TITLE: Utilizing raw rapeseed press cake in foods: A case study on sensory quality and profile of selected bitter compounds in snack bars

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ABSTRACT:

Today's canola quality rapeseed press cake (RPC) is a protein-rich co-product with potential as human food, but its application is limited due to antinutritional compounds and bitter taste. It remains, however, unknown how introduction of raw RPC to a food matrix affects sensory perception and which metabolites drive the sensation. Here, raw RPC from whole or dehulled seeds was introduced into snack bars at 0%, 7%, 14%, and 21%, and sensory responses were correlated to selected known RPC-derived bitter compounds. A trained panel evaluated 13 RPC-characteristic sensory attributes, and the bitter compounds sinapic acid, kaempferol 3-O-(2‴-O-sinapoyl-β-sophoroside) (KSS), KSS-hexose, selected bitter glucosinolates, and goitrin were quantified using targeted LC–MS/MS. Most dose-dependent sensory responses increased up to 14% RPC and then plateaued, whereas astringent mouthfeel increased almost linearly across the full dose range. Dehulling intensified several odor- and flavor-related attributes but did not increase bitterness or protein content in the final product. Principal component analysis linked bitterness and astringency positively with KSS, KSS-hexose, and goitrin. Dose-over-threshold analysis further showed that goitrin, but not progoitrin, reached concentrations relevant for bitterness perception. Together, the results demonstrate that raw RPC contributes distinct dose-dependent sensory attributes and that metabolite transformations in the food matrix shape final sensory profiles. These findings provide a basis for developing RPC-containing foods and for breeding rapeseed lines with improved sensory characteristics.

KEYWORDS:

Rapeseed press cake, sensory analysis, bitter compounds, dehulling, protein food, goitrin, kaempferol 3-O-(2‴-O-sinapoyl-β-sophoroside).

HIGHLIGHTS:

- This study presents the first sensory panel assessment of rapeseed press cake (RPC)-containing in food products (snack bars) made from whole and dehulled seeds.
- 13 RPC-characteristic sensory attributes are identified.
- Sensory profiles of the tasted snack bars differed significantly, influenced by the dosage of RPC and by the dehulling treatment. Bitterness and astringency are positively correlated with the RPC dosage.

- Goitrin, kaempferol 3-O-(2‴-O-sinapoyl-β-sophoroside) (KSS) and sinapic acid are RPC-derived bitter compounds that correlate with bitter taste of RPC-containing snack bars.
- Approximately 90% of glucosinolates introduced with the RPC are not detected in the snack bars, and goitrin levels in snack bars accounts for only ~10% of introduced progoitrin.
- Goitrin is – for the first time – reported to contribute to the perceived bitterness of an RPC-containing food product.

52 **1. INTRODUCTION**

53 The Planetary Health Diet calls for a substantial shift from animal- to plant-based protein
54 foods to achieve benefits both for human and planetary health (Willett et al., 2019). Rapeseed
55 (*Brassica napus L.*) is the world's second-largest oilseed crop and the rapeseed press cake
56 (RPC) that remains after oil extraction is a protein-rich (30-40%) side stream that today is
57 primarily used as feed and largely unexploited as protein source for human consumption
58 (Nwokolo & Bragg, 1978; Wiąz et al., 2005). Application of raw, unprocessed RPC from
59 today's double low elite lines in foods faces two challenges. Firstly, the raw RPC is not
60 approved as food in EU due to high levels of anti-nutritional glucosinolates (EFSA NDA
61 Panel, 2023; Thorsen et al., 2025). Secondly, consumers may regard RPC as feed with
62 undesirable bitterness as evidenced in a Polish study, in which consumers tasted biscuits
63 containing up to 20% RPC (Szydłowska-Czerniak et al., 2021).

64 Key metabolites associated with bitterness of seeds from rapeseed have been identified. This
65 includes the phenolic sinapine and sinapic acid, the former present at ~1-2% of rapeseed
66 seeds (Ismail et al., 1981; Schwarze, 1949). Schwarze (1949) reported that sinapine (the
67 choline ester derivative of sinapic acid) was key for bitterness of raw RPC. This was later
68 confirmed by Ismail et al. (1981), who reported that sinapine was accountable for ~37-50%
69 of the observed bitterness in water slurries of RPC, and that not only sinapine but also its two
70 precursors (sinapic acid and choline chloride) were perceived as bitter. More recently, Hald et
71 al. (2019) identified kaempferol glycosides e.g. kaempferol 3-O-(2‴- O-sinapoyl-β-
72 sophoroside) (KSS) as the major contributor of bitterness in a rapeseed protein isolate.
73 Walser et al. (2024) subsequently identified additional bitter KSS derivatives, although KSS
74 itself remained the primary contributor to the bitterness of the protein isolate. Some
75 glucosinolates (progoitrin, gluconapin, glucobrassicin) have been reported to have bitter taste
76 (Bell et al., 2018). A study on Brussels sprouts reported that progoitrin (2-(R)-OH-3-butenyl
77 glucosinolate), a major glucosinolate in rapeseed seeds, tastes bitter and that its hydrolysis
78 product goitrin (L-5-vinyl-2-thiooxazolidone) tastes extremely bitter (Fenwick et al., 1983).

79 Rather than using highly processed protein isolates for human consumption, it would be
80 desirable from a health and sustainability perspective to use unprocessed (raw) RPC (Dicken
81 et al., 2025; Lie-Piang et al., 2021). Upon preparation of food products containing RPC,
82 metabolites and enzymes from different ingredients are brought together in a food matrix,
83 which may result in notable changes to the metabolite composition, and thus sensorially alter
84 the final food product compared to that of the individual ingredients. Generally, we lack

knowledge about how the different RPC-derived bitter compounds affect sensory quality when in a food matrix.

Towards development of RPC-containing food products, it is important to identify sensory and metabolic bottlenecks in consumer acceptance. Thus, we conducted a sensory assessment with a trained panel to characterize how dose and dehulling treatment impact the intensity of rapeseed-derived sensory attributes in a food matrix. We formulated whole food snack bars with increasing proportion of raw RPC (from 0-21%) as food prototype and combined sensory evaluation with targeted metabolite analysis of known bitter compounds. Here, we report how the effect of increasing the proportion of raw RPC in whole food snack bars affect their sensory quality, and the perceived bitterness is aligned with the presence of key RPC-derived bitter compounds. The correlation between sensory perception and bitter compounds is discussed.

2. Materials and methods

2.1. Production of rapeseed press cake (RPC)

Both whole and dehulled 'canola quality' seeds (<25 µmol/g glucosinolates and <2% eruca acid) were provided by the commercial oil mill 'Bornholm's Oliemølle A/S', Aakirkeby, Denmark. For the dehulled seeds, remaining hulls were removed with an air blower. The whole and dehulled seeds were 'cold-pressed' (after pre-heating, no external heat was added, temperature reported by instrument outside press head between 70-80°C during pressing) in a OW 100 s-inox oil press from Ölwerk GmbH. The freshly produced RPC was stored in an air-tight container at 5°C until preparation of snack bars.

2.2. Production of snack bars

For the main experiment, seven types of experimental snack bars were produced with increasing proportions of raw RPC, from respectively, whole and dehulled seeds by the Copenhagen-based company 'Spora'. The base paste consisted of 33 g roasted almonds, 33 g brewed filter coffee, 33 g flax seed, 33 g pumpkin seed, 17 g chia seeds, 117 g Medjool dates and 83 g raisins. Aliquots of the base were proportionally substituted with RPC from cold-pressed whole or dehulled seeds to contain 7, 14 and 21% RPC (**Table 1**). The different pastes were moulded into rectangle bars of 10 g. The bars were packed in airtight containers and stored at 4°C until sensory analysis or were frozen (-20°C) until metabolite analysis.

2.3. Sensory descriptive analysis

115 **2.3.1. Sensory panel**

116 Ten participants were recruited from the external trained sensory panel of the Department of
117 Food Science at the University of Copenhagen. Inclusion criteria were that participants were
118 between 18 and 60 years of age, have no known food allergies, not to be pregnant or
119 lactating, and could understand and communicate in English. Each subject participated by
120 informed consent.

121 **2.3.2. Descriptive sensory profiling**

122 The trained panellists received a two-hour training session, which included vocabulary
123 development, attribute definition, reference selection and ranking of seven samples. A
124 subsequent training session included similar activities, refining attributes and intensity
125 scaling of samples. Training was deemed sufficient, as no significant interaction effects
126 between replicates and assessors were observed.

127 The descriptive sensory profiling was conducted in sensory booths designed according to
128 ISO/ASTM standards. Portions of 10 g, tempered samples (21°C), were monadically
129 presented in black plastic cups with lids, labelled with randomized three-digit codes. Sensory
130 responses were recorded on 15 cm anchored scales using FIZZ data acquisition software
131 (Biosystems, Couternon, France). Samples were presented in a complete randomised block
132 design over three replicated sessions. Still water at room temperature was provided for palate
133 cleansing.

134 **2.4. Chemical analysis**

135 **2.4.1. Extraction of metabolites**

136 Metabolites were extracted from snack bars and RPC by homogenizing 50 mg product by
137 shaking with chrome balls at 30 hz in a Retch Mixer Mill for 2x5 minutes with 300 µL 85%
138 methanol containing 5 µM p-hydroxybenzyl glucosinolate as internal standard. After
139 homogenization, samples were incubated in a thermomixer compact from Eppendorf at 800
140 rpm for 60 min at room temperature (21°C). Subsequently, the samples were centrifuged at
141 10,000 g for 5 min and the supernatant was used for subsequent analysis.

142 **2.4.2. Metabolite LC-MS/MS analysis of rapeseed press cake**

143 For the analysis of glucosinolates, flavonoids, phenylpropanoids and goitrin by liquid
144 chromatography coupled to tandem mass spectrometry (LC-MS/MS), RPC extracts were
145 diluted in deionized water 25- and 2500-fold, and energy bar extracts were diluted 5- and
146 500-fold. Four replicates for each sample were analysed, and analyte concentrations were

147 determined by comparing their signal against calibration standard curves. Calibration curves
148 were prepared for each analytical run using calibration standards prepared in deionized water
149 at concentrations 1, 10, 50, 100, 200, 500, 1000, 2000, 5000 and 10000 nmol/L. p-
150 hydroxybenzyl glucosinolate, which was added to the extraction solvent, was used as internal
151 standard to control for analyte losses during extraction and inter-injection variation during
152 analysis. Sinapine was detected in snack bars and RPC extract with a concentration above the
153 limit of quantification. Further dilution of the RPC extract resulted in a quenching of the
154 signal disproportionate to the dilution, suggesting that peak area is highly influenced by
155 matrix effects in our experimental setup, thus making the measurements unreliable.

156 For free amino acid analysis by LC-MS/MS, extracts from both RPC and snack bars were
157 diluted in deionized water 25-fold and subsequently 10-fold in 13C-, 15N-labelled Cell Free
158 Amino Acid Mixture (Merck Sigma-Aldrich). Samples were run in four replicates, except for
159 dehulled RPC for which only three replicates were used. Quantification of the individual free
160 amino acids was performed from the 13C, 15N-labeled amino acids used as internal standards
161 at known concentrations. See Supplementary Methods 1 and 2 for detailed descriptions of the
162 LC-MS/MS methods.

163 **2.4.3. Quantification of crude protein**

164 The total nitrogen content of raw RPC and snack bars were quantified by combustion in a
165 vario MACRO cube CHNS analyzer from Elementar. To estimate the samples' content of
166 crude protein, a nitrogen-to-protein conversion factor of 6.25 was used (FAO/WHO, 1991).

167 **2.5. Data analysis**

168 The sensory profiling data were analysed with IBM SPSS by different ANOVA models. For
169 evaluating individual sample differences in the GLM procedure a 2-way mixed-model
170 ANOVA was used with the factors samples(fixed) and assessors(random) and their
171 interaction. For analysing the effect of RPC dosage (7, 14, and 21%) and seed treatment
172 (whole or dehulled) a 3-way mixed model ANOVA was used with the factors dosage (fixed),
173 seed treatment (fixed) and assessor (random) and their 2-way interactions. In order to
174 compare the control sample, a separate ANOVA was run excluding the factor seed treatment.
175 In all ANOVA's, Tukey's test for comparisons of sample differences was used as post-hoc
176 test with a significance level of α=0.05.

177 Principal Component Analysis (PCA) was conducted in R version 4.5.2 (R Core Team, 2025)
178 with the package 'FactoMineR' (Lê et al., 2008). Mean values were used for each sample,

179 and the data was standardized before analysis. The analysis used sensory attributes with
180 significant differences as active variables and metabolites were added as supplementary
181 variables. The results were visualised as a biplot applying the scaling of variables
182 implemented in 'factoextra' (Kassambara & Mundt, 2020). All visualisations were done
183 using 'tidyverse' (Wickham et al., 2019).

184 **3. Results**

185 **3.1 Sensory evaluation of RPC-containing snack bars**

186 We formulated raw RPC-containing snack bars to investigate the sensory characteristics of a
187 raw RPC-containing food product. The snack bars were based on whole food ingredients
188 (including dates, raisins, nuts and coffee) with an increasing proportion (0%, 7%, 14%, and
189 21%) of raw RPC from whole or dehulled seeds (**Table 1**, **Figure 1**). A panel of trained
190 tasters generated a vocabulary of thirteen sensory attributes that characterized the sensory
191 impressions of raw RPC in the food product (**Table 2**). The panel subsequently rated the
192 intensity of these sensory attributes in snack bars with different doses of RPC from whole or
193 dehulled seeds (**Table 3**). The intensity of seven of eight dose-dependent attributes plateaued
194 in snack bars with >14% RPC (**Figure 2)**. Intensities of oxidized odor and bitter taste were
195 significantly higher in bars with 7% and 14% RPC than in the 0% control whereas cabbage
196 odor, cabbage flavor, bitter aftertaste and astringent afterfeel did not increase at 7% RPC but
197 first at 14% and 21%. Noticeably, the intensity of astringent mouthfeel was the only sensory
198 attribute that increased proportionally with dose. With respect to impact of dehulling, bars
199 containing RPC from dehulled seeds were rated higher for four sensory attributes (overall
200 odor intensity, cabbage odor, cabbage flavor and popcorn flavor) compared with bars
201 containing RPC from whole seeds (**Table 2**). Additionally, the intensity of the sensory
202 attributes burned odor, sour taste and ryebread flavor as well as pungent mouthfeel detected
203 in raw RPC, did not increase in snack bars in a dose- or dehulling-dependent manner,
204 showing that RPC does not impact these sensory attributes to the food product significantly.
205 Furthermore, the highest intensity scores (>10 out of 15) were recorded for overall intensity
206 of odor, oxidized odor, bitter taste, bitter aftertaste and astringent mouthfeel. These results
207 indicate that raw RPC markedly influenced the sensory profile of the snack bars in a dose-
208 dependent manner, with the most pronounced changes occurring at <14% RPC, and that
209 dehulling treatment has an impact on specific odor and flavor attributes in the snack bars.

210

211 **3.2.1. Metabolites associated with bitterness in raw RPC**

212 Next, we generated a list of known bitter compounds that are found in seeds of rapeseed
213 (phenolics, flavonoids, glucosinolates and their hydrolysis products) (**Supplementary Table**
214 **1**). In the raw RPC, sinapic acid concentration was approximately 7.5–10 µmol/g, with whole
215 seed RPC generally at the higher end of this range. In the snack bars, sinapic acid levels
216 increased with proportion of RPC (**Figure 3A**). Bars made with 21% RPC from dehulled
217 seeds contained sinapic acid in proportion to the amount of RPC added, whereas all other
218 tested snack bars contained less-than-expected sinapic acid (**Figure 3A**). Unfortunately,
219 attempts to quantify sinapine in the samples were unsuccessful most likely due to severe
220 matrix effects.

221 We quantified the key bitter compounds KSS and a KSS-hexose, known from rapeseed protein
222 isolates, to investigate how introducing RPC into snack bars affected the profile of these
223 compounds. We found that KSS and KSS-hexose were present in both raw RPC and snack bars
224 and that peak area of both compounds increased with increasing dosage of RPC in snack bars
225 (**Figure 3A**). No KSS or KSS-hexose was detected in control snack bars without RPC.
226 Surprisingly, the peak area of KSS and KSS-hexose in snack bars was much higher than
227 expected based on the peak areas observed in analysis of raw RPC (respectively, 5-9-fold or 5-
228 7-fold higher). This suggests that KSS and KSS-hexose may be generated from higher order
229 derivatives during preparation of the snack bar or be more easily extractable from the snack
230 bar food matrix.

231 We quantified three glucosinolates (progoitrin, gluconapin, glucobrassicin) selected for their
232 abundance and documented bitter taste as well as one hydrolysis product, goitrin (derived
233 from progoitrin) also with reported bitter taste (**Supplemental Table 1**). In raw RPC from
234 whole seeds, the sum of quantified glucosinolates was 4.08 µmol/g, and of these progoitrin
235 was the most abundant glucosinolate with a concentration of 2.54 µmol/g, followed by
236 gluconapin at 1.05 µmol/g and glucobrassicin at 0.49 µmol/g, respectively (**Figure 3B**). The
237 level of goitrin was 0.82 µmol/g (**Figure 3C**). In the raw RPC from dehulled seeds, the total
238 level of quantified glucosinolates was 12.74 µmol/g, i.e. ~3 fold higher than RPC from whole
239 seeds, with progoitrin being most abundant with a concentration of 7.26 µmol/g, followed by
240 gluconapin at 3.61 µmol/g and glucobrassicin at 1.87 µmol/g, respectively (**Figure 3B**). The
241 level of goitrin was 0.195, i.e. ~4x lower than the level found in RPC from whole seeds
242 (**Figure 3C)**. This shows that RPC from whole seeds have less of the selected glucosinolates

243 than RPC from dehulled seeds but show signs of significant glucosinolate hydrolysis taking
244 place prior to incorporation into the food matrix.

245 In the snack bars with 7%, 14%, or 21% raw RPC from whole seeds, we observed progoitrin
246 levels of 0.046, 0.057, and 0.137 µmol/g, respectively, whereas the snack bars with RPC
247 from dehulled seeds contained 0.023, 0.064 and 0.101 µmol/g, respectively (**Figure 3B**).
248 These results show that upon preparation of the snack bars and thereby incorporation of RPC
249 into a food matrix, extensive hydrolysis of glucosinolates occurs. Unexpectedly, only small
250 amounts of goitrin were detected in the snack bars, accounting for 7.3-15.6% of the
251 progoitrin added to the snack bars (**Figure 3C**).

252 **3.3 Correlation between sensory scores and content of RPC-derived metabolites**

253 Next, we investigated whether the variation in intensities of sensory attributes between
254 samples correlated with the content of RPC-derived bitter compounds in the snack bars.
255 Based on a principal component analysis, we generated a biplot combining dose- and
256 dehulling-dependent sensory attributes with measured metabolites added as supplementary
257 variables. The PCA biplot indicated that the snack bars formed two distinct clusters along
258 PC1 (86.7%): 0–7% RPC and 14–21% RPC, based on correlations among dose- or dehulling
259 treatment-dependent sensory attributes and RPC content (Figure 4). All attributes were
260 observed to be driving this separation, except the popcorn flavor. The overall odor intensity,
261 bitter taste, bitter aftertaste, astringent mouthfeel and astringent afterfeel was positively
262 associated with content of KSS, KSS-hexose and goitrin in the snack bars (**Figure 4**). The
263 content of sinapic acid, gluconapin and glucobrassicin showed weaker association with any
264 dose- or dehulling-dependent sensory attributes than the bitter compounds goitrin, KSS, and
265 KSS-hexose, suggesting that in the food matrix these metabolites are not key for the intensity
266 of sensory attributes characteristic of RPC. The cabbage odor was associated with the content
267 of progoitrin in snack bars. Over PC2 (7.52%), we observed a trend of separation between
268 RPC from whole or dehulled seeds. When comparing snack bars with the same dose of RPC
269 from whole or dehulled seeds, bars containing press cake from dehulled seeds were more
270 strongly associated with popcorn flavor and, to a lesser extent, oxidized odor. In conclusion,
271 variation in the sensory profile of the snack bars was primarily associated with RPC
272 concentration and the levels of goitrin, KSS, and KSS-hexose, while the modest dehulling-
273 related separation observed along PC2 was not associated with the metabolites quantified in
274 this study.

275 **3.4. Dose-over-thresholds values**

276 A bitterness threshold - the concentration at which half of a tasting panel can perceive a
277 compound's bitterness - can be used to calculate the DoseoverThreshold (DoT) factor, which
278 indicates whether the compound is present at levels high enough to influence a food's
279 flavour. For progoitrin, the DoT factors for all snack bars were <0.1, while for goitrin, the
280 DoT factors were in the range 0.3-1.4 (dependent on dose and dehulling treatment, **Table 4**).
281 This observation suggests that goitrin, but not progoitrin, contribute to RPC-derived
282 bitterness in the snack bars.

283 **3.5. Protein and amino acids**

284 We analyzed crude protein and free amino acids in the RPC and snack bars. Raw RPC from
285 whole and dehulled seeds contained 28% and 32% crude protein, respectively
286 (Supplementary Figure 1). The free amino acid profile of both materials was dominated by
287 glutamate (3-3.5 µmol/g), followed by alanine (~1.0 µmol/g) and aspartate (0.5-0.8 µmol/g)
288 (Supplementary Figure 2). Concentrations of individual free amino acids were generally
289 slightly higher in RPC from dehulled than from whole seeds.

290 Incorporation of RPC into snack bars at 7-21% did not substantially affect total crude protein
291 content, which remained at approximately 15-17% across all formulations (Supplementary
292 **Figure 1**). The free amino acid composition of the snack bars differed markedly from that of
293 the raw RPC. In all snack bar samples, proline was the predominant free amino acid (3.3-6.6
294 µmol/g), followed by alanine (1.8-2.3 µmol/g) and glutamine (0.7-0.9 µmol/g), whereas the
295 remaining amino acids were present at lower concentrations. Although incorporation of RPC
296 resulted in variation in concentration of several individual amino acids, no clear dose or
297 dehulling treatment-dependent effect was observed. These show that inclusion of RPC in
298 snack bars did not alter the crude protein content of the snack bars and only had a limited
299 effect on the profile of free amino acids.

300

301 **4. Discussion**

302 The present work adopts a novel approach to characterize the sensory contribution of raw
303 RPC in a food matrix and - by combining sensory and targeted metabolomic data - generates
304 new insights on how bitter rapeseed-derived metabolites contribute to sensory attributes. The
305 sensory contribution of RPC was dominated by oxidized and cabbage odors, bitter taste and

306 cabbage flavor as well as astringent mouthfeel and bitter aftertaste. The intensity of most
307 RPC-derived attributes was dose-dependent, with astringent mouthfeel notably increasing its
308 intensity almost linearly with dose. For other dose-dependent attributes, addition of more
309 than 14% RPC to the snack bars resulted in only a minimal increase in perceived intensity.
310 The dose-response plateaus may reflect sensory adaptation. The non-linear effects may arise
311 from matrix interactions, by which some bitter compounds may be partially masked as their
312 concentration rises in the dense and sweet snack bar food matrix. Notably, retainment of the
313 seed hull (i.e. using RPC from whole seeds) did not increase bitterness. This indicates that
314 metabolites enriched primarily in the embryo are responsible for RPC-derived sensory
315 attributes detectable in the snack bar food matrix. In fact, bars with RPC from dehulled seeds
316 were perceived to be more intense for some attributes. A possible explanation is that hull
317 fibres bind metabolites responsible for sensory attributes. Alternatively, the hulls themselves
318 may serve as a dilution factor of RPC, reducing effective concentration. Furthermore, the
319 concentration of protein was not notably higher in snack bars with RPC from dehulled seeds
320 versus whole seeds. Together, the results indicate that there is no apparent advantage in terms
321 of sensory impact of RPC or content of crude protein in dehulling seeds prior to oil pressing.

322 The measurements of selected known bitter compounds show a tight correlation of intensity
323 of bitter taste and the peak area of KSS, KSS-hexose and the concentration of sinapic acid
324 and goitrin (**Figure 4**), suggesting that these metabolites contribute to perceived bitterness of
325 raw RPC in the snack bar food matrix. The observation that certain snack bars had a DoT-
326 factor values above 1 for goitrin provides the first evidence that goitrin may contribute to
327 RPC-derived bitterness in an RPC-containing food matrix. This finding is consistent with
328 earlier observations reported for goitrin in Brussels sprouts (Fenwick et al., 1983). At highest
329 RPC levels (21%) intensity of bitter taste and bitter aftertaste did not rise proportionally with
330 the increased concentration of these metabolites, presumably due to masking of the additional
331 bitterness by the complex food matrix. Our study provides evidence that other compounds
332 beside previously the reported sinapine and choline chloride (Ismail et al., 1981) may
333 contribute to perceived bitterness of raw RPC.

334 For the selected glucosinolates and sinapic acid, the measured concentrations in the snack
335 bars were lower than expected if dilution were the only factor. The observation that only
336 ~10% of the RPC-derived progoitrin was recovered as goitrin is noticeable. Goitrin is key for
337 the anti-thyroid effect of RPC (Galanty et al., 2024) and unlike inorganic thiocyanate ions - a
338 hydrolysis product of several glucosinolates - the goitrogenic effect is not alleviated by

adequate iodine status (Thorsen et al., 2025). Possible explanations could be that goitrin has been further metabolized/derivatized or that goitrin is not efficiently extracted from the matrix with the employed protocol. The finding that the peak areas of KSS and KSS-hexose in the bars were far higher than expected from raw RPC levels (5-9x) suggests that higher order kaempferol conjugates were hydrolyzed into KSS or KSS-hexose during preparation of the food, or that the metabolites were more efficiently extracted from the snack bar food matrix compared to the raw RPC. Our targeted metabolite analysis indicates that goitrin, sinapic acid and kaempferol glycosides in rapeseed is at least partly responsible for the RPC-derived bitterness, astringency and overall odor intensity of up to mid-range doses. The lack of correlations between the dose-dependent sensory attributes like cabbage and popcorn flavor as well as oxidized odor of RPC and the selected bitter compounds, highlights a need for further studies using untargeted metabolomics.

Our study illustrates the need for chemical characterization of the final food product itself, since metabolites in the raw RPC undergo chemical changes when added to the food matrix. Furthermore, the results may be used by food scientists and chefs as inspiration to introduce RPC in their recipes and by breeders looking to breed new rapeseed varieties with improved sensory characteristics e.g. by breeding for reduced level of bitter compounds in the seed.

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The authors declare no conflicting interests.

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Niels Christian Holm Sanden: Conceptualization, Methodology, Project administration, Funding Acquisition, writing – review & editing.

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Wender L.P. Bredie: Writing, Conceptualization, Formal Analysis, Supervision,

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437 **TABLES:**

438 **Table 1: Overview of different snack bars used in study.** Snack bars contained increasing
439 content (0-21%) of rapeseed press cake (RPC) from either whole or dehulled seeds.

Sample code	% RPC (w/w)	RPC type
Control	0	None
Whole_7	7	Whole
Whole_14	14	Whole
Whole_21	21	Whole
Dehulled _7	7	Dehulled
Dehulled _14	14	Dehulled
Dehulled _21	21	Dehulled

440

441

Table 2: Vocabulary describing the sensory attributes characteristic of raw rapeseed press cake.

Sensory attribute	Definition	Scale range	Reference material
Odour			
Overall intensity	Strength of the sample's odor	Weak - strong	None
Burned	Odor of burnt or over-toasted food	A little – a lot	Burned toasted white sandwich bread
Cabbage	Odor reminiscent of raw, shredded white cabbage	A little – a lot	Shredded white cabbage
Oxidized	Odor associated with stale, rancid, or cardboard-like notes	A little – a lot	Aged almond oil
Taste/flavor			
Sour	Taste associated with citric acid solution	A little – a lot	Citric acid (2 g/L water)
Bitter	Taste associated with quinine solution	A little – a lot	Quinine (1 g/L water)
Ryebread	Flavor characteristic of untoasted rye bread	A little – a lot	Untoasted ryebread slice
Cabbage	Flavor reminiscent of raw, shredded white cabbage	A little – a lot	Shredded white cabbage
Popcorn	Flavor reminiscent of freshly popped, unsalted popcorn	A little – a lot	Unsalted fresh popcorn
Mouthfeel			
Astringent	Dry, puckering sensation on the tongue and inner cheeks and gums	A little – a lot	2 black tea bags (Medova) infused in 250 mL water
Pungent	Irritating, sharp, mustard-like sensation in the nasal and oral cavity	A little – a lot	Fresh arugula leaves
After impressions			
Bitter aftertaste	Lingering bitterness remaining after swallowing/ spitting, comparable to the aftertaste of black tea	A little – a lot	Quinine (1 g/L water)
Astringent afterfeel	Lingering dry, puckering sensation after swallowing/ spitting	A little – a lot	2 black tea bags (Medova) infused in 250 mL water

Table 3: Statistical analysis of whether dose and dehulling treatment affect sensory attributes. Sensory panel mean intensity values for snack bars containing different dosages of rapeseed press cake (RPC) made from, respectively, whole or dehulled seeds and attributes showing product differences. Abbreviations: 'Whole' snack bars with RPC from whole seeds; 'Dehulled': snack bars with RPC from dehulled seeds.

	Dosage				Dehulling Treatment		Dosage		Dehulling Treatment		Dosage x Dehul. Tr.	
Sensory attribute	0%[a]	7%	14%	21%	Whole	Dehulled	F(2,18)	*p*-value	F(1,9)	*p*-value	F(2,138)	*p*-value
Odor												
Overall intensity	**7.0b**	**8.4b**	**10.3a**	**10.8a**	**9.4b**	**10.2a**	6.570	**0.007**	8.821	**0.016**	2.518	0.084
Burned	8.1	8.3	8.2	8.6	8.7	8.0	0.140	0.870	0.744	0.411	1.342	0.265
Cabbage	**4.0b**	**5.5b**	**8.9a**	**8.6a**	**7.0b**	**8.4a**	8.770	**0.002**	9.038	**0.015**	0.828	0.439
Oxidized	**3.7c**	**6.4b**	**10.1a**	**7.9b**	7.5	8.8	6.316	**0.008**	3.613	0.090	0.165	0.848
Taste/Flavor	7.4	7.7	7.9	7.7	7.2	8.3	0.062	0.940	4.998	0.052	2.333	0.101
Sour												
Bitter	**5.3c**	**7.6b**	**10.0a**	**10.9a**	9.1	9.9	10.937	**<0.001**	1.408	0.266	1.065	0.347
Ryebread	8.0	8.6	7.9	7.8	8.4	7.8	1.074	0.363	1.258	0.291	0.064	9.938
Cabbage	**5.2b**	**6.8b**	**9.9a**	**9.6a**	**7.7b**	**9.8a**	8.034	**0.003**	36.510	<**0.001**	0.028	0.973
Popcorn	**4.0b**	**6.2a**	**7.0a**	**6.0a**	**5.6b**	**7.2a**	0.933	0.411	5.434	**0.045**	0.266	0.766
Mouthfeel												
Astringent	**5.0d**	**6.7c**	**8.5b**	**10.2a**	8.0	8.9	7.692	**0.004**	2.013	0.190	2.480	0.087
Pungent	5.5	6.2	6.5	6.5	6.3	6.5	0.072	0.931	0.089	0.773	0.489	0.615
After impressions												
Bitter aftertaste	**6.3b**	**7.8b**	**10.1a**	**10.0a**	9.4	9.3	3.806	**0.042**	0.038	0.850	0.103	0.902
Astringent afterfeel	**6.0b**	**7.3b**	**9.1a**	**9.8a**	8.4	9.1	3.808	**0.042**	1.304	0.283	0.837	0.435

Table 4: Dose-over-Threshold factors (DoT-factors) for goitrin and progoitrin in snack bars containing rapeseed press cake (RPC). A DoT-Factor of more or equal to 1 suggests that the particular compound should be detectable as bitter based on the perception in an aqueous solution. The bitterness threshold values are extracted from observations by Fenwick et al. (1983). 'Dehulled' and 'Whole' refers to the source of RPC used in the formulation of the particular snack bar.

Dosage	**7%**		**14%**		**21%**	
	Dehulled	**Whole**	**Dehulled**	**Whole**	**Dehulled**	**Whole**
Goitrin	0.39	0.29	1.01	0.70	1.37	1.13
Progoitrin	0.02	0.00	0.05	0.01	0.08	0.03

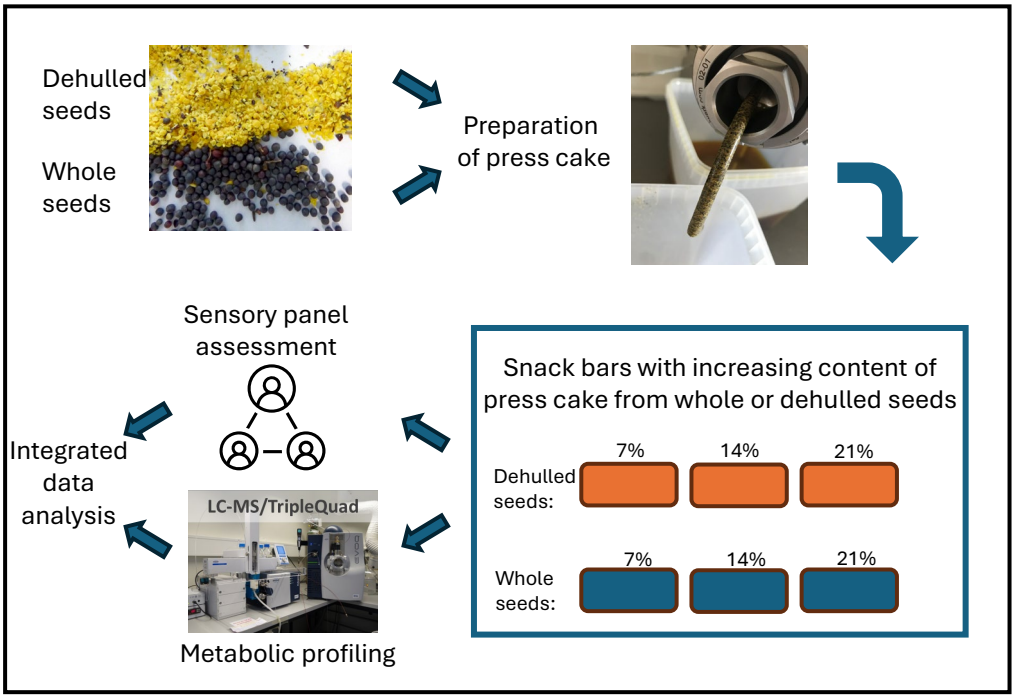

Figure 1: Workflow of study from preparation of press cake to integrated data analysis.

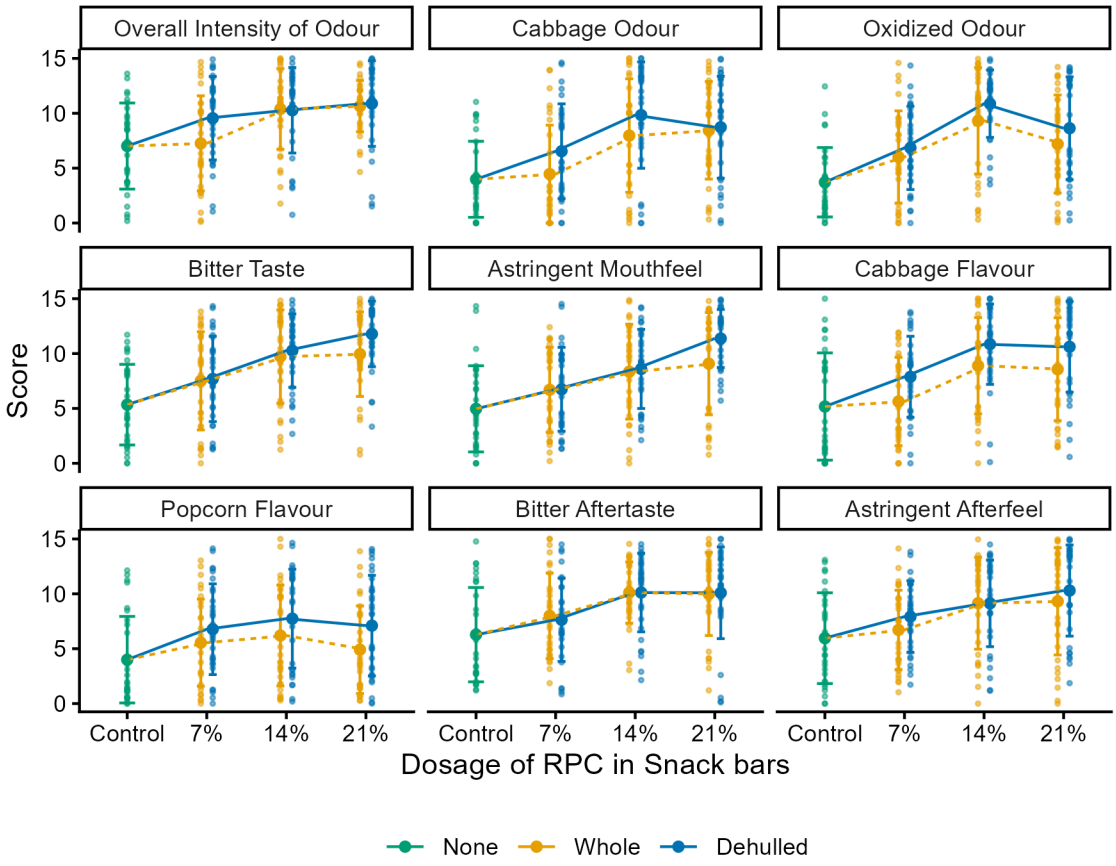

Figure 2: Intensity of sensory attributes in snack bars with press cake from whole or dehulled seeds. For Overall intensity of Odour, the scale goes from 0-15 where 0 is weakest odour and 15 is stronger odour. For all other sensory attributes, 0 is a little and 15 is a lot of the particular attribute. Small dots represent individual sensory measurements, large dots represent means of sensory scores, and error bars represent standard deviations.

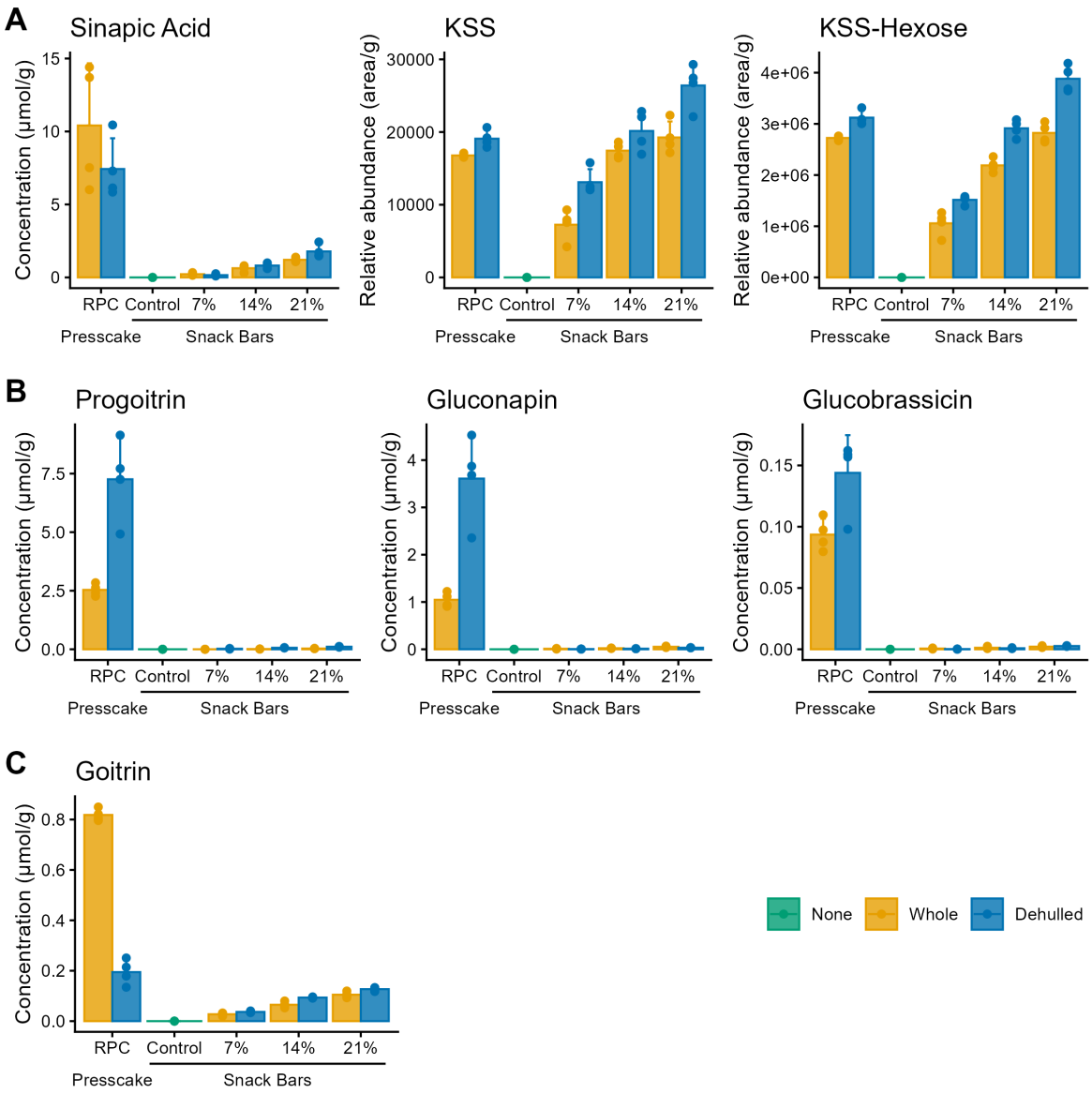

Figure 3: Quantification of known bitter compounds in raw rapeseed press cake (RPC) and snack bars supplemented with RPC. Note that the values for KSS and KSS-Hexose are peak areas normalized to sample weight. The percentages refer to the dosage of RPC included in the snack bars (mass percentage). The top of the bars are averages of four technical replicates and error bars show the standard deviation for the technical replicates. KSS: kaempferol-3-o-sinapoylsophoroside, KSS-Hexose: kaempferol-3-o-sinapoylsophorosideglucoside.

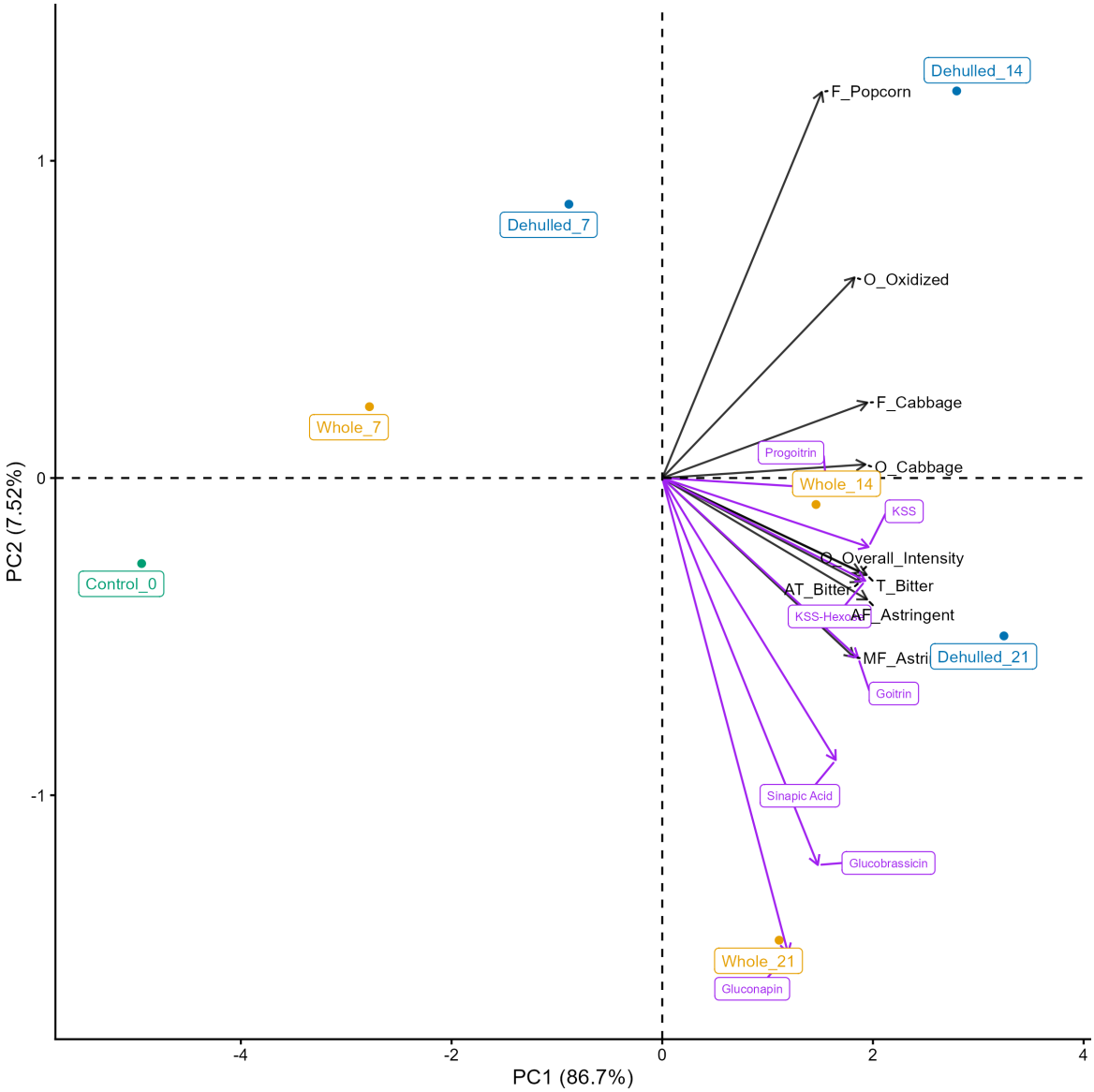

Figure 4: Biplot of sensory attributes with bitter metabolites projected as supplementary variables. Snack bars corresponding to sample IDs are detailed in **Table 1.** Abbreviations: O: Odour, T: Taste, F: Flavour, MF: Mouthfeel, AT: Aftertaste, AF: After Mouthfeel.

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Supplementary Methods:
Supplementary Method 1: Detection and quantification of glucosinolates, flavonoids, phenylpropanoids and goitrin by LC-MS/MS

Supplementary Method 2: Detection and quantification of free amino acids by LC-MS/MS

Supplementary Method 1: Detection and quantification of glucosinolates, flavonoids and goitrin by LC-MS/MS

The method was modified from Crocoll et al. (2016) to include non-glucosinolate metabolites as well as to adjust and optimize parameters for the LC-MS/MS system in use. Briefly, chromatography was performed on a 1290 Infinity II UHPLC system (Agilent Technologies). Separation was achieved on a Kinetex XB-C18 column (100 x 2.1 mm, 1.7 µm, 100 Å, Phenomenex, Torrance, CA, USA). Formic acid (0.05%, v/v) in water and acetonitrile (supplied with 0.05% formic acid, v/v) were employed as mobile phases A and B, respectively. The elution profile was: 0-0.5 min, 3% B; 0.5-4.0 min, 3-45% B; 4.0-4.2 min 45-100% B, 4.2-5.1 min 100% B, 5.1-5.2 min, 100-3% B and 5.2-6.2 min 3% B. The mobile phase flow rate was 400 µL/min. The column temperature was maintained at 40°C. The liquid chromatography was coupled with Ultivo triple quadrupole mass spectrometer (Agilent Technologies) equipped with a Jetstream electrospray ion source (ESI). The instrument parameters were optimized by infusion experiments with pure standards, when available. The ion spray voltage was set to 4000V in positive mode and 3500V in negative mode. Dry gas temperature was set to 325°C and dry gas flow to 12 L/min. Sheath gas temperature was set to 400 °C and sheath gas flow to 12 L/min. Nebulizing gas was set to 50 psi. Nitrogen was used as dry gas, nebulizing gas and collision gas. Multiple reaction monitoring (MRM) was used to monitor precursor ion → fragment ion transitions. MRM transitions were determined by direct infusion experiments of reference standards except for kaempferol-3-O-sinapoyl-sophoroside (KSS) and kaempferol-3-O-sinapoylsophoroside-hexose (KSS-Hexose), where purified putative compounds were used, isolated from RPC by preparative LC. Both Q1 and Q3 quadrupoles were maintained at unit resolution. MRM transitions, fragmentor voltage and collision energies are detailed in Supplementary Table 2. Mass Hunter Quantitation Analysis for QQQ software (Version 10, Agilent Technologies) was used for data processing.

Supplementary Method 2: **Detection and quantification of free amino acids by LC-MS/MS**

The same LC-MS system equipped with Zorbax Eclipse XDB-C18 column (150 x 3.0 mm, 1.8 µm, Agilent Technologies) was used for amino acid analysis. Formic acid (0.05 %, v/v) in water and acetonitrile (supplied with 0.05 % formic acid, v/v) were employed as mobile phases A and B, respectively. The elution profile was: 0.00-0.75 min, 3 % B; 0.75-4.10 min, 3-65 % B; 4.10-4.20 min, 65-100 % B; 4.20-4.95 min, 98 % B; 4.95-5.00 min, 100-3 % B; and 5.0-6.0 min 3 % B. The mobile phase flow rate was 400 µL/min. The MS instrument parameters were optimized by infusion experiments with pure standards. The ion spray voltage was set to 2500 V in a positive ion mode. Dry gas temperature was set to 325 °C and dry gas flow to 11 L/min. Sheath gas temperature was set to 350 °C and sheath gas flow to 12 L/min. Nebulizing gas was set to 40 psi. Nitrogen was used as dry gas, nebulizing gas and collision gas. MRM was used to monitor precursor ion → fragment ion transitions. MRM transitions and other parameters such as fragmentor voltage and collision energies were optimized using reference standards. Both Q1 and Q3 quadrupoles were maintained at unit resolution. MRM transitions, fragmentor voltage and collision energies are detailed in Supplementary Table 3. Mass Hunter Quantitation Analysis for QQQ software (Version 10, Agilent Technologies) was used for data processing See supplementary table 1 for an overview of the quantified metabolites.

Supplementary Table 1. Overview of specialised metabolites identified using LC-MS.

Formal name	Trivial name / abbreviation	Sensory description
Glucosinolates		
2-(R)-2-Hydroxy-3-butenylglucosinolate [a]	Progoitrin	Bitter [1]
3-Butenyl glucosinolate[a]	Gluconapin	Bitter (pungent, cabbage-like) [1]
3-Indolylmethylglucosinolarte[a]	Glucobrassicin	Bitter ("unpleasant") [1]
Hydrolysis-product from glucosinolate		
L-5-vinyl-2-thiooxazolidone[a]	Goitrin	Extreme bitter taste [1]
Phenolics		
4-Hydroxy-3,5-dimethoxycinnamic acid (sinapic acid)[a]	Sinapic acid	Bitter, astringent [2, 5]
Sinapic acid choline ester (Sinapine)	Sinapine	Bitter [2, 5]
Kaempferol derivatives		
Kaempferol-3-O-sinapoylsophoroside [b]	KSS	Bitter, astringent [3]
Kaempferol-3-O-sinapoylsophoroside-hexose[b]	KSS-Hexose	Bitter, astringent [4]

Method of quantification: [a] Absolute; [b] Relative (Peak area).
Sensory descriptors: [1] Bell et al., 2018; [2] Fenwick et al., 1983; [3] Hald et al., 2019; [4] Walser et al., 2024, [5]Ismail et al. (1981).

Supplementary Table 2: MRM transitions, fragmentor voltages and collision energies used for LC-MS/MS.

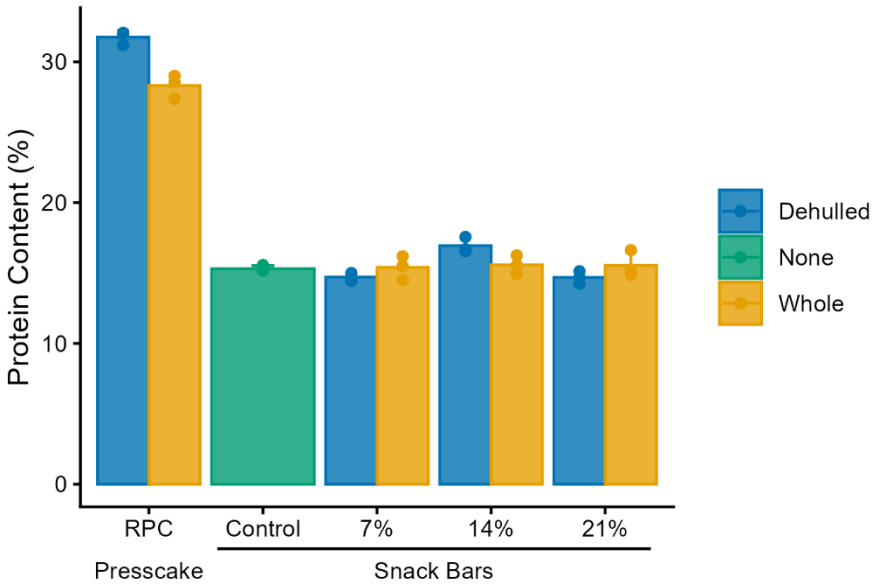
Analyte	Retention Time [min]	Q1 [m/z]	Q3 [m/z]	Fragmentor [V]	CE [V]
2(R)-2OH3but $[M-H]^-$	1.35	388	195	103	20
	1.35	388	97[Qt]	103	20
	1.35	388	75	103	36
3Butenyl $[M-H]^-$	2.28	372	259	103	16
	2.28	372	97[Qt]	103	20
	2.28	372	75	103	36
Goitrin $[M+H]^+$	2.25	130	96	53	5
	2.25	130	85	53	13
	2.25	130	70[Qt]	53	5
I3M $[M-H]^-$	3.08	447	259	112	24
	3.08	447	205	112	20
	3.08	447	97[Qt]	112	24
KSS $[M-H]^-$	3.22	815	623	175	32
	3.22	815	609	175	32
	3.22	815	591	175	36
	3.22	815	429	175	44
	3.22	815	284[Qt]	175	52
KSS-Hexose $[M-H]^-$	2.67	977	815[Qt]	175	32
	2.67	977	623	175	48
	2.67	977	609	175	48
	2.67	977	591	175	48
	2.67	977	446	175	56
	2.67	977	284	175	68
pOHB $[M-H]^-$	2.06	424	259	92	20
	2.06	424	182	92	20
	2.06	424	97[Qt]	92	24
Sinapic acid $[M+H]^+$	3.35	225	175[Qt]	73	12
	3.32	225	147	73	20
	3.32	225	119	73	20
Sinapine $[M+H]^+$	2.46	310	251[Qt]	97	16
	2.46	310	207	97	28
	2.46	310	175	97	32

Qt = quantifier ion, additional transitions were used for identification. Q = quadrupole. CE = collision energy.

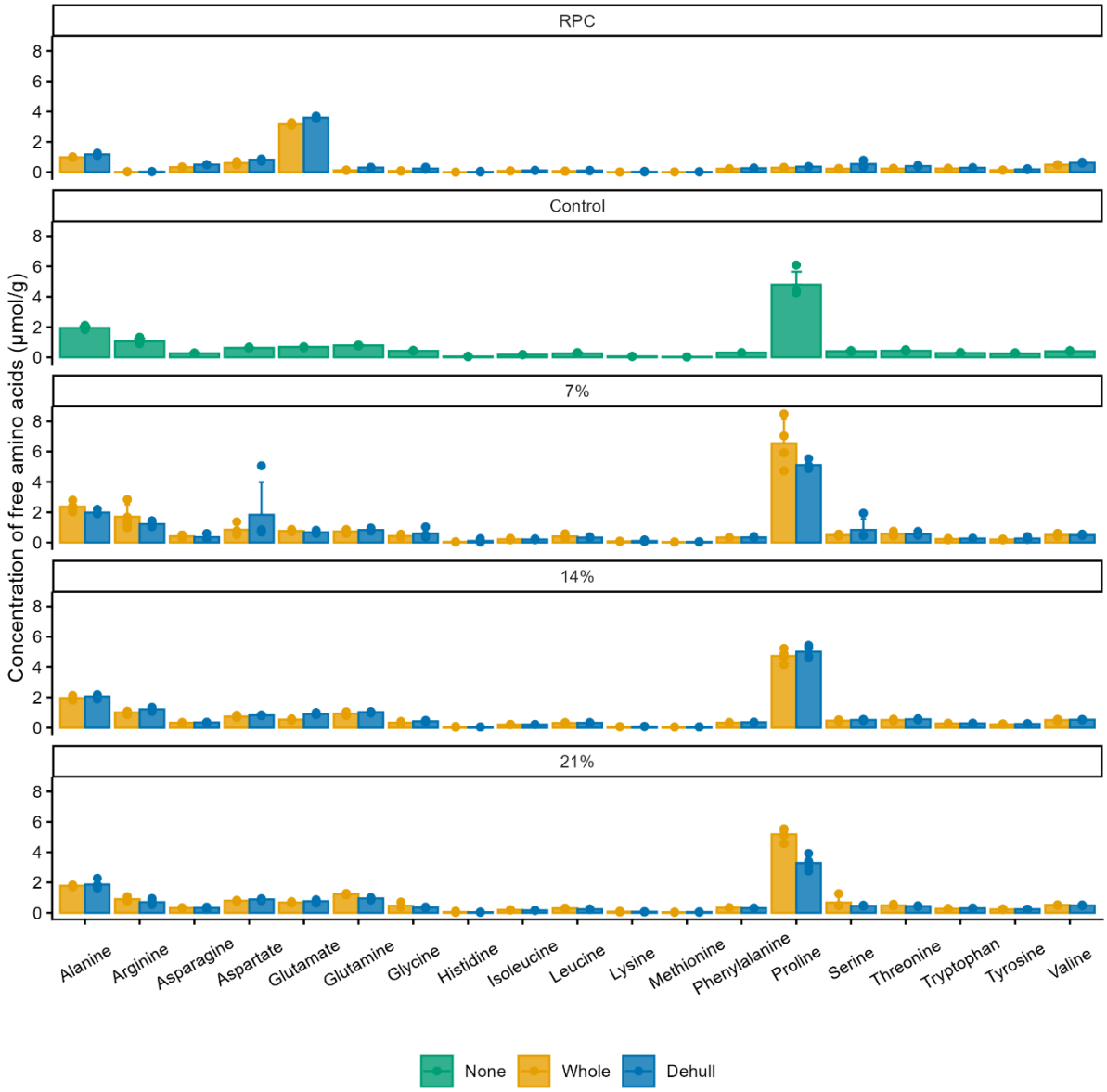
Supplementary Table 3: MRM transitions, fragmentor voltages and collision energies used for amino acid LC-MS/MS analysis.

Analyte	Retention Time [min]	Q1 [m/z]	Q3 [m/z]	Fragmentor [V]	CE [V]
13C-15N-Ala $[M+H]^+$	1.43	94	47	63	8
13C-15N-Arg $[M+H]^+$	1.36	185	75	63	28
13C-15N-Asn $[M+H]^+$	1.42	139	77	63	12
13C-15N-Asp $[M+H]^+$	1.42	139	77	63	12
13C-15N-Cys $[M+H]^+$	1.38	126	79	63	8
13C-15N-Cystine $[M+H]^+$	1.38	249	156	63	8
	1.38	249	77	63	28
13C-15N-Gln $[M+H]^+$	1.46	154	136	63	4
13C-15N-Glu $[M+H]^+$	1.47	154	107	63	8
13C-15N-Gly $[M+H]^+$	1.40	79	50	63	4
	1.40	79	33	63	4
13C-15N-His $[M+H]^+$	1.32	165	118	63	12
13C-15N-Ile $[M+H]^+$	2.77	139	92	63	8
13C-15N-Leu $[M+H]^+$	3.04	139	92	63	8
13C-15N-Lys $[M+H]^+$	1.31	155	90	63	16
13C-15N-Met $[M+H]^+$	2.18	156	109	63	8
13C-15N-Phe $[M+H]^+$	3.50	176	129	63	12
13C-15N-Pro $[M+H]^+$	1.56	122	75	63	16
13C-15N-Ser $[M+H]^+$	1.40	110	63	63	8
13C-15N-Thr $[M+H]^+$	1.44	125	78	63	8
13C-15N-Trp $[M+H]^+$	3.73	218	200	63	4
	3.73	218	156	63	8
	3.73	218	127	63	28
13C-15N-Tyr $[M+H]^+$	2.54	192	145	63	12
13C-15N-Val $[M+H]^+$	1.77	124	77	63	8
Alanine $[M+H]^+$	1.43	90	44	63	8
Arginine $[M+H]^+$	1.36	175	70	63	28
Arginine $[M+H]^+$	1.36	175	60	63	12
Asparagine $[M+H]^+$	1.42	133	74	63	12
Aspartate $[M+H]^+$	1.42	134	74	63	12
Cysteine $[M+H]^+$	1.38	122	76	63	8
	1.38	122	59	63	20
Cystine $[M+H]^+$	1.38	241	152	63	8
	1.38	241	74	63	28
Glutamate $[M+H]^+$	1.47	148	102	63	8
	1.47	148	84	63	12
	1.47	148	56	63	32
	1.47	147	130	63	4
Glycine $[M+H]^+$	1.40	76	48	63	4

	1.40	76	31	63	4
Histidine $[M+H]^+$	1.32	156	110	63	12
	1.32	156	93	63	24
Isoleucine $[M+H]^+$	2.77	132	86	63	8
Leucine $[M+H]^+$	3.04	132	86	63	8
Lysine $[M+H]^+$	1.31	147	84	63	16
Methionine $[M+H]^+$	2.18	150	104	63	8
	2.18	150	61	63	20
	2.18	150	56	63	12
Phenylalanine $[M+H]^+$	3.50	166	120	63	12
	3.50	166	103	63	28
Proline $[M+H]^+$	1.56	116.1	70	63	16
	1.56	116	43	63	0
Serine $[M+H]^+$	1.40	106	60	63	8
	1.40	106	42	63	24
Threonine $[M+H]^+$	1.44	120	74	63	8
	1.44	120	56	63	0
Tryptophane $[M+H]^+$	3.73	205	188	63	4
	3.73	205	146	63	8
	3.73	205	118	63	28
Tyrosine $[M+H]^+$	2.54	182	136	63	12
	2.54	182	123	63	0
Valine $[M+H]^+$	1.77	118	72	63	8
	1.77	118	55	63	0



Supplementary Figure 1: Concentration of crude protein in raw rapeseed press cake and snack bars. The top of the bars are averages of the technical replicates and error bars show the standard deviation for the technical replicates.



Supplementary Figure 2. Free amino acids in raw rapeseed press cake and snack bars containing varying doses of RPC from 'whole' or 'dehulled' seeds. The top of the bars are averages of the technical replicates and error bars show the standard deviation for the technical replicates.