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Aluru Madhuri
Department of Food Safety and Quality Testing Laboratory, Indian Institute of Food Processing Technology, Thanjavur, Tamil Nadu, India

Suman Thamburaj
Department of Food Biotechnology, Indian Institute of Food Processing Technology, Thanjavur, Tamil Nadu, India

R Jaganmohan
Department of Food Product Development, Indian Institute of Food Processing Technology, Thanjavur, Tamil Nadu, India

Dr. R Vidyalakshmi
Professor and Head, Department of Food Safety and Quality Testing Laboratory, Indian Institute of Food Processing Technology, Thanjavur, Tamil Nadu, India

Corresponding Author:
Dr. R Vidyalakshmi
Professor and Head, Department of Food Safety and Quality Testing Laboratory, Indian Institute of Food Processing Technology, Thanjavur, Tamil Nadu, India

ATR-FTIR spectroscopy for adulteration detection of corn flour in cassava flour

Aluru Madhuri, Suman Thamburaj, R Jaganmohan and Dr. R Vidyalakshmi

Abstract
Corn flour which acts as a major adulterant in cassava flour during the manufacturing of tapioca (cassava) sago plays a major role in effecting the farmers economically. Nutritionally, there is a considerable difference between cassava and corn flour. Even though, there are some available microscopic methods to detect adulteration of different flours, ATR-FTIR was recognized as a beneficial tool for detecting adulteration of corn flour in cassava flour. Cassava flour, Cornflour, and different percentages of spiked cassava flours (10%, 20%, 30%, 40%, 50%) were subjected to proximate in parallel with FT-IR spectroscopy. The spectroscopy results showed that Cassava flour was detected from corn flour by the nearly disappearing of specific bands at 1743.65 cm^{-1}, 1643.35 cm^{-1}, 1544.98 cm^{-1} which are present in corn flour. On increasing the adulteration, the intensity of peaks at these specific bands was kept on increasing. As FTIR is a fast and non-destructive technique, it can be employed in finding adulteration of tapioca sago.

Keywords: Corn flour, cassava flour, FTIR, adulteration

1. Introduction
Adulteration is the biggest problem facing the entire world. It affects different people in different ways i.e., Farmers and dealers in the economic aspect, consumers in the health aspect, etc. (Banti, 2020) [3] It is being observed in each commodity including "Tapioca Sago"
Tapioca sago is produced from the roots of cassava. Depending on the region, the name of this crop varies, mandioca or manioca (Brazil), cassada or cassava (Africa and Southeast Asia), tapioca (India and Malaysia), yucca (Central America) (Agbemafle, 2019) [1].
Nearly 2.7 lakh hectares area of land in India is being cultivated with cassava and the production is 71 lakh tonnes. Cassava yields an average of 22 tonnes per hectare. It has the highest starch content (25 to 35%) and is mostly processed for starch and sago. Tamil Nādu is the leading producer, processor, and exporter of cassava in India followed by Kerala, Andhra Pradesh. (Periyasamy, 2021) [14] (Chennakrishnan *et al*., 2020) [5].
A mixture of gelatinized and ungelatinized starch created by heat-moisture treatment forms the spherical pearls. To make tapioca pearls, the starch is wetted until it reaches a moisture content of 50%. Continuous mechanical shaking disintegrates the wet starch and forms spherical particles. Dry heat drying or roasting at 250-300 °C is then applied to the particles. (Immawan *et al*. 2018) [10].
To decrease the moisture content of finished products, the pearls are cooled before being exposed to a second drying phase at a lower temperature (50-80 °C).
From the newspaper's reviews, in the past five years, many complaints were raised by the farmers regarding the adulteration of cassava flour during the manufacturing of sago. Sago manufacturing industries importing cheap quality corn grits and corn flour from other countries for usage as an adulterant in sago due to its high starch content. Due to this adulteration, it is reported that nearly 50% cost per tonne of sago is being reduced which is affecting the farmers economically. There is a need to find a solution to prevent this. So far, many microscopic methods are available for the detection of adulterants in flours. In the present research, a method has been developed by using a spectroscopic method called Fourier Transform Infra-red (FTIR).
FTIR is a spectroscopic technique widely used to characterize food ingredients and to detect possible food adulterants based on their unique spectral fingerprints (Valand *et al*., 2020) [18].

It ranges in 2 different regions i.e., middle range (450 to 4000 cm^{-1}, FT-MIR) or near range (4000 cm^{-1} to 10000 cm^{-1}, FT-NIR). As of our knowledge currently, there is no systematically established relationship between FTIR spectral features on Corn and Cassava flour, despite the appeal of FTIR as a quick, non-destructive methodology. (Arslan *et al*. 2020) [2] (Nezami *et al*. 2021) [12].

2. Materials and Methods

2.1 Sample preparation

Fresh cassava root was obtained from the local market, Thanjavur. Roots were subjected to peeling followed by chopping into a small piece (5×0.5×0.2 cm) and drying in a hot air oven at 55 degrees Celsius till the moisture content reach less than 8% (Hansethsuk, 2003) [8]. Then, the dried cassava was subjected to milling followed by sieving through an 80-mesh size. Flour is stored by packing in a plastic bag. Dried corn grits were procured from the local market, subjected to milling followed by sieving through 80 mesh size, and stored in plastic bags. Tapioca sago samples of 5 types 20 were procured from local market, Tanjore, Tamil Nadu.

2.2 Proximate analysis

The moisture, proteins, carbohydrates, fats, fibre, and ash content in corn flour and cassava flour were determined by A.O.A.C (2016).

2.3 Fourier transforms infrared (FTIR) spectrometric analysis

All spectra of the selected samples were measured using ATR-FTIR ((Shimadzu IR tracer-100) spectrophotometer. All spectra were recorded in the transmittance mode. The transmission technique was applied to conduct 45 scans for each of the sample in the spectral range of 4000-400 cm^{-1}. Samples to be analysed are placed directly on the diamond crystal of ATR-FTIR.

There is minimal sample preparation since it is ATR-FTIR. Flours were kept in a hot air oven at 50 degrees Celsius until the initiation of FTIR analysis, to prevent the absorption of moisture from the atmosphere (He *et al*., 2021) [9].

2.4 FTIR measurements

FTIR spectra of corn flour, cassava flour and spiked samples (10% to 50%) were recorded.

Table 1: Assignments of FT-IR absorption bands of based on nutritional property.

Nutritional factor	Wave number (cm^{-1})	Functional group and vibrational mode
Moisture content	3000-3500	-H & -OH
Protein	1600-1700	-C=O (-Amide I)
Moisture and protein content	1200-1600	-NH bending, -CH stretching } Amide II
Fat	2500-3000 1700-1800	-C-H(CH_2) stretching C=O stretching
Ash	835-2520	-

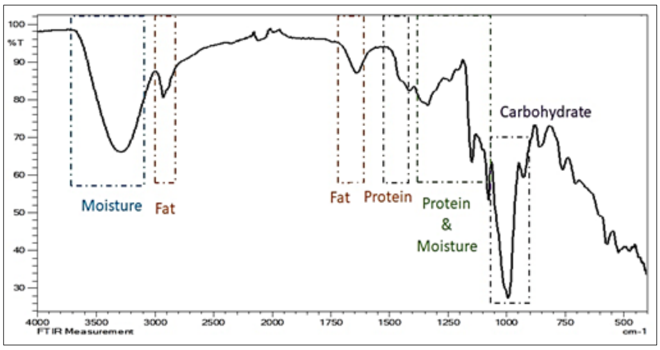

Fig 1: Graphical representation on distribution of nutrient components.

3. Results and Discussion

The proximate analysis of corn flour, cassava flour and different ratios of spiked samples were evaluated as shown in table 2. The acquired results showed that there was a significant difference in nutrient content among all the samples (Paterne *et al*., 2019) [13]. There was an increase order in moisture content, protein content, fat, ash and energy levels on increasing the spiking percentage of corn flour which is an adulterant in cassava flour i.e. corn flour > 50% > 40% > 30% > 20% > 10% > cassava flour (Chisenga *et al*., 2019) [6]. Total carbohydrate content was more in cassava when compared to corn flour and it follows the decreasing order as follows; corn flour < 50% < 40% < 30% < 20% < 10% < cassava flour. (Qamar *et al*., 2016) [15].

Table 2: Proximate composition of different spiked percentages of cassava and besan flour.

Samples	Moisture	Protein	Fat	Total carbohydrates	Ash %	Energy
Cassava	8.85 ± 0.05^{e}	1.17 ± 0.02^{f}	0.20 ± 0.01^{g}	88.34 ± 0.07^{a}	1.42 ± 0.005^{f}	359.92 ± 0.10^{d}
Corn	10.9 ± 0.1^{a}	8.31 ± 0.11^{a}	3.33 ± 0.01^{a}	74.92 ± 0.16^{g}	2.53 ± 0.01^{a}	362.91 ± 0.39^{a}
10% AD	9.15 ± 0.01^{d}	2.39 ± 0.03^{e}	0.31 ± 0.005^{f}	86.71 ± 0.01^{c}	1.43 ± 0.01^{f}	359.25 ± 0.10^{e}
20% AD	9.16 ± 0.02^{d}	2.41 ± 0.009^{e}	0.47 ± 0.01^{e}	86.42 ± 0.04^{c}	1.53 ± 0.01^{e}	359.59 ± 0.07^{de}
30% AD	9.415 ± 0.005^{c}	2.69 ± 0.03^{d}	1.225 ± 0.01^{d}	85.07 ± 0.06^{d}	1.585 ± 0.005^{d}	362.12 ± 0.03^{b}
40% AD	9.495 ± 0.005^{c}	3.57 ± 0.04^{c}	1.48 ± 0.005^{c}	83.83 ± 0.03^{e}	1.61 ± 0.005^{c}	362.98 ± 0.01^{a}
50% AD	9.88 ± 0.02^{b}	4.11 ± 0.01^{b}	1.53 ± 0.005^{b}	82.78 ± 0.04^{f}	1.68 ± 0.005^{b}	361.41 ± 0.07^{c}

Note: Values having the different letters are significantly different from each other (p< 0.05%). Each value is a mean of 3 replicates i.e. n = 3.

From FTIR analysis, difference among the pure and spiked samples has been adopted on the basis of molecularity. The major difference between corn and cassava flour lies in its protein content. From the figure 3, it is clearly visible that, there are 3 regions with wavenumber 1743.65 cm^{-1}, 1643.35 cm^{-1}, 1544.98 cm^{-1} representing Amide I (-C=O) stretching, Amide II (-NH bending and -CN stretching), Amide II stretching respectively. The peak at 1743.65 cm^{-1} is clearly present in pure corn flour and it almost gets disappeared on increasing the concentration of cassava flour. The other two peaks at 1643.35 cm^{-1}, 1544.98 cm^{-1} were observed as strong, sharp peaks in pure corn flour, whereas medium, broad peaks in cassava flour.

Since the protein content is increasing in the order corn flour > 50% > 40% > 30% > 20% > 10% > cassava flour, the intensity of the peak was increased in the same order. (Barth, 2007) [4] (De Girolamo *et al*. 2020) [7]. The next nutritional component that varies most between corn and cassava is their fat content. From the graph, it is clearly visible that the region from 2500-3000 cm^{-1} and 1700-1800 cm^{-1} which represents the fat content in the sample were also increased in intensity of peak on increasing the percentage of spiking of adulterant i.e. corn flour. (Sutsuga *et al*. 2018) [17] (Sujka *et al*. 2017) [16]. Another important region from 3000-3500 nm^{-1} which representing moisture content was also clearly explanatory from the spectrograph. Since the moisture content follows the order corn flour > 50% > 40% > 30% > 20% > 10% > cassava flour (Nesakumar *et al*. 2018) [11], the size of that band is less in cassava flour when compared with pure corn flour and its size was kept on increasing with increasing the spiked percent of corn flour which is an adulterant.

3.1 FTIR analysis on tapioca sago samples procured from local market: After analysing the procured samples from market, FTIR results showed that 3 of the 20 samples were found to be adulterated with corn because of the presence of unique peak at the wave number 1743.65 cm^{-1} and two strong, sharp peaks at 1643.35 cm^{-1} and 1544.98 cm^{-1} which are supposed to be medium and broad.

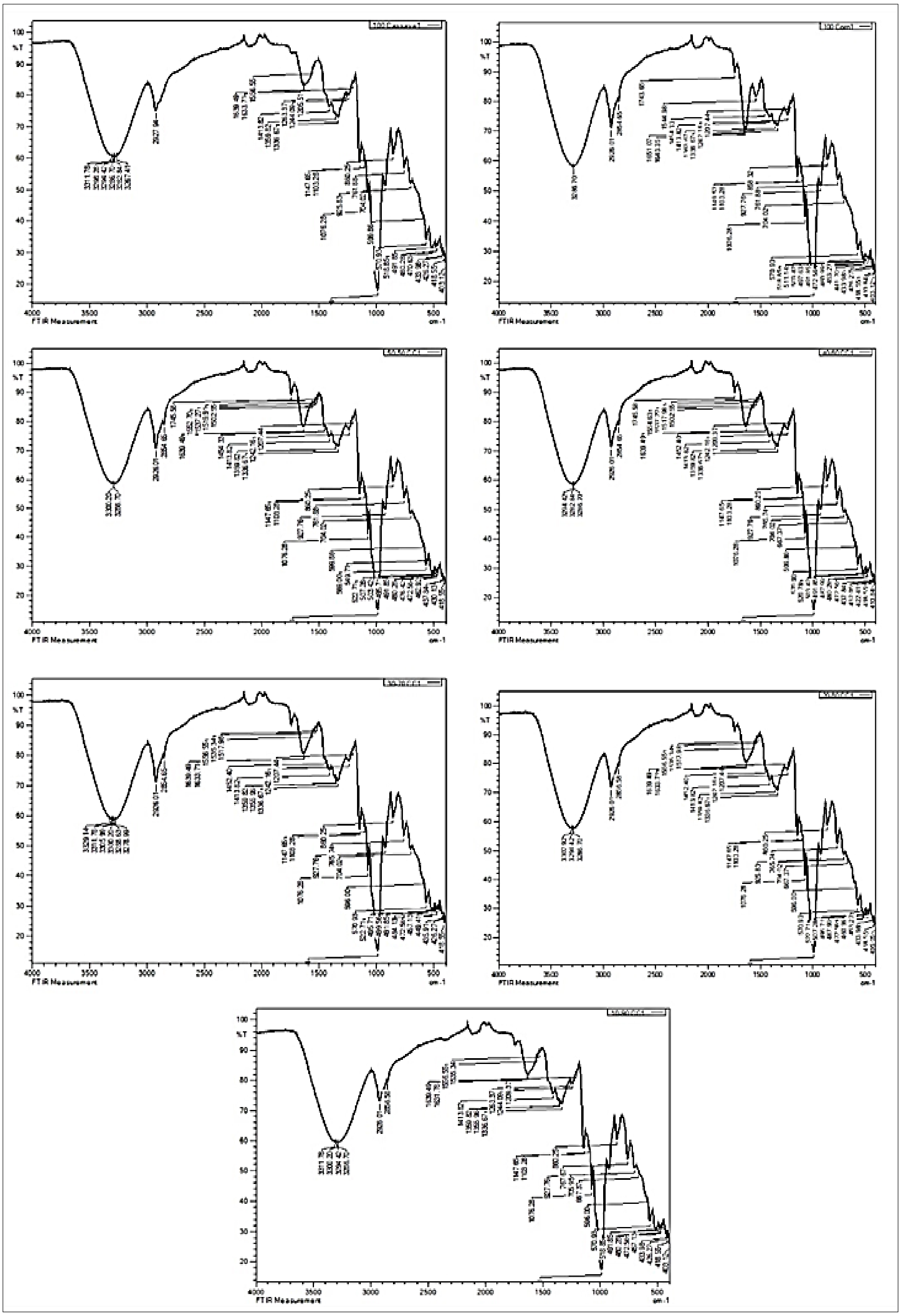

Fig 2: FTIR spectra of different spiked ratios of cassava and corn flour.

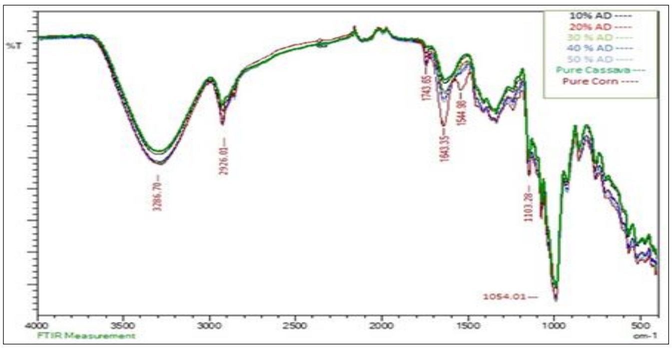

Fig 3: Overlapped infrared spectra of pure and spiked samples.

4. Conclusion

The current study clearly shows that the nutritional properties showed significant difference among corn flour, cassava flour and spiked samples. On the basis of results, it is concluded that FTIR provides a better means to visualize the chemical composition of corn, cassava and spiked samples. It is very quick, reliable and cheaper analytical technique which can be affectively be used for detection of corn flour(adulterant) in cassava flour by identifying unique spectral figure prints. It is non-destructive technique, provides precise measurement which requires no external calibration, increases speed, collecting a scan every second has minimum sample preparation. By applying chemometrics to those identified spectral fingerprints, it can be a developed method which can be able to reveal the quantity of adulterant being added.

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