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Enhancement of *Curcuma Longa* and *Zingiber Officinale* Bioactives Extraction through High-Pressure Processing (HPP) Method for Functional Food and Beverages

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Abstract: Surveys indicated food and beverages enhanced with bioactive compounds from natural plants (functional foods and beverages) have huge advantages on human health and promote a good state of mental and physical well-being. However, extraction of the bioactive compounds poses several challenges such as extreme temperature, long extraction time and toxic solvent used, hence degrading the integrity of the bioactive compounds. The aim of this research is to optimize the operating conditions of a green extraction method using High Pressure Processing (HPP) for the plants of *Curcuma Longa* and *Zingiber Officinale*. The operating condition parameters selected are pressure (200 MPa, 600 MPa), extraction time (5, 10, 15 mins) and solid-to-liquid ratio (1:15, 1:7, 1:4.3 % w/w). The nutritional analysis was conducted for total phenolic content (TPC) and the functional activities analyzed were total anti-oxidant (TAO) and total anti-inflammatory (TAI). The findings indicate, for *C. Longa*, the optimum HPP operating conditions for maximum TPC (8.033 ug/ml) and maximum TAO (1.432 mg/ml) are 600 MPa, 10 min extraction time and 1:4.3% w/w solid: liquid ratio. However, if turmeric TAI (94.1%) is targeted, the best HPP operating conditions are 200 MPa, 10 min extraction time and 1:15% w/w solid: liquid ratio. Meanwhile, for *Z. Officinale*, 200 MPa seems to be the best operating pressure to retain the highest nutritional content and functional activities. However, depending on the targeted content or activity, the best extraction time varies from 5 min to 15 min and the best solid: liquid ratio varies between 1:4.3% to 1:15%. HPP research demonstrates that an optimized green extraction method suitable for the application of functional food and beverages is possible and could be enhanced for targeted properties of interest, such as anti-oxidant and anti-inflammatory.

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Keywords: High Pressure Processing (HPP), *Curcuma Longa*, *Zingiber Officinale*, Total Phenolic Content, Total Antioxidant, Total Anti-Inflammatory

1. Introduction

Plants have huge advantages in producing the active compounds for many purposes such as for the development of pharmaceuticals (insulin), drugs delivery system (e.g. plant polysaccharides) [1][2], functional foods (e.g. carrot juice with *L. acidophilus* as a probiotics drink) [3] as well as for food additives (e.g. aroma, colour) [4]. Extraction method is required to extract a specific compound from plants using suitable extraction techniques. Various methods for the food preservation technique such as Microwave Extraction (ME), Supercritical Fluid Extraction (SFE), Ultrasound, Pulsed Electric Field (PEF), Subcritical Water Extraction and High-Pressure Processing (HPP) have been developed to enhance the yield or efficacy of bioactive components [5]. Among these methods, HPP entails applying low temperatures (0–50 °C) and pressure (100–900 MPa) to the food in a short amount of time (a few seconds to several minutes). There are several of HPP products on the market, including juices, fresh meat, ham, and oysters. Because the treatment largely preserves the original raw character of juices as well as their quality, promotes safety, and extends shelf life, the practice of HPP of raw cold prepared fruit and vegetable juices is rapidly expanding.

High pressure processing (HPP) is a physical processing technology that offers several benefits to food products. It extends the shelf life of food, enhances product texture, alters the physicochemical properties of food, regulates enzyme activity, and effectively deactivates microorganisms [6],[7],[8]. There are four conditions used in this analysis which are (200 MPa; 5 mins, 200 MPa; 10 mins, 200 MPa; 15 mins, and 600 MPa; 10 mins). HPP is an effective method for extraction of heat sensitive materials. HPP increases the yields and mass transfer rates of herbal products by cell wall breakdown as compared to the conventional extraction methods such as Soxhlet, Heat Reflux, Ultrasonic, Microwave, and Supercritical Carbon Dioxide Extractions [9].

The effectiveness of HPP in controlling microbes is influenced by various parameters, including pressure, temperature, exposure time, product characteristics, packaging type, and the organism's tolerance to pressure changes. The pressure applied in HPP can range from 200 to 900 MPa, but the minimum pressure required to inactivate vegetative microorganisms at room temperature is 400 MPa. Consequently, the typical pressure range employed in commercial HPP systems is between 400 and 600 MPa.

HPP does not adversely affect the structure of low-molecular-weight compounds that contribute to nutritional and sensory qualities, as the covalent bonds within these compounds remain stable at pressures below 2000 MPa. However, at pressures above 400 MPa, intermolecular and intramolecular bonds in biological systems start to break, leading to microbial inactivation while having minimal impact on the chemistry of the food [10]. When dealing with pressure-resistant organisms like bacterial spores, higher processing temperatures can be employed in conjunction with HPP. Cheng et al., 2019 found that in High Pressure Extraction (HPE) pressure different have the effect on total phenolic content (TPC), flavonoid, and gallic acid concentration in Djulis hull plant where, the highest TPC has highest at 600 MPa followed by 450 MPa, 300 MPa and 150 MPa [11].

The extraction time in High Pressure Processing (HPP) is significant because it directly affects the efficiency and extent of extraction of desired compounds from food or plant materials. The duration of the extraction process determines the extraction efficiency, which refers to the amount of target compounds extracted from the raw material. Generally, longer extraction times allow for more thorough extraction, leading to higher yields of desired compounds.

HPP aims to achieve consistent processing effects across the entire product. An optimal solid to liquid ratio helps in homogenizing the pressure throughout the sample. When the solid to liquid ratio is properly balanced, the pressure is evenly distributed, ensuring that all parts of the food product

experience the desired pressure conditions for pathogen destruction and other desired effects. The physical process of dissolving bioactive components into the solvent occurs when the solvent ratio is increased. With a higher proportion of solvent, there is increased contact between the bioactive compounds and the solvent, resulting in higher leaching rates. In other words, a greater concentration difference between the solid's interior and the solvent leads to a faster extraction rate [12].

2. Materials and Methods

The method consists of extraction of *C. Longa* and *Z. Officinale* by the High-Pressure Processing (HPP) (Model: Hiperbaric 55) technique at different conditions to obtain bioactive compounds such as *curcumin* and *gingerols*. Then, continued with centrifugation of the bioactive compounds for both samples by using the Centrifuge MPW-380R machine. After that, the supernatants were separated from the pellet and further used for the functional analysis. Last but not least, the analysis is to measure the Total antioxidant, Total phenolic content and Total anti-inflammatory of functional beverages. The result will be compared between conditions in HPP for the optimization of concentration bioactive compounds in different operating conditions of HPP.

2.1 Materials

The research work was carried out at the laboratory in Universiti Tun Husssien Onn Malaysia (UTHM) campus Pagoh. All solvents, chemicals and reagents were prepared and provided by Material Laboratory and Upstream Bioprocess Laboratory at UTHM. *C. Longa* and *Z. Officinale* used in this research work were purchased at a fresh market in Pekan Pagoh, Johor.

2.2 Collection and Preparation of samples

Raw materials (*C. Longa* and *Z. Officinale*) were obtained from the market at Pagoh for the extraction by using High Processing Pressure (HPP) method. Firstly, the materials were washed, removed its foreign matter and dried at room temperature. The initial weight of washed materials was measured to obtain its initial weight before sliced to let them dry in the oven at 50 °C for three days. After three days, the samples were collected and calculating its moisture content by weighted the dried materials using the weighing scale. All collected *C. Longa* and *Z. Officinale* were grounded into fine powder by using Hammer Grinding (PX-MFC90D). Then, the samples filled in the 80 mL bottles by following the Table 1.

Table 1: Samples Set Conditions filled with different weights (5 g, 10 g and 15 g) of *C. Longa* and *Z. Officinale*

Set Conditions	5 g		10 g		15 g	
	Turmeric	Ginger	Turmeric	Ginger	Turmeric	Ginger
1	✓	✓	✓	✓	✓	✓
2	✓	✓	✓	✓	✓	✓
3	✓	✓	✓	✓	✓	✓
4	✓	✓	✓	✓	✓	✓
	4	4	4	4	4	4
Total	24 bottles					

The set conditions are referred to the technique extracted by using High Processing Pressure (HPP). The condition of pressure and time are shown by Table 2.

Table 2: Set Conditions with its Parameters in HPP

Set Condition	Pressure (MPa)	Time (min)
Condition 1	200	5
Condition 2	200	10
Condition 3	200	15
Condition 4	600	10

Summaries of the experimental conditions described in Table 3

Table 3: Summary of Experimental Parameter in HPP

Summary of Experimental Parameter in HPP	
Material	*Curcuma Longa, Zingiber Officinale*
Sample Solid Weight (g)	5 g, 10g and 15g
Extraction Pressure (MPa)	200 MPa, 600 MPa
Extraction Time (min)	5 min, 10 min and 15 min

2.3 Extraction of *C. Longa* and *Z. Officinale* using HPP

Extraction of *Curcuma Longa* and *Zingiber Officinale* conducted by using the High-Pressure Processing (HPP) method. The sample bottles were introduced in the HPP machine by following the condition shown in Table 2 and HPP's parameters are described in Table 3. Then, followed by centrifugation of *C. Longa* and *Z. Officinale* by using Centrifuge MPW-380R machine. The parameters in centrifuge the samples were at (10,000 rpm; 15 mins; 24 °C). Two layers were formed, the upper one was the supernatant (liquid) and the bottom was the pellet (solid). Supernatant liquid separated from the pellet that used for the analysis of total antioxidant, total phenolic content and total anti-inflammatory activity.

2.4 Determination of Total Phenolic Content (TPC) for Nutritional Analysis

TPC of *C. Longa* and *Z. Officinale* were measured using Folin-Ciocalteu Reagent in the Lowry Assay method. Gallic acid was used as a standard. Standard gallic acid solution was prepared by dissolving 10 mg of gallic acid in a 10 mL methanol producing 1 mg/mL solution. Various concentrations of the standard solutions (0.01, 0.02, 0.03, 0.04 and 0.05 mg/mL) were prepared. To make 7.5% of Na_2CO_3, 7.5 g of sodium carbonate was diluted into 100 mL of distilled water. 100 µL of gallic acid solution, 5 mL of 10% Folin-Ciocalteu reagent and total volume of 7% Na_2CO_3 were added in each test tube obtaining 10 mL of total solutions. Test tube was covered with aluminium foil. The blue colored mixture was shaken to produce a homogenous mixture by using a vortex mixer and left to incubate in the water bath for 30 minutes in temperature of 40°C. Then, measured the solutions in the UV-Vis Spectrophotometer at the wavelength of 765 nm against blank. The absorbance was used to plot the calibration curve to determine the level of phenolic compound in the samples. For determination of samples extracted, 100 µL of sample extracts, 5 mL of 10% Folin-Ciocalteu reagent and total volume of 7% Na_2CO_3 were added in each test tube obtaining 10 mL of total solutions by using the micropipette. Then, test tubes were shaken by using vortex mixer and incubated in the water bath in 40°C for 30 minutes. The absorbance of the extracted was obtained using UV-Vis Spectrophotometer with the wavelength of 765 nm against methanol as a blank sample.

2.5 Determination of Total Antioxidant (TAO) for Functional Properties

The total antioxidant activity was determined by using DPPH (2, 2- diphenyl-1-picryl-hydrazyl) assay method. L-ascorbic acid (10 mg) as standard and samples weighed and dissolved in methanol

solution (10 mL) with the concentration of 1 mg/mL using vortex mixer to obtain homogenous mixtures. Then, five concentrations (0.01, 0.02, 0.03, 0.04 and 0.05 mg/mL) were prepared which act as standard calibration curve solution. 4 mg of DPPH assay weighed and dissolved to 100 mL absolute methanol to obtain 0.1 mM solution. Continued with 1 mL of 0.1 mM DPPH solution filled into the test tube by using a 1000 µL micropipette containing 1 mL of sample solution and 3 mL methanol. Hence, the total solution was 5 mL then placed in a dark environment for 30 minutes. In addition, a blank test tube filled with methanol as a blank sample. The colour turned from purple to yellow indicated the appearance of antioxidant activity in the sample. A UV- Vis Spectrophotometer was used to measure the absorbance of each test tube at 517 nm.

2.6 Determination of Total Anti-Inflammatory (TAI) for Functional Properties

An anti-inflammatory is evaluated by using BSA denaturation assay. With 0.1 M phosphate buffer saline (pH-6.4), the stock solution of test sample extracts and standard samples was pipetted in various test tubes. The reaction mixture (5 mL) was made up of 0.02 mL of extract, 4.78 mL of phosphate-buffered saline (PBS, pH 6.4), and 0.2 mL of 1% bovine albumin. The reaction mixture was incubated for 15 minutes in a water bath (37 °C) following heated to 70 °C for 5 minutes. Lastly, after cooled the sample at room temperature, turbidity was measured at 660 nm using UV-Vis Spectrophotometer. The Eq. 1 used to evaluate the percentage of inhibition of denaturation.

$$\%\ Inhibition\ of\ denaturation = \left(1 - \frac{Absorption\ of\ test\ sample}{Absorption\ of\ control\ sample}\right) \times 100 \quad Eq.1$$

3. Results and Discussion

3.1 Sample Extraction

Curcuma Longa and *Zingiber Officinale* rhizome plant samples were successfully extracted using HPP that used filtered water as an extraction medium. In Figure 1, before HPP extraction, solid state is homogenous and liquid state is clearly seen separated with 2 phases only (based on the visual colour observation). However, after HPP extraction, 3 phases are developed, dark brown top liquid phase, middle whitish phase (more liquid + solid) and bottom brown solid phase (more solid + liquid).

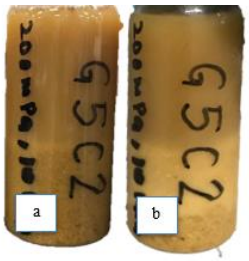

Figure 1: Sample Ginger (*Zingiber Officinale*) at Condition 2 (Pressure 200 MPa, Time: 10 min, solid-to-liquid ratio: 1:15% w/w) (a) before HPP extraction; (b) after HPP extraction

3.2 Nutritional Analysis of TPC

Total phenolic content was determined in the samples of *Curcuma Longa* and *Zingiber Officinale* for the study of nutritional analysis using Folin-Ciocalteu reagent (FCR). TPC analysis was conducted. Gallic acid was used as a standard to determine the concentration of TPC in *Curcuma Longa* and *Zingiber Officinale*. The absorbances were read at a wavelength of 760 nm against blank. The FCR reagent oxidizes phenols in turmeric and ginger extracts and changes the colour from yellow to dark blue colour indicated there are present phenolic compounds in the samples. Table 5 shows different concentrations of TPC for turmeric and ginger at different HPP operating conditions. For the 4 operating conditions of turmeric, the minimum value of TPC is 5.653 µg/mL at condition 3 (5 g, 200 MPa, 15 min) and the maximum concentration 8.033 µg/mL at condition 4 (15 g, 600 MPa, 10 min). Meanwhile, for ginger, the minimum value of TPC is 7.180 µg/mL at condition 3 (5 g, 200 MPa, 15 min) and the

maximum concentration 10.289 µg/mL at condition 1 (15 g, 200 MPa, 5 min). For turmeric, the higher the pressure and solid: liquid ratio, the greater the TPC value.

Table 5: TPC concentrations (µg/ml) for turmeric (*Curcuma Longa*) and ginger (*Zingiber Officinale*) at different HPP operating condition

Operating Condition		TPC concentration (µg/mL)	
		Turmeric	Ginger
Condition 1	5 g, 200 MPa, 5 min	6.446	7.521
	10 g, 200 MPa, 5 min	7.279	9.169
	15 g, 200 MPa, 5 min	7.947	**10.289***
Condition 2	5 g, 200 MPa, 10 min	6.412	7.639
	10 g, 200 MPa, 10 min	6.780	9.210
	15 g, 200 MPa, 10 min	7.032	10.215
Condition 3	5 g, 200 MPa, 15 min	**5.653^**	**7.180^**
	10 g, 200 MPa, 15 min	7.630	8.783
	15 g, 200 MPa, 15 min	7.660	13.515
Condition 4	5 g, 600 MPa, 10 min	6.460	7.330
	10 g, 600 MPa, 10 min	8.019	9.159
	15 g, 600 MPa, 10 min	**8.033***	10.088

(^) minimum values of TPC; (*) maximum values of TPC.

3.2.1 Effect of Pressure on TPC

Effect of pressure in HPP were conducted between pressure (200 MPa and 600 MPa) in the fixed extraction time (10 min). As shown in the Figure 2, the TPC values were compared for different condition which are condition 2 (200 MPa) and condition 4 (600 MPa) indicated that *Zingiber Officinale* has the highest amount of TPC at (200 MPa, 15 g) with 10.215 µg/mL compared to (600 MPa,15 g) give 10.088 µg/mL. Percent reduction in *Zingiber Officinale* only 1.25%. Hence, it is assumed the bioactive ingredient having degradation due to temperature changes while conducting experiment. Meanwhile, different for *Curcuma Longa* where at 600 MPa, concentration TPC showed highest at 8.033 µg/mL compared to 7.032 µg/mL at 200 MPa with the same 15 g solid sample.

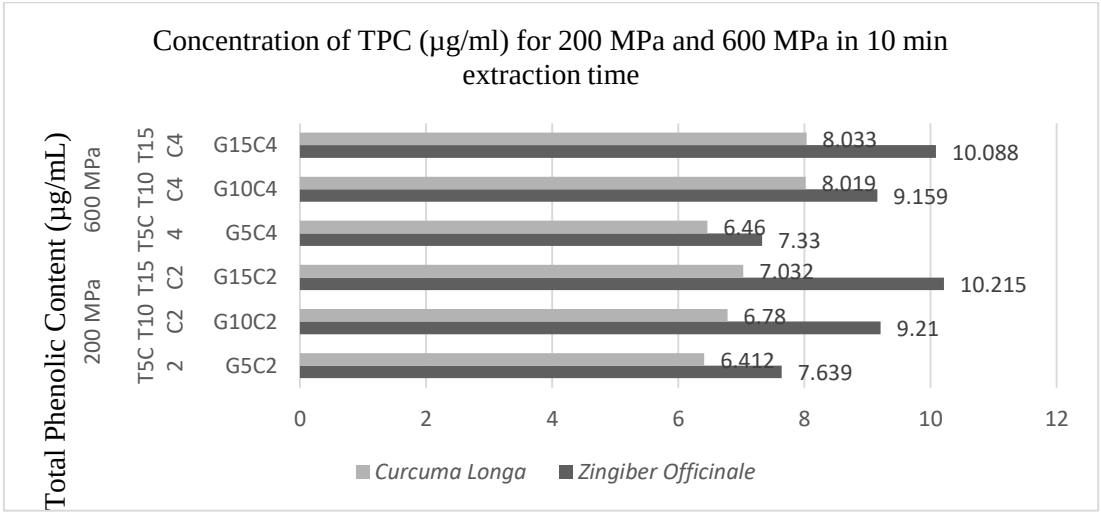

Figure 2: Concentration of TPC (µg/ml) for 200 MPa and 600 MPa in 10 min extraction time for *Curcuma Longa* and *Zingiber Officinale*

HPP is a new preservation technology for the thermal treatment. Hence, the study of HPP in turmeric and ginger for the total phenolic content is still developed and need further study. Thus, the result for this TPC will compared with other plants extract instead of turmeric and ginger. The specific bioactive component in TPC is strongly impacted by pressure in HPP. The rise in TPC caused by HPP could potentially be explained by the alteration of the molecular structure of phenolic compounds during high-pressure application. At very high pressure, phenolic compounds' hydrogen bonds can be altered and modified [13]. Past researched found that peel of 'Golden Delicious' apples in plant extract has significantly increased as much as 41.7% after treated with 600 MPa, 35°C, 5 mins on the contrary treated with 400 MPa have the reduction in TPC [14].

Reduction of TPC after HPP might be associated with the remaining activity of polyphenoloxidase (PPO) [15]. The processing and storage of apples can produce important losses of nutrients and bioactive compounds due to the action of food enzymes such as polyphenoloxidase (PPO) and peroxidase (POD) that are involved in different detrimental reactions. Additionally, the application of pressure can lead to an increase in membrane permeability, which in turn can facilitate the release of phenols and prevent additional thermal oxidation. Other than that, the pressure-induced breaking of covalent bonds can cause the depolymerization of proanthocyanidins resulting to breakdown into polyphenolic units.

3.2.2 Effect of Extraction Time on TPC

Effect of extraction time in HPP were conducted at 5,10 and 15 mins for the fixed pressure 200 MPa. Effect of different extraction time in TPC shown by Figure 3 illustrated that at 15 g *Zingiber Officinale*, the TPC is increased with the increase in extraction time which is ranging from 10.289 µg/mL to 10.215 µg/mL to 13.515 µg/mL but different with *Curcuma Longa*, the TPC is fluctuated with the increased in extraction time from concentration of 7.947 µg/mL to 7.032 µg/mL to 7.66 µg/mL. Choi et al. also found that the TPC is significantly increased in Dried Turmeric for High-Pressure Processing in different extraction times (5, 10 and 15 min). Prasad et al., 2009 in their research constructed that TPC did not show significant changes when the extraction time was increased from 2.5 to 30 minutes. According to the Pascal theory, during the High-Pressure process, the pressure uniformly and instantly spreads throughout the entire material. This rapid pressure equilibrium between the inside and outside of the cells allows for a quick equalization. As a result, the solvent diffuses rapidly, leading to a high extraction speed, and the extraction yield can reach its maximum value in a short period of time.

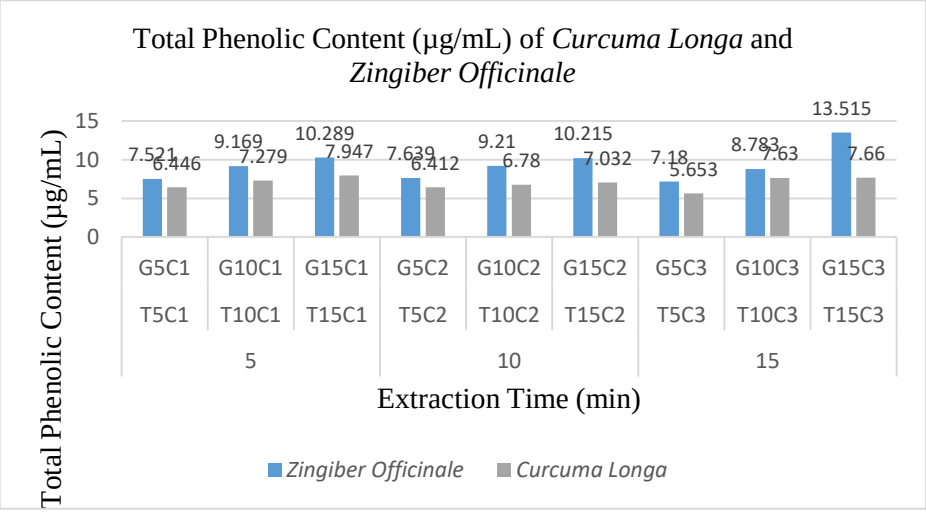

Figure 3: Concentration of TPC (µg/mL) for *Curcuma Longa* and *Zingiber Officinale* at different Extraction Time (5, 10 and 15 min) at 200 MPa Pressure measured using Uv-Vis Spectrophotometer at 765 nm

3.2.3 Effect of solid-to-liquid Ratio on TPC

Effect of solid-to-liquid ratio in HPP were conducted at 1:15% w/w, 1:7% w/w and 1:4.3% w/w for the fixed pressure 600 MPa. Concentration of TPC showed significantly increased with the increase in solid-to-liquid ratio from 1:15, 1:7 and to 1:4.3 g/mL for both samples as shown by the Figure 4. *Curcuma Longa* vary from 6.46 µg/mL, 8.019 µg/mL to 8.033 µg/mL same goes to *Zingiber Officinale* where it rising from 7.33 µg/mL, 9.159 µg/mL to 10.088 µg/mL respectively. Prasad et al., 2009 have found that when the solid to liquid ratio increased from 1:25 to 1:50, the total phenolic content and extraction yield increased from 12.1±0.1 to 14.2±0.2 mg/g DW and 13.5±0.4 to 14.8±0.6%, respectively in longan fruit pericarp. In situations where there is a higher amount of solid compared to liquid, it is likely that more solvent can enter cells, alongside a greater permeation of phenolic compounds into the solvent.

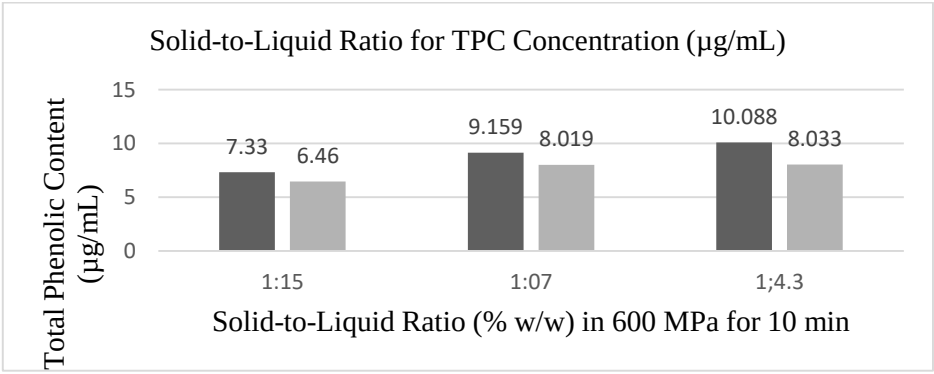

Figure 4: Concentration of TPC (µg/mL) for *Curcuma Longa* and *Zingiber Officinale* at different solid-to-liquid ratio at 600 MPa Pressure and 10 min extraction time measured using Uv-Vis Spectrophotometer at 765 nm

3.3 Functional Activity

Total Antioxidant and Total Anti-Inflammatory were determined in the samples of *Curcuma Longa* and *Zingiber Officinale* for the study of functional properties. The DPPH assay at concentrations ranging from for both samples. L-ascorbic acid was used as standard calibration curve. The standard calibration of L-ascorbic acid for total anti-oxidant activity measured by using Uv-Visible Spectrophotometer at 517 nm.

3.3 Quantification of Total Antioxidant

The DPPH (2, 2- diphenyl-1-picryl-hydrazyl) in total antioxidant (TAO) was used to evaluate the potential free radical scavenging activity of the ethanolic extract of the different concentration of *Curcuma Longa* and *Zingiber Officinale* samples. Table 6 shows different concentrations of TAO for turmeric and ginger at different HPP operating conditions. For the different operating conditions of turmeric, the minimum value of TAO is 0.014±0.85 mg/mL at condition 4 (5 g, 600 MPa, 5 min) and the maximum concentration 1.423±1.76 mg/mL at condition 4 (15 g, 600 MPa, 10 min). Meanwhile, for ginger, the minimum value of TAO is 1.068±1.55 mg/mL at condition 3 (10 g, 200 MPa, 15 min) and the maximum concentration 1.655±1.92 mg/mL at condition 3 (15 g, 200 MPa, 15 min).

Table 6: TAO (mg/ml) for turmeric (*Curcuma Longa)* and ginger *(Zingiber Officinale*) at different operating conditions

Operating Conditions		Total Antioxidant (mg/mL)	
		Turmeric	Ginger
Condition 1	5 g, 200 MPa, 5 min	0.042±0.87	0.192±0.96
	10 g, 200 MPa, 5 min	0.866±1.40	1.064±1.53
	15 g, 200 MPa, 5 min	1.321±1.71	1.622±1.89
Condition 2	5 g, 200 MPa, 10 min	0.044± 0.87	0.418±1.11
	10 g, 200 MPa, 10 min	0.702±1.34	1.124±1.58
	15 g, 200 MPa, 10 min	1.389±1.80	1.642±1.90
Condition 3	5 g, 200 MPa, 15 min	0.045±0.89	0.038±0.86
	10 g, 200 MPa, 15 min	0.566±1.20	**1.068±1.55^**
	15 g, 200 MPa, 15 min	1.310±1.71	**1.655±1.92***
Condition 4	5 g, 600 MPa, 10 min	**0.014±0.85^**	0.006±0.84
	10 g, 600 MPa, 10 min	0.712±1.34	1.077±1.54
	15 g, 600 MPa, 10 min	**1.423±1.76***	1.628±1.89

Mean value (means ± standard deviation); (^) minimum value of TAO; (*) maximum value of TAO

3.3.1 Effect of Pressure on TAO

Effect of pressure in HPP for TAO were conducted between pressure (200 MPa and 600 MPa) for the fixed extraction time (10 min). From Figure 5 shows the concentration of total antioxidant based on different pressure condition (200 MPa and 600 MPa) of *Curcuma Longa* (Turmeric) and *Zingiber Officinale* (Ginger), it was observed that at 600 MPa for turmeric T15C4 gives the highest concentration antioxidant with 1.423±1.76 mg/mL comparison with T15C2 at 200 MPa which is 1.389±1.80 mg/mL.

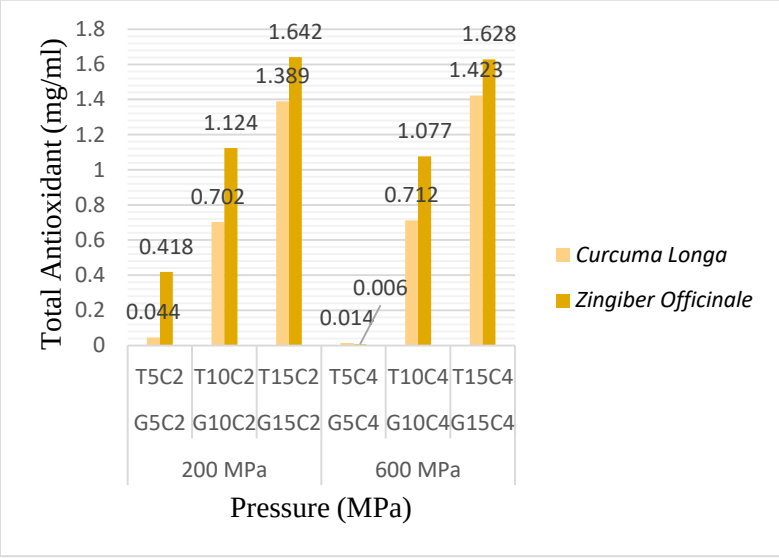

Figure 5: TAO (mg/mL) for *Curcuma Longa* and *Zingiber Officinale* at 200 MPa and 600 MPa for the fixed 10 min extraction time

Meanwhile, ginger sample give the highest antioxidant activity at 200 MPa pressure with G15C2 (1.642±1.90 mg/mL) compared to 600 MPa pressure G15C4 (1.628±1.89 mg/mL). It was observed that for ginger, there are less significant difference with only decreased in 0.85% difference at 200 MPa compared to 2.39% difference for turmeric at 600 MPa. It shows that from the graph bar, the concentration of total antioxidant for *Curcuma Longa* and *Zingiber Officinale* samples increase with the increase in pressure. Same researched from Fernández-jalao et al., 2018, Fernandez determined the

antioxidant activity of apple 'Golden Delicious' on different pressure conditions (400, 500 and 600 MPa). The result indicated that at 600 MPa, the DPPH (µmol TE/g dw) give the antioxidant activity of 30.01±2.0 compared to 400MPa and 500 MPa where gives the value 22.83±0.8 and 21.00±0.8. Same researched from Cheng et al., 2019, Djulis hull plant extracts from HPE-150, HPE-300, HPE-450 and H-600 MPa showed higher antioxidant capacity, at 57.1%, 62.7%, 63.8%, and 69.8%, respectively.

The process of High-Pressure Processing involves three distinct stages: pressure boost, maintaining, and relief. In the pressure boost stage, the pressure rapidly increases from atmospheric pressure to the desired processing pressure within a short time. This sudden pressure rise disrupts cells, promoting increased mass transfer of phenolic compounds. The level of pressure and resistance encountered during mass transfer directly affect the extent of phenolic compound transfer. In the maintaining stage, the extraction solvent efficiently diffuses into the cells due to the conditions set in the previous stage. Finally, in the relief stage, the pressure is reduced from the operating pressure back to atmospheric pressure. This pressure reduction significantly alters the hydrogen bonds, ionic bonds, and hydrophobic interactions that govern the cellular structure. Consequently, the cells can expand, leading to the formation of porous, broken, or loosely structured configurations. These structural changes ultimately enhance the diffusion of bioactive compounds, contributing to improved total antioxidant activity.

3.3.2 Effect of Extraction Time on TAO

Effect of extraction time in HPP for TAO were conducted at 5, 10 and 15 mins for the fixed pressure 200 MPa. Extraction time is also considered as an important role in extracting bioactive compounds. Figure 6 showed that with the increasing extraction time 5,10 and 10 min, the total antioxidant (mg/mL) also increase for both turmeric and ginger. The TAO for ginger ranging from 1.622 (mg/mL)/15g, 1.642 (mg/mL)/15g to 1.655 (mg/mL)/15g with rising in times. Same goes to turmeric from 1.321 (mg/mL)/15g, 1.389 (mg/mL)/15g and 1.310 (mg/mL)/15g.

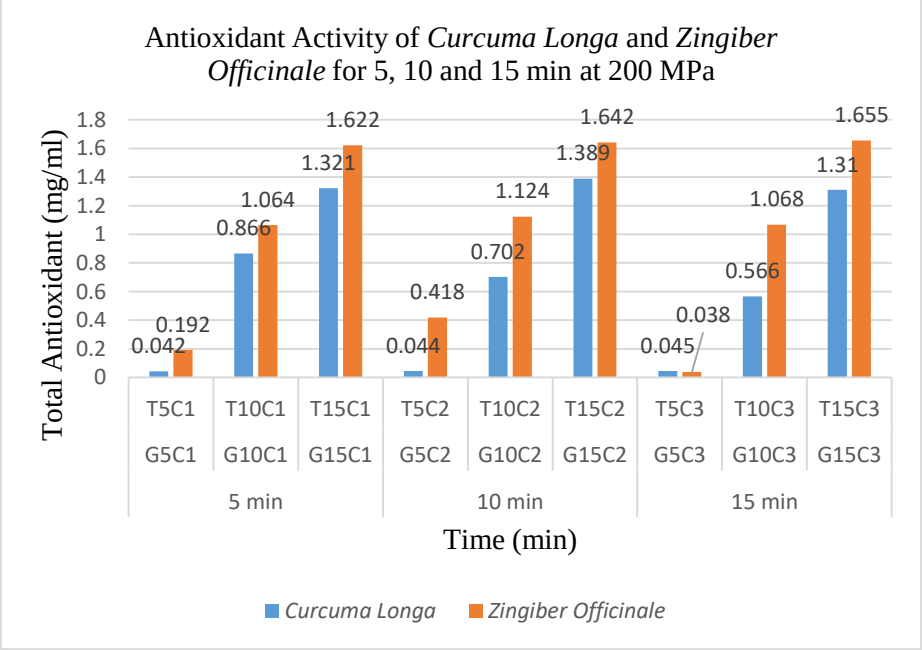

Figure 6: TAO (mg/mL) of *Curcuma Longa* and *Zingiber Offinale* at different Extraction Time (5, 10 and 15 min) at the same Pressure Condition (200 MPa)

But for T15C3, the value significantly decreased about 5.7% compared with T15C2. Choi et al., 2020 found that at 15 min extraction time, DPPH is significantly increased to 7.35 ±0.30 mg VCE/g dried turmeric, as compared to a 5 min extraction (6.97±0.52 mg VCE/g). Prolonged extraction time allows for increased contact between the extraction solvent and the source material. This prolonged contact enables more efficient diffusion of antioxidants and other bioactive compounds into the solvent, resulting in higher antioxidant activity.

3.3.3 Effect of solid-to-liquid Ratio on TAO

Effect of solid-to-liquid ratio in HPP for TAO were conducted at 1:15% w/w, 1:7% w/w and 1:4.3% w/w for the fixed pressure 600 MPa.

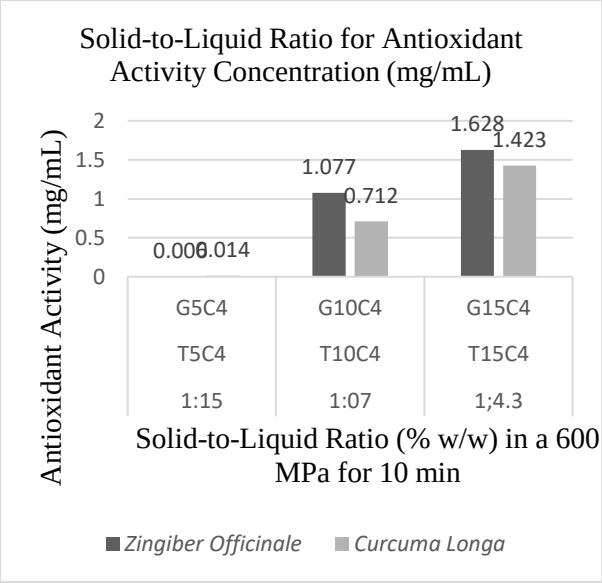

Figure 7: TAO (mg/mL) for *Curcuma Longa* and *Zingiber Officinale* at different Solid-to-Liquid Ratio at 600 MPa Pressure and 10 min Extraction Time

The solid to liquid ratio also affects the overall processing efficiency in terms of throughput. Higher solid to liquid ratios may require longer processing times or higher pressure levels to achieve desired effects due to the increased resistance to pressure transmission. Solid-to-liquid ratio in graph bar shown by Figure 7 indicated that for ginger (*Zingiber Officinale*), the antioxidant ranging from 0.006 mg/mL, 1.077 mg/mL to 1.628 mg/mL as well as for turmeric (*Curcuma Longa*) from 0.014 mg/mL to 0.712 mg/mL to 1.423 mg/mL with increasing solid-to-liquid ratio.

3.4 Quantification of Total Anti-Inflammatory

Inhibition of protein denaturation was evaluated with the method of BSA denaturation assay. In this study, *Curcuma Longa* and *Zingiber Officinale* extracts were dissolved in absolute methanol to obtain anti-inflammatory activity (%) at different conditions in HPP. Salicylic acid was used as a positive control and anti-inflammatory of samples were determined by using Uv-Visible Spectrophotometer at 660 nm.

Table 7: Percentage Inhibition of Protein Denaturation (%) of *Curcuma Longa* and *Zingiber Officinale* at different conditions in HPP extraction method

Samples	Inhibition of Protein Denaturation (%)
T5C1	76.9±0.00
T10C1	74.7±0.00
T15C1	72.2±0.01
T5C2	**94.1±0.00***
T10C2	85.1±0.01
T15C2	60.9±0.01
T5C3	91.8±0.00
T10C3	89.7±0.01
T15C3	84.3±0.00
T5C4	90.9±0.00
T10C4	76.6±0.03
T15C4	**55.9±0.01^**

G5C1	79.9±0.01
G10C1	64.2±0.01
G15C1	48.7±0.01
G5C2	**81.2±0.00***
G10C2	53.3±0.01
G15C2	35.7±0.01
G5C3	80.8±0.01
G10C3	37.8±0.01
G15C3	**24.3±0.00^**
G5C4	73.4±0.01
G10C4	51.7±0.00
G15C4	42.4±0.00
Salicylic Acid	95.6±0.04

Mean value (means ± standard deviation); (^) minimum value of TAI; (*) maximum value of TAI

3.4.1 Effect of Pressure on TAI

Effect of pressure in HPP for TAI were conducted between pressure (200 MPa and 600 MPa) for the fixed extraction time (10 min). As illustrated in Figure 8, the percentage of anti-inflammatory activity in the BSA denaturation assay represented salicylic acid used as a positive control. At 200 MPa, the inhibition protein denaturation at *Zingiber Officinale* (ginger) for G5C2 is decreased with the increase in pressure from 200 to 600 MPa from 81.2% to 73.4% at the same 5g ginger. Along with *Curcuma Longa* (turmeric) ranging from 94.1% to 90.9%. It is shown that, for 600 MPa, the anti-inflammatory for both samples have little effect on anti-inflammatory activity.

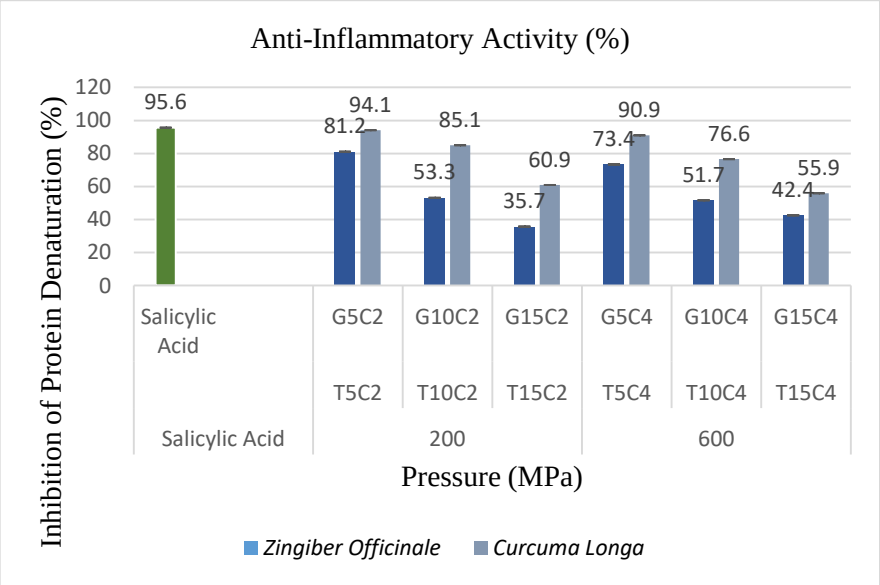

Figure 8: Total Anti-Inflammatory Activity (%) for *Curcuma Longa* and *Zingiber Officinale* at different Pressure (200 MPa and 600 MPa) with the same processing Time (10 mins) by using HPP

3.4.2 Effect of Extraction Time on TAI

Figure 9 indicated that percent anti-inflammatory activity of *Curcuma Longa* and *Zingiber Officinale* were compared with Salicylic Acid. The result showed at 15 g solid weight, the ginger showed significantly decreased anti- inflammatory with the rising in extraction time (min) where the value ranged from 48.7% to 35.7% to 24.3% in 5, 10, 15 min. Turmeric also shows significantly decreased and increased in anti-inflammatory activity that give value from 72.2% to 60.9% to 84.3% in 5,10 and 15 min extraction time. Choi, et al from their researched found that at rising extraction time from (5-15 min), *curcumin* compound that are beneficial for the anti-inflammatory activity were decreased from 438.83±1.14, 400.32±8.23, 390.72±1.97 µg/g of dried Turmeric.

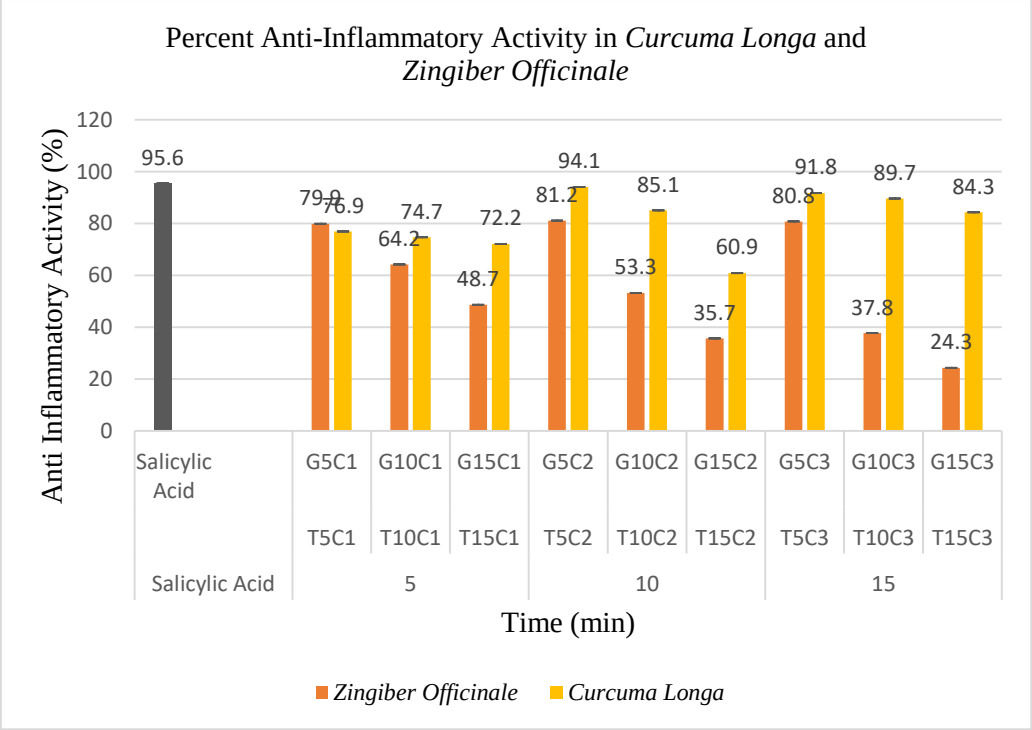

Figure 9: Total Anti-Inflammatory (%) for *Curcuma Longa* and *Zingiber Officinale* at different Extraction Time (5, 10 and 15 min) at 200 MPa Pressure

3.4.3 Effect of solid-to-liquid ratio on TAI

Figure 10 indicated that with high a solid-to-liquid ratio, the anti-inflammatory activity was decreased for both samples (*Curcuma Longa* and *Zingiber Officinale*). *Curcuma Longa* compared to salicylic acid (95.6%) anti-inflammatory activity, were vary from 73.4% to 51.7% to 42.4% as well as for *Zingiber Officinale* that give results from 90.9% to 76.6% and to 55.9% anti-inflammatory. In general, an increased ratio of solids to liquids offers a greater amount of solvent, facilitating its penetration into cells and creating favorable conditions for the diffusion of phenolic compounds into the solvent. This is primarily due to enhanced solubility and concentration gradients.

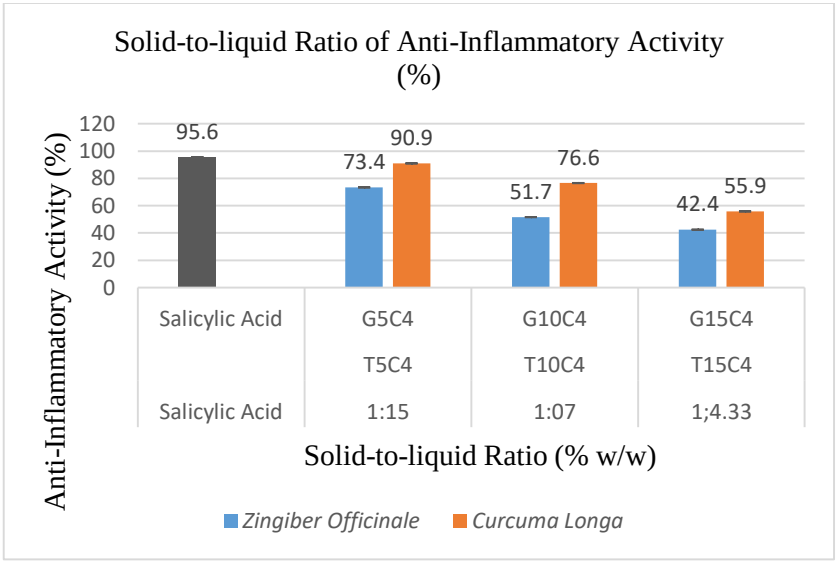

Figure 10: Total Anti-Inflammatory Activity (%) for *Curcuma Longa* and *Zingiber Officinale* at different Solid-to-liquid Ratio at 600 MPa Pressure and 10 min Extraction Time

3.5 Enhancement of High Pressure Processing Conditions

The enhancement of HPP operating conditions for Total Phenolic Content (TPC), Total Anti-oxidant (TAO) and Total Anti-Inflammatory (TAI) of *Curcuma Longa* and *Zingiber Officinale* is presented in Table 8. For the extraction of total phenolic content and total anti-oxidant, the best operating conditions are 600 MPa, 10 mins and 1:4.3% w/w solid:liquid ratio. However, to extract turmeric at its best anti-inflammatory activity, the best operating conditions are 200 MPa, 10 mins and 1:15% w/w solid:liquid ratio. Meanwhile, in ginger, 200 MPa seems to be the best operating pressure to retain the highest nutritional and functional activity. However, depending on the targeted parameter (TPC, TAO or TAI), the extraction time varies from 5 min to 15 min and the solid:liquid ratio varies between 1:4.3% to 1:15%.

Table 8: Summary of Enhancement HPP Operating Conditions of *C. Longa and Z. Officinale* for different nutritional and functional parameters

Optimum HPP Operating Conditions	*Curcuma Longa*			*Zingiber Officinale*		
	TPC	TAO	TAI	TPC	TAO	TAI
	8.03 ug/mL	1.432 mg/mL	94.1%	10.29 ug/mL	1.655 mg/mL	81.2%
Pressure (MPa)	600 MPa	600 MPa	200 MPa	200 MPa	200 MPa	200 MPa
Extraction Time (min)	10 min	10 min	10 min	5 min	15 min	10 min
Solid-to-Liquid ratio % (w/w)	1:4.3	1:4.3	1:15	1:4.3	1:4.3	1:15
(g:g)	15:65	15:65	5:75	15:65	15:65	5:75
Remarks	For the extraction of total phenolic content and total anti-oxidant, the best operating conditions are 600 MPa, 10 mins and 1:4.3% w/w solid:liquid ratio. However, to extract turmeric at its best anti-inflammatory activity, the best operating conditions are 200 MPa, 10 mins and 1:15% w/w solid:liquid ratio.			In ginger, 200 MPa seems to be the best operating pressure to retain the highest nutritional and functional activity. However, depending on the parameter, the extraction time varies from 5 min to 15 min and the solid:liquid ratio varies between 1:4.3% to 1:15%.		

4. Conclusion

It can be concluded that, the plants of *Curcuma Longa and Zingiber Oficinale* s' bioactive compounds were successfully extracted by using High Pressure Processing (HPP) method at different operating conditions. The total phenolic content, total antioxidant and total anti-inflammatory has been successfully conducted using methods described in methodology for *Curcuma Longa* and *Zingiber Officinale*. The nutritional content and functional properties of *Curcuma Longa* were found to be highest in conditions of 600 MPa, 10 min extraction time and 1:4.3% w/w of solid-to-liquid ratio. But, for anti-inflammatory, the highest conditions are in 200 MPa, 10 min and 1:15% w/w solid-to-liquid ratio. Next, for *Zingiber Officinale*, it was seen that, at conditions of 200 MPa pressure potential for

extracting the bioactive compound. Apart from that, extraction time and solid-to-liquid ratio are varies depending on the conditions from 5 min to 10 min and from 1:4.3% w/w to 1:15% w/w.

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