620. [https://doi.org/10.1007/s11746-010-](https://doi.org/10.1007/s11746-010-1543-8)  
838 1543-8
- 839 Levasseur, A., Benoit, I., Asther, M., Asther, M., Record, E., 2004. Homologous  
840 expression of the feruloyl esterase B gene from *Aspergillus niger* and  
841 characterization of the recombinant enzyme. *Protein Expr. Purif.* 37, 126–133.  
842 <https://doi.org/10.1016/j.pep.2004.05.019>
- 843 Licata, M., La Bella, S., Lazzeri, L., Matteo, R., Leto, C., Massaro, F., Tuttolomondo,  
844 T., 2018. Agricultural feedstocks of two Brassica oilseed crops and energy  
845 cogeneration with pure vegetable oil for a sustainable short agro-energy chain in

- 846 Sicily (Italy). *Ind. Crops Prod.* 117, 140-148.  
847 <https://doi.org/10.1016/j.indcrop.2018.02.032>
- 848 Lomascolo, A., Uzan-Boukhris, E., Sigoillot, J.-C., Fine, F., 2012. Rapeseed and  
849 sunflower meal: a review on biotechnology status and challenges. *Appl.*  
850 *Microbiol. Biotechnol.* 95, 1105–1114. [https://doi.org/10.1007/s00253-012-4250-](https://doi.org/10.1007/s00253-012-4250-6)  
851 6
- 852 Mailer, R.J., 2008. Anti-nutritional components, fibre, sinapine and glucosinolate  
853 content, in Australian canola (*Brassica napus* L.) meal. *J. Am. Oil Chem. Soc.* v.  
854 85, 937–944. <https://doi.org/10.1007/s11746-008-1268-0>
- 855 McClements, D.J., Decker, E., 2018. Interfacial antioxidants: a review of natural and  
856 synthetic emulsifiers and coemulsifiers that can inhibit lipid oxidation. *J. Agric.*  
857 *Food Chem.* 66, 20–35. <https://doi.org/10.1021/acs.jafc.7b05066>
- 858 Milkowski, C., Strack, D., 2010. Sinapate esters in brassicaceous plants: biochemistry,  
859 molecular biology, evolution and metabolic engineering. *Planta* 232, 19–35.  
860 <https://doi.org/10.1007/s00425-010-1168-z>
- 861 Naczki, M., Shahidi, F., 1990. Carbohydrates of canola and rapeseed. In: Shahidi F.  
862 (eds) canola and rapeseed. Springer, Boston, MA. [https://doi.org/10.1007/978-1-](https://doi.org/10.1007/978-1-4615-3912-4_12)  
863 4615-3912-4\_12
- 864 Odinot, E., Fine, F., Sigoillot, J.-C., Navarro, D., Laguna, O., Bisotto, A., Peyronnet, C.,  
865 Ginies, C., Lecomte, J., Faulds, C., Lomascolo, A., 2017. A two-step  
866 bioconversion process for canolol production from rapeseed meal combining an  
867 *Aspergillus niger* feruloyl esterase and the fungus *Neolentinus lepideus*.  
868 *Microorganisms* 5, 67. <https://doi.org/10.3390/microorganisms5040067>
- 869 Ouerghemmi, I., Bettaieb Rebey, I., Rahali, F.Z., Bourgou, S., Pistelli, L., Ksouri, R.,  
870 Marzouk, B., Saidani Tounsi, M., 2017. Antioxidant and antimicrobial phenolic  
871 compounds from extracts of cultivated and wild-grown Tunisian *Ruta*

- 872 *chalepensis*. J. Food Drug Anal. 25, 350–359.  
873 <https://doi.org/10.1016/j.jfda.2016.04.001>
- 874 Oueslati, S., Ksouri, R., Falleh, H., Pichette, A., Abdelly, C., Legault, J., 2012. Phenolic  
875 content, antioxidant, anti-inflammatory and anticancer activities of the edible  
876 halophyte *Suaeda fruticosa* Forssk. Food Chem. 132, 943–947.  
877 <https://doi.org/10.1016/j.foodchem.2011.11.072>
- 878 Pion, F., Ducrot, P.-H., Allais, F., 2014. Renewable alternating aliphatic-aromatic  
879 copolyesters derived from biobased ferulic acid, diols, and diacids: sustainable  
880 polymers with tunable thermal properties. Macromol. Chem. Phys. 215, 431–439.  
881 <https://doi.org/10.1002/macp.201300702>
- 882 Ramos-de-la-Peña, A.M., Contreras-Esquivel, J.C., 2016. Methods and substrates for  
883 feruloyl esterase activity detection, a review. J. Mol. Catal. B Enzym. 130, 74–87.  
884 <https://doi.org/10.1016/j.molcatb.2016.05.004>
- 885 Reano, A.F., Chérubin, J., Peru, A.M.M., Wang, Q., Clément, T., Domenek, S., Allais,  
886 F., 2015. Structure–activity relationships and structural design optimization of a  
887 series of *p*-hydroxycinnamic acids-based bis- and trisphenols as novel sustainable  
888 antiradical/antioxidant additives. ACS Sustain. Chem. Eng. 3, 3486–3496.  
889 <https://doi.org/10.1021/acssuschemeng.5b01281>
- 890 Record, E., Asther, Mi., Sigoillot, C., Pagès, S., Punt, P.J., Delattre, M., Haon, M., van  
891 den Hondel, C.A.M.J.J., Sigoillot, J.-C., Lesage-Meessen, L., Asther, Ma., 2003.  
892 Overproduction of the *Aspergillus niger* feruloyl esterase for pulp bleaching  
893 application. Appl. Microbiol. Biotechnol. 62, 349–355.  
894 <https://doi.org/10.1007/s00253-003-1325-4>
- 895 Shahidi, F., Ambigaipalan, P., 2015. Phenolics and polyphenolics in foods, beverages  
896 and spices: antioxidant activity and health effects – A review. J. Funct. Foods 18,  
897 820–897. <https://doi.org/10.1016/j.jff.2015.06.018>

**Manuscript**

- 898 Shahidi, F., Yeo, J., 2016. Insoluble-bound phenolics in food. *Molecules* 21, 1216.  
899 <https://doi.org/10.3390/molecules21091216>
- 900 Siger, A., Czubinski, J., Dwiecki, K., Kachlicki, P., Nogala-Kalucka, M., 2013.  
901 Identification and antioxidant activity of sinapic acid derivatives in *Brassica*  
902 *napus* L. seed meal extracts: main phenolic compounds in rapeseed. *Eur. J. Lipid*  
903 *Sci. Technol.* 115, 1130-1138. <https://doi.org/10.1002/ejlt.201300077>
- 904 Sørensen, A.-D.M., Lyneborg, K.S., Villeneuve, P., Jacobsen, C., 2015. Alkyl chain  
905 length impacts the antioxidative effect of lipophilized ferulic acid in fish oil  
906 enriched milk. *J. Funct. Foods* 18, 959–967.  
907 <https://doi.org/10.1016/j.jff.2014.04.008>
- 908 Sripad, G., and Narasinga Rao M.S., 1987. Effect of methods to remove polyphenols  
909 from sunflower meal on the physicochemical properties of the proteins. *J. Agric.*  
910 *Food Chem.*, 1987, 35, 962–967. <https://doi.org/10.1021/jf00078a025>
- 911 Suriyarak, S., Bayrasy, C., Schmidt, H., Villeneuve, P., Weiss, J., 2013. Impact of fatty  
912 acid chain length of rosmarinate esters on their antimicrobial activity against  
913 *Staphylococcus carnosus* LTH1502 and *Escherichia coli* K-12 LTH4263. *J. Food*  
914 *Protection* 76, 1539-1548. <https://doi.org/10.4315/0362-028X.JFP-12-254>
- 915 Szydłowska-Czerniak, A., Rabiej, D., Krzemiński, M., 2018. Synthesis of novel octyl  
916 sinapate to enhance antioxidant capacity of rapeseed-linseed oil mixture: octyl  
917 sinapate as a new lipophilic antioxidant. *J. Sci. Food Agric.* 98, 1625–1631.  
918 <https://doi.org/10.1002/jsfa.8637>
- 919 Szydłowska-Czerniak, A., Trokowski, K., Szlyk, E., 2011. Optimization of extraction  
920 conditions of antioxidants from sunflower shells (*Helianthus annuus* L.) before  
921 and after enzymatic treatment. *Ind. Crops Prod.* 33, 123–131.  
922 <https://doi.org/10.1016/j.indcrop.2010.09.016>

**Manuscript**

- 923 Tong, J., Ma, B., Ge, L., Mo, Q., Zhou, G., He, J., Wang, Y., 2015. DicaFFEoylquinic  
924 acid-enriched fraction of *Cichorium glandulosum* seeds attenuates experimental  
925 type 1 diabetes via multipathway protection. *J. Agric. Food Chem.* 63, 10791–  
926 10802. <https://doi.org/10.1021/acs.jafc.5b04552>
- 927 Ugolini, L., Cinti, S., Righetti, L., Stefan, A., Matteo, R., D'Avino, L., Lazzeri, L.,  
928 2015. Production of an enzymatic protein hydrolyzate from defatted sunflower  
929 seed meal for potential application as a plant biostimulant. *Ind. Crops Prod.* 75,  
930 15–23. <https://doi.org/10.1016/j.indcrop.2014.11.026>
- 931 Vafiadi, C., Topakas, E., Nahmias, V.R., Faulds, C.B., Christakopoulos, P., 2009.  
932 Feruloyl esterase-catalysed synthesis of glycerol sinapate using ionic liquids  
933 mixtures. *J. Biotechnol.* 139, 124–129.  
934 <https://doi.org/10.1016/j.jbiotec.2008.08.008>
- 935 Vuorela, S., Meyer, A.S., Heinonen, M., 2003. Quantitative analysis of the main  
936 phenolics in rapeseed meal and oils processed differently using enzymatic  
937 hydrolysis and HPLC. *Eur. Food Res. Technol.* 217, 517–523.  
938 <https://doi.org/10.1007/s00217-003-0811-3>
- 939 Wang, S., Tateyama, S., Kaneko, D., Ohki, S., Kaneko, T., 2011. Synthesis of well-  
940 defined hyperbranched polymers bio-based on multifunctional phenolic acids and  
941 their structure–thermal property relationships. *Polym. Degrad. Stab.* 96, 2048–  
942 2054. <https://doi.org/10.1016/j.polymdegradstab.2011.10.003>
- 943 Weisz, G.M., Kammerer, D.R., Carle, R., 2009. Identification and quantification of  
944 phenolic compounds from sunflower (*Helianthus annuus* L.) kernels and shells by  
945 HPLC-DAD/ESI-MSn. *Food Chem.* 115, 758–765.  
946 <https://doi.org/10.1016/j.foodchem.2008.12.074>
- 947 Wildermuth, S.R., Young, E.E., Were, L.M., 2016. Chlorogenic acid oxidation and its  
948 reaction with sunflower proteins to form green-colored complexes: chlorogenic

- 949 acid oxidation. *Compr. Rev. Food Sci. Food Saf.* 15, 829–843.  
950 <https://doi.org/10.1111/1541-4337.12213>
- 951 Zago, E., Durand, E., Barouh, N., Lecomte, J., Villeneuve, P., Aouf, C., 2015a.  
952 Synthesis of lipophilic antioxidants by a lipase-B-catalyzed addition of peracids to  
953 the double bond of 4-vinyl-2-methoxyphenol. *J. Agric. Food Chem.* 63, 9069–  
954 9075. <https://doi.org/10.1021/acs.jafc.5b03551>
- 955 Zago, E., Lecomte, J., Barouh, N., Aouf, C., Carré, P., Fine, F., Villeneuve, P., 2015b.  
956 Influence of rapeseed meal treatments on its total phenolic content and  
957 composition in sinapine, sinapic acid and canolol. *Ind. Crops Prod.* 76, 1061–  
958 1070. <https://doi.org/10.1016/j.indcrop.2015.08.022>
- 959 Zhang, K., Lu, K., Qu, C., Liang, Y., Wang, R., Chai, Y., Li, J. 2013. Gene silencing of  
960 *BnTT10* family genes causes retarded pigmentation and lignin reduction in the  
961 seed coat of *Brassica napus*. *Plos One.* 8, e61247.  
962 <https://doi.org/10.1371/journal.pone.0061247>

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#### 967 **Figures captions**

968

969 **Fig. 1.** HPLC chromatogram of industrial SFM methanolic extract at 326 nm. Main identified  
970 phenolics: 3-*O*-caffeoylquinic acid (3-CQA) (1), 4-*O*-caffeoylquinic acid (4-CQA) (2), 5-*O*-  
971 caffeoylquinic acid (5-CQA) (3), caffeic acid (CA) (4), 5-*O*-coumaroylquinic acid (5-CoQA)  
972 (5), 5-*O*-feruloylquinic acid (5-FQA), (6), 3,5-di-*O*-caffeoylquinic acid (3,5-diCQA) (7) and  
973 4,5-di-*O*-caffeoylquinic acid (4,5-diCQA) (8).

974

975 **Fig. 2.** Hydrolysis kinetics of the main CAEsters in industrial SFM, as a function of enzyme  
976 type: AnFaeB (**A**); ChIE (**B**), and enzyme concentration. CAEsters: 4- and 5-*O*-caffeoylquinic  
977 acid (4- and 5-CQA, **1**); 3-*O*-caffeoylquinic acid (3-CQA, **2**); 3,5-di-*O*-caffeoylquinic acid (3,5-  
978 diCQA, **3**), 4,5-di-*O*-caffeoylquinic acid (4,5-diCQA, **4**) and caffeic acid (CA, **5**). Hydrolysis  
979 conditions: pH6, 50°C. Before starting the enzymatic hydrolysis, the reaction mixture was pre-  
980 incubated for 30 min for homogenization. Values are mean  $\pm$  SD (n=2) and expressed as caffeic  
981 acid equivalents (CAE). For each series, values followed by same superscript letters are not  
982 significantly different,  $p \leq 0.05$ .

983

984 **Fig. 3.** Time course of 4- and 5-*O*-caffeoylquinic acid (4- and 5-CQA), caffeic acid (CA) and  
985 total caffeic acid derivatives (TCAD) concentrations during enzymatic hydrolysis with **AnFaeB**  
986 (pH6, 30 nkat/g meal) (**A**) and **ChIE** (pH6, 10 nkat/g meal) (**B**), applied to non-industrial SFM  
987 at 50°C. Before starting the enzymatic hydrolysis, the reaction mixture was pre-incubated for 30  
988 min for homogenization. Initial PC: Initial phenolic concentration in non-industrial SFM.  
989 Values are mean  $\pm$  SD (n=2) and expressed as caffeic acid equivalents (CAE). Values followed  
990 by same superscript letters are not significantly different,  $p \leq 0.05$

991

992 **Fig. 4.** Time course of 4- and 5-*O*-caffeoylquinic acid (4- and 5-CQA), caffeic acid (CA) and  
993 total caffeic acid derivatives (TCAD) concentrations during enzymatic hydrolysis with **AnFaeB**  
994 (pH6, 30 nkat/g meal) (**A**) and **ChIE** (pH6, 10 nkat/g meal) (**B**), applied to non-industrial SFM  
995 **methanolic extract** at 50°C. Before starting the enzymatic hydrolysis, the reaction mixture was  
996 pre-incubated for 30 min for homogenization. Initial PC: Initial phenolic concentration in non-  
997 industrial SFM methanolic extracts. Values are mean  $\pm$  SD (n=2) and expressed as caffeic acid  
998 equivalents (CAE). Values followed by same superscript letters are not significantly different,  $p$   
999  $\leq 0.05$ .

1000

1001 **Fig. 5.** Time course of sinapine (SNP), sinapic acid (SA), 1,2-di-*O*-sinapoyl- $\beta$ -D-glucose (1,2-  
1002 diSG) and total sinapic acid derivatives (TSAD) concentrations during enzymatic hydrolysis  
1003 with **AnFaeA** (pH5.5, 39 nkat/g meal) applied to non-industrial RSM at 55°C (**A**), and to non-

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1004 industrial RSM methanolic extract at either 55°C (**B**) or 37°C (**C**). Before starting the enzymatic  
1005 hydrolysis, the reaction mixture was pre-incubated for 30 min for homogenization. Initial PC:  
1006 Initial phenolic concentration in non-industrial RSM (**A**) or in non-industrial RSM methanolic  
1007 extract (**B**, **C**). Values are mean  $\pm$  SD (n=2) and expressed as sinapic acid equivalents (SAE).  
1008 Values followed by same superscript letters are not significantly different,  $p \leq 0.05$ .

1009

1010 **Supplementary data. Fig. S1.** Chromatograms obtained after the enzymatic hydrolysis of the  
1011 industrial SFM with 20 nkat AnFaeB/g meal (**A**) and 30 nkat ChIE/g meal (**B**) at  $t = 0$  min (red)  
1012 and  $t = 240$  min (black) at 326 nm. 3-*O*-caffeoylquinic acid (3-CQA) (**1**), 4-*O*-caffeoylquinic  
1013 acid (4-CQA) (**2**), 5-*O*-caffeoylquinic acid (5-CQA) (**3**), caffeic acid (CA) (**4**), 5-*O*-  
1014 coumaroylquinic acid (5-CQA) (**5**), 5-*O*-feruloylquinic acid (5-FQA)(**6**), 3,5-di-*O*-  
1015 caffeoylquinic acid (3,5-diCQA) (**7**) and 4,5-di-*O*-caffeoylquinic acid (4,5-diCQA) (**8**).

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1017 **Supplementary data Fig. S2.** Time course of 3-*O*-caffeoylquinic acid (3-CQA), 3,5-di-*O*-  
1018 caffeoylquinic acid (3,5-diCQA) and 4,5-di-*O*-caffeoylquinic acid (4,5-diCQA) during  
1019 enzymatic treatment with **ChIE** (pH6, 10 nkat/g meal) applied to non-industrial SFM at 50°C  
1020 (**A**), and to non-industrial SFM methanolic extract at 50°C (**B**). Before starting the enzymatic  
1021 hydrolysis, the reaction mixture was pre-incubated for 30 min for homogenization. Initial PC:  
1022 Initial phenolic concentration in non-industrial SFM. Values are mean  $\pm$  SD (n=2) and  
1023 expressed in caffeic acid equivalents (CAE). Values followed by same superscript letters are not  
1024 significantly different,  $p \leq 0.05$ .

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1026 **Supplementary data Fig. S3.** HPLC chromatograms obtained from the enzymatic hydrolysis of  
1027 the non-industrial RSM (**A**) and the corresponding methanolic extract (**B**) with 39 nkat  
1028 AnFaeA/g meal at  $t = 0$  (red) and  $t = 240$  min (black) at 323 nm. Sinapoyl glucose isomer (**1**),  
1029 Sinapine (SNP) (**2**), Kaempferol-based sinapic esters (**3**), *trans*-SA (**4**), *cis*-SA (**5**), di-sinapoyl-  
1030 gentiobioside isomers (**6**), di-sinapoyl glucose isomer (**7**), tri-sinapoyl gentiobioside isomers (**8**)  
1031 and 1,2-di-*O*-sinapoyl- $\beta$ -D-glucose (**9**).

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1033 **Supplementary data Fig. S4.** Chemical structure,  $^{13}\text{C}$ -NMR (125 MHz, DMSO- $d_6$ ) and  $^1\text{H}$ -  
1034 NMR (500 MHz, DMSO- $d_6$ ) data for 1,2-di-*O*-sinapoyl- $\beta$ -D-glucose

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1036 **Supplementary data Fig. S5.** Mass spectrum (negative mode) and fragmentation scheme for  
1037 1,2-di-*O*-sinapoyl- $\beta$ -D-glucose

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**Table 1.** Lipid and moisture content of SFM and RSM samples<sup>#</sup>.

	Lipid content (% DM)	Moisture (% DM)
NI-SFM	1.5 ± 0.0	7.3 ± 0.8
I-SFM	1.9 ± 0.3	10.5 ± 0.7
NI-RSM	1.4 ± 0.0	13.1 ± 0.3
I-RSM	1.5 ± 0.06	10 ± 0.1

<sup>#</sup> Values are mean ± SD (n=3).

**Table 2.** Phenolic content of Non-Industrial and Industrial SFM<sup>#</sup>

	<b>NI-SFM</b>		<b>I-SFM</b>	
	Before alkaline hydrolysis	After alkaline hydrolysis	Before alkaline hydrolysis	After alkaline hydrolysis
Chlorogenic acid ( $\mu\text{mol CAE/g DDM}$ )	$55.8 \pm 0.9^b$	n.d.	$41.4 \pm 2.5^c$	n.d.
Caffeic acid ( $\mu\text{mol/g DDM}$ )	$1.2 \pm 0.0^e$	n.d.	$2.1 \pm 0.5^d$	n.d.
TCAD* ( $\mu\text{mol CAE/g DDM}$ )	$71.7 \pm 0.5^a$	n.d.	$57.4 \pm 1.5^b$	n.d.

<sup>#</sup>Values are mean  $\pm$  SD (n=3). Values followed by same superscript letters are not significantly different,  $p \leq 0.05$ .

\* TCAD: total caffeic acid derivatives in SFM, expressed in  $\mu\text{mol CAE/g DDM}$ . CAE: caffeic acid equivalents. DDM: defatted dry matter.

**Table 3.** Phenolic content of Non-Industrial and Industrial RSM<sup>#</sup>.

	<b>NI-RSM</b>		<b>I-RSM</b>	
	Before alkaline hydrolysis	After alkaline hydrolysis	Before alkaline hydrolysis	After alkaline hydrolysis
Sinapine ( $\mu\text{mol SAE/g DDM}$ )	$31.7 \pm 0.7^c$	--	$21.6 \pm 0.2^d$	--
Sinapic acid ( $\mu\text{mol/g DDM}$ )	$3.9 \pm 0.1^e$	$62.5 \pm 1.8^a$	$2.2 \pm 0.0^f$	$46.8 \pm 3.0^b$
TSAD* ( $\mu\text{mol SAE/g DDM}$ )	$64.2 \pm 1.2^a$	$62.5 \pm 1.8^a$	$44.8 \pm 0.2^b$	$46.8 \pm 3.0^b$

<sup>#</sup> Values are mean  $\pm$  SD (n=3). Values followed by same superscript letters are not significantly different,  $p \leq 0.05$ .

\* TSAD: total sinapic acid derivatives in RSM, expressed in  $\mu\text{mol SAE/g DDM}$ . SAE: sinapic acid equivalents. DDM: defatted dry matter.

**Table 4.** Concentration of caffeic acid (CA), CAEsters and total caffeic acid derivatives (TCAD) before and after hydrolysis of NI-SFM methanolic extract with AnFaeB and ChIE at 50°C<sup>#</sup>.

	Initial content in the meal	AnFaeB	ChIE
4- and 5-CQA ( $\mu\text{mol CAE/g DDM}$ )	$55.8 \pm 0.9^c$	--	$2.0 \pm 0.1^h$
3-CQA ( $\mu\text{mol CAE/g DDM}$ )	$8.9 \pm 0.4^e$	--	$7.8 \pm 0.1^f$
3,5-di-CQA ( $\mu\text{mol CAE/g DDM}$ )	$4.0 \pm 0.2^g$	--	$0.6 \pm 0.0^j$
4,5-di-CQA ( $\mu\text{mol CAE/g DDM}$ )	$1.8 \pm 0.1^g$	--	--
CA ( $\mu\text{mol/g DDM}$ )	$1.2 \pm 0.0^i$	$68.1 \pm 1.7^{ab}$	$55.4 \pm 0.3^{cd}$
TCAD* ( $\mu\text{mol CAE/g DDM}$ )	$71.7 \pm 0.5^a$	$68.1 \pm 1.7^{ab}$	$65.8 \pm 0.4^b$
CA yield (%) based on initial TCAD in meal	--	95.0	77.2
CA yield (%) based on TCAD at t = 0 of enzyme incubation <sup>§</sup>	--	100.4	84.2

\* TCAD: total caffeic acid derivatives; CAE: caffeic acid equivalent. DDM: defatted dry matter. Values are mean  $\pm$  SD (n=2). Values followed by same superscript letters are not significantly different,  $p \leq 0.05$ .

<sup>#</sup> 10 min incubation for AnFaeB, 60 min incubation for ChIE.

<sup>§</sup> The substrate was pre-incubated for 30 min in MOPS buffer before adding enzyme (t = 0 of enzyme incubation).

**Table 5.** Maximal amount and yield of sinapic acid (SA) after enzymatic treatment of NI-RSM and the corresponding methanolic extract with AnFaeA.

	NI-RSM 55°C	NI-RSM methanolic extract, 55°C	NI-RSM methanolic extract, 37°C
TSAD* at t = 0 of enzyme incubation <sup>§</sup> ( $\mu\text{mol SAE/g DDM}$ )	53.1 $\pm$ 1.0 <sup>c</sup>	59.2 $\pm$ 2.3 <sup>b</sup>	65.6 $\pm$ 2.3 <sup>a</sup>
Maximum SA released after 240 min incubation with enzyme ( $\mu\text{mol/g DDM}$ )	41.3 $\pm$ 0.3 <sup>d</sup>	32.3 $\pm$ 0.4 <sup>e</sup>	30.6 $\pm$ 1.2 <sup>e</sup>
SA yield (%) based on initial TSAD in meal	64.3	50.3	47.7
SA yield (%) based on TSAD at t = 0 of enzyme incubation <sup>§</sup>	77.7	54.6	46.6

\* TSAD: total sinapic acid derivatives; SAE: sinapic acid equivalent. DDM: defatted dry matter. Values are mean  $\pm$  SD (n=2). Values followed by same superscript letters are not significantly different,  $p \leq 0.05$

<sup>§</sup> The substrate was pre-incubated for 30 min in MOPS buffer before adding enzyme (t = 0 of enzyme incubation).

Fig. 1

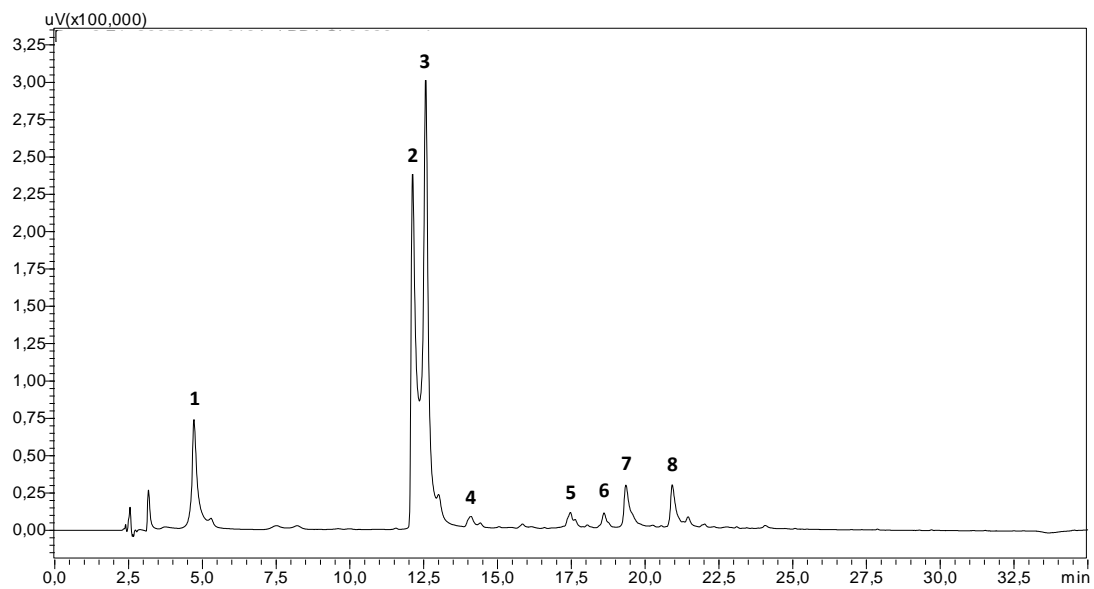
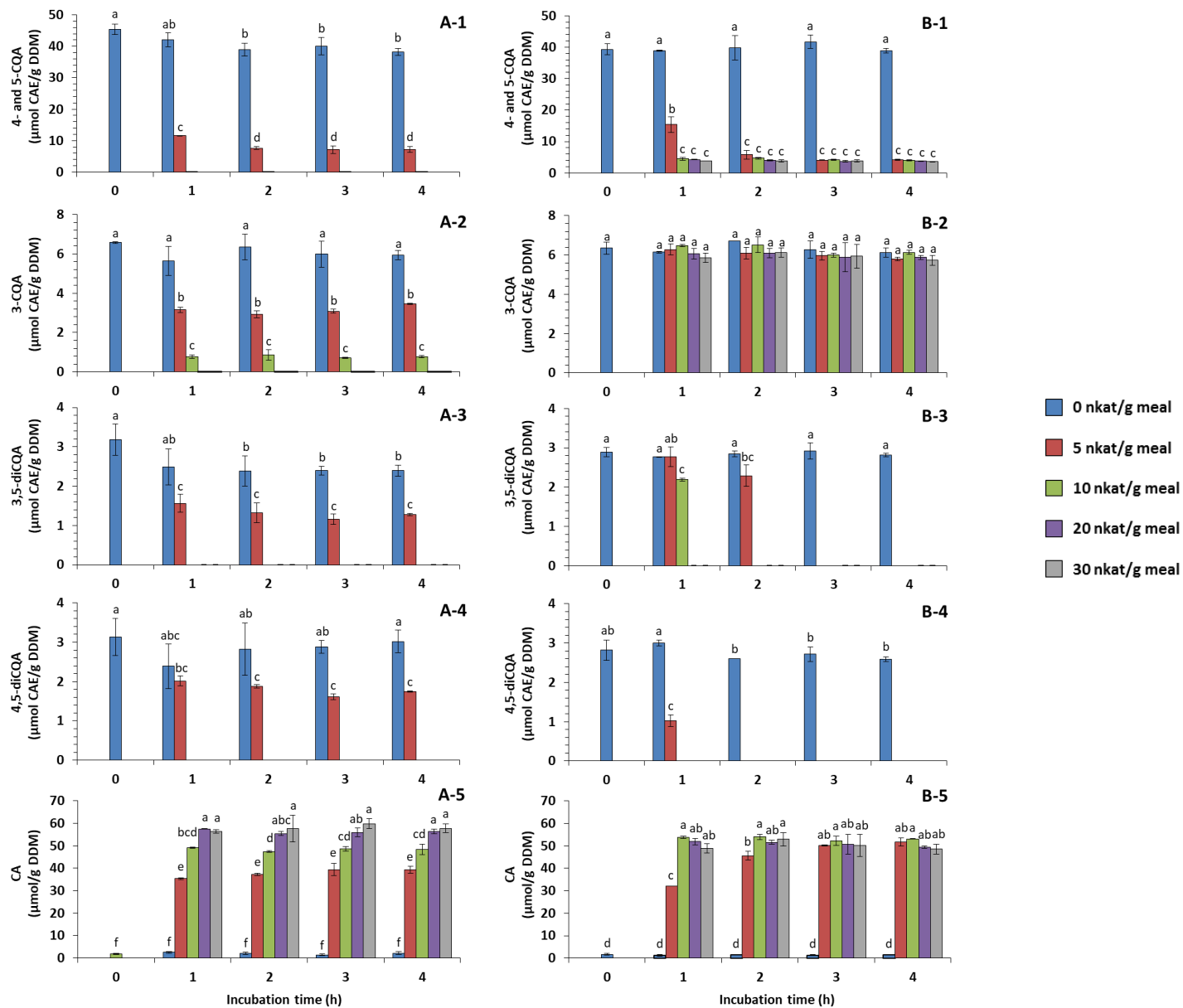


Fig. 2



**Fig. 3**

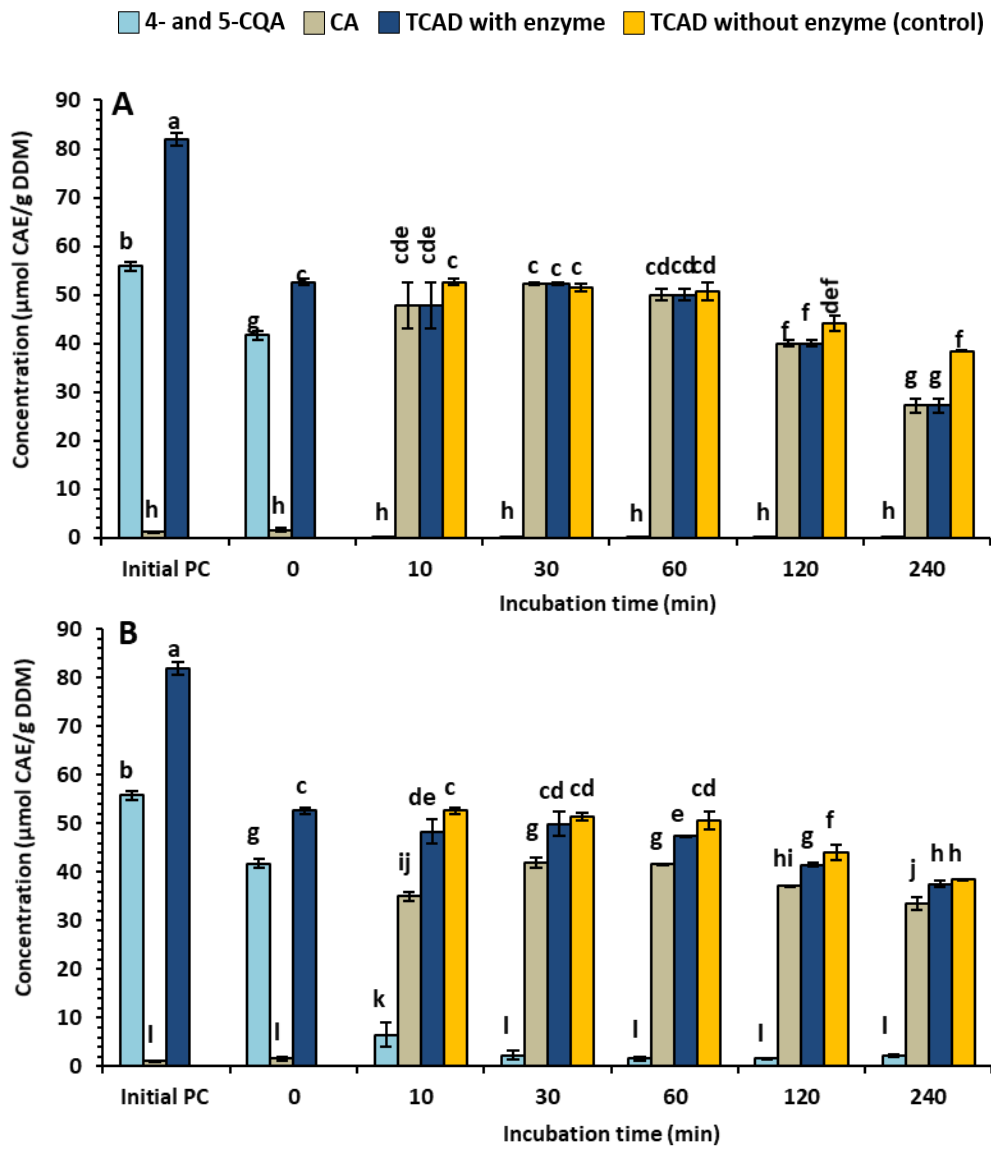


Fig. 4

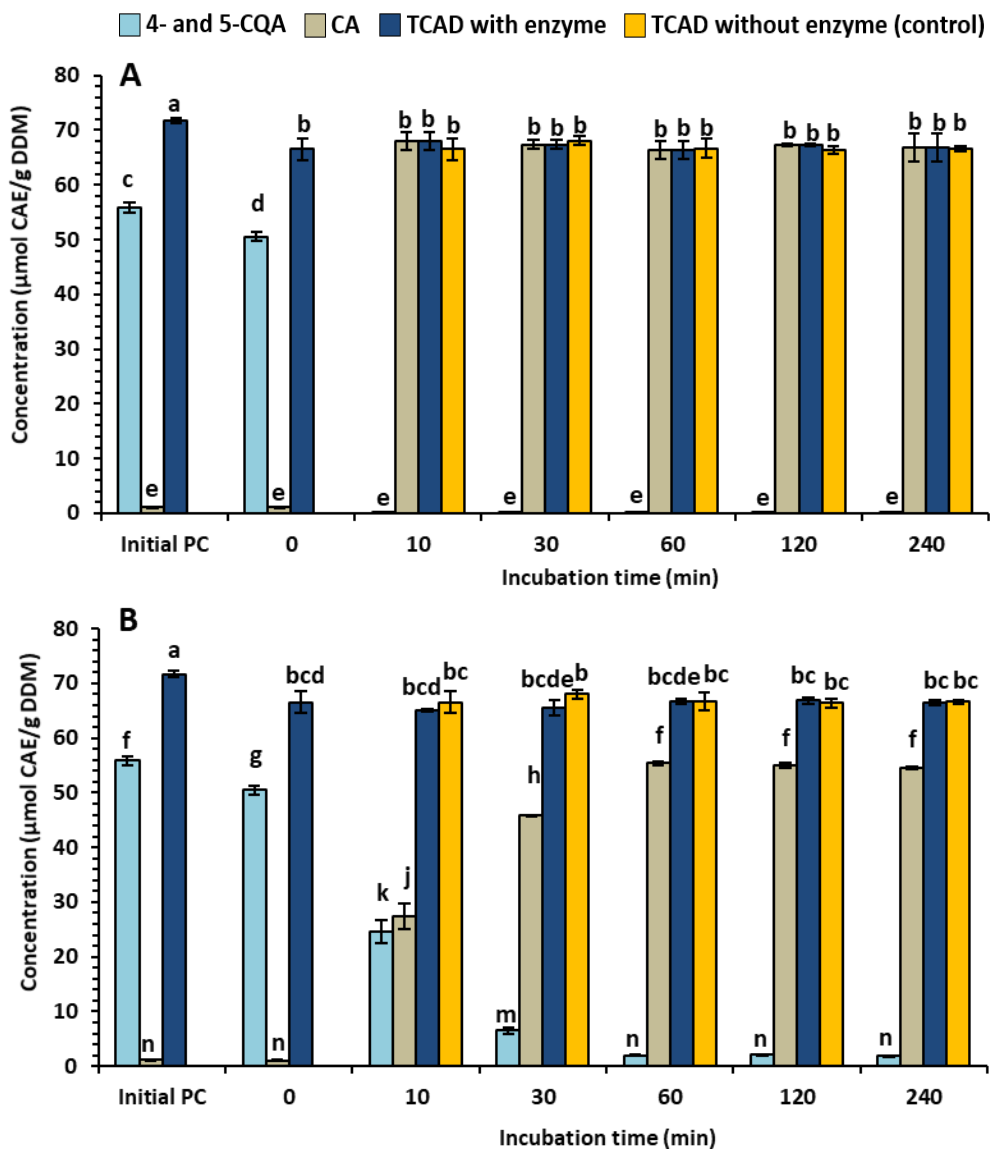
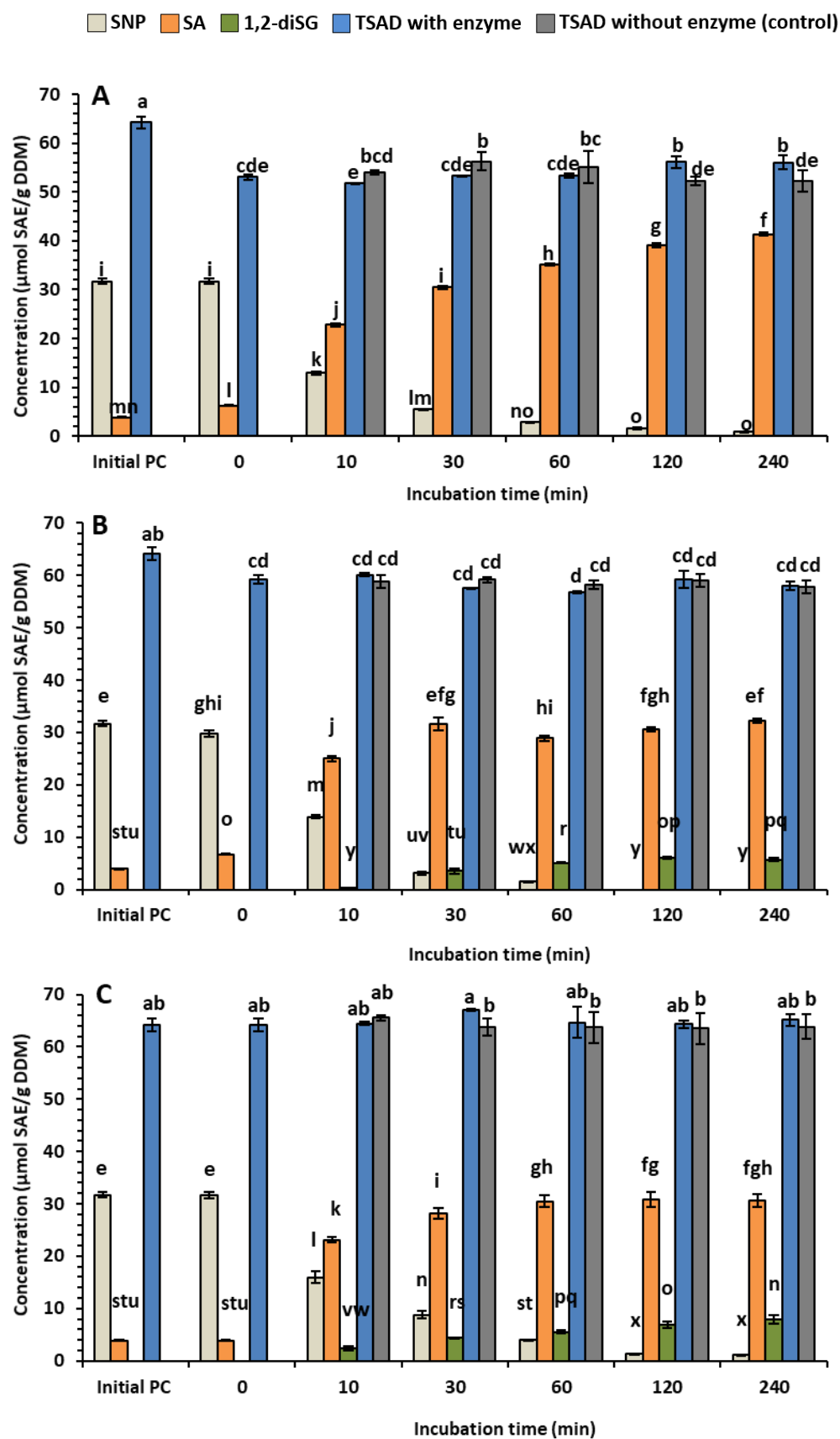
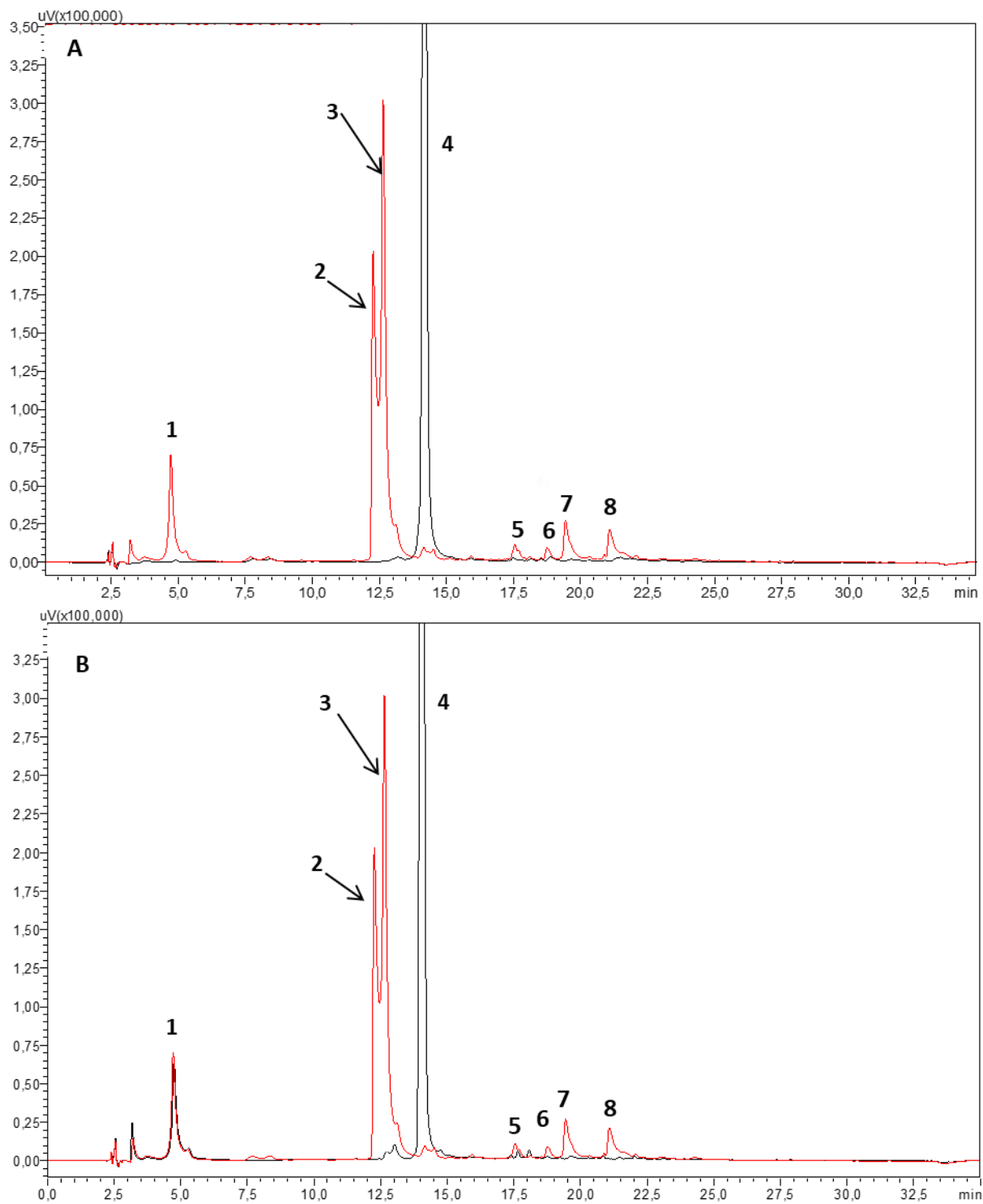


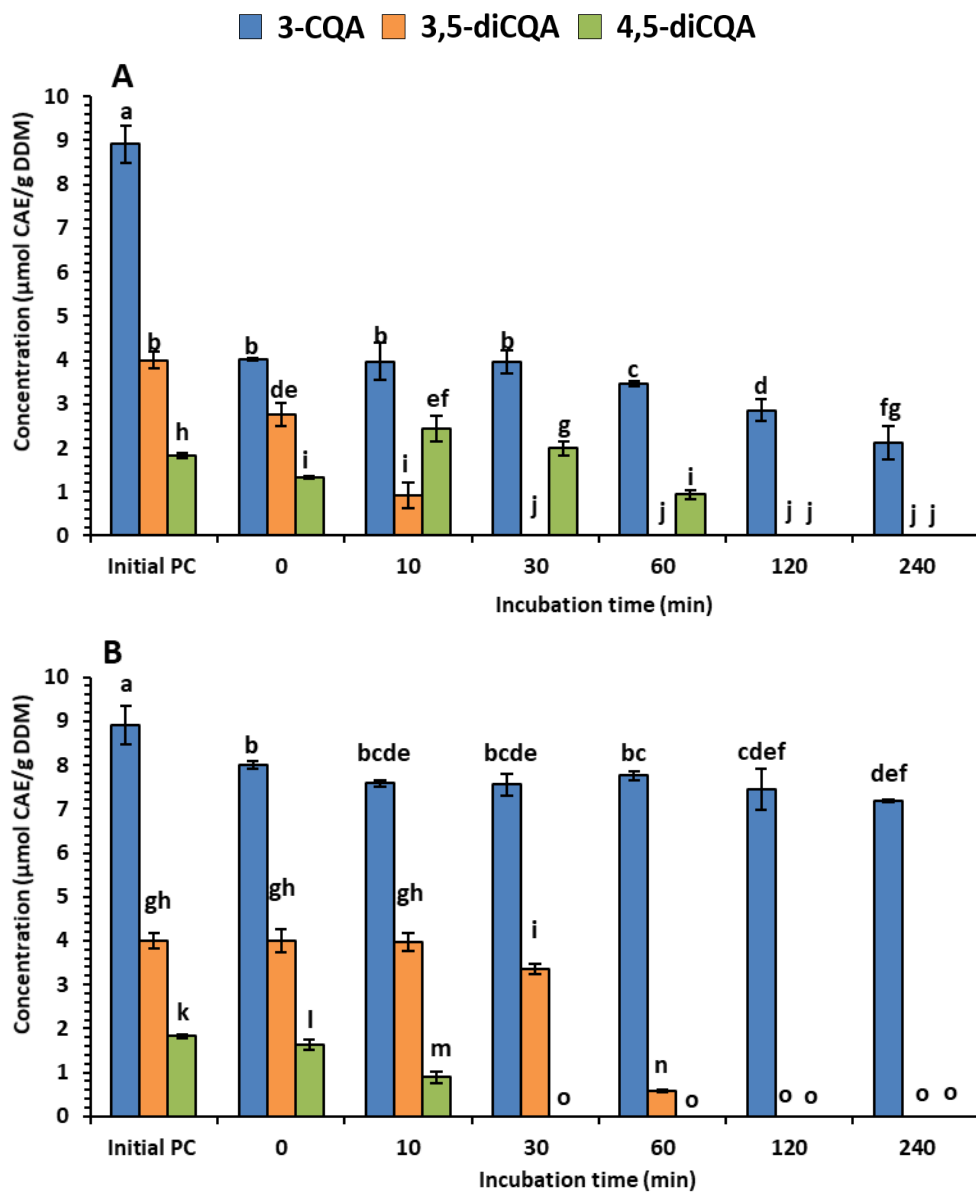
Fig. 5



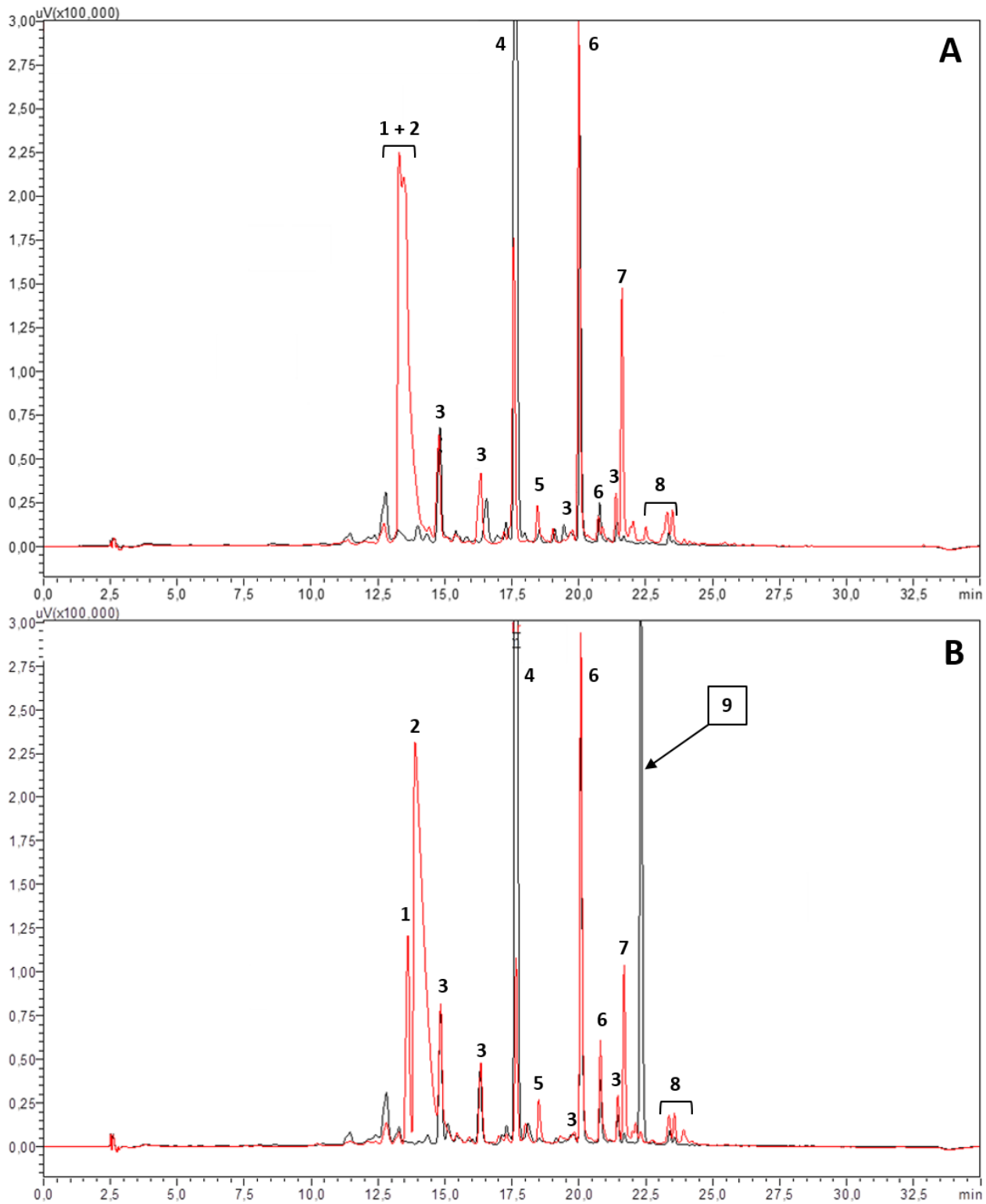
### Supplementary data. Fig. S1



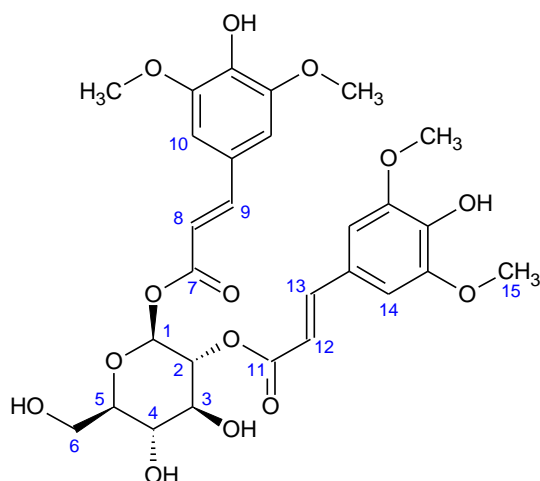
## Supplementary data. Fig. S2



### Supplementary data. Fig. S3



## Supplementary data. Fig. S4



n°	( $\delta$ /ppm) $^{13}\text{C}$	type	( $\delta$ /ppm) $^1\text{H}$	Multiplicity
1	92.0	CH	5.76	d*, $J = 8.4$ Hz
2	72.7	CH	4.90	dd*, $J = 8.4, 9.2$ Hz
3	74.1	CH	3.57	t*, $J = 9.2$ Hz
4	69.8	CH	3.31	m*
5	78.1	CH	3.40	m
6	60.4	CH <sub>2</sub>	3.71	m
			3.52	dd, $J = 5.4, 12.0$ Hz
7	165.1	CO	-	-
8	113.4	CH	6.47	d, $J_{\text{trans}} = 15.8$ Hz
9	147.4	CH	7.56	d, $J_{\text{trans}} = 15.8$ Hz
10	106.6	CH	7.00	s*
11	165.7	CO	-	-
12	114.6	CH	6.52	d, $J_{\text{trans}} = 15.8$ Hz
13	145.9	CH	7.54	d, $J_{\text{trans}} = 15.8$ Hz
14	106.3	CH	6.98	s
15	56.2	CH <sub>3</sub>	3.78-3.77	s

Supplementary data. Fig. S5

