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Traditional processing techniques impacted the bioactivities of selected local consumed foods

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ABSTRACT

Epidemiological and clinical studies have evidenced the crucial role of vegetables and mushrooms for our health. Vegetables and mushrooms are usually cooked before consumption which induces many physicochemical changes in foods. A change in the phytochemical content might alter the health benefits of the food. In this regard, the impact of domestic cooking particularly boiling, steaming and stir-frying at different time intervals (under-cooked, well-done and overcooking) on the phenolic content and antioxidant potential of locally grown *Allium cepa* L., *Brassica chinensis* L., *Pleurotus sajor-caju* and *Moringa oleifera Lam* leaves and pods were investigated. The anti-proliferative effects of the fresh and cooked vegetables were assessed on the hepatocellular carcinoma (HepG2) cell line. The results demonstrated a general increase in the phenolic content of *A. cepa, P. sajor-caju, M. oleifera* leaves and pods, but a decrease in *B. chinensis* after cooking. Both the raw and the cooked vegetables and mushroom demonstrated notable free radical scavenging abilities, potent ferric reducing antioxidant power and strong iron chelating activity across the different processing methods. Cooking significantly increased reducing potential of *M. oleifera* leaves and *P. sajor-caju*, while a significant decline was observed for *B. chinensis* and *M. oleifera* pods after thermal treatments. Boiling and stir-frying caused significant loss in the chelating potential of *B. chinensis* ($p<0.05$). In addition, cooking enhanced the anti-proliferative properties of *B. chinensis, P. sajor-caju* and *M. oleifera* leaves against liver cancer cells. The present data provide a new insight into the influence of conventional cooking methods on locally cultivated foods, highlighting the importance of choosing the appropriate processing technique to maximize bioaccessibility of the beneficial bioactive compounds.

Abbreviations: ANOVA, analysis of variance; DMEM, Dulbecco's Modified Eagle's Medium; FBS, fetal bovine serum; FDW, freeze dry weight; FRAP, ferric-ion reducing antioxidant power; GAE, gallic acid equivalent; MTT, dimethylthiazol diphenyltetrazolium; QE, quercetin equivalent; TFC, total flavonoid content; TPC, total polyphenol content.

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Introduction

Epidemiological data and clinical studies have highlighted the strong association between plant-based foods and reduced risk of chronic including cancers [1,2]. Indeed, besides being excellent sources of nutrients, plant-based foods contain non-nutritive bioactive constituents, which have been reported for their array of prophylactic and therapeutic properties [3,4]. Among these non-nutrients, polyphenol is the major and most studied group. The protective role of polyphenols has mainly been attributed to their antioxidant and anti-inflammatory properties, involved in the prevention of various chronic disorders, including cancer [5]. Carcinogenesis is a multistage event, resulting from culminating genetic alterations. This chronic disease is characterised by a number of hallmarks, which provides unique targets and diverse possibilities for preventive and therapeutic strategies [6]. Given that 30 to 50% of cancers are preventable through lifestyle modifications [7], the physiological function of foods beyond nutrition, is a promising approach to cancer prevention.

Whilst some vegetables can be eaten raw, most of them need to be cooked. The latter results in chemical and physical alterations of the food which increases the palatability, digestibility, bioaccessibility and bioavailability [8,66]. Thermal processing also inactivates microorganisms and anti-nutritional components as well as enhances flavor and shelf-life [9]. Nevertheless, cooking may also cause the loss of certain thermolabile nutrients, allow the development of harmful and less bioactive compounds, such as some Maillard reaction products, trans fatty acids, heterocyclic aromatic amines, and diterpenoids [10,8,11]. In addition, culinary processes have been reported to induce substantial changes in the phenolic content of vegetables. Thermal energy may help soften the cellular matrix of vegetables and facilitate the extractability and release of bound phenolic compounds. At the same time, some phenolic structures may as well be thermolabile and be degraded or lost during cooking [8,66]. Studies also report the interaction between phenolic compounds and other food nutrients, such as proteins, lipids and fibres [12]. With high temperatures, these compounds take part in the Maillard reaction, forming melanoidins, hence decreasing the phenolic content of the food. As a result, the bioavailability of polyphenols and its associated health benefits are strongly influenced by cooking techniques.

The Mauritius economy has made great strides over the past three decades, and the country has witnessed drastic socioeconomic as well as environmental changes [63]. This has brought along alterations in the population's lifestyle, which has been accompanied by a relatively high incidence of non-communicable diseases, including cancer. In consequence, research towards local plant-based food as therapeutic solutions against these diseases triggered great concern. A wide variety of Mauritian grown foods have been documented for their phytochemical richness and attractive pharmacological activities, among them *Allium cepa* L, *Brassica chinensis* L, *Moringa oleifera* Lam and *Pleurotus sajor-caju* [13–15]. These locally cultivated plants and mushroom have prompted great interest for their strong antioxidant properties and high level of phenolic compounds [13–15]. Moreover, mushrooms grown locally *Pleurotus sajor-caju,* have shown great promise in attenuating hepatocarcinogenesis, in addition to effectively reducing oxidative damage and inducing antioxidant enzymes *in vivo* [15]. Nevertheless, these foods have been tested in their raw state, while most of them are normally cooked before consumption. Applying thermal energy to these functional foods may bring along physicochemical alterations, which in turn can modify their biological attributes. In view of the limited data available on the positive health effects of these foods after cooking, this study aims at evaluating the effect of traditional cooking methods on the antioxidant and antiproliferative potentials of selected Mauritian functional foods.

Materials and methods

Chemicals and reagents

All chemicals and solvents used were of analytical grade. Folin-Ciocalteau reagent, sodium carbonate, ferric chloride and ferrous sulfate were bought from Loba Chemie Pvt, Ltd (India). Quercetin, gallic acid, EDTA, aluminum chloride hexahydrate, 2, 4, 6-tripyridyl-s-triazine, ferrous chloride tetrahydrate, DPPH, ferrozine and dimethylthiazol diphenyltetrazolium (MTT), were purchased from Sigma-Aldrich (Germany). Fetal bovine serum (FBS), L-glutamine, streptomycin penicillin and Dulbecco's Modified Eagle's Medium (DMEM) were purchased from Gibco.

Sample collection

Brassica chinensis L. and *Allium cepa* L. were purchased from commercial gardens at Vacoas, central region of Mauritius while *Moringa oleifera* Lam. leaves and pods were collected from a domestic backyard at Flacq, eastern part of the island of Mauritius. All plants were authenticated by the herbarium of Mauritius Sugar Industry Research Institute, Réduit. Locally cultivated edible mushroom, *Pleurotus sajor-caju* strain CC 201, was purchased from the Mushroom Unit of the Food and Agricultural Research and Extension Institute. The samples were cleaned and subjected to different processing techniques.

Samples preparation

All the samples were washed with tap water, dried on a paper towel and only the edible parts were kept. Each vegetable and mushroom were cut into equal pieces to achieve the same texture at the same cooking time. *A. cepa, B. chinensis*, and *P. sajor- caju* were cut in thin slices in parallel cutting. The immature *M. oleifera* pods were cut in sticks of 4 cm. Each

Table 1
Local vegetables and mushrooms under study and the different cooking methods employed.

Family	Food used Latin name	Vernacular name	Part of plant	Cooking technique	Cooking time (min) Under-cooked	Well-done	Over-cooked
Brassicaceae	*Brassica chinensis* L.	Chinese Cabbage	Leaves and stem	Boiling	4	8	12
				Steaming	5	10	15
				Stir-frying	3	4	5
Liliaceae	*Allium cepa* L.	Onion	Bulb	Boiling	5	15	30
				Stir-frying	5	15	30
Moringaceae	*Moringa oleifera* L.	Drumstick	Leaves	Boiling	4	8	12
				Steaming	5	10	15
				Stir-frying	3	6	9
			Immature Pods	Boiling	10	20	30
				Steaming	10	20	30
Polyporaceae	*Pleurotus sajor-caju* (CC201)	Oyster Mushroom	Fruiting body	Boiling	5	10	15
				Steaming	5	10	15
				Stir-frying	4	8	12

food sample was then divided into equal portions (500 g for *A. cepa, B. chinensis, M. oleifera* leaves and *P. sajor- caju*, and 200 g for *M. oleifera* pods) and subjected to three common cooking methods based on the Mauritian culinary practices, namely boiling, steaming and stir-frying. All cooking techniques were conducted at three different time intervals to mimic under-cooked, well-done and over-cooked conditions (Table 1). In addition, a raw/ uncooked portion was also investigated.

Boiling

Each food sample was added to boiling distilled water (1:1 w/v), and allowed to boil for different time periods (Table 1). After the boiling time, both the cooking water and the sample were rapidly cooled on ice to prevent further cooking from residual heat.

Steaming

A domestic food steamer (HS6000, Black & Decker, U.S.A.) was used for this process. The food samples were placed on perforated trays and allowed to cook for different time periods (Table 1). The food sample was rapidly cooled on ice.

Stir-frying

In a non-stick pan (Tefal, France), each food sample was stir-fried, without oil, at moderate heat for a different time period (Table 1). After cooking, the food sample was rapidly cooled on ice.

Extraction procedure

Each food sample was pulverized using a food processor (Blixer2, Robot Coupe, France) before being subjected to an ultrasound assisted extraction. Two extracting solvents, namely distilled water and hydro- methanol (70%, methanol v/v) were used. The different samples under study were homogenized with each extracting solvent (1:1 w/v), using a sonicator probe system (Q700, QSonica, U.S.A) at amplitude 40% and frequency 20 kHz, for a time period of 30 min, at a temperature not exceeding 40 °C. The filtrate was then centrifuged at 5000 rpm for 6 min. The residue was exhaustively extracted using the same procedure for seven times. All the supernatants were pooled together and filtered under vacuum. Methanol was evaporated under vaccum at 38 °C using a Flash Evaporator (LABORATA 4003, Heidolph, Germany) prior to lyophilisation.

Estimation of phenolic content

Total phenolic content (TPC)

The Folin-Ciocalteu assay adapted from Ramlagan et al. [16]. Briefly, for the TPC, 0.125 mL of extract was added to 1.75 mL of distilled water, followed by 0.125 mL Folin- Ciocalteu solution. After 3 min of incubation at room temperature, 0.5 mL of 20% sodium carbonate was added. The solution was then placed at 40 °C for 40 min. Absorption of the blue color formed was read at 685 nm (Biotek Synergy HT, USA). The experiment was performed in triplicates in three independent assays. The TPC of the samples was expressed as mean (mg Gallic acid equivalent (GAE)/ g freeze dry weight (FDW)) ± standard error of the mean (SEM).

Total flavonoid content (TFC)

The aluminum chloride assay from Lamaison and Carnet [17] were used to estimate the Total Flavonoid Content (TFC). In a 96-well plate 100 μL of methanolic sample was added to 100 μL of 2% methanolic aluminum chloride hexahydrate ($AlCl_3 \cdot 6H_2O$). The reaction time was 10 min and the absorbance of the yellow coloration was read at 440 nm.The TFC was expressed as mean (μg Quercetin equivalent (QE)/g FDW) ± SEM

Antioxidant activities

Ferric-ion reducing antioxidant power (FRAP)

The ferric-ion reducing antioxidant power (FRAP) of the samples under study was assessed according to Ramlagan et al. [16]. The FRAP reagent was freshly prepared from mixing 100 mL of 0.25 M acetate buffer (pH 3.6) to 10 mL of 20 mM ferric chloride and 10 mL of 10 mM 2,4,6-tripyridyl-s-triazine. The final reaction mixture contained 25 µL of extract, 75 µL of distilled water and 750 µL of FRAP reagent. Absorbance was read at 593 nm. The experiment was repeated with ferrous sulfate (source of Fe^{2+}) at different concentrations, which allowed the generation of a standard curve. Results were reported in mmol Fe^{2+}/g FDW.

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity

The DPPH free radical scavenging ability of the different samples was evaluated according to an adapted method from Ramlagan et al. [16]. A total of 100 µL of 100 µM methanolic DPPH solution was added to 100 µL of the methanolic sample. After 30 min incubation at room temperature, absorbance was read at 517 nm. Gallic acid was used as positive control.

Eq. (1) was used to calculate the percentage inhibition.

$$\text{Chelating/ Scavenging } (\%) = (A_0\text{-}A_1)/A_0 \times 100 \quad (1)$$

Where A_0 is the absorbance of the negative control; i.e., the reaction mixture only without sample and A_1 is the absorbance of the test sample.

The concentration of extract required to achieve 50% antioxidant effect relative to the negative control (AA_{50}) was calculated from the dose-dependent curve using GraphPad Prism 6.01 software (GraphPad, Inc., San Diego, CA, USA).

Iron chelating

The method from Ramlagan et al. [16] was used to evaluate the iron (II) chelating effect of the samples under investigation. Briefly, the reaction mixture contained 40 μL of extract, 10μL of 0.5 mM FeCl2·4H2O and 50μL of distilled deionized water. After 5 min incubation at 25 °C, 10μL of 2.5 mM ferrozine was added and absorbance reading was taken at 562 nm. EDTA was used as positive control. The metal chelation activity of the samples was calculated as per Eq. (1) and results were expressed as mean AA_{50} concentration ± SEM.

Cell culture

The human hepatocellular carcinoma (HepG2) cell line, purchased from American Type Culture Collection, was cultured in DMEM supplemented with 10% FBS, 2 mM L-glutamine, 100 U/L streptomycin-penicillin and 1 mM sodium pyruvate. The cells were maintained at 37 °C in an atmosphere of 5% CO_2 and 95% humidity.

Cytotoxicity screening

The cytotoxic effect of the samples was investigated on HepG2 cell line using the MTT cell viability assay. Briefly, the cell monolayer was trypsinized and seeded at a density of 8000 cells per well in a 96 well plate. After acclimatizing overnight, the cells were treated with different concentrations of extracts (0.01 to 2 mg/mL) in 1% FBS growth medium, for 24 h and 48 h. Following the treatment incubation period, the media was removed and 200 μL of 0.5 mg/mL MTT solution was added to each well. A total of 100 μL of dimethyl sulfoxide was added to dissolve the formazan after 1 h incubation and absorbance was read at 570 nm (Biotek Synergy HT, USA). The proliferative response of the treated cells was normalised to the corresponding negative controls and the concentration required to inhibit 50% of cell growth relative to control (IC_{50} value) was determined using GraphPad Prism 6.01 software (GraphPad, Inc., San Diego, CA, USA). Etoposide and Paclitaxel were used as positive controls.

Statistical analysis

All assays were conducted in triplicates and expressed as mean ± SEM. The AA_{50} and IC_{50} values were achieved using the 'log (Inhibitor) vs. Response' model in GraphPad Prism, version 6.01 from GraphPad Software (San Diego, CA, USA). Normality of data was tested using the Shapiro-Wilk test and Levene's tests were used to check for homogeneity of variance. An independent *t*-test was used to compare the two extraction methods. The effect of the different cooking methods (boiling, steaming and stir-frying) on the foods under study was statistically compared through one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test. TPC, TFC and cytotoxicity were analysed by two-way ANOVA using cooking methods and extraction methods as factors, followed by Dunnett's multiple comparison test. All confidence limits used were based on 95% ($p<0.05$).

Results and discussion

Effect of cooking on total polyphenol content

In general, cooking enhanced the phenolic content of *A. cepa, P. sajor- caju, M. oleifera* leaves and pods, but decreased the amount of polyphenol in *B. chinensis* (Fig. 1). Steaming *B. chinensis* for 5, 10 and 15 min decreased the TPC by 27.3%, 32.3% and 32.5% respectively as compared to the uncooked vegetable (Fig. 1(B)). In contrast, the phenolic level of *M. oleifera* leaves increased by 22.9%, 21.5% and 14.3% after boiling for 4, 8 and 12 min respectively and increased by 32.6%, 18.2% and 8.5% after 5, 10 and 15 min steaming with respect to the raw leaves (Fig. 1(C)). The TPC of *M. oleifera* pods was enhanced by 88.6% and 58.8% after 20 min boiling and steaming for respectively (Fig. 1(D)). Similarly, studies performed on different vegetables demonstrated that the effect of domestic cooking on TPC varied across the different foods analysed [18,19,9]. Şengül et al. [19] reported the loss in TPC in beets, turnip, black radish and red cabbage after steaming, whilst the polyphenol level increased in red radish, kale, broccoli and white cabbage after the same processing technique. Since the polyphenolic composition of food is highly variable, thermal processing has varying effects on the total level of phenolic compounds of the different vegetables. Maillard and Besset [20] has described the change in polyphenol at high temperatures through three mechanisms. Firstly, the lignin bonds to phenolic acids may be broken, releasing the bound phenolics. Secondly, the lignin itself may be degraded at high temperatures, increasing the phenolic acid content and lastly, high temperatures may degrade polyphenol compounds.

Cooking time had a varied effect on the TPC of some vegetables. An increase in the boiling and stir-frying time for *A. cepa* (Fig. 1(A)) and stir-frying time for the *P. sajor-caju* (Fig. 1(E)) was proportional to a rise in phenolic compounds. The TPC of *A. cepa* aqueous extracts was increased by 13.4% and 41.4% after boiling for 15 min and 30 min, and was increased by 41.6%, 59.2% and 122.7% after stir-frying for 5, 15 and 30 min, respectively. Stir-frying *P. sajor-caju* raised the phenolic level by 2.4%, 44.4% and 205.3% as compared to its raw sample.

This effect may be partly ascribed to the cell wall lysis and the enhanced extraction from the intracellular matrix. In addition, the thermal energy might foster the release of bound polyphenols as well as breakdown of complex phenolic polymers, resulting in the formation of new compounds. Ferracane et al. [21] observed an overall higher concentration of caffeoylquinic acids in artichoke due to the formation of dicaffeoylquinic acid isomers, after boiling, steaming and frying, which resulted in the redistribution of caffeoylquinic acid isomers through intramolecular transesterification at high temperatures. Depending on cooking technique, temperature and processing time, diverse products like Maillard reaction products may be formed from the non-enzymatic browning reaction [11]. Hence, Maillard reaction products may have potentially interfered with the spectrophotometric Folin-Ciocalteu method, leading to overestimation of phenolic compounds. Furthermore, it should be noted that Folin-Ciocalteu reagents interact and interfere with certain non-phenolic substances, such as ascorbic acid and reducing sugars, which may affect the TPC measurement results [22].

Thermal processing has been associated with a loss in phenolic compounds [8]. *B. chinensis* TPC decreased by 54.5%, 48.8% and 26.2% as compared to the uncooked sample after boiling for 4, 8 and 12 min respectively (Fig. 1(B)). Although cooking decreased TPC of *B. chinensis*, prolonging the boiling duration increased the phenolic content. This might suggest the breakdown of polyphenols from conjugate forms, as well as polymerisation and the formation of complex structures of phenolic compounds [8]. In general, studies conducted on the influence of cooking on *Brassicaceae* vegetables showed that boiling decreased the polyphenol level of these vegetables [67,23,24]. Wachtel-Galor and colleagues reported a decrease of more than 60% after boiling *B. chinensis* for 5 and 10 min [24]. Similarly, Managa et al. [18] described a loss in total phenolics in *Brassica rapa* subsp. *chinensis* after 15 min boiling. Chen et al. also reported a significant decrease in phenolic compounds after boiling three different cultivars of *Brassica rapa* subsp. *chinensis* for 10 and 15 min but no difference was noted between the cooking times [72]. The loss in polyphenols was mostly attributed to a decrease in flavonoids. Greater thermal stability from deacylated flavonoid glycosides (deacylated kaempferol-3-O-diglucoside-7-O-glucoside and deacylated quercetin-3-O-diglucoside-7-O-glucoside) was observed as compared to their acylated compounds (kaempferol-3-O-caffeoyldiglucoside-7-O-glucoside and quercetin-3-O-caffeoyldiglucoside-7-O-D-glucoside) [67]. On the other hand, Podsędek et al. [23] observed a reduction of approximately 60% of anthocyanins and 70% of hydroxybenzoic acids after boiling cabbage for 20 min. They also noted the thermal stability of phenolic acids and an increase in hydroxycinnamic acids after steaming.

In contrast, the TPC of *M. oleifera* leaves and pods, which significantly increased upon short steaming and boiling time ($p<0.05$), subsequently decreased as cooking duration was prolonged, which might suggest the degradation of phenolics during higher temperatures. Subramaniam et al. [25] also reported a significant increase of 64.71%, 70.59% and 69.12% in *M. oleifera* leaves TPC after boiling for 5, 10 and 20 min respectively, but no notable differences were reported between the cooking techniques.

It is also worth mentioning that in this study the vegetables were analysed with the cooking water. Hence, soluble phenolics released from the cellular matrix or formed during cooking may leach in the water, resulting in transfer of polyphenol to the water. In the same line, Podsędek et al. [23] reported that phenolic losses in boiled red cabbage were proportional to the amount of water used. A loss of 24 to 32% of phenolics in different varieties of red cabbage, when doubling the amount of water, was observed. The phenomenon was attributed to a loss in hydrophilic phenolics.

Moreover, a significant difference was observed between the aqueous and hydro-methanolic extraction methods, except for *M. oleifera* pods ($p<0.05$). All the different processed foods were extracted, with two different solvents, namely, water and hydro-methanolic solution. The latter were used to optimize the recovery of the phytochemicals during the extraction

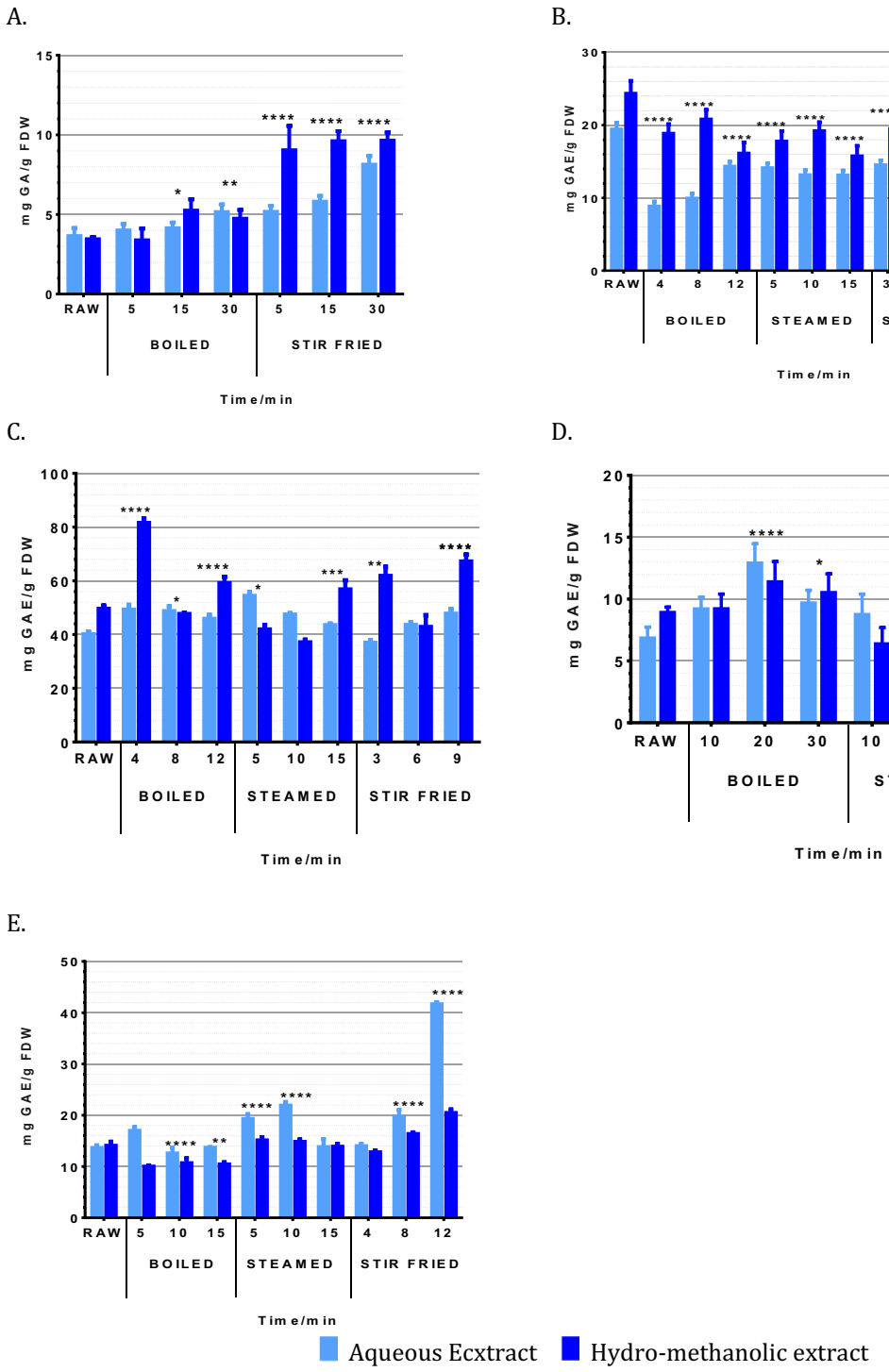

Fig. 1. TPC of aqueous and hydro-methanolic (A) *A. cepa*, (B) *B. chinensis*, (C) *M. oleifera* leaves (D) *M. oleifera* pods and (E) *P. sajor- caju* CC201 raw and cooked extracts. Results expressed as mean ± SEM mg GAE/ g FDW of three independent assays performed in triplicate. Significance was assessed using two-way ANOVA followed by Dunnett's multiple comparison; the cooked extracts were compared to the raw extract, with extraction methods and cooking techniques as factors; $^{*}p< 0.05$, $^{**}p< 0.01$, $^{***}p< 0.001$ and $^{****}p< 0.0001$.

method. Phytochemicals are non-uniformly distributed across vegetables. Hence, the recovery and type of phytochemical will vary across extraction techniques used. Polyphenols have demonstrated better extractability towards organic solvents. The polarity of the extraction solvent, the ratio solvent mixtures, the physical characteristics and affinity of the different compounds present in the food will determine the type and amount of phenolic compounds extracted [26]. For example, Falleh et al. [27] reported a greater polyphenol content in *Mesembryanthemum edule* L. shoots in the methanolic extract as compared to the ethanolic extract, however, the ethanolic extract had a significantly higher antioxidant activity than the latter, suggesting different affinity and type of polyphenol to the two different solvents used. The proportion of phenolic compounds depends on solvent polarity, which normally increases in the following order: methanol> ethanol> ethyl acetate> butanol > water [28]. The more polar a compound, the higher the polar to nonpolar ratio should be, and vice versa [26]. Babbar et al. reported methanol as the most effective polyphenol extraction solvent for vegetables [62]. Another study on 37 raw vegetables observed 70% methanol as the most efficient solvent for the extraction of polyphenolic antioxidants [29]. Therefore, for a comprehensive understanding on the phytochemicals and their biological activities of the selected local foods after cooking, a hydro-methanolic extraction was carried out. On the other hand, from a nutritional perspective, a solvent extraction might not mimic the bioaccessible compounds after household processing, in this view an aqueous extraction was also performed.

Since the phenolic composition varies from plant to plant, it was interesting to consider the effects of processing on the different classes of phenolic compounds. Previous studies have highlighted the high content of flavonoids of the foods under study [13–15]. In this regard, the total flavonoid content was also assessed before and after processing. These phytochemicals have been reported as heat- susceptible compounds [8], and therefore, domestic processing may have a considerable influence on dietary flavonoid content. In the present study, the TFC of *B. chinensis, M. oleifera* pods and *Pleurotus* mushroom decreased, while an increase in TFC was observed in *A. cepa* and *M. oleifera* leaves after cooking (Fig. 2). *B. chinensis* TFC gradually decreased by 30.8%, 53.8% and 62.6% after steaming time was increased from 5 to 15 min as compared to its raw sample. The loss in flavonoids after 4 min boiling (33% with respect to the uncooked sample) was progressively recovered as boiling time was prolonged to 8 and 12 min (26.4 and 14.3% respectively with respect to the raw sample) (Fig. 2(B)). On the other hand, boiling and steaming *M. oleifera* pods for 30 min enhanced TFC by 30.0% and 40.0% (Fig. 2(D)).

In addition, a significant increase in flavonoids was noted after boiling and stir-frying of *A. cepa* and *M. oleifera* leaves for the hydro-methanolic extracts ($p< 0.01$). However, long boiling time (30 min) decreased the flavonoid level of *A. cepa*. Similarly, Ioku et al. [30] observed an increase in quercetin 4′-O-β -glucoside after boiling *A. cepa* for 5 min. The compound significantly decreased after 20 and 40 min boiling. They also observed that this quercetin derivative was transferred to the boiling water without degradation. Moreover, Lombard et al. [31] reported a loss of 18% in flavonoid, mainly 3,4′-O-quercetin diglucoside and 4′-O-quercetin glucoside, after boiling *A. cepa* for 5 min and discarding the cooking water. Likewise, Rodrigues et al. [32] reported a loss in flavonoid after mild boiling (30 min) and the loss was exacerbated as boiling time was prolonged (60 min). They measured the amount of quercetin 3,4′-diglucoside and quercetin 4′-glucoside after boiling *A. cepa*; and observed 37% transfer of quercetin glycosides passed on to the water after 30 min cooking, of which 37% were diglucosides and 39% were monoglucosides. No degradation of quercetin was reported at this particular time point but degradation of quercetin was noted for longer boiling time. In contrast, other studies have described 18 to 75% loss in quercetin in the *A. cepa* tissue after boiling for 3 to 60 min [33].

In general, flavonoids exist in nature as glycosides, aglycones and methylated derivatives [68]. Consequently, the thermostability of flavonoids will greatly depend on the acylation and/ or glycosylation of these compounds [67]. Hence, a decrease in TFC could be the result of degradation of thermolabile and low molecular weight flavonoids, better extractability, breaking of chemical bonds, formation of lower molecular weight compounds and inter-chemical conversion between the different compounds [8]. Literature reports significant changes in flavonoid proportion after cooking, related to deacylation and deglycosylation via hydrolysis, leading to detection of higher levels of non-acylated kaempferol and quercetin glycosides [18,34]. For instance, Rohn et al. observed the stability in quercetin aglycones and degradation in quercetin glycosides, mainly quercetin diglycosides into quercetin monoglycosides, after thermal processing [69]. In the same vein, Wu et al. [34], reported thermal resistance of acylated kaempferol tri- and tetra-glycosides after domestic cooking of broccoli, while a greater degree of loss for non-acylated kaempferol diglucosides was noted. In contrast, Managa et al. [18] observed an increase in non-acylated kaempferol after stir-frying *B. chinensis*, which was attributed to deglycosylation. The latter also reported an increase in aglycone compounds into several simpler compounds after boiling and steaming.

The present data provide useful and suggestive estimates of phenolic and flavonoid content, nevertheless, for a substantive understanding of the consequence of processing on the phytochemical content of local foods, qualitative and quantitative chemical characterization are warranted.

Effect of cooking on the antioxidant potential

Considering the multiple mechanisms of action of antioxidants [35] three independent methods namely FRAP, DPPH radical scavenging and iron chelating assays were performed in this study.

The reducing power of the extracts ranged from 0.154 ± 0.001 to 13.2 ± 0.2 mmol Fe^{2+}/g FDW (Table 2). Overall, cooking significantly increased the FRAP of *A. cepa, M. oleifera* leaves and *P. sajor-caju*, while a significant decline was observed for *B. chinensis* and *M. oleifera* pods after thermal treatments ($p<0.05$). The FRAP of *A. cepa* increased by 45.9%, 116.6% and 149.8% after 5, 15 and 30 min stir-frying respectively. The antioxidant activity of *M. oleifera* leaves was increased by 223.0%,

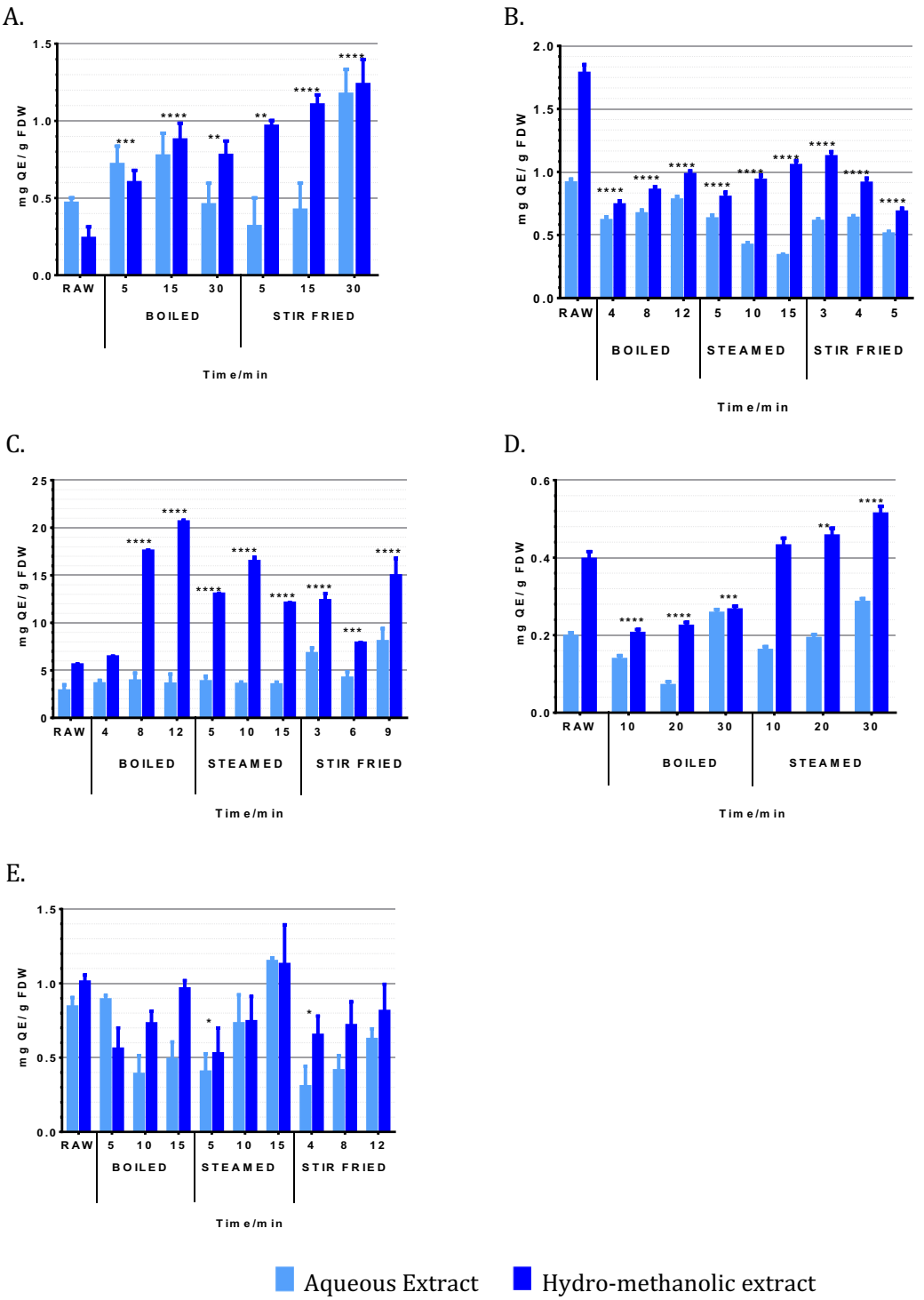

Fig. 2. TFC of aqueous and hydro-methanolic (A) *A. cepa*, (B) *B. chinensis*, (C) *M. oleifera* leaves (D) *M. oleifera* pods and (E) *P. sajor- caju* CC201 raw and cooked extracts. Results expressed as mean ± SEM mg QE/ g FDW of three independent assays performed in triplicate. Significance was assessed using two-way ANOVA followed by Dunnett's multiple comparison; the cooked extracts were compared to the raw extract, with extraction methods and cooking techniques as factors; $^{*}p< 0.05$, $^{**}p< 0.01$, *** for $p< 0.001$ and $^{****}p< 0.0001$.

Table 2
Effect of cooking on the FRAP of selected locally grown foods.

Food	Extraction method	Cooking time	Cooking Techniques					
			Raw	Boiled		Steamed		Stir-Fried	
				FRAP (mmol Fe^{2+}/g FDW)	Δ (%)	FRAP (mmol Fe^{2+}/g FDW)	Δ (%)	FRAP (mmol Fe^{2+}/g FDW)	Δ (%)
Allium cepa (Onion)	Aqueous	Raw	3.1 ± 0.6						
		Under-cooked		3.0 ± 0.7	−3.6	N.A.		4.5 ± 1.3	+45.9
		Well-done		2.9 ± 0.9	−4.6	N.A.		6.7 ± 1.6**	+116.6
		Over-cooked		8.1 ± 0.8***	+163.2	N.A.		7.7 ± 1.6***	+149.8
	Hydro-methanolic	Raw	5.0 ± 0.9						
		Under-cooked		9.0 ± 2.3*	+79.4	N.A.		7.2 ± 1.0	+43.5
		Well-done		6.2 ± 1.6	+24.1	N.A.		9.6 ± 1.6**	+92.2
		Over-cooked		4.3 ± 0.5	−14.8	N.A.		9.4 ± 1.7*	+88.0
Brassica chinensis (Chinese Cabbage)	Aqueous	Raw	1.1 ± 0.1						
		Under-cooked		0.9 ± 0.1	−15.5	0.6 ± 0.14**	−44.6	0.65±0.1**	−40.9
		Well-done		1.1 ± 0.1	−4.6	0.5 ± 0.10***	−53.6	0.69±0.04*	−37.3
		Over-cooked		0.9 ± 0.3	−22.7	0.7 ± 0.11*	−34.6	0.69±0.1*	−37.3
	Hydro-methanolic ####	Raw	1.9 ± 0.4	1.4 ± 0.2*	−25.7	1.1 ± 0.1***	−39.0	1.6 ± 0.1	−17.1
		Under-cooked							
		Well-done		1.6 ± 0.2	−16.6	1.4 ± 0.1*	−25.7	1.7 ± 0.1	−11.2
		Over-cooked		1.1 ± 0.2***	−43.3	1.6 ± 0.10	−16.0	1.5 ± 0.1	−18.7
Moringa oleifera (Drumstick) leaves	Aqueous	Raw	2.5 ± 0.1						
		Under-cooked		8.2 ± 0.7****	+223.0	7.6 ± 0.1****	+201.3	6.6 ± 0.4****	+
		Well-done		6.4 ± 0.7****	+151.0	4.3 ± 0.3****	+70.5	4.7 ± 0.3***	+85.2
		Over-cooked		8.3 ± 0.2****	+225.8	5.3 ± 0.2****	+110.8	3.4 ± 0.2	+32.2
	Hydro-methanolic ##	Raw	8.5 ± 1.0						
		Under-cooked		6.4 ± 0.8*	−25.0	10.6 ± 0.8*	+23.7	8.3 ± 0.6	−2.5
		Well-done		8.4 ± 0.3	−1.8	11.7 ± 0.4***	+36.7	10.4 ± 0.1*	+21.5
		Over-cooked		13.2 ± 0.3****	+54.1	6.6 ± 0.5*	−22.7	9.4 ± 0.5	+10.1
Moringa oleifera (Drumstick) pods	Aqueous	Raw	1.0 ± 0.2						
		Under-cooked		0.6 ± 0.03	−37.4	0.91±0.22	−8.1	N.A.	
		Well-done		0.5 ± 0.06*	−46.5	0.78±0.22	−21.2	N.A.	
		Over-cooked		0.6 ± 0.07	−35.4	0.60±0.20	−39.4	N.A.	
	Hydro-methanolic	Raw	1.2 ± 0.2						
		Under-cooked		0.66±0.09***	−45.5	0.97±0.19	−19.8	N.A.	
		Well-done		0.71±0.07**	−41.3	0.77±0.08**	−36.4	N.A.	
		Over-cooked		0.79±0.16**	−34.7	0.60±0.09***	−50.4	N.A.	
Pleurotus sajor-caju (Oyster Mushroom)	Aqueous	Raw	0.27±0.02						
		Under-cooked		0.70±0.02****	+159.3	0.75±0.03****	+177.8	0.15±0.01****	−44.4
		Well-done		0.52±0.01****	+92.6	0.76±0.03****	+181.8	0.18±0.01***	−33.3
		Over-cooked		0.69±0.02****	+155.6	0.62±0.03****	+129.6	0.19±0.01***	−29.6
	Hydro-methanolic ###	Raw	0.67±0.03						
		Under-cooked		0.65±0.02	−3.0	0.96±0.05****	+43.3	1.0 ± 0.03****	+49.3
		Well-done		0.80±0.02*	+19.4	1.36±0.07****	+103.0	1.09±0.04****	+62.7
		Over-cooked		1.02±0.05****	+52.2	1.77±0.08****	+164.2	1.12±0.04****	+67.2

FRAP values expressed in mmol Fe^{2+}/g FDW ± SEM ($n = 3$) of three independent assays performed in triplicate.
Significance was assessed using one-way ANOVA followed by Dunnett's multiple comparisons; the cooked extracts were compared to the raw extract;.
* $p < 0.05$,.
** $p < 0.01$,.
*** for $p < 0.001$ and.
**** $p < 0.0001$.Significance between the extraction methods was assessed using an independent T-test;#$p < 0.05$,.
$p < 0.01$,.
$p < 0.001$ and.
$p < 0.0001$.Δ (%): Relative percentage change in antioxidant activity of the cooked extract compared to the respective uncooked extract.N.A.: Not Applicable.

151.0% and 225.8% after 4, 8 and 12 min boiling respectively, and was increased by 201.3%, 70.5% and 110.8% after 5, 10 and 15 min steaming, with respect to the raw leaves. Stir-frying also retained and enhanced *M. oleifera* leaves ferric reducing potential, however, as cooking time increased the antioxidant activity progressively decreased from 159.1%, 85.2% to 32.2%. The FRAP of *P. sajor-caju* significantly increased by 159.3%, 92.6% and 155.6% and of 177.8%, 181.8% and 129.6% after boiling and steaming for 5, 10 and 15 min respectively ($p<0.05$), whereas a decrease of −44.4%, −33.3% and −29.6% after 4, 8 and 12 min stir-frying was noted.

In contrast, increasing steaming time of *M. oleifera* pods gradually resulted in loss of FRAP by 19.8% (10 min), 36.4% (20 min) and 50.4% (30 min) as compared to the uncooked pods. A loss in reducing antioxidant potential was also observed after *B. chinensis* was boiled (15.5%, 4.6% and 22.7% after 4, 8 and 12 min respectively) and steamed (44.6%, 53.6% and 34.6% after 5, 10 and 15 min respectively). The difference in loss of FRAP between the two cooking methods could be attributed to a loss in water-soluble antioxidant compounds [36].

Different FRAP profiles were observed for cooked aqueous and hydro-methanolic extracts. A longer stir-frying time increased the FRAP of hydro-methanolic *M. oleifera* leaves extracts, while decreased the FRAP of the aqueous corresponding extracts (Table 2). The same trend could be observed for the stir-fried *P. sajor-caju* extracts. In contrast, longer boiling time decreased FRAP of hydro-methanolic *A. cepa* extracts, while increasing the reducing antioxidant capacity of the aqueous *A. cepa* extracts. This could suggest the formation of novel molecules of different polarities. Longer stir-frying time for *M. oleifera* leaves and *P. sajor-caju* may possibly increase slightly non-polar compounds with reducing power, whereas boiling *A. cepa* for longer period may result in more polar compounds with reducing properties.

The antioxidant trend observed for DPPH free radical scavenging potential (Table 3) and iron (II) chelating activity (Table 4) did not mirror entirely that of the FRAP assay.

Cooking had a great influence on the free radical scavenging properties of the foods under study. Short stir-frying time showed the greatest scavenging abilities for *A. cepa* with AA_{50} values of 920.23 ± 104.9 µg/ mL and 2101.0 ± 49.3 µg/ mL for the aqueous and hydro-methanolic extracts respectively Thermal treatments significantly reduced the scavenging properties of *M. oleifera* pods ($p<0.05$). Boiling and steaming for 30 min decreased *M. oleifera* pods free radical scavenging activity by 92.3% and 305.7% respectively. Likewise, boiling and stir-frying caused significant loss in antioxidant capacities of *B. chinensis* ($p<0.05$). A loss of 210.4% in free radical scavenging antioxidant activity was noted after *B. chinensis* was boiled for 4 min. This effect may possibly be attributed to degradation of redox-active compounds after heat treatments.

In contrast, steaming and stir-frying significantly increased the scavenging capacities of *P. sajor-caju* ($p<0.05$). A gradual increase in the antioxidant activity of *P. sajor-caju* from 8.9%, 13.2% to 22.6% was noted as steaming time was extended from 5, 10 to 15 min respectively This may suggest possible formation of beneficial compounds after these conventional processing techniques.

Cooking time also played a significant role on the scavenging capacities of the vegetables. For instance, a gradual decrease in antioxidant potential was observed as boiling, steaming and stir-frying time was extended for *M. oleifera* leaves. Only short boiling (4 min) and steaming (5 min) time periods retained the free radical scavenging activity of *M. oleifera* leaves (Table 3). A similar trend was observed after boiling *A. cepa*. The scavenging activity of *A. cepa* was enhanced by 70.1% after boiling for 5 min, however, as boiling time was prolonged to 15 and 30 min, the antioxidant activity decreased to 23.0% and 34.3% respectively, with respect to its raw sample. This may suggest the possible release of antioxidant compounds from the cellular matrix after a short thermal processing period, which degrades after higher heat treatments.

The food extracts also showed the ability to compete for Fe^{2+} ions and induce iron chelation (Table 3). Among the foods tested, *B. chinensis* was observed as the most effective iron chelator, with the boiled 4 min aqueous extract as the most potent extract (AA_{50}: 94.7 ± 12.9 µg/ mL). Nevertheless, increasing the boiling time decreased the antioxidant activity. Similar trends were observed for *M. oleifera* leaves which showed the highest chelating activity after boiling for 4 min, with an increase of 47.8% in chelating activity as compared to the raw sample. This increase was gradually reduced to 21.7% and 218.2% (with respect to the raw leaves) when boiling time was increased to 8 and 12 min respectively. Steaming, on the other hand, did not retain the chelating potential of *M. oleifera* leaves and lead to 116.0%, 142.0 and 274.8% loss in this antioxidant mechanism as compared to the uncooked vegetables. The different cooking techniques also demonstrated opposite effects on the chelation activity of *M. oleifera* pods and *A.cepa*. Boiling decreased the chelating potential of the pods by −59.2%, −75.3% and −54.8% (10, 20 and 30 min) whereas steaming retained and enhanced the same activity by 47.6%, 54.5% and 36.2% (10, 20 and 30 min). A gradual increase in iron chelating activity was observed, for the aqueous extracts of *A.cepa* as the boiling time was prolonged. In contrast, longer stir-frying time significantly reduced the antioxidant potential of *A. cepa* ($p<0.01$). In addition, cooking improved the overall iron chelating abilities of *P. sajor-caju*. Prolonging the boiling and stir-frying time enhanced the chelation capacities of the *P. sajor-caju*. Steaming for 10 min raised the antioxidant capacity by 35.18% (aqueous extract) and 79.22% (hydro-methanolic extract) in comparison to the uncooked mushroom. However, as steaming time was extended (≥15 min, aqueous extracts), iron chelating potential of *P. sajor-caju* decreased.

According to the literature, the effect of thermal procedures on antioxidant capacity of vegetables will vary on the food matrix and extractability, the cooking methods and time, as well as the analytical techniques conducted [18,8,19,9]. Similar to our investigation, studies on the antioxidant capacities of the vegetables reported varying results, either decline, unchanged or enhancement in antioxidant capacities, across different foods via several cooking techniques analysed [37,19,24]. In the present study, cooking had a notable negative effect on the antioxidant properties of *B. chinensis*. All cooking techniques decreased the ferric reducing power of this vegetable, whereas only boiling and stir-frying affected the free radical scavenging properties of the food. In contrast, Managa et al. [18], reported a significant increase in FRAP after subjecting

Table 3
Effect of cooking on the DPPH free radical scavenging capacities of selected locally grown foods.

Food	Extraction method	Cooking time	Cooking Techniques					
			Raw	Boiled		Steamed		Stir-Fried	
				AA_{50} (µg/ mL FDW)	Δ (%)	AA_{50} (µg/ mL FDW)	Δ (%)	AA_{50} (µg/ mL FDW)	Δ (%)
Allium cepa (Onion)	Aqueous	Raw	4407.7 ± 172.0						
		Under-cooked		7497.3 ± 580.8**	+70.1	N.A.		920.2 ± 104.9**	+79.1
		Well-done		5420.0 ± 591.3	−23.0	N.A.		1205.6 ± 184.9**	+72.6
		Over-cooked		5920.7 ± 758.8	−34.3	N.A.		688.6 ± 93.3**	+84.4
	Hydro-methanolic	Raw	3764.7 ± 264.5						
		Under-cooked		4994.3 ± 65.7	−32.7	N.A.		2101.0 ± 49.3	+44.2
		Well-done		4813.7 ± 398.9	−27.9	N.A.		5538.7 ± 667.1	−47.1
		Over-cooked		8018.7 ± 500.2***	−113	N.A.		N.D.	
Brassica chinensis (Chinese Cabbage)	Aqueous	Raw	570.2 ± 83.5						
		Under-cooked		1770.0 ± 120.9****	−210.4	406.6 ± 67.4	+28.7	1496.0 ± 157.9****	−162.4
		Well-done		1402.0 ± 22.8***	−145.9	458.9 ± 8.5	+19.5	1069.0 ± 69.5***	−87.5
		Over-cooked		1483.0 ± 48.9****	−160.1	517.2 ± 15.3	+9.3	373.2 ± 71.3	+34.5
	Hydro-methanolic [#]	Raw	337.0 ± 22.5						
		Under-cooked		565.0 ± 66.1	−67.7	400.3 ± 55.1	−18.8	522.0 ± 43.9	−54.9
		Well-done		558.1 ± 10.6	−65.6	456.6 ± 9.4	−35.5	724.9 ± 21.9**	−115.1
		Over-cooked		731.9 ± 79.7***	−117.2	516.9 ± 15.4	−53.4	925.0 ± 8.4****	−174.5
Moringa oleifera (Drumstick) leaves	Aqueous	Raw	142.4 ± 3.5						
		Under-cooked		126.9 ± 6.8	+10.8	100.1 ± 9.4	+29.7	154.7 ± 4.8	−8.7
		Well-done		217.3 ± 0.9	−52.7	211.9 ± 2.3	−48.9	237.4 ± 36.7*	−66.7
		Over-cooked		326.9 ± 15.2***	−129.6	240.2 ± 8.8*	−68.7	589.4 ± 40.1****	−314
	Hydro-methanolic	Raw	26.9 ± 2.0						
		Under-cooked		35.0 ± 1.7	−30.1	43.1 ± 3.0	−60.6	170.6 ± 13.8	−535.3
		Well-done		115.2 ± 10.0	−329	63.8 ± 6.2	−137.6	399.7 ± 0.2****	−1388.3
		Over-cooked		280.5 ± 4.4***	−944.3	168.0 ± 20.3	−525.7	904.1 ± 86.3****	−3266.5
Moringa oleifera (Drumstick) pods	Aqueous	Raw	820.4 ± 18.6						
		Under-cooked		1769.0 ± 19.8**	−115.6	2022.0 ± 116.2***	−146.5	N.A.	
		Well-done		1826.0 ± 75.3***	−122.6	2020.0 ± 44.4***	−146.2	N.A.	
		Over-cooked		1578.0 ± 63.4**	−92.3	3328.0 ± 284.4****	−305.7	N.A.	
	Hydro-methanolic	Raw	1523.0 ± 151.2						
		Under-cooked		2008.0 ± 199.3	−31.8	1217.0 ± 92.9	+20.1	N.A.	
		Well-done		2002.0 ± 186.6	−31.5	1670.0 ± 53.6	−9.7	N.A.	
		Over-cooked		2394.0 ± 14.2**	−57.2	1229.0 ± 77.8	+19.3	N.A.	
Pleurotus sajor-caju (Oyster Mushroom)	Aqueous	Raw	952.7 ± 66.3						
		Under-cooked		800.4 ± 25.7****	+16.0	868.2 ± 8.1**	+8.9	488.3 ± 8.0****	+48.7
		Well-done		1207.0 ± 41.6****	−26.7	827.1 ± 15.7***	+13.2	739.7 ± 16.4****	+22.4
		Over-cooked		831.6 ± 8.9***	+12.7	737.1 ± 6.2****	+22.6	435.8 ± 6.5****	+54.3
	Hydro-methanolic	Raw	614.7 ± 12.7						
		Under-cooked		485.9 ± 43.6*	+21.0	359.9 ± 27.8****	+41.5	343.3 ± 16.7****	+44.2
		Well-done		738.1 ± 55.1*	−20.1	351.4 ± 45.3****	+42.8	265.7 ± 79.4****	+56.8
		Over-cooked		740.7 ± 47.2*	−20.5	365.3 ± 46.1****	+40.6	256.0 ± 17.4****	+58.4

DPPH free radical scavenging assay results expressed in AA_{50} values µg FDW/ mL ± SEM ($n = 3$) of three independent assays performed in triplicate.
Significance was assessed using one-way ANOVA followed by Dunnett's multiple comparisons; the cooked extracts were compared to the raw extract;.
* $p < 0.05$,.
** $p < 0.01$,.
*** for $p < 0.001$ and.
**** $p < 0.0001$.Significance between the extraction methods was assessed using an independent T-test;.
[#] $p < 0.05$.Δ (%): Relative percentage change in antioxidant activity of the cooked extract compared to the respective uncooked extract.N.A.: Not Applicable.N. D: Not detected.

Table 4
Effect of cooking on the iron (II) chelating potential of selected locally grown foods.

Food	Extraction method	Cooking time	Cooking Techniques					
			Raw	Boiled		Steamed		Stir-Fried	
				AA_{50} (µg/ mL FDW)	Δ (%)	AA_{50} (µg/ mL FDW)	Δ (%)	AA_{50} (µg/ mL FDW)	Δ (%)
Allium cepa (Onion)	Aqueous	Raw	1578.0 ± 490.8						
		Under-cooked		14,003.0 ± 313.0****	−787.4	N.A.		3138.0 ± 642.4	−98.9
		Well-done		20,013.0 ± 229.8****	−1168.3	N.A.		3129.0 ± 384.6	−98.3
		Over-cooked		3344.0 ± 752.5	−111.9	N.A.		4095.0 ± 412.6*	−159.5
	Hydro-methanolic	Raw	3076.0 ± 568.7						
		Under-cooked		3127.0 ± 904.1	−1.7	N.A.		3813.0 ± 792.7	−24.0
		Well-done		2084.0 ± 475.3	+32.2	N.A.		6758.0 ± 41.7**	−119.7
		Over-cooked		831.6 ± 143.8	+73.0	N.A.		4739.0 ± 538.8	−54.1
Brassica chinensis (Chinese Cabbage)	Aqueous	Raw	183.0 ± 50.0						
		Under-cooked		94.7 ± 12.9	+48.3	730.4 ± 28.8****	−299.1	287.5 ± 66.7	−57.1
		Well-done		149.3 ± 26.0	+18.4	265.9 ± 9.7	−45.3	350.4 ± 63.4	−91.5
		Over-cooked		224.3 ± 23.7	−22.6	132.9 ± 10.6	+27.4	612.8 ± 102.8****	−234.9
	Hydro-methanolic##	Raw	154.5 ± 13.7						
		Under-cooked		946.8 ± 100.2*	−512.8	1180.1 ± 226.0**	−663.8	691.2 ± 115.4	−347.4
		Well-done		1228.3 ± 114.3**	−695.0	2244.3 ± 371.1****	−1352.6	457.9 ± 38.2	−196.4
		Over-cooked		1358.0 ± 217.0***	−779.0	1463.7 ± 150.4***	−847.4	326.6 ± 6.2	−111.4
Moringa oleifera (Drumstick) leaves	Aqueous	Raw	1129.1 ± 49.2						
		Under-cooked		589.4 ± 297.5*	+47.8	2439.0 ± 26.6****	−116.0	510.8 ± 193.4**	+54.8
		Well-done		1374.7 ± 28.2	−21.7	2732.0 ± 183.8****	−142.0	389.2 ± 34.4**	+65.5
		Over-cooked		3593.3 ± 79.0****	−218.2	4232.0 ± 164.9****	−274.8	195.4 ± 79.3***	+82.7
	Hydro-methanolic	Raw	4044.0 ± 49.0						
		Under-cooked		3217.3 ± 49.8	+20.4	3068.3 ± 15.4	+24.1	4192.3 ± 111.7	−3.7
		Well-done		2376.3 ± 16.3	+41.2	2775.3 ± 106.2	+31.4	4518.7 ± 19.9	−11.7
		Over-cooked		6147.7 ± 45.6	−52.0	N. D		N.D.	
Moringa oleifera (Drumstick) pods	Aqueous	Raw	682.0 ± 191.6						
		Under-cooked		1085.9 ± 360.2	−59.2	357.3 ± 89.7	+47.6	N.A.	
		Well-done		1195.3 ± 351.4	−75.3	310.3 ± 38.2	+54.5	N.A.	
		Over-cooked		1055.7 ± 393.2	−54.8	435.3 ± 92.2	+36.2	N.A.	
	Hydro-methanolic	Raw	708.3 ± 155.8						
		Under-cooked		866.1 ± 75.8	−22.3	818.8 ± 177.0	−15.6	N.A.	
		Well-done		744.8 ± 119.9	−5.2	542.8 ± 47.0	+23.4	N.A.	
		Over-cooked		781.9 ± 244.0	−10.4	1039.6 ± 170.2	−46.8	N.A.	
Pleurotus sajor-caju (Oyster Mushroom)	Aqueous	Raw	1260.0 ± 49.2						
		Under-cooked		3081.0 ± 297.5****	−144.5	1529.0 ± 26.6	−21.3	468.5 ± 193.4**	+62.8
		Well-done		471.6 ± 28.2**	+62.6	913.7 ± 183.8	+27.5	496.4 ± 34.4**	+60.6
		Over-cooked		454.4 ± 79.0**	+63.9	1238.0 ± 164.9	+1.7	503.4 ± 79.3**	+60.0
	Hydro-methanolic	Raw	869.0 ± 26.6	763.6 ± 49.8	+12.1	313.0 ± 15.4****	+64.0	435.7 ± 111.7**	+49.9
		Under-cooked		669.8 ± 16.3	+22.9	180.6 ± 106.2****	+79.2	591.0 ± 19.9*	+32.0
		Well-done		621.8 ± 45.6	+28.4	397.3 ± 95.2***	+54.3	345.3 ± 45.8***	+60.3
		Over-cooked							

Iron chelating results expressed in AA_{50} values µg FDW/ mL ± SEM ($n = 3$) of three independent assays performed in triplicate.
Significance was assessed using one-way ANOVA followed by Dunnett's multiple comparisons; the cooked extracts were compared to the raw extract;.
* $p < 0.05$,.
** $p < 0.01$,.
*** for $p < 0.001$ and.
**** $p < 0.0001$.Significance between the extraction methods was assessed using an independent T-test;.
$p < 0.01$.Δ (%): Relative percentage change in antioxidant activity of the cooked extract compared to the respective uncooked extract.N.A.: Not Applicable.N.D: Not detected.

B. chinensis to the same processing techniques. Moreover, the *M. oleifera* leaves in this study demonstrated an increase in antioxidant level after thermal processing. This corroborated with Subramaniam et al. [25] results, who reported an increase of approximately 10-fold in DPPH radical scavenging abilities of *M. oleifera* leaves after boiling, and an increase of 5-fold in FRAP after the same cooking method. The antioxidant capacities of *A. cepa* also demonstrated varied changes across the cooking techniques applied, as well as differences in the analytical methods employed (Tables 2–4). Likewise, Juániz et al. [38] reported higher DPPH radical scavenging activity in griddled *A. cepa*, however, a decline in ABTS radical antioxidant capacity after the same cooking technique was noted. In contrast to the other food tested, cooking had a positive effect on the antioxidant potential of the *P. sajor-caju* under test. In accordance, Ng and Tan [36] noted a rise in FRAP after boiling and steaming *Pleurotus* mushroom.

The findings demonstrated that individual antioxidant compounds respond differently to different cooking techniques. The alterations in the chemical structure of bioactive molecules upon thermal processing can significantly modify the biological properties of traditional ingredients. Accordingly, it has been reported that, polymerisation of procyanidins and the loss of sugar moieties during deglycosylation of phenolic glycosides were indicated as factors for promotion of antioxidant activity [21]. Nevertheless, the position and the number of hydroxyl groups attached to the phenolics are crucial determinants for the antioxidant properties of a compound [38].

Furthermore, food processing promoted the progressive chemical or enzymatic oxidation of antioxidant compounds [72] . The formation of novel molecules with antioxidant and/ or prooxidant activities might as well be the consequence of domestic processing and might explain the altered antioxidant potential of the local foods under study after such procedures. Depending on the rate of the reactions, the food and processing conditions, intermediate oxidation state products can result in higher radical scavenging efficiency. In the case of polyphenols, this could be attributed to an increased capacity to donate a hydrogen atom from the aromatic hydroxyl group and/or the ability of the aromatic structure to support electrons [39]. For instance, catechin enzyme reaction products, at early-stage reaction, exhibit higher antioxidant efficiency, which progressively decreases as oxidation continues and prior to the formation of Maillard reaction products [40]. In addition, reducing browning reaction products resulted in higher antioxidant potential, while short heat treatment caused reduction of the original antioxidant activity as demonstrated in tomato juice [41]. It should also be noted that thermal treatment can inactivate oxidative enzymes responsible for oxidation of antioxidant molecules, which may result in a rise in antioxidant level [42].

Nevertheless, it is fundamental to consider that the total antioxidant potential of a food may be the consequence of multiple compounds rather than a single compound, which may even operate in synergy. Also, each compound has different physical properties (polarity, thermostability, etc.…), which will be affected differently by different food processing techniques and temperature. Interactions between the components in the food matrix might as well affect the total antioxidant activity of a food subjected to domestic processing.

Effect of cooking on the cytotoxic potential of the foods under study

The extracts under study were tested for their anti-proliferative properties on a human liver cancer cell line (HepG2). Etoposide and paclitaxel, used as positive controls, reported IC_{50} values of 2.53 µg/mL and 20.7 ng/mL respectively after 48hr treatment.

The cooked *B. chinensis* and *P. sajor-caju* extracts significantly inhibited the growth and survival of the liver cancer cells as compared to the raw extracts ($p< 0.05$) (Fig. 4(A) and 4(D) respectively). In contrast, thermal processing substantially decreased the cytotoxic effect of *A. cepa, M. oleifera* leaves and pods compared to their raw samples (Fig. 4). The raw *A. cepa* extracts (both aqueous and hydro-methanolic extracts) exerted a dose-dependent cytotoxic effect on HepG2 cells with calculated IC_{50} of 1.540± 0.208 mg FDW/ mL and 1.567± 0.206 mg FDW/ mL after 48 h treatment respectively. However, the cooked *A. cepa* samples did not demonstrate any cytotoxic effect and cell viability (expressed as a percentage of negative control) remained above 80% after 24 h and 48 h for all tested concentrations regardless of extraction methods.

B. chinensis

The *B. chinensis* extracts showed a dose and time dependent cytotoxic effect. A significant difference was observed between the aqueous and the hydro-methanolic extraction methods ($p<0.05$). In general, the steamed and stir-fried *B. chinensis* extracts significantly inhibited HepG2 cell growth as compared to its raw sample. The most potent sample was the steamed 15 min aqueous extract with IC_{50} value: 280.2 ± 7.4 µg FDW/ mL. Increasing the steaming time significantly increased the anti-proliferative ability of this vegetable ($p<0.001$) (Fig. 4(A)). On the other hand, longer stir-frying time decreased the cytotoxic potential of the cooked vegetable.

Similarly, a previous study reported an increase in antiproliferative potency of *Brassicaceae* vegetables after cooking on breast cancer cells [43]. The study observed that cell proliferation was inhibited in breast cancer cells in a dose-dependent manner following exposure to differently processed broccoli extracts. The steamed 3 min and microwaved 2 min freeze dried broccoli showed greater anti-proliferative activities in MCF-7 cells as compared to the fresh freeze-dried extracts. In addition, a negative correlation was made between the TPC and TFC of the cruciferous vegetables and their cytotoxic effect on cancer cell lines [43], which is in connection with the results obtained for *B. chinensis* in this work. However, these studies also reported a high content in sulfur-rich compounds, namely glucosinolate [44] and isothiocyanate [43]. Other works also observed stir-frying and steaming as best cooking techniques for the retention and/ or the enhancement

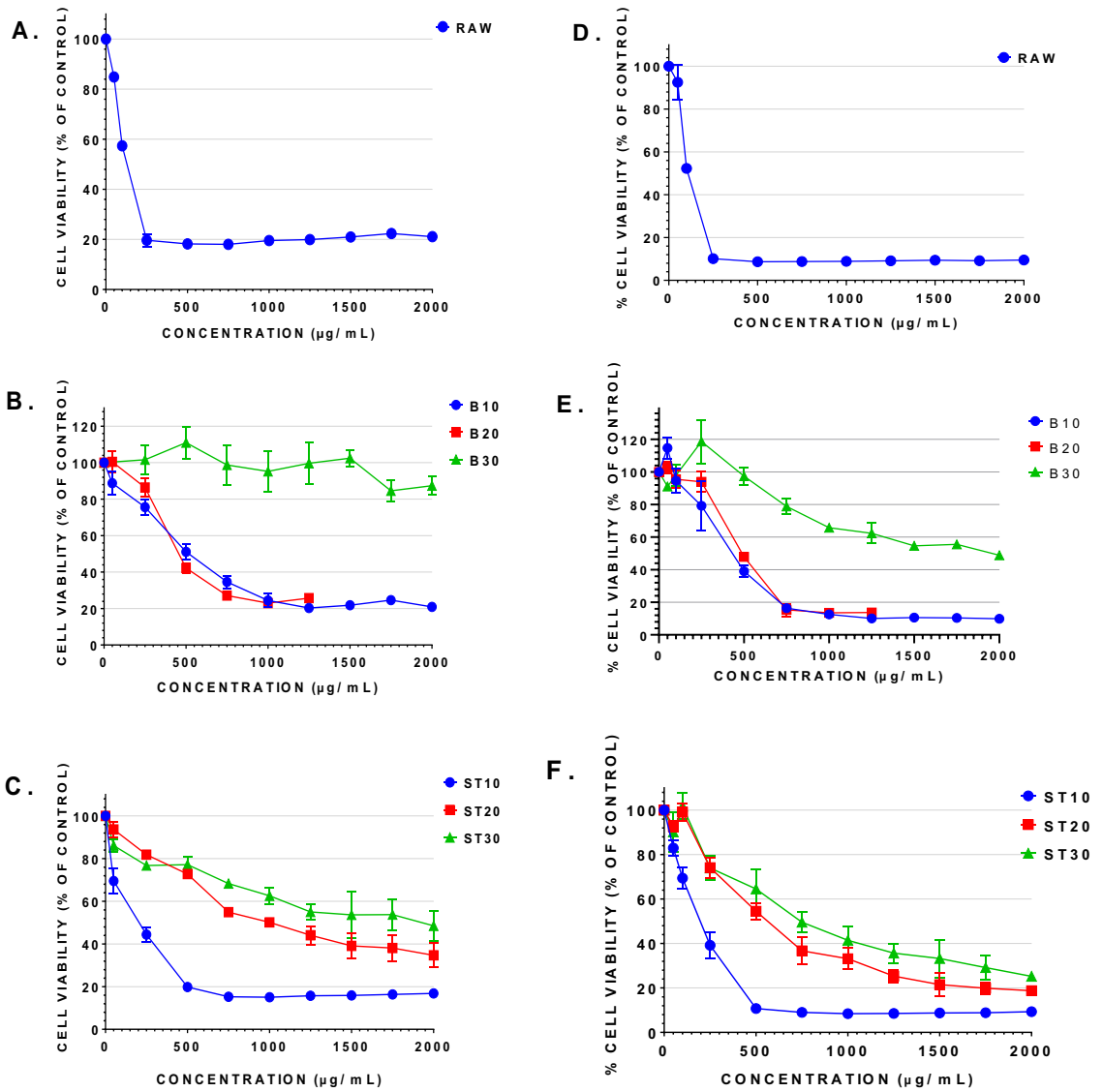

Fig. 3. Dose dependent effect of *M. oleifera* pods aqueous extracts post (A) to (C) 24 h **and (D) to (F)** 48 h **treatment**. The results were expressed as the mean percentage of negative control ± SEM of three independent assays in triplicate B10, B20, B30: boiled 10, 20 and 30 min; ST10, ST20, ST30: steamed 10, 20 and 30 min.

of glucosinolates and isothiocyanates [34][45]. These compounds have garnered much attention these last years for their potent chemopreventive and anti-tumor properties [46–48]. Thermal processing of *Brassica* vegetables normally reported the influence of glucosinolate– myrosinase system. In addition, the formation of glucosinolate hydrolysis product into the reactive bioactive isothiocyanate compounds [49] during moist cooking, could have increased the anti-proliferative potential of *B. chinensis*. Consequently, the major anti-proliferative effect of cooked *Brassicaceae* vegetables could be attributed to glucosinolates and isothiocyanates, however, this shall be confirmed through further experimentations.

P. sajor-caju

The present study demonstrated a significant difference in the anti-proliferative potential of the uncooked and cooked *P. sajor-caju* ($p<0.05$) (Fig. 4(D)), with an insignificant difference between the cooking methods, i.e., boiling, steaming and stir-frying ($p<0.05$). The most anti-proliferative *P. sajor-caju* extracts were the boiled 10 min sample with IC_{50} value of 537.2 ± 30.3 µg/mL and 426.0 ± 15.2 µg/mL for the aqueous and the hydro-methanolic extract respectively. Long boiling period (≥10 min) was observed as the best cooking method to enhance the cytotoxic potency of the *P. sajor-caju* in comparison to other cooking techniques. The longest steaming and stir-frying duration also showed increased anti-proliferative capacities as opposed to shorter cooking time. Increasing stir-frying time of the mushroom from 4, 8 to 12 min decreased the IC_{50} from 1550.7 ± 147.5 µg/mL, 912.2 ± 72.4 µg/mL to 813.0 ± 66.9 µg/mL, thus increasing the anti-proliferative potency.

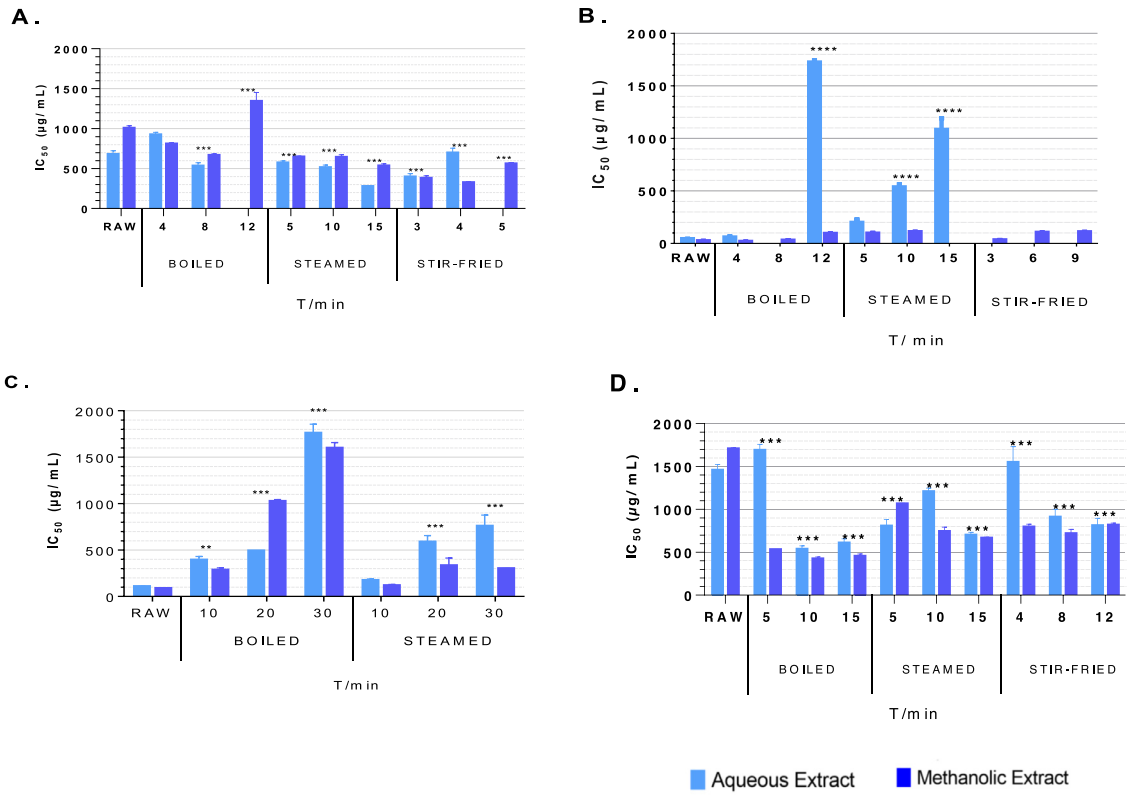

Fig. 4. IC_{50} values of the different raw and cooked (A) *B. chinensis*, (B) *M. oleifera* leaves, (C) *M. oleifera* pods and (D) *P. sajor-caju* aqueous and hydro-methanolic extracts after 48 h **treatment against HepG2 cell line.** The results were expressed as the mean percentage of negative control ± SEM of three independent assays in triplicate. No IC_{50} could be detected for the *B. chinensis* boiled 12 min and stir-fried 5 min aqueous extract, and *M. oleifera* leaves boiled 8 min and the stir-fried aqueous extracts within the concentration range tested. Significance was assessed using two-way ANOVA followed by Turkey's multiple comparisons, with extraction methods and cooking techniques as factors, the cooked extracts were compared to the raw extract: $^{**}p<0.01$, $^{***}p<0.001$.$^{****}p<0.0001$.

The chemopreventive actions of *P. sajor-caju* have been documented [70]. Moreover, preclinical investigations on uncooked locally cultivated mushrooms demonstrated potent chemopreventive properties against liver cancer [15]. The mushroom was also reported to possess an important level of L-ergothioneine, a naturally occurring amino acid, with potent antioxidant properties [[15],[50]]. The present study indicated a significant increase in the anti-proliferative properties of cooked *P. sajor-caju* extracts as compared to the raw extracts on liver cancer cells ($p<0.05$). The boiled mushroom extracts showed marked cytotoxicity as compared to steaming and stir-frying (Fig. 4(D)). This enhancement in biological activity could suggest a better extractability of the bioactive compounds, including ergothioneine, after boiling. In the same line, Yen et al. [51] described a superior yield in ergothioneine after a hot water extraction from *Pleurotus eryngii* and *Pleurotus citrinopileatus* [51]. Thus, this may indicate boiling as the best cooking method for enhancing the chemopreventive actions of locally grown *P. sajor-caju*.

A. cepa

In contrast to *P. sajor-caju* and *B. chinensis*, cooking significantly limited the anti-proliferative activities of *A. cepa*. Raw *A. cepa*, both aqueous and hydro-methanolic extracts, demonstrated marked cytotoxicity on HepG2 cells at the tested concentrations. However, after cooking the anti-proliferative potential of *A. cepa* on this cell line significantly decreased ($p<0.05$). As per literature, high consumption of *Allium* vegetables has been associated to reduced risk of gastric [52], upper aerodigestive tract [[65]], breast [[64]] and colorectal cancer [34]. In general, *A. cepa* chemopreventive actions have been attributed to its unique composition of sulfur and flavonoids compounds [53]. Hence, the elevated TFC of cooked *A. cepa* was expected to increase the cytotoxicity of *A. cepa* on cancer cells, but a negative correlation was observed between the two parameters. In the same line, the antiplatelet activity of *A. cepa* was reduced after cooking [54]. This may suggest the degradation of compounds with pharmacological benefits upon heat treatments. However, the formation of therapeutic compounds upon thermal processing of *A. cepa* should also be noted. For instance, a novel compound, 8-C-(*E*-phenylethenyl) quercetin, was found in *A. cepa*/beef soup and was observed to induce autophagic cell death of colon cancer cells through activation of ERK pathway [55].

M. oleifera *leaves and pods*

Similar to *A. cepa*, the strong anti-proliferative activity detected with the raw *M. oleifera* pods extracts decreased proportionally with increased cooking time (Fig. 4(C)). The most cytotoxic *M. oleifera* pods extracts were the raw extracts with IC_{50} values: 93.4 ± 2.0 and 85.1 ± 4.1 µg FDW/ mL after 24 h, and 104.7 ± 3.5 and 84.17 ± 9.6 µg FDW/ mL after 48 h for the aqueous and hydro-methanolic extract respectively. Short steaming time for *M. oleifera* pods had a similar cytotoxic effect to the uncooked sample. However, longer steaming and boiling durations decreased the anti-proliferative potential of this vegetable Fig. 3. Similar to the pods, *M. oleifera* leaves had lower antiproliferative capacities after cooking. The hepatocarcinoma cells were more sensitive to the raw *M. oleifera* leaves extracts compared to the cooked samples and showed both a concentration-dependent and a time-dependent reduction on cell viability, with IC_{50} values of 167.6 ± 11.7 µg/ mL and 43.3 ± 2.3 µg/ mL after 24 h and 48 h incubation respectively for the aqueous extracts; and 239.2 ± 12.7 µg/mL and 23.0 ± 3.7 µg/mL after 24 h and 48 h incubation respectively for the hydro-methanolic extracts. Boiling for 4 min showed a similar cytotoxic effect to the uncooked leaves extract with IC_{50} values of 59.3 ± 13.9 µg/ mL (aqueous extract) and 16.7 ± 3.5 µg/mL (hydro-methanolic extract) after 48 h treatment. However, as boiling time was increased, the antiproliferative effect of *M. oleifera* leaves on HepG2 decreased. Similarly, short steaming time induced higher antiproliferative activity than longer steaming period. Nevertheless, the shortest (5 min) steaming time showed less activity than the shortest boiling time (4 min) by approximately 3-fold. Similar to boiling and steaming, the shorter stir-frying duration, the higher the cytotoxic effect on this cell line. To the best of our knowledge, the anti-proliferative properties of cooked *M. oleifera* have never been reported previously. Nevertheless, several preclinical studies confirmed the anticancer potential of *M. oleifera* extracts and bioactive components on oesophageal cancer [56], liver cancer [57], breast cancer, colon cancer [58], ovarian cancer [59] and prostate cancer [60].

According to previous reports, the antiproliferative activities of foods may be considered: active: $IC_{50} \leq 50\,\mu g/mL$; moderately activity: $50\,\mu g/mL < IC_{50} \leq 200\,\mu g/mL$; weakly active: $200\,\mu g/mL < IC_{50} \leq 1000\,\mu g/mL$; inactive: $IC_{50} > 1000\,\mu g/mL$ [61]. Accordingly, the preliminary screened food extracts, even after being subjected to domestic processing (except for *A. cepa*), retain interesting anti-proliferative properties .

Conclusion

The conventional processing methods had significant and varied effects on the polyphenol content, antioxidant and antiproliferative activities of the locally grown foods. Foods like *B. chinensis, P. sajor-caju, M. oleifera* leaves and pods had similar or higher antioxidant and antiproliferative properties after thermal processing. The data suggest that the cooking procedures should be carefully considered and selected to maximize their benefits. For instance, short boiling and steaming period may enhance the prophylactic properties of *M. oleifera* leaves, whereas, longer cooking time may help extract the beneficial compounds from *P. sajor-caju* cellular matrix and hence contributing to its health effects. The changes in the phenolic composition during cooking need to be further investigated using chromatographic techniques coupled with mass spectrometry and other analytical techniques to understand the different transformation processes including deacylation, deglycosylation and hydrolysis of the polyphenols. This will allow the recommendation of the most appropriate cooking technique of each food responsible for optimal health benefits of consumers.

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Declaration of Competing Interest

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

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