

Research Notes: Harnessing the nutritional benefits of pineapple pulp fiber to enhance antioxidant potential of wheat flour

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Abstract

Pineapple pulp fiber is highly palatable but is usually wasted during the processing of pineapple juice resulting in a loss in the pineapple value chain. It is known to contain both soluble and insoluble dietary fiber, antioxidants, vitamins, bromelain, and many important minerals. Changing lifestyles associated with most western diets characterized by excess intake of calories has led to increased incidence of type 2 diabetes mellitus in Africa. Moreover, hyperglycemia in diabetes mellitus is linked to increased generation of reactive oxygen species (ROS) leading to oxidative stress. ROS are known to damage cellular macromolecules including oxidation of lipids and nucleic acids. The need to formulate dietary formulas to achieve both reductions of calories with enhanced antioxidant potential inspired us to design a wheat flour-based formula enriched with pineapple pulp fiber of up to 10% of its weight. Pineapple pulp fiber-enriched wheat flour positively impacts the pineapple value chain. This study aimed at determining the antioxidant potential of the pineapple pulp fibre enriched wheat flour. Pineapple pulp fiber-enriched wheat flour was prepared by mixing 10 g of dry and milled pineapple pulp with 90g of all-purpose wheat flour. The total polyphenolic content, total flavonoid content, and ferric reducing antioxidant power of the enriched and non-enriched wheat flour were determined using spectrophotometric method. The results were statistically analysed using t-tests at a $p < 0.05$ level of significance. Enrichment of wheat flour with pineapple pulp fiber significantly increased the total polyphenolic content ($p = 0.001$), total flavonoids content ($p = 0.002$), and Ferric Reducing Antioxidant Power (FRAP) ($p = 0.001$) of the flour. These results indicate that pineapple pulp fiber has a potential to enhancing the nutritional quality of wheat flour through added antioxidant properties.

Background

Pineapple (*Ananas comosus*) originated from South America. It belongs to the *Bromeliaceae* family, and genus *ananas*. It is the third most important tropical fruit in the world after banana and citrus (Malézieux et al., 2003). Uganda is a leading pineapple producer in East Africa, due to the favourable climatic conditions (Nyamwaro et al., 2018). The major areas of pineapple production in Uganda include the central, and south-western regions (Bua et al., 2013). In Uganda, farmers grow pineapple as a major source of cash income by converting the pineapple into juice and wine thereby accumulating a lot of waste as pineapple pulp fibre (Bua et al., 2013). Processing pineapple into juice is the fastest growing value addition strategy of pineapple use in Uganda however large amounts of pineapple pulp is discarded, with all the nutritious dietary fiber (Sebuuwufu et al., 2019).

The major sources of waste in pineapple processing include the peel (30–42%), cores (9–10%), stems (2–5%), crowns (2–4%) (Ketnawa et al., 2012). The pineapple waste accounts for half of its weight (Ketnawa et al., 2012). The waste is both of liquid and solid form containing fermentable sugars alongside other nutrients. The liquid waste contains 7.3 and 0.22% sugar and citric acid respectively while the solid waste contains 8.2% glucose, 12.22% fructose in addition to fiber (Abdullah and Mat, 2008).

Change in food habits of mainly the urban population, particularly the increasing consumption of calorie foods coupled with a decrease in the consumption of fiber-rich vegetables and fruits, is causing increasing health problems associated with obesity, hypertension, coronary disease, and diabetes (Pena, 2007). Refined wheat flour is one of such high-calorie foods whose consumption has greatly increased in form of home-baked confectioneries and fast food (Auma *et al.*, 2019, Swaminathan *et al.*, 2021). There is an urgent need for innovations in wheat-based product development without losing essential nutrients. In our previous study (Sebuuwufu *et al.*, 2019) we showed that during the processing of pineapple into juice, essential dietary fiber derived from the pulp is usually discarded. The presence of dietary fiber and polyphenolic compounds in pineapple makes it a good candidate for the enrichment of food to contain fiber and antioxidant properties. Ackom and Tano-Deborah (2012) reported that pineapple pulp fiber can be used to supplement calorie-high flour (Ackom and Tano-Debrah, 2012). In addition, our previous study confirms the suitability of pineapple pulp fibre enrichment in wheat flour for human consumption based on its microbial sensitivity rheological and sensorial properties (Sebuuwufu *et al.*, 2019). Therefore, the aim of this study was to determine antioxidant potential of the pineapple pulp fibre enriched wheat flour.

Methods

Collection of pineapples

Pineapple samples were obtained from farmers in southwestern Uganda as previously reported (Sebuuwufu, 2017). They were transported to the Biochemistry Laboratory at Mbarara University of Science and Technology in dry and opaque polythene bags to avoid light-mediated degradation of polyphenols during transit.

Preparation of the pineapple pulp powder

The pulp powder was processed according to the method described by Ackom and Tano-Debrah (2012) with modifications. In brief the pineapple fruit was cleaned, peeled and sliced, and blended. The juice was squeezed out of the fiber and filtered using a clean mucilage cloth. To modify the pH of the pulp fibre and reduce its sugar content to allow it to dry without sticking together, samples of the pulp fibre were then soaked in varying concentrations of food-grade calcium hydroxide for 15 minutes, followed by 15 minutes soaking in water. The concentration of the solution was varied from 0.01 M - 0.1 M to obtain neutral pH. The pulp samples were then air-dried at room temperature and thereafter milled into fine dry powder. The pulp powder was finally sieved (30 microns sieve) to produce a powder with uniform particle size.

Preparation of pineapple fiber-enriched wheat flour

Pineapple pulp fiber-enriched wheat flour was prepared by mixing pineapple pulp fiber and all-purpose fine white wheat flour (Mandella Millers Uganda) in a ratio of 1:9 (10%) determined from our previous study (Sebuuwufu *et al.*, 2019). The ratio of 10% for fortification was arrived at following finding of the

sensorial analysis, rheological properties and antimicrobial sensitivity data from our previous study (Sebuwuufu et al (2019)). The mixture was then homogenized using a blender.

Extraction of crude phenolic compounds

Total phenolic compounds in pineapple pulp fibre enriched wheat flour were extracted according to the method of Moore *et al.* (2009). Briefly, 5 g of pineapple pulp fibre enriched wheat flour was blended with 20 mL of 80 % chilled laboratory-grade ethanol for 10 min. After centrifugation at 2500 *g* for 10 min, the supernatant was removed and extraction was repeated once. Supernatants were pooled, evaporated at 45 °C to <5 mL, and reconstituted in 10 mL of water. The extracts were stored at -40 °C until use.

Determination of total polyphenol content

The total polyphenolic content of the pineapple pulp fibre enriched wheat flour samples were determined according to Yu et al (2002) (Yu *et al.*, 2002). Briefly, 50 µl of pineapple pulp fibre enriched wheat flour extract was added to 250 µl of the Folin–Ciocalteu reagent, 750 µl of 20% sodium carbonate, and 3ml double-distilled water. The mixture was incubated in the dark at room temperature for 2 hours, and the absorbance was read at 765 nm using a Pico 200 microliter UV/Vis spectrophotometer (Pico drop Limited, UK). Reactions were conducted in triplicate, and results are expressed as milligrams of gallic acid equivalents per gram (mgGAE/g) of pineapple pulp fibre enriched wheat flour. The experiment was repeated after a week and mean values of results computed for analysis.

Determination of total flavonoids content

Flavonoid contents of wheat samples were determined using the aluminum chloride colorimetric method as described by Chang *et al.* (2002). Briefly 0.5 mL of pineapple pulp fibre enriched wheat flour extract was mixed with 1.5 mL of 95 % ethanol, followed by 0.1 mL of 10 % aluminum chloride, 0.1 mL of 1 M sodium nitrate, 0.1M sodium hydroxide, and 2.8 mL of double-distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 510 nm using a Pico 200 microliter UV/Vis spectrophotometer. The flavonoid content was calculated using a standard calibration of quercetin solution and expressed as micrograms of quercetin equivalent (QE) per gram of sample. The experiment was repeated after a week and mean values of results computed for analysis.

Extraction of antioxidants from pineapple pulp fibre enriched wheat flour

The extraction of antioxidants was conducted according to Moore *et al.* (2006). In short, 0.5 g of pineapple pulp fibre enriched wheat flour was extracted with 5 ml of 50 % acetone in a screw-capped tube in the dark at ambient temperature overnight. The tube was centrifuged at 2000 *g* for 5 minutes and the supernatant obtained.

Ferric reducing antioxidant power (FRAP) assay

The ferric reducing antioxidant power of the sample was determined spectrophotometrically following the method of Benzie and Strain (1996) (Benzie and Strain, 1996). The method is based on the reduction of

Fe³⁺+2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ) complex (colorless complex) to Fe²⁺-tripyridyl triazine (blue colored complex) formed by the action of electron-donating antioxidants at low pH. The Ferric reducing antioxidant power (FRAP) reagent was prepared by mixing 300.00 mM acetate buffer, 10.0 ml TPTZ in 40.0 mM HCl, and 20.0 mM FeCl₃.6H₂O in the proportion of 10:1:1 at 37°C. Exactly 3.995 ml of freshly prepared working FRAP reagent was pipetted using 1-5 ml variable micropipette and thoroughly mixed with 5.0 µl of the extract.

An intense blue color complex was formed when ferric tripyridyltriazine (Fe³⁺+TPTZ) complex was reduced to ferrous (Fe²⁺) form and the absorbance at 593 nm was recorded using Bio-wave 2 pico drop spectrophotometer against a reagent blank (3.995 ml FRAP reagent +5 µl double distilled water) after 30 min incubation at 37°. All the determinations were performed in triplicates and the average absorbance was recorded. A calibration curve was made by plotting the absorbance at 593 nm versus different concentrations of FeSO₄. The concentrations of FeSO₄ were, in turn, plotted against the concentration of standard antioxidant Trolox.

The FRAP values were obtained by comparing the absorbance change in the test mixture with those obtained from increasing concentrations of Fe³⁺ and expressed as mg of Trolox equivalent per gram of sample. The experiment was repeated after a week and mean values of results computed for analysis.

Data statistical analysis

The raw data obtained were entered in a Microsoft Excel datasheet and analyzed using Statistical Package for Social Sciences (SPSS) version 20.0. Data were tested for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests. Since the data were normally distributed, variations in antioxidant properties of pineapple pulp-fiber enriched and non-enriched wheat flour were compared using independent samples t-test.

Results

Total polyphenolic content of pineapple pulp fiber enriched wheat flour

On comparing the total polyphenolic content (TPC) of pineapple pulp fiber-enriched wheat flour (PPFWF) and all-purpose wheat flour (AWF) we found that the total polyphenolic content of pineapple pulp fiber-enriched wheat flour (0.033 ± 0.002 mgGAE/g) was significantly (p<0.001) higher than that of the all-purpose wheat flour (0.004±0.001 mgGAE/g) as shown in Figure 1.

Total flavonoid content of pineapple pulp fiber enriched wheat flour

A significantly higher level of total flavonoids content was recorded in PPFWF (0.0489±0.0020mgQE/g) when compared to AWF (0.0255±0.0002 mgQE/g) as shown in Figure 2.

Ferric reducing antioxidant power (FRAP) of pineapple pulp fiber enriched wheat flour

The mean FRAP of PPFWF ($0.0385 \pm 0.0034 \text{ mg FES O}_4\text{E/g}$) was significantly ($p < 0.001$) higher than that of AWF ($0.0017 \pm 0.001 \text{ mg FES O}_4\text{E/g}$) as shown in Figure 3.

Discussion

Pineapple pulp fiber is highly palatable and contains numerous amounts of essential nutrients and dietary antioxidants, yet it is usually wasted during the processing of pineapple juice. This results in a loss in the pineapple value chain. There are many value-added products of pineapple waste utilizing both solid and liquid waste. Waste fibre has been used as a carbon source in fermentation to produce lactic acid used in food processing (Abdullah and Mat, 2008). It is a useful source of bromelain, proteolytic enzymes that have found use as a meat tenderizer, digestive aid, cleansing agent, antibiotic potentiating agent (Rowan et al., 1990, Pavan et al., 2012, Upadhyay *et al.*, 2010). It is also a source of cellulose nanocrystals which can be used to reinforce nanocomposites, prebiotics, a source of alginolytic enzymes including laccase and manganese peroxidase, and various organic acids (dos Santos Bazanella et al., 2013, Dorta and Sogi, 2017). On the other hand, with increased urbanization, there is increased adoption of western food habits that include high consumption of refined wheat flour, a high-calorie food. Wheat consumption has been associated with an increased incidence of the redundant sigmoid colon in Uganda, most especially among male young adults (Tumusiime, 2007). Diet is one of the major contributors to the development of type 2 diabetes. In a study done by Lang et al. (2020) found that an increase of 100kcal/day per capita sugar consumption increases the prevalence of diabetes mellitus by 1.62%-fold (Banerjee and Vats, 2014). Dietary fiber intake lowers the risk of developing coronary heart disease such as stroke, hypertension, diabetes, obesity, and gastrointestinal disorders (Howarth et al., 2001, Ruhee and Suzuki, 2018, Narayan et al., 2014). This is posing a risk in the development of type II diabetes mellitus, among other high-calorie diet related challenges. In this study, the antioxidant potential of the pineapple pulp fibre enriched wheat flour was evaluated. There was an observed increase in the antioxidant potential of the wheat flour on enrichment with pineapple pulp fiber

An increase in total polyphenolic content, total flavonoid content, and ferric reducing antioxidant power of wheat flour due to its fortification with pineapple pulp fiber indicates that the pineapple pulp fiber is suited for this purpose. This increases the potential of this formulated feed to reduce the risk factors of type II diabetes mellitus as has been found with hypoglycemic, antioxidant, and lipid-lowering properties of dietary fiber (Post et al., 2012), and polyphenolic compounds (Lin et al., 2016). Our present results agrees with the study of Saikia and Mahanta (2016) who reported that processed pineapple fiber had a high total flavonoid and total polyphenolic content and high ferric reducing antioxidant power. The pineapple is an excellent source of edible dietary fiber with many other vital macro and micronutrients. Another important component of pineapple pulp fiber is dietary antioxidants (Martínez et al., 2012, Saura-Calixto, 2011), which are very vital in combatting reactive oxygen species (ROS), that are implicated in oxidative stress, one of the major contributors to the complications of diabetes mellitus.

The increase in antioxidant properties (FRAP) is attributed to increasing in both polyphenolic and flavonoid contents of wheat flour since both increased on enrichment with pineapple pulp fiber. The high

FRAP may be due to a high content of Vitamin A, Vitamin C, Vitamin E, and Vitamin B as well as metal cations including copper, magnesium, and iron which are known antioxidants in the pineapple (da Silva et al., 2013, Hossain et al., 2015). The pineapple peel contains ferulic acid, a precursor of vanillic acid used in the production of vanillin, the main component of vanilla flavor (Tang and Hassan, 2020). Steingass et al. (2015) also reported an abundance of more than 60 different types of polyphenolic compounds in the pineapple. A mixture of all these antioxidants in the pineapple could be responsible for increased total polyphenolic, total flavonoid, and ferric antioxidant capacity of wheat flour enriched with pineapple pulp fiber.

Conclusion

This study further confirms our previous findings on suitability of using pineapple pulp fibre in enriching wheat flour with significant increase in antioxidant properties such as total polyphenol content, total flavonoid content, and ferric reducing antioxidant power in pineapple pulp fibre enriched wheat flour. These results indicate that pineapple pulp fiber has a potential to enhancing the nutritional quality of wheat flour through added antioxidant properties.

Recommendations

We recommend further studies to determine the *in vivo* effect of pineapple pulp fiber enriched wheat flour on hyperlipidemia, oxidative stress and glycemic control in context of diabetes mellitus using animal model.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

JO DT GKR and PMA conceived and designed experiments and wrote the manuscript, JO, NK, and K M, did laboratory work. All authors carried out data analysis

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Figures

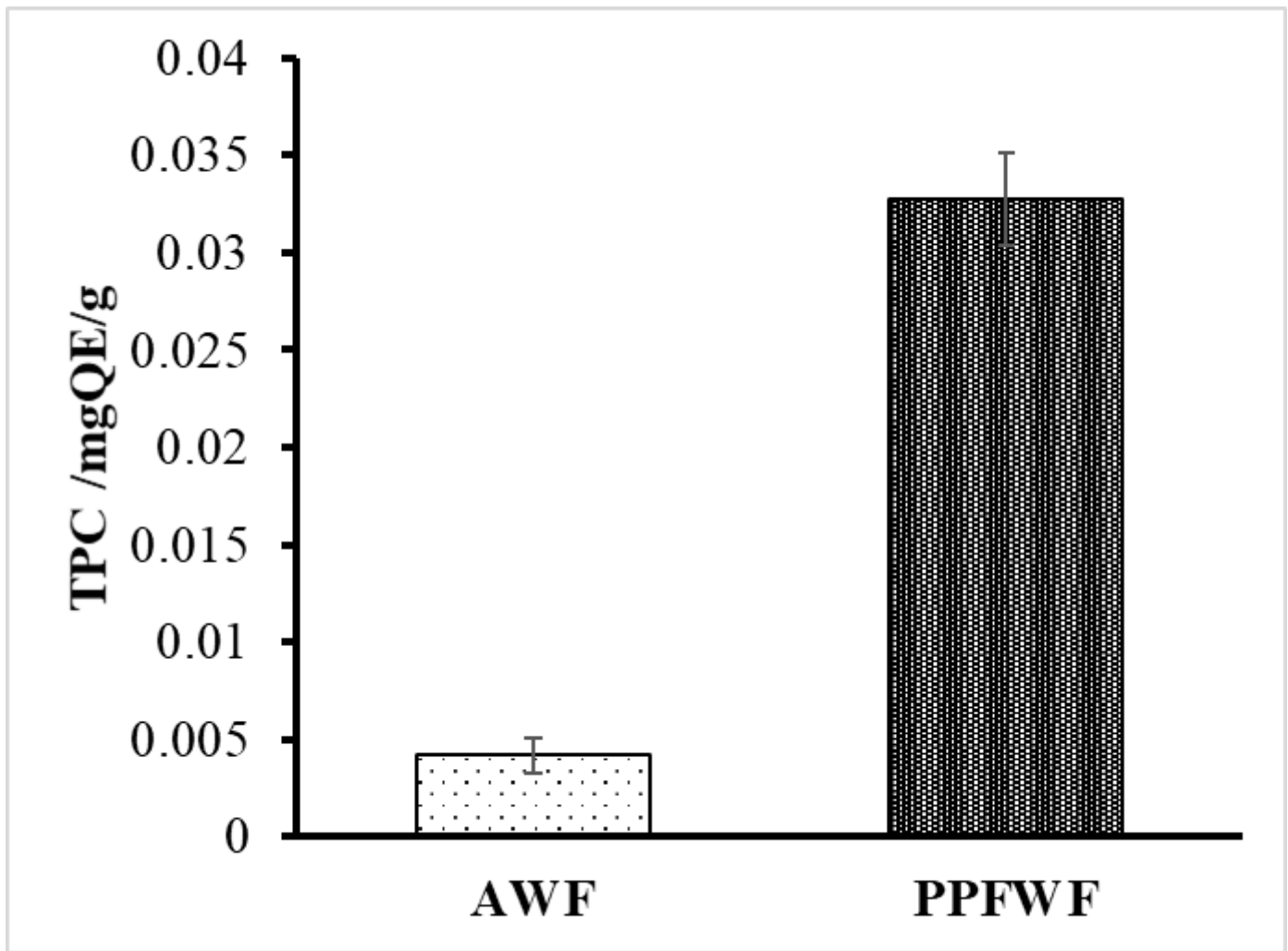


Figure 1

Effect of 10% pineapple pulp fiber enrichment of wheat flour on its total polyphenolic content. Data are shown as mean \pm S.D (n=3).

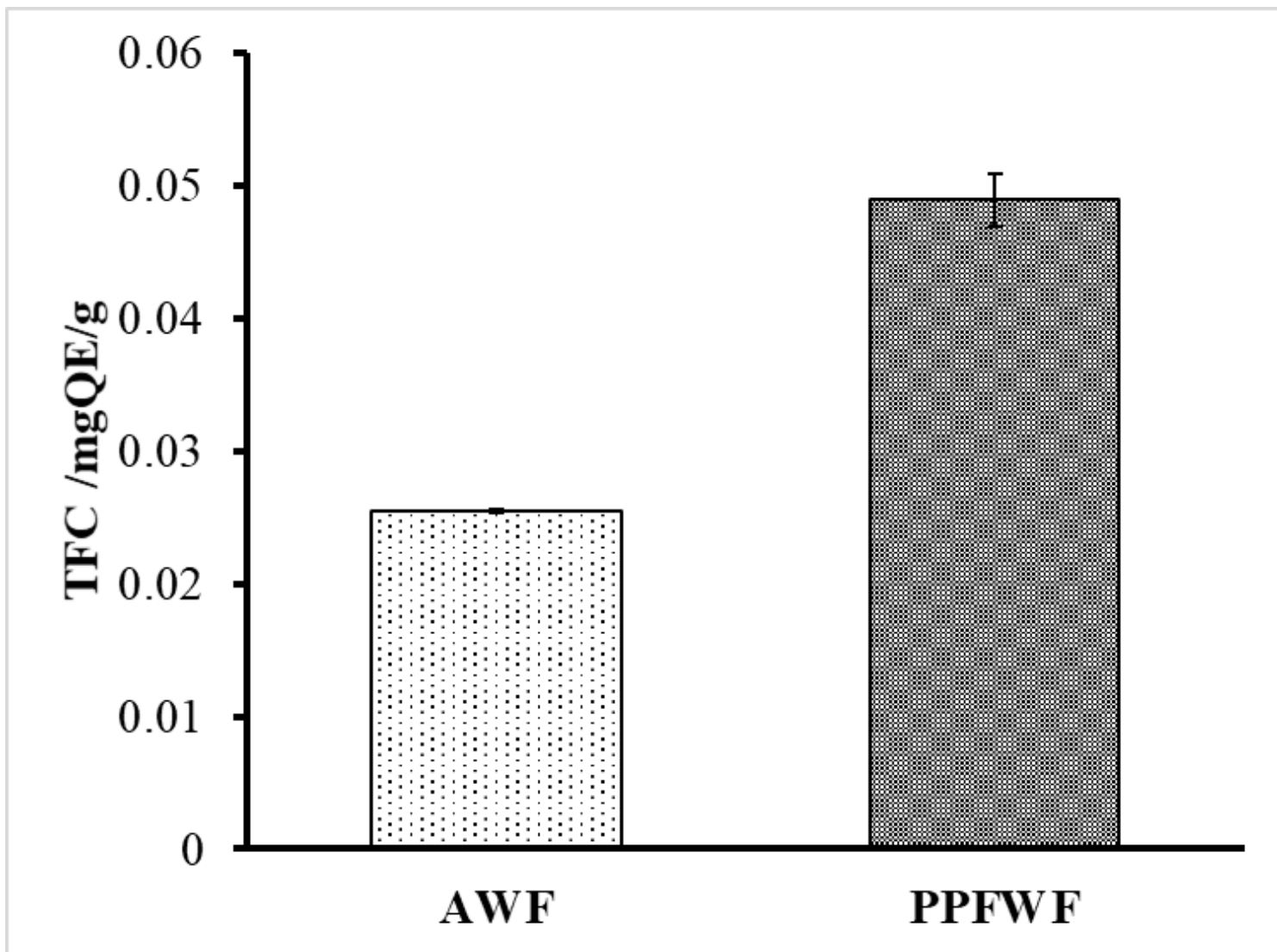


Figure 2

Effect of 10% pineapple pulp fiber enrichment of wheat flour on its total flavonoids content. Data are shown as mean \pm S.D (n=3).

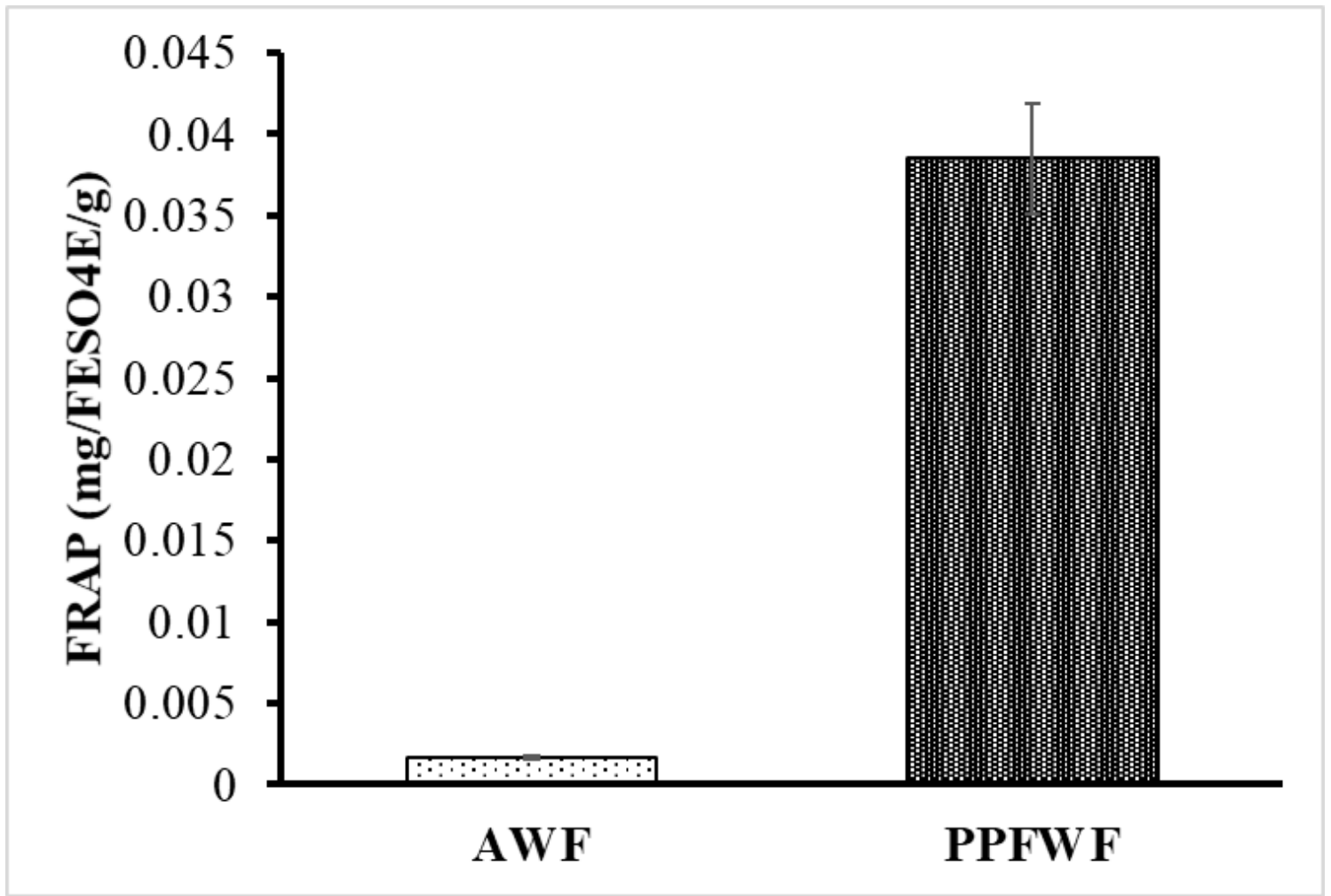


Figure 3

Effect of 10% pineapple pulp fiber enrichment of wheat flour on ferric reducing antioxidant power. Data are shown as mean \pm S.D (n=3).

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