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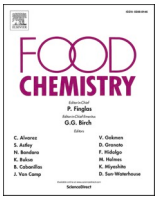

Influence of different processing method on lignan content of selected Malaysian plant-based foods

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ABSTRACT

This research assessed the influence of pickling, fermentation, germination, and tea brewing on lignan content of a variety of food highly consumed in Malaysia. Lignans have been measured by a validated LC-MS/MS method. Secoisolariciresinol (SECO) was the most abundant compound in fermented and germinated samples. Pickling significantly decreased larisiresinol content by approximately 86 %. Fermentation increased lignan content in a mixture of flaxseed and mung beans (799.9 ± 67.4 mg/100 g DW) compared to the unfermented counterpart (501.4 ± 134.6 mg/100 g DW), whereas the fermentation of soybeans and mung beans did not significantly affect the SECO content. Germination increased lignan content, which reached its peak on day 6 of germination for all the tested matrixes. In tea brew, lignans concentration increased with brewing time reaching its highest concentration at 10 min of brewing. The results of this study expand the knowledge on the effect of processing on lignan content in food.

1. Introduction

Lignans are a class of diphenolic compounds derived from combining two phenylpropanoid C6-C3 units at the β and β′ carbon atoms (Imai, Nomura, & Fukushima, 2006). Secoisolariciresinol (SECO) is the most abundant lignan, whereas matairesinol (MATA), pinoresinol (PINO), and lariciresinol (LARI) occur as minor components (Heinonen, Nurmi, Liukkonen, Poutanen, Wähälä, Deyama, & Adlercreutz, 2001). Oilseeds, nuts, cereals, vegetables (*Brassica* species), fruits, and beverages (tea, coffee, beer, and wine) are the major sources of lignans in the European diet (Khalesi, Irwin, & Schubert, 2015), whereas leafy vegetables and beverages (tea and coffee) are the main contributors to lignan daily intake in Malaysia (Kasim et al., 2018). Some epidemiological studies have suggested that lignans reduce the risk of cardiovascular diseases and other chronic diseases (e.g., breast cancer), as well as reduce body weight and fat accumulation (Park & Velasquez, 2012).

In Malaysia, The Malaysian Non-Communicable Disease Surveillance highlighted the rapid rise of obesity due to, among other factors, the low intake of fruits and vegetables. This vital challenge must be tackled because it will aid in preventing undesirable health conditions in society. In Malaysia, there is a research gap on the factors affecting the consumption of fruits and vegetables (Tan, Dunn, Samad, & Feisul, 2011). These dietary changes lead to a high prevalence of obesity. According to the Malaysian Adults Nutrition Survey (MANS) 2014, the consumption of "processed foods" to which salt and condiments are added, had increased, and it appears among the top ten most consumed foods (MANS, 2014). The intake of these food groups is positively associated with lignan intake, thus may lower the risk of obesity.

Food processing is known to affect the content of lignans in foods (Meagher & Beecher, 2000) and may influence bioavailability and antioxidants of several compounds (Verghese, Willis, Boateng, Gomaa, & Kaur, 2021). Bioprocessing refers to a group of sustainable technologies for extracting, purifying, and producing food and food ingredients through enzymes and/or microorganisms (Kennes, 2018). Fermentation is one of the bioprocessing methods traditionally applied to extend food shelf-life. 'Tempeh' is a traditional process of fermentation of soybeans, which provides many health benefits, including enhancing digestibility. Tempeh is a precooked fungal fermented bean, or cereal, bound together by the mycelium of a living mould (mostly *Rhizopus* spp.) (Nout & Kiers, 2005).

Pickles are made by naturally fermenting fruits and vegetables. Pickling is the process of preserving edible products by immersing them in brine containing vinegar (acetic acid) and salt, followed by a heat treatment. When vegetables are brined at salt concentrations of up to

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about 8 % (w/v) and allowed to ferment naturally, the brine solution supports fermentation by a sequence of different types of microorganisms (Montaño, Sánchez, Beato, López-López, & De Castro, 2016).

Germination is the physiological process through which seeds produce an embryonic root and stem within a specified time frame (Paulsen, 2008). It noticeably modifies the seed microstructure, digestibility, and profiles of active compounds (Di Stefano, Tsopmo, Oliviero, Fogliano, & Udenigwe, 2019).

Tea is the most popular beverage globally. Malaysians drink an average of 160 mL of tea per day, making it the second most popular beverage after plain water (MANS, 2014) and the highest contributor to lignan intake in the Dutch population (Milder, Arts, van de Putte, Venema, & Hollman, 2005; Milder, Feskens et al., 2005). Tea consumption is linked to positive effects on human health, with polyphenols being the responsible constituents (Isemura, Pervin, Unno, Saito, & Nakamura, 2022).

To properly tackle the challenge of growing obesity in the Malaysian population, a dietary approach based on diets rich in lignans may be a suitable strategy. For it to be successful, an accurate database of lignan content in the food items most consumed by Malaysian is necessary and, at the moment, lacking. In addition, new bioprocessing techniques are gaining popularity worldwide and are commonly used in the Malaysian diet. Studying the effect of those techniques on lignan content may help optimizing the conditions for the highest lignan intake. Thus, the current work aimed to determine the effect of pickling, fermentation, germination on the lignan content of various foods the Malaysian population mostly consumes. In addition, the tea brewing process has been investigated, given its high contribution to lignan intake in the diet. For this purpose, the lignans were extracted and hydrolyzed, and the extracts were analyzed with LC–MS/MS for lignan content and composition.

2. Materials and methods

2.1. Chemicals and reagents

Analytical grade acetonitrile (HPLC grade), methanol, acetone, sodium acetate, sodium hydroxide, glacial acetic acid, formic acid, analytical standards of matairesinol, secoisolariciresinol, lariciresinol, pinoresinol, secoisolariciresinol diglucoside and glycocholic acid (glycine-1 ^{13}C, 13C-GA) were purchased from Sigma-Aldrich Chemie NV (Zwijndrecht, Netherlands). For the enzymatic hydrolysis, β-glucuronidase with an activity of ≥ 300,000 units/g solid and a sulfatase activity of ≥ 10,000 units/g solid type H-1 from *Helix pomatia* were purchased from Sigma-Aldrich Chemie GmbH (Schnelldorf, Germany). The *R*hizopus oryzae fungus for fermentation of flaxseed, mung beans, and soybeans was obtained from Startercultures.eu/starterculturen.nl.

2.2. Sample collection

The selection of plant-based foods was based on data from The National Health Morbidity Survey, Malaysia Adult Nutrition Survey (MANS) conducted in 2014 (MANS, 2014). The most consumed plant-based foods (leafy green vegetables, green vegetables, legumes, other types of vegetables, and beverages) with unknown lignan content and composition were selected. Fresh shallot, celery, green Chinese mustard, long bean were purchased at three different locations (fresh products). In addition, for fresh products, a minimum of 100 g or 3 units were sampled at each location: an outlet of a nationwide supermarket chain, a local grocery, and an open-air street market. Flaxseed, mung beans, and soybeans were purchased at the local market. The commercial Malaysian black tea sample used was from the brand “Boh Tea”. Flaxseed has high lignan content (3 mg/g) (Milder, Arts et al., 2005; Milder, Feskens et al., 2005), and therefore, it was selected to evaluate lignan extractability.

Pickling, germination, fermentation, and brewing were applied to specific subsets of the samples, as indicated in Table S1.

2.3. Sample treatments

Celery, green Chinese mustard, and long bean were cut into cubic pieces of 2 cm whereas shallot was sliced into slices of 2 mm of thickness. Then, the samples were freeze-dried using the freeze-dryer (Christ, Alpha 1–4 LD Plus, Salmenkipp, the Netherlands). The final moisture content of freeze-dried samples were between 1 and 4 %. Subsequently, the samples were stored at −20 °C (Liebherr, Comfort, the Netherlands) until further analysis.

2.3.1. Preparation of pickled samples

Shallot, celery, green Chinese mustard, and long bean samples were selected for pickling using a traditional domestic fermentation. Salt (25 g) was dissolved in water (200 mL) and vinegar (200 mL) with a final concentration of 1:16, w/v and mixed (74 °C, 15 min). Fresh samples were rinsed with water, cut, and placed in a clear jar (Fido 250 mL clamp jar, Bormioli Rocco, IKEA, Sweeden) with the cooled pickling solution. Then, the samples were stored indoors (10–20 °C) for natural pickling. Samples were collected after 5 weeks of pickling.

2.3.2. Preparation of fermented samples

Mung beans and soybeans were fermented following a procedure previously described with some modifications (Widaningrum, Ermi, & Endang Yuli, 2015). Samples were rinsed and macerated to water (1:3, w/v, 12 h, room temperature (RT)). Following maceration, the samples were drained, dehulled, and split in half. The dehulled samples were placed in boiling water (1:2, w/v, 30 min, 100 °C). After boiling, the samples were drained, cooled down (<36 °C), and transferred to a container with vinegar (20 mL). Afterwards, the samples were inoculated with the fungi *Rhizopus oyrzea* at the level of 0.1 % (w/w) of boiled samples. Finally, the samples were packed in perforated polyethylene bags, and they were incubated (24–48 h, 25–30 °C) or until the formation of a compact white mycelium.

For the preparation of the mixture of mung beans and flaxseed, a similar procedure was followed. In brief, mung beans were rinsed and macerated in water (12 h, RT). After removing the excess water, the mung beans were dehulled and split in half, drained again, and boiled in demineralized water (1:2, w/v, 30 min). Subsequently, the flaxseed was rinsed, drained, and boiled in demineralized water (1:2, w/v, 3 min). Flaxseed and mung beans were mixed (1:1, w/w) to form a white layer of compact mycelium around the beans. The sample was let to cool down (36 °C). The fermentation followed the same procedure as the fermentation of mung and soybeans described above.

2.3.3. Preparation of germinated samples

Mung beans, soybeans, and flaxseed were selected for the germination process. Firstly, the mung beans and soybeans were rinsed and were macerated in water (1:3, w/v, 12 h, 20 °C). Flaxseed was soaked with water for 1 h. After removing the excess water, the seeds were placed into a container (60 × 150 mm, 20 g) and kept in the dark (RT) for germination. The sprouts were rinsed with water every 12 h to avoid microbial growth. The germination period lasted 7 days, and samples were collected every day. The collected sprouts were freeze-dried and milled using a commercial waring blender (model 34BL 99, Dynamic Corporation, USA) into a fine powder (0.5 mm sieve) and stored in the dark (RT) until further analysis.

2.3.4. Preparation of tea brews

Tea brew was prepared by placing one tea bag in boiling tap water (1:100, w/v). The tea was infused for different time intervals (2, 5, 7, 10 min), and the various samples were let to cool down before 50 mL of each sample was filtered through a 1.2 μm Acrodisc filter (Gelman Sciences, Ann Arbor, USA). For each brewing period, two different tea bags were used. The samples were stored at −20 °C until analysis.

2.4. Lignan extraction

Fresh, pickled, fermented and germinated samples were freeze-dried, ground and stored at −20 °C until analysis. Lignans were extracted according to Milder et al., Norskov et al. and Penalvo et al. with slight modifications (Figure S1) (Milder, Arts, Venema, Lasaroms, Wähälä, & Hollman, 2004; Nørskov & Knudsen, 2016; Peñalvo, Haajanen, Botting, & Adlercreutz, 2005). In brief, dry samples (20 mg) were extracted in alkaline conditions (1 mL, 0.3 M NaOH in methanol/water, 8/2, v/v) and were spiked with ^{13}C-GA (10 μL, 50 μg/mL). Afterwards, the samples were vortexed for 2 min and incubated in a vortex incubator (60 °C, 1 h). The samples were cooled, and the pH was adjusted to 5 with glacial acetic acid. The samples were further vortexed for 1 min prior to centrifugation (4 °C, 20,800 g, 15 min). The supernatant was transferred to a plastic tube and evaporated under a nitrogen flow at 60 °C. This extraction was not performed for tea samples. The supernatants from extractions and the tea samples were subjected to hydrolysis by adding 0.6 mL of β-glucuronidase/ sulfatase (2 mg/mL in 50 mM sodium acetate buffer, pH 5) to the dried samples and to brew tea (1 mL) using a vortex incubator (37 °C, 16 h). The hydrolysis was stopped by adding 0.5 mL of 0.4 % formic acid, and the samples were centrifuged (4 °C, 20,800 g, 15 min). After centrifugation, 300 μL of acetonitrile was added to the supernatant. Finally, the samples were filtered through a Whatman 0.45 μm PTFE filter (Gelman Sciences, Ann Arbor, USA) and transferred to an amber HPLC vial for LC-MS/MS analysis.

2.5. LC–MS/MS analysis of lignans

Sample analysis was carried with a Nexera UPLC system (Shimadzu Corporation, Kyoto, Japan) coupled with a LCMS-8050 triple quadrupole mass spectrometer (Shimadzu Corporation, Kyoto, Japan). The UPLC unit consisted of a SIL-30AC autosampler, a LC-20ADXR solvent delivery module, DGU-20ASR degassing unit, a CTO-20AC column oven, and a FCV-20AH$_2$ valve unit. The samples (5 μL) were injected on an Acquity Premier BEH C18 Column, 1.7 μm, 2.1 × 100 mm BEH, connected to an Acquity UPLC BEH C18 VanGuard Pre-column, 130 Å, 1.7 μm, 2.1 mm × 5 mm (Waters Chromatography B.V., 4879 AH Etten-Leur, the Netherlands). The flow rate was set at 0.3 mL/min and the column temperature at 40 °C. The mobile phases consisted of 0.1 % formic acid (solvent A), acetonitrile with 0.1 % formic acid (solvent B) with the following elution profile (t in [min]/[%B]): (0.0/5), (0.5/5), (2.0/25), (5.0/50), (7.0/95), (8.5/95), (8.6/5) and (12.5/5). MS data was collected for 12.5 min. Negative ionisation mode was used for the MS analysis of all analytes except pinoresinol, which was analyzed in positive mode. The voltage of the turbo ion-spray ionization was 4.0 kV. The temperature of the electrospray ionization probe, desolvation line, and heat block were set at 300 °C, 250 °C, and 400 °C, respectively. The pressure of the collision-induced dissociation gas was 4 kPa, whereas the flow rates of the drying gas, nebulizer gas, and heating gas were set at 10 mL/min, 3 mL/min, and 10 mL/min, respectively. The electrode voltage of Q1 pre-bias (collision cell energy entrance potential), Q2 collision cell (collision energy), Q3 pre-bias (collision cell energy exit potential), parent and fragment ion *m/z* of the multiple reaction monitoring transitions were optimized (Table 1) using the support software (LabSolutions, Shimadzu Corporation, Kyoto, Japan). The dwell time ranged from 3 msec to 8 msec for single reaction monitoring (SRM) of the analytes. The signal of the most abundant fragment ion was selected for quantitation.

2.6. Method validation

2.6.1. Calibration curves

All standards were dissolved in 100 % acetonitrile and kept at −20 °C. A working solution (50000 ng/mL) consisting of all the lignans and ^{13}C-GA in water/acetonitrile (1:1, v/v) was prepared on the day of the analysis and used for the preparation of the calibration curves. A standard curve in the range of 1000–10000 ng/mL (LARI), 12.5–10000 ng/mL (MATA), 200–5000 ng/mL (PINO); and 125–7500 ng/mL (SECO). Calibration curves were obtained by plotting the peak area against the concentration of the calibration standard and consisted 6 to 8 points depending on the analytes. Each calibration standard was injected three times, and the average value of the peak areas was used to construct the calibration curves. The standard curves showed good linearity with regression coefficients not lower than 0.997. In addition, the coefficient of variations (CV %) of calibration curves intra-day was determined.

The limit of quantification (LOQ) and the limit of detection (LOD) were calculated from the standard deviation of the intercept (S) and slope (s) of the calibration curve (LOQ = 10 × S/s and LOD = 3.3 × S/s).

Table 1
Compound-dependent LC-MS/MS parameters.

Compound	Precursor ion (*m/z*)	Product ion (*m/z*)	Dwell time (ms)	Q1 Pre Bias (V)	Collision Energy (CE) (V)	Q3 Pre Bias (V)
LARI	359.1000	329.1500	8	−28.0	28.0	15.0
		175.1000	8	−21.0	−21.0	24.0
		159.9000	8	−25.0	−25.0	–32.0
MATA	357.1000	83.0000	8	−18.0	28.0	14.0
		121.9500	8	28.0	35.0	26.0
PINO	358.7000	269.9000	3	−19.0	–23.0	−13.0
		226.9000	3	−11.0	−39.0	−25.0
SECO	361.0000	165.0000	5	27.0	25.0	18.0
		346.0500	5	28.0	21.0	27.0
		121.0500	5	27.0	41.0	12.0
^{13}C-GA	465.3000	75.0000	5	23.0	42.0	27.0
		402.4000	5	23.0	36.0	11.0
		382.5000	5	18.0	41.0	18.0

* Q1 Pre Bias = collision cell energy entrance potential; CE = collision energy, Q3 Pre Bias = collision cell energy exit potential; LARI = lariciresinol; MATA = matairesinol; PINO = pinoresinol; SECO = secoisolariciresinol; ^{13}C-GA = glycocholic acid (glycine-1 ^{13}C).

2.6.2. Extraction efficiency

^{13}C-GA was used to evaluate the extraction efficiency of lignans by spiking the plant samples after adding the extraction solvent and the tea samples after filtration with a ^{13}C-GA solution (10 μL, 50 μg/mL). The efficiency was calculated as a concentration of ^{13}C-GA measured by LC-MS/MS compared to the estimated ^{13}C-GA concentration (357.14 ng/mL). Lignan content values were corrected according to the determined extraction efficiency percentages.

2.6.3. Matrix effect

The matrix effect (ME), as an indicator of the ionization suppression or enhancement, was calculated as follows: ME(%)= (B/A), where A is the peak area for each analyte in a standard solution of known concentration and B is the peak area for each analyte spiked in an extracted sample at the same concentration. Samples from various matrices were spiked with a lignan standard solution (10 ul, 2.5 μg/mL) was used to assess the matrix effect.

2.7. Statistical analysis

Data were presented as mean ± standard deviation (SD) from duplicate analyses of two independent samples. Results were statistically analyzed among samples using one-way analysis of variance (ANOVA) and Tukey HSD multiple comparison post-hoc test. P-values < 0.05 were regarded as statistically significant. Statistical analyses were performed using IBM SPSS Statistics version 25 software (IBM Corp).

3. Results and discussion

3.1. Selection of plant food

Eight foods including flaxseed were selected for this study. Plant foods were selected that are most commonly consumed according to the data from The National Health Morbidity Survey, Malaysia Adult Nutrition Survey (MANS) conducted in 2014. The chosen foods were among the ones for which the highest intake (g/day) is reported in each of the following food groups: leafy green vegetables, green vegetables, legumes, other types of vegetables, and beverages. Pickling, germination, fermentation, and brewing, was applied to specific subsets of the samples as indicated in Table S1.

3.2. Chromatography

A mixture of lignan standards (1250 ng/mL) was used to optimise the chromatographic conditions. Good chromatographic separation was achieved for SECO (retention time: 6.2 mins), LARI (6.4 mins), PINO (6.9 mins) and MATA (7.3 mins) (Figure S2). The short analysis time allows the screening of large sample sets. After several injections of the solution with the lignan standard mix, the analysis of blank samples showed no carry-over effects.

3.3. Mass spectrometry

The optimisation of the MRM conditions (Table 1) was performed by flow injection analysis of individual lignan standard solutions (20 μg/mL-50 μg/mL). The negative ionisation mode was the most suitable for analysing SECO, LARI and MATA in agreement with other studies (Milder et al., 2004; Nørskov & Knudsen, 2016; Peñalvo et al., 2005). However, PINO generated signal only when positive ionisation mode was used. LARI generated lower signal compared to the signal of the other analytes in comparable concentration. This is demonstrated by the different concentration range compared to those for PINO, MATA and SECO. Thus, the positive mode was used for PINO, which was discovered to be different from other studies.

3.4. Method validation

3.4.1. Quantitation

Calibration curves of lignan standards were used to quantify the concentrations of lignans in the samples and were constructed from calibration points for SECO, PINO, MATA and LARI (Table S2). The calibration curves consisted of 8 points in SECO, PINO and MATA, while LARI consisted 6 points. The results showed an excellent correlation between peak area and concentration of the lignan standard solutions within the used concentration range for each lignan. The LOD was 1.06 ng/mL and LOQ was 3.23 ng/mL, for LARI;, the LOD was 0.26 ng/mL and LOQ was 0.79 ng/mL for MATA; the LOD was 0.13 ng/mL and LOQ was 0.38 ng/mL for PINO; and the LOD was 0.20 ng/mL and LOQ was 0.60 ng/mL for SECO (Table S3).

The coefficient of variations (CV %) of calibration curves intra-day were range of 1.4–3.4 % (PINO), 1.9–2.6 % (MATA), 4.8–6.9 % (SECO) and 2.3–2.5 % (LARI) (Table S4).

3.4.2. Extraction efficiency

The extraction efficiency of ^{13}C-GA varied vary from 70.9 to 108.2 %, and the CV values ranged from 0.3 to 19.9 % (Table S5). The ^{13}C-GA efficiency values were used to normalise the concentrations of the extracted lignans. The findings indicate that the extraction method implies that signal suppression due to matrix effects is the primary source of the deviation from the expected target of 100 %. In general, the acceptable range of extraction efficiency is 70 – 120 % (Commission, 2017).

3.4.3. Matrix effect

An overview of matrix effects (ME) in selected samples is depicted in Table S6. The ME were range of 99.1 – 125.4 % (PINO), 101.1 – 126.6 % (MATA) and 87.8 – 112.9 % (SECO). Our findings denoted a slight MS enhancement (ME > 100 %). The result indicated the influence of possible ionization competition between the analyte and components in the sample matrix (Taylor, 2005).

3.5. Influence of pickling on lignan contents

Fig. 1 shows the lignan contents in fresh celery, Chinese mustard, long beans, and shallot and the lignans content after 5 weeks of storage when pickled. The level of lignans in fresh samples ranged from 0.5 to 2.0 mg/100 g DW. Only LARI was detected in these samples. LARI in celery showed the most significant decrease (about 86 %) after pickling compared to the other products. Moreover, the LARI content significantly dropped by approximately 80 % in the pickled compared to the fresh Chinese mustard. No LARI as detected in shallot and long beans after pickling.

So far, little is known about the changes in the lignan content during the pickling process. One study has reported that only PINO was identified in pickled olive (Fayek, Farag, & Saber, 2020). Nevertheless, a plethora of studies found that phenolic content decreased after pickling. Brining with salt and pickling with vinegar over three weeks led to a decline of up to 69 % in the phenolic contents of vegetables (Chan et al., 2014).

The decline of lignan contents in our findings was in line with the reported decline in phenolics described above. Given the relatively high thermo-stability of lignans (Gerstenmeyer, Reimer, Berghofer, Schwartz, & Sontag, 2013), its decline in the plant tissues could partially be explained by either degradation by endogenous polyphenol oxidase (Fang, Hu, Liu, Chen, & Ye, 2008) or leaching out to the brine (Yamaguchi et al., 2001) after prolonged storage in acid and salt solution. Further investigation on lignan stability in acid and salt solution is needed to fully understand the mechanism of lignan decrease in pickled vegetables. Nevertheless, the observation that lignan content could decrease during the pickling process is important when developing products high in lignans.

3.6. Influence of fermentation on lignan contents

A mixture of flaxseed and mung beans has been used since the flaxseed seed coat's polysaccharides at the epidermal cell layer becomes viscous with water (Emaga, Rabetafika, Blecker, & Paquot, 2012) and forms mucilage, a gel-like layer that forms when the seed is soaked in water. The mucilage halted the formation of a compact white mycelium; hence the fermentation was unsuccessful. Therefore, we used mung beans as a supplementary ingredient with a ratio of 1:1 to reduce the mucilage viscosity and form a white layer of compact mycelium around beans, binding them into a dense, firm cake.

The mixture of fermented flaxseed and mung beans presented by far the highest lignan content among the analyzed samples (799.9 ± 67.4 mg/100 g DW, Table 2). This represents a 37 % increase compared to the unfermented mixture. Moreover, both fermented and unfermented soybeans exhibited 0.3 mg/100 g DW, being the lowest lignans content of the analyzed foods.

MATA, SECO, and PINO were detected in the mixture of both unfermented and fermented flaxseed and mung beans samples. SECO represented the major contributor of total lignans. Lignan levels were not significantly different in the fermented and unfermented soybean and mung bean samples.

To the best of our knowledge, no data are available on the changes in the lignan content during fermentation using *Rhizopus oyrzae*. Nevertheless, several studies found that fermentation influenced phenolic content. A study proved the fermentation by *Rhizopus oryzae* significantly increased phenolic acids content from rice bran (Schmidt,

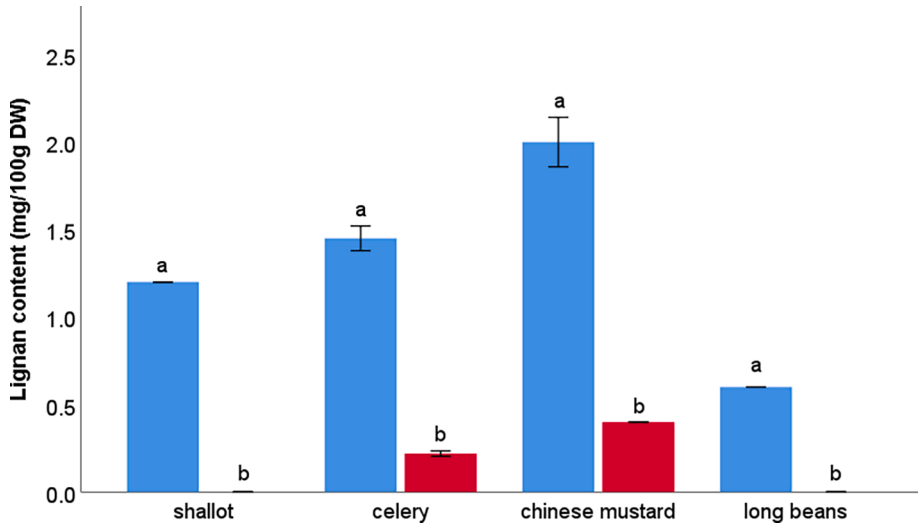

Fig. 1. Lignan (LARI) content of selected foods before (blue bars) and after 5 weeks of pickling (red bars) (mean ± SD, n = 2). In each sample type, different lowercase letters indicate significantly different lignan content between fresh samples and samples pickled. LARI = lariciresinol. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Lignan content (mg/100 g DW) in unfermented and fermented samples (mean + SD, n = 2).

Sample	MATA	SECO	PINO	total lignans
Soybeans				
unfermented		0.3 ± 0.1		0.3 ± 0.1
fermented		0.3 ± 0.0		0.3 ± 0.0
mung beans				
unfermented		0.4 ± 0.0		0.4 ± 0.0
fermented		0.4 ± 0.1		0.4 ± 0.1
Flaxseed + mung beans				
unfermented	0.1 ± 0.1	501.0 ± 134.4	0.4 ± 0.1	501.4 ± 134.6
fermented	0.1 ± 0.1	799.1 ± 67.3*	0.8 ± 0.1*	799.9 ± 67.4*

*indicates a significant difference in lignan content between the fermented and the unfermented sample. MATA = matairesinol; SECO = secoisolariciresinol; PINO = pinoresinol.

Gonçalves, Prietto, Hackbart, & Furlong, 2014).

In grains, bioactive compounds, including lignans, are mainly located in the outer parts of the grain (Hano et al., 2017). Prior to the fermentation of soybeans and mung beans, the hulls were removed, leaving the relatively lignan-depleted cotyledons, which may explain the low lignan content of unfermented and fermented beans.

Conversely, flaxseed was not dehulled; therefore, the lignans were higher in unfermented and fermented flaxseed. Endogenous enzymes and indigenous microbes are found concentrated in the outer layers of grains (Dornez, Joye, Gebruers, Delcour, & Courtin, 2006), and the microbes frequently express significant hydrolytic enzyme activities. Moreover, these cell wall-degrading enzymes' actions produced during fermentation may cause an increase in the release of phenolic compounds (Huynh, Van Camp, Smagghe, & Raes, 2014).

All in all, these findings demonstrate that fermentation with filamentous fungi could be considered a potential process for increasing the release of lignans, thereby contributing to the production of food products with added value.

3.7. Influence of germination on lignan contents

Fig. 2 illustrates the change in lignan contents during germination of mung beans, soybeans, and flaxseed. SECO, MATA, PINO, and LARI were detected in all samples. SECO was the most abundant lignan in flaxseed. Overall, the lignan content increased during germination and reached its peak on day 6. Subsequently, the lignan content decreased at day 7 of germination. Germinated flaxseed showed the highest lignan content, which reached approximately 1500 mg/100 g DW, while germinated mung beans showed the lowest lignan content (0.3 mg/100 g DW). As far as we know, these are the first data on the effect of germination on soybean and mung beans. The highest level of lignans was found on day 6 for all germinated samples.

A study showed that the SECO content was increased by 4.5-fold in 8-day germinated flaxseed compared to ungerminated flaxseed (Wang et al., 2016). Similarly, Makowska demonstrated that the total amount of lignans in triticale variety of Madilo grain increased by about 17 % – 32 % in germinated grain over 72 h (Makowska, Waśkiewicz, & Chudy, 2020). Moreover, another study reported that lignan content in germinated rye grain was 80 % higher than in native grain (Katina et al., 2007).

The germination process is known to activate endogenous enzymes in seeds, resulting in noteworthy changes in the lignan content (Szokol-Borsodi, Sólyomváry, Molnár-Perl, & Boldizsár, 2012). The biosynthesis of lignans begins with two coniferyl alcohol (CA) molecules as precursors (Hano et al., 2006). The enzymes phenylalanine ammonia lyase (PAL), chalcone synthase (CHS), dirigent-protein oxidase (DPO), pinoresinol-lariciresinol reductase (PLR), and glucosyl transferase (GT) are involved in the biosynthesis of lignans. PAL, and CHS enzymes are up-regulated during the germination of flaxseeds; hence it might partly explain the accumulation of lignans (Wang et al., 2016).

3.8. Influences of black tea brewing on lignan concentration

Fig. 3 illustrates the content of total lignans in tea brews over four different brewing times (2, 5, 7, 10 min). As expected, the content of total lignans in the brew increased with prolonged brewing. The total lignan concentration was around 0.03 mg/100 mL at 2 min and increased more than twofold at 10 min of the brewing (approximately 0.1 mg/100 mL). The lignan concentration increased 5 and 7 min of brewing, but no significant difference was observed between 7 and 10 min.

PINO was the most abundant lignan detected in our tea sample. The presence of PINO in tea brew is in agreement with Milder, Arts, Venema, Lasaroms, Wähälä, & Hollman (2004). Moreover, a comprehensive study of plant foods commonly consumed in the Netherlands reported

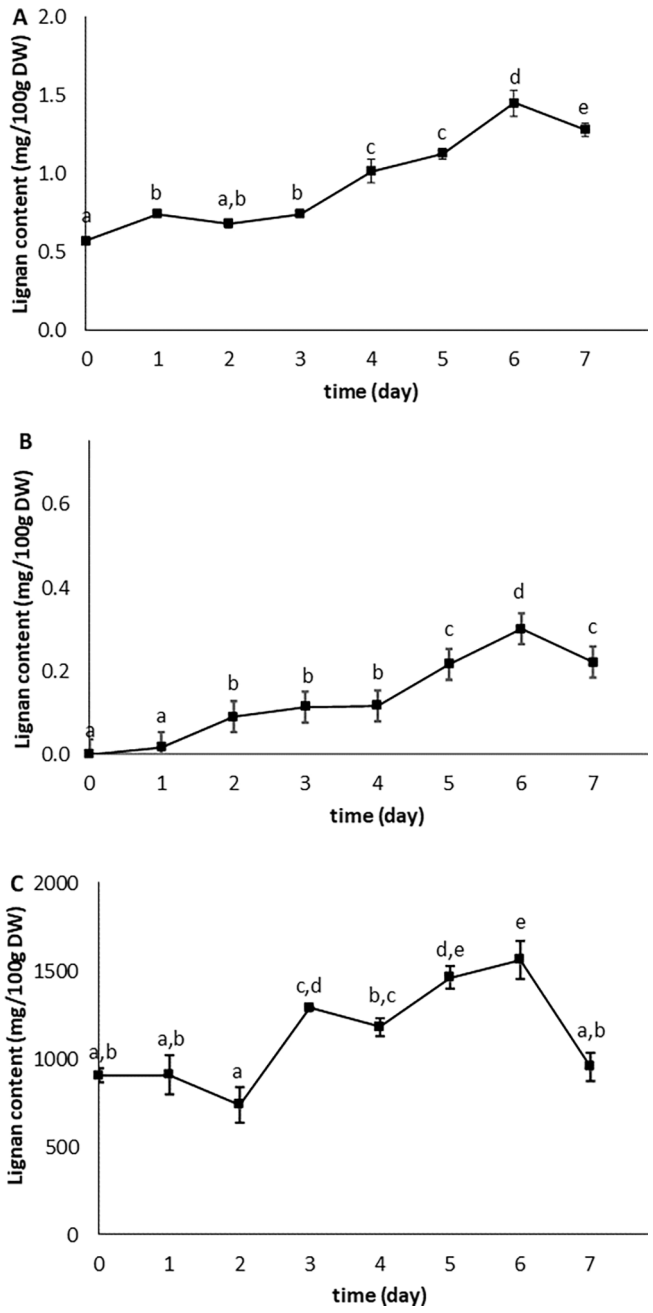

Fig. 2. Lignan content during germination of A) soybean, B) mung bean, and C) flaxseed (mean ± SD, n = 2). Different lowercase letters indicate a significant difference in lignan content.

that the lignan concentration in black tea brews ranged between 0.06 and 0.08 mg/100 mL after five minutes of brewing (Milder, Arts et al., 2005; Milder, Feskens et al., 2005). These two aforementioned studies are in agreement with our findings. Despite no report on the influence of tea brewing time on the lignan concentration could be found, a study on flavonoids and other polyphenols of commercial tea bag products (typical of those used in the UK, US, continental Europe, and the Middle East) reported that total phenolics, flavonoids, catechins, and theaflavins in brews were up to 35–55 % of the total available phenolics and that longer brewing times gave higher polyphenol concentration in tea brew (Lakenbrink, Lapczynski, Maiwald, & Engelhardt, 2000).

In addition, it has been reported that tea infusion time and temperature both contribute to the concentration of bioactive compounds in the tea brew (Damiani, Bacchetti, Padella, Tiano, & Carloni, 2014). A

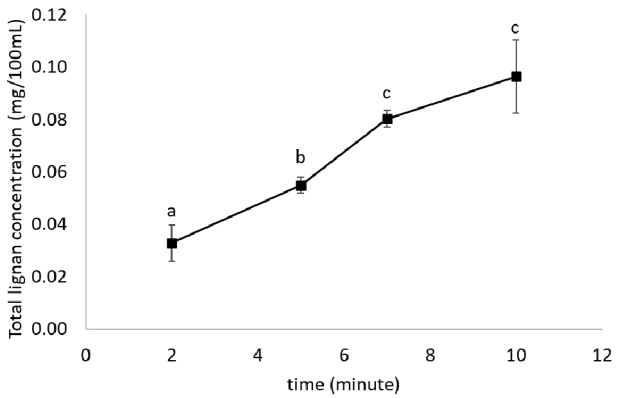

Fig. 3. Total lignan concentration during the brewing time of black tea (mean ± SD, n = 2 of independent brewing). Different lowercase letters indicate a significant difference in total lignan concentration.

study on tea brewing parameters showed that the longer the brewing time, the higher the concentration of total polyphenols (Kowalska et al., 2021). The aforementioned studies corroborate our findings, revealing a continuous increase in lignan concentration with prolonged brewing. A higher concentration content might be achieved when brewing tea for longer than 10 min.

Water temperature used to make the infusion may affect the content in thermo-labile compounds. However, heating at 100 °C for 10 to 40 min has been reported not to change the lignan concentration, indicating that brewing temperature may have a modest effect on lignan content of brewed tea (Gerstenmeyer et al., 2013).

4. Conclusion

In summary, fermentation and germination increased the lignan content in food products, as did a prolonged brewing for tea. Further research into the causes of lignan accumulation in fermented foods it is necessary to establish the optimal design of fermentation conditions. The data produced widen the range of known lignan content data in foods. In addition, the selection of foods was based on data from the Malaysia Adult Nutrition Survey with unknown lignan content to investigate whether these foods could be a potential source of lignan. On top of that, this study provided insight into how different processing methods, especially bioprocessing techniques, can affect lignan content, thus providing a basis for further investigations.

Globalization is affecting Malaysia, causing a change from traditional diets and lifestyles to more Westernized eating patterns, leading to an increase in obesity and non-communicable diseases. The health of the Malaysian population is likely to deteriorate further as the transition to a more developed economy has been fast over the last decade. Therefore, the bioprocessing approaches we used may enrich foods with lignans, increasing total lignan intake in the general population with potential beneficial effects on health. This discovery would encourage the consumption of fermented and germinated food as a better source of lignans for a healthy diet. Moreover, we could promote how to brew tea to get an optimal lignan content. These approaches are low-cost yet time-saving, and the food can be simply prepared at home.

Other processing approaches to increase lignan content of foods need to be investigated. A national strategy needs to be in place to help prevent, treat and manage obesity and non-communicable diseases in Malaysia, despite the fact that research on these areas is very much in its infancy.

CRediT authorship contribution statement

U.K. Hussain Zaki: Conceptualization, Investigation, Formal

analysis, Writing – original draft. **C. Fryganas:** Conceptualization, Investigation, Formal analysis, Writing – review & editing. **L. Trijsburg:** Writing – review & editing. **E.J.M. Feskens:** Writing – review & editing. **E. Capuano:** Supervision, Conceptualization, Investigation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2022.134607.

References

Chan, E. W. C., Tan, Y. P., Chin, S. J., Gan, L. Y., Kang, K. X., Fong, C. H., & How, Y. C. (2014). Antioxidant properties of selected fresh and processed herbs and vegetables. *Free Radicals and Antioxidants, 4*(1), 39–46.

Commission, E. (2017). SANTE/11813/2017—Guidance Document on Analytical Quality Control and Method Validation Procedures for Pesticide Residues and Analysis in Food and Feed. *Official Journal of the European Union, 46*, 1-42.

Damiani, E., Bacchetti, T., Padella, L., Tiano, L., & Carloni, P. (2014). Antioxidant activity of different white teas: Comparison of hot and cold tea infusions. *Journal of Food Composition and Analysis, 33*(1), 59–66.

Di Stefano, E., Tsopmo, A., Oliviero, T., Fogliano, V., & Udenigwe, C. C. (2019). Bioprocessing of common pulses changed seed microstructures, and improved dipeptidyl peptidase-IV and α-glucosidase inhibitory activities. *Scientific Reports, 9*(1), 1–13.

Dornez, E., Joye, I. J., Gebruers, K., Delcour, J. A., & Courtin, C. M. (2006). Wheat-kernel-associated endoxylanases consist of a majority of microbial and a minority of wheat endogenous endoxylanases. *Journal of Agricultural and Food Chemistry, 54*(11), 4028–4034.

Emaga, T. H., Rabetafika, N., Blecker, C. S., & Paquot, M. (2012). Kinetics of the hydrolysis of polysaccharide galacturonic acid and neutral sugars chains from flaxseed mucilage. *Biotechnology, Agronomy and Society and Environment, 16*(2), 139–147.

Fang, Z., Hu, Y., Liu, D., Chen, J., & Ye, X. (2008). Changes of phenolic acids and antioxidant activities during potherb mustard (Brassica juncea, Coss.) pickling. *Food Chemistry, 108*(3), 811–817.

Fayek, N. M., Farag, M. A., & Saber, F. R. (2020). Metabolome classification via GC/MS and UHPLC/MS of olive fruit varieties grown in Egypt reveal pickling process impact on their composition. *Food Chemistry, 339*, Article 127861.

Gerstenmeyer, E., Reimer, S., Berghofer, E., Schwartz, H., & Sontag, G. (2013). Effect of thermal heating on some lignans in flax seeds, sesame seeds and rye. *Food Chemistry, 138*(2–3), 1847–1855.

Hano, C., Corbin, C., Drouet, S., Quéro, A., Rombaut, N., Savoire, R., & Lainé, E. (2017). The lignan (+)-secoisolariciresinol extracted from flax hulls is an effective protectant of linseed oil and its emulsion against oxidative damage. *European Journal of Lipid Science and Technology, 119*(8), 1600219.

Hano, C., Martin, I., Fliniaux, O., Legrand, B., Gutierrez, L., Arroo, R., & Lainé, E. (2006). Pinoresinol–lariciresinol reductase gene expression and secoisolariciresinol diglucoside accumulation in developing flax (Linum usitatissimum) seeds. *Planta, 224*(6), 1291–1301.

Heinonen, S., Nurmi, T., Liukkonen, K., Poutanen, K., Wähälä, K., Deyama, T., Adlercreutz, H. (2001). In vitro metabolism of plant lignans: new precursors of mammalian lignans enterolactone and enterodiol. *Journal of Agricultural and Food Chemistry, 49*(7), 3178-3186. doi: 10.1021/jf010038a.

Huynh, N. T., Van Camp, J., Smagghe, G., & Raes, K. (2014). Improved release and metabolism of flavonoids by steered fermentation processes: A review. *International Journal of Molecular Sciences, 15*(11), 19369–19388.

Imai, T., Nomura, M., & Fukushima, K. (2006). Evidence for involvement of the phenylpropanoid pathway in the biosynthesis of the norlignan agatharesinol. *Journal of Plant Physiology, 163*(5), 483–487.

Isemura, M., Pervin, M., Unno, K., Saito, K., & Nakamura, Y. (2022). Health Effects of Tea Consumption *Nutrition Guide for Physicians and Related Healthcare Professions,* 303-308.

Kasim, N. B. M., Ahmad, M. H. B., Shaharudin, A. B. B., Naidu, B. M., Ying, C. Y., & Aris, T. B. (2018). Food choices among Malaysian adults: Findings from Malaysian Adults Nutrition Survey (MANS) 2003 and MANS 2014. *Malaysian Journal of Nutrition, 24*(1).

Katina, K., Liukkonen, K. H., Kaukovirta-Norja, A., Adlercreutz, H., Heinonen, S. M., Lampi, A. M., & Poutanen, K. (2007). Fermentation-induced changes in the nutritional value of native or germinated rye. *Journal of Cereal Science, 46*(3), 348–355. https://doi.org/10.1016/j.jcs.2007.07.006

Kennes, C. (2018). Bioconversion processes (Vol. 4, pp. 21): MDPI.

Khalesi, S., Irwin, C., & Schubert, M. (2015). Flaxseed Consumption May Reduce Blood Pressure: A Systematic Review and Meta-Analysis of Controlled Trials–3. *The Journal of Nutrition, 145*(4), 758–765.

Kowalska, J., Marzec, A., Domian, E., Galus, S., Ciurzyńska, A., Brzezińska, R., & Kowalska, H. (2021). Influence of Tea Brewing Parameters on the Antioxidant Potential of Infusions and Extracts Depending on the Degree of Processing of the Leaves of Camellia sinensis. *Molecules, 26*(16), 4773.

Lakenbrink, C., Lapczynski, S., Maiwald, B., & Engelhardt, U. H. (2000). Flavonoids and other polyphenols in consumer brews of tea and other caffeinated beverages. *Journal of Agricultural and Food Chemistry, 48*(7), 2848–2852.

Makowska, A., Waśkiewicz, A., & Chudy, S. (2020). Lignans in triticale grain and triticale products. *Journal of Cereal Science, 93*, Article 102939.

MANS. (2014). The National Health and Morbidity Survey 2014 *Malaysia Adult nutrition Survey* (Vol. Volume III).

Meagher, L. P., & Beecher, G. R. (2000). Assessment of data on the lignan content of foods. *Journal of Food Composition and Analysis, 13*(6), 935–947.

Milder, I. E., Arts, I. C., van de Putte, B., Venema, D. P., & Hollman, P. C. (2005). Lignan contents of Dutch plant foods: A database including lariciresinol, pinoresinol, secoisolariciresinol and matairesinol. *British Journal of Nutrition, 93*(3), 393–402. https://doi.org/10.1079/BJN20051371

Milder, I. E., Arts, I. C., Venema, D. P., Lasaroms, J. J., Wähälä, K., & Hollman, P. C. (2004). Optimization of a liquid chromatography− tandem mass spectrometry method for quantification of the plant lignans secoisolariciresinol, matairesinol, lariciresinol, and pinoresinol in foods. *Journal of Agricultural and Food Chemistry, 52*(15), 4643-4651. doi: 10.1021/jf0497556.

Milder, I. E. J., Feskens, E. J. M., Arts, I. C. W., de Mesquita, H. B. B., Hollman, P. C. H., & Kromhout, D. (2005). Intake of the Plant Lignans Secoisolariciresinol, Matairesinol, Lariciresinol, and Pinoresinol in Dutch Men and Women. *Journal of Nutrition, 135*(5), 1202–1207. https://doi.org/10.1093/jn/135.5.1202

Montaño, A., Sánchez, A. H., Beato, V. M., López-López, A., & De Castro, A. (2016). Pickling (pp. 369-374): Elsevier.

Nørskov, N. P., & Knudsen, K. E. B. (2016). Validated LC-MS/MS Method for the Quantification of free and bound lignans in cereal-based diets and feces. *Journal of Agricultural and Food Chemistry, 64*(44), 8343–8351.

Nout, M., & Kiers, J. (2005). Tempe fermentation, innovation and functionality: Update into the third millenium. *Journal of Applied Microbiology, 98*(4), 789–805.

Park, J. B., & Velasquez, M. T. (2012). Potential effects of lignan-enriched flaxseed powder on bodyweight, visceral fat, lipid profile, and blood pressure in rats. *Fitoterapia, 83*(5), 941–946.

Paulsen, M. R. (2008). *Measurement and maintenance of soybean quality Soybeans* (pp. 151–192). Elsevier.

Peñalvo, J. L., Haajanen, K. M., Botting, N., & Adlercreutz, H. (2005). Quantification of lignans in food using isotope dilution gas chromatography/mass spectrometry. *Journal of Agricultural and Food Chemistry, 53*(24), 9342–9347.

Schmidt, C. G., Gonçalves, L. M., Prietto, L., Hackbart, H. S., & Furlong, E. B. (2014). Antioxidant activity and enzyme inhibition of phenolic acids from fermented rice bran with fungus Rizhopus oryzae. *Food Chemistry, 146*, 371–377.

Szokol-Borsodi, L., Sólyomváry, A., Molnár-Perl, I., & Boldizsár, I. (2012). Optimum yields of dibenzylbutyrolactone-type lignans from cynareae fruits, during their ripening, germination and enzymatic hydrolysis processes, determined by on-line chromatographic methods. *Phytochemical Analysis, 23*(6), 598–603.

Tan, A. K., Dunn, R. A., Samad, M. I. A., & Feisul, M. I. (2011). Sociodemographic and health-lifestyle determinants of obesity risks in Malaysia. *Asia Pacific Journal of Public Health, 23*(2), 192–202.

Taylor, P. J. (2005). Matrix effects: The Achilles heel of quantitative high-performance liquid chromatography–electrospray–tandem mass spectrometry. *Clinical Biochemistry, 38*(4), 328–334.

Verghese, M., Willis, S., Boateng, J., Gomaa, A., & Kaur, R. (2021). Effect of Food Processing on Antioxidant Potential, Availability, and Bioavailability. *Annual Review of Food Science and Technology, 12*, 307–329.

Wang, H., Wang, J., Guo, X., Brennan, C. S., Li, T., Fu, X., & Liu, R. H. (2016). Effect of germination on lignan biosynthesis, and antioxidant and antiproliferative activities in flaxseed (Linum usitatissimum L.). *Food Chemistry, 205*, 170–177. https://doi.org/10.1016/j.foodchem.2016.03.001

Widaningrum, F. N., Ermi, S., & Endang Yuli, P. (2015). Introductory Study on Processing of Fermented Jack Bean (Canavalia Ensiformis). *Indonesian Journal of Agricultural Postharvest Research, 12*(3), 129–136. https://doi.org/10.21082/jpasca.v12n3.2015.129-136

Yamaguchi, T., Mizobuchi, T., Kajikawa, R., Kawashima, H., Miyabe, F., Terao, J., & Matoba, T. (2001). Radical-scavenging activity of vegetables and the effect of cooking on their activity. *Food Science and Technology Research, 7*(3), 250–257.