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SHORT COMMUNICATION

Microscopic detection of adulteration of Bengal gram (*Cicer arietinum*) flour with other legume flour based on the seed testa macrosclereids

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Abstract Besan, the flour of Bengal gram (*Cicer arietinum* Linn), a legume, is a popular ingredient of many culinary dishes in India. Because of its high demand, its flour is often adulterated by that of other legumes, such as, *Lathyrus sativus* (lathyrus) or *Pisum sativum* (pea) by unscrupulous traders. There are chemical methods of paper chromatography and HPLC by which the adulteration with the flour of *L. sativus* can be established but they are sophisticated techniques. At present, there are no chemical methods by which the adulteration with the flour of *P. sativum* can be detected. Microscopy is a simple technique and has been used for detection of adulteration of a number of food grains. Microscopic observation of the flour of these three legumes showed that their seed testa macrosclereids are distinct in their shape and size. The macrosclereids of *C. arietinum* are longer with a mean length of 155.6 microns and are bent at one end. Those of either *P. sativum* or *L. sativus* are shorter with a mean length of 61.8 and 72 microns respectively, with flat ends and with a different morphology. The seed testa macrosclereids of other edible legumes also show differences in morphology. Thus, it was observed that microscopic observation of the besan flour for the macrosclereids of other legumes is a powerful but simple means by which detection of adulteration of besan with the flour of *P. sativum* or *L. sativus* or by other edible legumes can be made. These observations make the subject matter of this manuscript.

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Legumes occupy an important place in the diet of people of many countries in the tropics and sub-tropics (Singh and Singh 1992). They are a rich source of protein and form a major component of a vegetarian diet. Their widespread usage is also because they mature rapidly, can be grown in times of drought, can be easily transported because of their low moisture content and they are less susceptible to spoilage because their seed coats are impervious to water (Rao 2008). Bengal gram also called chickpea or gram (*Cicer arietinum* Linn), is a major pulse crop in India (Pratape et al. 2004) and accounts for nearly 40% of the total pulse production. India is the largest producer of Bengal gram in the world (FAOSTAT 2008). Besan, the flour of Bengal gram is a popular ingredient of many dishes in India (Mridula et al. 2010). Because of its high demand, adulteration of dehusked legume flour, particularly that of besan (*C. arietinum*) flour with that of cheaper flour such as, *Lathyrus sativus* or *Pisum sativum* occurs in the country by unscrupulous traders (FAC 2002, 2007, 2008a, b, 2009a, b). While the adulteration with the flour of *P. sativum* is a deceitful act, the same with that of *L. sativus* can be hazardous to health due to the presence of a neurotoxin β-*N*-oxalyl-α, β-diaminopropanoic acid in this legume seed. There are chemical methods of paper chromatography and high performance liquid chromatography by which the presence of this amino acid can be detected (IS 2005; Thippeswamy et al. 2007), thus indirectly establishing adulteration of besan with lathyrus flour. There are however no chemical methods by which the adulteration with the flour of *P. sativum* can be detected Samples of besan deliberately adulterated and also market samples of besan

and also the seed husks of other edible legumes were treated and observed microscopically. It was observed that the adulteration of besan with the flour of other edible legumes can be detected by microscopic examination since the macrosclereids of one legume, particularly that of *C. arietinum, L. sativus* and *P. sativum* are distinctly different from another. The preparation of the samples and the microscopic observations are presented in this manuscript.

Material and methods

Legumes examined in the study The following edible legume seeds were purchased from the local market: *C. arietinum* (chick pea, gram or Bengal gram), *P. sativum* (pea), *L. sativus* (lathyrus), *Phaseolus mungo* (black matpe), *Lens esculenta* (laird lentils), *Cajanus cajan (*tur dahl), *Phaseolus vulgaris* (rajma), *Phaseolus aureus* (mung beans) and *Glycine max* (soya).

Sample preparation

Preparation of adulterated besan (C. arietinum) with the flour of L. sativus or P. sativum Whole grains of the three legume seeds with husks were taken and finely powdered. The flour of either of the adulterants was added to besan at 0.25, 0.5, 1.0, 2.5 and 5% concentration and thoroughly mixed. A modified AOAC Official method 978.10 (2005) was used to prepare the sample for microscopic observation. About 2 g of the sample powder was taken in a beaker; 200 ml of 1.25% sulfuric acid was added and boiled for 30 min with bumping granules. The liquid was then filtered through nylon cloth placed on a funnel and the residue was washed with near-boiling water. The residue was transferred to the beaker and was boiled with 200 ml 1.25% sodium hydroxide for 30 min. It was filtered through the nylon cloth placed on a funnel and the residue was again washed with near-boiling water. The residue free from extraneous organic matter was then suspended in 50% aqueous glycerine solution and viewed microscopically.

Preparation of sample of other edible legumes for microscopic examination A sample of the whole legumes was soaked in water to loosen the seed coat or testa. The testa was then finely ground using a pestle and mortar. A slide was prepared in 50% aqueous glycerine solution and viewed microscopically.

Preparation of market samples of besan (C. arietinum) for microscopic detection of adulteration Market samples of besan that are under dispute for alleged adulteration are received by this laboratory from judicial courts of other states of the country, as it is one of the four appellate laboratories of the country under the Directorate General of Health Services, Govt. of India for dispute samples under

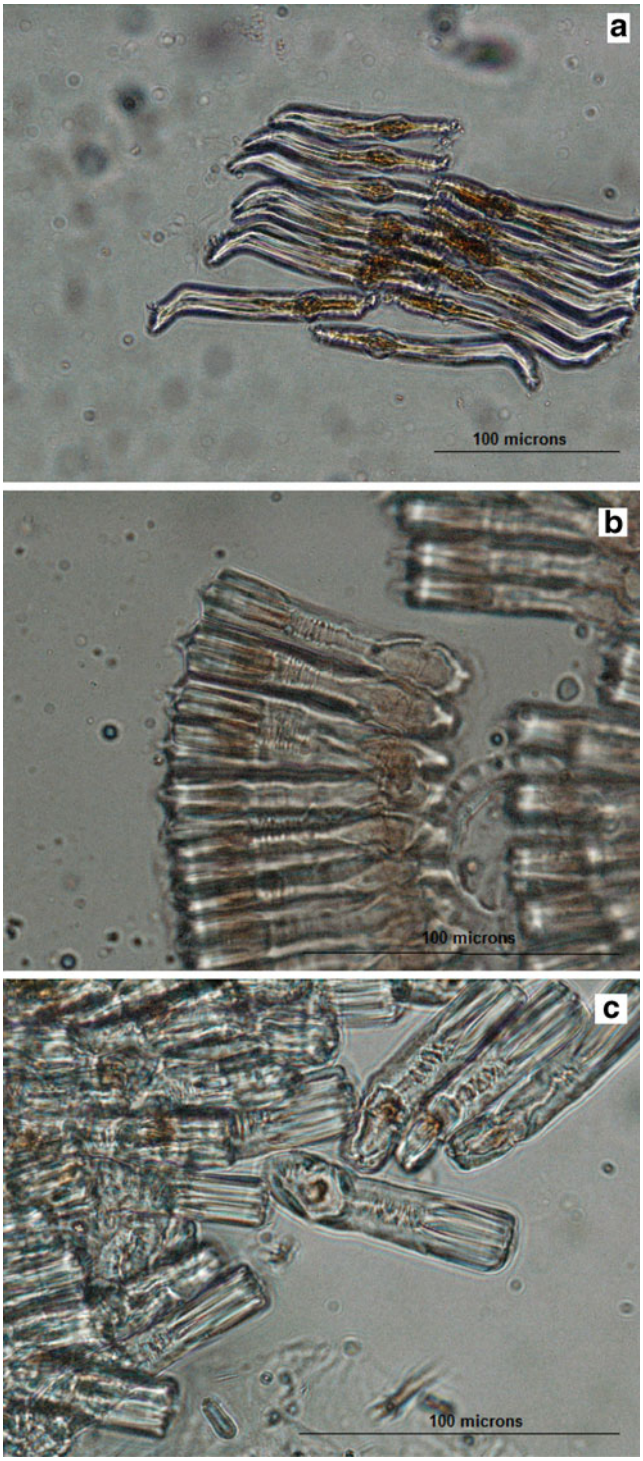

Fig. 1 Macrosclereids of **a**. *C. arietinum* (magnification 200X), **b**. *P. sativum* (magnification 400X) and **c**. *L. sativus* (magnification 400X)

Table 1 Measurement of the length and breadth (at the widest point) of the macrosclereids (microns) of the seed coat (testa) of edible legumes (n=50)

	Length		Breadth	
	Mean	SD	Mean	SD
Lathyrus sativa	72.0	12.91	10.7	2.63
Pisum sativum	61.8	9.42	15.9	2.88
Phaseolus mungo	59.8	6.86	11.1	1.66
Cajanus cajan	73.2	9.08	9.1	2.31
Glycine max	42.1	0.55	14.0	3.42
Phaseolus aureus	45.8	3.20	12.6	2.31
Phaseolus vulgaris	42.0	5.48	18.8	3.53
Lens esculenta	43.7	4.44	14.3	2.92

the Prevention of Food Adulteration Act 1954 and Rules thereof (2008). These samples were examined in this study. On receipt, samples are well mixed and treated as described in the section on preparation of adulterated besan samples.

Microscopy

The size and shape of the testa macrosclereids was examined by an Olympus light microscope Model BX51, equipped with 4, 10, 20, 40 and 100 X objectives and 10X wide field eyepieces and with a DP12 microscope digital camera system. Eyepiece micrometer was used to take measurements of the macrosclereids.

Results and discussion

Microscopic observations of macrosclereids of the seed husk of the adulterant lathyrus and pea and that of besan and their measurements are presented in Fig. 1 and in the Table 1. The macrosclereids are lignified and appear dark brown to black in color due to the high deposition of tanniferous content. The macrosclereids of *C. arietinum* are elongated being bent at one end, narrow at both ends but sinuous in the middle ranging in length from 37.6 to

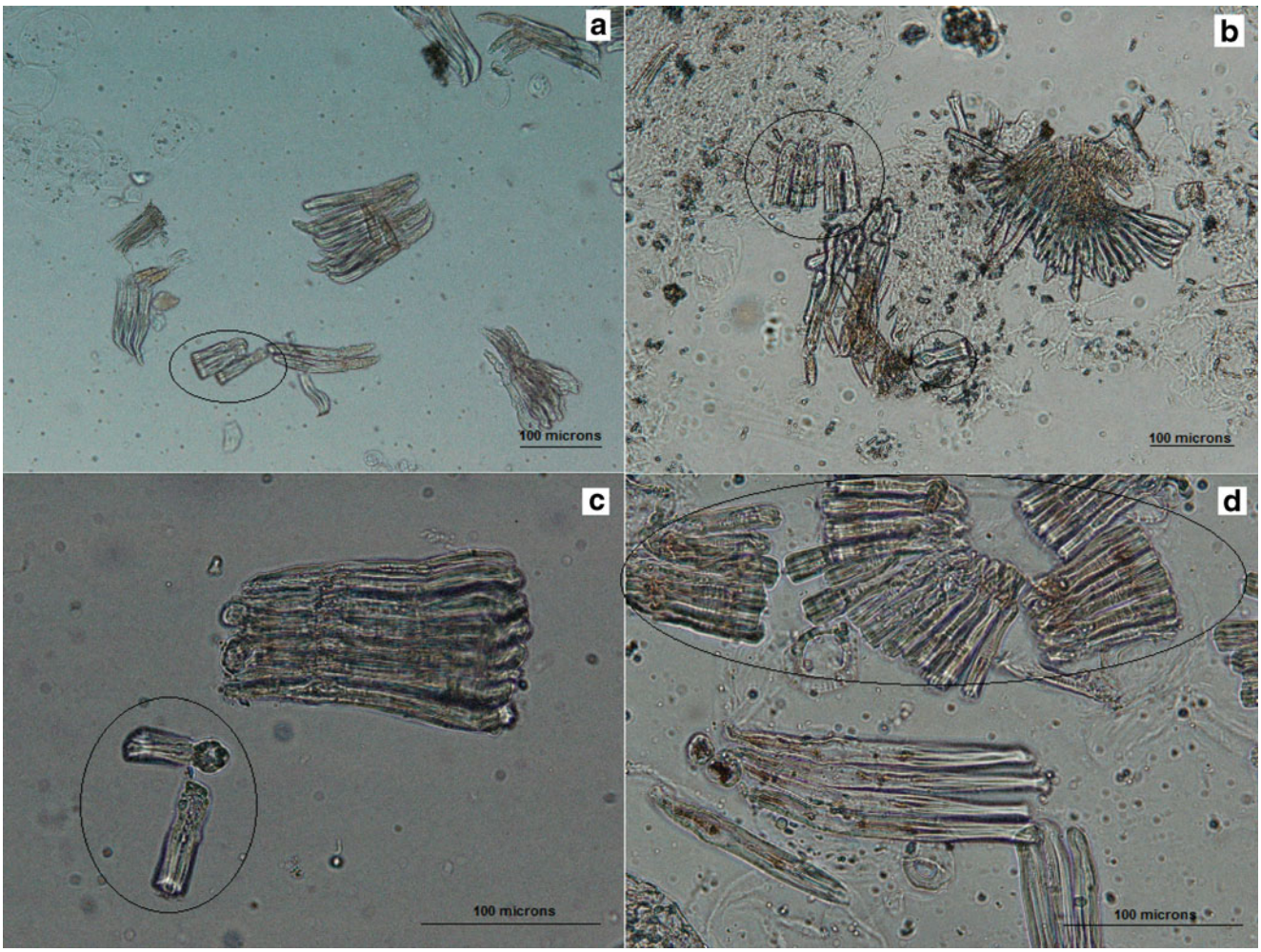

Fig. 2 Detection of admixture of besan (*C. arietinum)* flour with other legume flour (marked by black circles) by microscopic examination of the macrosclereids (**a** and **b** magnification = 100X, **c** and **d** magnification = 200X)

155.6 μ with a mean of 94.9 μ, and a mean breadth of 11.1 μ. The macrosclereids of *L. sativus* are usually shorter than that of *C. arietinum* with an average length of 72 μ, being broad at one end, narrowing at the other and constricted at the middle. A papilla like structure is usually observed at the center of the broad end. The *P. sativum* macrosclereids are similar to those of *L. sativus* but do not show any papilla like structure. Adulteration of market samples of besan could be detected microscopically as shown in Fig. 2. Macrosclereids of seed husk different from that of *C. arietinum* were present, indicating adulteration of the flour. Observations of the deliberately adulterated samples showed that the adulterants could be detected even when added at 0.25% concentration to the besan.

Gram dhal of *C. arietinum,* also called gram, Bengal gram or chickpea is prepared by splitting large sized well-developed seeds in a mill and separating the husk. Gram dhal is used for making flour or besan by grinding in hand mills or roller mills (Wealth of India 1950). The admixture of besan with *P. sativum* and *L. sativus* flour cannot be detected by external examination. Unlike in the case of cereals where the admixture of one cereal with another can

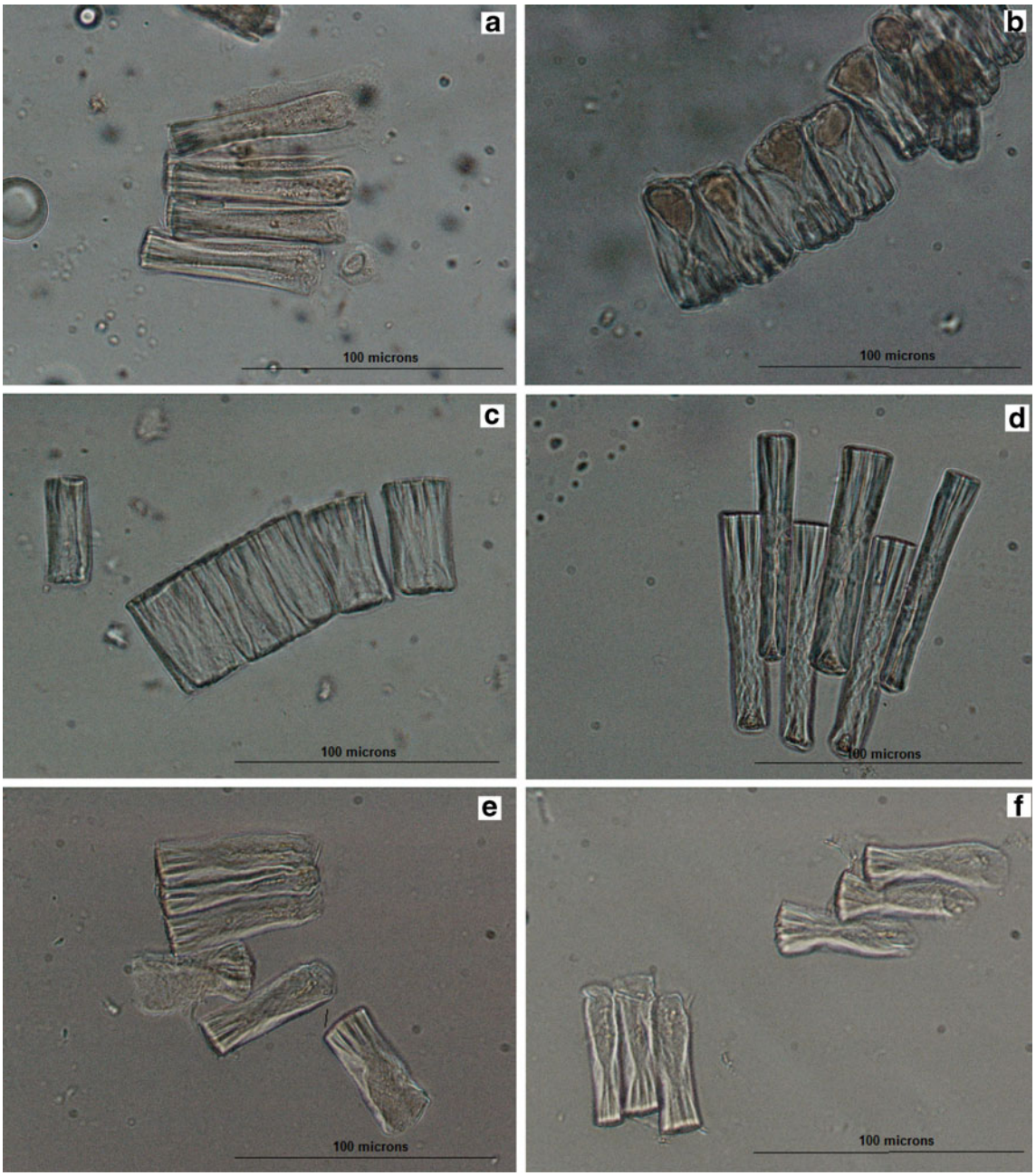

Fig. 3 Macrosclereids of the seed coats of edible legumes (magnification 400X) **a**. *P. mungo*, **b**. *P. vulgaris*, **c**. *C. cajan*, **d**. *G. max*, **e**. *L. esculenta*, **f.** *P. aureus*

be detected by observing the iodine stained starch granules (Kirk and Sawyer 1999), the starch grains of all legumes are similar and hence their observation cannot be used to distinguish the admixture of flour with another microscopically. Detection of the adulterant *L. sativus* in a sample of besan at more than 1% level is possible by chemical means, by the detection of the marker amino acid β-N-oxalyl amino l-alanine (BOAA) by paper chromatography (IS 2005). BOAA can also be detected by liquid chromatography and the LOD is 3.5±0.1 ppm (Thippeswamy et al. 2007) but this latter method requires costly infrastructure and analyst competence. In the case of adulteration with the flour of *P. sativum*, there is no chemical test by which this can be detected.

The plant sclereids range from isodiametric to much elongated cells and may be branched. They may occur as isolated cells (idioblasts), groups of cells or as extensive tissues. Five major kinds of sclereids are described namely, brachysclereids, macrosclereids, osteosclereids, astrosclereids and trichosclereids (McGraw-Hill 2007). In the present investigation the macrosclereids of other edible legumes were also studied and found to be of varying sizes with certain characteristic traits by which the macrosclereid of one legume could be differentiated from another (Fig. 3). Gupta et al. (1985) have also made similar observation of anatomical differences of *C. arietinum* and *L. sativus*. Macrosclereids of *P. mungo* are short with a mean length of 59.8 μ, with a constriction of the lumen. Those of *P. vulgaris* are short and stumpy with a sharp constriction of the lumen in the middle and a mean length of 42 μ. The macrosclereids of *C. cajan* are similar to those of *P. mungo* but do not show a definite lumen. The sclereids of G.max and *P. aureus* are similar and only vary in length. Sclereids of *L. esculenta* are curved at the middle, bulbous at both ends and have a mean length of 43.7 and breadth of 14.3 μ. The morphology of the macrosclereids of the seed husks of all these legumes is different from that of besan.

Thus, in the present study, it is seen that a simple technique of the microscopic observation of the macrosclereids of the seed husk that is present in the besan flour can be used to detect adulteration. While in the case of adulteration with *L. sativus*, this adulteration can be confirmed by chemical/chromatographic methods, presently microscopy is the only method by which adulteration with pea flour can be detected. However, it is realized that the adulteration can be detected microscopically only if during the processing of the legumes to flour, particles of the seed husk are also inadvertently included. We have been able to detect seed husk macrosclereids in the market besan (*C. arietinum* flour) samples in spite of the efficient pulse processing technologies developed (Pratape et al. 2004). The minute size of the macrosclereids of these legumes makes the possibility of their inclusion in the flour high enough to detect and establish adulteration. Although, adulteration of besan with other edible legume seeds has not been observed or reported, microscopy is again a method that can be used to detect it as the legume macrosclereids are distinct in their appearance.

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