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Physicochemical characterization of annatto seeds (*Bixa orellana*) sold in Ouagadougou and their oils extracted using chemical processes

Mah Alima Esther Traoré*[1], Adama Lodoun[1], Pingdwindé Marie Judith Samadoulougou-Kafando[1], Nestor Beker Dembélé[2], Kiswendsida Sandrine Léticia Dayamba[2], Charles Parkouda[1]

[1]*Food Technology Department (DTA), Institute for Research in Applied Sciences and Technologies (IRSAT), National Center for Scientific and Technological Research (CNRST), Burkina Faso*

[2]*Agrosilvopastoral Sector, Manga University Center, Norbert Zongo University, Manga, Koudougou, Burkina Faso*

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ABSTRACT

Annatto (*Bixa orellana*) is a dye plant with multiple uses. In Burkina Faso, there is significant and growing consumption of these seeds in many sectors, especially in popular restaurants. The objectives of this study were to evaluate the physicochemical and nutritional characteristics of annatto seeds and their oils sold in Burkina Faso. Annatto seeds were collected from markets in the city of Ouagadougou and analyzed with standard methods. The results showed that the water content of the seeds varied from 8.84±1.6%/DM to 11.15±0.05%/DM, the ash content varied from 5.32±0.10%/DM to 7.71±0.10%/DM, the pH of the seeds ranged from 6.83±0.15 to 7.23±0.06, the protein content ranged from 7.21±0.11%/DM to 8.57±0.60%/DM, the lipid content ranged from 1.19±0.15%/DM to 2.62±0.36%/DM, the dietary fiber content ranged from 33.36±0.09%/DM to 41.65±0.24%/DM. The energy value ranged from 328.40±4.60 to 380.44±1.96 Kcal/100g. They were rich in various minerals, with high calcium and magnesium values. The vitamin C content of the seeds was approximately 0.2mg/100g and their beta-carotene content ranged from 0.40 to 1.99 mg/100g. As for the oil extracted from the seeds, the results showed that the unsaponifiable content ranged from 5.793±0.00% to 20.349±0.00%, the acid index varied from 0.280±0.00% (mg KOH/g) to 3.625±0.99% (mg KOH/g), and the refractive index varied from 1.495±0.00 to 1.509±0.00. The annatto seeds sold in Burkina Faso that were analyzed have good physicochemical properties, making them suitable for use in food, cosmetic, or pharmaceutical applications.

***Corresponding author:** Mah Alima Esther Traoré ✉ esthertraore1@gmail.com

INTRODUCTION

Bixa orellana L., commonly known as annatto or roucou, is a shrub belonging to the Bixaceae family, native to tropical America (Ezéchiel Akakpo *et al.*, 2019). It is known worldwide for its seeds, which are the most important natural source of carotenoid pigments, mainly bixin and norbixin. For thousands of years, the indigenous peoples of Latin America have used annatto as a food coloring, cosmetic, and for its medicinal properties in the treatment of various ailments (Vilar Dde *et al.*, 2014). Annatto extract, designated by the code E160b, is a leading natural colorant in the food (cheese, margarine, snacks), cosmetics, and pharmaceutical industries due to its yellow to orange-red color and nutritional profile (Scotter *et al.*, 2000). Annatto is known for its use in food, cosmetics, dyeing, and even pharmacopoeia for its antioxidant, antibacterial, and anti-inflammatory properties (Raddatz-Mota *et al.*, 2017). Annatto seeds are increasingly used in Burkina Faso in many sectors, although the majority come from elsewhere (Traoré *et al.*, 2025). Annatto is used in cooking as a natural coloring agent to make dishes more appealing (Ouédraogo *et al.*, 2025). Despite the commercialization of the seeds in Burkina Faso and the level of consumption, their biochemical and nutritional composition remains poorly documented in Burkina (Traoré *et al.*, 2025). Also, the chemical compounds present in annatto seeds may vary from one agroecological zone to another (Ezéchiel Akakpo *et al.*, 2020). Furthermore, the added value of the annatto industry also lies in the production of oil, extracted from the seeds, which is rich in lipophilic antioxidant compounds such as tocotrienols, carotenoids, and essential fatty acids (Vilar Dde *et al.*, 2014). However, the quality and composition of this oil can vary considerably depending on the genotype, growing conditions, extraction method, and storage conditions (Batista *et al.*, 2022, Cardenas-Conejo *et al.*, 2023). The quality of these commercialized annatto products is rarely controlled, exposing them to risks of alteration, contamination, or degradation, thus affecting their efficacy and safety. Producers and consumers lack accurate information, which significantly limits their integration into the daily diet. In light of this, the aim of this study was to characterize the biochemical and nutritional parameters of the seeds, as well as the properties of the oil extracted from them. The study sought to inform researchers and users of annatto with a view to reviving its production and use in Burkina Faso.

MATERIALS AND METHODS

Sampling and coding of samples

Annatto seeds were collected from several markets in the city of Ouagadougou in Burkina Faso. The samples taken from the markets were coded and packaged in plastic bags in accordance with good hygiene practices to avoid contamination, then sent to the laboratory for analysis. The sample codes are listed in Table 1.

Table 1. Codification of annatto samples

Codes	Description	Collection site
GR-GM	Annato seeds from the Grand Market	Grand Market
GR-MG	Annato seeds from Gounghin Market	Gounghin Market
GR-MT	Annato seeds from Toecin Market	Toecin Market
GR-MCAII	Annato seeds from the Cité An II Market	Cité An II Market
GR-MN	Annatto seeds from Nabi-Yaar Market	Nabi-Yaar Market

Physicochemical analyses

Determination of the proximate composition of annatto seeds

The moisture content of annatto seeds was determined by weighing before and after drying at a temperature of 130°C in accordance with ISO 712-1 (ISO712-1, 2024). The ash content was determined by differential weighing after the samples were placed in an oven overnight in accordance with ISO 2171 (ISO2171, 2023). The pH was determined using the potentiometric method according to the AOAC method.

Two grams of crushed annatto seeds were weighed and placed in tubes. Thirty milliliters of distilled water was added to each tube. After homogenization, the pH was determined using a digital pH meter (WTW pH 340) previously calibrated with standard buffer solutions pH = 4, pH = 7, and pH = 10 (Konings *et al.*, 2016). Titratable acidity was determined according to ISO 750 (ISO750, 1998). The principle of this method is based on the neutralization of organic acids with soda in the presence of thymolphthalein.

The lipid content of the samples was determined using the Soxhlet method, in accordance with ISO 659 (ISO659, 2009). Extraction was carried out by hot maceration followed by rinsing of the sample with hexane by distillation. The lipid content was determined by weighing after evaporation of the hexane. This collected oil will then be analyzed to determine its physicochemical characteristics.

Protein determination was performed using the Kjeldahl method in accordance with ISO 20483. The protein content was determined based on the nitrogen content in the sample in the presence of Kjeltabs catalyst pellets and concentrated sulfuric acid (H2SO4) (96%) (ISO20483, 2013).

The energy value of annatto seeds was determined using the Merrill and Watt coefficients adopted by the FAO based on the protein (P), carbohydrate (C), and lipid (L) contents (Merrill and Watt, 1973). The energy value of the sample was obtained using the following equation:

Energy value (kcal/100g DM)= (P × 4 kcal) + (C × 4 kcal) + (F × 9 kcal)

P: protein content (g/100g DM); G: carbohydrate content (g/100g DM); L: fat content (g/100g DM).

Total sugars were determined using the orcinol method described by Strecker and Montreuil (Strecker and Montreuil, 1979).

Crude fiber was measured using the method described by AOAC (Konings *et al.*, 2016). 0.4g of crushed annatto seeds were weighed into a flask. 100ml of 0.25N sulfuric acid was added to the contents, homogenized, and brought to a boil for 30 minutes under reflux cooling.

Next, 100 ml of 0.31N soda was added to the contents and the mixture was boiled for 30 minutes under reflux cooling. The extract obtained was filtered through ashless filter paper and the residue was washed several times with hot water until the alkalis were completely removed. After removal, the residue was dried in an oven at 105°C for eight hours, cooled in a desiccator, and then weighed. The residue obtained was incinerated in an oven at 550°C for three hours, then cooled in a desiccator, and finally the ashes were weighed.

Determination of lycopene and vitamin C content

The beta-carotene and lycopene content of annatto seeds was determined by UHPLC using a technique described by Savadogo. Carotenoids soluble in organic solvents are directly detectable by Thermo Scientifica UHPLC chromatography (coupled with a UV-Visible detector) after extraction by maceration in chromatographic-grade hexane (Savadogo *et al.*, 2023). The ascorbic acid content of annatto seeds was determined by UHPLC based on a technique described by Van de Velde. Vitamin C is water-soluble and directly detectable by Thermo Scientifica UHPLC chromatography (coupled with a UV-Visible detector) after extraction in a diluted formic acid solution (Van de Velde *et al.*, 2012).

Mineral content of annatto seeds

The minerals contained in annatto seeds, namely calcium, magnesium, zinc, iron, manganese, and copper, were analyzed by flame atomic absorption spectrometry in accordance with the method described by Jorhem (2000).

Physicochemical characterization of oil extracted from annatto seeds

The quality control of the oil extracted from annatto seeds was determined to assess its purity. The oil's refractive index was determined according to ISO 6320 (ISO6320, 2017). The acid value is the number of milligrams of sodium hydroxide (NaOH) required to neutralize the free acids contained in 1g of annatto oil. It was determined according to ISO 1242 (ISO1242, 2023). The unsaponifiable matter of a fatty substance is the set of products present in that fatty substance after it has been saponified by an alkaline hydroxide. The unsaponifiable matter content of the oil extracted from annato seeds was determined according to ISO 3596 (ISO3596, 2000).

RESULTS

Physicochemical characteristics of annatto seeds

Proximate composition of annatto seeds

Table 2 shows the proximate composition of annatto seeds. This includes moisture, ash content, pH, and crude fiber. The moisture content of the annatto samples ranged from 8.84±1.6%/DM to 11.15±0.05%/DM. The sample (GR-MN) had the highest value, while the sample (GR-GM) had the lowest.

The ash content of the annatto seeds ranged from 5.32±0.10%/DM to 7.71±0.10%/DM crude ash. Sample (GR-MT) had the lowest content, while (GR-MCAII) and (GR-GM) had the highest contents.

The pH of all samples was around neutral, ranging from 6.83±0.15S to 7.23±0.06. This means that there is a balance between the concentrations of [H3O+] and [OH-] ions.

The average crude fiber content of the annatto seed samples ranged from 33.36±0.09%/DM to 41.65±0.24%/DM. Statistical analysis revealed that there is a significant difference between the mean values of the different parameters.

The protein content values of the annatto seed samples analyzed ranged from 7.21±0.11%/DM to 8.57±0.60%/DM. Statistical analysis revealed a significant difference between the mean protein content values.

Table 2. Proximal composition and energy value of annatto seeds (Bixa orellana)

Codes	Moisture %	Ash (%) /DM	pH	Crude fiber (%) /DM	Protein (%) /DM	Fat (%) /DM	Carbohydrates (%) /DM	Energy value (kcal/100g) /DM
GR-MN	11.15±0.05^{e}	6.56±0.07^{b}	7.23±0.06^{b}	41.65±0.24^{d}	8.57±0.60^{b}	2.62±0.36^{b}	82.26±0.78^{a}	332.59±1.21^{b}
GR-MT	9.72±1.73^{b}	5.32±0.10^{a}	6.95±0.15^{a}	39.52±1.75^{cd}	7.56±0.29^{a}	1.70±0.35^{ab}	85.42±0.07^{b}	334.28±1.76^{bc}
GR-MG	10.33±0.06^{c}	5.33±0.15^{a}	6.83±0.15a	33.36±0.09^{a}	7.27±0.11^{a}	2.48±0.30^{b}	84.92±0.47^{b}	340.35±1.01^{c}
GR-MCAII	10.86±0.53^{d}	7.71±0.10^{d}	6.89±0.16^{a}	38.61±1.59^{bc}	7.36±0.11^{a}	2.29±1.12^{b}	82.64±1.31^{a}	328.40±4.60^{ab}
GR-GM	8.84±1.6^{a}	6.99±0.45^{c}	6.87±0.06^{a}	36.01±1.16^{b}	7.21±0.11^{a}	1.19±0.15^{a}	84.61± 0.49^{b}	380.44±1.96^{a}
Pr > F	0	0	0.04	0	0.002	0.057	0.001	0.008
Significant	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes

Key: Values with different letters in exponential notation in each column are significantly different at the 5% level (Tukey's test).

Table 3. Mineral content of annatto seeds

Sample codes	Ca (mg/100g)	Mg (mg/100)	Zn (mg/100)	Fe (mg/100)	Mn (mg/100)	Cu (mg/100)
GR-GM	440.355±0.62	474.461±0.79	1.246±0.02	68.127±7.79	3.164±0.21	0.059±0.06
GR-MG	361.097±0.51	397.786±0.66	1.289±0.03	9.483±1.20	2.472±0.37	0.151±0.39
GR-MCA	461.276±0.65	385.057±0.64	1.119±0.06	102.216±0.92	1.038±0.07	0.047±0.05
GR-MT	491.145±0.69	679.607±1.13	1.386±0.07	10.719±0.50	2.496±0.05	0.065±0.43
GR-MN	528.557±0.74	645.504±1.08	1.477±0.14	115.747±13.47	2.763±0.21	0.064±0.25

The fat content of the annatto seeds analyzed ranged from 1.19±0.15%/DM to 2.62±0.36%/DM. The lowest content was obtained in sample (GR-GM), while the highest content was recorded in sample (GR-MN). Statistical analysis revealed a similarity between the fat content of most samples, except for (GR-GM), which showed a significant difference from the others.

The carbohydrate content of the annatto seeds analyzed ranged from 82.26±0.68%/DM to 85.42±0.07%/DM. The lowest content was obtained in sample (GR-MN), while the highest content was recorded in sample (GR-MT). Statistical analysis revealed that there is a significant difference between the mean values of carbohydrate content.

The average energy value of the annatto seed samples analyzed ranged from 328.40±4.60 kcal/100g to 380.44±1.96 kcal/100g. Statistical analysis revealed a significant difference between the average values of crude fiber content.

Mineral, beta-carotene, and vitamin C composition of annatto seeds

The mineral content of the samples analyzed revealed significant variations. The GR-MN sample has a high calcium content and a high magnesium content. The GR-MN sample has a high zinc and iron concentration, while the GR-GM sample has a high manganese concentration and the GR-MCAII sample has a high copper concentration. These results are shown in Table 3.

The vitamin C and beta-carotene compositions are shown in Fig. 1 and are roughly the same for all samples, ranging from 0.21 to 0.27 mg/100g for ascorbic acid. Beta-carotene levels vary from 0.40 to 1.99 mg/100g. The GR-MT sample has the highest beta-carotene content (1.99 mg/100g), while the GR-MCAII sample has the lowest (0.40 mg/100g).

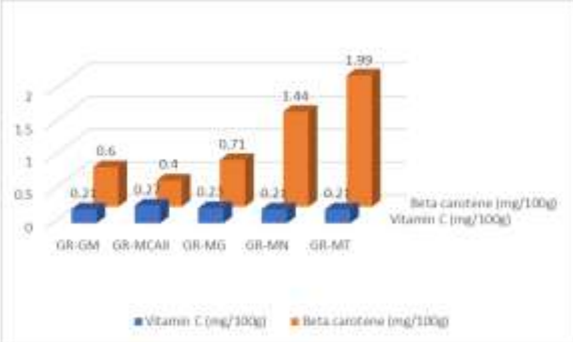

Fig. 1. Vitamin C and beta-carotene compositions

Physicochemical characteristics of oil extracted from annatto seeds

Table 4 shows the results of the physicochemical characteristics of the oil extracted from annatto seeds. These are the refractive index, acid index, and unsaponifiable content. The refractive index of annatto oil varied from 1.495±0.00 to 1.509±0.00 with significant differences. The acid value ranged from 0.280±0.00 (mg KOH/g) to 3.625±0.99 (mg KOH/g) with significant differences between the recorded average values. The unsaponifiable content ranged from 5.793±0.00% to 20.349±0.00%.

Table 4. Physicochemical characteristics of oil extracted from annatto seeds

Code	Refractive index	Acid value (mg KOH/g)	Unsaponifiables (%)
GR-MG	1.509±0.00^{c}	0.280±0.00^{a}	20.349±0.00^{c}
GR-MN	1.508±0.00^{c}	3.625±0.99^{c}	7.892±0.00^{ab}
GR-CAII	1.495±0.00^{a}	1.532±1.19^{b}	10.939±0.00^{b}
GR-MT	1.508±0.00^{c}	1.672±0.39^{b}	5.793±0.00^{a}
GR-GM	1.506±0.00^{b}	1.393±7.9E-05^{b}	11.544±0.00^{b}
Pr > F	0.000	0.002	0.003
Significant	Yes	Yes	Yes

DISCUSSION

Physicochemical characteristics of annatto seeds

The moisture content represents the proportion of water contained in the seeds and is influenced by several factors, including climatic conditions, the stage of seed maturity, drying methods, and even storage conditions. Proper management of these factors is essential to obtain seeds with better preservation and quality. The moisture content values obtained from our samples can be considered satisfactory when compared to coffee beans, millet, sorghum, and corn, for which the maximum moisture content is set at 13% for long-term storage at temperatures of 20 and 25°C, which are typical in hot regions (Cruz *et al.*, 2016). The water content results reveal values lower than those found in seeds other than legumes, which were 11.4% (Kapoor *et* *al.*, 1975). The low water content of our annatto seeds is interesting because it limits mold activity (Cruz *et al.*, 2016).

The ash content of the annatto seed samples ranged from 5.32±0.10%/DM to 7.71±0.10%/DM of crude ash. Among our samples, two had values similar to those reported in other studies on annatto, which found an average of 5.20%/DM (da Silva *et al.*, 2009; Kumar *et al.*, 2007). The lower ash content observed for the GR-MT sample could indicate a different mineral composition compared to the GR-MCAII and GR-GM samples, which have the highest contents. Based on the ash results obtained, it can be said that annatto is a food rich in minerals, comparable to Parkia biglobosa and Senegalia macrostachya seeds, which have ash contents of 4.57±0.13 and 4.11±0.03, respectively (Guissou *et al.*, 2020). Thus, its consumption can be a good source of minerals for the body.

The pH of the samples ranged from 6.83±0.15 to 7.23±0.06. This indicates certain homogeneity in the chemical composition of the seeds analyzed. This pH value could explain why annatto is used in many food preparations and does not alter the taste of the preparations in which it is used (Bastos *et al.*, 1999).

The average crude fiber content of the annatto seed samples analyzed ranged from 33.36±0.09%/DM to 41.65±0.24%/DM. These results are higher than those reported by other authors on annatto seeds, who found average values of 25.60% and 28.45%, respectively (Kumar *et al.*, 2007; Valério *et al.*, 2015). The difference between the samples suggests significant genetic or environmental variations (Ezéchiel Akakpo *et al.*, 2020). Thus, annatto seeds, which are rich in dietary fiber, could contribute significantly to good digestive health.

The results obtained for protein content are lower than those found in other studies on the chemical composition of annatto seeds, with contents of 11.50% and 14.35% (Kapoor *et al.*, 1975; Kumar *et al.*, 2007). This variation in the protein content of the seeds could be related to environmental conditions during seed development, but this low content is not a problem as annatto is generally used as a food coloring or additive.

The results for the fat content of annatto seeds sold in Ouagadougou are similar to those obtained by (Valério *et al.*, 2015), which are 2.22±0.11. However, our results are lower than those reported by (Dike *et al.*, 2016), who found 7.20±0.07. This difference could be due to the technique used to extract oil from annatto seeds. Certainly the lipid content is low, but the presence of carotenoid coloring makes this lipid unique and difficult to extract.

The carbohydrate content of the annatto seeds analyzed ranged from 82.26±0.68%/DM to 85.42±0.07%/DM. Carbohydrates are a source of energy for germination and seedling development, and serve as precursors for the synthesis of other important molecules such as lipids. Our results were significantly higher than those found in many studies, such as those by Valerio *et al.* and Dike *et al.*, who found 42.2±0.69 and 16.26±0.39, respectively (Dike *et al.*, 2016; Valério *et al.*, 2015). Annatto seeds are not a primary source of energy, but they can be combined with other, richer foods to help meet human energy needs as a dietary supplement.

The mineral content obtained shows marked heterogeneity for all minerals (calcium, magnesium, iron, manganese, and copper). However, the concentrations of calcium, manganese, copper, iron, and magnesium are higher than those reported for the biochemical composition of *Bixa orellana* seeds in several studies (Ezéchiel Akakpo *et al.*, 2019). These minerals play an essential role in the body by providing essential nutrients that are beneficial to the body. The vitamin C and beta-carotene contents obtained in this study show significant variations that could be explained by the degradation of ascorbic acid and beta-carotene over time, particularly due to the preparation and analysis of the samples, but also due to storage conditions during marketing. The significant variability could reflect genetic differences between annatto plants (Jansen and Cardon, 2005).

Physicochemical characteristics of oil extracted from annatto seeds

The annatto oil extracted from Bixa orellana seeds is bright red in color due to the pigments contained in the seeds. The carotenoid pigments, particularly bixin and norbixin, contained in the seeds give the oil remarkable coloring, antioxidant, and anti-inflammatory properties (Silva *et al.*, 2015).

The refractive index is a dimensionless quantity that characterizes the way light propagates in a medium. It is one of the physical parameters used in the identification of fats and oils or the detection of their impurities. The refractive index values of roucou oil ranged from 1.495±0.00 to 1.509±0.00 with significant differences. This variation in values could be partly explained by the difference in the concentration of soluble elements in the oil. The refractive index values obtained are slightly higher than those reported by (El-Naggar, 2016) for olive oil (1.466) and (Kouidri *et al.*, 2015) for argan oil. This difference could be due to variations in the chemical composition of the oil, influenced by factors such as climatic conditions and genetic variability. In addition, the high degree of unsaturation in annatto oil, as well as its high content of phenolic compounds, may be responsible for its high refractive index. Their presence is an asset because it gives this oil properties such as reducing the risk of inflammatory, degenerative, and cardiovascular diseases (Krinsky, 1994).

The acid index of a fatty substance allows the amount of free fatty acids present in a lipid to be assessed. It is a chemical criterion for purity, stability, and oil quality. It is also a means of controlling the level of hydrolytic, enzymatic, or chemical degradation of the fatty acid chains of triglycerides (Abaza *et al.*, 2002). The acid index of the oil extracted from annatto seeds was higher, especially for the GR-MN sample, than that of lupin oil (Hema *et al.*, 2023). Over time, or under poor storage conditions, triglycerides can undergo hydrolysis, releasing fatty acids that can increase the free acid index. The acid value is a key parameter that allows us to predict the course of action to be taken for oil neutralization and deodorization operations. Therefore, the lower the acidity, the better the quality of the oil (Diakite *et al.*, 2022).

The unsaponifiable content of an oil or fat corresponds to the fraction of lipid compounds that do not react with alkali hydroxides during saponification. These compounds remain insoluble in water but soluble in organic solvents after hydrolysis of triglycerides. The higher the unsaponifiable content, the richer the oil is in antioxidants and vitamins, making it more suitable for health and cosmetic use. This unsaponifiable fraction could consist of tocopherols (vitamin E) and traces of pigments (Diakite *et al.*, 2022). Our results show that the unsaponifiable content varied from 5.793±0.00% to 20.349±0.00%. These values are very high because the unsaponifiable content of natural fats is generally low, ranging from 0.3 to 1.5% (Morin and Pages-Xatart-Pares, 2012). These results were higher than those found in cottonseed oil samples, which ranged from 1.34 to 9.86 g/kg (Diakite *et al.*, 2022). These high unsaponifiable levels in annatto oil are largely due to the carotenoid pigments abundant in this product (Hansen *et al.*, 2015). These carotenoid compounds underpin the added value of this oil due to their strong antioxidant and anti-inflammatory activities (Raddatz-Mota *et al.*, 2017). Other factors could also explain this finding, including the extraction technique used.

CONCLUSION

This study provided insight into the physicochemical composition of Bixa orellana L. seeds sold in Burkina Faso and the oil extracted from the seeds. The study found that annatto seeds are rich in various nutrients, fiber, and minerals. They are therefore recommended as part of a healthy, balanced diet. The oil extracted from annatto seeds has shown great potential for use in medicinal and cosmetic applications, as evidenced by its high unsaponifiable content.

RECOMMENDATION(S)

We strongly recommend promoting this species in Burkina Faso by raising awareness of its nutritional qualities, encouraging producers to incorporate it into their crops, developing its value chain, and developing technologies appropriate to local needs.

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