

Effect of Drying Methods and Ultrasonication in Improving the Antioxidant Activity and Total Phenolic Content of Apple Pomace Powder

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Abstract

Although extrusion is a promising process to develop ready to eat cereals and snacks, thermal treatment to raw material during extrusion results in degradation of phenolic compounds. Therefore, an approach was made to enhance the total phenolic content (TPC) and antioxidant activity (AA) of apple pomace (AP) prior to extrusion process. In this study, AP powder was naturally fermented (F) for 12 h and then was subjected to ultrasonication (U) at various conditions [25, 37, and 50 μm ultrasonication amplitude (UA) for 1, 2, and 3 min of ultrasonication time (UT)]. AP was then dried in oven (O) and microwave (MW), separately and thus four drying methods, i.e. O_F-O_U , O_F-MW_U , MW_F-O_U , MW_F-MW_U were used in combinations. Full factorial design was used for experimental plan and results were analyzed using statistical software. It was observed that drying method significantly affected the TPC and AA of AP powder followed by UA. UT did not have any significant effect on TPC, and AA. Maximum TPC, and AA observed for the AP powder dried in MW after fermentation and ultrasonication (MW_F-MW_U) at 50 μm UA for 3 min UT were 372.98 mg GAE/100g DW, and 729.67 $\mu\text{mol TE}/100$ g DW, respectively. MW_F-MW_U drying exhibited a more prominent disrupted and porous structure of AP powder compared with that of O_F-O_U drying.

Keywords: apple pomace, fermentation, ultrasonication, microwave drying, total phenolic content, antioxidant activity

1. Introduction

Apple pomace (AP) is a solid residue (25%-30% of total fruit) obtained after the extraction of apple juice. AP is mainly composed of dietary fibers, carbohydrates, small amount of proteins, fat, and ash (Sudha, Baskaran, & Leelavathi, 2007). It also contains numerous phytochemicals in the form of simple sugars, pectin, and natural antioxidants (Bhushan, Kalia, Sharma, Singh, & Ahuja, 2008). The amount of total phenolic compounds (TPC) varies greatly in between flesh and peel of apple. Peel contains higher quantity of phenolic compounds. The procyanidins, catechin, epicatechin, chlorogenic acid, phloridzin and quercetin conjugates are commonly found in apple peels. In the apple flesh, catechin, procyanidins, epicatechin and phloridzin are found in much lower concentrations. Some of the phenolic compounds in AP have been correlated with antioxidant activities (AA) using various methods [2,2-diphenyl-1-picrylhydrazyl (DPPH), hydroxyl and superoxide anion radical scavenging activity, ferric reducing antioxidant power (FRAP)] and thereby confirming the AP as a valuable source of antioxidants (Diñeiro, Valles, & Picinelli, 2009).

As a common application, AP is used for direct disposal to soil in a landfill, and for pectin recovery usage (gelling agent, stabilizer and source of dietary fiber). These applications are not sufficient to utilize the several tons of AP produced every year; therefore, studies have got momentum to valorize the AP for other purposes also. AP as a rich source of antioxidant compounds could be used for increasing the stability of foods by preventing lipid peroxidation and also for protecting oxidative damage in living systems by scavenging oxygen radicals. Extruded snacks, where starch is the major component, will have more nutritional value if incorporated with fiber enriched flours containing antioxidants. But, on the other hand, high temperature extrusion process also results in loss of nutritive values. From preliminary experiments, it was observed that extrusion at optimum temperature reduced 25-30% TPC and AA of AP. Therefore, prior to extrusion, an effort is required to enhance

the nutritive values, specially, TPC, and AA in AP. In past few years, ultrasonication and fermentation have been used for accelerating the extraction of phenolic antioxidants from AP (Ajila, Brar, Verma, Tyagi, & Valéro, 2011; Ajila et al., 2012; Opalić et al., 2009; Vasantha Rupasinghe, Kathirvel, & Huber, 2011; Virot, Tomao, Le Bourvellec, Renard, & Chemat, 2010). Fermentation also brings about numerous biochemical, nutritional and organoleptic changes in the raw materials including the breakdown of certain constituents (Murekatete, Hua, Kong, & Zhang, 2012; Oboh & Amusan, 2009). Several methods such as heat treatment, aqueous extraction, microwave assisted extraction and far-infrared radiation have been studied to liberate and activate natural antioxidants (Candrawinata, Golding, Roach, & Stathopoulos, 2014; Grigoras, Destandau, Fougère, & Elfakir, 2013; Kim et al., 2008; Reis, Rai, & Abu-Ghannam, 2012). However, our comprehensive literature review revealed there has been very few detailed report on the use of microwaves and microwave drying to liberate phenolic compounds in plant materials and particularly none in case of AP. Therefore, keeping in view the above facts, the study was focused on enhancing the TPC and AA in AP powder using fermentation, ultrasonication and microwave (MW) drying technology.

2. Materials and Methods

Apple pomace (AP) powder provided by Tree Top, Inc. (Selah, WA) was stored at -20 °C. The initial moisture content of AP powder was 8.75% (wb). Protein, fat, ash, crude fiber and carbohydrate of AP powder were 4.14%, 2.79%, 2.11%, 22.06%, and 60.21%, respectively. For natural fermentation, AP slurry was prepared by adding 12.5 g AP powder in 100 ml distilled water. The fermentation was carried out in a controlled conditions with temperature 30±1 °C for 12 h. Fermented slurry was dried and further subjected to ultrasonication at 25 µm, 37 µm, and 50 µm amplitude for 1, 2, and 3 min. Sample to water ratio was kept constant as 5% (w/v) as a maximum concentration for ultrasonication from preliminary trials. Ultrasonicated sample was again dried and was stored at -20 °C for TPC and AA analysis. Two methods, i.e. hot air oven (50 °C) and microwave (MW) drying (90 W) were used for drying of sample till its constant weight. Four drying methods, i.e. O_F-O_U, O_F-MW_U, MW_F-O_U, MW_F-MW_U were used in combinations (Table 1). Hot air oven drying was denoted by 'O', and subscripts 'F', and 'U' indicated 'after fermentation', and 'after ultrasonication', respectively. For example, MW_F-O_U indicates microwave drying after fermentation and hot air drying after ultrasonication.

Table 1. Independent variable values of the process and their corresponding levels

S. No	Independent variable	Level	Values
1	Drying method	4	O _F -O _U , O _F -MW _U , MW _F -O _U , MW _F -MW _U
2	Ultrasonication amplitude, µm	3	25, 37, 50
3	Ultrasonication time, min	3	1, 2, 3

O: Oven drying, MW: Microwave drying, F: Fermentation, U: Ultrasonication.

One gram of AP powder was weighed and extracted with 10 ml of methanol. The methanol extract was centrifuged for 20 min at 2500 × g. The fresh supernatant solution was collected and used for the determination of total phenolic content and antioxidant activity. All measurements were taken with six replications.

2.1 Total Phenolic Content (TPC)

TPC of AP powder was determined using method (Singleton, Orthofer, & Lamuela-Raventós, 1999) with some modification. 50 µl methanol extract of sample was added with 3.5 ml distilled water and 150 µl Folin Ciocalteu reagent. The solution was vortexed and incubated for 30 min. Thereafter, absorbance of solution was measured at 760 nm against blank. Blank solution contained all the components that were present in the sample except the methanol extract. Gallic acid was used as positive control (standard) and linear regression curve between absorbance and concentration was drawn for the standard. This standard curve was used for calculating the concentration of sample and data was expressed in mg Gallic acid equivalent (GAE)/100 g dry weight (DW). This analysis was done in six replications.

2.2 Antioxidant Activity (AA)

Extinction of DPPH is a free radical scavenging activity which was measured using slightly modified spectrophotometric method described by (Brand-Williams, Cuvelier, & Berset, 1995). 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution was prepared by adding 7.9 mg of DPPH in 200 ml ethanol. 125 µl methanol extract was mixed with 2 ml ethanol and 0.5 ml of this solution was added with 3 ml DPPH. The

solution was vortexed and incubated for 30 min. Thereafter, absorbance of solution and control (DPPH) was measured at 517 nm against blank (ethanol). Results were expressed as μmol trolox equivalent (TE)/100 g dry weight (DW). Samples were analyzed in six replications.

2.3 Moisture Content (MC)

MC of AP powder was determined by air oven standard methods recommended by AOAC (1980). Initially 5 g of sample in triplicate was dried in hot air oven at 130-133 °C for 2 h. After drying, dried sample was again weighed. Following formula is used for calculating the MC.

$$\text{MC (wb, \%)} = \frac{W_i - W_f}{W_i} \times 100 \quad (1)$$

W_i = initial weight of sample (5 g),

W_f = weight of sample after drying, g

2.4 Microstructure Evaluation

The microstructure of control, oven dried and MW dried Apple pomace powder was examined using a scanning electron microscope (SEM) (Hitachi-S3400 N, Tokyo, Japan). Small amounts of samples were mounted on SEM specimen stubs by using double-sided adhesive tape. Each powder sample was coated with 10 Å thick layer of gold in a sputter coater before being scanned and photographed at 1000× magnification.

2.5 Statistical Analysis

Full factorial design was used for experimental plan and results were compared by analysis of variance (ANOVA) using SPSS (16.0) statistical software. All data were reported as mean \pm standard deviation of replicates. Tukey's tests were used to compare the significant differences of the mean values with the family error rate held at 0.05. Pearson correlation test was employed to correlate total phenolic content and antioxidant activity.

3. Results and Discussion

3.1 Effect of Drying Method on TPC and AA

It was depicted by ANOVA that drying methods (DM) had significant ($P < 0.05$) effect on TPC and AA followed by interaction effect of drying method and UA (Table 2). Table 3 shows that TPC, and AA increased significantly by 20.1%, and 47.9% on average when AP powder was MW dried after fermentation and ultrasonication as compare to oven drying during both the processes. Some other studies have also reported that the phenolic content was increased after microwave treatment of the plant materials (Boateng, Verghese, Walker, & Ogutu, 2008; Hayat et al., 2010; Omwamba & Hu, 2010). However, Sharma and Gujral (2011) found decrease in TPC of barley after microwave cooking. Microwave treatment of AP powder cleaved and liberated phenolic compounds, hence resulting in the increase of free phenolic compounds and enhancement of antioxidant activity. O_F - MW_U , and MW_F - O_U drying methods did not show any significant difference in TPC, while there was no significant difference in AA between MW_F - O_U and MW_F - MW_U drying methods. TPC of AP powder was found least during O_F - O_U drying that may be because of thermal degradation of TPC. When AP powder was MW dried after fermentation, and ultrasonication at 50 μm UA for 3 min UT, TPC and AA were increased by 27%, and 66.3%, respectively, as compare to oven dried during both the processes (Figure 1). Maximum TPC, and AA observed for the AP powder for MW_F - MW_U at 50 μm UA for 3 min UT were 16.7%, and 88.5% more than that of control (untreated AP powder), respectively. TPC value obtained from MW_F - O_U drying method was not significantly ($p > 0.05$) different than that of control AP powder. O_F - O_U and O_F - MW_U drying method did not show any significant difference in AA values with compare to that of control AP powder (Figure 1). Apart from enhancing the TPC and AA of AP powder, MW_F - MW_U drying saved 65% of drying time as compared to O_F - O_U drying (data not shown).

Table 2. Variance analysis for all dependent variables

Source	Dependent variables	Type III sum of square	df	Mean square	F value	P value
Drying method (DM)	TPC	47302.72	3	157.6757	110.67	0.000*
	AA	678833.34	3	226277.78	58.83	0.000*
Ultrasonication amplitude (UA)	TPC	8732.72	2	4366.36	30.65	0.000*
	AA	187686.93	2	93843.47	24.4	0.000*
Ultrasonication time (UT)	TPC	64.37	2	32.18	0.23	0.798
	AA	1460.91	2	730.45	0.19	0.827
DM*UA	TPC	3958.22	6	659.70	4.63	0.000*
	AA	159417.69	6	26569.62	6.91	0.000*
DM*UT	TPC	238.89	6	39.82	0.28	0.945
	AA	33112.20	6	5518.70	1.43	0.213
UA*UT	TPC	382.54	4	95.64	0.67	0.614
	AA	23506.46	4	5876.61	1.53	0.203
DM*UA*UT	TPC	584.38	12	48.69	0.34	0.978
	AA	53277.46	12	4439.79	1.15	0.332

*significant at 5 % level of significance.

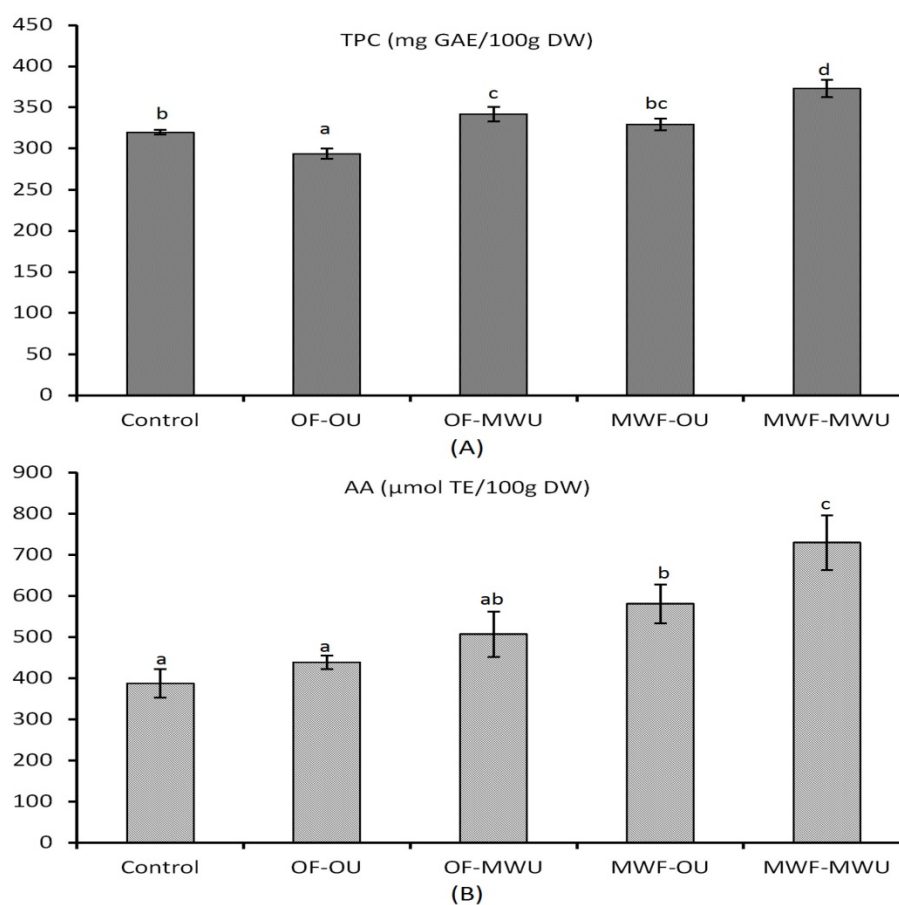


Figure 1. Effect of drying methods on TPC (A) and AA (B) of apple pomace powder at 50 µm UA for 3 min UT (O: oven drying, MW: microwave drying, F: fermentation, U: ultrasonication). Bars with different letters are significantly ($P < 0.05$) different

3.2 Effect of UA and UT on TPC and AA

It was explicated by ANOVA that there was significant ($P < 0.05$) effect of UA, and interaction of UA and drying method on TPC and AA of AP powder (Table 2). TPC and AA of AP powder increased significantly with increase in UA from 25 μm to 50 μm (Table 3). The reason may be that an increase in UA resulted in disruption of cell compartments facilitating the interaction of phenolic molecules with solvents at reasonably low temperatures. Alighourchi, Barzegar, Sahari, and Abbasi (2013) also observed an increase in TPC of pomegranate juice with increase in ultrasound power. For all drying methods and 3 min of UT, TPC increased nonlinearly with increase in UA from 25 μm to 50 μm , however, only during $\text{O}_F\text{-MW}_U$ and $\text{MW}_F\text{-MW}_U$ drying, TPC increased significantly ($p < 0.05$) with increase in UA from 25 μm to 37 μm and 37 μm to 50 μm , respectively (Figure 2). Except $\text{O}_F\text{-O}_U$ drying, all drying methods showed the significant ($p < 0.05$) difference in TPC values between 25 μm and 50 μm UA (Figure 2). With increase in UA, AA also increased for all drying methods except $\text{O}_F\text{-MW}_U$ method. For this drying method, AA first decrease with increase in UA from 25 μm to 37 μm and thereafter increased with UA up to 50 μm (Figure 2). $\text{O}_F\text{-O}_U$ and $\text{O}_F\text{-MW}_U$ drying method did not exhibit any significant ($p > 0.05$) change in AA of AP powder when UA increased from 25 μm to 50 μm for 3 min UT (Figure 2). A significant ($p < 0.05$) difference in AA of AP powder was observed between 25 μm and 50 μm UA during $\text{MW}_F\text{-O}_U$ drying, whereas, $\text{MW}_F\text{-MW}_U$ drying showed a continuous significant ($p < 0.05$) increase in AA with increase in UA in experimental range (Figure 2). When MW dried fermented and ultrasonicated AP powder was subjected to an increase in UA from 25 μm to 50 μm for 3 min UT, TPC, and AA were increased by 10.7%, and 80.4%, respectively. Maximum TPC and AA were found as 372.98 mg GAE/100 g DW and 729.67 $\mu\text{mol TE}/100\text{ g DW}$, respectively at 50 μm UA and $\text{MW}_F\text{-MW}_U$ drying method (Table 3).

Table 2 and Figure 3 shows that there was no significant ($p > 0.05$) change in TPC, and AA with increase in UT from 1 to 3 min, although maximum TPC and AA were found for 3 min of UT at 50 μm UA and $\text{MW}_F\text{-MW}_U$ drying (Table 3). The reason of this may be due to short time exposure of AP powder to the ultrasonication.

Table 3. Main effect of independent variables on responses

Independent variables	Values	TPC (mg GAE/ 100g DW)	AA ($\mu\text{mol TE}/100\text{g DW}$)
Drying method	$\text{O}_F\text{-O}_U$	291.62 \pm 10.29 ^a	417.95 \pm 25.26 ^a
	$\text{O}_F\text{-MW}_U$	326.96 \pm 12.54 ^b	478.45 \pm 49.19 ^b
	$\text{MW}_F\text{-O}_U$	318.92 \pm 10.43 ^b	578.25 \pm 54.69 ^c
	$\text{MW}_F\text{-MW}_U$	350.20 \pm 11.53 ^c	618.23 \pm 49.11 ^c
UA, μm	25	310.86 \pm 11.45 ^a	469.54 \pm 22.87 ^a
	37	332.88 \pm 9.97 ^b	571.18 \pm 26.85 ^b
	50	322.03 \pm 10.36 ^c	528.94 \pm 33.94 ^c
UT, min	1	323.01 \pm 10.06 ^a	527.37 \pm 19.66 ^a
	2	321.31 \pm 9.45 ^a	523.85 \pm 13.58 ^a
	3	321.45 \pm 10.66 ^a	518.43 \pm 13.69 ^a

Values within columns for individual variables with different superscript letters are significantly ($p < 0.05$) different. O: Oven drying, MW: Microwave drying, F: Fermentation, U: Ultrasonication.

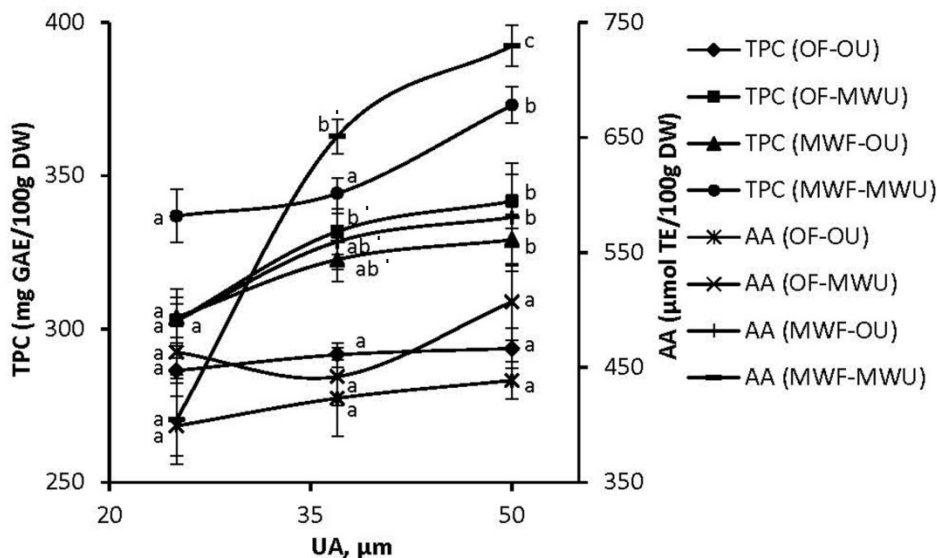


Figure 2. Effect of UA on TPC and AA of apple pomace powder for 3 min UT for all drying methods. Mean values in the same line with different letters are significantly ($p < 0.05$) different

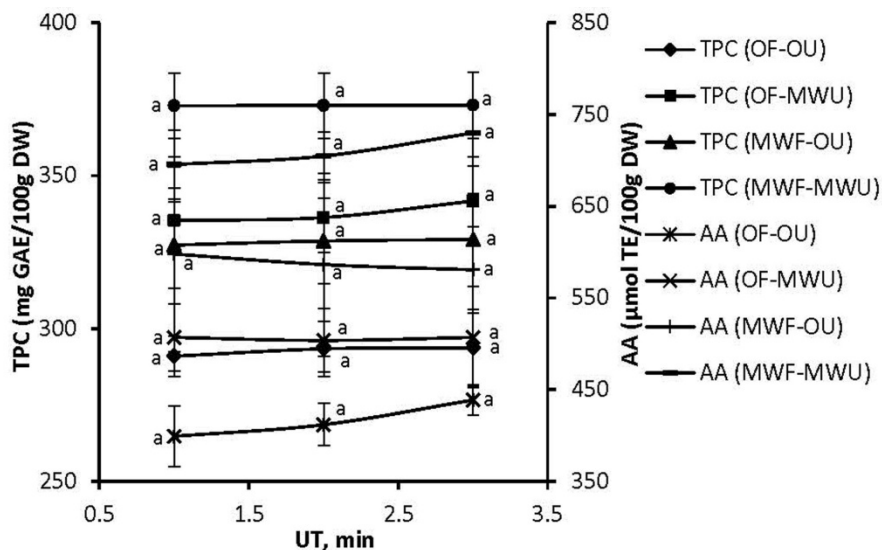


Figure 3. Effect of UT on TPC and AA of AP powder at 50 μm UA for all drying methods. Mean values in the same line with different letters are significantly ($p < 0.05$) different

3.3 Effect of Processing on Microstructures of AP Powder

The effect of the drying conditions on the microstructure of treated (fermented and ultrasonicated at 50 μm amplitude for 3 min) AP powder was carried out by means of SEM. The microphotographs of fresh, O_F-O_U and MW_F-MW_U AP powder are shown in Figure 4. Figure 4a shows the structure of fresh AP powder, which is a compact and less porous structure. The SEM images revealed that hot air drying (O_F-O_U) resulted in modification of the cellular structure (Figure 4b). MW drying after fermentation and ultrasonication (MW_F-MW_U) damaged the plant cell structure and caused more porosity (Figure 4c). Similar results were found by Giri and Prasad (2007) and Han, Yin, Li, Yang, and Ma (2010) for mushroom and apple slices, respectively. MW dried sample granules were more disrupted than hot air dried granules which caused increase in change into surface area and released more phenolics from bound structure.

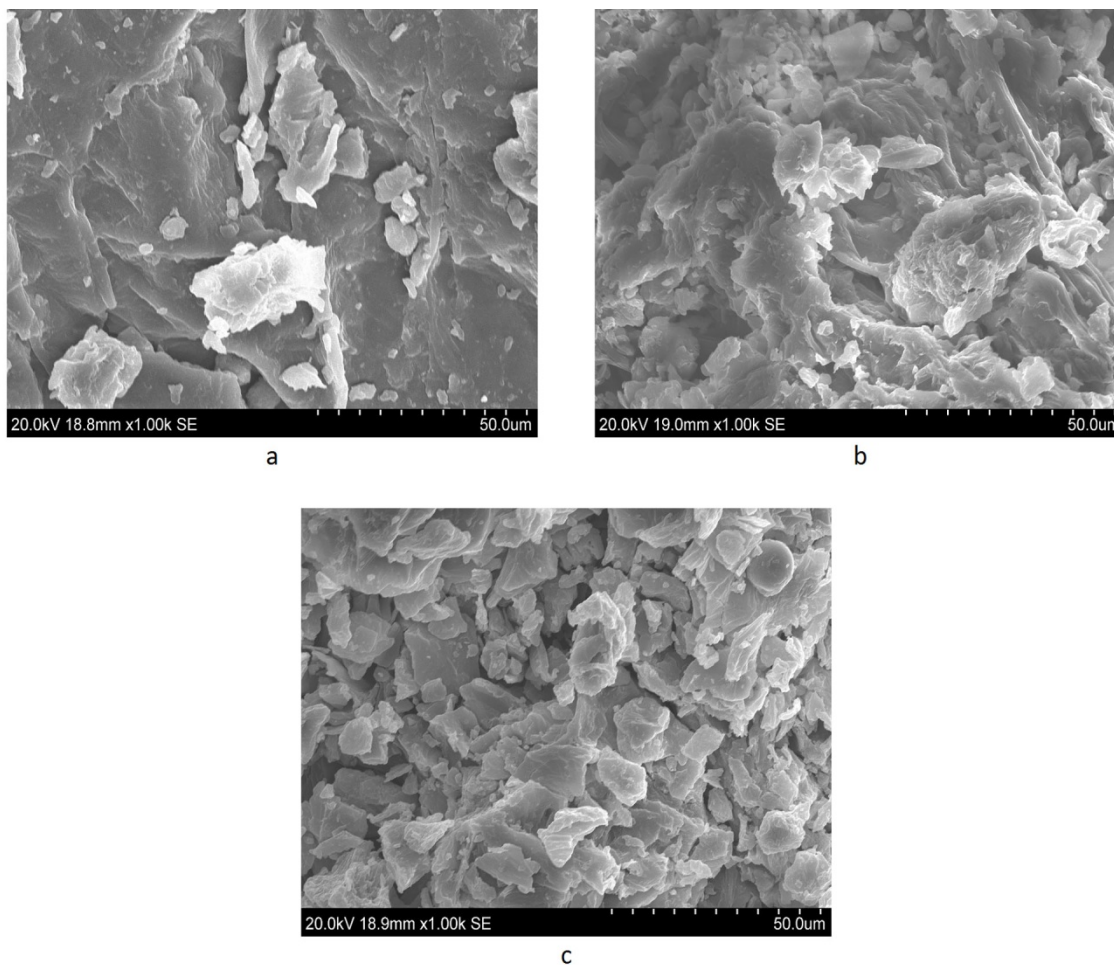


Figure 4. Scanning electron micrographs ($\times 1000$ magnification) of fresh (a), O_F-O_U (b), and $M_W_F-M_W_U$ (c) AP powder (12 h fermentation, ultrasonication at $50 \mu\text{m}$ amplitude for 3 min)

3.4 Relationship Between TPC and AA

The correlation coefficients between TPC and AA (DPPH assay), after fermentation and ultrasonic treatment of sorghum flour, are shown in Figure 5. Correlation experiments to predict the antioxidant properties have been performed by many authors. AP powder with higher levels of TPC had a greater antioxidant capacity (Sato et al., 2010; Savatović, Đilas, Tumbas, Čanadanović-Brunet, & Četković, 2005). The values of AA indicated a positive correlation with the values of TPC of AP powder at different drying method, and ultrasonication amplitude (UA). A linear correlation in each case was observed between AA and TPC. The correlation coefficient (r) were 0.9474, and 0.9960 at different drying method and UA, respectively, which indicated that TPC was the major factor accounting for the antioxidant activity of the sorghum flour.

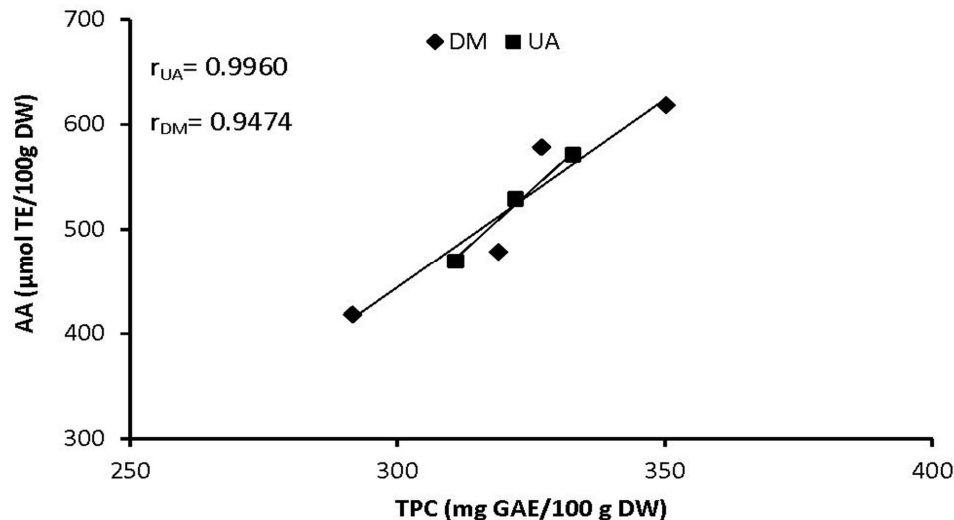


Figure 5. Correlation between TPC and AA of AP powder for different drying methods (DM) and ultrasonication amplitude (UA)

4. Conclusions

Drying method of AP after fermentation and ultrasonication played a significant role in enhancing the TPC, and AA in AP powder followed by UA. UT did not have any significant effect on TPC, and AA. Higher UA with MW drying during fermentation and ultrasonication process gave the higher values of TPC, and AA. From the microstructural analysis, MW drying caused more cell collapse and cell disruption resulting in release of phenolics from the bound structure. MW drying is also favorable for quick and efficient drying as compare to oven drying. AA of AP powder showed the linear correlation with TPC. More investigation is required to observe the effect of further increase in UA and UT on TPC and AA of AP powder.

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