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Full Length Research Paper

Phenolic compounds and antioxidant activities in some fruits and vegetables from Burkina Faso

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Levels of total phenolic compounds (TPC), proanthocyanidins (PAs) and antioxidant activities among sixteen fruits and vegetables commonly consumed in Burkina Faso were determined. Levels of TPC ranged from 0.21 to 3.33 mg of gallic acid equivalent per gram of fresh matter. The highest contents in TPC were found in hot chili pepper, okra, lemon, spinach, onion, while the highest contents in PAs were found in spinach (3.52 mg), onion (2.35 mg), okra (1.27 mg), hot chili pepper (1.11 mg), tomato (0.54 mg) and garlic (0.46 mg). Antioxidant activities ranged from 0 to 9 µmol per gram of fresh weight, trolox equivalent antioxidant capacity (TEAC). The highest values of TEAC were found in garlic (9.6 µmol), okra (3 µmol), spinach (2.2 µmol), tamarind (2.2 µmol) and onion (2.1 µmol). These data revealed that, some local fruits and vegetables from Burkina Faso are potential sources of bioactive compounds.

Key words: Antioxidant activity, phenolic compounds, fruits, vegetables, Burkina Faso.

INTRODUCTION

Fruits and vegetables are the major functional foods because they are the main sources of nutraceuticals such as vitamins, minerals and phenolic compounds (Tomás-Barberán and Espin, 2001; Szeto et al., 2002; Rupasinghe and Clegg, 2007). Epidemiological studies have shown the existence of a significant correlation between the intake of fruits and vegetables and the decrease of mortality and morbidity due to degenerative processes caused by oxidative stress (Birt et al., 2001; Dragsted et al., 2004). Antioxidants are substances that prevent or delay oxidative damage of lipids, proteins and nucleic acids caused by reactive oxygen species as well as free radicals. The main health damaging compounds, which are scavenged by antioxidants, include reactive free radicals such as superoxide, hydroxyl, peroxyl, alkoxyl and also non-radical species such as hydrogen peroxide, hypochlorous, etc. The most well known antioxidant constituents of fruits and vegetables, which may play these roles of prevention and protection, are vitamins C and E, carotenoids, minerals (selenium and zinc), some peptides and phenolic compounds (Tomás-Barberán and Espin, 2001; Rupasinghe and Clegg, 2007; Loarca-pina et al., 2010). These latter are the most ubiquitous antioxidants. Several data have revealed their high antioxidant capacities and their health promoting effects. Indeed, phenolic compounds have been reported to inhibit the development of cancerous tumours and to have anti-bacterial, anti-viral, anti-inflammatory, antispasmodic and anti diarrhoeic properties (Johnson, 2004; Dicko et al., 2006). Because of this promising evidence about the antioxidant action of these compounds, there is a need for local investigations on levels of phenolic compounds and antioxidant activities among fruits and vegetables currently consumed. In particular, these investigations are useful in developing countries where the health system facilities are precarious. Burkina Faso is a tropical country with a great potential of fruits and vegetables. These foods are not only sold in villages where they are produced but are brought mainly in towns for economical reasons.

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Abbreviations: ABTS, Diammonium salt; **TEAC,** trolox equivalent antioxidant capacity; **ABTS$^{\bullet+}$,** 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) radical; **GAE,** gallic acid equivalent.

Table 1. Total phenolic compounds (TPC), proanthocyanidins (PAs) and trolox equivalent antioxidant capacity (TEAC) of fruits and vegetables extracts.

Samples (fruits and vegetables)	TPC (mg GAE/100 g FW)		Pas (mg APE/100 g FW)		TEAC (µmol trolox/g FW)	
	Value*	SD**	Value	SD	Value	SD
Garlic (*Allium sativum)*	74.1	± 4.2	46.3	± 2.2	9.6	± 0.3
« Gombo » (*Hibiscus esculentus*)	252.6	± 14.6	127.1	± 5.6	3	± 0.2
Cabbage (green) (*Brassica oleracea)*	49.3	± 2.6	11.8	± 0.60	1.1	± 0.1
Cucumber (*Cucumis sativus)*	21.7	± 0.9	11.8	± 0.65	ND	
Eggplant (*Solanum melongena)*	34.8	± 0.8	29.4	± 1.48	0.7	± 0.0
Eggplant (local) (*Solanum aethiopicum)*	74.1	± 1.8	30.5	± 1.62	1.7	± 0.1
Lemon (*Citrus limonum)*	203.5	± 7.4	3.4	± 0.21	1.3	± 0.1
Onion (bulb) (*Allium cepa)*	155.9	± 6.5	234.7	± 12.73	1.5	± 0.1
Onion (leaves) (*Allium cepa)*	103.3	± 6.2	45.6	± 2.35	2.1	± 0.1
Parsley (*Petroselinum crispum)*	21.8	± 1	16.1	± 0.93	1.9	± 0.1
Pepper (*Capsicum frutescens)*	333.5	± 16.2	111.5	± 4.58	1.6	± 0.1
Spinach (*Spinacia oleracea)*	182.5	± 8.6	352	± 11.6	2.2	± 0.1
Sweet pepper (green) (*Capsicum annuum)*	ND***		ND		0.6	± 0.0
Tamarind (*Tamarindus indica)*	44.2	± 2.1	14.3	± 0.9	2.2	± 0.1
Tomato (sauce) (*Solanum lycopersicum)*	56.3	± 2.9	54.3	± 2.8	1.2	± 0.1
Zucchini (*Cucurbita pepo)*	100.2	± 5.1	7.9	± 0.4	0.4	± 0.0

*Values are presented as the mean of 3 replicates ± SD. **SD = Standard deviation; ***ND = not detected value.

The aim of this study is to determine levels of phenolic compounds and antioxidant activities among local commonly consumed fruits and vegetables from the central region of Burkina Faso. This study will contribute to the assessment of the levels of bioactive compounds in locally consumed foods.

MATERIALS AND METHODS

Plant materials

The selection of fruits and vegetables (Table 1) was based on the data of the main consumed foods in Burkina-Faso kindly provided by the National Center of Nutrition and on the harvest season of the samples. The samples were purchased from three big markets of Ouagadougou, which could be considered in the first steps of the fruits and vegetables distribution chain in Ouagadougou. The sampling was done during the end of the rainy season, between September and December 2007 to take into account all the targeted foods. Fruits and vegetables sold in the markets are from the downtown gardens and the nearest villages of the central region surrounding the town. Sixteen samples selected at the varietal level including common cucumber (green, elongated), cabbage (green), eggplant (local, green), eggplant (occidental, purple, teardrop shape), garlic (white bulb), Okra (locally named «gombo», green, average length), lemon (yellow), onions (red skin, bulb and leaves), parsley (dark green, curly leaves), hot pepper (scotch Bonnet Burkina yellow), spinach (flat leaves), sweet pepper (green), tamarind (sweet-sour taste), tomato (sauce, red, plum shape) and zucchini (green, elongated) were analyzed. The ripeness and the quality of the samples were judged according to their physical aspect (Moyer et al., 2002).

Chemicals

Gallic acid (3,4,5-trihydroxybenzoic acid) was obtained from Aldrich (Steinheim, Germany); 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS), Folin-Ciocalteu reagent and 6-hydroxy-2,5,7,8 tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma Chemical Co. (St Louis, MO, USA). Apple ciders procyanidin oligomers (average degree of polymerization = 7,4) were kindly provided by Dr. Stephanie Prigent (Wageningen University, Wageningen, The Netherlands) and Dr. Catherine M. G. C. Renard (INRA, Rennes, France). These procyanidins (DP ≈ 7.4) were purified by RP-HPLC and characterized by thyolisis-HPLC (Guyot et al., 1998; Prigent, 2005). All the other chemicals were of analytical grade.

Extraction of phenolic compounds

The samples for each item of fruit or vegetable were collected and cleansed. Then edible parts in equal amounts of each provenance were pooled, mixed and homogenized (Pellegrini et al., 2003). About 1 g of the homogenized sample was extracted by continuous stirring with 1% (v/v) HCL in methanol (Dicko et al., 2005) at 25°C for 15 min, followed by centrifugation at 4 000 *g* for 15 min at 25°C. The supernatant was then collected and the residue was extracted again with 3 ml of HCl/methanol as described earlier and the two supernatants were combined. The different extracts were analyzed directly without another operation, for their contents in total phenolic compounds, proanthocyanidins and their antioxidant activities.

Determination of phenolic compounds

The amount of total phenolic compounds was determined using Folin-Ciocalteu's method (Singleton et al., 1999) adapted to a 96-well plate microtiter assay. To 20 µl of extract, 80 µl of Folin-Ciocalteu reagent were added, followed by 5 min incubation. Then 80 µl of 20% (W/V) sodium carbonate solution was added. After 30 min of incubation, the absorbances were measured at 760 nm. Gallic acid was used as standard ($y = 0.0012x - 0.0004$; $r^2 = 0.9902$). Proanthocyanidins (PAs) were quantified with an adaptation to a 96-well plate assay as described by Dicko et al. (2005). To 50 µl of the extract was added 700 µl of an HCl-Butanol solution 30% (v/v). The mixture was put in tightly closed Eppendorf tubes and vortexed for 1 min. Then, the tubes were heated at 100°C for 2 h. After cooling, 200 µl aliquots were put in triplicate in a 96 multi-well plate and the absorbances were read at 550 nm. Apple procyanidins (DP ≈ 7.4) treated as indicated earlier were used as a standard ($y= 0.0006x + 0.0024$; $r^2= 0.9869$).

Determination of antioxidant activities

The trolox equivalent antioxidant capacity (TEAC) method was used to determine the antioxidant capacity. A stable stock solution of 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) radical ($ABTS^{\bullet+}$) was produced by reacting a 7 mM of aqueous solution of ABTS with 2.45 mM potassium persulfate, as final concentrations. This mixture was put in dark at 25°C for 16 h before use. The working solution was obtained by diluting the stock solution in 50 mM sodium phosphate, pH 7.4 to an absorbance between 0.7 and 0.90 at 734 nm. The ability of phenolic compounds to scavenge the radical $ABTS^{\bullet+}$ was monitored as described by Riedl and Hagerman (2001). Control assays were performed by replacing the phenolic extract with the acidic methanol (extraction solution). Reactions were monitored up to 30 min by measuring the absorbances at 1 mn interval. The results were expressed as TEAC that is, µmol of trolox equivalent ($y = 2.7004x + 0.05901$; $r^2 = 0.9984$) per gram of fresh matter.

Statistical analysis

All spectrophotometric assays were carried out in triplicate in 96-well microtiter plates using a multi-well plate reader (µquant Bio-Tek Instrument, Inc) interfaced with a personal computer. Slopes of absorbances were automatically recorded using KC junior software 1.31.5 (Bio-Tek Instrument, INC, USA). Excel 2003 (Windows) was used for all the other statistical calculations. Significant difference in mean between total phenolic compounds (TPC) and proanthocyanidins (PAs) levels was tested by student's t-test, $P < 0.05$ implies significance.

RESULTS AND DISCUSSION

Levels of phenolic compounds

Levels of phenolic compounds were expressed in terms of gallic acid equivalent (GAE) which corresponds to the mean response of all the major phenolic compounds in fruits and vegetables (Georgé et al., 2005). Among the screened fruits and vegetables, the highest levels of phenolic compounds were found in hot chili pepper (333.5 mg GAE/100 g, FW), okra, lemon, spinach and onion bulb (Table 1). Brat et al. (2006) have also determined total phenolics of green and red bell peppers (8.2 and 26.8 mg/100 g FW respectively), citrus genus fruits (30 to 45 mg/100 g), onion (76.1 mg/100g FW), garlic (59.4 mg/100 g, FW) and eggplant (65.6 mg/100 g, FW). These contents are lower than those found in fruits and vegetables in the present study except for bell peppers. The differences may be due to genetic and environmental factors including the climate, the soil mineral composition and the carrying and storage conditions (Tomás-Barberán et al., 2001). The particularity of hot chili pepper is its high content of the monophenolic compounds capsaicins such as capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homodihydrocapsaicin and homocapsaicin (Reilly et al., 2001). Hot chilli pepper is usually used in sauce as spice in combination with other ingredients. Marinating sauces are slightly heated and that might decrease phenolic antioxidant contents. Nevertheless, some studies still reported high levels in total phenolics for hot pepper-rich sauces (Thomas et al., 2010). Spinach and onion are known to contain high levels of flavonoids. Onion is particularly rich in quercetin, catechin, kaempferol and myricetin (Yang et al., 2004) and spinach is reported to display high levels of flavonoid derivatives of glucuronic acid (Bergman et al., 2001). Ortho-C acid was determined to be the major phenolic compound with a concentration of 2.78 mg/100 g for raw spinach (Bunea et al., 2008). The level reported by these authors is slightly higher than our results. The level of phenolic compounds in tomato found in the present study is comparable to data reported by Toor and Geoffrey (2005) who measured a similar content for different fractions of this fruit. It has been reported that flavonoids are the major phenolics in tomatoes. For okra and "gombo", we did not record previous reports on its content in phenolic compounds. Since the present study revealed its high level of phenolic compounds, it could be interesting to perform further analysis of its qualitative composition.

Levels of proanthocyanidins

The HCL/butanol assay used here for the determination of proanthocyanidins is more specific than many other tests such as the vanillin assay (Makkar, 2000; Santos-Buelga and Scalbert, 2000). The interferences which might result from flavan-4-ols conversion into proanthocyanidins (PAs) or from chlorophylls have been importantly destroyed during the heating step (Santos-Buelga et al., 2000; Prigent, 2005). Levels of PAs among screened fruits and vegetables from Burkina Faso ranged from 3.4 to 352 mg/100 g in sweet pepper and spinach Table 1). The results show that, spinach, onions, «gombo» and pepper have the highest contents in PAs (352; 234.7; 127.1 and 111.5 mg/100 g FW, respectively). Among the fruits mentioned in the literature, apple has been particularly reported to be a plant food which has very high content in PAs. It has even been reported, by butanol/HCl test, to display values up to 800 mg/100 g (FW/FW) of PAs for cider apples (Guyot et al. 1998). Whereas spinach has the highest level of PAs, these compounds were not detected in sweet pepper. A middle positive correlation (r = 0.5) was found between the content in phenolic compounds and proanthocyanidins of the samples. Levels of phenolic compounds and proanthocyanidins were not significantly different (P= 0.05, but for p= 0.07).

Nevertheless, they were not highly correlated, showing that PAs are diversely distributed in fruits and vegetables content in fruits and vegetables is not always sufficient to make an approximation of its total phenolic contents.

Antioxidant activities

The results of the measured antioxidant capacities of the samples are displayed in Table 1. The highest TEAC values were found in garlic, followed by «gombo», spinach and hot pepper. The lowest values were encountered in cucumber, zucchini and eggplant. Tamarindus showed a good TEAC value compared with the other fruits and vegetables. Concerning onion (bulb), its TEAC value was intermediate, whereas a low value was found for tomato.

The antioxidant capacity of most of the antioxidant compounds found in fruits and vegetables such as ascorbic acid, α-tocopherol, carotenoïds and organo-sulfur compounds is equivalent to their reducing capacity. The use of a single solvent for the extraction makes possible to take into account some eventual synergic interactions between antioxidants. The ranking order of antioxidant activities is similar to that found by Pellegrini et al. (2003) and Proteggente et al. (2002) for the common samples with this study (cabbage, eggplant, cucumber, onion (bulb), pepper, spinach, tomato, zucchini). The TEAC values are a little bit lower than those reported by Proteggente et al. (2002) but more close to data reported by Pelligrini et al. (2003) except the TEAC values of pepper, spinach and zucchini which are lower. The high TEAC value of garlic is in line with previous studies which had already reported its biological properties mainly due to the organo-sulfur compounds such as the S-alkyl-L-cysteine sulphoxides (Borek, 2001; Corzo-Martinez et al., 2007). The TPC value of garlic is the eighth behind those of hot pepper, lemon and onion, while its TEAC value is the highest. That denotes the important contribution of the organo-sulfur compounds and the other non phenolic antioxidant compounds in garlic antioxidant capacity expression. Onion (bulb), another vegetable of *Lilliaceae* family, expressed a TPC value among the highest, while its TEAC value was intermediate, in agreement with the fact that, flavonoids are abundant in onion, even more than in garlic (Corzo-Martinez et al., 2007). Spinach is known to be rich in flavonoids, phenolic acids and pigments such as lutein and chlorophyll which are also antioxidant compounds (Bergman et al., 2001; Lanfer-Marquez et al., 2005). These compounds may be responsible for the particularly high TEAC value of this vegetable. «Gombo» sample expresses a high TEAC value as well as high TPC and PAs values compared with the other vegetables (Table 1). We can thus, presume a good correlation between total polyphenols and antioxidant activity of okra. Its high antioxidant activity justifies well its use by the populations as antispasmodic. However, there is a lack of studies focused on the characterisation of this vegetable antioxidant compounds. The TEAC value of tamarindus sampled at the marked is similar to Lamien-Meda et al. (2008) result for the fruit directly harvested from tree. However, because of its high content in sugars and organic acids this sour-sweet taste fruit is susceptible to browning reactions during the postharvest handlings, causing variable modifications in phenolics contents. The consumers are not always beneficially affected by the post harvest modifications of the fruits and vegetables. That is an important reason, particularly when evaluating antioxidants intake in a group of people, why the bioactive contents and antioxidant activities are often assessed on samples that can reflect the consumer consumption of these substances. The low value of TEAC for tomato was also reported (Proteggente et al., 2002; Pelligrini et al., 2003). As proposed by Pellegrini et al. (2003), it may be due to a limit of the applied assay or to an incomplete extraction (Pelligrini et al., 2003).

The weak correlation between TEAC and TPC values indicates a diversity of content in antioxidant compounds; even for different varieties of the same species, it has been reported that, significant correlation may not appear (Kalkönen et al., 1999; Deepa et al., 2006; Saxena et al., 2007). That also confirms that in evaluating antioxidant activity, the Folin-Ciocalteu assay for total phenolic compounds is not enough to draw a conclusion on the antioxidant potential of fruits and vegetables.

Conclusions

The present study allowed estimating levels of phenolic compounds and antioxidant activities among fruits and vegetables widely available in Burkina Faso. The major fruits and vegetables which displayed high antioxidant activities were garlic, okra, spinach, hot chili pepper, onion (bulb and leaves) and lemon. Therefore, these fruits and vegetables may be considered as good sources of bioactive compounds. Their consumption as sources of nutraceutics could be recommended particularly in groups of people where the incidence of oxidative stress-induced diseases is quite important (cancers, AIDS and so on). For an optimal use of these significant sources of natural antioxidants, more research may be envisaged to characterize the antioxidant compounds and to determine the effects of post harvest factors and culinary process on their final antioxidant activities.

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