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DETERMINATION OF FUNCTIONAL GROUPS AND NUTRIENTS IN A LOCAL READY- TO -USE SUPPLEMENTARY FOOD BY FOURIER TRANSFORM INFRARED SPECTROSCOPY

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Abstract

Development of new functional foods must ensure presence of adequate nutrients to mitigate acute malnutrition. This study consists of an innovative approach for simultaneous detection of chemical bonds and organic content such as carbohydrates, fibres, lipids, and proteins of a local Ready-to-Use Supplementary Food (RUSF) by Fourier transform infrared (FTIR) spectroscopy and proximate analysis techniques. The RUSF was prepared by mixing peanut butter with soy bean oil into thin slurry. Icing sugar, baobab powder, extruded sorghum powder, and extruded soy meal powder were added into the peanut butter- soy bean oil slurry and thoroughly blended a the peanut butter making machine until a brown thick RUSF paste was produced. The RUSF was thoroughly mixed and a sample enough to cover the diamond crystal on the FTIR was placed onto the diamond crystal after zeroing the machine by scanning the air at mid-infrared region (4000-400 cm^{-1}). Obtained experimental FTIR wave numbers and literature-based wave ranges were used to assign chemical bonds and identify nutrients in the local RUSF. The proximate analysis fat content by Analysis of Association of Official Analytical Chemists (AOAC) Soxhlet method (AOAC, 2016), protein content using the VELPA SCIENTIFICA automatic distillation and titration system (Model UDK159), carbohydrate content through the carbohydrate by difference method (AOAC, 2004), and moisture by Adams analyser. FTIR analysis detected various different functional groups like amine groups, quinones, alcohols, aliphatic amines, alkanes, alkenes, alkyl halides, carboxylic acids, esters, ethers, ketones, peroxides, nitro compounds, phenols, and triglycerides. Carbohydrates, fibres, lipids, proteins, and water were detected by both FTIR and proximate analysis. This research underscored the potential of FTIR spectroscopy and proximate analysis as tools for rapid assessment and identification of nutrients in food science. This study concluded that the RUSF contained nutrients that could reduce and manage malnutrition and non-communicable diseases.

Key words: Fourier Transform Infrared, mid infrared spectroscopy, functional group$_3$, moderate acute malnutrition, Ready-to-Use Supplementary Food

Introduction

Ready-to-Use Supplementary Food (RUSF) refers to large-quantity lipid-based nutrient supplements for treatment of moderate acute malnutrition. RUSFs are fortified with micronutrients. They contain essential fatty acids and quality protein to ensure that a child's nutritional needs are met. RUSFs are advantageous as they do not require additional water or fuel to cook the product and they have low microbial count and longer shelf life. These products are available in different packaging options such as porches, sachets, and plastic jars with lids. These products include Plumpy Sup, eeZee RUSF, Nutributter, Plumpy Doz, Plumpy Doz- corn formula, Plumpy Up, Plumpy Soy, Growell Child, Gowell Mum, Plumpy Sup, Plumpy Sup corn formula, Plumpy Mum, and eeZee cup (Nutriset, 2014). RUSFs are supposed to be modifiable, low cost, palatable, safe, and nutrient dense for prevention or treatment of acute malnutrition (Manary, 2006). RUSFs consist of peanut butter, milk powder, vegetable oil, sugar, vitamins, and minerals. RUSFs contain all the energy and nutrients required to facilitate rapid catch-up growth especially in treatment of children from 6 months to 23 months with moderate acute malnutrition, appetite, and without medical complications. Some of the commercially available products are packaged in individual sachets that provide 500 kcal per sachet. It is used as is without any other processing. After being opened, the sachet can be used throughout the day.

Many local RUSFs were developed in the world using local nutrient dense ingredients such as legumes, fruits, and vegetables, which contain lots of carbohydrates, fats, and fatty acids, proteins, minerals, phytochemicals, and vitamins. It is necessary to analyse such RUSFs to determine if all these organic compounds still exist in the RUSF matrix. Zimbabwe has plenty of nutrient dense and medicinal plants which could be utilised as valuable ingredients for production of several traditional and modern functional foods.

Fourier transform infrared (FTIR) spectroscopy is an essential analytical technique for characterisation of samples in the forms of fibres, films, gases, liquids, pastes, powders, and solutions (Nandiyanto *et al*, 2019). FTIR is an essential tool for simultaneous determination of organic components, including chemical bonds, as well as organic content such as carbohydrates, lipids, and proteins (Nandiyanto *et al*., 2019).

During infrared spectroscopy, infrared radiation is passed through a sample. Some of the infrared radiation is absorbed by the sample while some of it is passed through (transmitted). The outcome is a spectrum that represents the molecular absorption and transmission that creates a fingerprint of the sample. Each sample has unique molecular structure such that two molecular structures with unique combination of atoms do not produce the exact same infrared spectrum. An infrared spectrum represents a unique fingerprint of a sample with absorption peaks corresponding to the frequencies of vibrations between the bonds of the atoms making up the material. The FTIR spectrum can be obtained by the result of absorption versus wave number or transmission versus wave number data. The Infrared spectrum is divided into three wave regions, namely, the far-IR spectrum (<400cm-1), the mid- IR spectrum (400-4000cm^{-1}), and the near-IR spectrum (4000-13000cm^{-1}) but the mid-IR spectrum is used most frequently in sample analysis (Nandiyanto *et al.,* 2019). FTIR can identify unknown materials, determine the quality and consistency of a sample and can determine the quantity of components in a mixture (Nurwahidah *et al*., 2019). Fourier transform infrared spectroscopy – attenuated total reflectance (FTIR–ATR) analysis allows for the identification of the position, intensity, and shape of infrared peaks,[17] which can reveal chemical bonds and quantify chemical structures of macromolecules such as carbohydrates, lipids, fiber, moisture, bioactive compounds, antinutrients, and other compounds of interest. FTIR can provide compositional information in terms of carbohydrates, fat, moisture, and protein content in foods (van de Voort, 1992). Identification of molecules and nutrients in unknown substances is supported by Messerschmidt and Harthcock (1988), who stated that over the last 100-plus years a great number of infrared spectra have been measured, and the peak positions of known molecules derived from these spectra can be used to identify the molecules in an unknown sample.

Despite the widespread use of FTIR in Food Science researches, there's a pronounced research gap concerning its application in detecting nutrition composition of foods. Conventional methods, such as proximate analysis, although accurate, require longer time and more labor that makes them less feasible for large-scale or rapid analyses. Such limitations necessitate the urgency need for innovative, efficient techniques that can offer both accuracy and speed (Sirega *et al.,* 2024). This study aims to devise a rapid and reliable method for detecting local RUSF's proximate contents using Fourier Transform Infrared (FTIR) spectroscopy integrated with proximate analysis.

There are plenty of molecular fragments considered to be functional groups attached to organic structures or backbones, for example, -C-X, i.e., the halogens (X=F, CL, Br, and I), hydroxy (X=OH), oxy or ether (X=OR, where R = alkyl), and amino (X=NH2, =NH or ΞN). With the exception of carbonyl functionality, these three basic functional groups cover most of the common occurrences in simple organic compounds (Coates, 2000). The infrared spectrum is effective in diagnostics which makes it possible to differentiate functional group structures for primary, secondary, and tertiary aromatic amines (Coates, 2000). Carbonyl compounds are chemically important and essential in the interpretation of the entire spectrum as they define two related families of organic compounds called aldehydes ad ketones

The C=O absorption is one of the most characteristic in the entire spectrum. In essence, the ketone is considered the root compound, with the aldehyde being a unique case where the carbonyl group is terminal and only has one substituent, the other being a single hydrogen atom. All other carbonyl compounds are derived from the base ketone structure where one or both alkyl (or aryl) substituents are replaced by another functionality, e.g., from a single hydroxy group like in carboxylic acids, to two ether groups, as in the case of an organic carbonate (Coates, 2000). Thiols and thio-substituted compounds can be diagnosed by FTIR. Thiols and thio-substituted compounds are direct analogs of the equivalent oxygenated compounds such as alcohols and ethers (Coates, 2000).

The objectives of this study included the determination of the chemical bonds and functional groups in the local RUSF by FTIR spectroscopy and proximate analysis in order to detect and identify nutrients in the local RUSF. There was need to determine the proximate profile of RUSF by detecting key nutrients such as carbohydrates, fats, fibre, moisture, and proteins through their characteristic spectral signatures.

According to our knowledge, this is the first systematic diagnosis and collection of typical unique frequencies for a local RUSF manufactured from local Zimbabwean ingredients. The analysis results were compared with official published literatures to assign chemical bonds, absorption frequencies, functional groups, and identify organic nutrients in the local RUSF. The nutrients were found to be suitable for managing and curing acute malnutrition in humans.

Materials and methods

A local RUSF was developed from local Zimbabwean ingredients and analysed for presence of carbohydrates, fibre, fats, moisture, and proteins by FTIR and proximate analysis techniques. Soy beans were sampled by the snowball method and bought from farmers in Mazowe district, Mashonaland Central in Zimbabwe and extruded using a single screw extruder in Harare. The soy beans extrudates were ground into fine soy bean powder by a hammer meal in Harare. Produced soy meal powder was packed in an air tight plastic container for later use. Red sorghum was sampled by the snowball method and bought from farmers in Guruve district, Mashonaland Central in Zimbabwe and extruded using a single screw extruder in Harare. The sorghum extrudates were ground into fine sorghum powder in a hammer mill in Harare. Produced sorghum powder was packed in an air tight plastic container for later use. Baobab powder was randomly purchased from local villagers in Lower Guruve and placed in air tight containers for later production of the local RUSF. Peanut butter, icing sugar, and soy bean oil were purchased form OK supermarket in Harare. All ingredients were stored at ambient temperature prior to local RUSF production.

Peanut butter, baobab, icing sugar, extruded soy meal, extruded sorghum powder, and soy bean oil were used to manufacture the peanut-based RUSF paste. The RUSF was prepared by mixing peanut butter with soy bean oil into thin slurry in a peanut butter making machine. Icing sugar, baobab powder, extruded sorghum mealie meal, and extruded soy meal powder were mixed and added to the peanut butter- soy bean oil slurry and thoroughly blended by the peanut butter making machine until a brown thick nutrient rich RUSF paste was formed. The RUSF paste was packed in plastic jars with lids for storage and analysis.

The RUSF's functional groups were detected using Fourier Transform Infrared (FTIR) spectroscopy (Perkin Elmer, UATR TWO, Massachusetts, USA). The FTIR spectra were obtained using a Two FTIR interfaced with an ATR sampling accessory that had a single bounce diamond crystal. A spectrum in the absorbance mode was obtained by measuring from $4000cm^{-1}$ to $400cm^{-1}$ through accumulation of 64 scans at a spectral resolution of $4cm^{-1}$. The FTIR instruments use a HeNe laser as an internal wavelength calibration standard (referred to as the Connes Advantage) which means that the instruments are self -calibrating and do not need to be calibrated by the user (Nurwahidah *et al.,* 2019). Air was scanned without a sample on the diamond crystal as the reference background spectrum before each RUSF sample measurement. Enough RUSF paste to

cover the diamond crystal was deposited onto the diamond crystal to obtain the spectrum. All spectra were processed by PerkinElmer Spectrum TM software (version 5.2.1), Massachusetts, USA). Characteristic absorption band number and their functional groups were detected. FTIR identified the types of chemical bonds and functional groups present in the local RUSF. Spectral features present were strictly due to the sample. The wavelength of the light absorbed was the salient feature associated with chemical bonds as observed in the annotated spectrum. Interpretation of the infrared absorption spectrum determined the chemical bonds in the local RUSF paste. The report was generated using a template that included the spectrum and data base referral results. Obtained transmittance spectra and absorption band numbers were compared with published standard references in literature to identify chemical bonds, functional groups, and nutrients in the local RUSF in table 1.

The proximate composition of the macronutrients protein, lipids, dietary fiber, and moisture of local RUSF was measured according to the methods proposed by the AOAC (Association of Official Analytical Chemists, 2000; 2005; 2016) and the carbohydrate content was estimated by difference.

The moisture analyser (Adams, Model PMB53, South Africa) was used to determine moisture content of the RUSF. The sample was placed onto the moisture analyzer scale and the weight was recorded. The heater automatically switched on and the heat supplied vaporized water from the samples. When moisture content stabilised, the moisture analyzer indicated the percentage moisture content on the analyzer's screen. Moisture measurement was based on thermos-gravimetric principles according to weight loss of the sample due to heating. Drying temperature was pegged at 105 ^{O}C with constant temperature drying. The moisture content was recorded using the same method for all samples.

The protein content was determined using VELPA SCIENTIFICA automatic distillation and titration system (Model UDK159), method number 7. One gram of the RUSF was weighed in three digestion tubes and two tablets of catalyst CT0006650 were added into each test tube followed by 12 ml of concentrated sulphuric acid. The test tubes were shaken gently and placed into the digestion block (Velpa Scientifica, model UDK159) at 420 ^{O}C for 60minutes for heating and digestion. The test tubes were left to cool to 50 ^{O}C - 60 OC.

Distillation and titration of the digested samples were carried out by the automatic distillation unit (Velpa Scientifica, model UDK159). The distilled water used for dilution per sample was 50 ml: 30 ml boric acid and 50 ml sodium hydroxide. Hydrochloric acid was used as the titrant. The volume of titrant and the protein content were displayed on the machine screen and recorded.

To measure the fat content of the RUSF samples, AOAC Soxhlet method (AOAC, 2016) was used. Crude fat determinations were done using Soxhlet apparatus (Simtronics, India) based on the Soxhlet extraction method. Borosilicate quick-fit round bottomed flasks (500 ml) were cleaned with detergent and 300 ml of clean water. The flasks were rinsed with warm distilled water and then 99.99 % ethanol and dried in a pre-heated air circulating box oven (Scientific, Model 279D, South Africa), at 110 ^{0}C to a constant weight. Samples of 15 g were weighed and placed into a muslin extraction thimble and covered with glass wool. These were inserted into the extraction column with the condenser connected to running cold tape water. Approximately 500 ml of pet ether solvent was added into the round bottomed quick-fit flask. An electro-thermal heating mantle set at 70 ^{0}C heated the flask for 3 hours. Evaporated solvent was cooled by running cold tape water in the condenser and refluxed back into the quick-fit round bottomed flask. After extraction, the extraction thimble was removed and the flask was placed in a water bath at 70 ºC to evaporate the petroleum ether. The sample was dried in an air circulating box oven (Scientific, Model 279, South Africa) at 105 ºC for 30 minutes cycles to remove residual solvent and re-weighing until the sample weight was constant. The flask with the sample was cooled in a desiccator and weighed on an analytical balance (Adams, Model PW54, and South Africa). The weight of the fat was expressed as the percent of the initial sample using the equation below:

$$\text{Crude fat (\%)} = \frac{\text{Weight of extracted fat sample}}{\text{Weight of sample}} \times 100$$

The carbohydrates in the local RUSF were determined by the difference method. The sum of the percentages of ash, fat, moisture, and protein were deducted from the total weight of the sample. The equation below was used:

Carbohydrate (%) = [100 - (Moisture (%) + Ash (%) + Fibre (%) +Fat (%) +Protein (%)] (AOAC, 2004).

The method of Association of Official Analytical Chemists (AOAC, 2000 with modifications) was used to determine the crude fibre of the RUSF. Samples were defatted by the Soxhlet process and placed into a 500 ml borosilicate round bottomed flask containing 1.0 g of porcelain boiling chips. A volume of 200 ml of boiling 1.25 % (v/v) sulphuric acid was added and the flask was quickly placed onto a hot heating mantle and then connected to a condenser with running cold tape water. Contents in the flask were boiled for 3 minutes and then allowed to digest through reflux for 30 minutes. The digested sample was filtered through a very fine cloth and subsequently washed with hot distilled water three times to remove acidity. The sample was washed back into the conical flask using 200 ml of 1.25 % (w/v) sodium hydroxide solution. A condenser was connected to the round bottomed flask on the heating mantle and permitted to reflux for 30 minutes. The sample was filtered on a fine cloth by washing with distilled water to remove alkalinity. The residue was transferred into a clean and dry porcelain crucible with a spoon end spatula. Remaining pieces of the sample were washed into the crucible by using 50 ml of ethanol. The crucible containing the sample was dried in a box an air circulating oven (Scientific, Model 279, and South Africa) at 110 ^{O}C for 6 hours until a constant weight was achieved on an analytical balance. The contents in the crucible were ignited in a pre-heated muffle furnace (Scientific, Model283B, South Africa) at 650 ^{O}C for 30 minutes and allowed to cool in a desiccator. The cool sample was weighed and the difference in weight between the ashed samples and the digested samples was considered as the crude fibre content (Southgate, 1969). Ash content was calculated using he equation below:

$$\text{Crude fibre (\%)} = \frac{\text{weight after ignition}}{\text{Weight of sample}} \times 100$$

Results

All FTIR absorption band numbers represent the chemical groups of the components present in the local RUSF. The table shows that some low to medium absorption band numbers lie in the region of 600 cm^{-1}–1800 cm^{-1} and this region is the finger print region of macronutrients. Absorption band numbers around 1680 cm^{-1} – 1770 cm^{-1} belong to the C═O band that is assigned to lipids and carbohydrates (Hong *et al*., 2021). For protein analysis, the determination of the secondary structure of this macronutrient was mainly based on the analysis of the amide I band between 1700 cm^{-1} and 1600 cm^{-1}.

Table 1. FTIR analysis data and interpretation of a local RUSF.

Peak number	Absorption Frequency X(cm^{-1})	Y (% T)	Group Frequency (cm^{-1})	Bond	Functional group
1	3314.83	95.22	3200-3570	O-H stretch	Alcohol, hydroxy groups
2	3008.14	92.66	3000-3100	C-H symmetric stretch	Alkene
3	2923.28	59.80	2935-2915	C-H stretch, asymmetric	Alkene
4	2853.57	70.34		C-H stretch	Alkane
5	1744.26	59.06	1670-1820	C= O stretching	Ester carbonyl of triglycerides
5	1744.26	59.06	1725-1750	C=O stretching	Ester
6	1650.44	89.99	1620-1680	C=C / HOH	Alkene/ water
8	1650.44	89.99	1590-1650	N-H bend	Primary/ secondary amino
9	1650.44	89.99	1600-1650	C=C stretch	Conjugate ketone or quinone
7	1537.44	92.94	1400-1600	C=C stretch	Aromatic compound – diketones
8	1460.75	81.43	1515-1560	N-O stretch	Nitro compounds, nitrosamine
9	1376.98	85.79	1350-1480	C-H bending symmetric	Phenol/ tertiary alcohol
9	1376.98	85.79	1345-1385	N-O stretch	Nitro compounds
10	1235.83	80.26	1000-1300	C-O stretch	Alcohols, Aromatic ethers, Alkyl aryl ether
11	1159.29	67.96	1159-1164	C-O stretch	Ester, Tertiary alcohol
11	1159.29	67.96	1130-1190	C-N stretch	Secondary amine

12	1068.18	70.53	1080-1360	C-N stretch	Amine
12	1068.18	70.53	1050-1150	C-O- stretch	Alcohols, Ethers, carboxylic acids
12	1068.18	70.53	1020-1090	C-N stretch	Alcohols, Ethers, carboxylic acids
13	1052.34	71.89	1020-1090	C-O stretch	Primary alcohol, alkyl - substituted ether
14	989.90	73.02	980-960	CH=CH trans	Primary alcohol, alkyl - substituted ether
15	909.16	81.90	890-915	C-H out of plane bend	Aromatic ring.
16	867.16	85.66	820-890	C-O-O stretch	Peroxides, Alkenes
17	721.15	75.29	600-800	C-CL, C-H rocking	Peroxides, Alkenes
18	521.86	71.76	500-600	C-I stretch	Peroxides, Alkenes
19	471.38	72.19	470-500	S-S stretch	Peroxides, Alkenes

The spectrum of the local RUSF is presented in figure 1. Different absorption band numbers and absorbances showed presence of various bonds and functional groups in the local RUSF. Distinct absorption band numbers of the local RUSF absorption frequencies were compared with reference literature by Coates (Coates, 2000). These were used to assign functional groups and identify bonds and nutrients in the local RUSF.

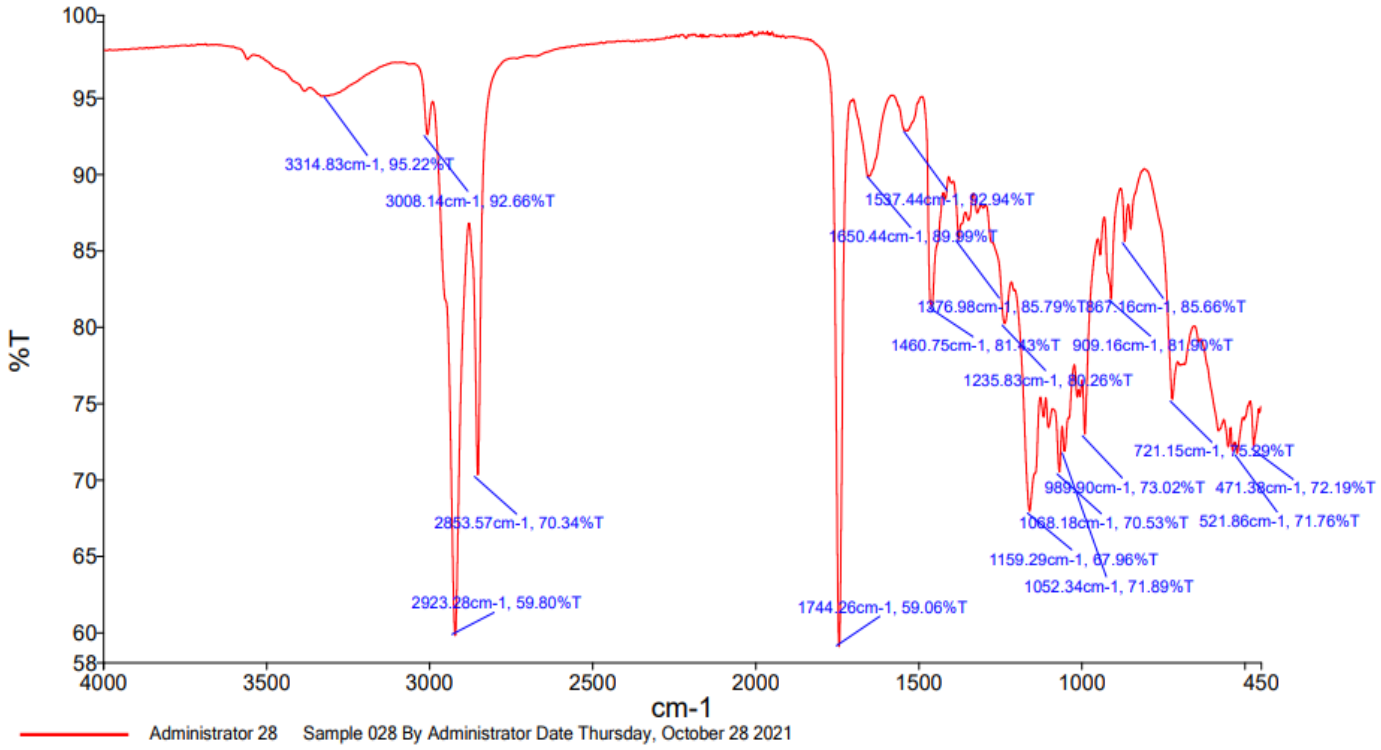

Figure 1. FTIR spectrum of the local RUSF scanned at infra region 4000-450 cm^{-1}. (PerkinElmer Spectrum IR Version 10.6.2).

The local RUSF produced 19 distinct absorption band numbers as indicated by figure 1. Absorption band number 3314.83 cm^{-1} showed presence of O-H stretch of alcohol and *hydroxyl* groups. Absorption band number 3008.14 cm^{-1} represented C-H symmetric stretch of alkenes and 2923.28cm^{-1} corresponded to C-H stretch and symmetric bonds of an alkene. Absorption band number 2853.57 cm^{-1} showed the presence of C-H stretch and symmetric C-H bonds of an alkane. The 1744.26 cm^{-1} absorption band number indicated C=O stretch of esters. Absorption band number 1650.44 cm^{-1} revealed the N-O stretch bonds of an alkane and O-H stretch bond of water. It also had N-H bend of primary and secondary amino groups as well as C=C bonds of conjugate ketone or quinone. Absorption band number 1537.44cm^{-1} represented C=C stretch bonds of aromatic compounds called diketones and 1460.75cm^{-1} absorption band number corresponded to N-O stretch bonds of nitrosamine nitro compounds. The 1376.98cm^{-1} absorption band number represented C-H symmetric bonds of phenol and tertiary alcohols and N-O nitro stretch bonds of nitro compounds.

Absorption band number 1235.83 cm^{-1} showed the presence of C-O stretch and symmetric C-H bonds of alcohols, aromatic ethers, and alkyl aryl ethers. Absorption band number 1159.29cm^{-1} represented C-O stretch bonds of esters and tertiary alcohol and C-N stretch bonds of Secondary amines. Absorption band number 1068.18cm^{-1} represented C-O stretch bonds of alcohols, ethers, and carboxylic acids and C-N stretch bonds of amines and secondary amines. The absorption band number 1052.34cm^{-1} indicated C-O stretch bonds of primary alcohol and alkyl -substituted ethers. Absorption band number 989.90cm^{-1} showed presence of CH=CH stretch bonds of alkenes. Absorption band number at 909.16cm^{-1} showed presence of C-H out of plane bend bonds of aromatic rings. Absorption band number 867.16 cm^{-1} represented C-O-O stretch bonds of alkenes and peroxides while absorption band number 721.15cm^{-1} corresponded to C-H rocking bonds of aliphatic compounds and C-CL bonds of alkyl halides. Absorption band number 521.86cm^{-1} showed presence of C-I bonds of aliphatic iodo- compounds while absorption band number 471.386 cm^{-1} indicated the presence of S-S bonds of polysulfides, alkenes, and alkyl halides. FTIR analysis showed the presence of different functional groups such as carboxylic acids, aromatics, alkanes, alcohols, phenols, aliphatic amines, alkenes, and primary as well as secondary amine groups in the local RUSF.

Table 2. Proximate results of the local RUSF

Analytical parameter	**Result**
Moisture (g/100 g)	2.4 ± 0.13
Carbohydrates (g/100 g)	44.7 ± 1.02
Proteins (g/100 g)	15.5 ± 0.14
Fat (g/100 g)	31.2 ± 0.09
Crude fibre (g/100 g)	2.2 ± 0.06

The proximate analysis of the local RUSF showed that the local RUSF prototype contained carbohydrates, crude fibre, fats, proteins, and moisture.

Discussion

Spectroscopy methods with physicochemical tests allow for rapid and reliable identification of macronutrients and bioactive compounds. FTIR spectral analysis can diagnose presence of many functional groups. Detectable groups include aldehydes (H–C=O: C–H stretch), alkenes(–C=C– stretch), alcohols, carboxylic acids, esters, and ethers (C–O stretch), aromatics (C–C stretch in–ring), carboxylic acids (C=O stretch) or (O–H bend), carbonyls (C=O stretch), primary and secondary amines (N–H wag), carbohydrates, polysaccharides, and nitrates (Muruganantham *et al.,* 2009). In other studies, functional groups like alcohols, alkanes, alkene, alkyl halides, alkynes, amines, aromatic compounds, ether, nitrile, and nitro compounds were diagnosed by FTIR spectroscopy by Pongpiachan (Pongpiachan, 2014). Ragavendran (Ragavendran *et al.,* 2011) analysed leaf extract of Aerva lantana by FTIR and found functional groups of amines, carboxylic acids, halogens, polysaccharides, organic hydrocarbons, and sulphur derivatives in the extract display. This shows that FTIR is an essential tool that can produce individual unique spectrum according to unique chemical bonds in each sample and different functional groups would not produce similar spectra. This means that FTIR can be used to identify unknown materials, determine quality of a sample, and determine the number of components in a mixture (Nurwahidah *et al., 2019*) which is important in food and medicinal product development. Detection and characterisation of constituents of functional foods is essential in medicinal foods research such as the local RUSF.

Obtained experimental wave number and literature-based wave ranges were used to assign chemical bonds and to identify nutrients in the local RUSF. Experimental wave number 1159.29 cm^{-1} and literature range of 1159 cm^{-1} to 1164 cm^{-1} were used to assign C-O bonds of carbohydrates and proteins and C-OH groups of serine, threonine, and tyrosine residues of cellular proteins. Possible nutrient type was collagen and protein (serine, threonine, and tyrosine) (Fung *et al.,* 1996). This concurred with Yang (Yang *et a*l., 2005) who stated that C-O bonds from the stretching mode of C-OH groups represented serine, threosine, and tyrosine of proteins. The -C-O-C was assigned to cellulose and polysaccharides (Shetty *et al.,* 2006). This showed that the local RUSF had both carbohydrates and proteins that could fight protein- energy malnutrition and the polysaccharides

provided some fibre to fight constipation. This qualified the local RUSF as an important potential functional food to reduce malnutrition in Zimbabwe.

Experimental wave number 1235.83 cm^{-1} and literature range of 1230 cm^{-1} to 1238 cm^{-1} were used to assign the overlapping of the protein amide iii and the nucleic acid phosphate (PO2-) vibration that is composed of amide iii and phosphate vibration of nucleic acids (Chiriboga *et al*., 1998).

The fingerprint FTIR spectroscopy absorption bands of proteins are the stretching vibration of amide I and amide II (Brauner *et al.,* 2005). The former is attributed to C=O and ring stretching vibration in the range of 1690–1600 cm^{-1}, and the latter is attributed to C–N stretching vibrations in the range of 1600–1500 cm^{-1}. This supported by experimental wave number 1650.44 cm^{-1} and literature range of 1649 to 1652 cm^{-1} that were used to assign the unordered random coils and turns of amide I where protein was the nutrient present (Eckel *et al*., 2001). This observation is supported by the fact that pure soy protein isolate has typical infrared absorption bands at 1636 cm^{-1} to 1680 cm^{-1} and 1533 cm^{-1} to 1559 cm^{-1} that are attributed to the -NH- bonds of amide i at 1640 cm^{-1} and at 1550 cm^{-1} for amide ii in peptides bonds forming the backbone of proteins. The absorption band at 1241 cm^{-1} to 1472 cm^{-1} was attributed to the C=O and C-N stretching and N-H bending of amide iii vibrations (Su *et al*., 2008). This concurs with the observations that the bands observed in rice at 1250 cm-1 and 1360 cm^{-1} corresponded to the amide-III protein band ranges, as referenced in previous studies of rice, (Ji *et al.*, 2020; Wei *et al.*, 2021) and the C–N stretching mode of proteins (Coates, 2002). Wave numbers 1650.44 cm^{-1} and 1235.83 cm^{-1} showed that the local RUSF contained some soy proteins from the extruded soy meal powder that was used in the local RUSF formulation. Proteins were confirmed to be 15.5 ± 0.14 g/100 g by proximate analysis (Masheka *et al.,* 2023) in the local RUSF. Proteins are essential for prevention of protein- energy malnutrition.

Esters in the local RUSF were associated with experimental wave number 1744.26 cm^{-1} and literature range of 1744 cm^{-1} to 1750 cm^{-1} were used to assign the ester group(C=O) vibration of triglycerides and the possible nutrient type assumed was fat (Wu *et al.,* 2001). This agreed with the carbonyl ester triglycerides obtained from the RUSF FTIR spectrum. It can be concluded that the RUSF contained some fats that can supply some energy and could be the source of essential polyunsaturated linoleic fatty acids that cannot be produced by the human body.

The vibrations of $-CH_3$, the deformation of $-CH_2$, and the C═O bond (Reigar *et al.,* 2024) were associated with lipids. These agreed with a previous research that showed that experimental wave number 2853.7 cm^{-1} and literature range of 2853 cm^{-1} to 2860 cm^{-1} was used to assign the CH_2 bond of lipids. The asymmetric CH_2 stretching mode of the methylene chains was found in membrane lipids and the assumed possible nutrient type was fat (Fung *et al*., 1996). The presence of fats was supported by experimental wave number 2923.28 cm^{-1} and literature range of 2923 cm^{-1} to 2930cm^{-1} that were used to assign the C-H stretching bends in malignant and normal tissues and the assumed possible nutrient type was fat (Wu *et al*., 2001). In addition, the availability of fats in the local RUSF was confirmed to be 31.2 ± 0.09 g/ 100 g by proximate analysis (Masheka *et al*., 2023).

A broad absorption band in the range of between 3650 cm^{-1} and 3250 cm^{-1} indicated the presence of hydrogen bonds. This band confirmed the existence of hydrate (H_2O), hydroxyl (- OH), ammonium, or amino group. The hydroxyl compound was followed by the presence of spectra at frequencies of 1600 cm^{-1} to 1300 cm, 1200 cm^{-1} to 1000 cm and 800 cm^{-1} to 600 cm^{-1} that confirmed the presence of water **(**Coates, 2000**)** in the local RUSF. Previous studies showed that water molecules, being infrared-active, exhibit absorption in two distinct regions, approximately 1300 cm^{-1} −2000 cm^{-1} and 3500 cm^{-1} −4000 cm^{-1}. The analysis pinpointed several regions associated with moisture content, including 640 cm^{-1}, 710 cm^{-1}, 860 cm^{-1}, 940 cm^{-1}, and numerous regions around 1200 cm^{-1} −1800 cm^{-1} (particularly 1630 cm^{-1}). (Troen *et al*., 2020) The results for 1630 cm^{-1} align well with Nesakumar et al. that assigned 1637 cm^{-1} to the moisture (Nesakumar *et al.,* 2018). Presence of water concurs with the proximate analysis of the local RUSF where moisture was found to be below 2.5 % (Masheka *et al*., 2023). Low moisture increased the shelf life of the local RUSF due to low water activity.

This innovative application for quantifying proximate composition of the local RUSF using FTIR holds promise for versatile applications in agriculture, dietary guidance, and the advancement of nutritional research as well as contributing to enhancing our understanding of FTIR spectra in the context of macronutrients. Understanding the nutrient composition helps the community make informed dietary choices. Knowledge of the health benefits associated with the identified nutrients in the local RUSF can encourage the use of the supplementary food. Knowledge about the value

of local ingredients in nutrition encourages promoting local food products supply chains, strengthens community resilience, supports and improves the livelihoods of local farmers. The results of this research enhance provision of information on the nutrient content of the local RUSF to educate the community about its health benefits and promote its use as a dietary supplement. Future research endeavors can leverage this approach for a broader spectrum of food samples and nutritional components, expanding FTIR spectrometry applicability and impact in the field of food quality control, nutritional studies, and culinary arts. The FTIR analysis provided valuable insights into the nutritional profile of the new supplementary food, for example, FTIR was used to investigate the relationship between nutrition and protein structure in food (Deng et al., 2020) and guide the treatment of food to maintain the nutrition... By sharing this information, we can empower the community to enhance their diets with locally available, nutrient-rich foods.

Conclusion

This study showcases the potential of FTIR spectroscopy coupled with proximate analysis as robust tools for rapid and accurate assessment techniques to detect nutrients in the local RUSF. FTIR spectroscopy is a quick analytical technique that confirmed the presence of key functional groups in foods such as aromatics, alkanes, alcohols, phenols, aliphatic amines, alkenes, amine groups, carboxylic acids, ethers, and esters in the local RUSF. FTIR in conjunction with the proximate analysis detected carbohydrates, fibres, lipids, and proteins in the local RUSF. This showed that the local RUSF had both carbohydrates and proteins that could fight protein- energy malnutrition and the polysaccharides provided some fibres that could reduce constipation. All detected nutrients qualified the local RUSF as a potentially good functional food that can be utilised to reduce moderate acute malnutrition**.** This research underscores the potential of FTIR spectroscopy and proximate analysis as rapid and accurate proximate assessment in food science and culinary arts.

Conflict of interest

The authors declare that there is no conflict of interest reported in this work.

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