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A Research on Making Pulses More Palatable and Useful Through Irradiation with 2-6µm Mid-Infrared Ray

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ABSTRACT: *Pulses are a good protein source. However, two main issues with pulses are I) their high protein content can be problematic for renal and some cardiac disease patients and II) chemicals are added to enhance their taste, aroma and shelf life. We have overcome these issues by subjecting pulses to mid-infrared rays (mid-IR) generated from our recently invented 2-6 µm mid-IR generating atomizer (MIRGA). This technology provides a straight-forward and economically viable approach to enhance the pulses' palatability and shelf life, along with altering their nutritive value. This suits the diverse dietary needs of a wide demographic. The effect of mid-infrared ray on the pulses' physicochemical attributes and shelf life are validated and described here.*

KEYWORDS: MIRGA, 2-6 µm mid IR, pulses, irradiation, sensory attributes, palatability, nutrition, enhancement.

INTRODUCTION

Pulses are widely used in cuisine, therapeutic and industrial applications. However, for patients with renal diseases, dietary avoidance is necessary in order to avoid the protein-load; furthermore, value adding to pulses is an expensive process. In this research, we applied different doses of 2-6 µm mid-IR and economically altered the pulses' nutritive values as well as their sensory attributes, in an economical manner and without adverse effects. The aim of the study is to alter the pulses inherency characteristics in order to suit different needy clinical patients and also for disease prevention. Research on technology to add value to pulses is scarce, herein we tried and successfully demonstrated the same.

In general, terahertz and mid-infrared wavelength rays are popularly known to transmit through nearly any material without causing biological harm. **Pereira *et al.*, 2011,** have summarized an overall picture of how these radiations can be safely generated, detected and applied. Specifically, mid-infrared is of high interest since many biomolecular compounds have strong resonances in this region and it is ideally suitable to exploit this area. The precision, sterility, and versatility of mid-infrared is opening up huge opportunities as explained by **Toor *et al*., 2018**. Thus, in order to enhance the sensory value of pulses like green gram and dehusked split, we have applied 2-6 µm mid-infrared (mid-IR) rays externally over the packaged pulses and succeeded to enhance their taste and aroma.

MATERIAL AND METHOD

Design of the MIRGA and emission of 2-6µm mid-IR has been presented in detail by **Umakanthan *et al*., 2022a; Umakanthan *et al*., 2022b; Umakanthan *et al*., 2023c; Umakanthan *et al*., 2023d**. MIRGA *(patent no.: 401387)* is a 20 ml pocket sized atomizer *(Supplementary file – figure F1)* containing inorganic water based solution in which approximately two sextillion cations and three sextillion anions are contained. During spraying, depending on pressure (vary with the user) applied to plunger, every spraying generates 2-6µm mid-IR (Fig 1). Every time spraying emits 0.06ml which contains approximately seven quintillion cations and eleven quintillion anions. *(details about MIRGA available in supplementary text T1) (Method of MIRGA spraying in Supplementary file – video V1)*

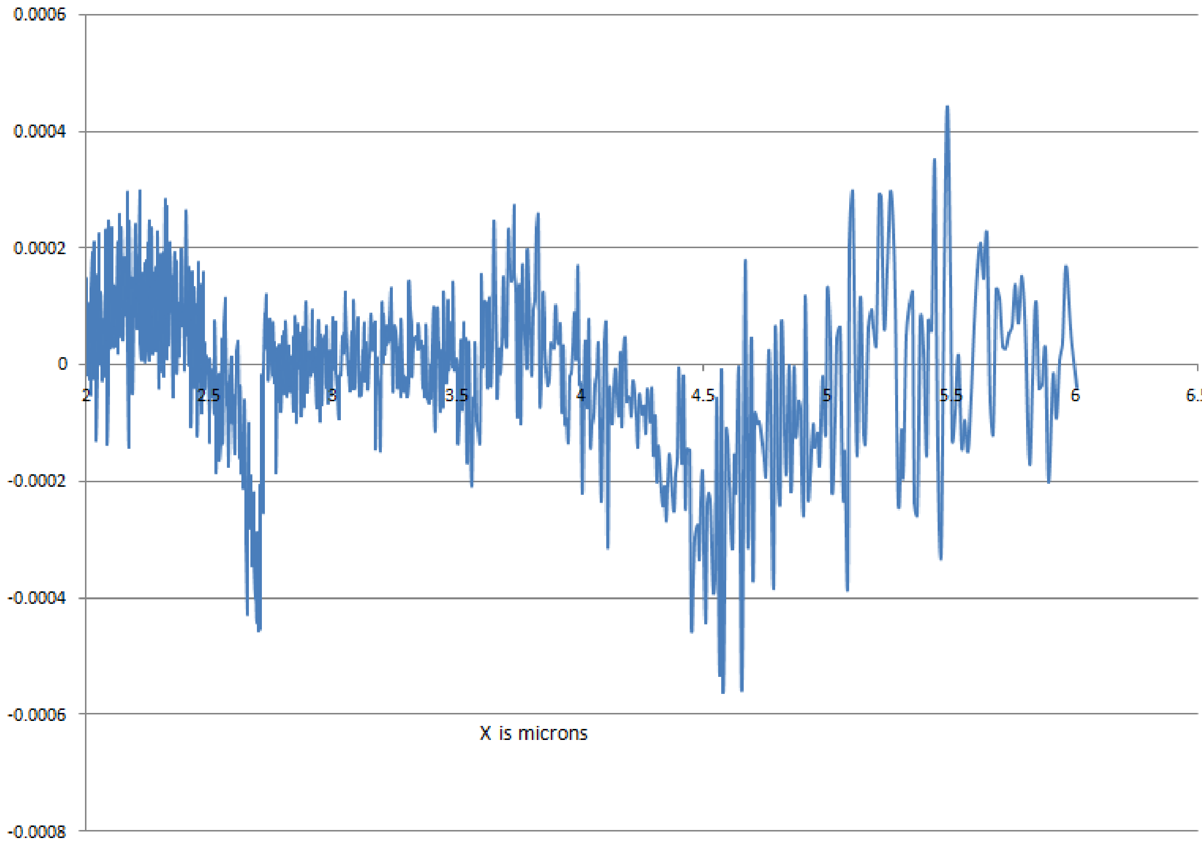


Fig 1: Estimation of 2-6 µm mid-infrared while spraying MIRGA atomizer

The inorganic compounds used in the generation of MIR are a perspective for biomedical applications **(Tishkevich *et al*., 2019; Dukenbayev *et al*., 2019)**. It is also a new synthesis method for preparation of functional material (2-6 µm mid-IR) **(Kozlovskiy *et al*., 2021; El-Shater *et al*., 2022)**. It is well known that the combination of different compounds, which have excellent electronic properties, leads to new composite materials, which have earned great technological interest in recent years **(Kozlovskiy and Zdorovets, *2021;* Almessiere *et al*., 2022)**.

Two sensory panels were utilized to evaluate the results: a 6-member trained sensory expert panel and a 15-member consumer panel. The test samples were commercially available green gram (*Vigna radiata*) and dehusked split chickpeas (*Cicer arietinum*) packaged in >50µm thickness polythene bag. The control and trial samples were from the same source. Different batches of each brand and sample of marketed green gram and chick pea were individually tested, and never mixed.

The instruments used to demonstrate this research finding are: chemical compound transformation – Gas chromatography–mass spectrometry (GC-MS); chemical bond changes – transform infrared spectroscopy (FTIR); structural changes – Powder X-ray diffraction (PXRD); configuration – Transmission electron microscopy (TEM); proton resonances – Proton nuclear magnetic resonance (1H-NMR); and Calorific value – Bomb calorimeter method. *(instrumentation details in Supplementary text T2)*

Spraying method was externally from 0.25 to 0.50 meter over the packaged (polyethylene) pulses *(method of spraying video in Supplementary video V1)*. This distance allows the MIRGA solution to form ion clouds, oscillation and 2-6 µm mid-IR generation. The ray can penetrate the packaging and act on the contained pulses. Closer spraying does not generate energy.

Thirteen volumes of 100 g each of green gram were randomly sampled from bulk 2 kg package. The samples were repackaged in >50µm thickness polythene bags. The first packet served as non-sprayed control (C) sample for instrumental analysis and was subjected to evaluation by an expert sensory panel before and after cooking. The remaining packets were numbered from 1 to 12. The 1 to 12 numbered samples were correspondingly given 1 to 12 times MIRGA spraying on one or either sides of the packet (for example, packet 1 received one spraying, packet 2 received 2 sprayings). The 12-sprayed sample was expected to denature the pulses natural characteristics due to the input of excess mid-IR energy.

All the sprayed samples and control were subjected to sensory test. The experiment was explained to the trained panelists. They were then asked to rate their preference on a sensory evaluation questionnaire for different traits, using 9 point Hedonic scale: 1 - Dislike extremely, 2 - Dislike very much, 3 - Dislike moderately, 4 - Dislike slightly, 5 - Neither like nor dislike, 6 - Like slightly, 7 - Like moderately, 8 - Like very much, 9 - Like extremely **(Everitt, 2009; O'Mahony *et al.*, 2014)**. The response sheets we completed individually without panel interaction. The untrained panel members were requested to describe the results in the fewest words possible, for maximum clarity.

Depending on the hedonic scoring, the control, sprayed sample having enhanced sensory attributes and the sample having deteriorated sensory attributes were selected and subjected for instrumentation. Also the selected samples were individually cooked, in proportion to the pulse weight, specific quantity of water and table salt (same source) were used by the same chef. Another green gram batch of 13 packets (control and packets receiving 1-12 sprayings) were kept at room temperature 32–41^{0}C to determine shelf-life enhancement. The same trials were repeated using various quantity (100, 250, 500 and 1000 gm) of packed green gram of other brands.

The same method was followed in dehusked split chickpeas and the samples collected were subjected to various instrumentations.

RESULTS AND DISCUSSION

Sensory profiling: Before and after cooking, the control samples had a standard taste and aroma. However, as the spraying number reached 3 in green gram, and 4 in chick pea, the taste, aroma shelf life and palatability increased. After 12 and 10 sprayings respectively in green gram and chick pea, the samples became unpalatable *(Table 1)*. These sensory attribute changes were perceivable within 1–5 minutes of spraying.

Nutritive value estimation: Table 2 shows that, in both the pulses, carbohydrates increased by 5-30%, protein reduced by 14-56% and fat increased by 14-58%. But 12 spraying increased the fat by 133% in green gram.

Table 1: Sensory scoring and shelf life trial results comparison – Green gram and Chick pea

Spraying number	Raw (uncooked)		Cooked		Hedonic scale point	Non trained panel ranking
	Taste, Aroma, Consistency	Shelf life	Time taken (mins)	Taste, Aroma, Consistency		
Green gram						
Control	Normal	9-12 months	25	Normal	5	Neither like nor dislike
3 sprayed	Enhanced, soft, smooth	30-40% enhanced (brand-dependent)	45	Enhanced, smooth texture	8	Like very much
12 sprayed	Reduced, become hard	50-70% reduced (brand-dependent)	40	Reduced, pasty	2	Dislike very much
Dehusked split Chickpeas						
Control	Normal	11-13 months (brand-dependent)	20	Normal	5	Neither like nor dislike
4 sprayed	Enhanced, became very soft and dissolved faster in mouth	30-40% enhanced (brand-dependent)	17	Enhanced, smooth texture	8	Like very much
10 sprayed	Tasteless, became very soft and sticky	50-70% reduced (brand-dependent)	13	Reduced, pasty	1	Dislike extremely

The table shows that the 2-6 µm mid-IR enhanced the taste, aroma and shelf life without the addition of chemicals.

Table 2: Nutritive analysis comparison - Green gram and Chick pea

Green gram				
S.No	**Parameters**	**Control**	**3 sprayed**	**12 sprayed**
1.	Carbohydrates %	64	83.2	63.8
2.	Energy (Kcal/ 100 gm)	345	342	248.8 (28% reduction)
3.	Protein %	23	22.9	19.1 (17% reduction)
4.	Total fat %	1.1	1.1	2.56 (133% increase)
Dehusked split Chickpeas				
S.No	**Parameters**	**Control**	**4 sprayed**	**10 sprayed**
5.	Carbohydrates %	60.5	63.5 (5% increased)	62
6.	Energy (Kcal/ 100 gm)	360	371	372
7.	Protein %	18.5	10.3 (56% reduction)	9.43 (51% reduction)
8.	Total fat %	5.85	5 (14% reduction)	2.45 (58% reduction)

Spraying caused the nutritive values both enhanced and reduced. A patient can choose the nutritionally modified pulse according to the need. One time spraying and beyond 12 will also further modify the nutritional status which may fit to patients need. However further elaborate study is needed.

Instrumentation results *(raw data and detailed interpretations in Supplementary file – D1 and T2)*

GC-MS

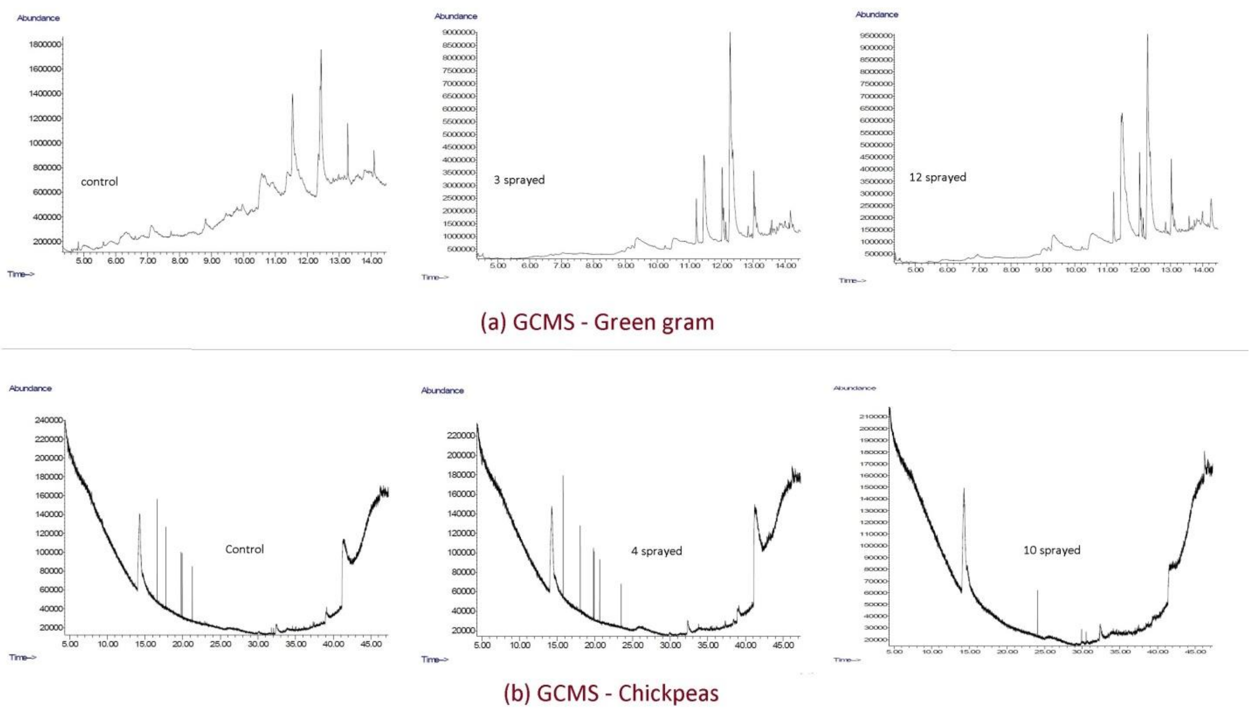


Fig 2: GCMS spectra

(a) Green gram *(Fig 2a)*

Control sample contains Ethyl iso-allocholate, Curan, 16,17-didehydro, and other molecules such as Ethyl iso-allocholate, 2,6-Bis[2-[2-S thiosulfuroethylamino] ethoxy] pyrazine, etc. However, most of the peaks from the control are disappearing after MIRGA spraying.

3 sprayed sample: There were the unique peaks of n-Hexadecanoic acid, Methyl 10-trans, 12-cis-octadecadienoate, Pyrrolidine which were not present in the control. There was higher amount of 9,12-Octadecadienoic acid (Z, Z) than GG12 sample. Thus the 3 sprayed sample was found to be soft and smooth when tasted.

12 sprayed sample has shown all unique peaks such as Pentadecanoic acid, Deoxyspergualin, 7,10-Octadecadienoic acid, methyl ester, which could be responsible for tastelessness, hardness, and stickiness. The given difference in the peaks detected in 12 sprayed and 3 sprayed samples are responsible for faster cooking (i.e., 5 mins lesser) in the case of 12 sprayed sample.

(b) Chickpeas *(Fig 2b)*

Control sample contains 1-Octanol, Ethyl 2-[1,3-bis(4-amino-3-furazanyl)-2-triazeno] acetate & 1,6;3,4-Dianhydro-2-deoxy-β-d-ribo-hexopyranose etc. However, most of the peaks from the control sample are disappearing in MIRGA sprayed samples, except Acetamide, N-(6-acetylaminobenzothiazol-2-yl)-2-(adamantan-1-yl).

4 sprayed sample: There were unique peaks of 5-Methyl-4'-hydroxy-2-benzylidene-coumaran-3-one, Cyclopropene, and Topotecan, which made the samples to be very soft and dissolved in mouth fastly.

10 sprayed sample has shown all unique peaks such as Iron, dicarbonyl(η5-2,4-cyclopentadien-1-yl) (pentamethyldisilanyl), 9,12-Hexadecadienoic acid, methyl ester, and Hexasiloxane which are responsible for tastelessness and stickiness.

FTIR

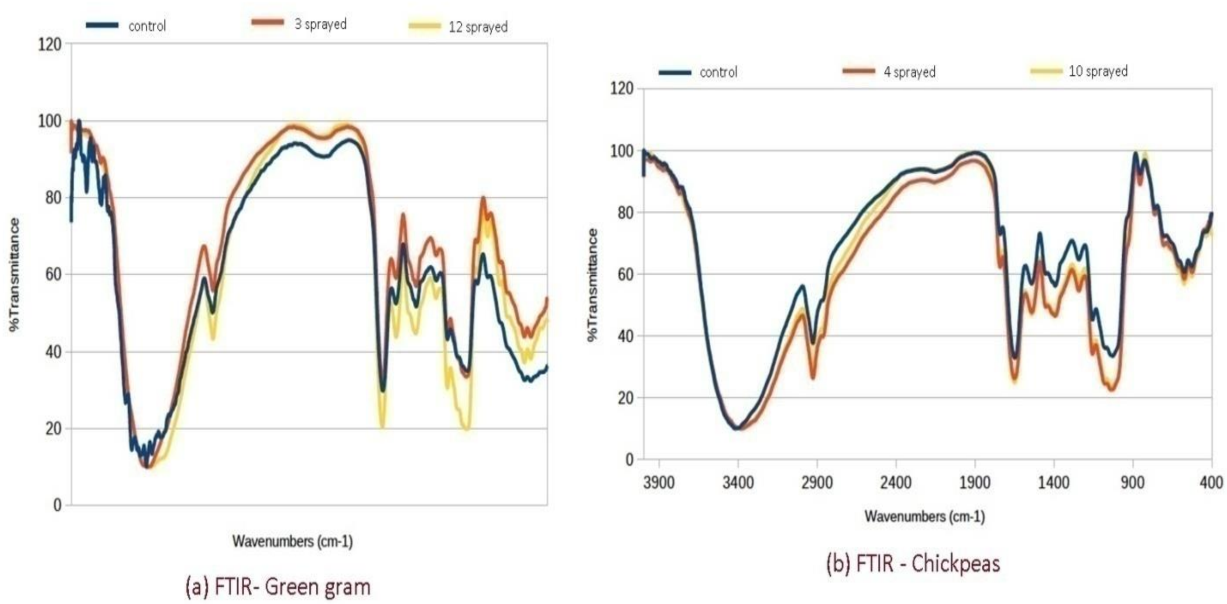


Fig 3: FTIR spectra

(a) Green gram *(Fig 3a)*

Control sample has key peaks at these wavenumbers (cm^{-1}): 3485 (O-H, N-H) with shoulders at 3587, 3543, 3533, 3391; 2934 (N-H), 2095 (N=C=S), 1644 (C=N), 1546 (N-O), 1392 (C-H), 1238 (C-O), 1156 (C-O), 1004 (unk), 927 (tentatively C=C), 524 (C-Br). The volatile compound 2-acetyl-1-pyrroline (2AP) is one aroma compound present in green gram. Other major aroma compounds in green gram are hexanol, benzyl alcohol, γ-butyrolactone, 2-methyl-2-propenal, and pentanol.

3 sprayed sample: Compared to control, this sample does not show the multiple shoulders on the peak centered at 3422 cm^{-1}. It is suggested this key change in the FTIR spectrum reflects the change in taste and aroma of the 3 sprayed sample where a combination of the aroma compounds mentioned above are enhanced.

12 sprayed sample: Compared to the 3 sprayed sample, this sample does not show the multiple shoulders on the peak centered at 3422 cm^{-1} and an additional peak at 853 cm^{-1} (C=C). These differences in the FTIR spectrum reflect the change in the composition of aroma compounds that render the 12 sprayed sample tasteless.

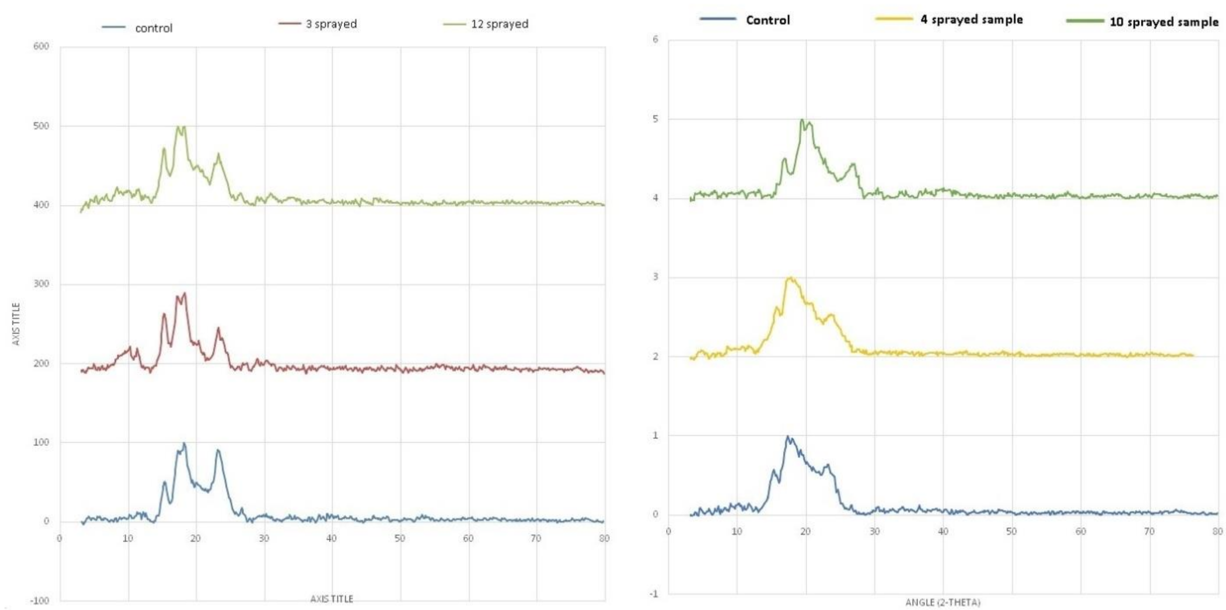
(b) Chick pea *(Fig 3b)*

Control: shows peaks at (cm^{-1}): 3418 (O-H, N-H), 2928 (C-H), 2852 (C-H), 1736 (C=O), 1648 (C=C), 1541 (N-O), 1392 (C-N), 1243 (C-O), 1155 (C-N), 1027 (C-N), 854 (C=C), 574 and 522 (alkyl halide).

4 sprayed sample: The subtle difference between the FTIR of the control and 4 sprayed samples is the slight symmetrical shoulder around the 3418 cm^{-1} (O-H, N-H) peak. It is possible this is an indicator of the change in the composition of the aroma compounds (key volatile compounds that may give a 'beany' aroma and flavor notes are pentanol, hexanal, 2-hexenal, hexanol, heptanal, furan-2-pentyl, 2-octenal, nonanal, 2,4 decadienal, and 2,4- undecadienal) (Shiva Shariati-Ievari, 2013; IR Spectrum Table by Frequency Range, Sigma Aldrich).

10 sprayed sample: The slight shift in the peak from 3418 to 3384 cm^{-1} indicates a change in the composition of aroma compounds that leads to the tastelessness of this sample.

PXRD



(a) PXRD - Green gram (b) PXRD - Chickpeas

Fig 4: PXRD spectra

(a) **Green gram** *(Fig 4a)*

Control sample: One broad peak is observed between 13.0^{o} and 28.0^{o}. Three prominent peaks within this range are observed at15.4^{o}, 18.3^{o} and 23.3^{o}. Other minor peak is observed are at 2θ= 26.7^{o}.

3 sprayed sample: First broad low-level peak is observed between 6.0^{o} and 13.0^{o}. Peaks within this range are observed at 10.3^{o} and 11.3^{o}. Following this is a broad high-level peak observed between 13.0^{o} and 25.0^{o}. Peaks within this range are observed at 10.3^{o} and 11.3^{o}. Three prominent peaks within this range are observed at 15.3^{o}, at split-peaks 17.3^{o}and 18.3^{o}, and lastly at 23.1^{o}. Other minor peaks are observed are at 2θ= 27.4^{o} and 28.9^{o}.

12 sprayed sample: One broad peak is observed between 13.0^{o} and 28.0^{o}. Three prominent peaks within this range are observed at 15.4^{o}, at split-peaks 17.3^{o}and 18.2^{o}, and at 23.2^{o}. Other minor peak is observed are at 2θ= 26.8^{o}.

3 sprayed and 12 sprayed show very similar peak patterns, particularly the split-peaks around 17.0^{o} and 18.0^{o}. 3 sprayed also show the most number of peaks observed in its XRD. Peak intensity

around 15.0^{o} increases with the increase in spraying while peak around 23.0^{o} decrease with the increase of spraying. Changes of intensity of peaks indicate change of crystalline phases present in samples with 12 spraying.

PXRD patterns of all the samples agree with the signature peaks of green gram. Peaks at 15.0^{o}, 18.0^{o}, and 23.0^{o}agree with the crystal pattern as observed by **Zou *et al*., 2019, Qian *et al.*, 2013** and **Xu, 2014**. Mix of amorphous and crystalline phases is observed due to the semi-crystalline nature of starch.

(b) Chick pea *(Fig 4b)*

Control sample: PXRD pattern exhibited a broad peak between the range 2θ=13.03^{o}and 26.27^{o} Three prominent peaks are observed in the directions 2θ= 15.29^{o} split peaks between 17.25^{o}and 19.15^{o}, and 23.18^{o}.

4 sprayed sample: exhibited a broad peak between the range 2θ=12.35^{o}and 25.26^{o} Three prominent peaks are observed in the directions 2θ= 15.16^{o} split peaks between 17.50^{o}and 19.96^{o}, and 23.18^{o}

10 sprayed sample: exhibited a broad peak between the range 2θ=12.45^{o}and 25.14^{o}. Three prominent peaks are observed in the directions 2θ= 15.33^{o} split peaks between 17.22^{o}and 20.33^{o}, and 23.28^{o}.

The three spectra exhibited prominent peaks at comparatively same directions. Peak intensities of samples Control and 10 sprayed are relatively lower than that of the 4 sprayed.

TEM

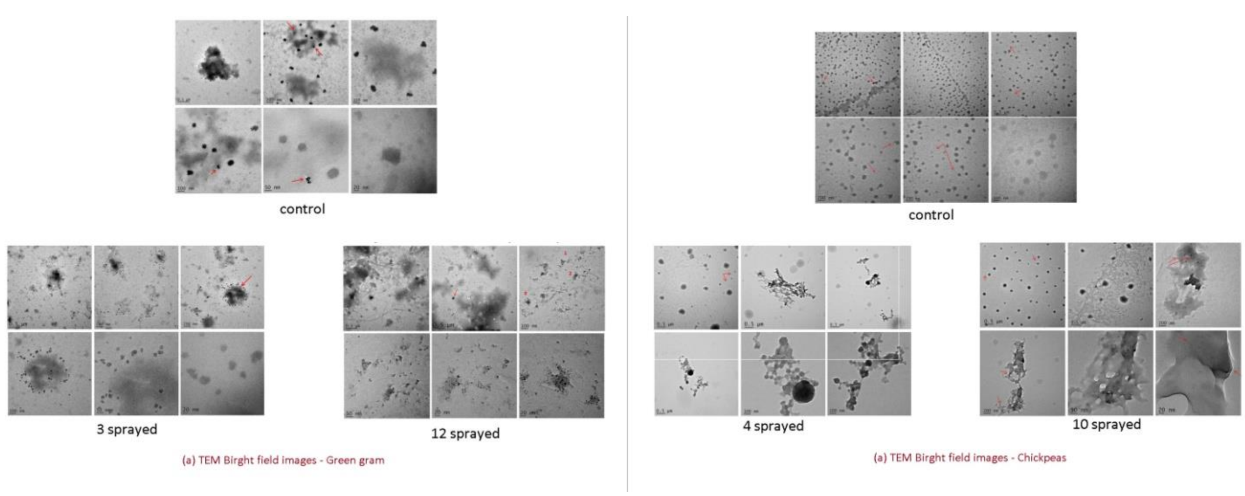


Fig 5: TEM images

(a) Green gram *(Fig 5a)*

With respect to the control, while the 3 sprayed sample mostly maintains the matrix structure of the control, the 12 sprayings affected the sample structure more heavily. This mainly concerns the loss of structure of aggregate types observed in the control (and mostly maintained in the 3 sprayed sample), and the far larger (in the 12 sprayed sample, with respect to control and 3 sprayed samples) numerical abundance of nanoparticles. Concerning the latter, despite they show size range comparable to that of control, their spatial arrangement is evidently different (clustered in the control, close each other but not clustered in the 12 sprayed sample). Finally, diffraction patterns indicate that neither 3 nor 12 sprayings affect significantly the atomic arrangement of this sample, with respect to control.

(b) Chick pea *(Fig 5b)*

With respect to the control, both 4 and 10 sprayed samples maintain the general structure of sample matrix. This concerns both types of particulates mainly observed, the droplet-like larger one, and the dark nanoparticles one. Also, diffraction patterns indicate that both sprayings do not affect significantly the atomic arrangement of the sample matrix. The most evident effect of both 4 and 10 sprayings seem to be instead related to forcing the aggregation status of the sample materials. Indeed, while in the control sample neither aggregates nor clusters are documented, but in the 4 and 10 sprayed samples both aggregates and clusters are observed, with increasing number from 4 to 10 sprayings. The image documentation suggests that a mechanism similar to a “dragging” of the overall material composing the sample have occurred, with intensity (and effects) increasing from 4 to 10 sprayings.

13C Solid state NMR

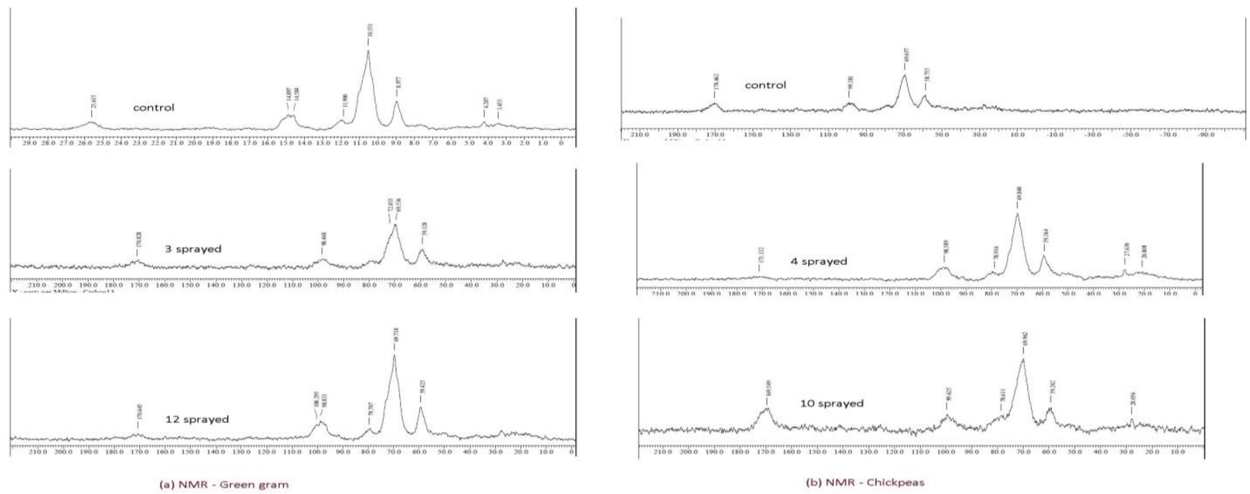


Fig 6: 13C solid state NMR spectra

(a) Green gram *(Fig 6a)*

One of the main compositions of beans in general is carbohydrates, and there is no significant change in the –CO–phenylalkane at 27.874 ppm in control, 3 and 12 sprayed samples. So we used this peak as a reference to normalize the integral values in all the three sample data sets. With respect to the control, the ethers at 59.486 ppm and 79.342 ppm, the cyclic ether at 98.711 ppm, the acetic acid ester at 170.097 ppm increased in the 3 sprayed sample. But in the 12 sprayed sample, theses integrals dropped in value again. The monosaccharide ribose (carbohydrates) at 69.779 ppm exhibits an opposite trend. These behaviors may be interpreted as to the 3 sprayed sample being more favorable than the control sample, and the 12 sprayed sample being less favorable. (**Badertscher *et al*., 2009; Martino *et al*., 2012)**

(b) Chick pea *(Fig 6b)*

One of the main compositions of chickpeas in general is carbohydrates, and there is no significant change in the chemical shift of the monosaccharide at 69.657 ppm in control, 4 and 10 sprayed samples, and we used this peak as a reference to normalize the integral values in all the three data sets. With respect to the control, the cyclic ether at 99.381 ppm and the carbonyl groups–CH3 at 170.462 ppm dropped in value in the 4 sprayed sample. But in the 10 sprayed sample, these integrals increased significantly. These behaviors are interpreted as to the 4 sprayed sample being more favorable than the control sample, and the 10 sprayed sample being less favorable. **(Badertscher *et al*., 2009; Rembold *et al.*, 2014)**

Compared with control data, all the instrumentation data illustrated that, MIRGA depending on number of sprayings had altered the chemical bonds, thereby structure, chemical composition, leading to enhanced/ decreased inherent characters of pulses.

Action of MIRGA emitted 2-6 µm mid IR on Pulses

Invention background, definition, technique of mid-IR generation from MIRGA, toxicological study on MIRGA, safety of the MIRGA sprayed usables and primeval and future scope of MIRGA have been described by **Umakanthan *et al*., 2022a** and **Umakanthan *et al*., 2023d** *(detailed discussion on MIRGA available in supplementary text T2).* Depending on number of MIRGA spraying (energy given), a receptor's chemical bond configurations and subsequent physical and chemical characters can be altered to our desire. This has been achieved using MIRGA spraying in coffee and tea **(Umakanthan *et* al., 2022a)**, cocoa **(Umakanthan *et al*., 2022b)**, table salt **(Umakanthan *et al*., 2023c)** and terminalia **(Umakanthan *et al*., 2023d)**.

In pulses, research on safe improvement of sensory attributes is minimal. Methods viz., cultivar selection, oxidation temperature control, soaking and thermal water treatment, germination, solvent extraction, fermentation, enzymatic treatment and ultrafiltration, and addition of sugars, salts, acids, flavorings are present day technologies applied to reduce/ remove/ mask the naturally present off-flavors in pulses, and however enhancement of flavor is really a need **(Roland *et al.*, 2017)**. In food industry, the focus is higher on yield than on nutritional improvement **(Cooper *et al*., 2017)**. In 2050, it is expected that global population grow to 10 billion with dietary shift from meat towards food products **(Poore *et al.*, 2018; Lucas *et al.*, 2019)**. Nutritional improvement of crops has been practiced through selective breeding of crops. Although biofortification methods are developing, they have their own advantages and challenges **(Lockyer *et al*., 2018; Jha *et al*., 2020; Shahzad *et al*., 2021)**. Whereas 2-6 µm is the safest zone, living cells vibrational frequencies coincide with this range, causing chemical bond changes and lead to physiochemical changes, hence favorable changes.

Depending on geographical area of cultivation and agropractice, the nutritive value differs in pulses. In this research, we improved the protein, carbohydrate and fat by MIRGA spraying which produce the sustainable effect and outcome in 1-5 minutes by altering the chemistry of pulses as evidenced by the laboratory data.

The data showed the alterations in chemical bonds, morphology, and chemicals compounds transformation. The applied 2-6 µm mid-IR is non-ionizing, hence safe **(Pereira *et al*., 2011)**, penetrate the intervening obscurants **(Prasad, 2005)**, coincide with the vibrational frequency of pulses **(Toor *et al*., 2018)**, alter its chemistry **(Mohan, 2004; Xu *et al.*, 2017)** and thereby physiochemical properties also **(Datta *et al*., 2014)** causing improved quality of pulses.

MIRGA manufacturing cost is USD 0.35 giving 300 sprayings which can improve the quality of 1 ton of any pulses.

CONCLUSION

In this study, green gram and chickpea were irradiated with 2-6µm mid-infrared. This irradiation has altered the physic-chemical inherency, nutritive values and enhanced the taste, aroma and shelf life, hence increased the commercial value. Since different doses of irradiation alter the nutritive value differently, we advocate customized servings of MIRGA sprayed pulses to renal and cardiac patients for those pulses are contraindicated. In the future, MIRGA technology may allow cultivation of sprayed seeds according to specific requirements, without the need for genetic modification.

Competing interest

In accordance with the journal's policy and our ethical obligation as researchers, we submit that the authors Dr.Umakanthan and Dr.Madhu Mathi are the inventors and patentee of Indian patent for MIRGA *(under-patent no.: 401387)* which is a major material employed in this study.

Data and materials availability

All data is available in the manuscript and supplementary materials.

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The authors received no specific funding for this research.

Author contribution

Umakanthan: Conceptualization, Methodology, Supervision, Validation.

Madhu Mathi: Data curation, Investigation, Visualization, Writing - Original draft preparation.

Umadevi: Project administration, Resources

Umakanthan, Madhu Mathi: Writing- Reviewing and Editing.

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