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Methodology for the detection of saffron in Gutkha

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ABSTRACT

Saffron is added in Gutkha due to its exotic and aesthetic value due to its flavor and colour. No specific methodology was available for detection of saffron in Gutkha as claimed by the manufacturer. Multistage methodology is described in this paper for the detection of Saffron in Gutkha comprising of physical method. Microscopic method and confirmation by Chemical and TLC method. The methodology described even can detect the saffron if added in powder form.

Key words: Saffron, Gutkha and *Crocus sativus* Linn.

INTRODUCTION

Gutkha is Pan Masala containing Tobacco in any form. Under Item No. 30 PFA Rules, 1955 the Pen Masala is described as "Pan Masala means the food generally taken as such or in conjugation with pan, it may contain Betel nut, lime, coconut, catechu, saffron, cardamon, dry fruits, mulethi, sabnermusa, other aromatic herbs and spices, sugar glycerine, glucose, permitted natural colours, menthol and non-prohibited flavours. It should be free from added colouring matter and any other ingredient injurious to health.

Saffron (kesar) means the dried stigma or tops of styles of Crocus Sativus Linnaeus. It shall be dark red in colour with a slightly bitter and pungent flavour, free from foreign odour and mustiness. It should be free from mould, living and dead insects, insect fragments and rodent contamination. The product shall be free from added colouring matter.

Physical Method

Principal

The detection of Saffron in Gutkha in based on identification of characteristic flaments of saffron by physical examination of the sample using a magnifying glass/or by microscopic examination (in case of powder saffron) for typical anatomical structure of Saffron filament.

Procedure

For whole stigma of saffron

Spread out the test sample on a filter paper and examine it with magnifying glass for presence of characteristic filaments of saffron (saffron is obtained from the flowers stigma) of the saffon plant (*Crocus sativus* Linn.).

For powdered saffron

Gutkha samples containing powdered saffron can be examined in the following three mediums.

- in distilled water
- in sodium or potassium hydroxide solution
- in aqueous iodine/iodine solution

Preparation or Reagent

- Distilled water
- 5% of potassium hydroxide

  Weigh 5 g of potassium hydroxide in a beaker, dissolve it completely in a little amount of water and finally make up the volume to 100 ml
- Aqueous Iodine/Iodine solution

To a 100 ml volumetric flask, and 2g of iodine, 4 g of potassium iodide and about 10 ml of water. Dissolve them completely and finally make up the volume to 100ml.

Apparatus

- Slides, Cover glasses, scalpel, needles, etc.
- Microscopes

Microscopic examination

Make a set of 3 slides for test sample and for pure saffron standard separately by taking a drop of water in 1st slide, a drop of KOH reagent in 2nd slide and drop of Aqueous Iodine/Iodine solution in 3rd slide. Then put the test sample on the slide with the ip of the scalpel or needle and mix it with the drop of solution placed on the slide and make thoroughly wet. Cover it with cover glass by pressing gently. The same steps are to be done for Standard Pure Saffron in second set of slides.

The slide prepared in water allows observations of all the elemental structure of powdered samples whereas the slide prepared in potassium hydroxide enables clarification of cellular elemental structure making the slide clearer and easier to observe the filaments structure of saffron if present and use of iodine/iodine solution helps in the even identifying the starch grains of Saffron which are stained blackish blue.

It is always desirable to compare the observations of test sample with pure saffron standard reference. However, anatomical structure of saffron powder under microscope may be as under.

- Fragment of the top extremity of the stigmas with large, hair like elongated papillas capable of reaching a length of 150µm.
- Epidemic debris of stigmas with small round papillas.
- Round pollen grains of large diameter (80µm to 100µm) with a thick, smooth cell wall and with a finely granular exine.
- Parenchymatous debris
- Debris of the epidemic of the style, consisting of long, thin walled and slightly sinuous cells.
- Debris of thin vascular bundles.

Confirmatory test for Saffron

Chemical method

Principal

The confirmatory test with di-phenyl amine reagent develop blue colour in presence of oxidizing agent (due to presence of active chemical "crocin" in Saffron). Thus, the presence of saffron is indicated by immediate development of blue colour, which turns to reddish brown.

Preparation of Reagent

Di-phenyl amine solution

Dissolve 0.1 g of di-phenyl amine to 20 ml concentrated sulhpuric acid and 4ml of water.

Apparatus

Porcelain dish with flat bottom

Extraction procedure

Take approx. 1 g of sample, add 20-25 ml of distilled water and kept it for about 15-20 minutes at room temperature with occasional shaking and then filter through with whatman No.1 filter paper. Similarly, pure saffron extract is also obtained for comparison purpose.

Reaction with di-phenyl amine solution

Place one drop of extracted sample in the porcelain dish and put one drop of di-phenyl amine solution. In the presence of saffron, it produces a blue colour immediately, which rapidly turns reddish brown.

It is always desirabe to compare the observations of test sample with pure saffron standard reference.

TLC Method

Principle

Saffron consists of dried stigmas of Crocus sativus L of which the principal constituents are water-soluble pigments. The aqueous extract of Gutkha samples containing Saffron when run on a TLC gives 4 yellow spots the characteristic of saffron at Rf values approx. 0.1, 0.2, 0.45, 0.65, whereas in Gutkha sample (without saffron) these sports will not appear. If the amount of saffron in Gutkha is less, only two prominent spots (Rf of 0.45 and 0.65) may appear.

Apparatus

- TLC plates
- Developing Tank

Solvent system

Ethyl acetate: Propanol: Water (13:52)

Spray solution (Anisaldehye reagent)

Prepare by mixing in the following order
10 ml of p-acetaldehyde
90 ml ethyl alcohol
10 ml Conc. Sulphuric acid

Procedure

Take approximately 1 gm of sample, add approx. 20-25 ml of distilled water and keep it for about 15-20 minutes at room temperature with occasional shaking and then filter it with whatman No. 1 filter along paper. The clear filtrate so obtained is spotted on TLC plate along with pure standard extract and the place is developed in the above-mentioned solving system (for approx. 10 cms).

Observations and Results

Observations in daylight

In Gutkha samples containing saffron, 3 prominent yellow spots are observed on visible examination of the plate. The freshly developed plates are to be examined for correct results, as the intensity of spots fades away with time.

Observations under UV light

On examination under UV light at 254 nm, 1 additional sport (closed to the solvent front) may become visible in Gutkha sample containing saffron.

After spraying with Anisaldehyde reagent

The plate when sprayed with Anisaldehyde reagent, the 4 spots of grayish green to violet colour appears in Gutkha samples containing saffron.

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