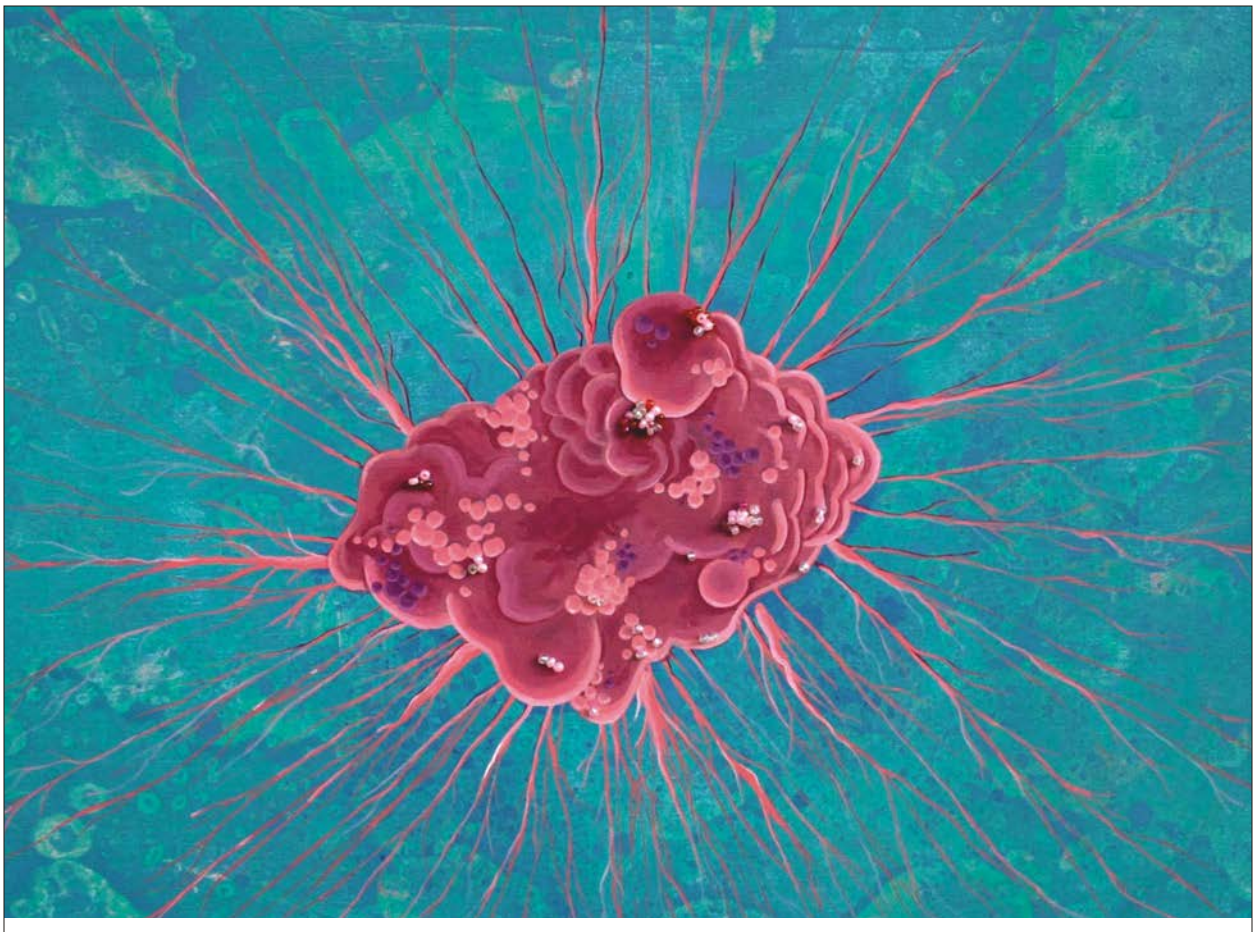

International Journal of Cancer Research

ISSN 1811-9727

Academic Journals Inc.

www.academicjournals.com

International Journal of Cancer Research 8 (4): 105-118, 2012
ISSN 1811-9727 / DOI: 10.3923/ijcr.2012.105.118

Determination of Processing Effects on Phytochemical Content, Antioxidants Activity and Chemopreventive Potential of Beets (*Beta vulgaris*) using a Colon Cancer Fisher 344 Male Rat Model

S. Appiah, M. Verghese, J. Boateng, L.A. Shackelford, B. Kanda, J. Patterson and L.T. Walker

Nutritional Biochemistry Laboratory, Department of Food and Animal Sciences, Alabama A and M University, Normal Alabama, 35762, USA

Corresponding Author: M. Verghese, Nutritional Biochemistry Laboratory, Department of Food and Animal Sciences, Alabama A and M University, Normal Alabama, 35762, USA

ABSTRACT

Beets (*Beta vulgaris*) have been reported to be a very nutritious vegetable which may provide health benefits against chronic diseases such as colon cancer due to phytochemicals present. The aim of this study was to investigate the effect of feeding Freeze Dried (FD), Cabinet Dried (CD) and pasteurized juice at 2 and 4% levels on Azoxymethane (AOM) induced Aberrant Crypt Foci (ACF) in Fisher 344 male rats and to determine the total phenolics and flavonoids content and antioxidants activity measured using FRAP and DPPH in steam blanched (SB), frozen, "individually quick frozen" (IQF), freeze dried and cabinet dried compared to fresh beets. Twenty eight rats were divided into 7 groups at 4 rats per group. The groups were fed control (C) diet (AIN-93G), C+2 and 4% FD, CD and beet juice. All rats received s/c injections of AOM in saline at 16 mg kg^{-1} b.wt. at 7 and 8 weeks. Rats were killed by CO_2 asphyxiation at the 17 week of age. Number of ACF, crypts/foci and total crypts were enumerated in the colon. Total phenolics, flavonoids and monomeric anthocyanin content as well antioxidant activity (using DPPH and FRAP) of beets (freeze dried, cabinet dried, "individually quick frozen", conventionally frozen steam blanched and fresh) were also determined. The total ACF incidence was significantly ($p<0.05$) higher in the control compared to beet fed groups. ACF reductions ranged from a low of 63% in rat fed 2% freeze dried beets to a high of 80% in rats fed 4% freeze dried beets. Total phenolics and flavonoids were significantly ($p<0.05$) higher in cabinet dried and freeze dried beets and higher antioxidant activity compared to the others (fresh, steam blanched, IQF and frozen). The results from the experiment indicates that feeding beets(cabinet dried, freeze dried or juice) reduced the incidence of AOM-induced ACF and therefore may be explored for its chemopreventive potential and other health benefits by the food industry.

Key words: Beets, cancer, processing, chemoprevention

INTRODUCTION

Epidemiological studies have discovered a strong link between increased consumption of fruit and vegetable and decreased risk of chronic diseases such as cancer, heart diseases and stroke (Van Duyn and Pivonka, 2000). Fruits and vegetables exhibit a wide range of phytochemicals that may lower the risk of colon cancer and other chronic diseases (Lako *et al*., 2007).

Despite advanced detection and treatment options that have developed over the years, cancer still continues to be a major health issue in the world in terms of morbidity and mortality (Greenlee *et al*., 2000). As reported by Greenlee *et al*. (2000), colorectal cancer deaths are higher compared to all other cancer deaths in the U.S. Moreover, rates are steadily increasing in other parts of the world. Colon cancer incidence is also reported to be higher in Australia, Europe and New Zealand (Johnson and Mukhtar, 2007). However, dietary modifications, to include more fruits and vegetables in the diet, may be an effective method for reducing colon cancer incidence (Van Duyn and Pivonka, 2000).

The sugar beet (*Beta vulgaris* L.) was one of the first crops developed using modern genetic principles (Panella and Lewellen, 2007). Taxonomically, the genus *Beta* is divided into four sections: *Corollinae, Nanae* (native to Greece), *Beta* (*vulgaris*) and *Procumbentes* (*Patellares*). The section *Beta* includes the cultivated beets (*Beta vulgaris*) which are divided into four Culti-groups namely leaf beets, garden beets, fodder beets and sugar beets (Lange *et al*., 1999). Beet (*Beta vulgaris* L.) is known to be a very nutritious vegetable and this is attributed to the natural antioxidants it contains (Cao *et al*., 1996). Phytochemicals in beets may exhibit complementary mechanisms of action in the prevention of cancer by scavenging oxidative agents, regulation of gene expression in cell proliferation, detoxification enzymes modulation and many more (Waladkhani and Clemens, 1998).

Postharvest processing of vegetables is done to ensure availability at all times. Fresh vegetables have poor shelf life hence, processing is necessary to maintain their availability. Although, this is thought to reduce the potency of antioxidants, fiber and other bioactive compounds (Nicoli *et al*., 1999), identifying the best technique can minimize the losses that may occur. While preservation is believed to be responsible for reduction of nutritional potency of vegetables, research on the effects of processing on phytochemical availability and chemopreventive potential of beets against colon cancer is limited. Hence, the objective of this study was to investigate the processing effects on Phytochemical Content and Chemopreventive potential of Beets (*Beta vulgaris*) using a colon cancer Fisher 344 male rat.

MATERIALS AND METHODS

Animal housing: Fisher 344 male weaning rats were obtained from Harlan, IN, housed in stainless steel wire cages at 2 rats per cage. The temperature and relative humidity were maintained at 21°C and 50%, respectively. Light and dark cycles were maintained at 12 h each. After a one-week acclimatization period, rats were divided into 7 groups (4 rats per group). All rats were given free access to potable water and fed control (AIN 93 G) and 2 and 4% levels of treatment diets (Fig. 1). Treatment diets were based on AIN 93 diet.

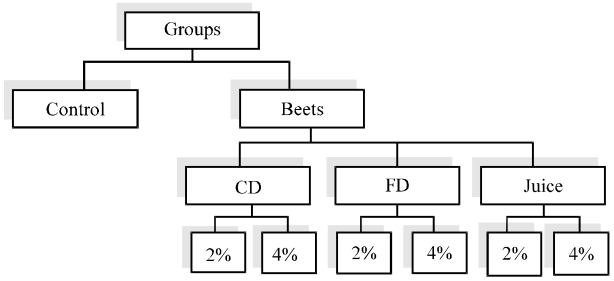

Fig. 1: Experimental design, CD: Cabinet dried, FD: Freeze dried, No. of rats per group = 4, No. of groups = 7

Diet preparation: Beets were obtained from the local farmers market and processed by different methods (i.e., freeze dried, cabinet dried and pasteurized) to prepare beet juice and powder. The powder was mixed in the diet at 2 and 4% levels. Diets were prepared and stored at refrigeration temperature (4°C) until fed. Modifications were made to maintain isocaloric nature of the diet. The groups receiving juice treatment were given C+2 and 4% beet juice instead of water.

Feed intake and body weights: Daily feed intakes and biweekly body weights were recorded throughout the experiment.

Carcinogen injection: For induction of ACF, all rats were given s/c injections of Azoxymethane (AOM) (NCI Chemical Repository, Kansas City, MO.) in saline at the rate of 16 mg kg^{-1} b.wt. at 7th week and another at 8th week of age.

Sample collection: Rats were killed by CO_2 asphyxiation at 17 weeks of age. Liver samples were removed and perfused with ice cold buffered saline (PBS), blotted onto filter papers, weighed and immediately frozen in liquid nitrogen and stored at -80 for further analysis. Colons were removed and flushed with phosphate buffer solution (0.1 M, pH 7.0) and prepared for counting ACF.

Enumeration of aberrant crypt foci (ACF): Each colon was divided into 2 equal segments (proximal and distal sections). Each respective segment was further divided into 2 cm segments, stained with 0.2% Methylene Blue for 5-10 min and examined under a light microscope. Enumeration of ACF's was performed as described by Bird (1987). ACF as well as crypts/focus were scored.

Cecal contents analysis: Ceca was flushed with potassium phosphate buffer 0.1 M, pH 7.2 and blotted on filter paper to measure cecal weight. Cecal contents were removed and pH noted.

Glutathione-S-transferase (GST) activity: Liver GST was assayed by a technique outlined by Habig *et al.* (1974).

Determination of catalase activity: Liver catalase was estimated in a UV recording spectrophotometer at 240 nm by monitoring the decomposition of H_2O_2 as described by Aebi (1984).

Determination of superoxide dismutase (SOD) activity: Liver superoxide dismutase was assayed by the technique of Fridovich (1989).

Preparation of beets extracts for total phenolics and flavonoids analysis as well as antioxidants activities using FRAP and DPPH: Beets were purchased from the local farmers market and Steam Blanched (SB) for 30 min using a steam blancher (Dixie Canner, Athens, GA) and then further processed by methods including Freeze Drying (FD) using a freeze dryer (Genesis, 35SQEL, SP Industries, Gardiner, NY), cabinet drying (CD) using a cabinet dryer (Proctor and Schwartz, 062:K23878, Horsham, PA, USA), conventional freezing (F) using a deep freezer (Kenmore, 25311351100, Washington, D.C.) and Individual quick freezing (IQF) method. Extracts were prepared from the FD, SB, CD, IQF, F and fresh forms of the beets by homogenizing 50 g of the beet samples in 150 mL of 80% methanol. The mixtures were then centrifuged at 4000 g for

10 min and the supernatant collected. This was then dried using a rotary evaporator (Buchi Rotavapor R-250) equipped with self cleaning dry vacuum system, model 2025 at 40°C, the residue was collected and stored at 80°C until determination of phenolics, flavonoids and anthocyanins.

Total phenolics determination: The total phenolic content of samples was analyzed by the Folin-Ciocalteu colorimetric method (Singleton *et al*., 1999).

Total flavonoids determination: A colorimetric assay (Kim *et al*., 2003) was used to quantify total flavonoid content.

Determination of ferric reducing antioxidants power (FRAP): The FRAP assay was conducted as described by Benzie and Strain (1999).

Free radical scavenging activity: The free radical scavenging activity of extracts was measured using the method of Brand-Williams *et al*. (1995) with some modifications.

Statistical analysis: Results are presented as Means±SEM. ANOVA was used to determine any significant differences among the treatment groups. Where significant ($p \leq 0.05$), means separated using Tukey's studentized range test. Statistical analysis was conducted using SAS, 9.1 (SAS, 2004).

RESULTS

Daily feed intake and weight gain in rats fed beets: There were significant differences seen among the rats fed the treatment and control diets. Rats fed the control diet had significantly lower feed intake compared to the treatment groups. However, there were no significant differences among the rats fed the beet diets. Among the treatment groups, the rats fed 4% beet juice had the highest feed intake (16.56 g) followed by the rats fed 4% FD, 2% beet juice, 2% FD, 2% CD and 4% CD (Table 1).

Cecal weight and cecal pH in rats fed beets: There were no significant differences in cecal weight or pH among the rats fed control and the beet diets (Table 2).

Aberrant crypt foci (ACF) incidence: Total ACF incidence was higher in the distal colon compared to the proximal both in the control and the beet diets. Lower incidence of ACF's was observed in the beet diets compared to the control both in the distal and the proximal colons. There

Table 1: Feed intake and weight gain in Fisher 344 male rats fed processed beets

Groups of beets	Feed intake (g day^{-1})	Weight gain (g/13 week)
Control	13.86±0.01^{b}	192.3±10.31^{b}
FD 2%	15.75±0.07^{a}	231.0±17.28^{a}
CD 2%	15.71±0.03^{a}	210.3±21.43^{a}
FD 4%	16.36±1.40^{a}	210.0±3.48^{a}
CD 4%	14.99±0.91^{a}	201.0±10.76^{a}
Juice 2%	16.23±0.12^{a}	199.7±5.76^{a}
Juice 4%	16.56±0.48^{a}	218.0±10.09^{a}

CD: Cabinet dried, FD: Freeze dried, Values are Means±SEM, n = 4, Values not sharing a common superscript are significantly different at $p<0.05$ using Tukey's studentized range test

Table 2: Beets effect on cecal weight and cecal pH of Fisher 344 male rats

Groups of beets	Cecal weight (g)	Cecal pH
Control	1.12±0.08[a]	8.25±0.03[a]
FD 2%	1.17±0.08[a]	7.94±0.26[a]
CD 2%	1.07±0.09[a]	7.95±0.11[a]
FD 4%	1.08±0.26[a]	7.95±0.07[a]
CD 4%	1.35±0.06[a]	7.82±0.09[a]
Juice 2%	1.58±0.06[a]	7.69±0.10[a]
Juice 4%	1.53±0.07[a]	7.79±0.07[a]

CD: Cabinet dried, FD: Freeze dried, Values are Means±SEM, n = 4, Values sharing a common superscript are non significant at $p<0.05$ using Tukey's studentized range test

Table 3: Effects of processed beets on AOM-induced total ACF in Fisher 344 male rats

Groups of beets	Proximal	Distal	Total ACF
Control	34.50±0.88[a]	103.25±1.86[a]	137.75±2.51[a]
FD 2%	17.25±0.88[b]	25.75±1.76[b]	43.00±4.50[b]
CD 2%	14.75±1.53[b]	27.75±0.58[b]	42.50±3.61[b]
FD 4%	9.25±1.52[c]	17.50±1.76[c]	26.75±5.13[d]
CD 4%	10.50±1.86[c]	19.75±1.86[c]	30.25±5.57[d]
Juice 2%	12.25±2.52[bc]	24.25±2.08[b]	36.50±6.56[c]
Juice 4%	9.50±0.58[c]	23.25±1.45[b]	33.75±3.61[cd]

CD: Cabinet dried, FD: Freeze dried, Values are Means±SEM, n = 4, Values not sharing a common superscript are significantly different at $p<0.05$ using Tukey's studentized range test

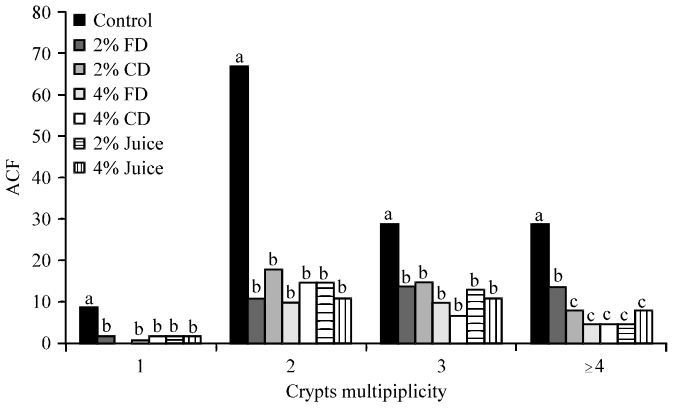

Fig. 2: Effects of feeding processed beets on crypts multiplicity, FD: Freeze dried, CD: Cabinet dried, n = 4, Values not sharing a common superscript are significantly different at $p<0.05$ using Tukey's studentized range test

was an average total ACF incidence reduction of 400% in the rats fed the treatment diets compared to the control (Table 3). The rats fed higher levels of beets (4% CD and FD) had significantly ($p<0.05$) lower ACF compared to the other treatment groups. Reductions in total ACF in rats fed beets diets ranged from a low of 63% in the 2% FD to a high of 80% in the rats fed 4% CD (Fig. 3).

Crypt multiplicity: Crypt multiplicity indicates the size of the ACF and shows cell proliferation. It is expressed as ACF with 1, 2, 3 and 4 or more crypt/focus. Figure 2 shows the effects of feeding processed beets on crypt multiplicity. ACF with 1 crypt were lower in rats fed both control and beet

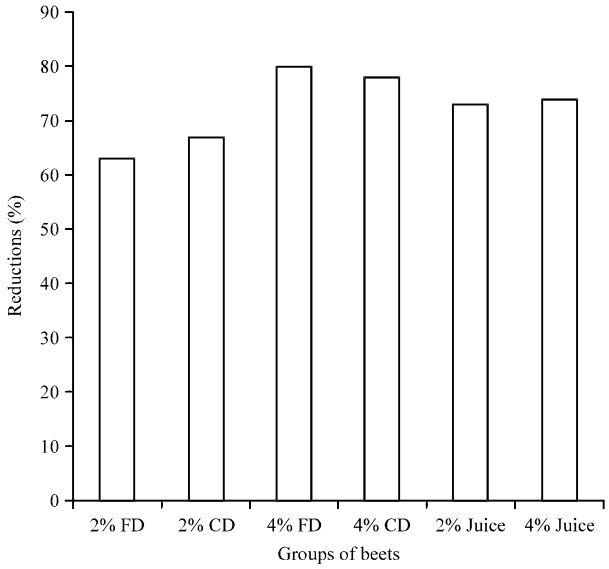

Fig. 3: Reductions (%) in total crypts compared to the control in the colon of Fisher 344 male rats fed processed beets, FD: Freeze dried, CD: Cabinet dried

diets compared to ACF with 2-4 crypts. Larger ACF (2, 3 and 4) were significantly higher ($p<0.05$) in the control compared to the treatment groups. ACF with 2 and 3 crypts were significantly ($p<0.05$) higher in the control compared to the treatment groups. However, larger ACF with multi crypts (≥4 crypts) were significantly lower in other treatment groups compared to the rats fed 2% FD (Fig. 2).

Total crypts: Total crypts give an indication of cell proliferation as it takes into account ACF and crypts multiplicity. The incidence of total aberrant crypts ranged from a high of 355 crypts in the rats fed the control to a low of 71 crypts (distal and proximal combined) in the rats fed 4% FD. Total crypts were higher in the distal colon compared to the proximal colon. Rats fed the beet diets had lower crypts compared to the rats fed the control diet both in the distal and the proximal colons. Among the rats fed the beet diets, the rat fed the 4% FD had the lowest number of total aberrant crypts in the distal colon as well as the total (45 and 71 crypts). Rats fed the 4% CD had the lowest aberrant crypts in the proximal colon (24 crypts). Among the rats fed beet diets, rats fed higher (4%) level had lower crypts compared to the rats fed the 2% level (Table 4).

Catalase, superoxide dismutases (SOD) and glutathione-S-transferases (GST) activities: Catalase activity was significantly higher ($p<0.05$) in the rats fed beets (about two folds) compared to the rats fed the control diet. Among the rats fed the beet diets, there were no significant differences ($p<0.05$) in catalase activity. However, the highest activity among the treatment groups was observed in the group fed 2% CD (98.98±0.02 μmol mL^{-1}) and the lowest was observed in the group fed 4% CD (84.61±0.03 μmol mL^{-1}). SOD activity was also significantly higher ($p<0.05$) in the rats fed the beet diets (over 2.5-3 folds) compared to the control group. There were no significant differences among the rats fed beet diets in the SOD activity. SOD activity (μmol mL^{-1}) however ranged from low of 51.21±0.001 in the rats fed the control to a high of 151.10±1.42 in the rats fed 4% beet juice. GST activity was numerically higher in the rats fed the 2% freeze dried beets

Table 4: Effects of feeding beets on AOM-induced total crypts

Groups of beets	Proximal	Distal	Total crypts
Control	84	271	355
FD 2%	56	76	132
CD 2%	39	77	116
FD 4%	26	45	71
CD 4%	24	52	76
Juice 2%	28	68	96
Juice 4%	25	68	93

CD: Cabinet dried, FD: Freeze dried, n = 4

Table 5: Effect of beets on selected hepatic enzyme activity in Fisher 344 male rats

Groups of beets	Catalase activity (μmol mL^{-1})	SOD activity (μmol mL^{-1})	GST activity (μmol mL^{-1})
Control	55.60±0.001^{b}	51.21±0.001^{b}	71.74±0.002^{c}
Juice 4%	95.29±0.04^{a}	151.103±1.42^{a}	101.56±0.01^{a}
Juice 2%	89.64±0.04^{a}	143.587±4.11^{a}	72.20±0.01^{c}
FD 4%	92.98±0.05^{a}	148.716±4.18^{a}	63.25±0.01^{c}
CD 4%	84.61±0.03^{a}	138.432±3.38^{a}	69.58±0.01^{c}
CD 2%	98.98±0.02^{a}	149.506±1.81^{a}	63.29±0.01^{c}
FD 2%	94.80±0.04^{a}	140.805±3.24^{a}	83.33±0.01^{b}

SOD: Superoxide dismutases, GST: Glutathione-S-transferase, CD: Cabinet dried, FD: Freeze dried, Values are Means±SEM, n = 4, Values not sharing a common superscript are significantly different at $p<0.05$ using Turkey's studentized range test

followed by the rats fed 4% juice. There were no significant differences in GST activity among the rats fed the control and beet diets. Catalase and SOD are essential antioxidants enzymes which work together to neutralize free radicals in body. SOD converts superoxide into hydrogen peroxide which is further converted to water and oxygen by catalase which is then excreted from the body. GST is a family of enzymes that works to conjugate, inactivate and excrete carcinogens (Table 5).

Total phenolics and flavonoids contents of fresh, branched, IQF, conventional freezing, freeze dried and cabinet dried beets: Total phenolic content in the beet samples was determined using the Folin-Ciocalteu method. Total phenolic content ranged from a low of 73.16±1.71 in the IQF to a high of 237.13±5.46 in the CD beets. There were no significant differences among the various processing methods applied to beets and the fresh form (CD, FD, SB and F) with the exception of IQF. The IQF beets had significantly lower phenolic content compared to the other processed and fresh beets. However, total flavonoid contents ranged from a low of 37.84±1.40 in the fresh beets to a high of 56.89±1.25 in FD. There were significant differences ($p<0.05$) in flavonoid content between processed and fresh beets. Total flavonoids were significantly ($p<0.05$) higher in the CD and FD compared to the other processed forms and the fresh beet. Fresh beet had significantly ($p<0.05$) higher flavonoid content compared to the SB, F and IQF beets. Among the FD and the CD beets, there were no significant differences in flavonoid content. Anthocyanin content in beets was however undetectable (Table 6).

FRAP and DPPH activities of fresh, branched, IQF, conventional freezing, freeze dried and cabinet dried beets: FRAP activity in the beets ranged from a low of 0.79±0.02 in the SB beets to a high of 4.96±0.06 in the CD beets. Percentage DPPH activity ranged from a low of 48% in the SB beets to a high of 78% in the FD. Processing effected the antioxidant activity of the beets

Table 6: Total phenolics and flavonoids in fresh and processed beets

Groups	TFC/CE/100 g beets	TPC/GAE/100 g beets
Fresh	37.84±1.40^{b}	203.62±2.44^{b}
SB	38.35±0.43^{b}	161.35±0.46^{c}
Frozen	38.60±2.23^{b}	111.06±2.92^{d}
IQF	42.60±2.00^{b}	73.16±1.71^{e}
CD	53.13±2.22^{a}	237.13±5.46^{a}
FD	56.89±1.25^{a}	230.62±11.91^{a}

TFC: Total flavonoids content, TPC: Total phenolic content, CE: Catechin equivalent, GAE: Gallic acid equivalent, IQF: Individually quick frozen, SB: Steam blanched, CD: Cabinet dried, FD: Freeze dried, Values are Means±SEM, n = 3, Values not sharing a common superscript are significantly different at $p<0.05$ using Turkey's studentized range test

Table 7: Effect of processing methods on antioxidant activity of fresh and processed beets using FRAP and DPPH

Beet forms	FRAP activity	DPPH activity (%)
Fresh	1.97±0.03^{d}	60
SB	0.79±0.02^{f}	48
Frozen	2.18±0.02^{c}	51
IQF	1.42±0.07^{e}	50
CD	4.96±0.06^{a}	63
FD	2.54±0.03^{b}	78

FRAP: Ferric reducing antioxidant power, DPPH: 2, 2-diphenyl-1-picrylhydrazyl, IQF: Individually quick frozen, SB: Steam blanched, CD: Cabinet dried, FD: Freeze dried, Values are Means±SEM, n = 3, Values not sharing a common superscript are significantly different at $p<0.05$ using Turkey's studentized range test

when measured with both the FRAP and the DPPH methods. There were significant ($p<0.05$) differences in antioxidant activity measured with FRAP among the fresh and the processed beets. FRAP activity of the fresh beets was significantly ($p<0.05$) higher compared to the SB and IQF but significantly ($p<0.05$) lower compared to frozen (F), CD and FD beets. Among the processed beets, higher FRAP activity was observed in the CD followed by the FD, F, IQF while the SB beets had the lowest FRAP activity. However, the antioxidant activity measured using DPPH showed the FD beets having the highest antioxidant activity (78%). Fresh beets had higher percentage DPPH (60%) compared to the SB (48%), F (51%) and the IQF (50%) but lower activity compared to FD and the CD. Among the processed beets, the SB had the lowest percentage DPPH activity (Table 7).

DISCUSSION

In this study, the objective was to investigate the chemopreventive potential of processed beets (cabinet dried, freeze dried and pasteurized juice) fed at of 2 and 4% level on Fisher 344 male Rats against azoxymethane induced Aberrant Crypt Foci (ACF) and to determine the effects of processing (blanching, freezing, freeze drying, "individual quick freeze" and cabinet drying) on phenolics and flavonoids contents and antioxidants activities measured by FRAP and DPPH of beets.

Experimental and epidemiological studies have reported a reduction in incidence of chronic diseases such as colon cancer through consumption of diets rich in bioactive compounds (Kim *et al*., 2003; Ou *et al*., 2002; Miller *et al*., 2000). Fruits and vegetables are rich sources of phytonutrients which have been reported to offer health benefits against chronic diseases (Cai *et al*., 2003). Phytonutrients in fruits and vegetables include fiber, antioxidants, polyphenols, indoles and allium

compounds. These compounds may offer health benefits through complementary or overlapping mechanisms which include modulation of detoxification enzymes, provide protection against oxidative stress, gene regulation in cell proliferation and several others (Netzel *et al.*, 2006; Waladkhani and Clemens, 1998).

Based on the results, weight gain (g/13 week) was significantly higher ($p<0.05$) in the rats fed the treatments diets (Table 1). This might be due to the higher feed intake in the rats fed the treatment diets compared to the control. Beets contain considerable amount of sugar and flavor compounds (Zarrabi, 2007) which might make the diets more palatable hence resulting in the greater feed consumption compared to the control which translated to the increased weight gain. A similar result was also reported by Boateng *et al.* (2007) and Kanda *et al.* (2012). They reported a significantly higher ($p<0.05$) weight gain and feed intake in rats fed selected fruit diets and juices compared to the control. Our results showed no significant differences ($p<0.05$) in cecal weight and cecal pH in the rats fed beets and the control (Table 2).

ACF numbers were significantly lower ($p<0.05$) in the treatment groups compared to the control (Table 3). Our results confirm published research from our laboratory and other researchers (Boateng *et al.*, 2007; Tache *et al.*, 2007) where feeding of selected fruits and onions reduced the incidence of ACF in rats. Studies suggest that fruits and vegetables contain macronutrients such as fiber and micronutrients such as trace minerals and vitamins which have been found to reduce the risk of cancer (Surh, 2003). In addition to the macro and micro nutrients, beets have been reported to contain dietary phytochemicals such as flavonoids which have been suggested to exhibit chemopreventive properties (Kujala *et al.*, 2002). Phytochemicals in beets may block the carcinogen (AOM) from reaching the sites of initiation (DNA and proteins) (Pereira *et al.*, 2011; Surh, 2003; Williams *et al.*, 2008), hence preventing the formation of ACF; an observation that may account for the lower incidence of ACF in the rats fed beet diets compared to the rats fed the control diets.

The antiproliferative properties of beets were also demonstrated by the reduced number of total crypts and crypt multiplicity in the rats fed the beet diets compared to the control (Table 4 and Fig. 3). Crypt multiplicity was higher in rats fed the control diet compared to the rats fed the beet diets. ACFs with higher crypt multiplicity (≥ 4) have the greatest possibility of progressing into tumors compared to ACF with lower crypts (≤ 3) which may possibly dissolve and disappear over time (Bird, 1987). Antiproliferative properties of fruits and vegetables might be due the presence of phytochemicals such as flavonoids which are antioxidants (Kameswaran and Ramanibai, 2008), hence have the ability to scavenge free radicals and inhibit the proliferation of damaged cells (Walle, 2007). However, the inhibition of growth of breast, colon, stomach, CNS and lung cancer tumor lines at concentrations of 12.5 to 200 $\mu L\ mg^{-1}$ of betanin in a dose dependant manner (Reddy *et al.*, 2005) clearly establishes the antiproliferative potential of beets which is a good source of betanin.

Beets are also known to contain betaine which has also been studied for its numerous health benefits. Betaine is reported to work in synergism with choline, folic acid, vitamin B-12 and methionine (SAM) as methyl group donor in DNA methylation (DeFelice, 2003). These properties of beets may as well be responsible for the lower incidence of ACF and crypt multiplicity observed in the results.

Results from our research also demonstrated that beets had a significant impact on selected hepatic enzymes (Table 5). Feeding beets enhanced liver catalase and superoxide dismutases activities (2 folds) but had no effect on GST activity in Fisher 344 male rats compared to the control. Similar results were observed in previous research by Sunkara *et al.* (2008) where, cranberry juice

resulted in an increase in the activities of CAT and SOD by 5-6 folds. Betacyanins and fiber content in beets have been suggested to enhance the activities of antioxidant and detoxification enzymes and increase the number of certain white blood cells responsible for the detection and elimination of aberrant cells (Murray *et al.*, 2005; Sengottuvelan *et al.*, 2006). Liver SOD works to convert xenobiotics into hydrogen peroxide which can still be harmful to the body (Sridevi *et al.*, 2007). Catalase further converts the hydrogen peroxide to water and oxygen which the body can readily excrete. However, GST is family of enzymes which work to promote the balance between endogenous prooxidants and antioxidants. Glutathione-S-transferases activity was similar among control and the rats fed the beet diets. This could be due to other mechanisms that need to be further investigated.

Total Phenolic Content (TPC) of unprocessed and processed beets ranged from 73.16±1.71 to 230.62±11.91 mg GAE/100 g beets (Table 7). Our results were higher than those reported by Jiratanan and Liu (2004) who found TPC range of 20-140 mg GAE/100 g beets. Stintzing and others also reported findings of TPC (242±13.4 to 660±35 mg GAE/100 g cactus) in various edible parts (pulp, seed and skin) of cactus pear (*Opuntia* sp.) which belong to the same family with beets. However, results of TPC in beets reported by Vinson *et al.* (1998) and Kujala *et al.* (2000) contradict our findings; they reported TPC in raw and cooked beets as 53.4±7.61 and 15.5±0.001-13.10±0.003 mg GAE/100 g beets, respectively.

Total Flavonoid Content (TFC) in beets (unprocessed and processed) ranged from 37.84±1.40-56.89±1.25 mg CE/100 g beets. Our results were similar to the findings from Chang *et al.* (2008) who reported TFC of 29.2±1.5-144.1±10.3 mg CE/100 g of fresh *Opuntia* (pulp, seed and peel). Tesoriere *et al.* (2005) reported TFC of 2.7±0.2 mg CE in cactus juice. Anthocyanin content in beet was undetectable. This confirms results by Kujala *et al.* (2000). This is attributed to the fact that both anthocyanins and betalain cannot co-exist in the same plant since they are responsible for different spectrum of coloration in plant. Beets are high in betalain, the color responsible for the red-yellow coloration in beets, while anthocyanins are responsible for orange, purple, blue etc. color in plants (Kujala *et al.*, 2002; Muchuweti and Chikwambi, 2008; Tiwari *et al.*, 2009).

The differences in polyphenolic content reported by several researchers may be affected by several factors (Shoji, 2007). Since, there is no definitive protocol in estimating the content of polyphenolic compounds in foods, researchers use several different methods and equipments for extraction of polyphenols. Research suggests that pH, temperature, light and other factors may affect the polyphenolic content in the same plants (Lamikanra *et al.*, 2005). One of the major factors that may contribute to differences in polyphenolic content is the extraction procedures. Methanolic extraction has been reported to yield higher polyphenolic activity compared to acetone and water (Sayago-Ayerdi *et al.*, 2007). Differences may also be due to the variety of the plants, climatic conditions and the soil in which the plant was cultivated, degree of maturity of the plant and several other factors (Penna *et al.*, 2001).

Researchers have reported several contradictory results regarding postharvest processing effects on the antioxidant contents in fruits and vegetables (Jiratanan and Liu, 2004; Shahidi and Naczk, 1995). Our TPC and TFC which were higher in the FD and CD beets compared to the fresh and other processed beets corresponded to higher free radical scavenging activity measured by DPPH and FRAP. This implies that antioxidant activity may be increased as a result of postharvest processing. Findings from our results may be attributed to the fact that phytochemicals in vegetables which are covalently bonded to an amine functional group and also esterified to glycosides require heat to release them and processing may therefore enhance bioavailability. This

confirmed the results by Jiratanan and Liu (2004) that reported that processing, especially drying of beets for longer periods yielded higher phenolic and flavonoid contents than unprocessed forms. Similar results were demonstrated by heat treatment of sweet corn yielding a higher phenolic content (Shahidi and Naczk, 1995) compared to the non-processed form. However, Jiratanan and Liu (2004) also reported that, antioxidant capacity of green beans remained the same after thermal processing. Our findings and that of other researchers suggest that, the effect of processing on antioxidants activities in vegetables may vary from species to species (Inocent *et al*., 2011).

CONCLUSION

From the results of the study, we found out that feeding processed beets both in the diet and juice (2, 4%) levels reduced the incidence of azoxymethane -induced aberrant crypts foci in Fisher 344 male rats. The results also suggest that processing may have different effects on phenolic contents in vegetables. These observations and that of other researchers suggest that postharvest processing effects may depend on the type of processing as well as may be species specific and on processing parameters.

End point tumor model and further clinical trials need to be investigated to make definitive conclusions. Studies should also be conducted to access the bioavailability of the phytochemicals from the beets. Further research is also needed to establish the processing methods required for particular vegetables in order to minimize of prevent the loss of antioxidants to positively impact health.

ACKNOWLEDGMENT

This study was supported by The Agricultural Experiments Research Station, Alabama A and M University, Normal, Al. 35762.

REFERENCES

Aebi, H., 1984. Catalase *in vitro*. Methods Enzymol., 105: 121-126.

Benzie, I.F. and J.J. Strain, 1999. Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods Enzymol., 299: 15-27.

Bird, R.P., 1987. Observation and quantification of aberrant crypt foci in murine colon treated with a colon carcinogen: Preliminary findings. Cancer Lett., 37: 147-151.

Boateng, J., M. Verghese, L. Shackelford, L.T. Walker and J. Khatiwada *et al*., 2007. Selected fruits reduce azoxymethane (AOM)-induced aberrant crypt foci (ACF) in Fisher 344 male rats. Food Chem. Toxicol., 45: 725-732.

Brand-Williams, W., M.E. Cuvelier and C. Berset, 1995. Use of a free radical method to evaluate antioxidant activity. LWT Food Sci. Technol., 28: 25-30.

Cai, Y., M. Sun and H. Corke, 2003. Antioxidant activity of betalains from plants of the Amaranthaceae. J. Agric. Food Chem., 51: 2288-2294.

Cao, E.H., X.Q. Liu, J.J. Wang and N.F. Xu, 1996. Effect of natural antioxidant tanshinone II-A on DNA damage by lipid peroxidation. Free Radic. Biol. Med., 20: 801-806.

Chang, S.F., C.L. Hsieh and G.C. Yen, 2008. The protective effect of *Opuntia dillenii* haw fruit against low-density lipoprotein peroxidation and its active compounds. Food Chem., 106: 569-575.

DeFelice, E., 2003. Nutrition and Health: Web Resource Guide for Consumers, Healthcare Providers, Patients and Physicians. Universe Inc., Lincoln, NE., USA., ISBN-13: 9780595296750, pages: 76.

Fridovich, I., 1989. Superoxide dismutases. An adaptation to a paramagnetic gas. J. Biol. Chem., 264: 7761-7764.

Greenlee R.T., T. Murray, S. Bolden and P.A. Wingo, 2000. Cancer statistics 2000. Cancer J. Clin., 50: 7-33.

Habig, W.H., M.J. Pabst and W.B. Jakoby, 1974. Glutathione-S-transferases. The first enzymatic step in mercapturic acid formation. J. Biol. Chem., 249: 7130-7139.

Inocent, G., D.M. Adelaide, E.L. Gisele, M.O.R. Solange, E.A. Richard and F. Elie, 2011. Impact of three cooking methods (steaming, roasting on charcoal and frying) on the beta-carotene and vitamin c contents of plantain and sweet potato. Am. J. Food Technol., 6: 994-1001.

Jiratanan, T. and R.H. Liu, 2004. Antioxidant activity of processed table beets (*Beta vulgaris* var. *conditiva*) and green beans (*Phaseolus vulgaris* L.). J. Agric. Food Chem., 52: 2659-2670.

Johnson, J.J. and H. Mukhtar, 2007. Curcumin for chemoprevention of colon cancer. Cancer Lett., 255: 170-181.

Kameswaran, T.R. and R. Ramanibai, 2008. The antiproliferative activity of flavanoidal fraction of *Indigofera tinctoria* is through cell cycle arrest and apoptotic pathway in A-549 cells. J. Boil. Sci., 8: 584-590.

Kanda, B., J. Boateng, L. Shackelford, S. Appiah, K. Campbell L.T. Walker and M. Verghese, 2012. Effects of processed peaches (*Prunus persica*) in reducing azoxymethane (AOM) induced Aberrant Crypt Foci (ACF) in Fisher 344 male rats. Int. J. Cancer Res., 8: 1-14.

Kim, D., O. Chun, Y. Kim, H. Moon and C. Lee, 2003. Quantification of phenolics and their antioxidant capacity in fresh plums. J. Agric. Food Chem., 51: 6509-6515.

Kujala, T.S., J.M. Loponen, K.D. Klika and K. Pihlaja, 2000. Phenolics and betacyanins in red beetroot (*Beta vulgaris*) root: Distribution and effect of cold storage on the content of total phenolics and three individual compounds. J. Agric. Food Chem., 48: 5338-5342.

Kujala, T.S., M.S. Vienola, K.D. Klika, J.M. Loponen and K. Pihlaja, 2002. Betalain and phenolic compositions of four beetroot (*Beta vulgaris*) cultivars. Eur. Food Res. Technol., 214: 505-510.

Lako, J., V. Trenerry, M. Wahlqvist, N. Wattanapenpaiboon, S. Sotheeswaran and R. Premier, 2007. Phytochemical flavonols, carotenoids and the antioxidant properties of a wide selection of Fijian fruit, vegetable and other readily available foods. Food Chem., 101: 1727-1741.

Lamikanra, O., K.L. Bett-Garber, D.A. Ingram and M.A. Watson, 2005. Use of mild heat pre-treatment for quality retention of fresh-cut cantaloupe melon. J. Food Sci., 70: C53-C57.

Lange, W., W.A. Brandenburg and T.S.M. De Bck, 1999. Taxonomic and cultonomy of beets (*beta vulgaris*). Bot. J. Linn. Soc., 81: 81-96.

Miller, H.E., F. Rigelhof, L. Marquart, A. Prakash and M. Kanter, 2000. Antioxidant content of whole grain breakfast cereals, fruits and vegetables. J. Am. Coll. Nutr., 19: 312S-319S.

Muchuweti, M. and Z. Chikwambi, 2008. Isolation and identification of anthocyanins in the fruit peels of starkrimson and marx red bartlett common pear cultivars and their bud mutants. Am. J. Food Technol., 3: 1-12.

Murray, M.T., J. Pizzorno and L. Pizzorno, 2005. The Encyclopedia of Healing Foods. Atria Books, New York, USA., ISBN-13: 9780743474023, pp: 164-166.

Netzel, M., G. Netzel, Q. Tian, S. Schwartz and I. Konczak, 2006. Sources of antioxidant activity in Australian native fruits. Identification and quantification of anthocyanins. J. Agric. Food Chem., 54: 9820-9826.

Nicoli, M.C., M. Anese and M. Parpinel, 1999. Influence of processing on the antioxidant properties of fruits and vegetables. Trends Food Sci. Technol., 10: 94-100.

Ou, B., D. Huang, M. Hampsch-Woodill, J.A. Flanagan and E.K. Deemer, 2002. Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays: A comparative study. J. Agric. Food Chem., 50: 3122-3128.

Panella, L. and R.T. Lewellen, 2007. Broadening the genetic base of sugar beet: Introgression from wild relatives. Euphytica, 154: 383-400.

Penna, C., S. Marino, E. Vivot, M.C. Cruanes and J.D. Munoz *et al*., 2001. Antimicrobial activity of argentine plants used in the treatment of infectious diseases isolation of active compounds from *Sebastiania brasiliensis*. J. Ethnopharmacol., 77: 37-40.

Pereira, P.C., A.F. Vicente, M.F. Mesquita and A.S. Cabrita, 2011. Dietary fiber and DMBA-induced rat mammary carcinomas. Int. J. Cancer Res., 7: 289-299.

Reddy, M.K., R.L. Alexander-Lindo and M.G. Nair, 2005. Relative inhibition of lipid peroxidation, cyclooxygenase enzymes and human tumor cell proliferation by natural food colors. J. Agric. Food Chem., 53: 9268-9273.

SAS, 2004. Statistical Analysis Software. Version 9.1. SAS Institute, Cary, New Jersey, USA.

Sayago-Ayerdi, S.G., S. Arranz, J. Serrano and I. Goni, 2007. Dietary fiber content and associated antioxidant compounds in roselle flower (*Hibiscus sabdariffa* L.) beverage. J. Agric. Food Chem., 55: 7886-7890.

Sengottuvelan, M., S. Rajagopal and N. Nalini, 2006. Modulatory influence of dietary resveratrol during different phases of a 1, 2-dimethylhydrazine induced mucosal lipid-peroxidation, antioxidant status and aberrant crypt foci development in rat colon carcinogenesis. Biochimica Biophysica Acta, 1760: 1175-1183.

Shahidi, F. and M. Naczk, 1995. Food Phenolics: An Overview in Shahidi. In: Food Phenolics Sources, Chemistry, Effects and Application, Naczk, F. (Ed.). Technomic Publishing Company Inc., Lancater, PA., pp: 1-5.

Shoji, T., 2007. Polyphenols as natural food pigments: Changes during food processing. Am. J. Food Technol., 2: 570-581.

Singleton, V.L., R. Orthofer and R.M. Lamuela-Raventos, 1999. [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol., 299: 152-178.

Sridevi, M., G. Chandramohan and K.V. Pugalendi, 2007. Protective effect of *Solanum surattense* Leaf-extract on blood glucose, oxidative stress and hepatic marker enzymes in STZ-diabetic rats. Asian J. Biochem., 2: 247-255.

Sunkara, R., M. Verghese, V. Panala, R. Field, J. Boateng, L.A. Shackelford and L.T. Walker, 2008. Chemopreventive potential of cranberries on azoxymethane induced aberrant crypt foci in Fisher 344 male rats. Int. J. Cancer Res., 4: 52-60.

Surh, Y.J., 2003. Cancer chemoprevention with dietary phytochemicals. Nat. Rev. Cancer, 3: 768-780.

Tache, S., A. Ladam and D. Corpet, 2007. Chemoprevention of aberrant crypt foci in the colon of rats by dietary onion. Eur. J. Cancer, 43: 454-458.

Tesoriere, L., D. Butera, M. Allegra, M. Fazzari and M.A. Livrea, 2005. Distribution of betalain pigments in red blood cells after consumption of cactus pear fruits and increased resistance of the cells to *ex vivo* induced oxidative hemolysis in humans. J. Agric. Food Chem., 53: 1266-1270.

Tiwari, B.K., C.P. O'Donnell, A. Patras, N. Brunton and P.J. Cullen, 2009. Anthocyanins and color degradation in ozonated grape juice. Food Chem. Toxicol., 47: 2824-2829.

Van Duyn, M.A. and E. Pivonka, 2000. Overview of the health benefits of fruit and vegetable consumption for the dietetics professional: Selected literature. J. Am. Diet. Assoc., 100: 1511-1521.

Vinson, J.A., Y. Hao, X. Su and L. Zubik, 1998. Phenol antioxidant quantity and quality in foods: Vegetables. J. Agric. Food Chem., 46: 3630-3634.

Waladkhani, A.R. and M.R. Clemens, 1998. Effect of dietary phytochemicals on cancer development (review). Int. J. Mol. Med., 1: 747-753.

Walle, T., 2007. Methoxylated flavones, a superior cancer chemopreventive flavonoid subclass. Semin. Cancer Biol., 17: 354-362.

Williams, D.S., M. Verghese, L.T. Walker, J. Boateng and L.A. Shackelford *et al*., 2008. Chemopreventive effects of flax seed oil and flax seed meal on azoxymethane-induced colon tumors in Fisher 344 male rats. Int. J. Cancer Res., 4: 28-40.

Zarrabi, M., 2007. Effect of sugar beet root aphid, *Pemphigus fuscicornis* (Homoptera: Pemphigidae), on sugar beet yield and quality in Iran. Pak. J. Biol. Sci., 10: 3462-3465.