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Detection and quantification of adulterated corn and soybean in ground coffee

Bui, T. P. Thao[1] , Truong, T.B. Lieu [1] and Dang, Q. Tuan [1,*]

[1]*Department of Food Technology, International University - Vietnam National University HCM city*
Quarter 6, Linh Trung ward, Thu Duc dist, Ho Chi Minh city, Vietnam
Phone: (84-8) 37244270; Fax (84-8) 37244271
**Corresponding author's E-mail address: dqtuan@hcmiu.edu.vn*

Abstract— Adulteration in commercial ground coffee has been a regular concern all over the world, especially when it is difficult to percept adulterations with the naked eyes. This study was performed in order to set up a simple and quick procedure to detect and quantify adulteration of corn and soybean in commercial roasted ground coffee products. The floatation, spot check and microscopic methods were applied to detect adulterants in ground coffee, while caffeine was used as a chemical maker to quantify the level of adulteration. Ground coffee samples were taken from national brands, local brands and street vendor shops. The flotation test using distilled water at room temperature appeared to be a simple, quick and easy method to detect the presence of corn and soybean: corn and soybean particles started to sink within 5 seconds, while pure coffee particles can float for more than 2 min. Additionally, microscopic examination using both compound and stereo-microscope appeared to be effective tools for detection of corn and soybean while examination by a spot check could confirm the presence of starch containing materials such as corn. Using corn as a model of the adulterant, then the caffeine content in ground coffee was well correlated with the amount of corn added, which allowed estimation of the adulteration quantity possible. The results from qualitative tests revealed that 88.9±10.5% of the commercial ground coffee samples were adulterated, either with corn, soybean or some non-coffee materials. Projection from the caffeine content regression showed that the level of adulteration in the surveyed samples was in a range from 10.0 up to 47.0%.

Keywords— **adulteration, caffeine, coffee, spot check, flotation test, microscopic test**

INTRODUCTION

Coffee is an important agricultural commodity in Vietnam, thus it is prone to be adulterated. The most common adulterants in coffee are corn and soybean. According to reports in the local mass media, the adulteration of coffee is so prevalent and it implies problems for the quality of coffee and the issue of food safety.

Many techniques have been developed in order to detect the adulteration in ground roasted coffee. Methods may be based on digital image processing (Sano et al., 2003), using a photothermal lens coupled with pH monitoring (Fontes et al., 2006), applying Infrared Spectroscopy (Reis et al., 2013) or high-performance liquid chromatography (Jham et al., 2007). Although effective, the methods employed were time demanding, expensive, laborious, requiring sophisticated machines and equipment, and, in most cases, not appropriate for routine analysis.

Besides that, physical evaluation of food has been widely used in food and feed quality control. It may include flotation technique, microscopic evaluation and spot check technique (Khajarern & Khajarern, 1999). Spot check test is a simple method for observation in color change for control of food quality and adulteration.

Flotation technique has been applied for samples of coffee from the market using different fluid such as water (cold and hot), organic compounds (carbon tetrachloride mixed with hexane) (Clarke and Wilson, 1978). Microscopic evaluation has been developed to analyze coffee included in other foods and drinks (Smith, 2001).

The objectives of this study were to develop a quick, inexpensive and effective procedure to detect and quantify the level of common adulterants such as corn and soybean in ground coffee, which could be widely acceptable not only by the food safety regulator, but also the producer and customer.

As for qualitative assessment, three simple techniques were suggested to apply such as floatation test, spot test and microscopic examination. In flotation test, the speed of sinking and time of floatation of coffee, corn and soybean in different fluids of different density (water, glycerin and table salt) were observed and recorded. Iodine solution in spot test was used to observe the change in color of the sample solutions to detect starch-containing materials and microscopic observation of morphology of each element of the samples by using stereo-microscope or compound microscope to confirm the adulterants.

As for quantitative assessment, caffeine was used as a chemical maker. By correlating the caffeine content and the amount of corn added, a regression equation was obtained to estimate the amount of corn and/or non-coffee materials in the commercial ground coffee samples.

MATERIALS AND METHODS

Coffee, corn and soybean samples

Seven roasted pure coffee samples, all of a robusta variety, were collected from districts in Ho Chi Minh City and coded as P1 to P7.

Nine commercial ground coffee samples were purchased from different local markets and they were of different labels, divided as national brand (NB- NB1, NB2, NB3), local brand (LB- LB1, LB2, LB3) and street vendor (SV- SV1, SV2, SV3).

Two samples of corn and soybean were bought from a local market and roasted to a degree of similar appearance to roasted coffee.

5 samples of ground coffee intentionally mixed with corn (an adulteration model) at a ratio of 20, 40, 50, 60 and 80% of corn to coffee (w/w). Each sample in this group was prepared in triplicate.

Materials and Chemicals

All solvents for spot test and flotation test including potassium iodine, Iodine crystals, table salt, and glycerin were purchased from a local chemical agent. Caffeine standard (98.0%), dichloromethane (99.5%) was purchased from a local chemical company.

Methods

Floatation test: Liquids of different density were made from mixing distilled water with table salt or glycerin in different ratios. The density of water at 1 atmosphere was taken from literature. Approximate 0.005g samples with 3 sieve sizes: 500 micron, 425 micron and 250 micron were put into 50mL solution in a test tube 50ml at 10ºC and room temperature (RT). The sinking speed and floating time of different materials, in different liquid mixtures were observed and described. A camera and a stop-watch were used to record the time of flotation.

Test color/spot check: using Iodine to detect starch in each sample through changing color from color of iodine solution turn into dark blue color. Coffee practically contains no starch, while corn contains large amount of starch. 50g samples were sieved through 35-mesh screen. 4g of the sieved sample were put in 40 mL water, stirred and heated up to 80°C for 20 minutes. The Iodine solution was made by adding few crystals of iodine to 2% Potassium Iodine solution. Three drops of the iodine solution were added and the color change observed.

Microscopic examination: Stereo-microscope (model Z2M-TZMC-7FH1, LW Scientific, US) with computer and camera connectivity was used to evaluate roasted pure coffee, corn and soybean, by observing their physical characteristics including shape, color, particle size, softness, hardness and texture, etc. In order to facilitate ingredient identification, samples would be sieved through 40-mesh screen. Stereomicroscopy was used with magnifying power 10X, 30X and 50X. Compound-microscope (model I4M-B04A-ISL3, LW Scientific, US) with computer and camera connectivity was used to observe internal cellular characteristic of roasted pure coffee, corn and soybean with magnifying power 40X and 100X. Samples were sieved through 40-mesh and 1-2g of the sieved samples were heated with 50mL of 8% KOH solution for 30-45 minutes.

Determination of moisture content: Oven method was used to determine moisture content of samples at 103± 2◦C in 2 hours for pure coffee, roasted corn, soybean and commercial ground coffee samples.

Determination of caffeine content: Raw coffee were ground and screened through 250 µm sieve. An accurately weighed amount of sieved coffee (approximately 50 mg) was dissolved in 25 mL of distilled water. The solution was heated gently for 5 minutes to extract caffeine completely from the solids. The solution was then filtered by a filter paper (Whatman No. 1) before being mixed with dichloromethane (by a volume 25 mL) for extraction of caffeine to an organic phase. The procedure was repeated twice more and the organic phases were combined (Belay et al., 2008).

Caffeine stock solution of (1.0 mg/mL) was prepared by dissolving 0.1 g of pure caffeine in 100 mL of dichloromethane. Then 7 caffeine working solutions were prepared by serial dilution of the stock in 50 mL volumetric flasks with dichloromethane.

The absorbance of the solution was measured by UV/Vis spectrophotometer (mode Genesys 10S, USA) at a wavelength of 273 nm against a dichloromethane blank (Phan et al., 2012). The value of caffeine content in various samples was calculated based on the regression equation.

Statistical Analysis

All treatments were done in triplicate. ANOVA and regression analysis were performed by using standard Software SPSS version 16.0.

RESULTS AND DISCUSSIONS

Floatation test

At RT (about 25°C), the sinking speed of material particles was faster than at temperature of 10°C. As observed, ground soybeans sink almost immediately in all solutions while ground corn started to fall quickly, just few seconds after dropping (both at RT and 10°C) or at most to 40 seconds. Pure ground coffee kept floating on the surface of liquid for over than 120 seconds, both at RT and 10^{o}C. The materials should be ground to an average size of 0.5 mm. In different solutions, the higher density makes the ground particles fall slower. In the distilled water (density 1g/mL), the particles felt faster than in the mixture of water/glycerin or the mixture of water/table salt (density 1.03, 1.06 and 1.09 g/mL). The size of particles, the nature and density of the solution did not have significant effect to distinguish corn and soybean from coffee. Besides, the soybean particles always sink faster than corn. This test showed that a limit of 120 seconds should be used to differentiate coffee from corn and soybean. The floatation

test is simple and easy to apply widely. Clean water at room temperature could be used as a good medium to differentiate coffee from corn and soybean.

The results from this test showed that out from nine commercial coffee samples collected, only one sample with national brand (NB1) contained no adulterant.

Color spot check test

In the iodine test, the color of corn samples turned dark blue, while the color of pure coffee and pure soybean did not change (Fig. 1). Corn contains large amount of starch, up 72% while coffee and soybean do not contain a starch (Mussato et al., 2011), so all samples containing corn will have color turned to dark blue. The results form observation revealed that 5 commercial coffee samples, of which two from local brand and all three street vendor, had color changed. The result was in accordance with the flotation test, and it confirmed that all these samples were adulterated and most probably with corn. Meanwhile all three national brand coffee samples were negative with the iodine test.

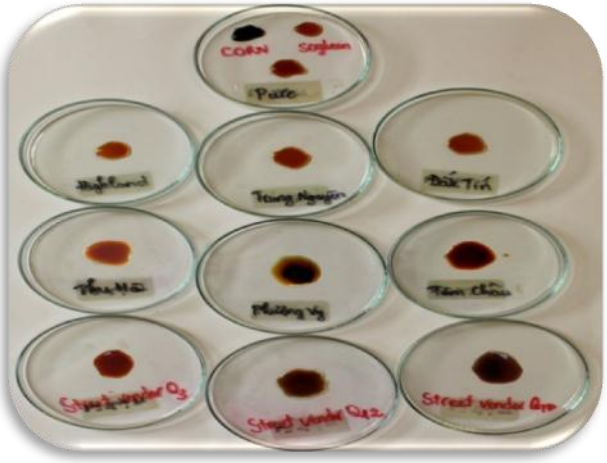

Figure 1: Color of commercial coffee samples in iodine test

Microscopic Examination

Different morphological features observed under microscope helped to discriminate coffee, corn and soybean. These characteristics were divided into 3 groups (Khajarern & Khajarern, 1999) as follwed.

Macroscopic features

After grinding, the fraction of roasted coffee was black, uniform and less crumble. The fraction of roasted soybean was brown and softer than coffee while the fraction of corn was slight dark and softest with many fragmentations.

Stereomicroscopic features

The fraction of roasted coffee was black or brown black, not translucent, with sharp contours, large cubic and hard texture.

The fraction of corn in many different sizes and smaller than coffee, contained hard woody part occurring in irregularly shaped lumps and was translucent yellow or white.

The fraction of roasted soybean was irregular and flat in shape with round edges, they appeared translucent, having a glazed or waxy surface, and varying color from cram to pale reddish brown. The texture was hard and brittle.

Histological features

For corn: cells were elongated, joined end to end with three basic forms. Rounded cells had polygonal and floury, branched, often rounded in shape, having yellow inside and white edge around. Long cells were filamentous and un-branched, with many small dots inside and lines were cut out inside which are features to discriminate with coffee. Cells had embryonic axis with larger cells in the rudimentary root.

For soybean: the hour-glass cells had the hour-glass liked shape, each being hexagonal. Polygon cells had rough-edged depressions with an irregular dot on a color less to tan, translucent background. The round large cells were irregular, shiny, semi translucent, round chunks, buff to tan or brown in denser portion; cell was divided into small particles inside which was the point to distinguish each other.

For coffee: the rounded cells were small cells with an irregular dot inside, attached to each other to create large of strip cells, buff to tan or brown in denser portion, translucent background. Long cells were filamentous and branched with some red dots and lines inside.

In the microscopic test, detection of morphology of soybean was observed in three samples (LB1, NB2 and NB3) and morphology of both of corn and soybean in 5 samples (LB2, LB3, SV1, SV2 and SV3). Neither corn nor soybean morphology was observed in the sample of NB1, which was confirmed be without adulteration by the flotation and spot check test previously.

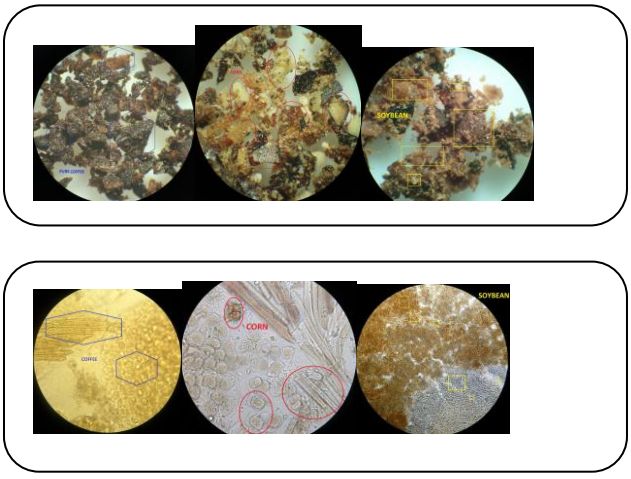

Figure 2: Morphology of roasted coffee, corn and soybean in stereomicroscope and histological features

Moisture content

According to national regulation standards, moisture content (MC) in roasted and ground roasted coffee should not be more than 5%. Except for one sample with quite

high MC (8.82%), the average MC of pure roasted coffee was around 5% (Fig. 3). As for commercial ground roasted coffee, the MC was lower than that of pure, whole bean roasted coffee (4.13%).

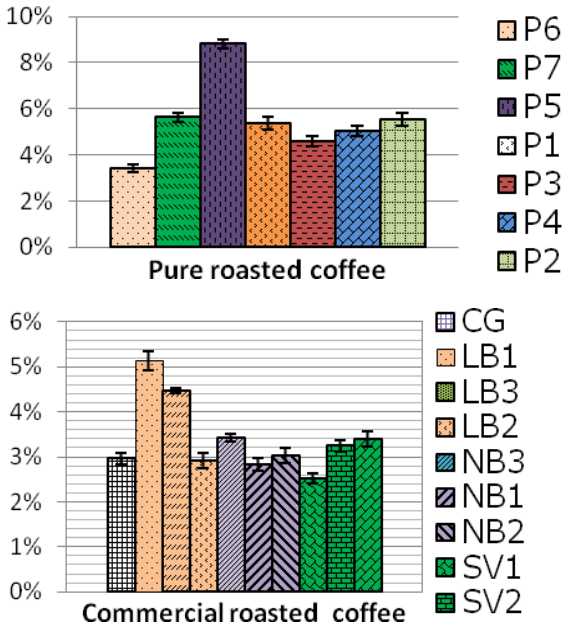

Figure 3: Moisture content of pure roasted coffee and commercial roasted coffee

Caffeine content

Statistically, the average amount of caffeine content in green Robusta coffee of is 2.2% and in roasted Robusta coffee 2.4%. However, many factors could affect caffeine content like planting, harvesting, processing, packaging and climate (Spiller, 1997) as well as post-harvesting processing conditions (drying, storage, roasting, grinding and extraction (Franca et al., 2009). In the pure coffee samples, all caffeine contents were higher than 1.0%, the highest % caffeine (d.b.) was 2.047±0.047% and the lowest 1.232±0.009% (Fig. 4). The average content of caffeine in 7 pure roasted coffee samples (Robusta) was 1.661%.

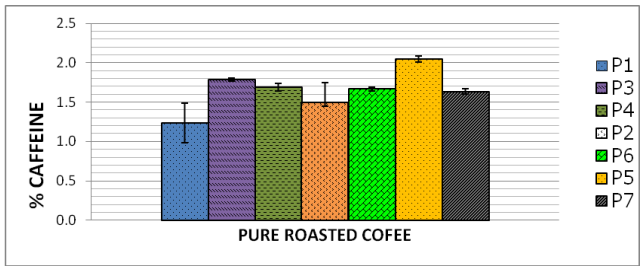

Figure 4: Caffeine content (%, d.b.) of pure roasted coffee

The commercial coffee products should have caffeine content more than 1% to comply to the local government regulation (TCVN 5251:2007). Out from 9 commercial roasted coffee samples examined in this study, only two (22%) complied with the regulation for caffeine content. It is worthy to note that the sample NB1, which was found to have no adulteration, was just a sample with the highest caffeine content (1.743±0.014%). Besides that, the caffeine content in the samples of local brand LB2, LB1, LB3 and street vendor SV1 was very low (lower than 0.5%), hence, it was in close relation to that fact that adulterants were detected in those products (Fig. 5).

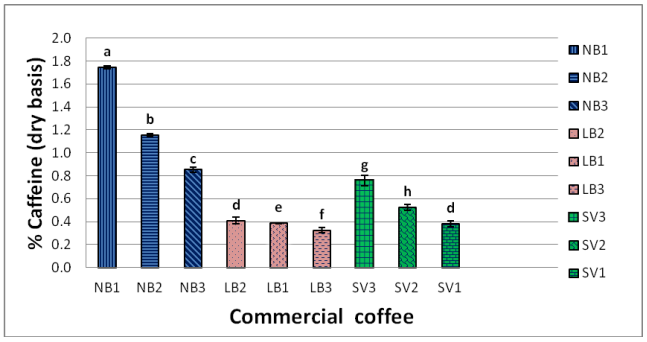

Figure 5: Caffeine content in commercial ground coffee

The caffeine content in intentionally adulterated coffee with corn was determined by measuring absorbance in the dichloromethane solution and compensating by the absorbance of the dichloromethane extract of corn, which was used as a control sample. The compounds extracted in dichloromethane from corn and soybean are lipids, not caffeine. A regression equation between % caffeine and % adulterated corn was established. It allowed an estimation of the amount of adulterated materials (non-coffee) present in the commercial ground coffee by projection.

The results of estimation indicated that the amount of adulteration in commercial ground coffee samples were from 10.38 to 47.45%, with the highest in the local brand coffee sample.

CONCLUSIONS

A procedure by combination of flotation test, spot check, microscope evaluation and collocation of caffeine content showed to be a quick and inexpensive method for detection and quantification of commonly employed adulterants such as corn and soybean in coffee.

The floatation test using clean water at RT should be applied first to detect corn and soybean. All non-coffee strange materials will sink within 5 second, while the coffee particles will flow for more than 2 min. This test is simple and easy to apply widely.

The spot check using iodine solution should follow to detect any starch-containing materials, such as corn. The results should be used to confirm the outcomes of the first test.

The microscopy was another way of detect a nature of adulterant. By observation under stereomicroscope and microscope-compound, morphology of coffee, soybean and corn were determined in each sample. This technique could require a certain technical training, and could be used in parallel or to confirm to the above techniques.

UV-Vis spectrophotometry is a quick and simple method for caffeine determination. Caffeine content in ground roasted coffee was highly correlated to the amount

of adulteration, so caffeine could be used as a maker to quantify the adulteration in commercial coffee. Most of adulterated coffee samples had low caffeine content, usually below regulation standards (less than 1%). The coffee adulteration was quite prevalent at a rate of 88.9±10.5%.

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