

Study of Fruit Raw Material by Fourier Transform Infrared Spectroscopy

Juliya Golubtsova

State Educational Institution of Higher Education

"Kemerovo Technological Institute of Food Industry (university)"

650056, Russia, Kemerovo, Stroiteley Boulevard, 47

Abstract

Fourier transform infrared (FTIR) spectroscopy allows identifying fruit raw material. The obtained individual infrared (IR) spectra and spectral characteristics (the intensity of absorption bands and the area under the spectral absorption curve) are strictly specific for each type of raw material and are determined by its structure and chemical composition.

The investigation of the FTIR application for the identification of fruit raw material in complex food systems showed that this technique can determine the presence of raw fruit in the product, but does not identify its type.

During a series of sequential experiments, IR spectra of anhydrous fruit raw material were obtained (strawberry, raspberry, cinnamon rose, gooseberry, cherry, banana, and kiwi). The spectra result from oscillatory motion of molecules, namely from transitions between vibrational levels of the main electronic state of molecules. The analysis of the spectra shows that their pattern is strictly specific for each type of raw material. However, all types of raw material have areas of absorption bands that are similar in location, but different in intensity.

Keywords: IR spectrum, identification, fruit raw material, quality, authenticity of raw materials.

INTRODUCTION

Identification as an activity has its own structure that includes goals and objectives, objects and subjects, tools and techniques [1].

Identification has similarities and differences with other types of evaluation activities: quality assessment, quality control and certification [2]. Common characteristics are tests for determination of the actual values and the compliance with various normative documents [3]. The differences lie in the list of criteria; in the subjects that define assessment activities; in the final result [4]. The final result of the identification is conformity assessment or identification of counterfeiting [5].

The term "identification" is treated differently. The analysis of normative documents showed that the term "identification" has the following definition [6].

Identification is a determination of compliance of provided product with requirements for this product (Certification of food and food raw materials in Russia, 1996).

GOST R51293-99 provides the following definition of identification: "Identification is a determination of compliance of specific products with its sample and/or description".

The Federal Law of January 2, 2000 No. 29-FZ defines identification as "establishment of the conformity of certain food, materials and products with the requirements of normative and technical documents and information about them contained in the accompanying documents and labels".

The clearest is the definition given in the Federal Law of December 27, 2002 No. 184-FZ (edited July 23,

2013) "On technical regulation": "Identification is the process of establishing the identity of the products with its essential features".

Essential features are identification criteria designed to establish identity and/or authenticity of goods [7].

Criteria may be expressed using complex and/or individual quality indicators or characteristics [8].

Identification criteria should be selected to meet the following requirements:

- Typicality for the particular type, name or homogeneous product groups;
- Objectivity and comparability;
- Verifiability;
- The difficulty of counterfeiting [9-10].

Goals and objectives of the research: the theoretical basis, scientific development and testing of specific identification method for fruit raw material and finished dairy products based on infrared spectroscopy.

METHODS

In accordance with the purpose and objectives of the work, the research objects were: fruit raw material:

Rubus idaeus (raspberry, cultivar "Nagrada"),
Fragaria vesca (perpetual strawberry, cultivar "Berskaya rannaya")

Ribes úva-crispa (gooseberry, cultivar "Cooperator")

Prunus fruticosa (steppe cherry, cultivar "Altayskaya lastochka")

Rosa majalis Herrm (cinnamon rose)

Actinidia deliciosa (Kiwi)

Músa paradisiaca (banana, cultivar "Extra");

- model fruit mixtures;
- fruit raw material processing products: samples of jam, directly-squeezed juices, syrup;
- developed dairy product – curd mousse with fruit filling (kiwi and banana);
- independently obtained samples of DNA from the samples of investigated fruit raw material, products of its processing and finished milk products (curd mousse with fruit filling).

Samples were taken according to requirements of GOST 26313-2014 "Products of processing of fruits and vegetables. Acceptance rules, methods of sampling", based on the rules of fruit examination.

The assessment of quality and authenticity of the fruit raw material was carried out using FTIR spectroscopy. The infrared spectrophotometer IRPrestige-21 (Shimadzu) with FTIR Silver Gate module and IRsolution software was used. The software includes modules for collecting and processing of data, quantitative analysis, generating of own spectra libraries, identification of compounds using own and standard spectra libraries, converting of formats of spectral files, processing of microscopic images, as well as a bibliography on IR-spectroscopy. Spectra of samples of fruit raw material were captured in the range of 600-4000 cm^{-1} , slot width 4 cm^{-1} , magnification 1, number of scans 40.

RESULTS AND DISCUSSION

During a series of sequential experiments, IR spectra of the anhydrous fruit raw material were obtained (strawberry, raspberry, cinnamon rose, gooseberry, cherry, banana, and kiwi). The spectra result from oscillatory motion of molecules, namely from transitions between vibrational levels of the main electronic state of molecules. The analysis of the spectra shows that their pattern is strictly specific for each type of raw material (Figs. 1-7, Table 1). However, all types of raw material have areas of absorption bands that are similar in location, but different in intensity.

The frequency range of 3800-2600 cm^{-1} usually contains valence fluctuation frequencies of OH-groups included in inter- and intramolecular hydrogen bonds, as well as CH_2 and CH_3 groups. The frequency range of 1800-1200 cm^{-1} mainly shows characteristic frequencies of the valence vibrations of -C=O and -C=C-, deformation vibrations of methyl and methylene groups as well as OH-groups.

Maximum absorption peaks are identified in the frequency range of 1100-1000 cm^{-1} for all types of fruit raw material. These peaks can be attributed to fluctuations associated with the C-O-H group in some phenolic compounds (for example, primary and secondary alcohols), which are present in large numbers in berries and fruits. Phenolic compounds are one of the many classes of secondary compounds that determine the biological value

of plants. The presence of phenolic compounds gives absorption bands caused by stretching vibrations of the free OH groups (frequency 3670-3580 cm^{-1}), intra- and intermolecular H-bonds in dimers and polymers (frequency 3400-3200 cm^{-1}) and fluctuations associated with C-O-H group: R-O-H (frequencies 1450-1250, 750-650 cm^{-1}), primary alcohols (frequencies 1075-1000; 1350-1260 cm^{-1}), secondary alcohols (frequencies 1125-1030; 1350-1260 cm^{-1}), tertiary alcohols (frequencies 1170-1100; 1410-1310 cm^{-1}), phenols (frequencies 1270-1140; 1410-1310 cm^{-1}); vibrations of carboxylic acid groups: valence vibrations of COOH groups (frequencies 1760; 1725-1700 cm^{-1}), free OH-groups (frequencies 3350-3500 cm^{-1}), bound OH-groups (frequencies 3300-2500 cm^{-1}), any OH-groups (frequencies 995-890 cm^{-1}), vibrations of C-O bonds (frequencies 1320-1210 cm^{-1}); vibrations of C-O-C groups in ethers of aromatic acids (frequency 1300-1250 cm^{-1}). The presence of carbohydrates is demonstrated by absorption bands caused by the valence vibrations of CH_2 -groups at frequency $\sim 2930 \text{ cm}^{-1}$. It should be noted that the description of intensity of the IR spectra bands requires analysis of absorption maximum intensity and integral intensity (area under the spectral absorption curve). The analysis of spectra shows that almost all kinds of investigated fruit raw material have absorption bands with various intensity in the specified frequency ranges.

The study of the obtained IR spectra of the fruit raw material has demonstrated that each type of raw material has different patterns, intensity of absorption bands and the area under spectral absorption curve. This is probably due to the characteristics of the chemical composition of the raw materials (Table 1). The highest intensity of the absorption maximum belongs to the cinnamon rose in the following frequency ranges: 3526.03; 2500...2774; 1709.97 and 3174.0 cm^{-1} , and is respectively equal to 28.29; 77.41...75.06; 59.02 and 57.31 rel. u.; however, the integral intensity equals to 8.94; 8.04...7.32; 15.78 and 21.17 rel. u. Cherry and kiwi fruits have average intensity of the bands in the absorption maximum (21.33...38.42 rel. u.), in the frequency ranges: 2218.23; 3212.58; 3311.92; 3507.70 cm^{-1} (for cherry) and 3113.24; 3470.09; 3625.37; 3879.98; 3933.99 cm^{-1} (for kiwi). There are also average values of the integral intensity (in the range 10.04...48.84 rel. u., except for the absorption frequency 3507.7 cm^{-1} for cherry) (Table 1). Raspberry and banana have very interesting IR spectrum: raspberry has the strongest absorption bands at frequencies 1027.14; 1094.65; 1333.83; 1724.44; 1220; 1437.03; 2806.55; 3061.16 cm^{-1} (integral intensities are 3508.17...473.69 rel. u.), medium absorption bands at frequencies 1532.51; 1827.63; 1992.55; 3737.64; 3875.16 cm^{-1} (integral intensities lie within 392.6...303.27 rel. u.). The weakest absorption band is located at 2263.56 cm^{-1} (integral intensity equals to 26.29 rel. u.).

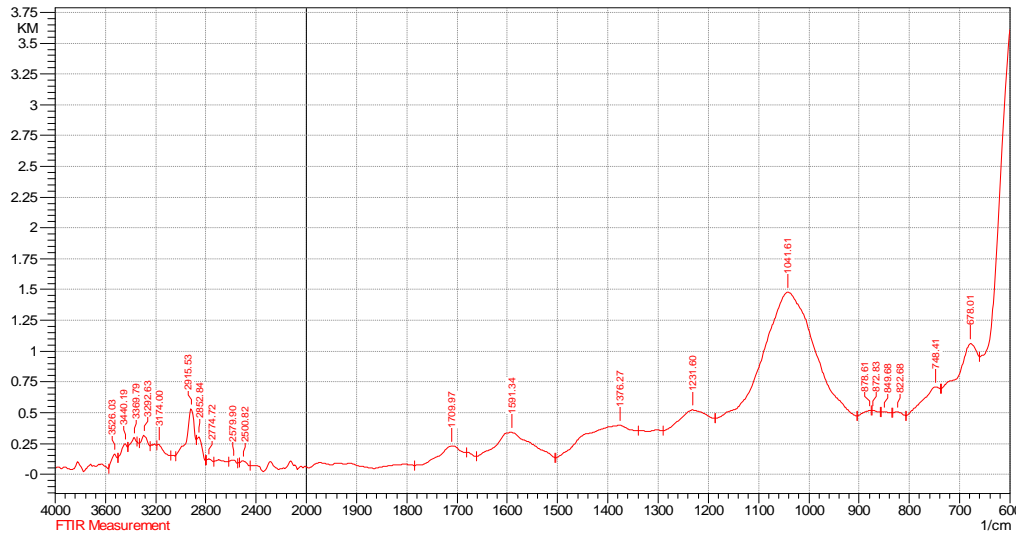


Figure 1. IR spectrum of cinnamon rose (anhydrous)

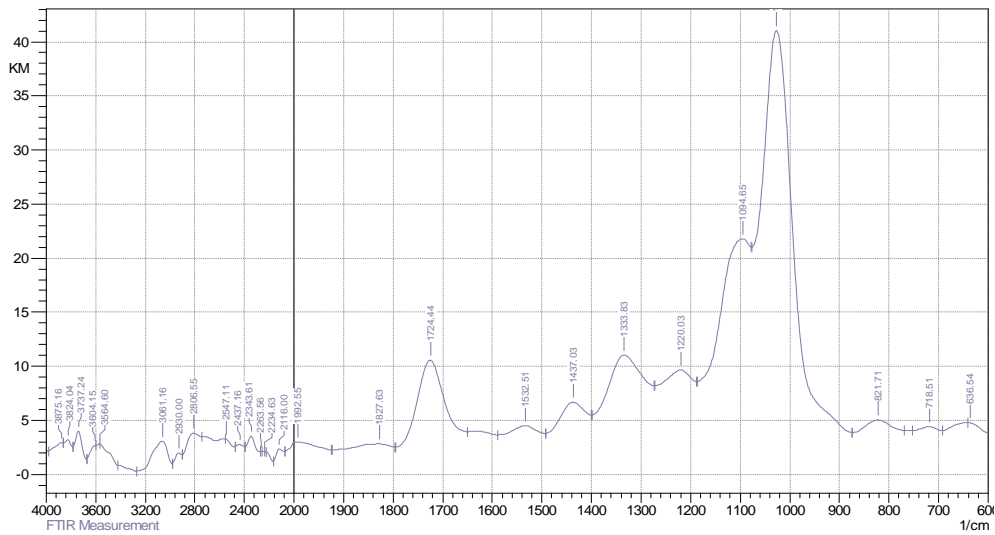


Figure 2. IR spectrum of raspberry (anhydrous)

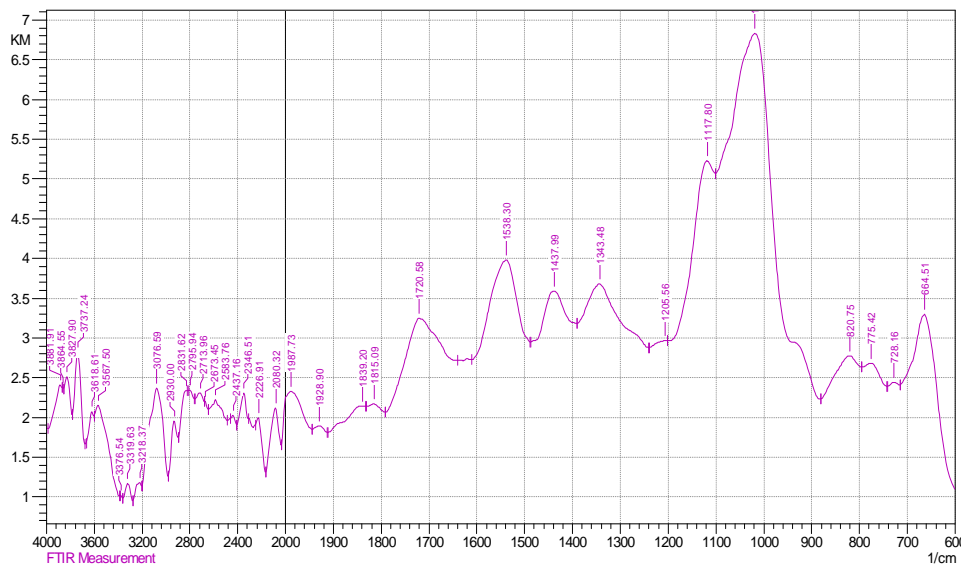


Figure 3. IR spectrum of perpetual strawberry (anhydrous)

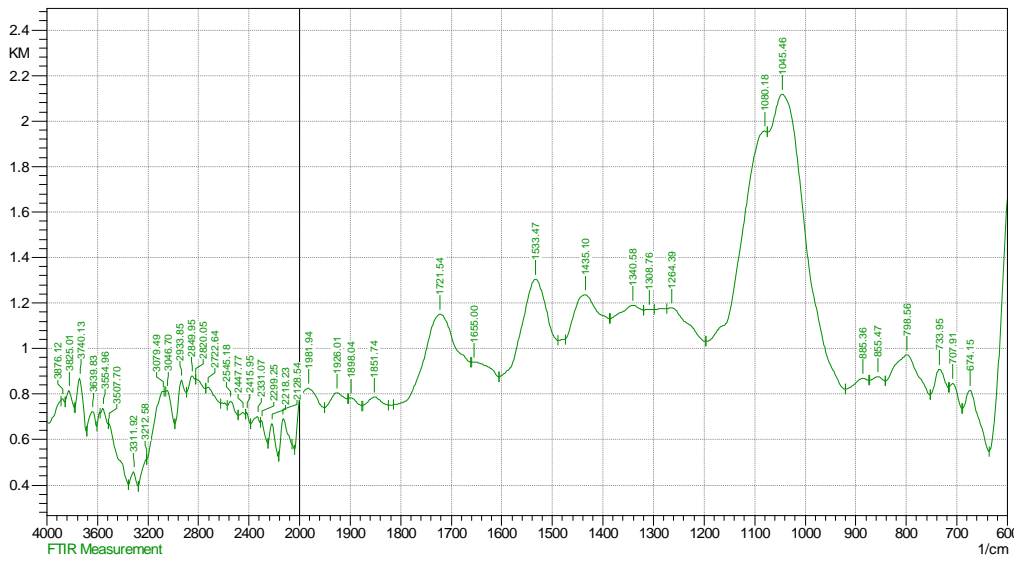


Figure 4. IR spectrum of cherry (anhydrous)

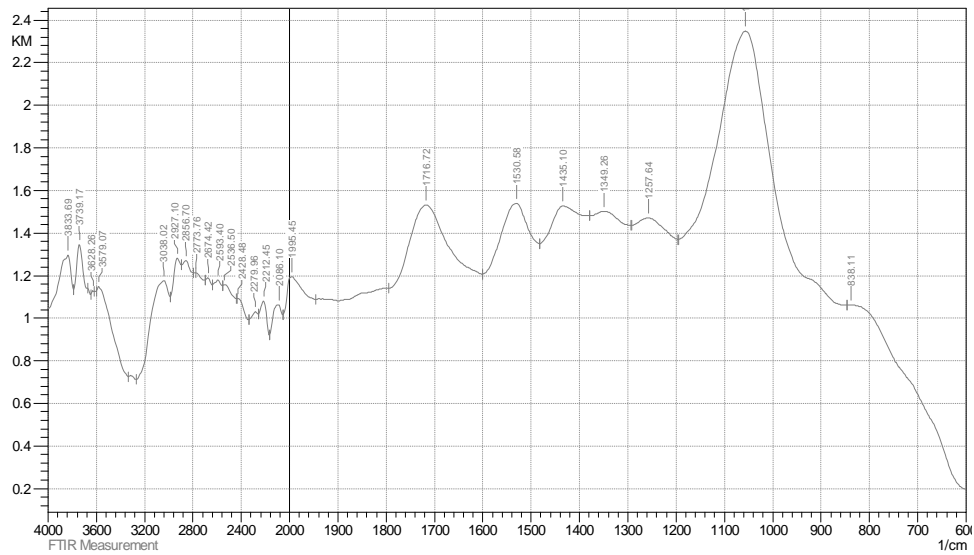


Figure 5. IR spectrum of gooseberry (anhydrous)

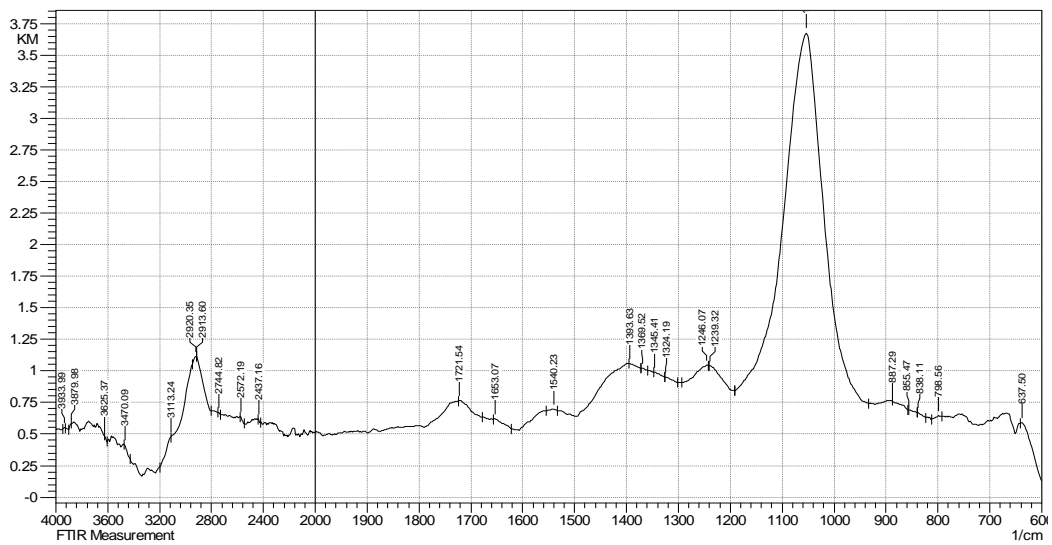


Figure 6. IR spectrum of kiwi (anhydrous)

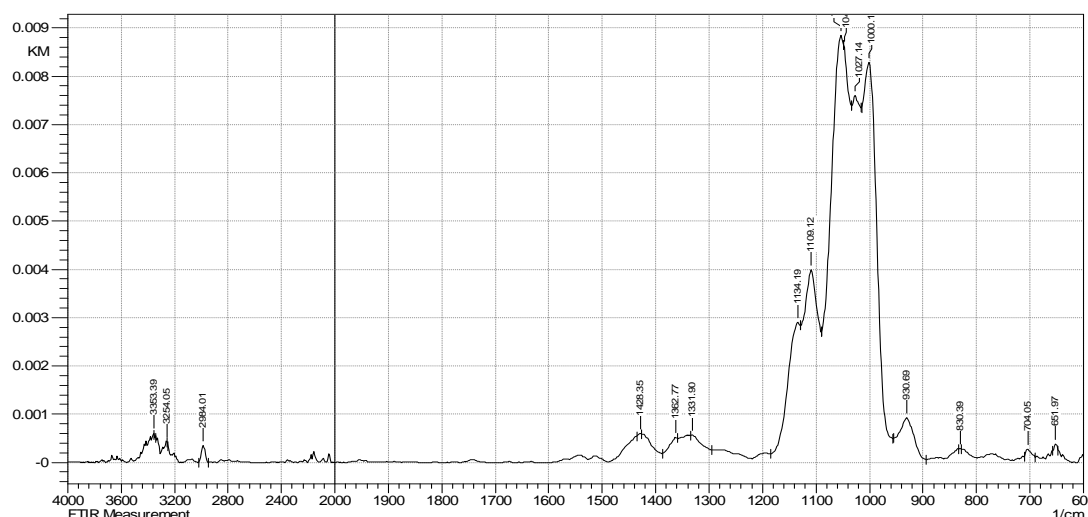


Figure 7. IR spectrum of banana (anhydrous)

Banana fruit has pronounced absorption bands; the intensity of the bands is more than 98 rel. u., while their integral characteristics are very low and do not exceed 0.25 rel. u. The absorption spectra of perpetual strawberry and gooseberry are characterized by low intensity bands. For

example, perpetual strawberry has band intensity in the range 6.59...9.53 rel. u. at 3218.37; 3319.63 and 3376.54 cm^{-1} ; integral intensity corresponds to the values 84.69...21.34 rel. u. (see Table 1).

Table 1. Some characteristics of the IR spectra of the investigated fruit raw material

Peak (frequency, cm^{-1})	Intensity of the absorption maximum (rel. u.)	Cumulative intensity (rel. u.)
Cinnamon rose (<i>Rosa majalis</i> Herrm)		
678.01	8.70725	66.430305
748.41	19.553586	41.569887
822.68	31.269642	12.895553
849.68	31.276178	11.143053
872.83	30.476614	8.859773
878.61	30.445458	14.010134
1041.61	3.340204	246.174615
1231.6	30.078721	47.689113
1376.27	39.932895	52.124933
1591.34	45.560376	37.242061
1709.97	59.019337	15.786634
2500.82	77.411696	8.041274
2579.9	76.311542	7.578493
2774.72	75.05806	7.32589
2852.84	49.797945	16.740072
2915.53	29.469227	51.134625
3174	57.311709	21.17245
3292.63	48.596357	24.563209
3369.79	50.349324	19.446385
3440.19	56.502771	15.412241
3526.03	68.292372	8.935276
	8.70725	66.430305
Raspberry (<i>Rubus idaeus</i> L.)		
636.54	0.001755	176.037953
718.51	0.003838	256.783837
821.71	0.00093	466.624305
1027.14	0.000000	3508.173291
1094.65	0.000000	1757.549117
1220.03	0.000000	753.885052

Peak (frequency, cm ⁻¹)	Intensity of the absorption maximum (rel. u.)	Cumulative intensity (rel. u.)
1333.83	0.000000	1107.387941
1437.03	0.000021	514.248622
1532.51	0.003166	392.630767
1724.44	0.000000	889.157835
1827.63	0.153933	328.418372
1992.55	0.097929	384.713817
2116	0.455442	182.490144
2234.63	0.798557	28.243824
2263.56	0.795464	26.293172
2343.61	0.029493	339.46726
2437.16	0.169092	219.214011
2547.11	0.054508	226.780974
2806.55	0.015035	506.966364
2930.00	1.006452	130.539651
3061.16	0.087926	473.698345
3564.6	0.146191	280.551487
3604.15	0.213834	155.429608
3737.24	0.009848	316.490875
3824.04	0.058539	228.177955
3875.16	0.120019	303.275353
Perpetual strawberry (<i>Fragaria</i>)		
664.51	0.050472	275.802574
728.16	0.360644	63.076563
775.42	0.208121	134.491493
820.75	0.167394	219.158593
1018.46	0.000015	1007.206006
1117.8	0.000591	406.698339
1205.56	0.107612	110.477831
1343.48	0.020955	491.266418
1437.99	0.025917	317.210528
1538.3	0.010464	414.667935
1720.58	0.056727	422.559982
1815.09	0.675873	84.350482
1839.2	0.715029	157.999814
1928.9	1.266267	57.61019
1987.73	0.470067	182.805719
2080.32	0.762125	234.345834
2226.91	0.995866	153.647687
2346.51	0.489795	205.860463
2437.16	0.948048	107.192716
2583.76	0.599226	326.794492
2673.45	0.623177	70.839642
2713.96	0.487365	175.079339
2795.94	0.450601	106.405662
2831.62	0.458505	153.116912
2930.00	1.105922	141.373909
3076.59	0.425844	408.976702
3218.37	6.597704	84.692086
3319.63	6.835605	88.287024
3376.54	9.533498	21.348328
3567.5	0.698718	352.952646
3618.61	0.833006	127.59061
3737.24	0.151446	245.226499
3827.9	0.306059	165.98451
3864.55	0.413738	32.083658

Peak (frequency, cm ⁻¹)	Intensity of the absorption maximum (rel. u.)	Cumulative intensity (rel. u.)
3881.91	0.389888	244.379929
Cherry (<i>Prunus fruticosa</i>)		
674.15	15.23591	36.922586
707.91	14.255737	21.003165
733.95	12.304544	30.779022
798.56	10.623038	79.320839
855.47	13.271715	26.790605
885.36	13.478714	39.366906
1045.46	0.762292	228.312007
1080.18	1.108837	172.188486
1264.39	6.62326	85.9341
1308.76	6.705179	24.870215
1340.58	6.438809	76.529669
1435.1	5.810805	102.396932
1533.47	4.964656	126.755943
1655	11.452609	49.677278
1721.54	7.08574	146.370365
1851.74	16.353585	39.27974
1898.04	16.484892	20.759512
1926.01	15.616704	35.509859
1981.94	14.969599	63.574386
2128.54	20.305006	67.031604
2218.23	21.335506	48.841734
2299.25	20.667005	39.99559
2331.07	19.834402	51.735909
2415.95	19.132696	27.632115
2447.77	19.035326	39.900053
2545.18	17.066098	62.389163
2722.64	14.777417	97.117083
2820.05	13.575034	66.252737
2849.95	13.169587	59.359746
2933.85	13.736922	71.81248
3046.7	15.243389	57.828958
3079.49	15.449822	90.36821
3212.58	30.694178	30.538254
3311.92	34.820961	33.067612
3507.7	21.278397	85.746919
3554.96	18.269799	43.179297
3639.83	18.919357	55.587979
3740.13	13.527	72.013428
3825.01	15.305926	59.725735
3876.12	16.554157	25.384366
Gooseberry (<i>Ribes úva-crispa</i>)		
838.11	8.687807	170.836216
1056.07	0.448805	551.716197
1257.64	3.386452	138.277246
1349.26	3.146635	125.22517
1435.1	2.963566	153.109899
1530.58	2.890646	164.461394
1716.72	2.93753	257.814458
1995.45	6.401311	118.945805
2086.1	8.63838	113.390294
2212.45	8.316844	93.229222
2279.96	9.305865	82.250958
2428.48	8.084668	98.190669

Peak (frequency, cm ⁻¹)	Intensity of the absorption maximum (rel. u.)	Cumulative intensity (rel. u.)
2536.5	6.950188	127.494984
2593.4	6.642593	95.618671
2674.42	6.469106	73.815377
2773.76	6.123389	91.050625
2856.7	5.360269	123.73107
2927.1	5.234291	111.768694
3038.02	6.665447	281.187965
3579.07	7.078951	254.466302
3628.26	7.392008	32.530474
3739.17	4.519203	140.187262
3833.69	5.058294	249.646592
Kiwi (<i>Actinidia deliciosa</i>)		
637.50	25.616102	15.797947
798.56	22.578802	12.241779
838.11	21.252347	10.658566
855.47	20.315285	11.182283
887.29	17.217013	22.560465
1054.14	0.020951	455.837433
1239.32	9.096275	46.238106
1246.07	9.021328	49.845238
1324.19	11.16027	22.45795
1345.41	10.172098	20.614504
1369.52	9.513237	12.707699
1393.63	8.731766	24.104778
1540.23	20.149664	15.329927
1653.07	23.842419	19.762447
1721.54	17.073755	32.933268
2437.16	23.913096	12.339752
2572.19	22.966567	20.160195
2744.82	21.206715	13.488541
2913.6	7.635589	99.901455
2920.35	7.625386	29.565103
3113.24	33.442463	30.802164
3470.09	38.423903	15.264697
3625.37	32.331937	10.80119
3879.98	26.013082	11.43776
3933.99	27.845552	10.042186
Banana (<i>Musa paradisiaca</i>)		
651.97	99.910762	0.003145
704.05	99.935339	0.004209
830.39	99.93229	0.001642
930.69	99.787323	0.031725
1000.13	98.109622	0.255105
1027.14	98.263871	0.129713
1047.39	98.030174	0.114425
1053.18	97.984255	0.242761
1109.12	99.084303	0.12885
1134.19	99.333919	0.079639
1331.9	99.869487	0.016032
1362.77	99.881373	0.009758
1428.35	99.861632	0.005079
2984.01	99.917728	0.011936
3254.05	99.901845	0.003391
3353.39	99.859867	0.005923

Table 2. Comparative evaluation of organoleptic, physical and chemical methods for identification of fruit raw material type

Methods	Measurable indicator	Effectiveness of type authentication
Organoleptic:	The appearance, taste, smell,color, consistency	Effective for identification of the type of raw material (non-recycled, fresh fruits and berries)
Physical and chemical:		
Titrimetric, photometric, fluorometric	Ascorbic acid content	Effective for identification of fruit groups used for preparation of juice or drink (pomaceous, citrus, grape), but cannot identify specific type of raw materials.
Ffluorometric method,high-performance liquid chromatography	Determination of vitamins B ₁ , B ₂ , B ₆ , A, E, K	Is not effective for determination of plant material type
Thermogravimetric technique	Dry substance content	Is not effective for determination of plant material type
Atomic absorption (1), molybdenum-vanadium (2), complexometric (3), titrimetric (4), fluorometric (5)	The content of mineral substances: sodium, potassium, calcium, magnesium, iron, manganese, copper, zinc, lead, cadmium, cobalt, nickel, chromium (1), phosphorus (2), calcium, magnesium (3), iodine (4), selenium (5).	Is not effective for determination of plant material type
Reversed-phase high-performance liquid chromatography	Organic acid content	Allows detection of the fruit or berry raw materials in products of their processing, but does not allow determination of raw material type in food
Spectrometric	Determination of carotenoid content	Allows identification of fruit or berry raw materials containing carotenoids in foods, but cannot determine the type of raw material
Potentiometric analysis	Titrateable acidity	Is not effective for determination of plant material type
Infrared spectrometry	IR spectra of fruit raw material	Effective for identification of fruit raw material and its presence in the product, but cannot determine the type of raw material in processed form and in food

IR spectroscopy is widely used for evaluation of authenticity and quality of raw materials, in particular the pollution of raw materials with technological elements (Ilyashenko *et al.*, 2012; Ilyashenko, 2011; Shukurov *et al.*, 2007a; Shukurov *et al.*, 2007b; Shukurov *et al.*, 2008); during identification of the components of plant material, in particular trioglycosides (Syedin *et al.*, 2014).

Our studies have shown that the FTIR technique allows obtaining individual IR spectra of fruit raw material. It was experimentally determined that the pattern of IR spectrum and spectral characteristics such as the intensity of absorption bands and the area under the spectral absorption curve are strictly specific for each type of raw material and allow identification of raw material type after the introduction of the standard sample spectrum into device library.

There is still the question about the application of this technique for identification of plant material in multicomponent food systems.

We attempted to identify types of fruit raw material in the multicomponent food system. The obtained IR spectra of the fruit raw material have been introduced into the database of the IR spectrometer. In order to explore the possibility of identification of fruit raw material type in a complex multicomponent system we prepared a model food system based on curd mousse "Activia" with the following composition: low-fat curd

cheese, skim milk, cream, milk protein concentrate, gelatine, yoghurt culture, bifidobacteria ActiRegularis (at least 1×10^7 CFU/g), 4.5% of fat, the manufacturer: Danon Industry LLC. The fruit raw material was crushed in a blender and then introduced into curd yoghurt product in the quantities of 5 wt% (for both one type of fruit raw material and their mixtures).

We used the curd mousse "Activia" with the following composition: low-fat curd cheese, skim milk, cream, milk protein concentrate, gelatine, yoghurt culture, bifidobacteria ActiRegularis (at least 1×10^7 CFU/g), 4.5% of fat, the manufacturer: Danon Industry LLC. The amount of fruit filler in yogurt, both separately and as a part of the fruit mixture, was 5 wt%.

The results show that using the large set of organic material in the library of IR spectrometer this method allows identification of fruit raw material in the food system, but it cannot identify the type of fruit raw material.

The software package IRsolution has chosen fruit raw material spectra with a probability of 724-765 of 1000 (positions 1-8) from its own library. However, it has identified cinnamon rose as core type instead of kiwi (selected position No. 1 in the library list of IR spectra).

When analyzing the yogurt with fruit mixture, we could not identify the type of raw fruit material (raspberry) using IR spectra libraries.

The software package has also identified fruit raw material with a probability of 785-702 of 1000 (positions 1-8), but it identified gooseberry instead of raspberry (the highlighted position No. 1 in the library list of IR spectra).

The literature sources contain practically no information about the possibility of using IR spectroscopy for assessment of the authenticity of plant material in the multicomponent food systems. There is work by I.A. Avilova and D.V. Hlystov (2014) on the possibility of using IR spectroscopy for determination of the quality of vegetable oils, authentication and identification of the manufacturer and control of technological process.

The assessment of the feasibility of the proven organoleptic, physical and chemical methods for identification of the type of raw and processed fruit material are shown in Table 2.

CONCLUSION

Thus, the FTIR spectroscopy allows identification of the fruit raw material. The obtained individual IR spectra and spectral characteristics (intensity of absorption bands and the area under the spectral absorption curve) are strictly specific for each type of raw material and are determined by its structure and chemical composition.

The investigation of the FTIR application for the identification of fruit raw material in complex food systems showed that this technique can determine the presence of raw fruit in the product, but does not identify its type.

REFERENCES

1. Spooner, D.M., Peralta, I.E., & Knapp, S. (2005). Comparison of AFLPs with Other Markers for Phylogenetic Inference in Wild Tomatoes [*Solanum L. Section Lycopersicon (Mill.) Wettst.*]. *Taxon*, 54(1), 43-61.
2. Subacius, S.M.R., & Bussab, W.O. (1998). Purine and Pyrimidine Composition in 5S rRNA and Its Mutational Significance. *Genetics and Molecular Biology*, 21(2), 255-258.
3. Tam, S.M., Mhiri, C., Vogelaar, A., Kerkveld, M., Pearce, S.R., & Grandbastien, M.A. (2005). Comparative Analyses of Genetic Diversities within Tomato and Pepper Collections Detected by Retrotransposon-Based SSAP, AFLP and SSR. *Theoretical and Applied Genetics*, 110(5), 819-831.
4. Thompson, J.D., Cuddy, K.K., Haines, D.S., & Gillespie, D. (1990). Extraction of Cellular DNA from Crude Cell Lysate with Glass. *Nucleic Acids Research*, 18(4), 1074.
5. Vazquez-Marrufo, G., Vazquez-Garciduenas, M., Gomez-Luna, B.E., & Olalde-Portugal, V. (2002). DNA Isolation from Forest Soil Suitable for PCR Assays of Fungal and Plant rRNA Genes. *Plant Molecular Biology Reporter*, 20, 379-390.
6. Vogelstein, B., & Gillespie, D. (1979). Preparative and Analytical Purification of DNA from Agarose. *Proceedings of the National Academy of Sciences*, 76(2), 615-619.
7. Watts, V.A., Butzke, C., & Boulton, R.B. (2003). Study of Aged Cognac Using Solid-Phase Microextraction and Partial Least-Squares Regression. *Journal of Agricultural and Food Chemistry*, 51(26), 7738-7742.
8. Xu, M.A., & Korban, S.S. (2002). A Cluster of Four Receptor-Like Genes Resides in the Vf Locus That Confers Resistance to Apple Scab Disease. *Genetics*, 162(4), 1995-2006.
9. Zhao, Y., Xu, Y., Li, J., Fan, W., & Jiang, W. (2009). Profile of Volatile Compounds in 11 Brandies by Headspace Solid-Phase Microextraction Followed by Gas Chromatography-Mass Spectrometry. *Journal of Food Science*, 74, 90-99.
10. Zhang, J., & Stewart, J.M. (2000). Economical and Rapid Method for Extracting Cotton Genomic DNA. *Journal of Cotton Science*, 4, 193-201.