Reviewer(s)' Comments to Author:

Reviewer: 1
Comments to the Author

The manuscripts: "Attenuation of Sinapic Acid and Sinapine-Derived Flavor- Active Compounds Using A Factorial-Based Pressurized High-Temperature Processing" indicated the extraction of flavor active compounds phenolic compounds from canola meal to improve the functional and physicochemical prosperity of the protein. It is important study and will have significant input for booming future alternative plant-based protein industries. However, the manuscript has many drawbacks to be accepted in the current form. Therefore, the authors should address the following comments before I recommend it for publication.

(1) **The manuscript provided enough background information about the importance of removing the flavor-active phenolic compounds from canola meal. However, there is no background information about the possible application of the phenolic compounds when they are produced as side stream in your proposed co-extraction process**.

Response: Thank you for your feedback. Base on the both reviewers comments, the introduction was re-written with the focus of improving the extractability of flavor-active phenolic compounds including canolol. Please find below the newly re-written introduction.

"**Introduction**

Currently, up to 60% of the world's dietary protein is provided by plant-based sources (Gorissen & Witard, 2018). With the current emphasis on sustainable ingredients, plant-based protein has garnered interest by the food and feed protein industry to meet consumer demands for new and alternative sources. Both canola and pea protein blends are reported to exceed the protein quality of meat and dairy and would play a key aspect in fulfilling the future protein demand for humans (Gläser et al., 2020; Hald et al. 2019). However, the presence of undesirable bitter complexes initiated by compounds such as glucosinolates, phytates, tannins, phenolics, and its high fibre content limits the use of canola meal in food sources (Khattab et al., 2010; Naczk et al., 1998). Moreover, the associations between the proteins and the tannins further contributes to the bitter taste in the protein products (Naczk et al. 1998). Recent advancements in canola industry have led to produce valuable protein isolates and other protein ingredients from canola meal. Hence, the residual meal after isolation of the protein fractions may impart as a value-added by product to produce bitter flavor-active phenolic compounds to introduce in the nutraceutical industry.

The phenolic compounds in canola can be categorized as free, esterified, and insoluble bound (with benzoic and/or cinnamic acid) (Alu'datt et al., 2017; Li & Guo, 2016b; Quinn et al., 2017). Kozlowska *et al.*, (1983) reported the content of insoluble and bound phenolic compounds in canola meal ranged from 32-50 mg/kg. The predominant free phenolic compounds in rapeseed meal were sinapic acid, vanillic acid, protocatechuic acid, syringic acid, *p*-coumaric acid, ferulic

acid, caffeic acid, and chlorogenic acid (Kozlowska et al., 1990). The predominant phenolic compounds in canola by-products are esterified, with sinapine accounting for over 80%, and sinapic acid occurring as the major free form (Li & Guo, 2016b; Quinn et al., 2017). The traditional processing methods require large amount of extraction solvents (for example 1 g meal requires 70 mL ethanol). This is considered environmentally undesirable even though up to 85% of the phenolics can be removed (Li & Guo, 2016b; Quinn et al., 2017). The abundance of sinapates and kaempferol derivatives present in the meal before and after solvent extraction warrants further investigation. Moreover, these bitter-flavoring phenolic compounds conjugate with other food ingredients including proteins, peptides, and lipids (Alu'datt et al., 2017). Consequently, the amount, bonding, and structure can have a profound effect on the extraction of these complex phenolic compounds; for example, their initial concentration determines the tannin-protein, protein-phenolic and lipid-phenolic-protein complexes (Alu'datt et al., 2017; Mišan et al., 2010).

The targeted removal and co-extraction of these bitter flavor-contributing compounds, especially sinapine, and kaempferol derivatives will contribute to further innovative processing of canola by-products. Furthermore, these value-added by products could be introduced as a source of nutraceuticals with high antioxidant activity (Alu'datt et al., 2017; Li & Guo, 2016b). Apart from sinapine, both sinapic acid and canolol are both reported as strong antioxidative, anti-radical and anti-mutagenic molecules (Cao et al., 2015; Chen, 2016; Morley et al., 2013). The formation of canolol is closely associated with high temperature processing as temperature-dependent parameters are necessary to improve the functional properties of canolol (Li & Guo, 2016a; Nandasiri et al., 2019). Hence, the isolation and purification of these flavor-active phenolic compounds and other antioxidative compounds would be an added advantage to the industry. Thus, a targeted efficient extraction method capable of releasing or separating the bitter-flavor active phenolic compounds from proteinaceous matter would be advantageous to the industry.

Both pressurized solvent extraction (PSE) and accelerated solvent extraction (ASE) have recently been applied by the natural product industry to extract phenolic compounds at a relatively high temperature (~200^{o}C), and pressure (~2000 psi) (Li & Guo, 2016a; Nandasiri et al., 2019). The higher phenolic extraction efficiency associated with these methods facilitate attenuation of the bitter-flavoring compounds in the meal, by impacting the extraction of the major sinapic acid derivatives, primarily sinapine and kaempferol derivatives (Li & Guo, 2016a, 2016b; Nandasiri et al., 2019). Thermal processing and the high pressure associated with ASE have many advantages including reduction in the surface tension and viscosity of the extracting solvents, which improves the solubility and mass transfer of targeted phenolics (Li & Guo, 2016a). ASE is also equipped with a closed chamber so that an inert supply with N_2 ensures the stability of the crude extracts with a higher yield of phenolic compounds (Nandasiri et al., 2019).

Previous research reported that structural alterations of phenolics resulted from the application of high pressure, and high temperature (Nandasiri et al., 2019), which generated canolol and flavor-active novel dimers and trimers (Harbaum-Piayda et al., 2010; Kraljić et al., 2015). These previous works discussed extraction yields and instability of these flavor-active phenolic compounds, however on a lab-scale, and further investigation is yet to be considered. A potential major drawback in converting them at both bench-top and industrial scale is absent so

far. Consequently, targeted extraction of bitter flavor-active phenolic co-stream ingredients from canola meal should substantially increase its value as a source of nutraceuticals. The present study investigated the pressurized temperature processing (ASE) as method of extraction of flavor-active phenolic compounds. Two different particle sizes (0.5 mm and 1.0 mm) and two extractants (methanol and ethanol) at different concentrations (30%, 40%, 60%, and 70% v/v) under high pressure (1500 psi) at three different temperatures (140, 160, and 180ºC) were examined in the current study. The present study investigated important parameters for extracting the bitter compounds, sinapine, sinapic acid, thomasidioc acid (TA), and major flavor-active kaempferol derivatives. Furthermore, the application of pressurized temperature processing *via* ASE with the targeted extraction of canolol was investigated. The targeted extraction has implications in co-processing of the canola meal for the production of value-added phenolic compounds."

(2) Have you measured the initial concentration of the phenolic compounds in the raw material?

Response: Thank you for your feedback. The initial concentration of the phenolic compounds has been conducted and already published in our previous manuscript with the focus of the antioxidant activity. However, our main focus was to identify the different extraction parameters on the extractability of flavor-active phenolic compounds.

Nandasiri, R., Eskin, N. A. M., & Thiyam−Höllander, U. (2019). Antioxidative Polyphenols of Canola Meal Extracted by High Pressure: Impact of Temperature and Solvents. *Journal of Food Science*, *84*(11), 3117–3128. https://doi.org/10.1111/1750-3841.14799

(3) Line 80-81: How did you confirm the purity of canolol?

Response: Purity of canolol was determined using the HPLC analysis. The purity was calculated as a ratio of canolol (peak area) to that of the combined area of the impurities.

(4) Line 103: What is your justification for selecting these extraction conditions?

Response: Thank you for your feedback. Up to date there has been many research studies conducted based on different solvents, and polarities. However, a complete study on the impact of solvent type, solvent polarity, extraction temperature, and particle size of the meal on the extractability of phenolic compounds is not completed to date. Therefore the current study investigated the extraction of bitter compounds using two different particle sizes of the meal (0.5 and 1.0 mm) with aqueous methanol and ethanol at different concentrations (30%, 40%, 60%, and 70%, v/v) under high pressure (1500 psi) and at three different temperatures (140, 160, and 180ºC) using ASE (ASE 300, Dionex, NY, USA).

(5) Extraction time has significant influence in ASE. What was the extraction time?

Response: Thank you for your feedback. The extraction time consist of preheating (2-min), heating (6-min), extraction static time (5-min*3) and purging 90 sec. with a total time of ~20 minutes. We have already published this information in our previous publication. I have attached the figure from the manuscript herewith.

Nandasiri, R., Eskin, N. A. M., & Thiyam−Höllander, U. (2019). Antioxidative Polyphenols of Canola Meal Extracted by High Pressure: Impact of Temperature and Solvents. *Journal of Food Science*, *84*(11), 3117–3128. https://doi.org/10.1111/1750-3841.14799

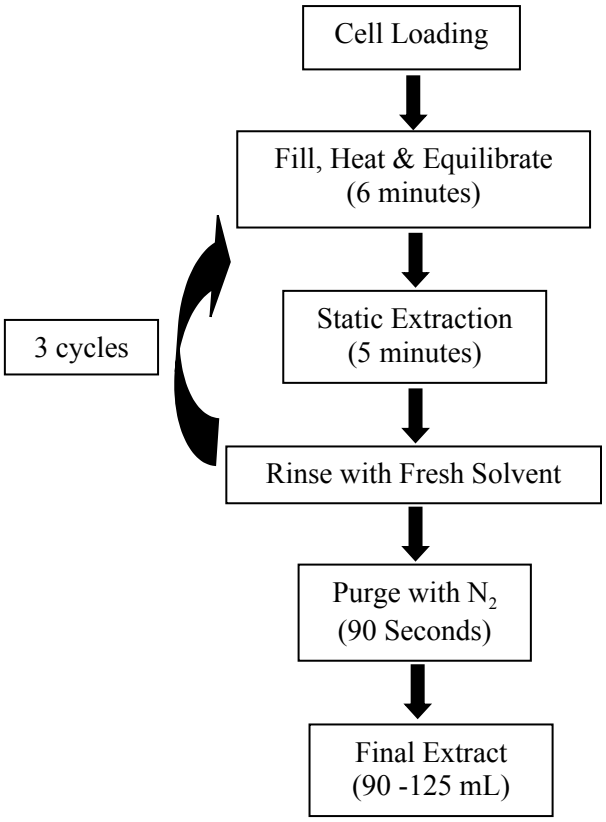

Figure 1: **Extraction of phenolic compounds using ASE**

(6) Why you did not consider time as a factor? Any Justification?

Response: Thank you for your feedback. The extraction time was optimized for the extractability of total phenolic content of the extractants, and therefore in the current study we did not consider the time as a factor. Please see above justification.

(7) The objective of your study was removing flavor compounds from canola meal to use the protein as plant-based protein. However, the study did not include any protein quality/functional properties analysis after the extraction. Since the extractions are conducted at high temperature, there could be denaturation/quality deteriorations of the protein. I suggest the authors to include this information to show whether the extraction method had effect on the quality of the protein or not.

Response: Thank you for your feedback. The focus of the manuscript was changed based on the reviewers comments and we have changed the scope of the manuscript to improvement of extractability of flavor-active phenolic compounds of canola meal via pressurized temperature extraction. Our future studies will be look at the protein quality and the quantity in terms of the phenolic extractability.

(8) Line 184-188: You need citation for this statement.

Response: Thank you for your feedback. We have added the necessary citations.

"The hydrolysis of sinapine to sinapic acid is considered the major structural-alteration pathway contributing to the flavor-active properties present in canola meal (Li & Guo, 2016a; Nandasiri et al., 2019; Siger et al., 2013)."

(9) Line 248-250: The dark brown color does not necessarily indicate the presence of high phenolic content. ASE usually produce milliard reaction product, which are mostly responsible for brown color of the extract.

Response: Thank you for your feedback. We have made the necessary changes including the justification of Maillard reaction products.

"The visually apparent darker brown/black colored extracts obtained at higher processing temperatures (180°C) by ASE was indicative of the presence of higher amounts of Maillard reaction products apart from the phenolics (Chen et al., 2014; Rubino et al., 1996)."

(10) I suggest to moving Tables 1a-f to supplementary material.

Response: Thank you for your feedback. We have made the necessary changes based on your comments and we will move the table 1 to supplementary materials section.

(11) The conclusion needs to rewrite after including information regarding the quality of the protein after the extraction process.

Response: Thank you for your feedback. We have re-arranged the conclusion based on the extractability of the flavor-active phenolic compounds and removing the section on the protein quality.

"The occurrence of major sinapates, namely sinapine, sinapic acid, and canolol and other active molecules including TA and kaempferol derivatives imparts flavor to canola meal. The targeted extraction and co-processing using ASE proved to be an efficient method for extracting these flavor-active molecules while attenuating the bitter molecules from the canola meal. The use of shorter extraction times (20 minutes), lower solvent usage, and improved concurrent and

targeted extractability of flavor-active phenolic molecules using ASE will enable the creation of co-streams of phenolic rich antioxidants. These phenolic rich antioxidative compounds from the meal characterize an additional potential source for use in the food and nutraceutical industries. These new co-streams can be piloted with canola protein industries to benefit the ongoing strong demand for alternative plant-based natural preservatives and shelf-life improving agents."

Reviewer: 2
Comments to the Author

Authors have reported the attenuation of Sinapic acid and sinapine-derived flavour-active compounds using a factorial bases pressurized High-Temperature Processing. Despite that it is well written manuscript, it is mandatory to clarify the main objective of this research, the type of experimental design as well as statistical analysis, and chemical characterization of the extract.

(1) Introduction section has to be rewritten to clarify the main objective of this research.

Response: Thank you for your feedback. Based on both reviewers comments we have re-written the introduction section. Please refer to the Reviewer 1 comments.

(2) The idea is to remove flavour-active compounds to the canola protein matrix or to revalorizate the by-products generated during this process since it could be rich in phenolics ?

Response: Thank you for your feedback. We have made the necessary changes to the manuscripts objectives.

"Previous research reported that structural alterations of phenolics resulted from the application of high pressure, and high temperature (Nandasiri et al., 2019), which generated canolol and flavor-active novel dimers and trimers (Harbaum-Piayda et al., 2010; Kraljić et al., 2015). These previous works discussed extraction yields and instability of these flavor-active phenolic compounds, however on a lab-scale, and further investigation is yet to be considered. A potential major drawback in converting them at both bench-top and industrial scale is absent so far. Consequently, targeted extraction of bitter flavor-active phenolic co-stream ingredients from canola meal should substantially increase its value as a source of nutraceuticals. The present study investigated the pressurized temperature processing (ASE) as method of extraction of flavor-active phenolic compounds. Two different particle sizes (0.5 mm and 1.0 mm) and two extractants (methanol and ethanol) at different concentrations (30%, 40%, 60%, and 70% v/v) under high pressure (1500 psi) at three different temperatures (140, 160, and 180ºC) were examined in the current study. The present study investigated important parameters for extracting the bitter compounds, sinapine, sinapic acid, thomasidioc acid (TA), and major flavor-active kaempferol derivatives. Furthermore, the application of pressurized temperature processing *via* ASE with the targeted extraction of canolol was investigated. The targeted extraction has implications in co-processing of the canola meal for the production of value-added phenolic compounds."

(3) In addition, the proposed method applies high temperature to the canola samples, and consequently the quality of the protein could be affected by this parameter. Utilization of the protein matrix after the treatment could be not useful. Authors have to clarify this important issue. Maybe the main objective of this research is not well addressed in the introduction section, and consequently all of this information is confused to the reader.

Response: Thank you for your feedback. The focus of the manuscript was changed based on the reviewers comments and we have changed the scope of the manuscript to improvement of extractability of flavor-active phenolic compounds of canola meal via pressurized temperature extraction. Our future studies will be look at the protein quality and the quantity in terms of the phenolic extractability. Both the objectives and the introduction section were re-written based on the reviewers response. Please refer to the previous response.

(4) In addition, the optimization of the phenolic recovery by ASE should be described in major details.

Response: Thank you for your feedback. Our results section on the impact of pressurized heat on flavor-active phenolic compounds discussed in detail about the optimization of phenolic recovery. Please find the detailed results section attached below.

4.3 Impact of pressurized heat on flavor-active phenolic compounds

"The literature generally supported that thermal processing affected sinapates. The high temperature (up to 200°C) and pressure (~1500 psi) of ASE facilitates the removal of the aglycone moieties attached to phenolic compounds by hydrolysis with minimal interference on its original composition (Yang et al., 2015). The application of ASE yielded comparatively higher amounts of phenolic compounds compared to conventional methods as well as ultrasound extraction (Li & Guo, 2016a; Nandasiri et al., 2019). This was attributed to the high pressure of ASE which increased the solubility of the targeted compounds and the diffusion rates as well as the mass transfer rates of the solutes (Li & Guo, 2016a). The concurrent extraction of ASE also facilitated the structural transformations of sinapine to sinapic acid and canolol, at elevated temperatures (Li & Guo, 2016a).

These transformations would enable the attenuation of bitter flavor-active phenolic compounds while improving its co-processing. For example, the decreasing content of sinapine, largely impacted by the increase in temperature (**Table 3**) is attributed to the decomposition or hydrolysis pathway (Khattab, et al., 2014; Oehlke et al., 2017). Results indicated that the concentration of sinapine decreased significantly ($p > 0.05$) from 9.75 mg/g DW to 5.12 mg/g DW with the increase in temperature from 140°C to 180°C with 70% (v/v) ethanol whereas, the concentration of sinapine further decreased from 12.1 mg/g DW to 5.12 mg/g DW with increase in temperature from 160°C to 180°C (**Table 3**). This confirms the transformation of sinapine at higher temperatures, either from the bound and free forms (Chen, et al., 2014; Khattab, et al., 2010). The thermal decomposition order of the phenolic compounds showed the following decreasing pattern; sinapine > sinapic acid > canolol (Khattab, et al., 2010).

High temperature pre-conditioning and thermal processing treatments can also significantly ($p < 0.05$) influence the structure of phenolic compounds besides sinapine, as well

as sinapic acid and canolol (Siger et al., 2013; Siger et al., 2015; Thiyam et al., 2009; Wroniak et al., 2016). Temperatures namely, 160°C and 180°C with a high pressure induced the hydrolysis of sinapine into sinapic acid which is consequently produces canolol by decarboxylation (Li & Guo, 2016a; Morley et al., 2013; Zago et al., 2015). In this study, the higher concentrations of sinapic acid and canolol produced by ASE confirm the conversion of sinapine to sinapic acid and canolol at the higher temperatures (**Table 3**). Thus, the combined treatment of ASE with microwave improved the quantity of phenolic compounds at relatively higher processing temperatures ranging from 160 to 180°C (Li & Guo, 2016a; Siger & Józefiak, 2016; Wroniak et al., 2016). The visually apparent darker brown/black colored extracts obtained at higher processing temperatures (180°C) by ASE was indicative of the presence of higher amounts of Maillard reaction products apart from the phenolics (Chen et al., 2014; Rubino et al., 1996).

The highest concentration of sinapic acid was attained at 160°C for both organic extractants (70% (v/v) methanol - 0.55 mg/g DW and 70% (v/v) ethanol - 0.63 mg/g DW) compared to 180°C (**Table 3**). A reduction in total sinapic acid and canolol content observed at temperatures above 160°C may be due to the loss of the *cis*-isomer of sinapic acid at temperatures higher than 140°C (Siger et al., 2015). Above 140°C, the *cis*-sinapic acid content decreased rapidly, and was undetectable at temperatures of 160°C and 180°C. Furthermore, both Harbaum-Piayda *et al.* (2010) and Kraljić *et al.* (2015) reported that canolol at high temperatures (>180°C) is converted into its other forms including dimers, trimers and oligomers. Therefore, a reduction in both sinapic acid and canolol is observed under higher processing temperatures. Spielmeyer *et al.* (2009) noted that the optimal temperature for extracting canolol was 160°C. Moreover, Morley *et al.* (2013) also reported that optimum roasting temperature for the formation of canolol is at the extraction temperature of 160°C. These findings are in agreement with our results, which also found that the highest level of canolol formation was at 160°C. In addition, Zago *et al.* (2015) reported 2-hour hydration of the meal before the treatment of super-heated steam (160°C) increased both the antioxidant activity and its total phenolic content (TPC) by 12% (22 mg SAEg/DM) compared to the non-hydrated meal further in agreement with our current findings. The authors suggest that the increase in its TPC may be due to the release of the bound phenolic compounds via the partial breakdown of the plant cell walls during the super-heated steam. The extraction conditions of ASE would facilitate similar properties yielding higher phenolic composition.

Khattab *et al.* (2014) reported that over 95% of sinapine was converted to sinapic acid using 70% (v/v) methanol by microwave extraction from canola meal. However, approximately 55% of sinapic acid was then decarboxylated to canolol with a yield of 4.2 g/kg. Thus, the relatively lower conversion rate of sinapic acid to canolol can be explained with the formation of other intermediaries of sinapic acid at higher extraction temperatures. The formation of TA at high temperature at acidic pH conditions with the precursor sinapic acid is a good example for the lower conversion rate of sinapic acid to canolol (Rubino et al., 1995).

In another note, Cai *et al.* (1999) reported that autoclaving of sinapic acid at 121°C for 15 minutes at 0.1 MPa pressure would also produce TA, which further confirms the processing conditions applied in ASE (200°C and 1500 psi) is ideal for the formation of TA at relatively higher temperature and pressure levels. The formation of these lignan derivatives and the Maillard reaction products at higher temperatures could directly influence the antioxidant activity as well as the total phenolic and flavonoid content of the extracts (Chen et al., 2014; Rubino et al., 1996). These lignans directly impact the flavor-profile at the higher processing temperatures and pressure conditions although the existing literature have not discussed this

aspect. The use of high pressure and temperature on the other hand is ideal for a short-time treatment to obtain these flavor-active compounds. Thus, shorter extraction time (~10-20 minutes) associated with the ASE provides the ideal environment for extracting these flavor-active minor compounds.

A recent sensory analysis conducted by Hald *et al.* (2019) further confirmed that the bitter flavor of canola meal by-products was due to the presence of kaempferol 3-*O*-(2‴-*O*-sinapoyl-*β*-sophoroside). They further reported that of these esterified products, KSS and KS were the most influential bitter compounds affecting the flavor profile (Hald et al., 2019; Yang et al., 2015). Further work by Siger *et al.* (2013) reported that other kaempferol derivatives are present in canola extracts including kaempferol 3-dihexoside-7-sinapoyl-hexoside (30 mg/100 g). They further reported that the concentration of these kaempferol derivatives increased with acid hydrolysis (Siger et al., 2013), which is relevant to the production and precipitation of protein concentrates.

On the contrary, most flavonoids are easily oxidized under aerated conditions, so the presence of an inert gas (N_2) is important to attenuate the oxidation (Nandasiri et al., 2019). Thus, an oxygen-free environment is essential for the extraction and the co-processing of the flavonoid-based flavor-active phenolic compounds. Apart from wet heat and high pressure, ASE's closed system equipped with inert gas (N_2) could facilitate the preservation of phenolic compounds and their antioxidant properties, which otherwise will be detrimental at such high and pressured conditions. Furthermore, this technique can readily recover highly reactive phenolic compounds and prevent their auto-oxidation. Frolov *et al.* (2013) reported that a closed system equipped with inert gas during ASE extraction minimized the rate of oxidative degradation by the complete evacuation of air from the extractants. Moreover, Li & Guo (2016a) stated that the formation and stability of canolol may be affected by shorter extraction times and the method of cooling after each extraction. The centrifugation of the extractants at 4°C immediately after each extraction step, in our method facilitated the higher recovery of phenolic compounds including canolol. Thus, an efficient cooling procedure is recommended soon after the thermal extraction to produce higher yields of flavor-active phenolic compounds including canolol, after ASE extraction. These extraction conditions correspondingly disfavor the Wessely-Moser regrouping thereby improving the extraction efficiency of flavor-active bitter-phenolics (Wang, 2010)."

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(5) The type of experimental design is not described.

Response: Thank you for your feedback. Our statistical analysis section describes the factorial design and the levels associated with each level.

3.6 Statistical analysis

"All the experiments were carried out in triplicates. Results were presented as mean ± standard deviation of triplicate analysis. Data points were checked for their normality and required transformations were carried out to obtain normalized data (Pallant, 2011). For the current experiment, logarithmic and square root transformations were conducted accordingly to obtain normalized data (Pallant, 2011). A factorial design consists with four independent factors including particle size (0.5 and 1.0 mm), type of extraction solvent (ethanol and methanol),

concentration (v/v) of the solvent (30%, 40%, 60%, and 70%) and extraction temperature (140, 160 and 180ºC). Data analysis was carried out using the general linear multiple regression model using the two-way analysis of variance (ANOVA). Multiple mean comparison was performed using Tukey's test at the level of significant of 0.05 ($p < 0.05$) (Pallant, 2011). To identify the correlation between each phenolic compound partial correlation analysis and a regression analysis was conducted for the major phenolic compounds to elucidate the structure-function relationship. All the data analysis tests were assessed by SPSS statistical software version 22 (IBM, New York, USA)."

(6) A table including the number of experiments and the combinations of the different independent variables have to be included.

Response: Thank you for your feedback. Our statistical analysis section describes the factorial design and the levels associated with each level and therefore we think we do not need to add an additional table to explain each level of the treatments.

(7) Tables summarizing the results of the statistical treatment are not well described.

Response: Thank you for your feedback. We strongly believe that our results and discussion section described the statistical analysis in detail with respect to both major sinapates and other minor components. I have attached the detailed analysis of statistics below.

4.1 Extraction efficiency of major sinapates

The hydrolysis of sinapine to sinapic acid is considered the major structural-alteration pathway contributing to the flavor-active properties present in canola meal (Li & Guo, 2016a; Nandasiri et al., 2019; Siger et al., 2013). Apart from sinapine, other sinapate derivatives including sinapic acid and canolol also contributes to the flavor properties of the canola meal (Morley et al., 2013; Thiyam et al., 2009; Thiyam et al., 2006). Furthermore, the decarboxylation of sinapic acid to canolol takes place at higher processing temperatures (Zago et al., 2015). Hence, the higher processing temperatures (>100ºC) are associated with the improved extractability of the bitter flavor-active phenolic compounds (Nandasiri et al. 2020). Thus, our findings demonstrated that both extraction temperature and extractant concentration appears to be the most important parameters for attenuating the major sinapates from the canola meal. Statistical analysis further illustrated that the extraction efficiency of these sinapates including sinapine, sinapic acid and canolol, were influenced by concentration of the extractant, type of solvent, and extraction temperature (**Table 1 a, b, c, d, e,** and **f**). It was previously reported that solvent concentration is an important factor affecting the rate and the degree of decarboxylation of sinapic acid (Li & Guo, 2016a; Nandasiri et al., 2019; Siger et al., 2013). Current study further confirmed that both the extractant concentration and the extraction temperature are the dominant factors attenuating the major sinapates. However, the particle size of the meal was the least important factor in extracting the flavor-active bitter molecules including the sinapates.

The extractability of sinapine, the major flavor-active phenolic compound present in canola meal (Thiyam et al., 2009) was primarily dependent on the extractant concentration and the extraction temperature. According to the model fit statistics both particle size ($p = 0.12$) and type of solvent ($p = 0.15$), had no significant effect on the extractability of sinapine (**Table 1a**).

This further confirms that the removal of sinapine was much less affected by the particle size of the dried canola meal compared to the type of solvent extractant (methanol, ethanol). A similar trend was observed for the extractability of sinapic acid, another flavor-active phenolic acid present in canola meal by-products. Except for particle size ($p = 0.81$), type of solvent ($p = 0.30$) and size*concentration interaction ($p = 0.24$), all other independent variables were significant ($p < 0.05$) (**Table 1b**) for extracting sinapic acid using the pressurized temperature processing. However, the extractability of canolol was mainly dependent on both the extractant concentration and type of extractant including the extraction temperature (**Table 1c**). The size of canola meal particles ($p = 0.11$) had a negligible effect on extractability of the canolol. The statistical analysis of the model accuracy was further conformed with the higher co-efficiencies of variances for all the major sinapates (sinapine - $R^2 = 0.998$, sinapic acid - $R^2 = 0.990$ and canolol - $R^2 = 0.982$).

The polarity of the extractant solvent could affect the extractability of phenolic compounds and its antioxidant properties (Teh & Birch, 2013). Furthermore, Li & Guo (2016a) reported that different polarities of the extractant solvents yield different distributions of major sinapates. The application of pressurized heat *via* ASE further facilitates the concurrent extraction of phenolic compounds and their transformations (Li & Guo, 2016a; Nandasiri et al., 2019). It was reported that the application of pressurized heat improves the H-bonding donor and accepting ability (Li & Guo, 2016a). Furthermore, the pressurized heat would further eliminate the number of hydroxyl groups and other attachments attached to the phenolic structure thereby improving the extractability of the phenolic compounds (Gaspar et al., 2008). The current study validated 70% (v/v) polarity of both ethanol and methanol extractants as the optimum concentration for extracting the major sinapates compared to their corresponding concentrations. Hence, the extractability of phenolic compounds increases with a decrease in the polarity index of the type of extractant (Terpinc et al., 2012). Considering the polarity index of both methanol (0.762) and ethanol (0.654) with having similar polarities confirms the current research findings. These results agree with previous reports where major sinapates including canolol was extracted at higher temperatures and when the optimum solvent concentration was 70% (v/v) (Li & Guo, 2016b, 2016a; Nandasiri et al., 2019; Thiyam et al., 2004; Zago et al., 2015).

The above results confirmed that the extractability of these three flavor-active sinapates were minimally affected by particle size ($p > 0.05$). Generally, the higher extraction efficiency of hydroxycinnamic acids is solely attributed to thermal degradation. For example, the generation of aroma compounds such as 4-vinylguaiacol (the product of the decarboxylation of ferulic acid), guaiacol and vanillin from ferulic acid and the bitter series *O*-caffeoyl-, *O*-feruloyl-, *O*-dicaffeoyl- and quinide derivatives derived from chlorogenic and quinic acids (Rahman et al., 2020).

4.2 Extraction efficiency of other flavor-active minor compounds

Apart from the major flavor-active sinapates, other classes of phenolics also serve as active bitter flavoring compounds such as kaempferol 3-*O*-*β*-sophoroside (KS) and, kaempferol 3-*O*-(2‴-*O*-sinapoyl-*β*-sophoroside) (KSS) (Hald et al., 2019; Yang et al., 2015). A sensory study conducted by Hald *et al.* (2019) demonstrated that protein isolates of rapeseed (canola) containing kaempferol 3-*O*-*β*-sophoroside (KS) exhibited a bitter taste above the low threshold concentration of 3.4 µmol/L confirming the as the key flavor-active molecule of the protein isolates. Kaempferol 3-*O*-*β*-sophoroside was also reported as a flavor-active phenolic compound found to be present in *Brassica* family (Yang et al., 2015). Extraction, identification, and quantification of these unique minor compounds would advance the avenues for biorefinery

approach as well as feed formulations targeting the removal of off flavors. Liquid chromatography (LC) coupled with mass spectrometry and tandem mass spectrometry (MS/MS-MS) identified this unique flavor-active molecule to be present in our extracts. The quantification of this molecule was done based on sinapic acid equivalents (SAE) to understand the impact of extraction parameters including concentration of the extractant, type of solvent, extraction temperature and the particle size.

The statistical analysis indicated that extractability of KSS was impacted by all the extraction parameters including concentration of the extractant, type of solvent, extraction temperature and the particle size, indicating the stability of this unique flavor active molecule (**Table 1e**). Nevertheless, post-hoc analysis using Tukey's test indicated that both 60% (v/v) and 70% (v/v) for both methanol and ethanol extractants had a minimal impact on the extractability of KSS (**Table 2a**). This further confirms that lower solvent polarities enable the extraction of this unique flavor active molecule, thereby attenuating the bitter off flavors from the meal. The application of less organic solvents and other harmful chemicals are often rewarded by the industries and the government, and often provide many economic benefits (Chen et al., 2014). However, the other kaempferol derivative, KS showed a different extractability compared to KSS. The extractability of KS was mainly depended on both solvent concentration and the particle size. Interestingly, both solvent type ($p = 0.26$) and extraction temperature ($p = 0.50$) had a minimal impact on its extractability (**Table 1d**). The results further indicated that this minor compound was relatively thermally stable than the other flavor-active compounds. Further, post-hoc analysis indicated that each concentration level had a significant impact on the extractability of KS (**Table 2a**). Hence, the use of smaller particle size meal with higher polarity aided a relatively higher concentration of KS. The above results confirmed that the extractability of these two unique flavor active minor compounds (KSS and KS) differed considerably. Thus, the results further confirmed the structural alterations in the phenolic compounds would affect the extractability parameters and may impact its flavor profile.

Thomasidioic acid (TA) is another flavor-active molecule but the structural alteration due to processing and extraction has not received much attention in recent years. Both Rubino *et al.* (1996) and Cai *et al.* (1999) reported that TA was not a natural phenolic compound but formed during the high temperature processing in the presence of oxygen at both acidic and alkaline pH. The formation of TA takes place in the acidic medium with the precursor sinapic acid with dehydrosinapic acid lactone as its intermediary product (Rubino, Arntfield, & Charlton, 1995). TA is categorized under the phenolic group of lignans. These lignans were reported to convert into hormone like compounds by the gut microflora inside the body, which protects the body against hormone dependent cancers (Ward, 1993). The quantification of this thermo-generative compound was conducted to understand the impact of each extraction parameter.

The statistical analysis indicated that extractability of TA was primarily depended on both extraction temperature and the concentration of the extractant (**Table 1f**). Both the size of the canola meal particles ($p = 0.48$) and the type of solvent ($p = 0.14$) had a minimum impact on the extractability of TA agreeing with the previous reports. On the contrary, at higher extraction temperatures, these lignan compounds further converts to other complex phenolic compounds including its dimers, trimers, and oligomers (Harbaum-Piayda et al., 2010; Morley et al., 2013; Oehlke et al., 2017; Siger et al., 2013). Consequently, the concentration of free TA would decrease with the formation of these phenolic derivatives. This was further confirmed via the statistical analysis showing that both 140 and 180^{o}C processing temperatures had no significant differences on the extractability of TA (**Table 2c**).

(8) Independent and dependent variables have to be identify in tables as well as along the manuscript.

Response: Thank you for your feedback. Our statistical analysis section describes the factorial design, independent and dependent variables, and the levels associated. Please find below the attached description from the statistical analysis section.

"A factorial design consists with four independent factors including particle size (0.5 and 1.0 mm), type of extraction solvent (ethanol and methanol), concentration (v/v) of the solvent (30%, 40%, 60%, and 70%) and extraction temperature (140, 160 and 180°C). Data analysis was carried out using the general linear multiple regression model using the two-way analysis of variance (ANOVA)."

(9) F- and p-values have to be included in the manuscript.

Response: Thank you for your feedback. The significance column indicates the p-values for each response variable. We will add the f-values into the manuscript considering your feedback.

Table S1a: Effect of particle size, solvent type, solvent concentration, and temperature on sinapine concentration

HPLC Analysis – Sinapine (LOG Transformation)	Sum of Squares	DF	Mean Square	F-value	Significance
Con	20.48	3	6.83	15281.07	0.00
Temp	0.39	2	0.19	431.92	0.00
Size * Con	0.17	3	0.06	127.19	0.00
Solvent * Con	0.58	3	0.20	435.75	0.00
Con * Temp	1.71	6	0.29	637.95	0.00
Size * Temp	0.53	2	0.27	598.06	0.00
Solvent * Temp	0.12	2	0.06	133.54	0.00
Size * Solvent * Con	1.65	3	0.55	1234.35	0.00
Size * Con * Temp	0.31	6	0.05	115.26	0.00
Solvent * Con * Temp	0.57	6	0.09	211.42	0.00
Size * Solvent * Temp	0.20	2	0.10	219.33	0.00
Size * Solvent * Con * Temp	0.18	6	0.03	68.80	0.00
Error	0.06	142	0.00		
Total	2203.18	190	6.83		
Corrected Total	27.19	189			
R^2 - 0.998					
Adj R^2 - 0.997					

DF: degrees of freedom; LOG: logarithmic; Con: concentration; Temp: Temperature; HPLC: high performance liquid chromatography; R^2: coefficient of correlation; Adj R^2 - adjusted coefficient of correlation

Table S1b: Effect of particle size, solvent type, solvent concentration, and temperature on sinapic acid concentration

HPLC Analysis – Sinapic acid (LOG Transformation)	Sum of Squares	DF	Mean Square	F-value	Significance
Con	21.36	3	7.12	2542.06	0.00
Temp	1.40	2	0.70	249.81	0.00
Solvent * Con	1.69	3	0.56	201.24	0.00
Con * Temp	2.84	6	0.47	169.04	0.00
Size * Solvent	0.80	1	0.80	283.99	0.00
Size * Temp	1.58	2	0.79	282.40	0.00
Solvent * Temp	1.87	2	0.93	333.64	0.00
Size * Solvent * Con	1.53	3	0.51	182.35	0.00
Size * Con * Temp	1.24	6	0.21	73.78	0.00
Solvent * Con * Temp	2.30	6	0.38	136.95	0.00
Size * Solvent * Temp	0.24	2	0.12	42.13	0.00
Size * Solvent * Con * Temp	2.05	5	0.41	146.60	0.00
Error	0.37	131	0.00		
Total	926.81	178			
Corrected Total	37.35	177			
R^2 - 0.990					
Adj R^2 - 0.987					

DF: degrees of freedom; LOG: logarithmic; Con: concentration; Temp: Temperature; HPLC: high performance liquid chromatography; R^2: coefficient of correlation; Adj R^2 - adjusted coefficient of correlation

Table S1c: Effect of particle size, solvent type, solvent concentration, and temperature on canolol concentration

HPLC Analysis – Canolol (LOG Transformation)	Sum of Squares	DF	Mean Square	F-value	Significance
Solvent	0.38	1	0.38	61.60	0.00
Con	25.94	3	8.65	1396.69	0.00
Temp	9.38	2	4.69	757.69	0.00
Size * Con	0.34	3	0.11	18.14	0.00
Solvent * Con	1.19	3	0.40	64.06	0.00
Con * Temp	1.19	6	0.20	32.12	0.00
Size * Temp	0.46	2	0.23	36.81	0.00
Solvent * Temp	0.36	2	0.18	28.66	0.00
Size * Solvent * Con	0.31	4	0.08	12.59	0.00
Size * Con * Temp	1.32	6	0.22	35.45	0.00
Solvent * Con * Temp	1.29	6	0.21	34.62	0.00
Size * Solvent * Con * Temp	1.79	8	0.22	36.21	0.00
Error	0.84	136	0.01		
Total	688.80	184			
Corrected Total	46.31	183			
R^2 - 0.982 Adj R^2 - 0.976					

DF: degrees of freedom; LOG: logarithmic; Con: concentration; Temp: Temperature; HPLC: high performance liquid chromatography; R^2: coefficient of correlation; Adj R^2 - adjusted coefficient of correlation

Table S1d: Effect of particle size, solvent type, solvent concentration, and temperature on kaempferol 3-*O*-*β*-sophoroside (KS) concentration

HPLC Analysis – KS (SQRT Transformation)	Sum of Squares	DF	Mean Square	F-value	Significance
Size	563.17	1	563.17	60.05	0.00
Con	22282.23	3	7427.41	791.91	0.00
Size * Con	546.82	3	182.27	19.43	0.00
Solvent * Con	1614.98	3	538.33	57.40	0.00
Con * Temp	1500.90	6	250.15	26.67	0.00
Size * Temp	578.81	2	289.41	30.86	0.00
Size * Solvent * Con	1506.08	3	502.03	53.53	0.00
Size * Con * Temp	349.79	6	58.30	6.22	0.00
Solvent * Con * Temp	972.49	6	162.08	17.28	0.00
Size * Solvent * Temp	239.68	2	119.84	12.78	0.00
Size * Solvent * Con * Temp	337.43	6	56.24	6.00	0.00
Error	1350.60	144	9.38		
Total	271600.79	192			
Corrected Total	32385.68	191			
R^2 - 0.958					
Adj R^2 - 0.945					

DF: degrees of freedom; SQRT: square root; KS: kaempferol 3-*O*-*β*-sophoroside; Con: concentration; Temp: Temperature; HPLC: high performance liquid chromatography; R^2: coefficient of correlation; Adj R^2 - adjusted coefficient of correlation

Table S1e: Effect of particle size, solvent type, solvent concentration, and temperature on kaempferol 3-*O*-(2‴-*O*-sinapoyl-*β*-sophoroside) (KSS) concentration

HPLC Analysis – KSS	Sum of Squares	DF	Mean Square	F-value	Significance
Size	302716.48	1	302716.48	69.16	0.00
Solvent	281870.43	1	281870.43	64.40	0.00
Con	2688694.01	3	896231.34	204.75	0.00
Temp	1431323.84	2	715661.92	163.50	0.00
Size * Con	2318117.40	3	772705.80	176.53	0.00
Solvent * Con	1952004.37	3	650668.12	148.65	0.00
Con * Temp	2138094.49	6	356349.08	81.41	0.00
Size * Temp	443444.35	2	221722.17	50.65	0.00
Size * Solvent * Con	1160887.26	3	386962.42	88.40	0.00
Size * Con * Temp	575877.71	6	95979.62	21.93	0.00
Solvent * Con * Temp	725714.48	6	120952.41	27.63	0.00
Size * Solvent * Temp	849788.95	2	424894.48	97.07	0.00
Size * Solvent * Con * Temp	586533.03	6	97755.51	22.33	0.00
Error	630321.94	144	4377.24		
Total	78186801.31	192			
Corrected Total	16226054.39	191			
R^2 - 0.961 Adj R^2 - 0.948					

DF: degrees of freedom; KSS: kaempferol 3-*O*-(2‴-*O*-sinapoyl-*β*-sophoroside); Con: concentration; Temp: Temperature; HPLC: high performance liquid chromatography; R^2: coefficient of correlation; Adj R^2 - adjusted coefficient of correlation

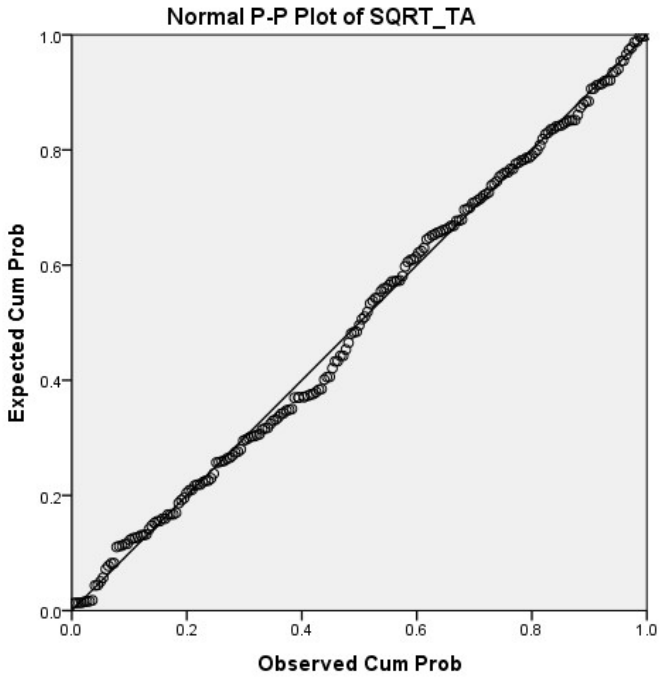
Table S1f: Effect of particle size, solvent type, solvent concentration, and temperature on thomasidioic acid (TA) concentration

HPLC Analysis – TA (SQRT Transformation)	Sum of Squares	DF	Mean Square	F-value	Significance
Con	3156.47	3	1052.16	239.34	0.00
Temp	143.54	2	71.77	16.33	0.00
Con * Temp	839.30	6	139.88	29.89	0.00
Solvent * Temp	188.80	2	94.40	210.59	0.00
Solvent * Con	2777.30	3	925.77	31.82	0.00
Size * Con	394.18	3	131.39	9.23	0.00
Size * Temp	81.15	2	40.58	21.47	0.00
Size * Solvent * Con	814.61	3	271.54	61.77	0.00
Size * Con * Temp	249.71	6	41.62	9.47	0.00
Solvent * Con * Temp	580.45	6	96.74	22.01	0.00
Size * Solvent * Temp	131.53	2	65.76	14.96	0.00
Size * Solvent * Con * Temp	130.10	6	21.68	4.93	0.00
Error	698.99	159	4.40	239.34	
Total	122771.70	213			
Corrected Total	15822.78	212			
R^2 - 0.956					
Adj R^2 - 0.941					

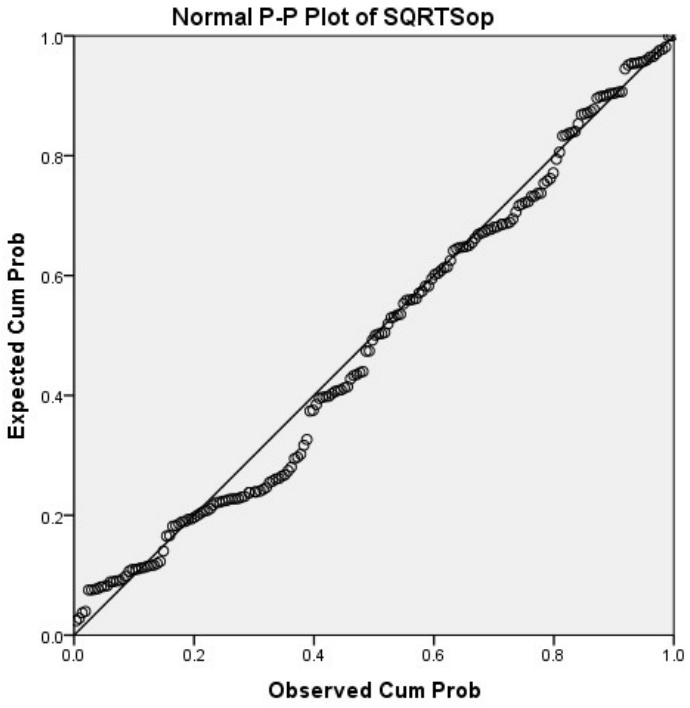
DF: degrees of freedom; SQRT: square-root; TA: thomasidioic acid; Con: concentration; Temp: Temperature; HPLC: high performance liquid chromatography; R^2: coefficient of correlation; Adj R^2 - adjusted coefficient of correlation

(10) Lack of fit test has also to be included in the tables to verify if the model is representative of the showed results.

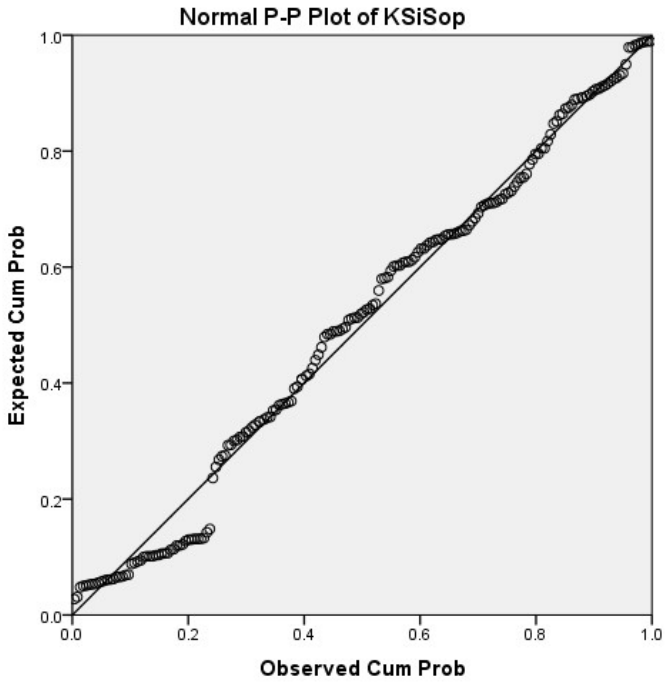
Response: Thank you for your feedback. We have used the normal probability plots to identify the lack of fits and the model accuracy for all the flavor-active phenolic compounds. I have indicated the normal probability plots for each compound attached below.



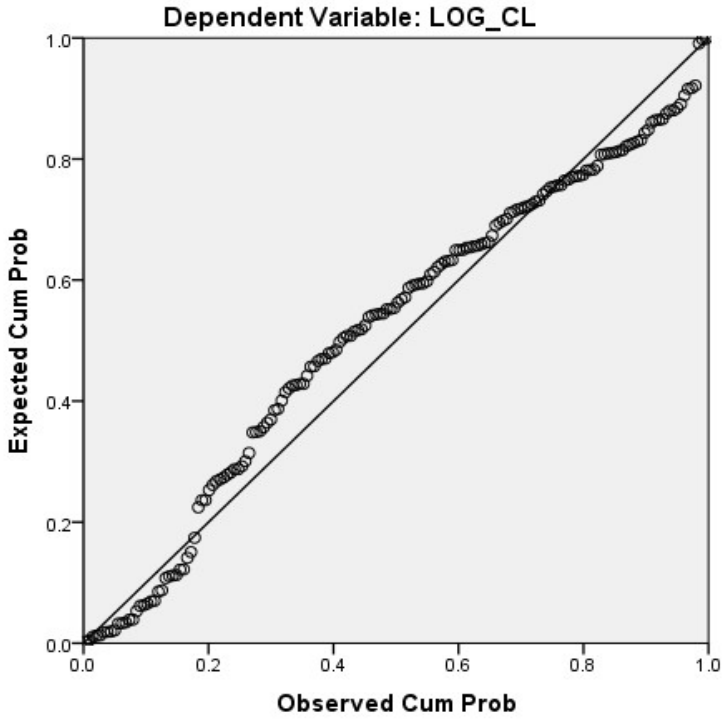
Normal probability plot for transformed data of TA - thomasidioic acid



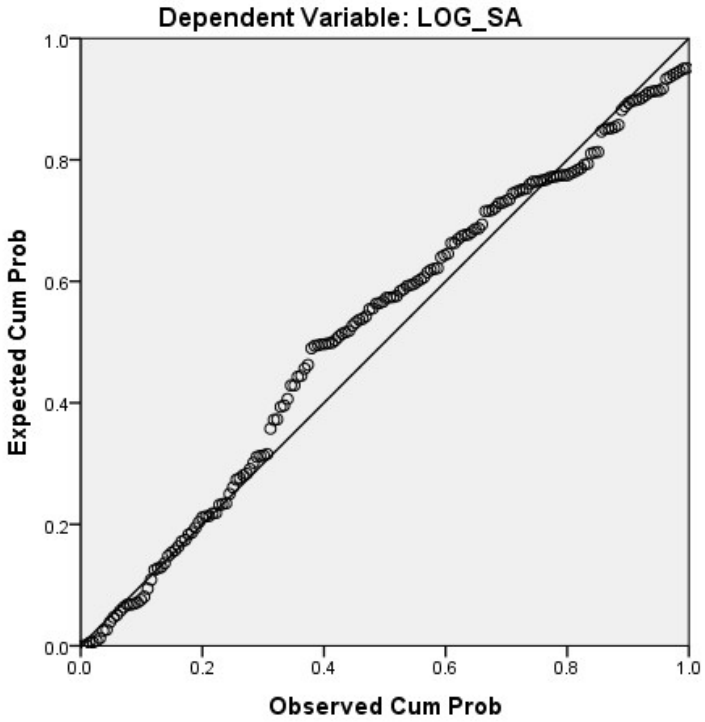
Normal probability plot for transformed data of Sop - kaempferol 3-*O*-*β*-sophoroside



Normal probability plot for KSiSop - kaempferol 3-*O*-(2‴-*O*-sinapoyl-*β*-sophoroside)



Normal probability plot for transformed data of CL – canolol



Normal probability plot for transformed data of SA – sinapic acid

(11) Analytical determination of phenolics has to be rewritten.

Response: Thank you for your feedback. The analytical determination of the phenolic compounds was primarily done using the HPLC for the major sinapates including sinapine, sinapic acid and canolol. Furthermore, the other flavor-active minor components were identified using the mass spectrometry/tandem mass spectrometry. Please find below the attached description from the methods section.

"HPLC analysis was adapted and carried out on a Kinetex® Biphenyl C_{18} 100 Å RP column (2.6 mm, 150 x 4.6 mm, Phenomenex, Canada) maintained at 30ºC with 0.4 mL/min flow rate, and 10 µL injection volume as Harbaum-Piayda *et al.* (2010) as described in Nandasiri *et al.* (2019). The mobile phase was consisted of 0.1% formic acid in water (A) and 0.1% formic acid in methanol (B). Chromatograms were acquired at 270 and 330 nm in triplicate by Chromeleon software Version 7.2 SR4 (Dionex Canada Ltd, Oakville, ON Canada). Calibration curves of sinapine, sinapic acid, and canolol were obtained from a series of standard solutions in methanol from 1.0 to 100 µg/mL (n = 11) with R^2 = 0.998 for sinapic acid, R^2 = 0.999 for canolol and R^2 = 0.999 for sinapine with detection limit of each compound at 0.001 mg/mL.

Structural elucidation of kaempferol-3-*O*-(2‴-*O*-sinapoyl-*β*-sophoroside), kaempferol-3-*O*-sophoroside, thomasidioic acid (TA) were tentatively identified by liquid chromatography with mass spectrometry and tandem mass spectrometry (LC-MS) using the HPLC method described above. Fractions were collected at one-minute intervals, and were dried (N_2) and analyzed by ESI-MS-MS/MS. Positive ion mode (ESI^+) was used, and spectra recorded on a Bruker Compact high resolution quadrupole time of flight mass spectrometer (Q-TOF-MS)

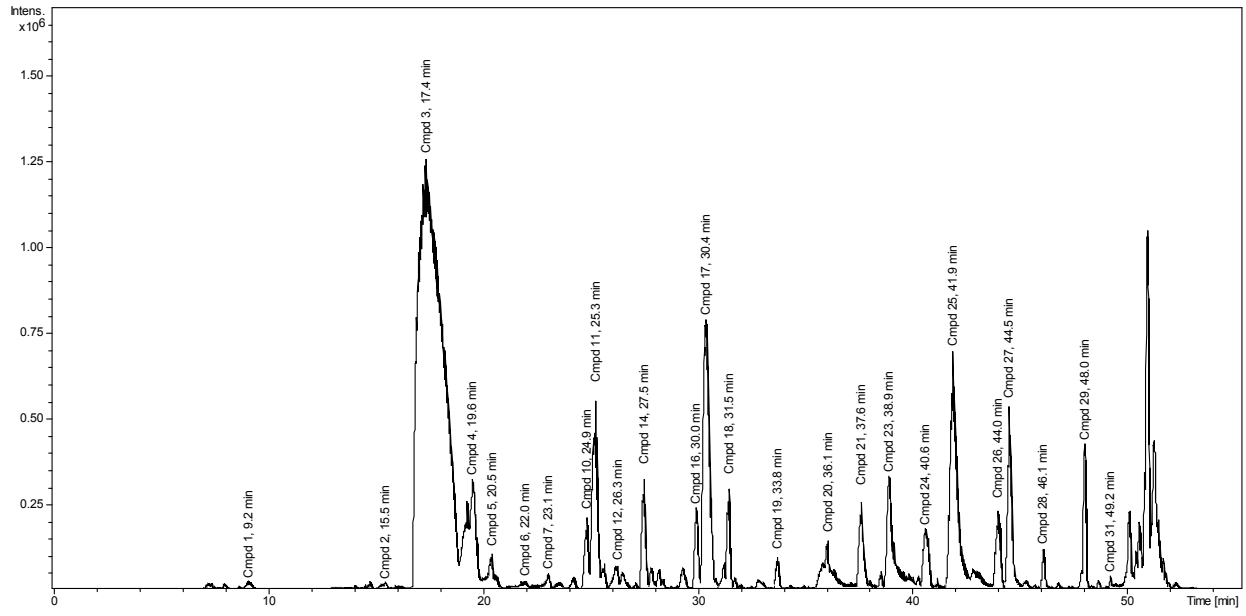
(Bruker Daltonics, Billerica, Massachusetts, USA). MS mode was applied during the formula generation and the mass range was from 50 m/z to 2500 m/z was used. The elute pump was operated at a maximum pressure of 10150 psi, with a capillary voltage of 3500V at a dry gas flow rate of 4.0 L/min with a drying temperature of 200°C. MS/MS tuning was carried out with 5.0 eV (ion energy) and 10.0 eV (collision energy). The obtained fragments were compared with the literature values in confirming the phenolic structures (Cai et al. 1999; Hald et al. 2019; Rubino et al. 1996)."

(12) More analytical parameters have to be included in this section

Response: Thank you for your feedback. However, we strongly believe the analytical parameters we have use for the current manuscript is enough considering the fact our main focus of the study was to determine the optimal conditions for individual flavor-active phenolic compounds using the pressurized temperature processing using ASE. For the authors knowledge this is the first publication to investigate the optimum extraction conditions for flavor-active phenolic compounds including KS, KSS and TA.

(13) It is mandatory to include a UV-chromatogram of the analysed extract numbering the peaks

Response: Thank you for your feedback. We have already included the UV-chromatogram of the analyzed extract with the specific numbers for identified and quantified phenolic compounds. We would like to put the UV-chromatogram to supplementary materials section to reduce the number of figures and tables.



Compound #	RT (min)
1 - Sinapine	17.4
2 - Sinapic Acid	23.1
3 - Kaempferol 3-*O*-(2”-*O*-Sinapoyl-*β*-sophoroside)	24.9
4 - Thomosidic Acid	25.3
5 - Kaempferol 3-*O*- *β*-sophoroside	30.4
6 - Canolol	33.8

Figure S1: UV-chromatogram of canola meal extracts treated with 70% (v/v) methanol extractant at 160ºC, 1.0 mm ASE

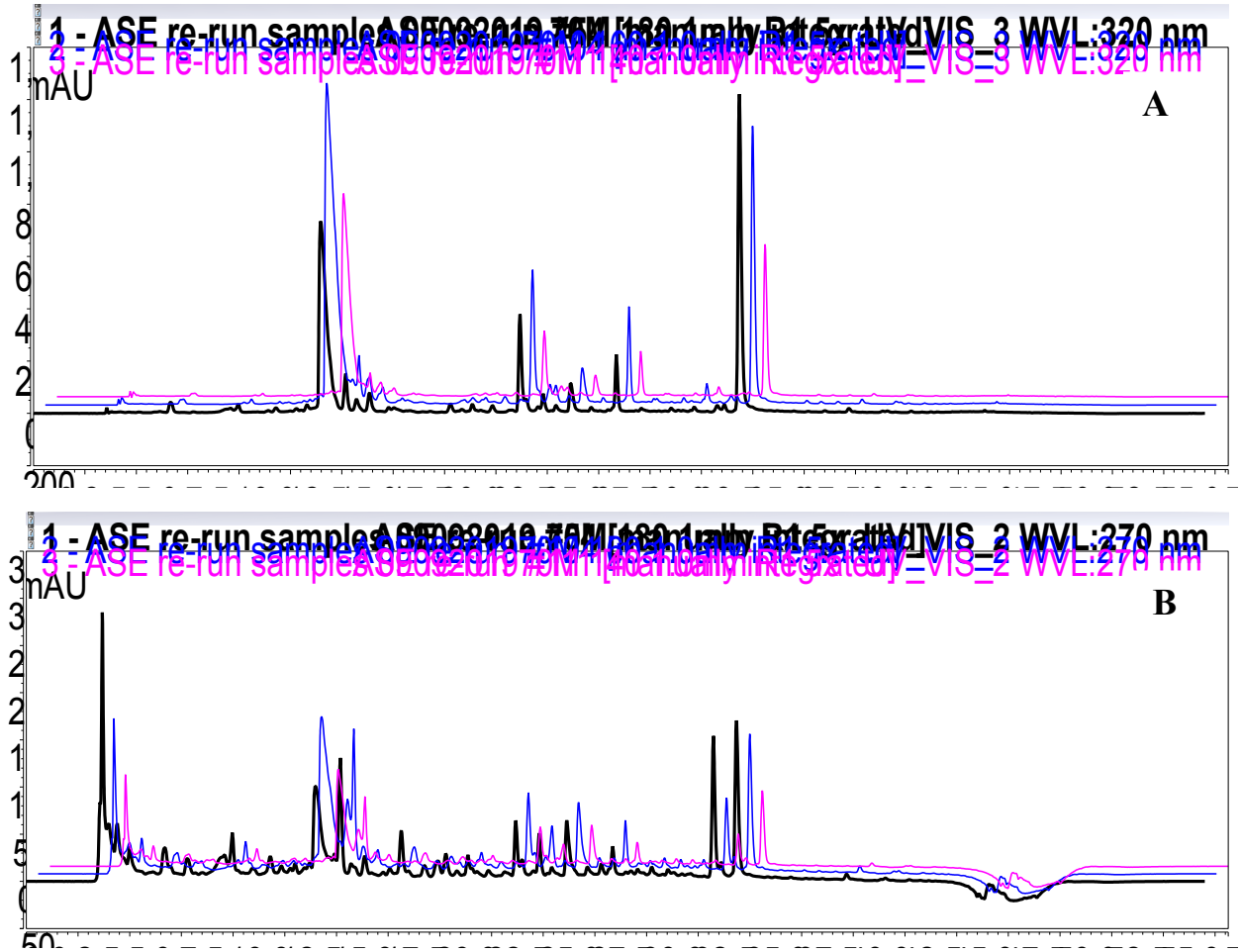


Figure S2: HPLC chromatogram of (**A**-320 nm, **B**-270 nm) 1.0 mm particle size for 70% (v/v) methanol extracts at different processing temperatures 140ºC (pink), 160ºC (blue) and 180ºC (black)

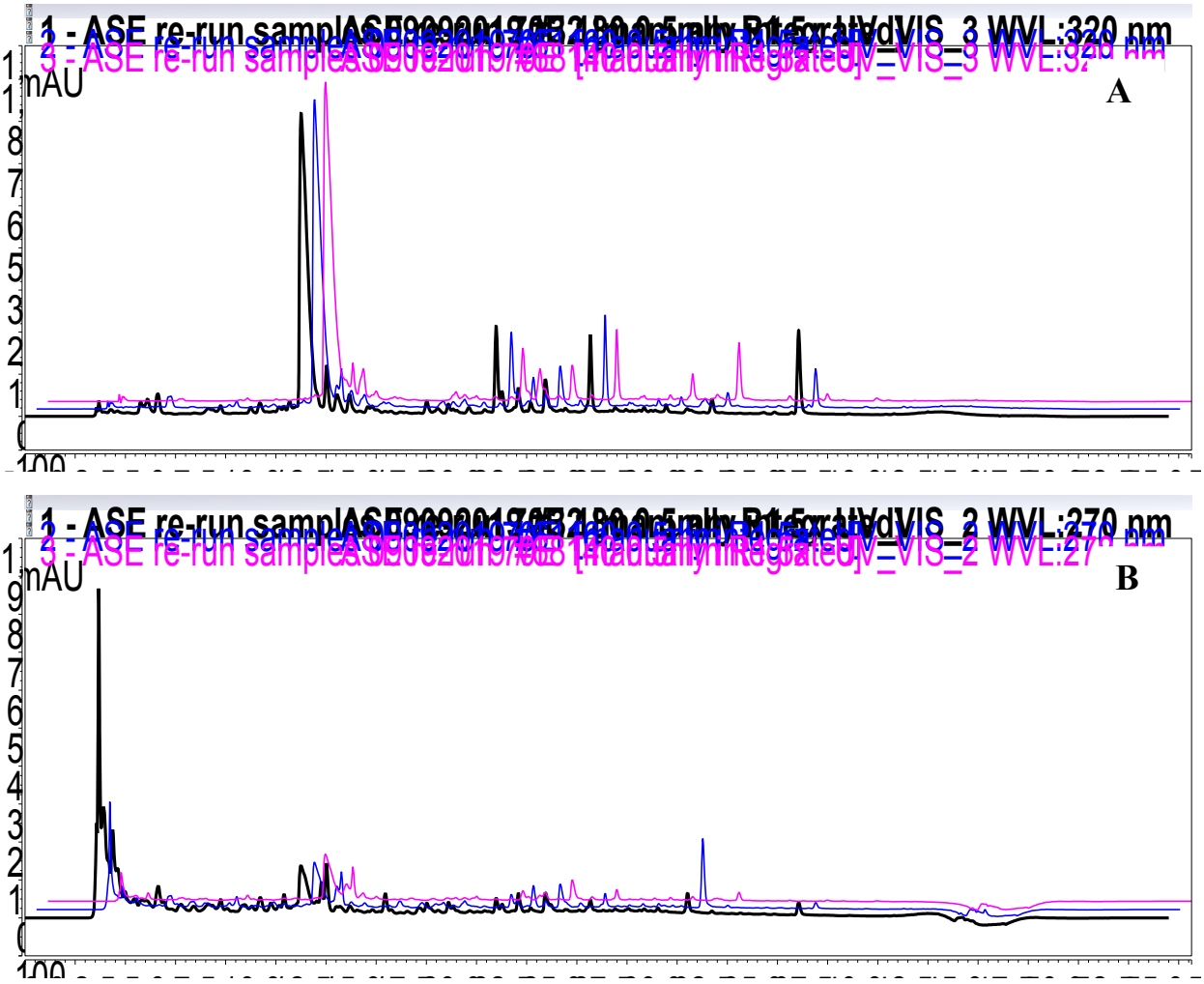


Figure S3: HPLC chromatogram of (**A**-320 nm, **B**-270 nm) 1.0 mm particle size for 70% (v/v) ethanol extracts at different processing temperatures 140ºC (pink), 160ºC (blue) and 180ºC (black)

(14) In addition, a figure including the mass spectra of the analysed compounds by MS have also to be included in the manuscript

Response: Thank you for your feedback. We have already included some of the MS spectral data for the standard compounds. We would like to put the MS spectral data diagram of the standard compounds to supplementary materials section to reduce the number of figures and tables.

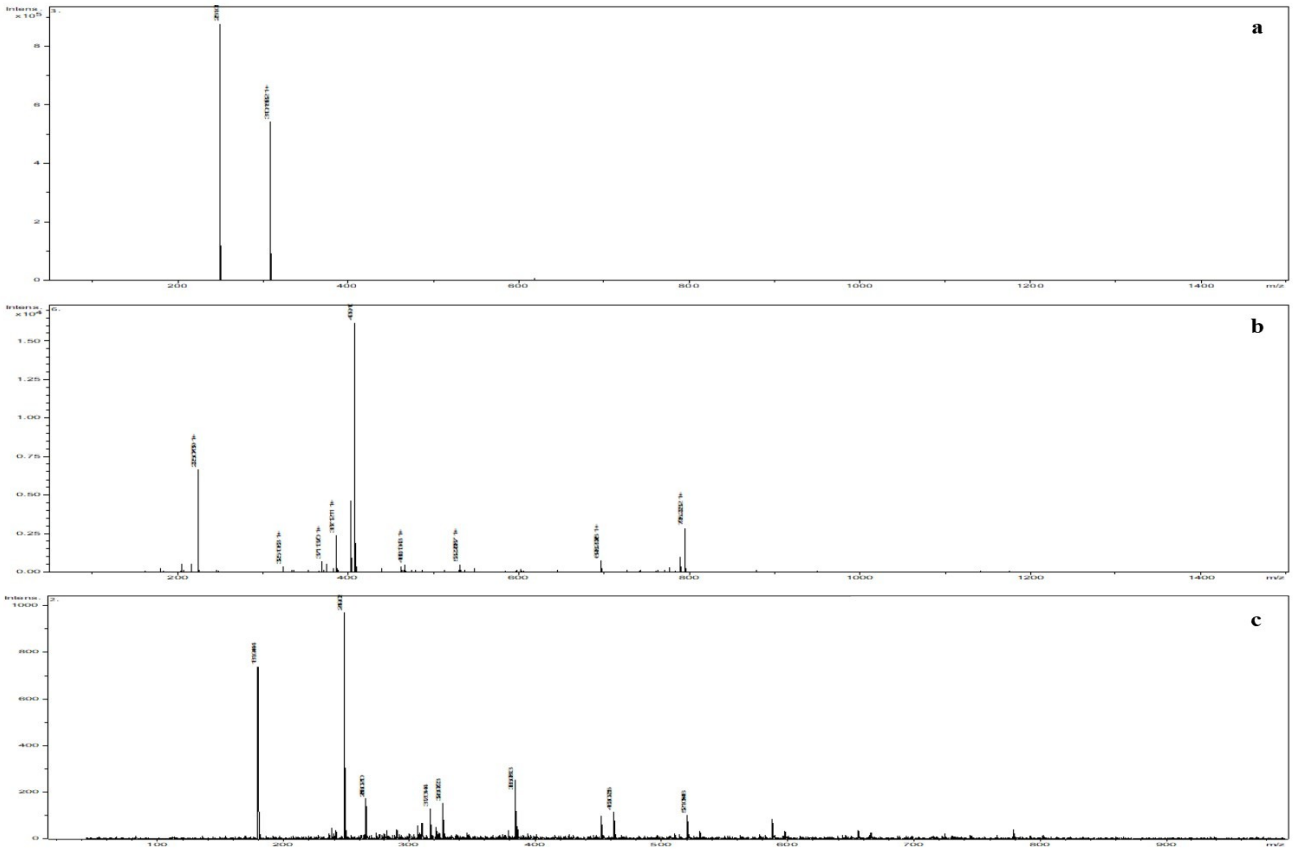


Figure S4: Positive electrospray ionization for sinapine (a), sinapic acid (b), canolol (c)

(15) Quantification carried out in samples is not described: detection and quantitation limits, inter- e intraday, calibration curves… have to be described if this method has not previously been published with analytical validation

Response: Thank you for your feedback. This method has been previously published in both the Journal of Food Science. Furthermore, a summary of the requested data is already available in the methods section.

"Calibration curves of sinapine, sinapic acid, and canolol were obtained from a series of standard solutions in methanol from 1.0 to 100 µg/mL (n = 11) with R^2 = 0.998 for sinapic acid, R^2 = 0.999 for canolol and R^2 = 0.999 for sinapine with detection limit of each compound at 0.001 mg/mL."

Nandasiri, R., Eskin, N. A. M., & Thiyam−Höllander, U. (2019). Antioxidative Polyphenols of Canola Meal Extracted by High Pressure: Impact of Temperature and Solvents. *Journal of Food Science*, *84*(11), 3117–3128. https://doi.org/10.1111/1750-3841.14799

Associate Editor Comments to Author:

Reviewers are advising for a re-submission of your manuscript. Please carefully address the reviewers' comments when re-submitting. Some other comments to consider:

(1) The presentation of the experimental results and statistical analysis need to be considerably improved.

Response: Thank you for your feedback. We strongly believe that our results and discussion section described the statistical analysis in detail with respect to both major sinapates and other minor components. I have provided the detailed explanation for the current question with the second reviewers comments on the statistical analysis.

(2) The authors need to indicate the type of experimental design, with the levels assayed for each input variable studied.

Response: Thank you for your feedback. Our statistical analysis section describes the factorial design and the levels associated with each level.

"A factorial design consists with four independent factors including particle size (0.5 and 1.0 mm), type of extraction solvent (ethanol and methanol), concentration (v/v) of the solvent (30%, 40%, 60%, and 70%) and extraction temperature (140, 160 and 180°C). Data analysis was carried out using the general linear multiple regression model using the two-way analysis of variance (ANOVA). Multiple mean comparison was performed using Tukey's test at the level of significant of 0.05 ($p < 0.05$) (Pallant, 2011)."

(3) They need to justify the value of time used and why this variable was not evaluated.

Response: Thank you for your feedback. The extraction time consist of preheating (2-min), heating (6-min), extraction static time (5-min*3) and purging 90 sec. with a total time of ~20 minutes. We have already published this information in our previous publication. I have attached the figure from the manuscript herewith. Furthermore, the extraction time was optimized for the extractability of total phenolic content of the extractants, with our previous work and therefore in the current study we did not consider the time as a factor.

Nandasiri, R., Eskin, N. A. M., & Thiyam−Höllander, U. (2019). Antioxidative Polyphenols of Canola Meal Extracted by High Pressure: Impact of Temperature and Solvents. *Journal of Food Science*, *84*(11), 3117–3128. https://doi.org/10.1111/1750-3841.14799

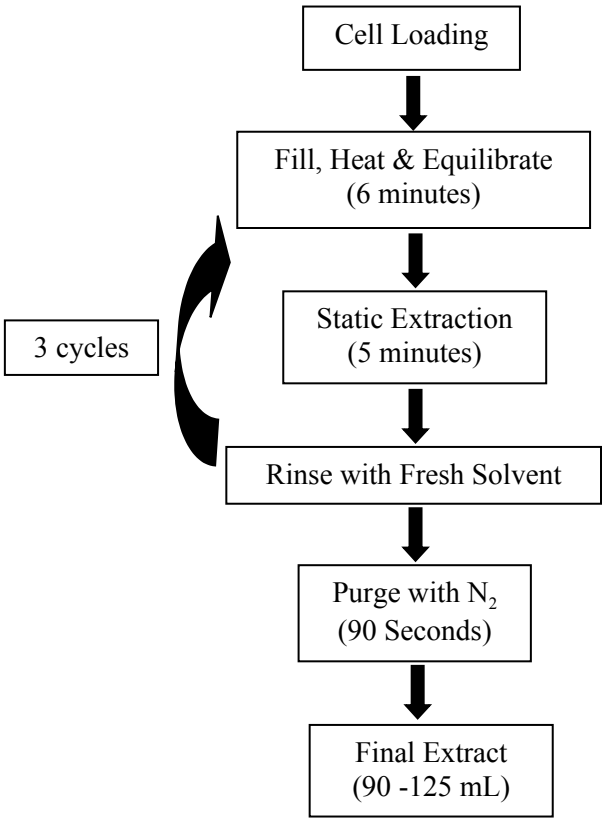


Figure 1: Extraction of phenolic compounds using ASE

(4) They need to start showing the experimental results obtained for each combination of factors (is this your table 3)? Are all your experimental data shown in Table 3? Then, the authors need to show the type of linear model that they are using to adjust the data, number of coefficients, etc.

Response: Thank you for your feedback. The Table 3 contains the results summary for all the treatment conditions including particle size (0.5 and 1.0 mm), type of extraction solvent (ethanol and methanol), concentration (v/v) of the solvent (30%, 40%, 60%, and 70%) and extraction temperature (140, 160 and 180^{o}C). Furthermore, the model fit analysis was interpreted in the **Table 1 a,b,c,d,e,f** for individual flavor-active phenolic compounds and all the requested information is given at the **Table 1 a,b,c,d,e,f**. The same model fit statistics was used for the analysis of antioxidant activity of the extractants and it is already published in the Journal of Food Science in year 2019.

Nandasiri, R., Eskin, N. A. M., & Thiyam−Höllander, U. (2019). Antioxidative Polyphenols of Canola Meal Extracted by High Pressure: Impact of Temperature and Solvents. *Journal of Food Science*, *84*(11), 3117–3128. https://doi.org/10.1111/1750-3841.14799

(6) Tables 1a-f can be merged in one single table where value for each coefficient of the model, p-values for each coefficient, as well as R2, adjusted R2 and lack of fit are shown.

Response: Thank you for your feedback. However, the model fit statistics don't follow the same pattern for all the flavor-active phenolic compounds. Therefore, we need to use separate entity for each flavor-active phenolic compound.

For example: I have illustrated the detailed statistical analysis for Canolol below. First table shows the model fit statistics for the main effects where you can see the particle size is the only factor not significant. Therefore, size factor was removed from the model. The next step was to identify the interaction effect of the main effects. The second table shows the all the two-way interaction effects between the main effects. As you can see the two-way interaction of size*solvent was not significant. Therefore, the size*solvent interaction was also removed from the model fit statistics to obtain the higher statistical power. The next step was to identify the three-way and four-way interactions among the main effects. As you can see the three-way interaction of size*solvent*temperature was not significant. Therefore, the size*solvent*temperature interaction was also removed from the model fit statistics. The final table shows the simplified table with the corrected model fit statistics with higher statistical power. Same procedure was used for all the flavor-active phenolic compounds to identify the individual model fit statistics. Therefore, we would require individual statistical table for each flavor-active compound and can not combine them into one table.

Tests of Between-Subjects Effects

Dependent Variable: LOG_CL

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	37.405[a]	7	5.344	105.646	.000
Intercept	639.110	1	639.110	12635.629	.000
Size	.131	1	.131	2.592	.109
Solvent	.610	1	.610	12.066	.001
Con	26.581	3	8.860	175.174	.000
Temp	8.744	2	4.372	86.434	.000
Error	8.902	176	.051		
Total	688.803	184			
Corrected Total	46.307	183			

a. R Squared = .808 (Adjusted R Squared = .800)

Tests of Between-Subjects Effects

Dependent Variable: LOG_CL

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	40.593[a]	24	1.691	47.063	.000
Intercept	628.864	1	628.864	17498.263	.000
Solvent	.469	1	.469	13.059	.000
Con	26.153	3	8.718	242.572	.000
Temp	9.271	2	4.635	128.983	.000
Size * Con	.428	3	.143	3.969	.009
Solvent * Con	1.173	3	.391	10.875	.000
Con * Temp	1.043	6	.174	4.836	.000
Size * Solvent	.005	1	.005	.143	.706
Size * Temp	.376	2	.188	5.237	.006
Solvent * Temp	.330	2	.165	4.592	.012
Error	5.714	159	.036		
Total	688.803	184			
Corrected Total	46.307	183			

a. R Squared = .877 (Adjusted R Squared = .858)

Tests of Between-Subjects Effects

Dependent Variable: LOG_CL

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Noncent. Parameter	Observed Power[b]
Corrected Model	45.465[a]	47	.967	156.228	.000	7342.707	1.000
Intercept	621.013	1	621.013	100294.837	.000	100294.837	1.000
Solvent	.381	1	.381	61.604	.000	61.604	1.000
Con	25.944	3	8.648	1396.689	.000	4190.066	1.000
Temp	9.383	2	4.692	757.691	.000	1515.381	1.000
Size * Con	.337	3	.112	18.142	.000	54.427	1.000
Solvent * Con	1.190	3	.397	64.064	.000	192.192	1.000
Con * Temp	1.193	6	.199	32.117	.000	192.705	1.000

Size * Temp	.456	2	.228	36.813	.000	73.626	1.000
Solvent * Temp	.355	2	.177	28.660	.000	57.320	1.000
Size * Solvent * Con	.310	3	.103	16.693	.000	50.079	1.000
Size * Con * Temp	1.317	6	.219	35.448	.000	212.690	1.000
Solvent * Con * Temp	1.286	6	.214	34.615	.000	207.693	1.000
Size * Solvent * Temp	.017	2	.009	1.381	.255	2.762	.293
Size * Solvent * Con * Temp	1.788	6	.298	48.139	.000	288.832	1.000
Error	.842	136	.006				
Total	688.803	184					
Corrected Total	46.307	183					

a. R Squared = .982 (Adjusted R Squared = .976)

Tests of Between-Subjects Effects

Dependent Variable: LOG_CL

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Noncent. Parameter	Observed Power[b]
Corrected Model	45.465[a]	47	.967	156.228	.000	7342.707	1.000
Intercept	621.013	1	621.013	100294.837	.000	100294.837	1.000
Solvent	.381	1	.381	61.604	.000	61.604	1.000
Con	25.944	3	8.648	1396.689	.000	4190.066	1.000
Temp	9.383	2	4.692	757.691	.000	1515.381	1.000
Size * Con	.337	3	.112	18.142	.000	54.427	1.000
Solvent * Con	1.190	3	.397	64.064	.000	192.192	1.000
Con * Temp	1.193	6	.199	32.117	.000	192.705	1.000
Size * Temp	.456	2	.228	36.813	.000	73.626	1.000
Solvent * Temp	.355	2	.177	28.660	.000	57.320	1.000
Size * Solvent * Con	.312	4	.078	12.589	.000	50.356	1.000
Size * Con * Temp	1.317	6	.219	35.448	.000	212.690	1.000
Solvent * Con * Temp	1.286	6	.214	34.615	.000	207.693	1.000
Size * Solvent * Con * Temp	1.794	8	.224	36.212	.000	289.695	1.000
Error	.842	136	.006				
Total	688.803	184					

Corrected Total	46.307	183					

a. R Squared = .982 (Adjusted R Squared = .976)

(7) I cannot follow the statistical analysis in Table 2. To which values are you applying the post-hoc test?

Response: Thank you for your feedback. The post-hoc analysis was conducted for the significant factors (main effects) for individual flavor-active phenolic compounds. If a factor (main effect) is not significant at the model fit statistics, the post hoc analysis was omitted.

For example, for canolol only solvent concentration and the extraction temperature main effects were significant. Therefore, we used only those main effects for the post-hoc analysis. We have indicated the post-hoc analysis tables for both the solvent concentration and the extraction temperature attached herewith. As the table indicates both 30% (v/v) and 40% (v/v) concentrations have no significance in the model for both Tukey and LSD post hoc analysis. However, for our statistical analysis we applied the Tukey's post-hoc analysis.

Multiple Comparisons

Dependent Variable: LOG_CL

	(I) Con	(J) Con	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	30	40	-.0090	.01633	.946	-.0515	.0334
		60	-.5963*	.01642	.000	-.6390	-.5536
		70	-.8984*	.01615	.000	-.9404	-.8564
	40	30	.0090	.01633	.946	-.0334	.0515
		60	-.5873*	.01668	.000	-.6307	-.5439
		70	-.8894*	.01641	.000	-.9320	-.8467
	60	30	.5963*	.01642	.000	.5536	.6390
		40	.5873*	.01668	.000	.5439	.6307
		70	-.3021*	.01651	.000	-.3450	-.2591
	70	30	.8984*	.01615	.000	.8564	.9404
		40	.8894*	.01641	.000	.8467	.9320
		60	.3021*	.01651	.000	.2591	.3450
LSD	30	40	-.0090	.01633	.581	-.0413	.0233
		60	-.5963*	.01642	.000	-.6288	-.5638
		70	-.8984*	.01615	.000	-.9303	-.8665
	40	30	.0090	.01633	.581	-.0233	.0413
		60	-.5873*	.01668	.000	-.6203	-.5543

		70	-.8894*	.01641	.000	-.9218	-.8569
	60	30	.5963*	.01642	.000	.5638	.6288
		40	.5873*	.01668	.000	.5543	.6203
		70	-.3021*	.01651	.000	-.3347	-.2694
	70	30	.8984*	.01615	.000	.8665	.9303
		40	.8894*	.01641	.000	.8569	.9218
		60	.3021*	.01651	.000	.2694	.3347

Based on observed means.

The error term is Mean Square(Error) = .006.

*. The mean difference is significant at the .05 level.

Multiple Comparisons

Dependent Variable: LOG_CL

						95% Confidence Interval	
	(I) Temp	(J) Temp	Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
Tukey HSD	140	160	-.3577*	.01426	.000	-.3914	-.3239
		180	-.5725*	.01431	.000	-.6064	-.5386
	160	140	.3577*	.01426	.000	.3239	.3914
		180	-.2148*	.01408	.000	-.2482	-.1815
	180	140	.5725*	.01431	.000	.5386	.6064
		160	.2148*	.01408	.000	.1815	.2482
LSD	140	160	-.3577*	.01426	.000	-.3858	-.3295
		180	-.5725*	.01431	.000	-.6008	-.5442
	160	140	.3577*	.01426	.000	.3295	.3858
		180	-.2148*	.01408	.000	-.2427	-.1870
	180	140	.5725*	.01431	.000	.5442	.6008
		160	.2148*	.01408	.000	.1870	.2427

(8) What does it mean (I) and (J)? This needs to be explained in the text.

Response: Thank you for your feedback. I and J are the mean comparison for each main effect. For example, as explained above pair wise comparison between the main effects were conducted do understand the post-hoc analysis. Each level of the main effect will be compared with other levels to understand the significance. As given above for the extraction temperature on the extractability of canolol was determined using pairwise comparison of each extraction temperature. For example 140 vs 160 and 180, 160 vs 140 and 180, 180 vs 140 and 160

(9) The authors need to draw conclusions from the study.

Response: Thank you for your feedback. We have already updated the conclusion accordingly in the manuscript.

"The occurrence of major sinapates, namely sinapine, sinapic acid, and canolol and other active molecules including TA and kaempferol derivatives imparts flavor to canola meal. The targeted extraction and co-processing using ASE proved to be an efficient method for extracting these flavor-active molecules while attenuating the bitter molecules from the canola meal. The use of shorter extraction times (20 minutes), lower solvent usage, and improved concurrent and targeted extractability of flavor-active phenolic molecules using ASE will enable the creation of co-streams of phenolic rich antioxidants. These phenolic rich antioxidative compounds from the meal characterize an additional potential source for use in the food and nutraceutical industries. These new co-streams can be piloted with canola protein industries to benefit the ongoing strong demand for alternative plant-based natural preservatives and shelf-life improving agents."

(10) It should not be a merely presentation of results from statistical analysis. What are the recommended conditions to maximize the extraction of these phenolics?

Response: Thank you for your feedback. We strongly believe that our results and discussion section described the statistical analysis in detail with respect to both major sinapates and other minor components. The optimum extraction conditions for individual flavor-active phenolic compound was explained and justified with the necessary references.

(11) An abstract is missing.

Response: Thank you for your feedback. We have already updated the abstract section accordingly in the manuscript.

"De-oiled canola sources of protein fractions contain flavor-active phenolic compounds. Conventional canola oil processing utilizes excess amount of toxic solvents and are associated with high intensity of bitter flavor-active phenolic compounds, limiting the use of the canola meal. Recent advances in the extraction and isolation of the bitter favor-active phenolic compounds from the by-products of canola meal protein isolates, however, would benefit the industry by producing a side-stream ingredient rich in phenolics. High temperature and pressure-aided processing, namely the accelerated solvent extraction (ASE) was investigated to extract the flavor-active bitter molecules from the canola meal. Extractability of flavor-active phenolic

compounds including the major sinapates, kaempferol derivatives and other thermo-generative compounds including thomasidioc acid (TA) was evaluated. The effects of temperature, solvent extractant and concentration, and the particle size of the meal, were examined on the extraction efficiency of these phenolic compounds. Extraction temperature (180ºC) was the primary determinant ($p<0.05$) for the attenuation of major sinapates including sinapine and sinapic acid. Both ethanol and methanol extractants at a concentration of 70% (v/v) significantly ($p<0.05$) extracted the flavor-active phenolic compounds. Pressurized high temperature through optimized ASE conditions attenuated the bitter undesirable flavor-active phenolic molecules from canola meal thereby facilitating a potential value-added phenolic-rich by-product"