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Inhibition of some enzymes implicated in diabetes mellitus by raw and blanched extracts of African lettuce (*Launaea taraxacifolia*)

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ABSTRACT: This study evaluates the impacts of blanching on α -amylase, α -glucosidase and lipase activities, and Fe²⁺-induced lipid peroxidation (LPO) inhibitory potentials as well as phenolic constituents of African lettuce (AL) leaves. The results revealed that both raw and blanched of AL extracts inhibited all the enzymes dose-dependently, but extract from raw AL had the highest α -amylase (IC₅₀ = 0.20 mg/mL), α -glucosidase (IC₅₀ = 0.27 mg/mL) and lipase (IC₅₀ = 0.45 mg/mL) inhibitory potential compared to that of blanched AL (α -amylase, IC₅₀ = 0.33 mg/mL; α -glucosidase IC₅₀ = 0.52 mg/mL, lipase IC₅₀ = 0.71 mg/mL). Raw AL extract exhibited highest lipid peroxidation (IC₅₀ = 1.01 mg/mL) inhibitory effect, scavenged DPPH (0.62 mg/mL) and OH (0.40 mg/mL) radicals than the blanched (LPO, IC₅₀ = 1.43 mg/mL; DPPH, 0.82 mg/mL; OH, 0.75 mg/mL). Furthermore, catechin, caffeic and ellagic acid, epigallocatechin, rutin, isoquercitrin and quercetin were identified in addition with gallic and chlorogenic acids and quercitrin in raw AL, while caffeic acid derivative (13.40 mg/g) was detected in blanched AL only. Conclusively, blanching reduced the antioxidant and antidiabetic potentials and phenolic contents of African lettuce leaves as evidence in the blanched AL.

Keywords: African lettuce, blanching, enzymes, antioxidant, polyphenols, diabetes mellitus.

Introduction

Diabetes mellitus is a known metabolic syndrome resulting from dysfunction of insulin production (Ryu *et al.*, 2013; Saravanan and Parimelazhagan, 2014). Deactivation/reduction of α -amylase, α -glucosidase and lipase activities have been established as control points in the management of diabetes mellitus (Ademiluyi *et al.*, 2015; Naik *et al.*, 2016). Consumption of dietary food rich in phytochemicals, as alternative therapeutics is a reliable practice in managing the disease (Ademiluyi *et al.*, 2015; Adefegha *et al.*, 2015; Adedayo *et al.*, 2015). In many homes today, consumption of vegetables is on the increase. The reason is because epidemiological studies have positively linked the intake of vegetables, known to be rich in bioactive compounds such as polyphenols, with effective management of diabetes mellitus.

According to Ryu *et al.* (2013) and Ademiluyi *et al.* (2015), polyphenols, a secondary metabolite phytochemicals, possessed insulin-like ability and could also prevent oxidation of biomolecules such as lipids, protein and genetic materials which, if left unchecked, can induce oxidative stress (Adedayo *et al.*, 2015; Oboh *et al.*, 2016). However, there are reports on the effects of processing, including blanching, on the biological effect of polyphenols.

African lettuce (*Launaea taraxacifolia*, Family: Asteraceae) is a tropical leafy vegetable, commonly consumed in Nigeria. The leaf is often used in folklore medicine as an alternative remedy in the treatment of several human ailments, including diabetes (Adebisi, 2004; Adinortey *et al.*, 2012), but information on the mechanism of action is scarce. In Nigeria, African lettuce is blanched, before consumption, purposely to increase the acceptability and palatability. However, till date, the effect of blanching on the functional/nutraceutical values of African lettuce has not being evaluated. This study aimed at evaluating the effects of blanching on α -amylase, α -glucosidase and lipase activities, and Fe²⁺-induced lipid peroxidation inhibitory perennials of African lettuce including their constituents phenolics

Materials and Methods

Chemical and reagents

Chemicals and reagents used in this study were of analytical grade and the water was glass distilled. Kenxin (Model KX3400C) model of refrigerated centrifuge and JENWAY UV-visible spectrophotometer (Model 6305; Jenway, Barlo World Scientific, Dunmow, United Kingdom) were used to centrifuge and measure absorbance respectively.

Sample processing

Fresh leaves of African lettuce (AL) were harvested from the University Medicinal Farm Garden between March and April 2018 and authenticated by A. A. Shorungbe (Voucher Number FUTA/BIO/131). The leaves were divided into two (2) portions; the blanched (BAL) (treated for 5 min at 80°C), and raw (RAL). Both portions (RAL and BAL) were dried at 30°C and blended. The extracts were prepared and the clear homogenate obtained was used.

Inhibition of α -amylase, α -glucosidase and lipase enzymes assays

Effect of the extracts on hog pancreatic α -amylase (0.5 mg/mL, EC 3.2.1.1) and α -glucosidase activities was determined using Worthington Biochemical Corp (Worthington, 1993) and Apostolidis *et al.* (2007) respectively. On lipase activity, Licia *et al.* (2006) method was used.

Antioxidant activity

The lipid peroxidation determination was according to the method of Ohkawa *et al.* (1979). The method of Halliwell and Gutteridge (1981) was used for hydroxyl radical scavenging ability of the extracts, while Puntel *et al.* (2015) method was used for Fe²⁺ chelating ability of the extracts

Spectrophotometric and HPLC-DAD analysis of phenolic in African lettuce

The total phenol content of the extracts was determined according to the method of Singleton *et al.* (1999) and presented as gallic acid equivalent. The total flavonoid content follows Meda *et al.* (2005), and calculated as quercetin equivalent. The quantification of phenolic compounds in the extracts was carried out according to the method described by Adedayo *et al.* (2016). using high performance liquid chromatography coupled with Diode-Array Detector (HPLC-DAD).

Data analysis

The results of three replicates reading were pooled together and expressed as mean $\pm$ standard deviation. One-way analysis of variance (ANOVA) and least significance difference (LSD) were carried out. Duncan multiple range tests were used to carry out post hoc analysis. Concentration needed to scavenge/inhibit 50% of radical/enzyme activity under the described assay conditions (IC₅₀) was calculated by nonlinear regression analysis. Significance was accepted at $p \leq 0.05$.

Results and Discussion

The effect of the extracts on some enzymes of carbohydrate metabolism are presented in Fig. 1A and 1B while IC₅₀ values are listed in Table 1. The extracts inhibited α -amylase activity, however, raw AL extract exhibited higher α -amylase inhibitory potential than the extract from blanched AL. In a similarly, extract from raw leaf of AL showed the highest α -glucosidase inhibitory potential than the extract from BAL. The same manner of effect was also observed on pancreatic lipase activity where extract from raw AL had higher inhibitory potential than the extract from blanched leaf of AL (Table 1)

Table 1. IC₅₀ values of α -amylase and α -glucosidase, Fe²⁺- induced lipid peroxidation inhibitory potentials, radicals (DPPH*, OH*) scavenging and Fe²⁺ chelating abilities of extracts from raw (RAL) and blanched (BAL) African lettuce (mg/mL)

Parameters	RAL	BAL
α -Amylase inhibition	0.20±0.01 ^a	0.33±0.04 ^b
α -Glucosidase inhibition	0.27±0.01 ^a	0.52±0.02 ^b
Lipase inhibition	0.45±0.03 ^a	0.71±0.07 ^b
Fe ²⁺ induced lipid peroxidation	1.01 ±0.03 ^a	1.43±0.01 ^b
DPPH* scavenging ability	0.62±0.02 ^a	0.82±0.02 ^b
OH* scavenging ability	0.40±0.05 ^a	0.75±0.03 ^b
Fe ²⁺ chelating ability	0.25±0.01 ^a	0.51±0.04 ^b

Values represent means of triplicate. Values with the same alphabet along the same row are not significantly different ($P > 0.05$)

Inhibition of carbo enzymes is one of the therapeutic approaches in managing diabetes as these delays carbohydrate digestion and ultimately glucose absorption (Kumar *et al.*, 2011; Ryu *et al.*, 2013; Adefegha *et al.*, 2014; Ademiluyi *et al.*, 2015). The inhibition of α -amylase and α -glucosidase by the vegetable extract, which is in line with several reports, could be attributed to the presence of phenolic compounds in the vegetables (Saliu and Oboh, 2013). More so, the higher α -amylase and α -glucosidase inhibitory potential of the RAL extract correspond to its phenolics, notably, caffeic and chlorogenic acids, and quercitrin (Oboh *et al.*, 2014; Abirami *et al.*, 2014; Adefegha *et al.*, 2015). Also, the synergistic effects of these phenolics could have influenced the strongest inhibitory effect of raw extract.

Reports on inhibition of enzyme, such as pancreatic lipase, which is responsible for more than 70% hydrolysis of dietary fats, is also pivotal in the management of metabolic disorder (Birari and Bhutani, 2007). Pancreatic lipase is responsible for the digestion and absorption of fatty composition of food (You *et al.*, 2012). Hence inhibition of pancreatic lipase is a valuable pathway for the treatment of diet-induced hyperglycaemia in humans. As shown in Fig. 1C and Table 1, AL extracts inhibited lipase activity, but RAL had the highest effect.

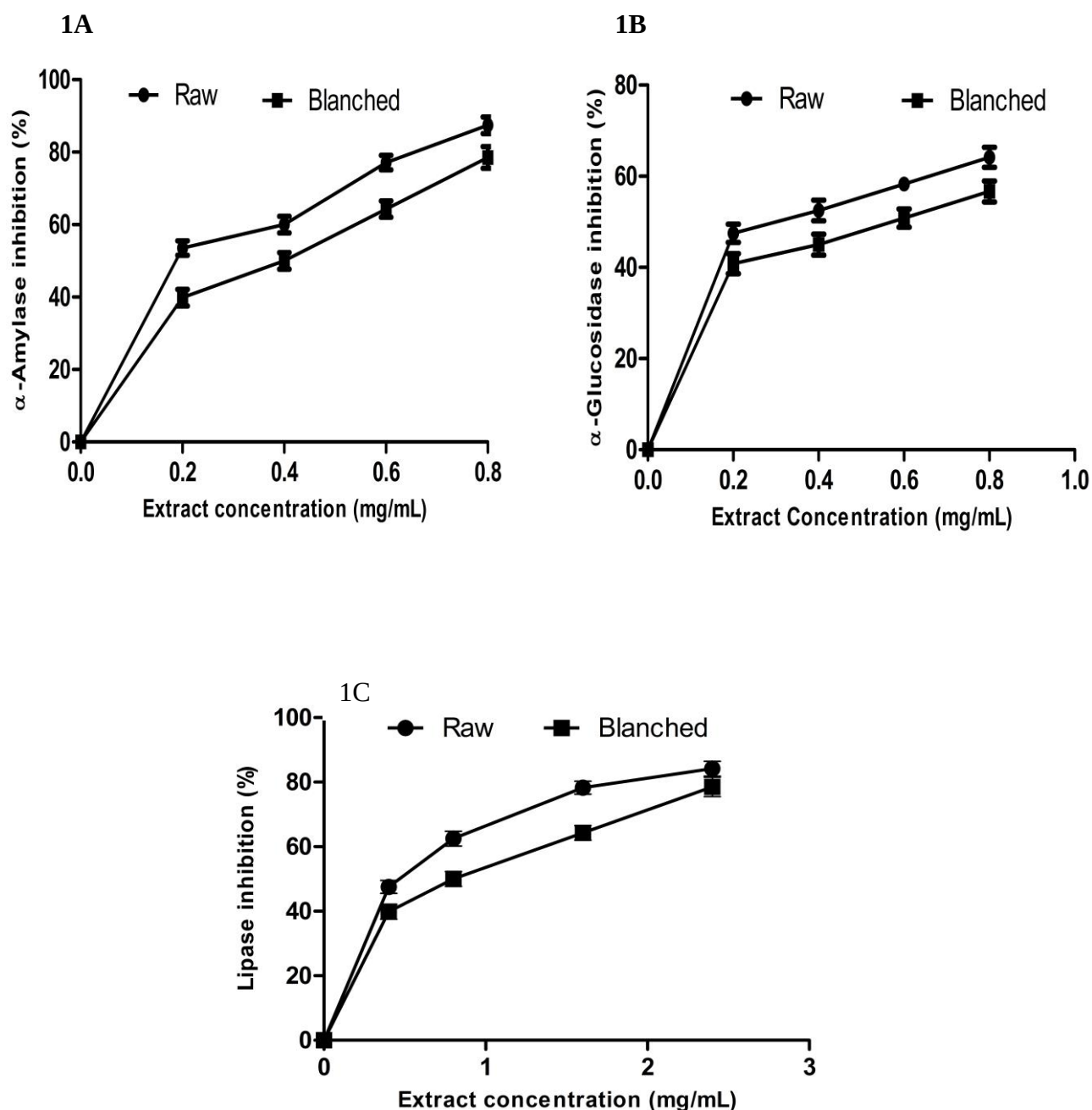


Figure 1: A- α -Amylase, B- α -Glucosidase and C-Pancreatic lipase inhibitory activity of extracts from raw and blanched African lettuce. Values represent mean $\pm$ standard deviation (N=3)

The extracts further inhibited MDA production, and scavenged hydroxyl and DPPH radicals, chelate Fe^{2+} (Fig 2A, B and 2 respectively), and the extract from raw leaf had the highest effect (Table 1). In addition. Oxidative stress is responsible for many incidence and progression of diabetes and its complications (Stephens *et al.*, 2009). The increased level of MDA could be due to iron's ability to break down lipid peroxide, which in turn causes radicals formation and favours lipid peroxidation (Bayir *et al.*, 2006; Ademiluyi *et al.*, 2014). Interestingly, the studied extracts inhibited lipid peroxidation in the tissue homogenate, scavenged OH radical and, also chelate Fe^{2+} , the effect that could be linked to the presence phenolic compounds (Obboh *et al.*, 2014; Adefegha *et al.*, 2015).

Consumption of dietary phenolic-rich foods such as the studied sample could be of use to strengthen endogenous antioxidant (Adefegha *et al.*, 2015; Oboh *et al.*, 2018). The antiradicals and Fe^{2+} chelating abilities of the studied extracts could be among the possible mechanisms through which the studied samples prevent lipid peroxidation.

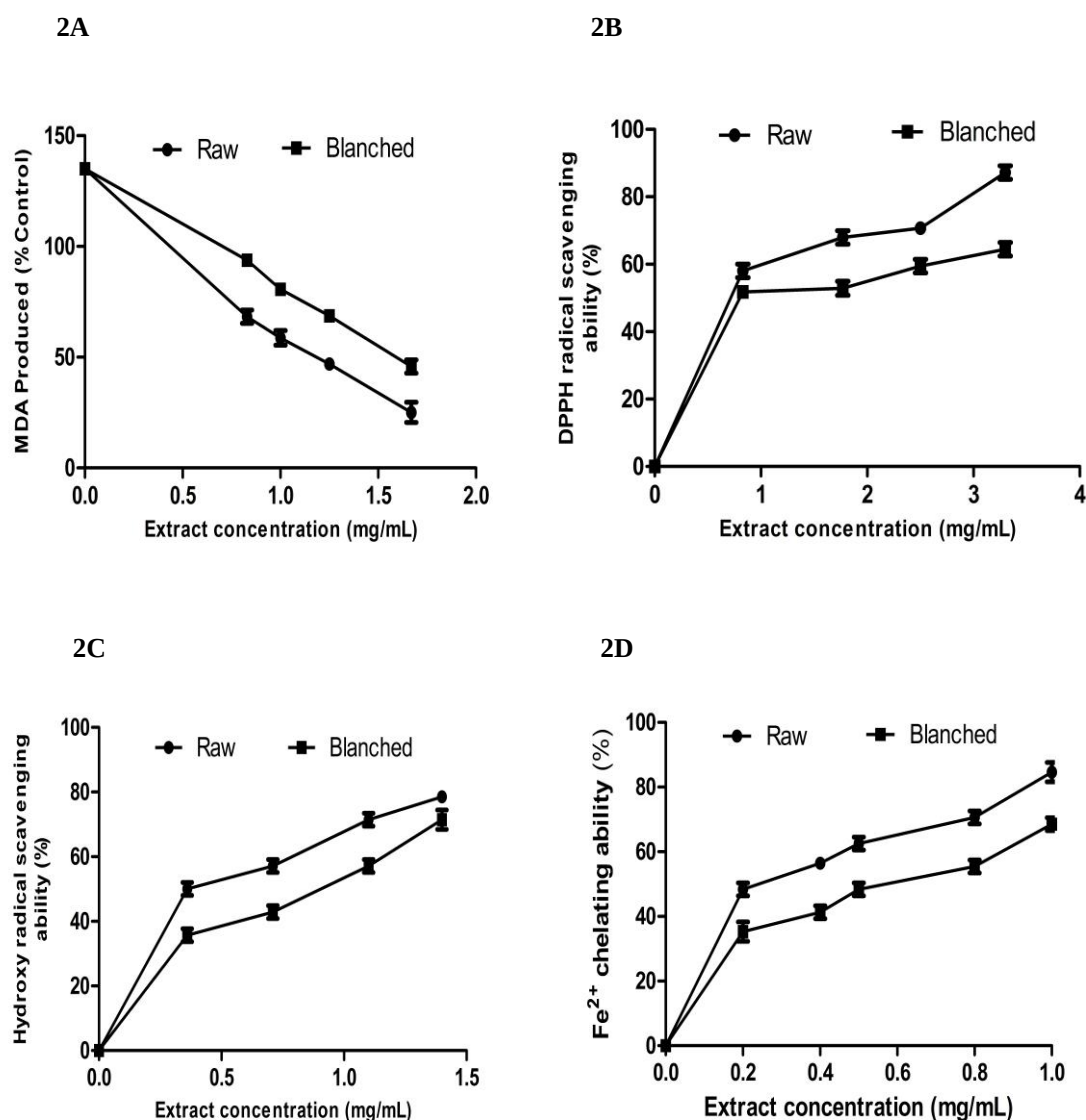


Figure 2A-Malonyladehyde content, (2B) DPPH radical and (2C) Hydroxyl (OH) radical scavenging abilities and, (2D) Fe^{2+} Chelating ability of raw AL (RAL) and blanched AL (BAL). Values represent means of triplicate.

HPLC-DAD analysis has advantage over colorimetric determination of phenolic compounds as it reveals accurate information of individual compounds. In this study, gallic acid, catechin, chlorogenic, caffeic and ellagic acid, epigallocatechin, rutin, isoquercitrin, quercitrin and quercetin were identified in the studied samples. On the account of total phenol and flavonoid contents (Table 2), Raw leaf's extract contained higher total phenol than the extract from blanched. Likewise, the total flavonoid content was higher in the RAL compared to that of BAL. The HPLC-DAD phenolic profiles of African lettuce (raw and blanched) (Tables 3 and Figure 6a and b) revealed the presence of phenolic

acids and flavonoids. Gallic and chlorogenic acids and quercitrin were present only in the RAL while caffeic acid was revealed only in BAL. Furthermore, raw is richer in caffeic acid, epigallocatechin, quercetin while ellagic acid, rutin, and isoquercitrin were abundantly present in blanched extract when compared (Table 2).

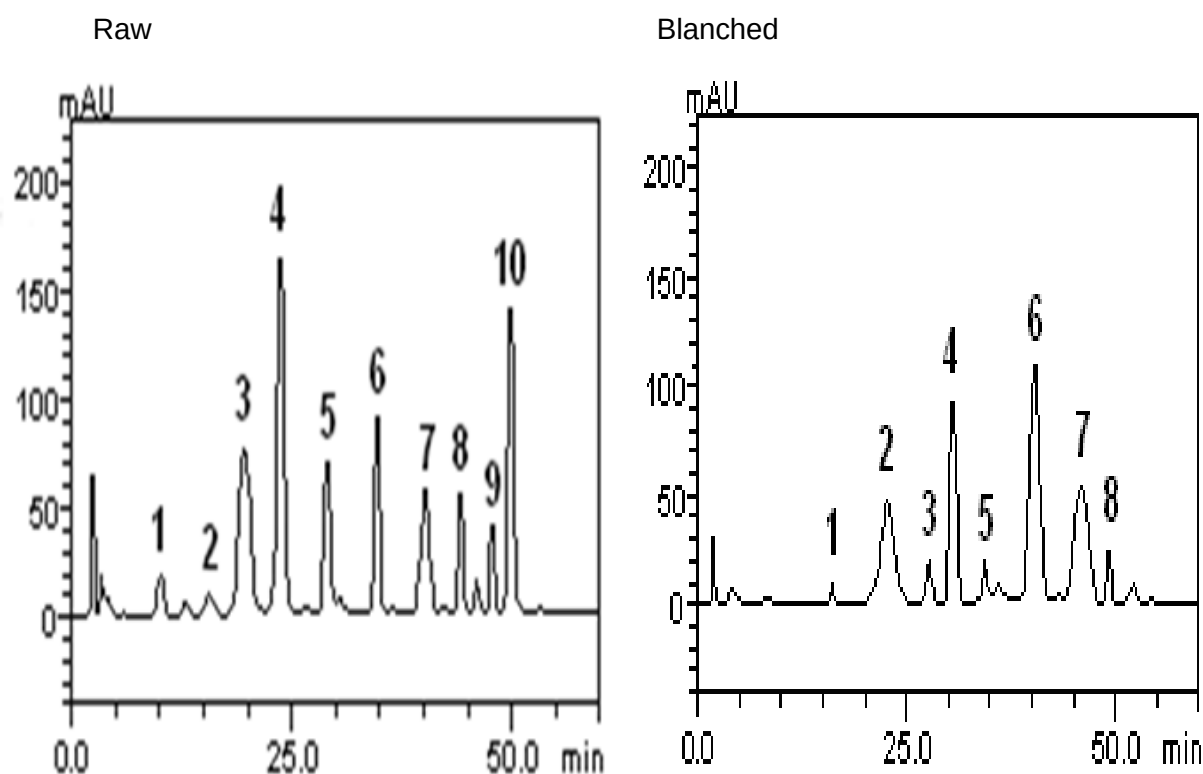


Figure 3. Representative high performance liquid chromatography profile of African lettuce leaves. (A) Raw leaves (RAW, peaks 1- 10 represents Gallic acid, catechin, chlorogenic acid, caffeic acid,, ellagic acid, epigallocatechin, rutin, isoquercitrin, quercitrin and quercetin respectively. (B) Blanched leaves (BAL, peaks 1- 8 represents catechin , caffeic acid , caffeic acid derivative , ellagic acid , epigallocatechin, rutin , isoquercitrin and quercetin respectively..

Several preclinical studies revealed that polyphenols offer protection against many human ailments most especially those triggered by oxidative stress such as diabetes and cardiovascular diseases (González-Castejón and Rodríguez-Casado, 2011) via chelation of metals and abstraction of free radicals whose formations have been linked to normal cellular metabolism (Amic *et al.*, 2003). However, thermal processing including blanching have been reported to increase/decrease the phenolic content and antioxidative ability of vegetables (Valverdú-Queralt *et al.*, 2011; Sharma and Gujral, 2011; Gerard and Roberts, 2014). The decrease/loss of some phenolic constituents (Table 2) and total phenolic contents (Table 3) in BAL extract could be accrued to the effect of the blanching process and consequently responsible for the decrease in the enzymes inhibition and antioxidant potentials. This loss of phenolics in the BAL extract is therefore in line with the report of (Ahmed and Ali, 2013), that blanching caused loss of phenolics in Cauliflower, and further stated that the loss could be as a result of covalent binding of amino acid with oxidized phenol and/or polymerization reaction. Phenolics in vegetables exist in two forms: free and those that form complexes with cell wall (bound phenolics). Hence, increased cooking temperatures and time causes cell walls disruption and breakdown of phenolic compounds (Miglio *et al.*, 2007; Ferracane, *et al.*, 2008; Bunea *et al.*, 2008; Ahmed and Ali, 2013; Sharma and Gujral, 2011)

Conclusion

Inhibition of key enzymes linked to diabetes and oxidative stress by AL, could be one of the mechanisms behind its use in folkloric medicine. However, this study has shown that blanching causes a reduction of its potentials, in relation to loss of some phenolic constituents as indicated in the results with BAL. Therefore, blanching could reduce functional and nutraceutical values of AL.

Conflict of Interest

No Conflict of interest

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