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STUDY ON PHYTOCHEMICALS PRESENT IN JUMLI SIMI AND EFFECT OF COOKING ON THEM

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Abstract

Bean is an indigenous crop of Jumla and Karnali zone as a whole. The indigenous bean is gaining popularity by the name Jumli simi. Indigenous bean is highly diversified with multicolour and multi size and shape grain and trailing type plants. Local selections have been given name as KBL (Karnali Bean Line) and number are given as KBL-1, KBL-2 and so on and have grouped based on their colors. The Jumli simi were collected from NFC, Thapathali and were grouped into 4 varieties on the basis of color (KBL-1, 2, 3 and 4). Then physiochemical and cooking properties of each variety were observed. Analysis of phytochemicals (phenols, flavonoids, tannins, anthocyanins) of each raw and cooked samples were carried out. Beans with darker colored seed coats exhibited highest phytochemical content and antioxidant activity. The effect of cooking caused reduction in phytochemicals and antioxidant activity.

Keywords: *Jumli simi, phytochemicals, soaking, cooking, antioxidant activity*

Introduction

The common bean (*Phaseolus vulgaris* L.) has undergone wide production, has extensive domestication and cultivation. It includes a wide array of edible dry bean seed types that differ in size, shape and color and are collectively known as dry beans (Jones 1999). Dry edible beans (*Phaseolus vulgaris* L.) are cultivated and consumed in great quantities throughout the world (Sathe 2002). Bean is an indigenous crop of Jumla and Karnali zone as a whole. It can be understood as local *Rajma* too. The indigenous bean is gaining popularity by the name Jumli simi. Indigenous bean is highly diversified with multicolour and multi size and shape grain and trailing type plants. Previously this crop was grown in uplands only, particularly near the forests or in the land recently brought under cultivation after cleaning the forest trees or mostly under upland condition. Bean in Jumla is grown after wheat in low land condition. In upland also bean is grown as sole crop during summer season. It is also grown with maize crop (ARS 2012; Bhujel *et al*. 2012). Bean is cultivated in a wide range of agro-climatic conditions from plains at 300 metre above sea level to the high hills at 4,000 metre above sea level in different season (Neupane *et al*. 2005). At present 2200 ha has been estimated to be covered by this crop producing 2300 MT during 068/069 in Jumla. Local selections have been given name as KBL (Karnali Bean Line) and number are given as KBL-1, KBL-2 and so on and have grouped based on their colors (ARS 2012). The superior genotypes include PB 0001, PB 0002, PB 0048 (ARS 2011).

Dry beans are a good source of protein, dietary fibre, starch, minerals and vitamins. They can be promoted as a healthy food, being nutrient dense and an excellent source of dietary fibre. A diet high in beans can potentially reduce the risk of developing a chronic disease (Wu *et al*. 2004). The inclusion of dry beans and other legumes in the daily diet has many beneficial effects in controlling and preventing metabolic diseases (Dilis and Trichopoulou 2009; Raju and Mehta 2009). The health benefits of beans have been attributed to the presence of fibre, bioactive proteins and peptides and secondary metabolites such as phenolic

compounds possessing antioxidant properties (Azarpazhooh and Joyce 2013).

Grain legumes research has received relatively little attention in Nepal. Jumli simi is an important cash generating legume in Jumla and adjoining hilly districts. The traditional farming of common beans has been carried out in Karnali zone as well as Jumla district and isn't given much importance since, it is consumed as pulse in Jumla. Despite its richness in phenolic components and antioxidant activity not much attention and research has been conducted in Nepal. Proper data on cooked legumes isn't available since much research hasn't been carried out (Bhujel *et al*. 2012).

The various types of beans are a staple food and a low-cost source of protein. The study of phenolic profiles and antioxidant activity of bean reflects the knowledge of varietal superiority (Neupane *et al*. 2005; Bhujel *et al*. 2012). The health benefits resulting from its consumption is necessary to be highlighted among the people. The research was carried out to study the phenolic content and antioxidant property of Jumli simi varieties.

Materials and Methods

Raw material collection and preparation

The Jumli simi were collected from Nepal Food Corporation (NFC), Thapathali, Kathmandu on February 2016. The bean varieties were manually separated based on their color and size and grouped KBL 1 (black), KBL 2 (red), KBL 3 (brown spotted) and KBL 4 (white). Each variety were soaked in distilled water overnight at room temperature. While the remaining raw ones were grinded with mortar and pestle into powder form, the powder were packed into air tight zip line bags and stored for further analysis. While soaked beans were cooked after the soaked water was thrown. Cooking was carried out in a boiling distilled water 100ºC in a standard laboratory hotplate until desirable softness was obtained when pressed between index finger and thumb. Then they were surface dried and wrapped around in an almunium foil and placed in air tight zip line bag until further analysis. Analysis was carried out in GoldenGate International College laboratory.

Physiochemical properties

The physical properties (i.e. 100 kernels weight, length-breadth ratio, bulk density, hydration capacity, swelling capacity and moisture content) were determined as per AOAC (2005); Singh *et al*. (2005).

Cooking properties

The cooking time of bean varieties, water uptake ratio, elongation ratio, gruel solid loss and cooked length-breadth ratio were determined as per Singh *et al*. (2005).

Chemical analysis

Total phenolics and flavonoids

The method described by Sadasivam and Manickam (2008) with slight modification was used for determining total phenolics. Powdered sample of 0.5g was taken and extracted using 10mL 80% ethanol which was left at 50°C for 3 hours then centrifuged at 3000 rpm for 15 minutes. The extract then was filtered and collected in a separate test tube while 10 ml ethanol was added to the residue and allowed to leave overnight followed by centrifugation for 15 minutes. The extracts then were combined and volume was made up to 25 ml by 80% ethanol. Evaporation until dry was carried out of a portion of alcoholic extract (10 ml) on a water bath; the residue obtained was dissolved with 10mL of distilled water and used for the determination for both phenolics and flavonoids determination. Total flavonoid determination was done as per the described protocol (Shen *et al*. 2009) and was expressed as RE (rutin equivalent).

Tannin content

Tannin was determined by Folin-Denis method as described by Sadasivam and Manickam (2008). The tannin content was calculated as tannic acid equivalent from the standard graph and value determined from the following equation:

1 O.D. = 0.1931 mg

O.D = optical density

Preparation of bean extract

1 g powdered sample was taken in a test tube and mixed with 3 ml 99% methanol, which was left in water bath at 60°C for 20 minutes. Then centrifugation at 3000 rpm was carried out for 20 minutes and the extract was separated in a separate test tube. The same process was repeated with the residue and two extracts were combined and the volume was made up to 10 ml with the addition of methanol solution. The extract was used for the

determination of monomeric anthocyanins and antioxidant activity (Dzomba *et al*. 2013).

Determination of the total monomeric anthocyanin

Preparation of buffer

Potassium chloride buffer (0.025M, pH 1.0) and Sodium acetate buffer (0.4M, pH 4.5) were prepared as per AOAC (2005).

Preparation of test solution

Samples were diluted 10 times using buffer of pH 1.0 and pH 4.5.

Determination of absorbance and calculation

Spectrophotometer (APEL PD-303 UV spectrophotometer) was used for the determination of sample absorbance at 510 and 700 nm (AOAC 2005). Monomeric anthocyanin content was determined as per AOAC (2005) and expressed as cyanidine-3-glucoside.

Anthocyanin pigment (mg/L) = A * MW* DF *10^3 * $(\mathcal{E} \times 1)^{-1}$

A = $(A_{510}-A_{700})$ pH 1.0 – $(A_{510}-A_{700})$ pH 4.5

Where, A_{500} it the absorbance at 500 nm

A_{700} is the absorbance at 700 nm

MW is the molecular weight of cyanidine-3-glucoside and is equal to 449.2 g/mol

DF is the dilution factor established

$\mathcal{E}$ is the molar extinction coefficient and is equal to 26,900 L x mol^{-1} x cm^{-1}

10^3 is the factor for the conversion of gram into milligram

Analysis of antioxidant activity

Antioxidant activity of bean extract was determined by free radical scavenging activity using the DPPH assay (Blois 1958) with slight modification. 0.5, 1.0, and 1.5 ml of sample were taken and volume was made upto 2 mL by addition of methanol (99.9%). 2 ml 0.1 mM DPPH was added to each extract and shaken vigorously and allowed to stand for 20 minutes in dark at room temperature. Absolute methanol was taken as control. Absorbance was determined after 20 minutes at 517 nm. Percentage inhibition activity was calculated from the following equation.

Antioxidant activity (%) = $(A_0-A_1)/ A_0$ x 100

Where, A_0 is the absorbance of the control

A_1 is the absorbance of the extract / standard

Experiments were carried out three times. Data were expressed as % scavenging activity.

Data analysis

All experiments were carried out in triplicate and average values were reported. The data obtained in this experiment were statistically analysed by using Genstat 5 Release 12.1 software. Experimental data obtained were statistically analysed using one way Analysis of variance (no blocking) at 5% level of significance. The means were compared by using least significant method.

Results

Physicochemical properties of Jumli simi

Means in the row with similar superscript were not significantly different ($p > 0.05$)

The highest hundred kernels weight was 27.4 ± 0.14^c of KBL 3. The highest L/B ratio was 1.95 ± 0.01^c of KBL 4. Bulk density was highest in both KBL 1 and KBL 3. KBL 4 had the highest hydration capacity, hydration index, swelling capacity, swelling index with values of 0.19^d g/seed, 0.85^d, 0.42 ± 0.01^c ml/seed and 1.83 ± 0.05^d.

The highest moisture content was of KBL 3 and lowest of that of KBL 4.

Cooking properties of Jumli simi

KBL 4 and KBL 1 required the minimum and maximum cooking time of 40 and 47.25±0.25 min, respectively. Gruel solid loss % varied among the beans from 8.88±1.92 to 11.69±0.15 %. Water uptake ratio was found highest in KBL 4 and lowest in KBL 1 with 1.88±0.00 and 1.74±0.00 values respectively. Cooked L/B ratio and Elongation ratio varied among the different varieties of Jumli simi.

Chemical composition of Jumli simi

The total phenols in the four varieties of Jumli simi was detected in the range of 77.27±4.36 as gallic acid (mg %) in KBL 4 to 371.83±8.49 as gallic acid (mg %) in KBL 1. There was significant difference found in the most varieties of the beans. The results obtained on total phenols were in good agreement with that reported previously for common beans (Carador-Martinez *et al.* 2002; Xu *et al.* 2007).

Table 1. Physicochemical properties of Jumli simi.

Parameter	KBL 1	KBL 2	KBL 3	KBL 4
100 Kernels weight (g)	20.03±0.58[a]	24.28±0.06[b]	27.4±0.14[c]	22.03±0.13[d]
L/B ratio	1.35±0.00[a]	1.66±0.02[b]	1.74±0.02[a]	1.95±0.01[c]
Bulk Density (g/mL)	0.90±0.00[a]	0.83±0.00[b]	0.90±0.00[a]	0.81±0.01[c]
Hydration Capacity (g/seed)	0.10±0.00[a]	0.16±0.00[b]	0.11±0.00[c]	0.19±0.00[d]
Hydration index	0.32±0.00[a]	0.65±0.00[b]	0.53±0.00[c]	0.85±0.00[d]
Swelling capacity (ml/seed)	0.11±0.00[a]	0.17±0.00[b]	0.12±0.00[a]	0.42±0.01[c]
Swelling index	0.35±0.00[a]	0.70±0.00[b]	0.57±0.00[c]	1.83±0.05[d]
Moisture content (%)	10.91±0.11[a]	11.65±0.45[b]	12.16±0.26[b]	10.52±0.33[a]

*Values expressed are mean ± standard deviation

Table 2. Cooking properties of Jumli simi.

Parameter	KBL 1	KBL 2	KBL 3	KBL 4
Cooking time (min.)	47.25±0.25	42.35±0.04	46.42±0.07	40.00±0.00
Water uptake ratio	1.74±0.00	1.78±0.00	1.82±0.00	1.88±0.00
Elongation ratio	1.06±0.00	1.04±0.00	1.15±0.00	1.24±0.00
Gruel loss (%)	11.69±0.15	9.57±0.02	10.73±0.05	8.88±1.92
Cooked L/B ratio	1.36±0.00	1.67±0.00	1.83±0.00	1.85±0.00

Total flavonoids of Jumli simi ranged from 85.26±4.92 as rutin (mg %) in KBL 4 to 252.81±7.65 as rutin (mg %) in KBL 2. Significant differences ($p>0.05$) in total flavonoids were found in all the varieties of Jumli simi.

Table 3. Chemical composition of Jumli simi

Parameters	KBL 1	KBL 2	KBL 3	KBL 4
Phenols (mg GAE[1]/100g)	371.73±8.49[a]	355.75±5.02[b]	368.83±8.93[a]	77.27±4.36[c]
Flavonoids (mg RE[2]/100g)	226.72±7.62[a]	252.81±7.65[b]	207.42±6.95[c]	85.26±4.92[d]
Tannins (mg TA[3]/100g)	278.67±8.66[a]	207.62±6.16[b]	263.04±7.59[c]	48.12±4.75[d]
Antioxidant activity (% DPPH[4])	83.49±4.91[a]	72.26±3.03[b]	77.7±3.2[a,b]	28.84±4.12[c]
Anthocyanin (mg/g)	0.46±0.02[a]	0.39±0.01[b]	0.37±0.02[b]	0.15±0.00[c]

All parameters are expressed on dry weight basis. Means ± standard deviation bearing similar superscripts in row are not significantly different ($p>0.05$).

[1]GAE = Gallic acid equivalent

[2]RE = Rutin equivalent

[3]TA = Tannic acid

[4]DPPH = 2, 2- diphenyl-1-1-picrylhydrazyl

The highest tannin content was observed in KBL 1 with the value of 278.67±8.66 (mg %) and lowest in KBL 4 i.e. 48.12±4.75 (mg %). Anthocyanin content showed no significant difference between KBL 2 and KBL 3. Anthocyanin content ranged from 0.15±0.00 mg/g in KBL 4 to 0.46±0.02 mg/g in KBL 1.

Effect of cooking on phenols, flavonoids and tannins

Total phenol, flavonoid and tannin content varied significantly ($p<0.05$) in raw and cooked beans (KBL 1, 2, 3 and 4 respectively. In cooked beans total phenolics was found highest in KBL 2 while lowest was in KBL 4. KBL 3 had the highest % loss in phenolics of about 47% while KBL 2 had 29% loss and KBL 4 had lowest 23% loss in phenolics in cooked samples. On the other hand, for the flavonoids the total flavonoid content for both raw and cooked was found highest in KBL 2. The highest % loss in total flavonoids was seen 48% in cooked KBL 3 and lowest 30% in KBL 2.

Table 4. Effect of cooking on phenols, flavonoids and tannins.

Conditions	Variety	Phenols (mg GAE[1]/100 g)	Flavonoids (mg RE[2]/100 g)	Tannins (mg TA[3]/100 g)
Raw	KBL1	373.73±8.49[a]	226.72±7.62[a]	278.67±8.66[a]
Raw	KBL 2	355.75±5.02[b]	252.81±7.65[b]	207.62±6.16[b]
Raw	KBL 3	368.83±8.93[a]	207.42±6.95[c]	263.04±7.59[c]
Raw	KBL 4	72.77±4.36[c]	85.26±4.92[d]	48.12±4.75[d]
Cooked	KBL 1	206.08±4.85[a]	122.78±3.69[a]	62.51±2.15[a]
Cooked	KBL 2	220.68±4.99[b]	178.87±4.20[b]	64.97±2.46[a]
Cooked	KBL 3	198.67±6.76[a]	109.43±4.95[c]	61.29±2.28[a]
Cooked	KBL 4	50.03±2.22[c]	60.15±2.70[d]	16.31±0.26[b]

The highest tannin content among the cooked beans was found highest 64.97 mg% in KBL 2 and lowest 16.31 mg% in KBL 4 whereas, highest % loss in tannin content was approximately 78% in KBL 1 and lowest was 67% in KBL 4.

Effect of cooking on anthocyanin

There were no significant differences in anthocyanin content between KBL 2 and KBL 3 in both raw and cooked, while there was significant difference among the rest of the samples. Among the raw Jumli simi, KBL 1 had the highest anthocyanin content (0.46±0.02) compared to others.

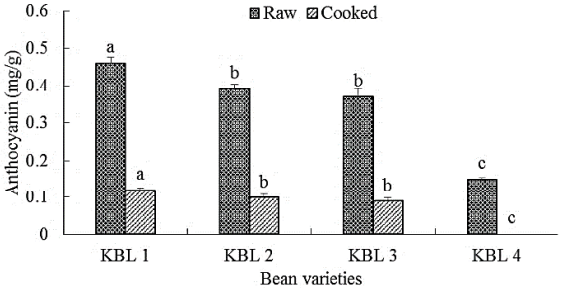

Figure 1. Effect of cooking on anthocyanin content of Jumli simi

Effect of cooking on DPPH radical scavenging capacity

Scavenging ability followed the order KBL 1> KBL 3> KBL 2> KBL 4. Statistical analysis showed KBL 3 had no significant differences with KBL 1 and 2 while rest of were significantly different from one another in case of raw samples. For the cooked, most of the samples showed significant difference. Scavenging activity on DPPH radical of Jumli simi was highest for raw variety 83.40% in KBL 1 while lowest was 28.84% in KBL 4. For the cooked sample the highest scavenging activity on DPPH radical was found highest again in KBL 1 72.03% and lowest in KBL 4 15.04%.

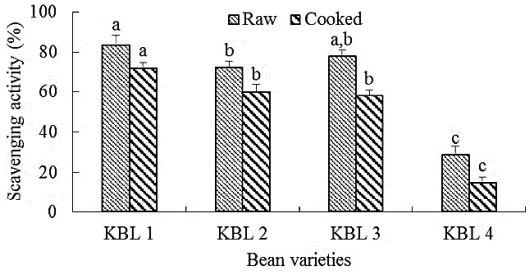

Figure 2. Effect of cooking on scavenging activity of Jumli simi

Discussion

Physicochemical properties of Jumli simi

Hydration capacity determines the extent to which seeds absorb water on soaking. Rakshit and Shimelis (2005) concluded that the legumes with higher hydration capacity, hydration index, swelling capacity and swelling index would require less cooking time, which is useful for saving fuel energy. The results of the present study are similar with those mentioned by previous workers (Wang *et al*. 2003; Rakshit and Shimelis 2005; FRD 2015) for other legumes. They reported that the legumes having the higher hydration and swelling capacity as well as index require less cooking time. A large hydration capacity leads to better cooking quality (less cooking time and texture) and quicker sprouting.

Cooking properties of Jumli simi

As KBL 2 and KBL 4, had comparatively higher swelling and hydration capacities, they required less cooking time. Cooking time of beans has been found to significantly decrease in beans that are soaked prior to cooking (Berrios *et al*. 1999; Rakshit and Shimelis 2005). The dark colored varieties seemed to have longer cooking time than the light ones. Similar findings relating to cooking properties of legumes have been reported in the FRD (2015). Longer cooking times result in a loss of nutrients so, the cooking time is of paramount importance for consideration. Akond *et al*. (2011)

and Dzomba *et al*. (2013) reported that the black beans had higher anthocyanin content, and similar result was obtained in the present study.

Chemical composition of Jumli simi

The results obtained on total phenols were in good agreement with that reported previously for common beans (Carador-Martinez *et al*. 2002; Xu *et al.* 2007). The value for total phenols was lower than that of dry bean produced in the United States (Wu *et al*. 2004). The differences between the above results and previous report may be attributed to the differences in the variety, growing conditions, solvent and temperature used during the extraction process (Xu *et al*., 2007).

The values corresponding to total flavonoids fell in the range as reported by Heimler *et al*. (2005) and Xu *et al*. (2007) while it was higher than as reported by Carador-Martinez *et al*. (2002). Growing location, post-harvest storage might contribute to the variations in flavonoids contents (Carador-Martinez *et al*. 2002). The values obtained for tannin content were in the same range as reported by Fernandez *et al*. (2005); Xu *et al*. (2007) while lower than that of Alonso *et al*. (2006).

The difference in result may be attributed partly to the differences in beans sources or determination methods (Xu *et al*. 2007). Tsuda *et al*. (1994) reported that white bean consisted of no antioxidant activity while red and black seed consisted of significant antioxidant activity (Carador-Martinez *et al*., 2002; Wu *et al.* 2004; Akond *et al*. 2011). This implies that the colored bean species consist of good antioxidant activity as compared to white beans species.

Effect of cooking on phenols, flavonoids and tannins

In cooked beans total phenolics was found highest in KBL. KBL 3 had the highest % loss in phenolics of about 47%. On the other hand, for the flavonoids the total flavonoid content for both raw and cooked was found highest in KBL 2. The highest % loss in total flavonoids was seen 48% in cooked KBL. This phenomenon of loss of total phenolics might be due to longer soaking time, during which some polyphenols (condensed tannin) in the seed coat were hydrolysed and diffused into the soaking water, breakdown of phenolics during processing. Although there are hundreds of varieties of dry edible beans in the world, data on phenolics in cooked legumes are very limited (Xu and Chang 2008).

Bressani and Elias (1980) observed that about 30-40% of phenolics could be removed from common beans by cooking and discarding the cooking water. Xu and Chang (2008) reported that about 75-79% of phenolics were leached into soaking and cooking water. Ismail *et al.* (2004) also reported that thermal treatment decreased the total phenolic content in all vegetables.

The tannin content values in cooked beans decreased significantly in comparison to their uncooked raw ones. Thermal processing might cause degradation of polyphenols and release bound phenolic compositions. These significant loss might be attributed to those water-soluble phenolics that were leached into soaking and cooking water before and during cooking as well as breakdown of phenolics during processing (Boateng *et al*. 2008). Xu and Chang (2008) reported that the loss in tannin content in common beans after being thermally processed ranged from 32-73% and the result obtained above is in this range.

Effect of cooking on anthocyanin

In case of cooked beans, anthocyanin was not detected in KBL 4. Cooking caused significant decrease in anthocyanin content compared to the raw ones. Xu and Chang (2009) reported that all thermal processing treatments significantly reduced the contents of anthocyanin in black beans. They indicated degradation of anthocyanin compositions upon thermal processing. The result obtained above was similar to the results by Boateng *et al*. (2008) and Xu and Chang (2009).

Effect of cooking on DPPH radical scavenging capacity

DPPH radical was found highest again in KBL 1. Free radical scavenging activity showed reduction upon cooking. Depletion in antioxidant capacity is related with reduction in bioactive compounds like phenols, flavonoids, and anthocyanin. Boiling is regarded as being destructive to antioxidant compounds (Kirshnaswamy and Raguramulu

1998). The changes in overall antioxidant properties of cooked beans can be attributed to leaching of water soluble antioxidant compositions, and the formation or breakdown of antioxidant compositions. Chutipanyaporn *et al.* (2014) reported increase in antioxidant activities upon cooking. The result obtained was similar to Xu and Chang (2009)

Conclusion

Local varieties of Jumli simi are rich in nutrients, and have significant antioxidant potential. Jumli simi is relatively nutritious due to their richness in polyphenols. The beans with higher hydration capacity, swelling capacity have lower cooking time. Dark colored beans were rich in phytochemicals and had higher antioxidant activity as compared to the light colored ones. Cooking of beans affected the phytochemical composition leading to reduction in phenols, flavonoids, tannins, anthocyanins and antioxidant activity.

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