Roasted barley addition to coffee: detection of the adulterant barley using ultraviolet and infrared spectroscopy

Habtamu Gebremichael Daba

The objectives of this research were to detect the adulterant roasted barley at different concentrations (5 to 20 \% w/w) in roasted and ground coffee using ultraviolet and mid infrared spectroscopy. Infrared spectroscopy transmission measurement of medium roasted coffee admixture (5 to 20 \% w/w barley) showed that the infrared spectra among 5 to 20 \% w/w barley addition to coffee were not resolved though it was possible to identify infrared spectra peak regions useful to discriminate the roasted barley from roasted coffee using caffeine, lipid amount and existing starch. The ultraviolet spectroscopy analysis showed that it can discriminate pure coffee from adulterated coffee, 5 to 20 \% w/w barley based on ultraviolet absorbance measurement. In conclusion, ultraviolet spectroscopy analysis resulted in discriminating pure roasted and ground coffee from the adulterated coffee as low as 5 \% w/ w barley addition. Mid infrared spectroscopy analysis could not resolve the addition of 5 to 20\% w/ w roasted and ground barley to pure coffee.

Key words: Adulterant, barley, coffee, infrared, spectroscopy and ultraviolet.

INTRODUCTION

Arabica coffee originated from Ethiopia and discovered by a goat herder named Kaldhi around 850 A.D. (Butt and Sultan, 2011). Coffee is the most traded next to petroleum in the world market, about 10 billion US dollar per year (Cordella et al., 2002). Annually, about 500 billion cups of coffee are consumed by about one third of the population of the world (Butt and Sultan, 2011). Economic adulteration of food affects the nature, quality, originality and nutritional value of food and thereby influences consumers’ expectations for quality food (Dennis, 1998).

Roasted coffee can be liable to adulteration by mixing it with cereals, coffee twigs, Robusta coffee, brown sugar (Jham et al., 2007). Higher cost of coffee and its physical characteristics resemblance to roasted and ground cereals, seeds, roots and parchments could be the reasons for its adulteration (Fontes et al., 2006; Varvolgyi et al., 2013). Moreover, the increase in coffee prices plays a role in economic adulteration of roasted coffee (Briandet et al., 1996).

Regulatory bodies, quality personnel and food chemists require fast, more reliable, low cost and less or no sample treatment analytical method for routine detection of adulterants in foods though most analytical methods are expensive, time taking and tired some (ALPDOĞAN et al., 2002).

Reis et al. (2013) studied detection of barley, corn, coffee husks and spent coffee grounds by using Fourier Transform Infrared (FT-IR). Ebrahim-Najafabadi et al. (2012) detected addition of barley to coffee using near infrared spectroscopy and chemometrics. Briandet et al. (1996) used infrared and chemometrics to detect adulterants in instant coffee. Souto et al. (2015) detected adulterants in roasted and ground coffee using Ultraviolet spectroscopy. Coffee adulteration detection instruments should be easily accessible by developing countries laboratories to carry out the routine analysis of detecting adulterants in roasted coffee. As to the author’s knowledge, there is literature gap on adulteration detection of barley in roasted coffee using ultraviolet (UV) spectroscopy; moreover there is no literature on roasted barley infrared spectra along with barley coffee mixture (5 to 20 \% w/w) spectra. Thus the less expensive ultraviolet and mid infrared (IR) spectrophotometer analysis of
roasted coffee for adulterant discrimination provides faster and reliable information regarding the adulterant barley in coffee. The aim of this study was to detect the adulterant barley in roasted coffee at different concentration (5 to 20% w/w) using ultraviolet and mid infrared spectrophotometer so as to discriminate barley adulterated coffee, roast and ground.

MATERIALS AND METHODS

Sample preparation

Pure Harar coffee was provided from Ethiopian Ministry of Agriculture and Natural Resources coffee quality inspection center and the six rowed barley was purchased from the local market (Addis Ababa, Ethiopia). The pure Harar coffee was roasted at medium roast in Probat®roaster at 200°C for 70 min, and finely ground in Mahlkonig® coffee grinder for 60 s, 0.15 <D< 0.5 mm. The barley was medium roasted at 250°C for 30 min, the roasted barley was also finely ground in Mahlkonig® coffee grinder for 60 seconds, 0.15 <D< 0.5 mm. The coffee and barley roasting, grinding and packaging were carried out at coffee quality inspection center laboratory (Addis Ababa, Ethiopia). For the study purpose, proportions of 5 to 20% w/w barley to coffee mixtures were prepared intentionally by mixing 95% coffee with 5% barley, 90% coffee with 10% barley and 80% coffee with 20% barley then for the homogeneity of the samples, each of the proportions were mixed thoroughly by a Stuart® electronic mixer (homogenizer), the stirrer wheel and containers were cleaned properly before and after each mixing. Then the roasted and ground pure coffee, pure barley and their mixtures were put into polyethylene bags for the analysis.

Infrared spectroscopy analysis

The coffee or coffee mixture was ground as fine powder with minimal moisture content then 1 milligram of the coffee barley mixture was mixed with 100 milligram potassium bromide (KBr), analytical grade and the mixing was carried out in small pestil and mortar, after a thorough mixing, it was put in Perkin Elmer die for compressing the sample to form pellets using a pressure for 5 min, a thin transparent pellet was formed. The spectra were recorded in KBr pellets using mid infrared spectrophotometer; the pellet was put in a confiner and placed in the mid infrared spectrophotometer compartment. Then the spectra were measured on a double ultraviolet / visible - near infrared spectrophotometer, Perkin Elmer lambda in 4000 cm⁻¹ to 400 cm⁻¹ with 32 scans with a resolution of 2 cm⁻¹ through transmission mid infrared measurement (Pujol et al., 2013). Then the graph was plotted using Microcal Origin® 6 software.

UV spectroscopy

Sample preparation for UV absorbance measurement (Wanyika et al., 2010; Komes et al., 2009)

0.25 g medium roast and finely ground Ethiopian Harar coffee or mixture with barley were weighed and dissolved in distilled water to net volume of 20 ml. The 20 ml sample solution were pipetted to 250 ml flask and 10 ml of 0.01 mol/l HCl, 2 ml of basic lead acetate solution were added and then made to the mark with distilled water, and then shaken up and filtered to clarify using Whatmann®filter paper.

50 ml of the filtered solution were pipetted and added to 100 ml flask then 0.2 ml 4.5 mol/l H₂SO₄ were added and made to the net volume with distilled water then shaken up and filtered to clarify using whatmann® filter paper. Then the absorbance of the samples were measured on UV spectrometer (Perkin-Elmer lambda 19, wavelength range 170 nm to 3200 nm, double monochromator) lambda at 274 nm using 10 mm quartz cuvette. Then the absorbance measurement of pure and adulterated coffee samples was recorded.

Measurements were in triplicates

Data analysis

Data obtained from UV spectroscopy was evaluated using one way ANOVA and significance of means were declared at p < 0.05 and mean separation was carried out with Least Significant Difference (LSD) comparison. SPSS software version 20 was used for the statistical analysis. UV absorbance graph was drawn using Microsoft Office Excel 2007. Graphs for IR measurements were plotted using Microcal Origin-6 software.

RESULTS AND DISCUSSION

Infrared spectra analysis

The qualitative transmission measurement of roasted and ground Harar coffee and coffee barley mixtures 5 to 20% w/w barley showed that the infrared spectra among 5 to 20% w/w barley addition to coffee were not resolved though it was possible to identify the peak regions useful to discriminate the roasted barley from roasted coffee IR spectra using caffeine molecule, lipid content and starch presence; this might be due to the amount of sample (1 mg) used to form pellets for IR analysis using potassium bromide since the PerkinElmer spectrophotometer clearly
resolved the pure roasted barley and coffee IR spectra but not the mixtures (5 to 20% w/w). The result obtained is in close agreement with the study of Varvolgyi et al. (2013) showed in their study that diffuse reflectance near infrared (NIR) spectroscopy could not discriminate 1%, 5%, 10% w/w barley except slight variation in 20% w/w barley. Gholizadeh et al. (2014) described that the total carbohydrate IR absorbance peak in barley was within the range of 1180 - 950 cm$^{-1}$. Moreover, Welna et al. (2013) stated that the peak between 1150 -900 cm$^{-1}$ in roasted coffee products were attributed to C-O-C group of polysaccharides, C-O stretching at 1070 cm$^{-1}$ and 1060 cm$^{-1}$.

Varvolgyi et al. (2013) found out high discrimination power of NIR spectroscopy in addition of barley 49 - 100% w/w barley in Robusta coffee. Welna et al. (2013) identified the peak at 1150 cm$^{-1}$ to be attributed to bulk carbohydrates in chicory, which could be the most significant in discriminating pure coffee and adulterated ones.

The sharp peak at 2925 cm$^{-1}$ in roasted coffee (Figure 1) was more intense than the sharp peak at 2925 cm$^{-1}$ in roasted coffee (Figure 2). Reis et al. (2013) showed that this peak at 2925 cm$^{-1}$ was attributed to C-H stretching of lipids due to higher lipid content in coffee than barley. Moreover, as it can be seen on the IR spectra, roasted barley (Figure 2) was devoid of a band at about 2852cm$^{-1}$.
next to the sharp peak at 2925 cm$^{-1}$, the band at about 2852 cm$^{-1}$ was attributed to C-H stretching of methyl (CH$_3$) group in the caffeine molecule and the peak was not observed in roasted barley IR spectra since barley has no caffeine in it. These two absorption bands were affected by lipids and caffeine in the roasted coffee but only by lipids in roasted barley that influence the absorption bands (Reis et al., 2013). The result of the study was in agreement with Reis et al. (2013).

The IR absorption region 2250 to 1850 cm$^{-1}$ was identified to be discriminative of the adulterant barley from the roasted coffee according to the study of Reis et al. (2013), this might be due to high amount of phenolics attached to non-degraded starch or it might partially be associated to non-degraded starch’s hydration water effect (Reis et al., 2013). The study also found out that the region between 2250 cm$^{-1}$ and 1850 cm$^{-1}$ could be used to discriminate roasted barley from roasted coffee IR spectra. Moreover, the absorption band at 1746 cm$^{-1}$ in roasted coffee was not evident in roasted barley IR spectra. Reis et al. (2013) also found out that the band 1745 - 1742 cm$^{-1}$ was weak in roasted barley due to its low lipid content, the band was formed by the C=O vibrations of triglycerides and aldehydes. This study also found that the band at 1650 cm$^{-1}$ in roasted coffee was not noticed in roasted barley IR spectra. Reis et al. (2013) described that decaffeinated coffee samples has shown less absorbance in the range 1700-1600 cm$^{-1}$.

According to the study of Reis et al. (2013), the absorbance band region 950-700 cm$^{-1}$ was identified as beneficial in the discrimination of roasted coffee from its adulterants due to the fact that coffee has no starch but barley does. This difference in the band region could be attributed to the alpha glycosidic links in barley and beta glycosidic links in coffee. The result of this study is in agreement of Reis et al. (2013).

The study found out that the absorbance peak region between 1746 cm$^{-1}$ and 1650 cm$^{-1}$ in roasted coffee IR spectra (Figure 3) had been absent in the IR spectra of roasted barley (Figure 2), with only a peak at 1643 cm$^{-1}$. Welna et al. (2013) stated that the peaks at 1700, 1658 and 1600 are attributed to the caffeine molecule and the absorbance band at 1643 cm$^{-1}$ were due to oxalate. The result of the study is in agreement with Welna et al. (2013).

Gholizadeh et al. (2014) stated that barley IR spectrum $1180 - 950$ cm$^{-1}$ was attributed to total carbohydrate peak moreover, there are other peaks of barley responsible for stretching of N-H and O-H, C-H, C=O carbonyl, amide I, II and for cellulosic compounds.

Reis et al. (2013) stated that sharp bands at 2924 - 2925 cm$^{-1}$ and 2852 cm$^{-1}$ along with absorptions at 1715-1745 cm$^{-1}$ and 760 cm$^{-1}$ were found in both roasted coffee (Figure 3) and barley (Figure 2).

Wang and Lim (2012) revealed that 2920 cm$^{-1}$ in pure roasted coffee was attributed for CH$_2$ assymetrical stretching of methyl groups, 2850 cm$^{-1}$ was related to C-H symmetrical stretching of methyl groups, 1739 cm$^{-1}$ was due to C = O stretching of polysaccharides /hemicelluloses and 1660 cm$^{-1}$ was due to C = C stretching band of lipids and fatty acids.

This study found that the peak 3433 cm$^{-1}$ in pure roasted coffee (Figure 3) shifted to 3419 cm$^{-1}$ in 10 % w/w barley (Figure 5) and to 3400 cm$^{-1}$ in 20% w/w barley

Figure 3. Infrared spectra for medium roasted pure coffee.
These might be related to many vibrations of OH groups and also minor influence of NH functional groups (Pujol et al., 2013). Welna et al. (2013) stated also that the broad band between 3420 - 3375 cm\(^{-1}\) in coffee products was assigned to O - H stretching of hydroxyl groups and water besides it was due to N-H stretching in amines I and II.

The band at 1094 cm\(^{-1}\) in pure roasted coffee (Figure 3) had shown slight change only in 20% w/w barley (Figure 4) with a band of 1084 cm\(^{-1}\). Welna et al. (2013) described as the most significant difference between pure coffee and adulterated ones occurred at 1150 cm\(^{-1}\) with respect to bulk carbohydrates in chicory (Welna et al., 2013), which can also be indicative of the adulterant barley in coffee. Briandet et al. (1996) also identified pure and adulterated freeze dried soluble coffee in the region 900 - 1100 cm\(^{-1}\) and used caffeine and chlorogenic acid as a primary source of variability in FTIR analysis. Reis et al. (2013) described that the sharp peak in the region 950 - 700 cm\(^{-1}\) in roasted barley had shifted to the bands for
the pure roasted coffee due to the difference in polysaccharide types present in coffee and barley, the presence of starch in barley, alpha glycosidic link however beta glycosidic links in roasted coffee.

The sharp peaks at 2955 cm⁻¹ and 2855 cm⁻¹ in pure roasted coffee (Figure 3) were attributed to the occurrence of large amount of lipids in coffee and Pujol et al. (2013) also revealed that the peaks were indicative for the presence of methyl and methylene presence, asymmetric and symmetric stretch of C-H bonds in aliphatic chain. Reis et al. (2013) stated that the spectra for roasted barley having low lipid showed change from the peak in roasted coffee, this study also found that the 20 % w/w barley (Figure 4) showed weaker spectral intensity than pure roasted coffee spectra (Figure 3). Absorption bands in 2250 - 1850 cm⁻¹ were the spectral region which Reis et al. (2013) described the region crucial to discriminate coffee and its adulterants due to the higher peak intensity in roasted barley than roasted coffee and this study found that the percentage transmittance for pure roasted coffee (Figure 3) was at about 50 and the percentage transmittance for 20% w/w barley (Figure 4) was at about 40, which showed increased absorbance for 20% barley than the absorbance for pure roasted coffee, the result found was in agreement with the study of Reis et al. (2013).

The sharp peak at 1746 cm⁻¹ in pure roasted coffee and coffee barley mixtures (10 and 20% w/w barley), in 5% w/w barley (Figure 6), the peak was 1745 cm⁻¹ and it was attributed to lipids, C=C vibrations (Pujol et al., 2013). Reis et al. (2013) stated that peak bands at 1745 - 1742 cm⁻¹ was more intense in roasted coffee but weaker in roasted barley due to low lipid content in barley.

The absorption bands in this study at 1060 cm⁻¹, 1164 cm⁻¹, 1246 cm⁻¹ and 1379 cm⁻¹ in pure roasted coffee, Figure 3, were correspond to chlorogenic acid according to Pujol et al. (2013). Meanwhile, Pujol et al. (2013) also stated that several vibrations in the range 900-1400 cm⁻¹ with C-H, C-O-C, C-N and P-O vibrations were attributed to characteristics of carbohydrates.

The 10 % w/w barley IR analysis showed that the peak at 3419 cm⁻¹ was not similar with the peak in pure roasted barley, 3433 cm⁻¹, which probably would be related to many vibrations of OH and minor effect of NH functional groups ( Pujol et al., 2013).

The broad band peak of 5% w/w barley (fig. 6) at 3400 cm⁻¹ was not similar with the peak for pure roasted coffee (Figure 3) 3433 cm⁻¹.

Ultraviolet absorbance measurement

The UV absorbance measurement at 274 nm using UV/VIS spectrophotometer in Table 1 revealed that the result obtained for pure coffee was significantly different

The UV absorbance measurement at 274 nm using UV/VIS spectrophotometer in Table 1 revealed that the result obtained for pure coffee was significantly different.
from the adulterated coffee (5 to 20% w/w barley) and this study found out that it was possible to identify the adulterant barley as low as 5% w/w barley in roasted and ground coffee. Fontes et al. (2006) stated that among the several approaches to detect adulterants in roasted coffee, the spectroscopic techniques UV-VIS to IR are to be mentioned.

Souto et al. (2015) used UV-Vis spectroscopy via hot water extraction in 239 - 405 nm range to discriminate adulterated coffee from pure roasted coffee based on molecular absorption of compounds found in coffee, coffee husks and sticks, this study also detected the adulterant barley in roasted coffee based on the maximum absorption wavelength of caffeine, 274 nm. This result was in agreement with Souto et al. (2015). As expected, the absorbance of the coffee barley mixture had decreased from 5% w/w barley addition to 20% w/w barley since barley does not contain any caffeine (Reis et al., 2013). The result of this study (Figure 7) had a potential to be used in adulteration detection of roasted coffee with roasted barley as it enabled the discrimination of low concentration (5% w/w barley) of adulterated coffee from pure roasted coffee. Varvolgyi et al. (2013) and Oliviera et al. (2009) emphasized the necessity of coffee adulteration detection at lower concentrations of the adulterants.

**Conclusion**

Ultraviolet spectroscopy analysis resulted in discriminating pure Harar coffee from the adulterated coffee as low as 5% w/w barley addition. However, infrared spectroscopy could not resolve among 5 to 20% w/w barley addition to coffee though it was possible to identify the peak regions useful to discriminate the roasted barley from roasted coffee Infra red spectra using caffeine, lipid amount and existing starch.

**REFERENCES**


