Potentials of Annatto (*Bixa orellana*) as Supplant of Red Pepper for Ethiopian Cuisine

Bekri Melka Abdo[1*] and Legese Shiferaw[2]
[1] Food Science and Nutrition Research, Wendo Genet Agricultural Research Center
*[2] Ethiopian Institute of Agricultural Research. *Correspondingauthor's E-mail: Bekrimelka2003@yahoo.com*
P.O box: 198 Shashemene, Ethiopia

Abstract

Arils of annatto seeds are used as a colorant for both edible and non edible products. This work was designed to determine the color strength of two genotypes of annatto and its application as colorant on Ethiopian sauce cuisine. Its color strength was described based on total carotenoid content in terms of bixin through spectrophotometric method. The pink flower genotype annatto was yielded higher total carotenoid content from the seed (3.23%) than the white flower genotype annatto (3.05%). The panelist response on the prepared sauce ('Shiro Wat') showed that acceptance of annatto colorant as of comparable with red pepper colorant.

Keywords: Annatto, Cuisine, Ethiopia, Red Pepper, Shiro Wat

Introduction

Annatto (*Bixa orellana*) belongs to the family Bixaceae and the genus Bixa. Under this genus, it is one of the five species known as orellana and native to Neotropics. It is commercially grown for the high appocarotenoid pigment content in its seed coat, particularly bixin (Rivera-Madrid, 2006). Annatto starts to bear the seed at the age of 2.5 to 3 years after planting and continues the production for 10 to 15 years (Kumaran, 2014). The fruit is obtained from September to December. On the basis of flower, fruit colour and shape, *Bixa orellana* has three types; one with white flowers and green capsules, second with purple flowers and brownish red capsules and third with pink flowers and red capsules (Akshatha *et al*, 2011). Two genotypes of *Bixa orellana* are found in Ethiopia, one at Wendo Genet Agricultural Research Center (white flower; Fig 1a) and the other at Tepi Agricultural Research Center (pink flower; Fig 1b).

Annatto has been used as a colorant for various food items, such as flavored milk products, fats and oils, butter, fine bakery wares, casings and decorations for meat, fish products, soft drinks, alcoholic drinks with less than 15% of alcohol, potato, cereal and flour or starch-based snacks, with acceptable bixin content from the range 10 mg/L | mg/kg to 25 mg/L | mg/kg (EFSA, 2016). Bixin is the principal coloring compound of annatto with diapocarotenoid moiety (Melka *et al*, 2017).

a. White flower, green capsule

b. Pink flower, red capsule

Figure 1. Genotypes of Annatto found in Ethiopia

The red pepper (*Capsicum annum*) of concern in this study is the main component of a mixture called '*Berbere'* or *paprika* composed of red pepper and other spices. It is used in some of the most popular dishes in Ethiopian cuisine. Besides its popularity in Ethiopia, red pepper can be used as raw material for extraction of oleoresin and paprika processing by the Ethiopian Spice Extraction Factory for export (Factsheet, 2020). Currently production of red pepper is under severe threat by several diseases and is damaged by moisture stress. These problems are causing major issues with the supply of red pepper, and threaten the sustainability of farmers' livelihood (Rutgers, 2010). As a result of these, it needs other colorants which have complementary function of red pepper to share the load when the shortage is occurred. In supporting this, annatto gives a range of colors from yellowish to red color with a slight peppery flavor on the arils of its seed and widely used for food colorants (Smith, 2014). The responsible compounds for color of both Annato and red peppers are carotenoids. Bixin (3) and Norbixin (5) are specific to annatto and Capsanthin (1) and Capsorbin (2) for red pepper. Capsacine (4) is the principal compound of pungency for red pepper. Within these similes, the total carotenoid content of the two genotypes of annatto were determined. Besides, the colorant feasibility of annatto was tested on Ethiopian sauce cuisine as a reference of red pepper.

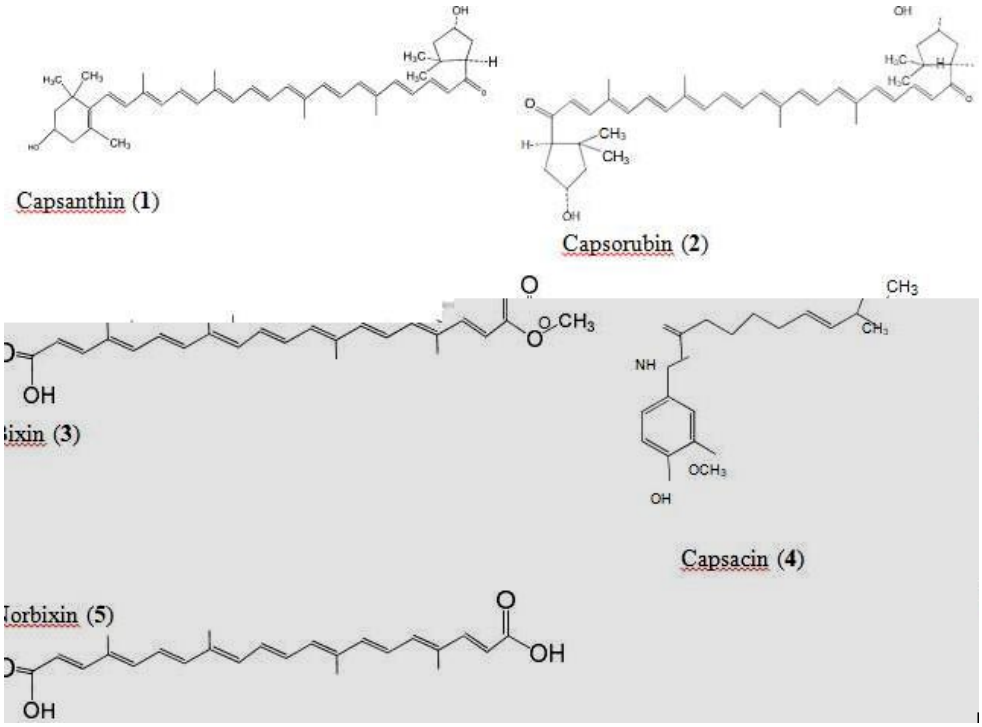

Figure 2. Structure of color and pungency responsible compounds in annatto and red pepper

Materials and Methods

Sample collection

A white flower, green capsule genotype of Annatto fresh fruits were collected from Wendo Genet Agricultural Research Center Gene Bank (1800 m a.s.l., N 07° 05' 576", E 038° 38' 015"). The red capsule pink flower genotype was collected from two locations of Tepi Agricultural Research Center (1205 ma.s.l., N 07° 11' 243", E 035° 25' 094") (Bebeqa and Tepi).

Sample extraction

The seeds of Annatto were pulped out from the capsule and were allowed to dry shade until maintained uniform moisture content. Dried seed samples were initially soaked in hexane for 6 h to remove fats and waxes. The residue was extracted with a mixture of solvent (chloroform and ethanol/ 1:1 ratio) by using Soxhlet apparatus for 5 h (Melka et al., 2017). The extract yield was determined based on the formula below:

$$\text{Extract yield (\%)} = \frac{\text{Mass of extract (NG)} \times 100}{\text{Mass of seed (NG)}}$$

Annatto seed extracts (5 mg) were weighed into a 50mL beaker. Chloroform (10 mL) solvent was added to the beaker. The mixture was stirred with a glass rod to dissolve the extract. The solutions were carefully transferred into a 100 mL volumetric flask. Chloroform was used to rinse the beaker and transferred to the flask. The volume of solution in the flask was made up to 100 mL mark with chloroform. The flask was then covered and placed in a hot water bath at 50 ^{o}C with periodic shaking until all the extract was completely dissolved. The solution was allowed to cool at room temperature. Chloroform was used as a blank and the absorbance of the solutions were read on UV-Vis spectrophotometer at λ max of 471 nm (Melka *et al.,* 2017). The color values were assessed based on determining total percent of carotenoids through the formula;

$$\text{Total carotenoids in terms of bixin (\%)} = \frac{\text{A.V. 100}}{\text{282.6 . W}}$$

Where A is absorbance of the extract, V is the total volume (mL); W is the weight of the extract (mg) and 282.6 is the extinction coefficient of bixin.

Preparation of sauce ('Shiro Wat')

Flour made of roasted pea ('Shiro'), cooking oil, tomato and slices of onion were used to prepare the sauce ('Shiro wat'). Hot water extract of annatto seeds and 'Berbere' (commercialized red pepper powder with flavor of spices) were used as a colorant. The sauces were prepared by a recognized cooker using both colorants. The prepared sauces were dished with 'Injera' (a sour fermented flat breed with slightly spongy texture). The cuisine was subjected to taste by panelists who were semi trained; 12 female and 21 males with the age range of 21-51 years. Color, taste and overall appearance of the sauces were scored with 9 points hedonic scale in which 9 extremely like, 8 like very much, 7 like moderately, 6 like slightly, 5 neither like nor dislike, 4 dislike slightly, 3 dislike moderately, 2 dislike very much and 1 dislike extremely which were supposed to show the degree of likeness. Panelists indicated their rating for each recipe by choosing the appropriate numerical score on well organized panelist questioner sheet. The distribution of panelist response frequency was calculated.

Statistical analysis

Extraction of the aril of seed of annatto genotypes and it total carotenoid test were performed with three replications. ANOVA for yield of extract and total carotenoid content of two genotypes of annatto was performed using CRD in three locations and analysis performed using SAS version 9. The mean, standard deviation and level of dislike were calculated from the 9 point hedonic score of the panelist.

Results and Discussion

The extract and total carotenoid yield performance of the two genotypes were characterized as originated location (Table 2). Pink flower with red capsule genotype had yielded higher carotenoid than the white flower with green capsule genotype. This result is in agreement with the work reported by Rivera-Madrid et al. (2006) with total carotenoid content in pink flower variant seeds were 2.25 times higher than the white flower variant. Overall both genotype seeds were comparable with the international export bench mark for annatto pigment which is more than 2.5% of pigment content (Heywood, 1993).

According to the panelist response (Table 3), all the mean of color, taste and overall appearance of annatto colored sauce ('Shiro Wat') was on the range of acceptance which was set 6 as a minimum acceptance value. Based on color parameters, the sauce of annatto colorant had more consumer preference than that of red pepper colorant. This shows that, annatto can be used for attractive colored cuisine. But the standard deviation of the taste was resulted higher and therefore a probability of the mean to happen below the minimum acceptance value.This indicated that to have a wide range of feeling among panelist for the taste of annatto colorant sauce. This might be caused by the impractical test of annatto colored sauce to the community. The wide range of taste of feeling could have to be narrowed though addition of flavor (spices) those are common to use on the preparation of red pepper colorant ('Berbere').

Table 1. Analysis of variance for mean square value of extract yield and total carotenoids of aril of seed of *Bixa Orellana*

Source of variation	DF	Extract yield	Carotenoid yield /extract	Carotenoid yield /seed
Genotypes	2	5.48**	141.24***	0.57***
Error	6	0.43	0.02	0.00
CV	-	6.34	0.44	0.47

****, **and * are significant at p<0.001, 0.01 and p<0.05 respectively; ns=non significant at p<0.05.*

Table 2. Effect of genotypes on mean extract yield and total carotenoid yield from aril of seed of *Bixa Orellana*

Location	Altitude (m.a.s.l.)	Var	Extract yield (%)	Total carotenoid yield from extract (%)	Total carotenoid yield from seed (%)
Bebeqa	1190	Pink Flower	11.44[a]	21.00[c]	2.40[c]
Tepi	1205	Pink Flower	10.78[a]	29.99[b]	3.23[a]
Wendo Genet	1800	White Flower	8.84[b]	34.47[a]	3.05[b]
LSD (0.05)	-	-	1.31	0.25	0.03

Means followed by the same letter under the same column are statistically non-significant at p<0.05 according to least significant difference (LSD) test.

Table 3. Distribution of panelist response frequency of two type colored recipe of 'Shiro Wat'

Scale	Grade	Frequency of response					
		Annatto			Red pepper		
		Color	Taste	Over All	Color	Taste	Over All
Like extremely	9	9	5	5	1	9	3
Like very much	8	16	8	14	11	11	14
Like moderately	7	6	11	7	16	8	14
Like slightly	6	1	5	4	3	5	1
Neither like nor dislike	5		1		1		1
Dislike slightly	4		1	2	1		
Dislike moderately	3	1					
Dislike very much	2		2	1			
Dislike extremely	1						
Total response		33	33	33	33	33	33
Mean		7.88	6.94	7.27	7.15	7.73	7.52
Standard deviation		1.17	1.73	1.57	0.97	1.07	0.83
Percent of dislike		3.03	9.09	9.09	3.03	0.00	0.00
Mean -SD		6.71	5.21	5.71	6.18	6.65	6.68

Conclusions

Integration with urban agriculture, annatto can be established easily as a green economy tree and its seeds are utilized for colorant application of different food items. Owing the test of annatto colorant recipe, we put forward the improvement of annatto colorant preparation with flavor additives and blending with small amount high pungent Chili (hot pepper). Overall, annatto can supplement the colorant application of red pepper with better advantages of availability, affordability and viability. Besides, conclusion can be drawn as annatto can be used as a colorant (red) of Ethiopian cuisine without pungency taste.

References

Akshatha V, Giridhar P and Ravishankar GA. 2011. Morphological diversity in *Bixa Orellana* L. and variations in annatto pigment yield. *Journal of Horticultural Science and Biotechnology,* 86(4):319-324.

EFSA. 2016. European Food Safety Authority. The safety of annatto extracts (E 160b) as a food additive. *Journal of EFSA*, 14(8):4544.

Factsheet. 2020. Spices, Herbs and Aromatics in Ethiopia. Netherlands-African Business Council.

Heywood VH. 1993. In: flowering plants of the world. New York Oxford University press (New York), 426.

Kumaran K. 2014. Production potential of annatto (*Bixa orellana* L.) as a source of natural edible dye. International Workshop on Natural Dye at Hyderabad.

Melka B, Bisrat D, Babu GN. 2017. Isolation, Characterization and Biological Activities of Food Colorants from *Bixa orellana. J. Pharmacovigil.,* 5:237.

Rivera-Madrid R, Escobedo-GM RM, Balam-Galera E, Vera-Ku M, Harries H. 2006. Preliminary studies toward genetic improvement of annatto (*Bixa orellana* L). *Scientia Horticulturae*109:165-172.

Rutgers T. 2010. An analysis of supply side constraints on Ethiopian red pepper and paprika capsicum production and export: a global value chain approach. Master thesis International Development Studies Utrecht University.

Smith K. 2014. Annatto and Color Removal. Wisconsin Center for Dairy Research, www.cdr.wisc.edu.