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Comparison of phenolic compounds and antioxidant capacities of traditional sorghum beers with other alcoholic beverages

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Thirty samples of sorghum beers “dolo” were selected from traditionally fermented household manufacturers from Burkina Faso. Dolo samples were screened for their total phenolic content, proanthocyanidins and putative antioxidant capacities, and were compared with industrial beers and wines. Total phenols were measured using the Folin-Ciocalteu method. Proanthocyanidins content were determined by the method of HCl-butanol hydrolysis. Antioxidant activities were evaluated both with 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and by the trolox equivalent antioxidant capacity (TEAC) using 2,2-azinobis(3-ethyl-benzothiazoline-6-sulfonic acid radical) (ABTS⁺). The average contents of total phenols and proanthocyanidins were 506 µg GAE/ml of dolo and 45 µg APE/ml of dolo, respectively. An average antioxidant capacity was found in 148 µmol of TEAC per litre of dolo or 2.2 µmol of TEAC per gram of dolo. Proanthocyanidins in dolo represent on average, 10% of total phenolic content. Results also showed that the red wines from different brands had higher levels of phenolic content and antioxidant capacities than dolo. Nevertheless, dolo displays higher levels of total phenolic content than lager beers and white wines. Sorghum grains as well as their derived food-products such as local beers are good sources of bioactive compounds comparable to other industrial alcoholic beverages.

Key words: *Sorghum bicolor*, dolo, beer, total phenols, proanthocyanidins, antioxidant capacity.

INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is an important food crop grown in arid and semi-arid regions of the world (Owuama, 1997). Sorghum beverages, whether alcoholic or non-alcoholic, are consumed in many African

countries (Muyanja et al., 2003). Dolo is a local beer manufactured from malted red sorghum grains in few steps, including malting, massing, yeast fermentation, filtration and conditioning (Figure 1). Dolo, like other African beers, has an economic and cultural importance in Africa, especially for women. Indeed, in Africa, more than half annual production of sorghum is processed into local beer. In Burkina Faso, the production of the sorghum as a staple food is on average one and half tons per year (FAO, 2011).

The potential of sorghum malt as an alternative source for lager beer brewing from barley malt has been described (Owuama, 1997). The production of alcoholic drinks constitutes the oldest industry of fermentation. The beers, while not being foods as such, are important

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Abbreviations: AA, Antioxidant activity; ABTS, 2,2'-azino-bis(3-ethylbenzthiazoline)-6-sulfonic acid; APE, apple procyanidins equivalent; BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; DPPH, 2,2-diphenyl-1-picrylhydrazyl; GAE, gallic acid equivalent; TEAC, trolox equivalent antioxidant capacity.

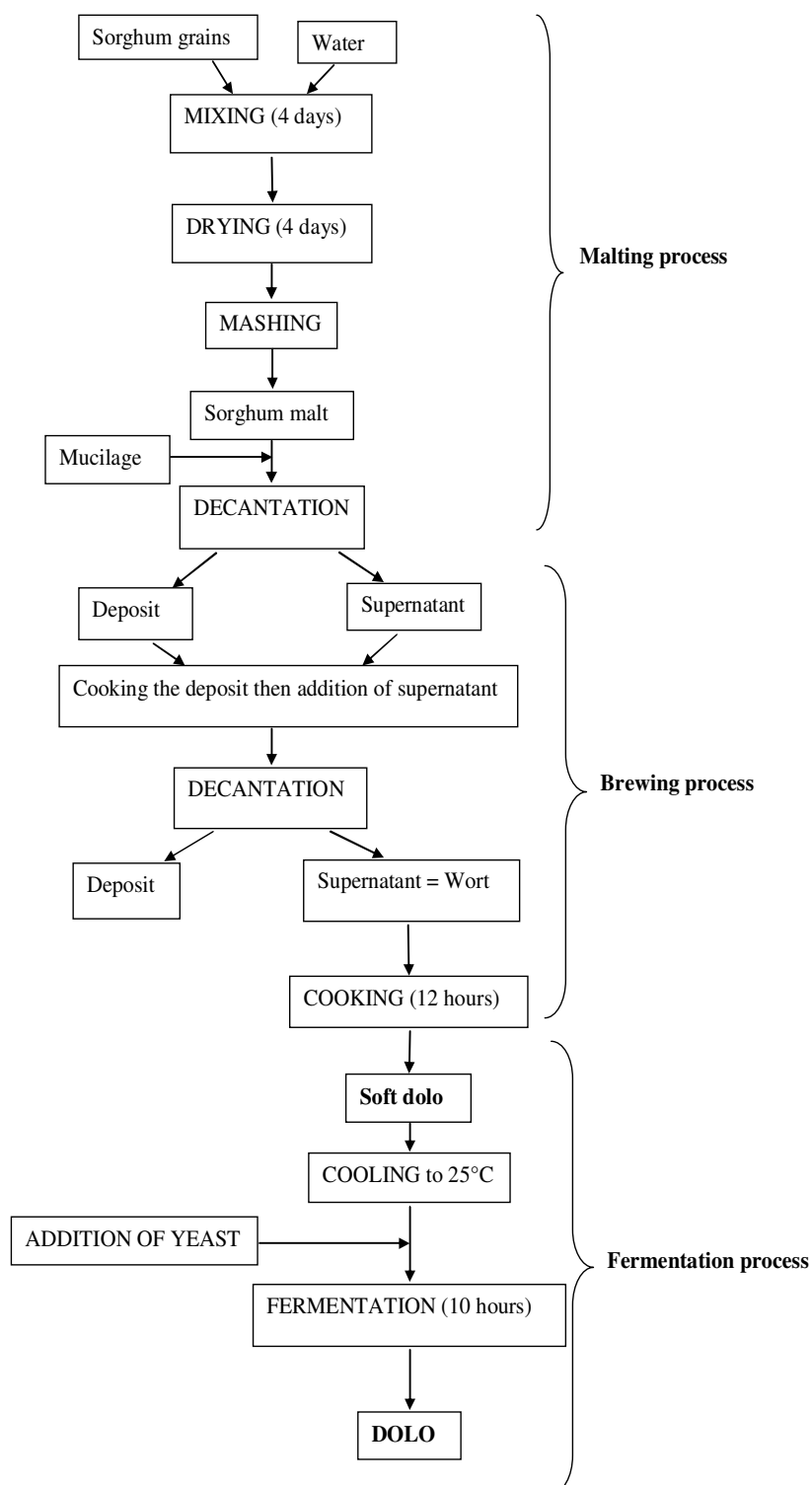


Figure 1. Steps of local sorghum beer "dolo" processing in Burkina Faso.

drinks in Africa. Sorghum beer is an opaque alcoholic beverage made from malted red sorghum grains; so it considerably differs from conventional clear lager type beers. Its opaque or pinkish-brown colour is correlated

with its contents in phenols, suspended particles and yeasts. Sorghum grain is known as a good source of phenolic compounds with a variety of genetically dependent types and levels, including phenolic acids,

flavonoids and proanthocyanidins (condensed tannins). Not all sorghums contain proanthocyanidins, but all contain phenolic acids and flavonoids. Pigmented sorghums contain anthocyanins, namely 3-deoxyanthocyanidins (apigininidins and luteolinidins) that could be potential food colorants. Some sorghums have a prominent pigmented testa that contains proanthocyanidins, which are polymers of flavan-3-ol units with variable degree of polymerization (Dykes and Rooney, 2006).

Nowadays, there is a prominent interest on food and drinks that contain antioxidant molecules such as phenolic compounds (Brohan et al., 2011; Djoulde et al., 2010). Indeed, phenolic compounds have been reported to inhibit the development of cancerous tumours, and to have anti-bacterial, anti-inflammatory, antispasmodic and anti-diarrhoeic properties (Awika and Rooney, 2004; Johnson, 2004). The potential for HIV infection and its treatment to be associated with an increased risk for cardiovascular disease is real and there may also be a role for antioxidants in improving this risk (Fawzi et al., 2004; Marston and De Cock, 2004; Tang et al., 2005). Most bioactivities of phenolic compounds are believed to be highly linked to their antioxidant activities. In spite of this promising evidence about the antioxidant action of the aforementioned compounds, there is a lack of local investigation on levels of phenolic content and antioxidant activities among local beers currently consumed in Africa. Since the environment and food processing have an impact on the levels of phenolic content, it is important to assess these parameters in some final sorghum foods and beverages (Tomás-Barberán and Espín, 2001). Several raw data on levels of phenolic content and antioxidant activities in sorghum grains are available (Dykes and Rooney, 2007). However, it is very scarce to find data on these compounds in sorghum end-products. Therefore, this study aimed at comparing the levels of phenolic content and antioxidant capacities between traditional beers, industrial beers and wines.

MATERIALS AND METHODS

Sampling

Thirty samples of dolo were bought in October 2009 in fifteen commercial suppliers of Ouagadougou, Burkina Faso. Local households are selected according to their geographical distribution. Samples were transported in a cooling box (0°C) to the laboratory. Only drinkable samples of 1 L each, manufactured not more than two days were collected. Some industrial beers and wines were also collected. Prior to analysis, samples were stored at -30°C.

Chemicals

Gallic acid (3,4,5-trihydroxybenzoic acid) was obtained from Aldrich (Steinheim, Germany); 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), Folin-Ciocalteu reagent, 2,2-diphenyl-1-

picrylhydrazyl (DPPH) and 6-hydroxy-2,5,7,8 tetamethylchroman-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 were purified by reverse-phase high performance liquid chromatography (RP-HPLC) and characterized by thiolysis-HPLC (Prigent, 2005). All the other chemicals were of analytical grade.

Phenolic content

The Folin-Ciocalteu method was used to measure total amount of phenolic content (Singleton et al., 1999). The original assay was adapted to a microtiter 96-wells plate system (Dicko et al., 2005a). Gallic acid (3, 4, 5-trihydroxybenzoic acid) was used as standard. To 20 μ L of beverage, 80 μ L of Folin-Ciocalteu reagent were added. After 5 min incubation at room temperature (20°C), 80 μ L of 20% (w/v) sodium carbonate solution was added and incubated. After 30 min of incubation, the absorbances were read at 760 nm. All tests were carried out in triplicate and results were expressed as gallic acid equivalent (GAE).

Proanthocyanidins (PAs) were quantified with an adaptation to a 96-well plate assay (Dicko et al., 2005a). It involved the hydrolysis of proanthocyanidins in a hot acid-alcohol medium into anthocyanidins. This method allows taking into account all the units of flavan-3-ols constituting the polymers (Prigent, 2005). The heating step destroys the anthocyanidins pigments generated by flavan-4-ols and eliminates part of the chlorophyll pigments. The routine assay is performed by mixing 50 μ L of the extract with 700 μ L of 30% HCl-butanol solution (v/v). The mixture was put in tightly closed 1.5 ml Eppendorf tube and vortexed for 1 min. Subsequently, the tube was heated at 100°C for 2 h and after cooling, 200 μ L aliquots were put in triplicate into a 96 multiwell plate and the absorbances were read at 550 nm. Apple procyanidins (DP ≈ 7.4) treated as aforementioned were used as a standard. Results were expressed as apple procyanidins equivalent (APE).

Antioxidant capacities

Since the evaluation of antioxidant capacity may vary according to the used methods, it was estimated both with 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and by the trolox equivalent antioxidant capacity (TEAC) assay using 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid radical). Trolox is a water soluble cyclic molecule homologous to α -tocopherol (vitamin E). A stable stock solution of ABTS^{•+} radical was produced by reacting a 7 mM of aqueous solution of ABTS with 2.45 mM potassium persulfate (as final concentrations). This mixture was stored in the dark at 25°C for 16 h before use. The daily working solution was obtained by diluting the stock solution in 50 mM sodium phosphate, pH 7.4 to an absorbance between 0.7 and 0.9 at 734 nm. The ability of phenolic compounds to scavenge the ABTS^{•+} radicals was monitored (Riedl and Hagerman, 2001). The control assays were performed by replacing sample with distilled water or ABTS^{•+}, with 50 mM sodium phosphate, pH 7.4. The decrease in absorbance was monitored over 30 min with 1 min interval. The decrease of absorbance over time was plotted and the differences between samples and controls assays were calculated. All tests were carried out in triplicate and results were expressed as TEAC, which is μ mol of Trolox equivalent per gram of fresh matter (Dicko et al., 2005a; Prior et al., 2005).

DPPH spectrophotometric (quantitative) assay was performed with some modifications (Brand-Williams et al., 1995). DPPH

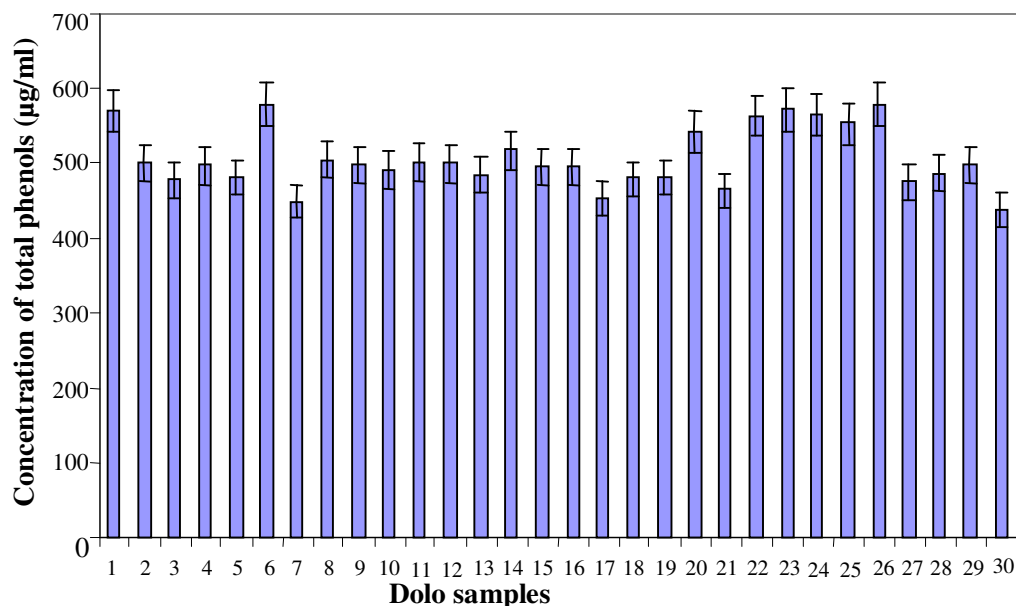


Figure 2. Comparison of content of total phenols in 30 samples of dolo.

method was widely used to test the ability of antioxidant compounds to act as free radical scavengers or hydrogen donors. This test is based on the capacity of stable free radical 2,2-diphenyl-1-picrylhydrazyl to react with hydrogen (H) donors, including phenols. It is used for the quantification of antioxidants in the complex of biological systems (Miliauskas et al., 2004). For the routine assay, a 100 µM DPPH solution was prepared by adding 4 mg of DPPH to 101.44 ml ethanol. The solution was vortexed vigorously until all DPPH was dissolved. Samples were diluted 10, 7, 5, 4, 3, or 2 folds in ethanol. Afterward, 1 ml of 100 µM DPPH in ethanol solution was added to 1 ml of diluted sample. The mixture was allowed to react at room temperature in the dark for 15 min. Control assays were prepared with 1 ml of sample solution and 1 ml of ethanol only; while the negative control was 1 ml of DPPH solution and 1 ml of ethanol. The absorbance values of the compounds changing from violet to yellow color were measured at 520 nm and converted to percentage antioxidant activity (AA%) using the formula:

$$AA\% = \frac{[Abs_{control} - (Abs_{sample} - Abs_{blank})]}{Abs_{control}} \times 100$$

Where, Abs_{sample} is the absorbance of the sample; Abs_{blank} is the absorbance of the blank and $Abs_{control}$ is the absorbance of the control. IC_{50} is defined as the concentration of the test sample leading to a 50% inhibition of the DPPH free radicals. IC_{50} value was calculated from the separate linear regression of plots of the mean percentage of the antioxidant activity against concentration of the test compounds (µM) obtained from three replicate assays. Moreover, L-ascorbic acid (vitamin C), butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) diluted to final concentrations of 100, 70, 50, 40, 30, 20, 10 µM in ethanol were used as positive controls.

Statistical analysis

Spectrometric assays were carried out in 96-well microtiter plates using a multiwall 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). All assays were carried out in triplicate and results were reported as means \pm standard deviation (SD) in triplicates. Microsoft Office Excel 2007 software for Windows was used for all the other statistical analyses.

RESULTS AND DISCUSSION

Levels of phenolic content

Levels of phenolic content were expressed in terms of gallic acid equivalent (GAE). The equation of the right-hand side of the proportioning of total phenolic content by the method of Folin-Ciocalteu gave $Y = 0.0012 X - 0.0004$ with $R^2 = 0.9902$. The total contents of phenols ranged between 437 and 578 µg GAE/ml of dolo (Figure 2). The average total phenolic content was 506 µg GAE/ml of dolo. Dolo samples showed significant differences in their content in total phenols. Screened beers, wines and dolo were also ranked according to their content in total phenols (Table 1). Total phenolic content of dolo were higher than Heineken lager beer, Flag special beer, Amsterdam beer and white wine, but they were lower than all red wines (Table 1).

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

Table 1. Comparison of total phenolic compounds, proanthocyanidins and antioxidant capacity of some beers, wines and dolo.

Alcoholic beverage	Total phenols ($\mu\text{g GAE/ml}$ of beverage)	PAs ($\mu\text{g APE/ml}$ of beverage)	DPPH: IC ₅₀ ($\mu\text{g/ml}$)	Rank*
Red wine: Les 3 rochers, Bordeaux**	2848 \pm 27	1208 \pm 16	(6.80 \pm 0.2) $\times 10^{-6}$	1
Red wine: Château de Guirauton, Graves, Grand vin de Bordeaux**	2514 \pm 21	1197 \pm 12	(4.55 \pm 0.1) $\times 10^{-5}$	2
Red wine: Merlot, Finca del Mar, Gandia**	2474 \pm 21	1164 \pm 11	(4.00 \pm 0.1) $\times 10^{-5}$	3
Red wine: Vin de pays d'OC. Merlot, Cepage Merlot**	2434 \pm 14	2175 \pm 20	(1.55 \pm 0.1) $\times 10^{-6}$	4
Red wine: J. P. Chenet, Premier de Cuvée, Merlot-Cabernet**	2354 \pm 11	1353 \pm 13	(1.48 \pm 0.2) $\times 10^{-4}$	5
Red wine: Finca del Mar, Cabernet Sauvignon, Vicente Gandia**	2101 \pm 20	1108 \pm 11	(1.35 \pm 0.1) $\times 10^{-4}$	6
Red wine: Cellier d'Or, Vin de table Français, Louis Eschenauer**	1781 \pm 11	997 \pm 3	(1.73 \pm 0.1) $\times 10^{-5}$	7
Dolo	506 \pm 40	45 \pm 5	(6.56 \pm 0.3) $\times 10^{-4}$	8
Amsterdam beer, Holland**	374 \pm 3	53 \pm 2	(5.23 \pm 0.2) $\times 10^{-4}$	9
Flag special beer, Luxe beer**	240 \pm 7	48 \pm 1	(2.22 \pm 0.1) $\times 10^{-4}$	10
Heineken beer, Heineken Lager beer**	206 \pm 2	34 \pm 1	(1.01 \pm 0.1) $\times 10^{-4}$	11
White wine: J. P. Chenet, Vin blanc de pays des Cotes de Gascogne**	187 \pm 1	49 \pm 2	(1.32 \pm 0.1) $\times 10^{-3}$	12

*Beverages were ranked according to their content in total phenolic compounds; **values are presented as the mean of 3 replicates \pm standard deviation (SD).

result from flavan-4-ols conversion into proanthocyanidins or from chlorophylls, may have been minimized during the heating step (Prigent, 2005; Santos-Buelga and Scalbert, 2000). Levels of proanthocyanidins were expressed in terms of apple procyanidins equivalent (APE). The equation of the right-hand side of the

proportioning of the proanthocyanidins by the HCl-Butanol method gave $Y = 0.0006 X + 0.0024$ with $R^2 = 0.9869$. Among dolo samples, proanthocyanidin contents ranged between 38 and 55 $\mu\text{g APE/ml}$ of dolo (Figure 3). The average content in proanthocyanidins was 45 $\mu\text{g APE/ml}$ of dolo. Thus, proanthocyanidins in dolo represent

on average 10% of total phenolic content. This finding may be due to the fact dolo is prepared in general with red sorghum grains and with pigmented testa layer rich in proanthocyanidins. There is a significant variation of the content of proanthocyanidins among dolo samples. The sprouted grains of sorghum contain on average

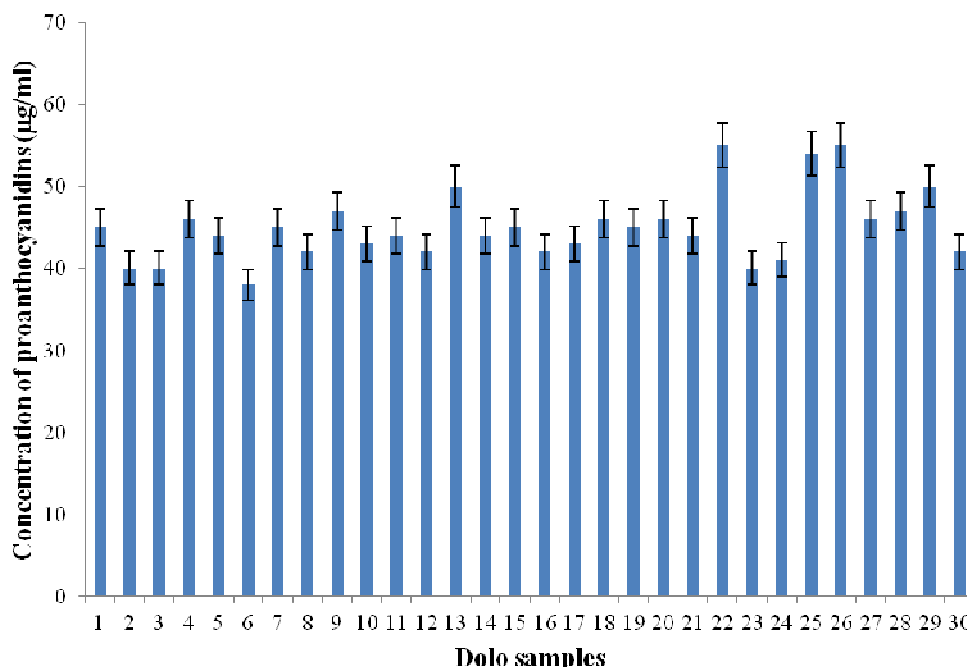


Figure 3. Comparison of content of proanthocyanidins in 30 samples of dolo.

0.23% of proanthocyanidins (Dicko et al., 2005a). It is noted that germination causes a light reduction in the content of proanthocyanidins. The samples of dolo contained on dry matter basis, more total phenolic content and proanthocyanidins than those of the germinated grains of sorghum. This is due to the fact that the beers are prepared from sorghum varieties whose content of total phenolic are very high. Germination in general causes a reduction in the content of total phenols. Proanthocyanidins compounds of dolo were higher than Heineken Lager beer, but lower than Flag special beer, Amsterdam beer, white wine and all red wine (Table 1).

Antioxidant capacities

The antioxidant activities of some samples were either determined with DPPH or the TEAC because it is practically difficult to monitor antioxidant activities of some samples both with the DPPH and the TEAC assays. Reduction of DPPH[•] by an antioxidant (DPPH[•] + A → DPPH-H + A[•]) or by radical species (DPPH[•] + R[•] → DPPH-R) results in a decrease of absorbance at 520 nm. Thus, the degree of discoloration of the solution indicates the scavenging efficiency of the added substance. DPPH is likely more selective than ABTS^{•+} in the reaction with H-donors. In contrast to ABTS^{•+}, DPPH does not react with flavonoids, which contain no OH-groups in B-ring, as well as with aromatic acids containing only one OH-group (Yokozawa et al., 1998). The DPPH test was suggested

to act by two mechanisms, the dynamic and static. In the first version, one measures the rate of DPPH decay observed after the addition of a phenolic-containing sample. In the static version, one determines the amount of DPPH scavenged by a tested sample. While the first assay characterizes the reactivity, the second is linked to the Stoichiometry of the reaction of DPPH with H-donor for individual substance or the quantity of active OH-groups in complex mixture (Yokozawa et al., 1998).

The Trolox equivalent antioxidant capacity (TEAC) of the samples ranged between 57 and 349 µmol/L of dolo, with an average of 148 µmol/L of dolo (Figure 4). This is equivalent to 2.2 µmol of trolox equivalent per gram of dolo. There is a great dispersion of the antioxidant capacity among samples. Dolo contains an antioxidant capacity lower than the grains of red sorghums which were ranging between 30 to 80 µmol of TEAC per gram of fresh matter (Dicko et al., 2005b). It is known that levels of phenolic content in beer decrease as function of time of the various stages of the beer preparation (Aron and Shellhammer, 2010). It appears that there is a poor correlation between levels of phenolic content and the antioxidant capacity of the dolo ($R^2 = 0.3$). The antioxidant capacity of the dolo may not be systematically linked to the occurrence of phenolic compounds. These results suggest that antioxidant capacities in dolo, in contrast to sorghum grains are not essentially governed by phenolic compounds. Dolo's antioxidant activities may be linked to other antioxidative compounds such as β-carotenes, tocopherols, peptides, etc (Miller and Rice-Evans, 1997). Dolo has higher antioxidant capacities than

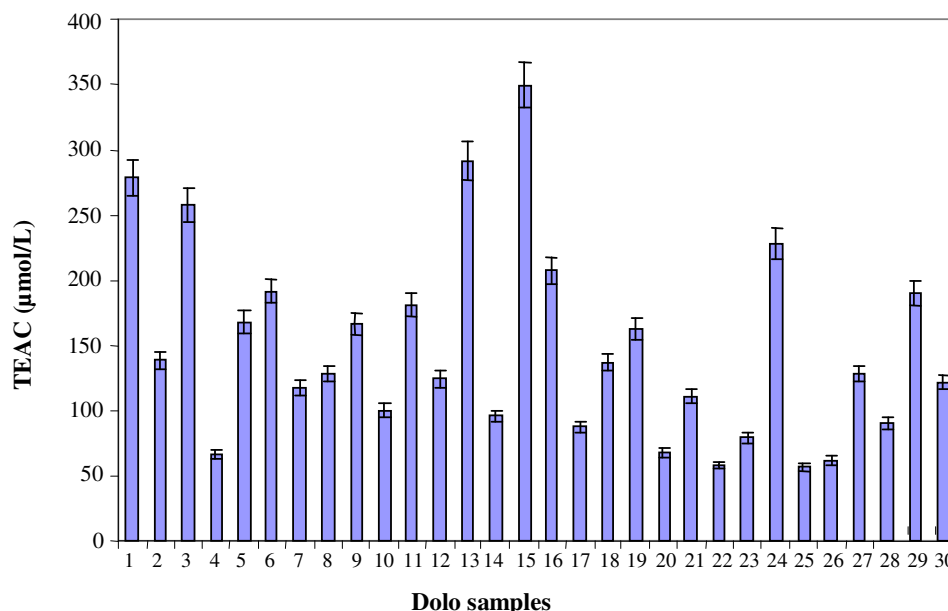


Figure 4. Comparison of antioxidant capacity in 30 samples of dolo.

some alcoholic beverages and lower antioxidant capacities than whisky and red wines (Pellegrini et al., 2003).

In the DPPH test system, the results were expressed as IC_{50} (Locatelli et al., 2009). The IC_{50} value of dolo was 6.56×10^{-4} $\mu\text{g/ml}$. The ascorbic acid, BHA and BHT used as standards showed IC_{50} values of 19.90, 5.3 and 18.6 $\mu\text{g/ml}$, respectively. The DPPH value of dolo was better than DPPH values of ascorbic acid, BHA and BHT. The DPPH radical scavenging capacity of dolo was also better than white wine, but there were less than Heineken lager beer, Flag special beer, Amsterdam beer and all red wines (Table 1). Moreover, the IC_{50} values were not correlated with the total phenolic content ($R^2 = 0.43$) and proanthocyanidins ($R^2 = 0.38$). However, without the inclusion of data from white wine, Heineken beer, Flag special beer, red wine "Vin de pays d'OC" and red wine "Cellier d'Or" the IC_{50} values have a good correlation with the total phenolic content ($R^2 = 0.95$) and proanthocyanidins ($R^2 = 0.91$). This deviation could be linked to the fact that the samples contained a lot of sulphites which interact with antioxidant assays. In fact sulphites are highly added in alcoholic beverages such as wines for conservation in tropical environment. This hypothesis is supported by the findings of Saxena et al. (2007) who reported a low correlation between phenolic compounds and antioxidants activities. Thus, compounds used for the conservation of alcoholic beverages could interact with the reagents and decreased significantly the correlation.

The samples of dolo had good DPPH radical scavenging capacity, but low antioxidant potential according to TEAC assay. These differences may be due to their different antioxidant mechanisms. The results of

the determination of the antioxidant capacity of a sample depend greatly on the methodology used. It appeared that it is important to compare different analytical methods varying in their oxidation initiators and targets in order to understand the biological capacity of an antioxidant (Cao and Prior, 1999). The antioxidant capacity could depend on the sample and type of phenolic compounds, which means that different phenolic compounds react in different ways. In contrast to fruits and vegetables (Sariburun et al., 2010), as well as malted sorghum grains (Dicko et al., 2005b), the phenolic compounds may not greatly govern the antioxidant behaviour of alcoholic beverages because of the presence of sulphites.

Conclusion

Analyses of the dolo samples were performed to determine levels of total phenolic content, proanthocyanidins and antioxidant capacities. The chemical substances present in this beer of sorghum highlight a beneficial antioxidant capacity of the dolo against the free radicals *in vitro*. Because of the putative beneficial properties of food containing antioxidants, it appeared that dolo constitutes a potential source of antioxidants. In addition, these findings showed that dolo samples display higher levels of phenolic content than lager beer and white wines.

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