Evaluation of Artichoke Bracts as a Potential Source of Bioactive Compounds, Bio-ethanol Production and Livestock Feeding

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ABSTRACT

Recycling of lignocellulosic agro-industrial residues waste is the key of the environmental sustainability. So, the goal of the present study was to utilize artichoke (*Cynara cardunculus* L.) bracts as a potential source of bio-active compounds or as a carbon source during bio-production of ethanol and to evaluate its nutritive value for livestock feeding by using *in vitro* gas production technique. The chemical composition, phenolic compounds, and radicals scavenging property of extracts were determined. Silage production and acid hydrolysis on conversion of lignocellulosic artichoke was studied. Also, the acid hydrolysate of lignocellulosic components as a carbon source was investigated during bio-production of ethanol using *Saccharomyces cerevisiae* and simultaneous saccharification fermentation process. The obtained results revealed variation in proximate composition and mineral content among treatments of artichoke bracts used here. The blanching processing of artichoke bracts had higher total phenolic content (935.43 mg GAE /100 g DW) and 89.64% inhibition of DPPH radical with an IC_{50} value of 6.23 mg/ml. The highest reducing sugar content was obtained by using sulphuric acid (3%) for 20 min at 120°C. Fermentation of the hydrolysates gave the highest ethanol yield of (10.02 g/L), which corresponds to volumetric productivity of ethanol being 1.52 g /L/h with fermentation efficiency of 97.39% and biomass of 4.64 g/L after 48 hr. The results of the present study suggest that adding sugar beet molasses to the fermentation medium enhanced production of bio-ethanol (14.01g/L). The results also showed that the nutritional value of the artichoke bracts is similar to that of good roughage as hay in terms of metabolizable energy (8.42 MJ/kg DM), net energy (3.15MJ/kg DM), short chain fatty acids (99.48 mM), microbial protein synthesis (76.49 g/kg) and organic matter digestibility (63.41 %).

Keywords: *Environmental sustainability, artichoke bracts, bio-ethanol production, lignocellulosic components, metabolizable energy.*

INTRODUCTION

Recently, more attention has been focused on utilization of and/or methodology tested in the improvement of crop residues and agro-industrial by-products for producing biofuel and using in livestock feeding. Crop residues and agro-industrial by-products play a more significant role in the nutrition of ruminants where they consist of proteins, sugars and lipids along with particular aromatic and aliphatic compounds and, therefore, they could be cheap and abundant sources of fine chemicals , and other bioactive components such as carotenoids, phytoestrogens (Llorach *et al*., 2002) and natural antioxidants, such as phenolic compounds, and functional compounds (Moure *et al*., 2001, Schieber *et al.,* 2001). Artichoke (*Cynara cardunculus* L. var. *scolymus*) is a perennial, rosette plant, widely cultivated for its nutritional value due to its particularly high content of bioactive compounds such as phenolic compounds, fructooligosaccharides, fibers and minerals. Artichoke plays an important role in human nutrition. Its health-protective potentials have been reported especially their hepatoprotective (Aktay *et al.,* 2000), anticarcinogenic (Wang *et al*., 2003), and hypocholesterolemic activities (Lupattelli *et al.,* 2004). It is considered as one of the most important agricultural economy crops in the countries bordering the Mediterranean basin including Egypt, where is the second largest producer of artichoke worldwide (about 390.672 ton annually, FAOSTAT, 2013). The artichoke residues can account for 80–85% of canning industry waste weight and

are consisting mainly of the leaves, stems and leaf bracts which are not suitable for human consumption but may be used as a nutritional additive in the production of animal feed or as manure (Ceccarelli *et al.,* 2010). However, the bracts are rich in lignocelluloses and contain bioactive compounds, as fructooligosaccharides (FOS), phenolics, and should be considered as a raw material for the production of food additives and nutraceuticals (López-Molina *et al*., 2005, Lattanzio *et al.*, 2009). Also, it can be used as nutritional additive for animal feed (Cajarville, *et al*., 2000, Tajodini *et al*., 2015), green forage for livestock and can also be used as alternative source for fuel generation (Ceccarelli *et al*., 2010). There has been an increasing interest in utilization from lignocellulosic agriculture-industrial residues as the most promising alternative sources for cost-effective bio-energy production. Environmental pollution, global warming, and the future of oil production are among major causes of public and private interests in natural bio-based resources as an alternative or substitute for fossil fuel oil (Iqbal *et al*., 2013).

Energy production from silage has also received much interest in recent years. Silage is an important way for farmers to enhance quality of the forage conservation (the preserved feed). The utilization of silage has been increasingly used as substrates or feedstock for biogas production in Europe and particularly in Germany (Weissbach, 2009). During the biogas process, biomass is converted through different steps into methane and carbon dioxide similar limitations as those observed with forage digestibility, by using additives as a silage-starter, will enhance of ethanol production in the biogas process (Digman *et al*., 2010).

Therefore, the main purpose of the present study was the evaluation of the artichoke bracts for bioactive compounds, bio-ethanol production and livestock feeding, as well as to investigate potential utilization of by-products artichoke silage as nutrient and carbon source for the ethanol fermentation process.

MATERIALS AND METHODS

Materials

Artichoke (*Cynara scolymus* L.) wastes (bracts) used in the present study were collected during the winter season of 2015 from Givrex company, Alexandria, Egypt. Three feedstuffs were used in this study : berseem hay (H), wheat straw (WS), and rice straw (RS) were obtained from the Experimental Farm of Agriculture College, Alexandria University.Table (1) shows the proximate analysis of these feedstuffs. Sugar beet molasses (SBM) was obtained from Delta Beet Sugar Company, Kafr Elsheikh, Egypt. Folin-Ciocalteu reagent, gallic acid, ascorbic acid and 1, 1-Diphenyl-2-picryl-hydrazyl (DPPH) radical were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents used were of analytical grade.

Table 1: Proximate analysis of hay, wheat straw and rice straw as dry matter basis

Component (%)	Hay	Wheat straw	Rice straw
Crude protein (N×6.25)	11.68±0.17	4.16±0.16	4.75±0.11
Crude ether extract	1.97±0.08	0.83±0.04	0.47±0.03
Crude fiber	31.13±0.52	37.16±0.49	32.88±0.56
Total ash	9.86±0.13	8.98±0.11	14.39±0.20
Nitrogen free extract*	45.36±0.72	48.87±0.80	47.51±0.78

* Calculated by difference.

Methods:

Preparation of artichoke bracts

Raw artichoke bracts (RAB): Fresh (raw) artichoke bracts were washed, drained and spread on aluminum trays and dried in a cabinet drier at 50 °C for 12 hrs. The dried bracts were ground to pass through 60 mesh sieve and kept in dry glass containers and stored at room temperature (20±2°C) until further uses.

Blanched artichoke bracts (BAB): Fresh artichoke bracts were blanched in boiling water for 5 min (1:5 w/v), cooled with tap water, drained, spread on aluminum trays and dried at the same condition for RAB. The dried bracts were ground and stored until further uses.

The powder of artichoke bracts (RAB & BAB) was extracted according to Mohdaly, *et al.* (2010), using methanol (1: 5 w/v). Extraction was carried out using shaking incubator at 22±2°C for 24 hr followed by filtration through Whatman No1 filter paper. The residues were re-extracted under the same conditions. The residues of BAB were dried at 40°C in an oven and stored as mentioned before until analyzed. The combined filtrates were evaporated in a rotary evaporator (Buchi, Switzerland) under vacuum at 40°C. The extracts obtained after evaporation of organic solvent were weighed to de-

termine the methanolic extract yield. The methanolic extract thus obtained was assessed for antioxidant activity and total phenolic content.

Preparation of silage samples

Silage of artichoke bracts (SAB):- Fresh artichoke bracts were directly packed in a plastic bag, compressed to remove the internal air and then sealed completely and kept in storage for the duration of the study. The samples were opened after 30 days (Megfas *et al.* 1993).

Silage of artichoke bracts with molasses (SABM):- Fresh artichoke bracts were treated with addition of molasses (2%), packed in a plastic bag and kept at ambient temperature for 30 days. Silage samples (SAB&SABM) were prepared for analysis by drying, grinding and stored at the same condition for RAB until further uses (Nasser, 2009).

Analytical methods:

Chemical composition

Proximate composition of RAB, BAB (after phenolic extraction), SAB, SABM, hay, wheat straw, and rice straw including moisture content, crude protein (N x 6.25), crude ether extract, crude fiber and total ash were carried out according to the AOAC (2000) procedures. Nitrogen free extract (NFE) was calculated by difference. Minerals (Mg, Ca, Fe, Mn, Zn and Cu) were estimated using Perkin Elmer Atomic Absorption Spectro-Photometer (Model 2380), England. Sodium and potassium were determined by flame photometer as described in the AOAC (2000). Reducing sugars were estimated by 3, 5 dinitrosalicylic method (Miller, 1959) using glucose as a standard and the total sugars content in the juice was estimated using phenol–sulphuric acid method (Dubois, *et al.,* 1956). The dietary fiber fractions including neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) cellulose and hemicellulose were analyzed using the methods of Goering & Van Soest (1970).

Total phenolics and antioxidant activity

The total phenolics in the methanolic extracts were assayed colorimetrically using the Folin-ciocalteu method (Lim *et al.,* 2006). Antioxidant activity of artichoke bracts was studied by evaluating the free radical scavenging activity of the 1, 1-Diphenyl-2-picryl-hydrazyl (DPPH) radical according to a modified method described by Brand-Williams *et al*. (1995). Briefly, 0.3 ml methanolic extract was added to 2.7 ml DPPH 0.1 mmol in methanol solution. The reaction mixture was vortex–mixed well and incubated for 30 min at room temperature in the dark. Absorbance was measured at 517 nm using Spectrophotometer. Antioxidant activity was expressed as percentage of inhibition of DPPH radical and calculated from the equation:

$$\text{Inhibition (\%)} = [(A_{DPPH} - A_{Sample}) / A_{DPPH}] \times 100$$

Where: A_{Sample} is the absorbance of sample; A_{DPPH} is the absorbance of the control (DPPH solution). Radical scavenging capacity was expressed as mg ascorbic acid equivalents (AAE) per 100 g sample. The IC_{50} value is defined as the concentration of sample (mg/ml) contained mg AAE for 50% inhibition of DPPH free radicals.

Measuring of ethanol

The produced ethanol was measured by gas chromatography (Shimadzu, GC-17A, Japan) using a RTX-1 column (20 m by 0.25mm) packed with 100% dimethyl polysiloxane and a flame ionization detector (Palo Alto, CA), N_2 as carrier gas at a flow rate of 30 ml/ min, and maintaining the injector and detector temperature at 150°C (Laopaiboon *et al.,* 2007).The ethanol yield (Y) was calculated as the actual ethanol produced and expressed as g ethanol per g total sugar utilized (g/g). The volumetric ethanol productivity (g/L/h) was calculated by ethanol concentration produced (g/L) divided by fermentation time giving the highest ethanol concentration (Laopaiboon *et al.,* 2009).

Bio-ethanol production:

Acid hydrolysate:

The samples of RAB and BAB (after phenolic extraction) were chemically treated with different sulphuric acid (3, 4 and 5 %) at the solid substrate ratio (1:10 w: v). Then it heated in an autoclave (Labtech, USA) at 120°C for 20 and 30 min. After cooling, autoclaved samples were filtered. Each hydrolysate was collected separately, and then neutralized with NaOH adjusting the pH between 5.0 and 5.5. Hydrolysate filtrates were then kept at 5°C until used for ethanol production. The remaining residue was washed and oven dried then kept for total sugars, and dietary fiber analysis (Massoud & Abd El-Razek, 2011).

Yeast activation:

The *Saccharomyces cerevisiae* was activated by adding 10 g of dry yeast to 50 ml of pre-cul-

ture broth containing 1g glucose, 0.4 g peptone, 0.15 g yeast extracts, 0.05 g KH_2PO_4, and 0.025 g $MgSO_4.7H_2O$, and incubated in a rotary incubator shaker, at 38°C and 200 rpm for 60 min before using it as an inoculums for ethanol production.

Fermentation method

One hundred milliliters of each of acid hydrolysate was added as a carbon source to flask containing 5g of yeast extract, 5g of peptone, 1.2g $(NH_4)_2SO_4$, 1g KH_2PO_4, and 0.5 g $MgSO_4.7H_2O$, to prepare one liter of the required fermentation medium for ethanol production .The pH value of the medium was adjusted to 6±0.3 before autoclaving at 121°C for 20 min.

The sterilized fermentation medium was incubated with 10 ml (10% inoculums size) of activated yeast, then, incubated in a shaking (Rotatory Incubator Innova 4230, Edison, NJ., USA) at 30°C and 200 rpm for 72 h (Wu *et al*., 2006). Through this period, both ethanol production and sugars consumption in the medium were periodically determined. At the end of the fermentation period, the temperature of broth was elevated to above the boiling point of ethanol to recover alcohol by distillation. The biomass in the fermentation broth, was separated by centrifugation, at 5000 xg for 20 min, dried at 70°C and weighed (Norris & Ribbons, 1970).

Measurement the *in vitro* gas production:

The *in vitro* gas production of RAB, BAB after phenolic extraction, hay, wheat straw, and rice straw was carried out using the method proposed by Menke & Steingass (1988). Approximately, 200 mg of sample were placed in triplicate in calibrated glass syringes. Rumen fluids were collected before morning feeding from three fistulated sheep fed berseem hay and commercial concentrate mixture twice a day. The rumen fluid was filtered through four layers of cheese–cloth and flushed with CO_2. The CO_2-flushed rumen fluid was added to the buffered mineral solution (1:2, v/v) which was maintained in a water bath at 39°C, and 30 ml of this mixture were introduced in each syringe for incubation. Syringes were shaken gently at each reading and the gas volume was recorded at 0, 3, 6, 9, 15, 24, 36, 48, 72 and 96 hr of incubation (Onodera & Henderson, 1980), Cumulative gas production (Y) at time (t) was fitted to the exponential model of Ørskov & McDonald (1979). The energy values and the percentages of organic matter digestibility of forages can be calculated from the gas produced according to Menke *et al*. (1979), Menke & Steingass (1988).

Statistical analysis:

Statistical analysis of data was done using a Co-Stat Software (2004) computer program and Student-Newman-Keuls test was used for testing the mean differences at 5% probability level (Steel *et al*., 1997).

RESULTS AND DISCUSSION

Chemical composition:

The data presented in Table (2) indicate a significant diversity in proximate composition and minerals of the artichoke bracts used which were documented for superiority of ethanol production. The

Table 2: Proximate composition and minerals content of the raw artichoke bracts (RAB), blanched artichoke bracts (BAB) after phenolic extraction and silage samples

Component	RAB	BAB	Silage	
			SAB	SABM
Moisture (%)	11.00[a]	11.01[a]	13.03[b]	13.31[b]
Total ash (%)*	7.03[a]	6.79[a]	8.22[b]	8.78[b]
Crude protein (%)*(N×6.25)	17.96[c]	17.59[c]	16.46[a]	17.05[b]
Crude ether extract (%)*	2.39[a]	2.01[a]	2.45[a]	2.42[a]
Crude fiber (%)*	24.58[a]	24.35[a]	29.96[c]	28.89[b]
Nitrogen free extract (%) **	48.04[b]	49.26[b]	42.91[a]	42.86[a]
Minerals (mg/100g)*				
K	2852	2594	3369	3595
Na	37.05	30.52	33.38	36.64
Ca	37.49	32.86	42.54	51.23
Mg	7.08	6.95	8.19	9.76
Fe	13.61	12.56	15.71	16.68
Mn	0.44	0.35	0.45	0.65
Zn	3.30	2.63	4.10	4.34
Cu	2.70	2.27	2.83	3.19

Means in the same row followed by the same letter(s) are not significantly different at 5% probability level. *On dry weight basis.

** NFE = 100 – (crude protein +crude ether extract +ash+ crude fiber).

SAB: silage of artichoke bracts, SABM: silage of artichoke bracts with molasses

composition of artichoke bracts were affected by treatment. The average of moisture content varied from 11.00% for RAB to 13.31% for SABM among studied samples. It was clear that the silage samples contained high level of ash and crude fiber and had low concentration of protein and nitrogen free extract compared to RAB and BAB. The crude ether extract content in the artichoke by-products showed similar values in both samples ranging from 2.01% to 2.39% where BAB had lower fat content. The highest nitrogen free extract (49.26%) was found in BAB followed by RAB (48.04%) while silage samples had the lowest value of nitrogen free extract. The obtained results are in agreement of data recorded by Hosseinzadeh *et al.* (2013), El-Sohaimy (2014), Abdel Magied *et al*. (2016) who found that the protein content of artichoke leaves was in the range of 8.05% to 21%, crude fat ranged from 2% to 4.11. On the other hand, crude fiber and total carbohydrates were found to be 63.76 and 76.34%, respectively.

The results of the mineral analysis in Table (2) indicate that the potassium was the major minerals followed by Ca, Na and Fe, then Mg in all samples. Meanwhile, Mn, Zn and Cu were found in lower concentrations. As noted from the data in Table (2), the ash of BAB had lower level of mineral content than that of RAB. These results are in agreement with those reported by Lutz *et al.* (2011), Mepba *et al*. (2007). They found that the cooking process caused a reduction in K, Na, Ca, Zn, Fe and P content of artichoke. However, these results are in disagreement with Said (2012) who found that the mineral content of dried artichoke bracts was found to be calcium (67.66 mg/100g), magnesium (18.71 mg/100g), sodium (0.05 g/100g) and potassium (3.88 g/100g). According to Parajó *et al*. (2004), ash is a drawback for ethanol production, when mineral components have neutralizing ability, which could increase the pH during acid hydrolysis, and higher temperatures or longer time would be required to achieve the desired hydrolyzing effect.

Total phenolic content and antioxidant activity:

Table (3) shows the extract yield, total phenolics and antioxidant activity of RAB and BAB. It was noted that methanolic extract yield was 8.24 and 10.75% in RAB and BAB, respectively. Total phenolic contents are presented in Table (3). It is clear that the blanching processing of artichoke bracts showed a higher total phenolic content of the methanolic extract (935.43 mg gallic acid equivalents (GAE) /100g DW) compared with raw artichoke bracts, RAB (516.20 mg GAE/100g DW). The increment of total phenolic content may be due to inactivating of polyphenol oxidase which catalyzes the oxidation of phenolics to quinones that subsequently induce the formation of secondary products (Wang *et al.,* 2003).The highest antioxidant capacity was found in BAB which showed 89.64% inhibition of DPPH radical with an IC_{50} value of 6.23 mg/ml compared with RAB. Ascorbic acid was used as a standard. Many of researches revealed that, a strong linear relationship was observed between total phenolics and antiradical capacity.

Table 3: Total phenolic content and antioxidant activity of artichoke bracts (on dry weight basis)

Component	Raw artichoke bracts (RAB)	Blanched artichoke bracts (BAB)
Total phenolic yield (g/100g)	8.24	10.75
Total phenolic content (GAE mg/100g sample.)	516.20 ±6.82	935.4± 37.61
Radical scavenging activity (% Inhibition)	56.78±4.12	89.64 ±5.63
Ascorbic acid equivalent (mg/g sample)	8.63±0.38	13.83±0.51
IC_{50} (mg /ml)	10.07±0.61	6.23±0.49

Based on these findings, the increment of phenolic content in cooked artichoke materials caused an increase of antioxidant capacity (Lutz *et al*., 2011). However, the results are in disagreement with Awad (2016) who showed that total phenolics of the artichoke bracts methanolic extract was 73.40 mg GAE/ g with IC_{50} value of 71.70 ug/ml. Gaafar & Salama (2013) reported that the free phenolic extract from bracts of artichoke might be of interest within the developing market of nutritional and health-protective potential, especially cancer.

Acid hydrolysis of artichoke bracts:

The effect of residence time and sulphuric acid concentration on total sugars of RAB and BAB

after phenolic extraction is summarized in Figure (1).The highest total sugar was obtained when the treatment was performed with 3 % w/v acid at 121 ºC for 20 min. After acid hydrolysis, total sugars increased to 22.06% in RAB and to 20.47% in BAB (after phenolic extraction). This can be attributed to hydrolysis of inulin during heat treatment to reducing sugars. Thereafter, a decrement was observed upon using high concentrations of sulphuric acid. Acid hydrolysis at higher concentration and longer hold time may cause formation of furfural and 5-hydroxymethylfurfural (HMF) from the dehydration of released sugars (Limayem & Steven, 2012).

Total sugars and lignocellulosic components of acid hydrolysate RAB, BAB after phenolic extraction and silage samples

The lignocellulosic compounds (cellulose, hemicelluloses and lignin) are the major carbohydrates in artichoke bracts. The data presented in Table (4) indicate significant differences in total reducing sugars and lignocellulosic compounds of RAB and BAB after phenolic extraction and acid hydrolysis compared with silage samples. Silage had the lowest total reducing sugars and the highest content of NDF and ADF. These results are in agreement with Megfas *et al*. (1993) who found that the NDF and ADF content of dried artichoke bracts increased during the ensiling period. The acid hydrolysis of the lignocellulosic components of RAB and BAB led to hydrolyze 67.52 - 66.87% of hemicelluloses, respectively. The advantages of the acid hydrolysis of lignocellulosic components of herbaceous material (grass), hardwoods and agricultural wastes produce syrup of monomeric sugars, and give better results by solubilizing the hemicellulose (Liao *et al*., 2007 , Balat, 2011).

Lignocellulosic hydrolysate of artichoke bracts as a carbon source of ethanol production

From the results in Tables (2) and (4), no statistical differences were found between the total sugars of RAB and BAB after phenolic extraction. It was noted that RAB was selected to be used for bio-ethanol production. The data presented in Table (5) indicate that ethanol content increased gradually from 8.77 g/L after 24hr of fermentation to 10.02 g/L after 48hr of fermentation in media having *S. cerevisiae*. However, the total and reducing sugars in the fermentation medium decreased continuously after the start of the fermentation then after 48hr it decreased slowly. Also, the biomass dry weight increased as fermentation progressed up to 48hr (4.64 g/L) and then remained constant. As it is noted from Table (5), adding sugar beet molasses as a carbon source in fermentation medium gave higher yield of ethanol (14.01g/L) with 0.49 g alcohol per g sugar and fermentation efficiency of 98.04% after 48h of fermentation comparing with using RAB. This was due to the high level of both total sugars and ash in molasses. The results of Massoud & Abd El-Razek (2011) showed that the lignocellulosic hydrolysate of the juice extracted from stalks containing 26% total sugars gave 12.40 g ethanol/L and fermentation efficiency (94.45%). Uppugundla *et al*. (2014) reported that the reason for lower ethanol yield of dilute acid corn stover is because most of the xylose is produced during the pretreatment. Therefore , waste water of ethanol fermentation medium after recovering of ethanol and removing biomass can be recommend for plant irrigation especially in areas where agricultural demand for water can be used as a carbon source in fermentation medium (Massoud & Abd El-Razek, 2011).

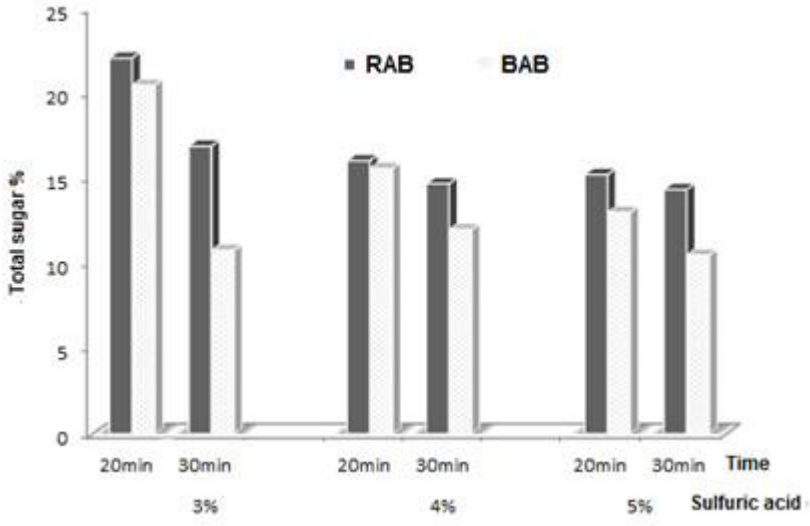

Fig. 1: The effect of residence time and sulfuric acid concentration on total sugars of raw artichoke bracts (RAB) and blanched artichoke bracts (BAB) after phenolic extraction

Table 4: Total sugars and lignocellulosic components of acid pre-treatment RAB, BAB after phenolic extraction and artichoke silage (g/100g Dry weight).

Parameter (%)	RAB		BAB		Silage	
	Unhydrolysate	Acid hydrolysate	Unhydrolysate	Acid hydrolysate	SAB	SABM
Total sugars	4.44	22.06b	4.01	20.47b	2.94a	4.124a
NDF*	47.2	46.39b	47.25	46.68b	52.74a	53.56a
ADF*	31.22	41.20a	31.16	41.22a	43.27b	43.86b
ADL*	4.27	9.16b	4.32	9.08b	6.35a	8.68b
Cellulose	26.95	32.03a	26.84	32.27a	36.91b	35.19b
Hemiocelullose	15.98	5.19a	16.09	5.33a	9.47b	9.70b

* (NDF), neutral detergent fiber, (ADF), acid detergent fiber and (ADL), acid detergent lignin.
RAB: raw artichoke bracts, BAB: blanched artichoke bracts after phenolic extraction, SAB: silage of artichoke bracts, SABM: silage of artichoke bracts with molasses. Means in the same row followed by the same letter(s) are not significantly different at 5% probability level.

Table 5: Ethanol production (%w/v) from acid hydrolysate of raw artichoke bracts

Parameter	Ethanol fermentation medium containing							
	Acid hydrolysate RAB				Acid hydrolysate RAB with molasses			
Time (hr)	0	24	48	72	0	24	48	72
Total reducing sugars (g/L)	22.62	3.15	1.81	1.79	29.50	3.08	1.28	1.07
Total sugar utilization (%)	-	86.07	92.00	92.09	-	89.56	95.66	96.37
Ethanol content (g/L)	-	8.77	10.02	10.05	-	12.53	14.01	14.00
Ethanol yield (g/g of sugar)	-	0.45	0.48	0.48	-	0.47	0.50	0.49
Maximum volumetric productivity of ethanol (g/L/h)	-	0.37	0.21	0.14	-	0.52	0.29	0.19
Biomass (g /L)	-	3.150	4.64	4.83	-	4.44	5.05	5.501
Fermentation Efficiency (%)*	-	88.23	94.11	94.51	-	92.99	98.04	96.07

* Fermentation Efficiency (FE) % = (Actual yield/Theoretical yield) × 100.
RAB: raw artichoke bracts.

The *in vitro* gas production

Results of least square means of cumulative gas production profiles are shown in Table (6) and Fig. (2), the cumulative volume of gas production increased with increasing time of incubation. There were significant differences among the substrates in terms of gas production at all incubation times. The data presented here showed that the highest values of gas production at 24 h of incubation were observed with hay and raw artichoke bracts, while the values of gas production of wheat straw and rice straw were low. The produced gas at 96 h ranged from 46-55 ml/200 mg DM. Total gas produced at 96 h of incubation was significantly ($P<0.05$) higher for hay and raw artichoke bracts than in the other substrates. Haddi *et al.* (2003) suggested that interactions between NDF, ADL, crude protein and ash contents influenced the kinetics of gas production. Kamalak *et al.* (2005) noted considerable variations among alfalfa varieties in terms of gas production at all incubation times according to the differences in the chemical composition of the varieties of alfalfa. Significant variations in chemical composition and *in vitro* rumen fermentation were observed among the ensiled acacia and leuceana with or without different levels of urea (Nasser, 2009). The present results indicate that the gas production from the soluble fraction (A) and insoluble fraction (B) ranged from 5.57-12.78 and 36.05 to 39.94 ml, respectively. The values of soluble fraction (A) of hay and raw artichoke bracts are higher ($P<0.05$) than the values of the other substrates, while the variation of the values of insoluble fraction (B) are not significant. Estimated gas production rate (C) varied from 0.05 to 0.08 ml/h. The highest values of (C) was for raw artichoke bracts while, the lowest values was for rice straw, respectively.

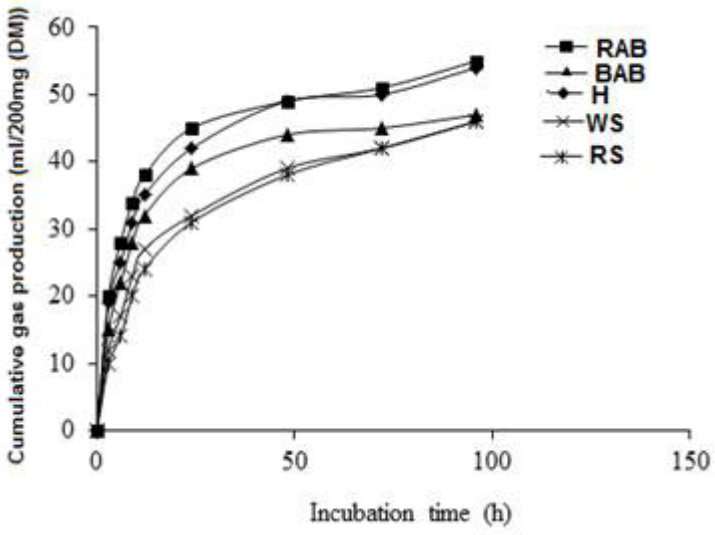

Fig. 2: Cumulative gas production for raw artichoke bracts (RAB), blanched artichoke bracts (BAB) after phenolic extraction, hay (H), wheat straw (WS) and rice straw (RS)

Table 6: Cumulative gas production (ml/200 mg DM) after 12, 24, 48, 72, 96 h of incubation and gas production parameters in raw artichoke bracts (RAB), blanched artichoke bracts (BAB) after phenolic extraction, hay (H), wheat straw (WS) and rice straw (RS)

Item	12 h	24 h	48 h	72 h	96 h	A*	B*	C*
RAB	38^{a}	45^{a}	49^{a}	51^{a}	55^{a}	11.89^{a}	39.94^{a}	0.08^{a}
BAB	32^{b}	39^{b}	44^{a}	45^{b}	47^{b}	6.96^{b}	38.09^{a}	0.08^{a}
H	35^{ab}	42^{ab}	49^{a}	50^{a}	54^{a}	12.78^{a}	38.98^{a}	0.07^{b}
WS	27^{c}	32^{c}	39^{b}	42^{b}	46^{b}	7.76^{b}	36.05^{ab}	0.05^{c}
RS	24^{c}	31^{c}	38^{b}	42^{b}	46^{b}	5.57^{b}	38.98^{a}	0.05^{d}

a, b, c, d means within the same column with different superscripts are significantly different (P<0.05) A*: gas production from the soluble fraction (ml), B*: gas production from insoluble fraction (ml),C* : gas production rate constant for the insoluble fraction (ml/h).

The intake of a feed is mostly explained by the rate of gas production (C) which affects the passage rate of feed through the rumen, whereas the potential gas production (A+ B) is associated with degradability of feed (Khazaal *et al*. 1995).

Energy contents, organic matter digestibility, short chain fatty acids and microbial protein

The predicted metabolizable energy (ME, MJ/kg DM), net energy (NE, MJ/kg DM), organic matter digestibility (OMD, %), short chain fatty acids (SCFA, mM) and microbial protein (MP, mg/kg DM) of hay, raw artichoke bracts, blanched artichoke bracts, wheat straw and rice straw, are presented in Table 6. The present data show that the ME and NE ranged from 6.44 to 8.42 and from 2.19 to 3.15 MJ/kg DM, respectively. The values of ME and NE were higher (P<0.05) for raw artichoke bracts, while the values for wheat straw and rice straw were low. The calculated organic matter digestibility from gas production values at 24 h was subsequently the highest in raw artichoke bracts (63.41 %) and the lowest in rice straw and wheat straw (45.03 and 46.06 %, respectively as shown from Table (6). The leaf fraction had higher fiber components, which is the reason for their lower *in vitro* dry matter digestibility (IVDMD).

Table 7: Metabolizable energy (ME), net energy (NE), organic matter digestibility (OMD), microbial protein (MP) and short chain fatty acids synthesis (SCFA) prediction in raw artichoke bracts (RAB), blanched artichoke bracts (BAB) after phenolic extraction, hay (H), wheat straw (WS) and rice straw (RS)

Items	ME (MJ/kg DM)	NE (MJ/kg DM)	OMD (%)	MB (g/kg OMD)	SCFA (mM)
RAB	8.42^{a}	3.15^{a}	63.41^{a}	76.49^{a}	99.48^{a}
BAB	7.61^{a}	2.99^{a}	58.00^{b}	69.96^{b}	86.16^{c}
H	7.98^{a}	2.82^{ab}	58.41^{b}		92.82^{b}
WS	6.58^{b}	2.23^{b}	46.06^{c}	55.56^{c}	70.62^{de}
RS	6.44^{b}	2.19^{b}	45.03^{c}	54.32^{c}	68.40^{e}

a, b, c, d, e, means within the same column with different superscripts are significantly different (P<0.05)

The limitation imposed by high fiber content is the reduction in dry matter digestibility leading to insufficient supply of energy. As a consequence, IVDMD was negatively correlated to NDF and ADF (Fadel Elseed *et al*. 2007). Microbial proteins and SCFA ranged from 54.32 to 76.49 g/kg OMD and 68.40 to 99.48 mM, respectively. Microbial proteins and SCFA were significantly ($P<0.05$) higher for raw artichoke bracts and hay than wheat straw and rice straw. The *in vitro* digestibility and gas production parameters were significantly correlated with chemical composition of shrubs (Nasser, 2009).

CONCLUSION

It can be concluded from the present study that blanched artichoke bracts (BAB) contained higher total phenolic content as well as the highest antioxidant capacity. These findings may suggest the utilization of these bioactive compounds in the area of food preparations. In addition, this by-product can be used as a major source of carbon during the production of ethanol. It also can be used instead of some other roughage by-products such as hay and rice straw as an alternative source for livestock feed. The present study deserves further investigation to maximize efficiency of utilization of agro-industrial wastes and their content of lignocellulosic biomass and thereby provide a renewable source of energy as well as to produce natural compounds which can be used as food additives.

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تقييم أوراق الخرشوف كمصدر محتمل لإنتاج المركبات النشطة حيوياً والإيثانول الحيوي وكعليقة للحيوانات

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يعتبر تدوير استخدام المركبات الليجنوسيليلوزية للمخلفات الزراعية والتصنيعية مفتاحاً لتنمية البيئة المستدامة، لذا كان هدف هذا البحث هو تعظيم الاستفادة من أوراق الخرشوف كمصدر محتمل لإنتاج المركبات النشطة حيوياً وكذلك كمصدر للكربون أثناء إنتاج الإيثانول الحيوي وتقييم قيمتها الغذائية لتغذية الماشية باستخدام تقنية الغاز معملياً. وقد تم تقدير التركيب الكيماوي والمواد الفينولية الكلية والنشاط المضاد للأكسدة لكسح الشوارد الحرة. ودراسة تأثير إنتاج السيلاج والتحلل الحامضي على المركبات الليجنوسليلوزية. كما تم تقييم نواتج التحلل الحامضي للمواد الليجنوسيليلوزية كمصدر كربوني أثناء عملية التخمر باستخدام خميرة الخباز لإنتاج الإيثانول الحيوي بطريقة الإسالة التخميرية اللحظية. تشير النتائج المتحصل عليها إلى وجود اختلافات فى كل من التركيب الكيماوي ومحتوى العناصر المعدنية بين أوراق الخرشوف المعاملة بالطرق المختلفة التي استخدمت فى هذه الدراسة. كما أدت معاملة السلق لأوراق الخرشوف قبل التجفيف إلى زيادة محتوى المواد الفينولية الكلية (٩٣٥,٤٣ ملجم حامض جاليك/١٠٠جم وزن جاف) والنشاط المضاد للأكسدة من خلال قيمة IC_{50} (٢٣,٦ ملجم عينة/مل). كما أوضحت النتائج إمكانية الحصول على أعلى محتوى من السكريات المختزلة باستخدام حامض الكبريتيك ٣٪ عند درجة حرارة ١٢٠°م لمدة ٢٠ دقيقة وباستخدام ناتج التحلل فى البيئة أعطى أعلى إنتاج من الإيثانول (١٠,٠٢ جم/ لتر) مع كفاءة إنتاجية للإيثانول (١,٥٢جم/ لتر/ساعة) وكفاءة تخمر تعادل ٩٧,٣٩ ٪ و كمية من الكتلة الحيوية حوالي ٤,٦٤ جم/ لتر بعد ٤٨ ساعة من عملية التخمر. كذلك أوضحت نتائج الدراسة إمكانية تحسين إنتاج الإيثانول (١٤,٠١ جم/لتر) بإضافة مولاس بنجر السكر إلى بيئة التخمر. كما تشير نتائج التقييم التغذوي إلى أن القيمة الغذائية لأوراق الخرشوف تضاهي القيمة الغذائية للأعلاف الجيدة مثل الدريس من منظور قيمة كل من الطاقة الميتابوليزمية (٤٢,٨ ميجا جول/كجم مادة جافة) والطاقة الصافية (٣,١٥ ميجا جول/كجم) والأحماض الدهنية قصيرة السلسلة (٤٨,٩٩ مليمول) والبروتين الميكروبي المخلق (٧٦,٤٩ جم/كجم) و معامل هضم المادة العضوية (٦٣,٤١ ٪).