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Article

General Characterization of Whey Protein Extracted through Various Techniques: A Comparative Analysis

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Abstract. A significant quantity of waste is generated by food processing industries worldwide leading to severe environmental and social challenges. Within the milk processing industry, the coagulation of insoluble proteins results in the production of copious amounts of effluents. This latter yields liquid rich in soluble proteins known as whey proteins. Various techniques, including the use of organic solvents, heat treatment, and lyophilization, are employed to extract these proteins. The objective of this research is to compare the extracted substances obtained with the commercially available product. Fourier-transform infrared spectroscopy (FT-IR) is employed as the analytical technique in this study. The FT-IR spectra of the extracted whey proteins exhibit characteristic peaks similar to those reported in the literature and the commercial product. These findings validate the effectiveness of the utilized extraction methods, and further steps should be considered to enhance the yield and quality of the extracted material. The findings provided insights into the suitability and quality of the extracted substances, paving the way for improvements in the extraction process. By maximizing the utilization of waste streams from the food transformation industry, this research aims to contribute to resource optimization and sustainability in line with environmental and societal considerations.

Keywords: Whey, protein, circular economy, waste valorization, food industry, recovery.

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1. Introduction

In Morocco, the production of milk has risen sharply from DH1.66 billion in 2003 to DH4.22 billion in 2019, an improvement of 154%. [1] About 90% (v/v) of whey is composed of water and is produced during the production process of cheese. It is the yellowish liquid that is left after the coagulation of the insoluble proteins with the acidification process or using rennet. [2], [3] It was generally disposed of as wastewater in the sewage, but after recognizing its valuable components, it was given a new life by valorizing it. [4]–[7] Besides the significant quantities of biochemical oxygen demand (BOD) and chemical oxygen demand (COD), which range between 30 to 50 and 60 to 80 g/L, respectively, it causes major environmental problems. [8],[9] It is considered to be the most polluting effluent in cheese production due to its high charge of organic and chemical content. [5],[10],[11] It mainly contains a high amount of proteins called "whey protein", which are β-lactoglobulin (β-LG), α-lactalbumin (α-LA), bovine serum albumin (BSA), and immunoglobulins, with an isoelectric point around 4.8–5.2. [12],[13] Whey Protein (WP) or "soluble whey protein" as some prefer to call it, is well known and appreciated for its nutritional value, [14],[15] which explains its incorporation in diverse ready-to-consume products as a supplement such as infant formula, whey protein-fortified beverages, yogurt, protein bars, desserts, and ice cream and cheese. [16][17] Whey is considered one of the most valuable sources of protein, offering an excellent profile of amino acids, particularly with a high concentration of branched-chain amino acids, which are essential for promoting muscle growth and mass. [18] Whey protein has also a potential to play the role of a vitamin carrier since it helped reduce vitamin B losses by 60% in its presence according to a study conducted in emulsions. [19] Whey protein can also be consumed in the form of supplements, which has proved to enhance muscle strength, reduce fatigue, and accelerate recovery. [20],[21] Another application of whey protein is in the food packaging industry as an edible coating due to its mechanical, optical, and barrier properties in addition to its natural ability to biodegrade. [22]

The physical recovery of whey protein is a complex process that has gone through many stages. Ultrafiltration (UF) is the biggest technology to recover the nearest native form of whey protein concentrate (WPC) and whey protein isolate (WPI). It requires using other membrane filtration to eliminate lipids using microfiltration and nanofiltration for demineralization. Despite the advances achieved in the recovery of powdered whey, it still faces some challenges in the formulation and processing parameters. Due to the presence of lactose in the crude product, the powder tends to stick and cake during the drying and storing of the final product, [23] as a result of the high processing temperature which causes the particles to agglomerate. [24] Some organic techniques have been explored on a laboratory basis following an eco-friendly extraction. [9], [25]

Based on previous studies, extraction methods play a pivotal role in the food industry, particularly in enhancing the efficiency and quality of protein recovery processes. Advanced extraction techniques, such as thermal, organic, and lyophilization methods, enable the recovery of whey proteins with minimal degradation, preserving their structural and functional integrity. By refining these methods, the industry can reduce production costs, improve sustainability, and enhance the economic viability of dairy products. These advancements not only meet the growing consumer demand for high-quality protein supplements but also position whey protein as a cornerstone in functional food and nutraceutical applications.

This article aims to undertake a comprehensive exploration of the different methods of whey protein recovery, focusing on a comparative analysis between commercially available whey protein products and those obtained from dairy industry effluents. Using Fourier Transform Infrared Spectroscopy (FTIR) as our main investigative tool, our aim is to delve deeper into the distinct chemical fingerprints and subtle structural nuances present in these different whey protein variants.

Through meticulous examination using FTIR techniques, we aspire to discern even the most minute compositional differences between these whey protein sources. In doing so, we aim to advance our understanding of the complex physicochemical characteristics underlying the diverse functionalities and applications of whey proteins in a wide range of industries and contexts. Furthermore, beyond simply highlighting disparities between commercially available whey proteins and their extracted equivalents, this study strives to elucidate broader implications for industrial processes and sustainable resource use. By shedding light on these disparities, we aim to provide valuable information that can inform strategies for optimizing production and extraction processes.

Fundamentally, our aim is to help refine production methods and improve the economic viability of the dairy industry. Through a nuanced analysis of the molecular patterns of whey proteins, we aspire to meet the evolving challenges of sustainable resource management while fostering innovation in industrial practices.

2. Material and Methods

2.1. Materials

Ultra-high temperature (UHT) milk was purchased from a local store in Sidi Maarouf, Casablanca- Morocco. UHT was used in this experiment since it does not contain any bacterial movement. Whey protein isolate (WPI) used in this study was purchased from ALLMAX, which has a nominal fat level of 0%, and a protein concentration of 27g per scoop and each scoop weighing 30g of powder. This specific product was chosen for its pure and refined formula with no artificial-added products. Another quantity of cheese whey was obtained from a vendor

specialized in artisanal homemade cheese using natural and traditional ways of extraction.

The ethanol used in the experiments came from Ethanol.SA, was of high purity with a confirmed ethanol concentration of 99.8%. This specific selection of reagents and commercial items aims to maintain the integrity and reproducibility of laboratory tests, ensuring that the study's results are based on precise and standardized techniques.

3. Methods

a. Preparation of milk whey

The UHT-treated milk was further heated to its boiling point. This was followed by the gradual addition of citrus juice until a pH of 5 was reached. The resulting solution was left to stand for 15 minutes, allowing casein to aggregate. After the incubation stage, the whey fraction was separated using a strainer and then stored in a refrigerated facility at 4°C. This procedure was conducted systematically in order to extract and preserve the whey component for future studies. With the aim of highlighting its potential application, these studies were achieved to investigate the biochemical composition and functional properties of the whey fraction, in order to highlight its potential applications in a range of food and pharmaceutical industries. In addition, further analyses were carried out to assess the effect of processing conditions on the physicochemical characteristics of the whey fraction, providing valuable insights for optimizing future extraction protocols in the future.

b. Thermal extraction of the whey protein

Thermally extracted whey protein was carefully produced by subjecting commercially available UHT milk to heat treatment, effectively reducing the risk for bacterial or fungal contamination. The experimental protocol involved placing two beakers containing whey previously prepared in the laboratory in an oven heated to 100°C for one hour. Following this heat treatment, a subtle, perceptible layer began to form on the surface of each beaker, indicating the formation of a film. Using laboratory tweezers, the films obtained were then carefully collected. An interesting phenomenon emerged during this process: the removal of a film led to the build-up of new films on the surface, with subsequent films becoming progressively thinner over time. This observation suggests a dynamic process of film regeneration and thinning, warranting further study of the underlying mechanisms and potential applications of these whey protein films in fields, such as food packaging and biomedical applications.

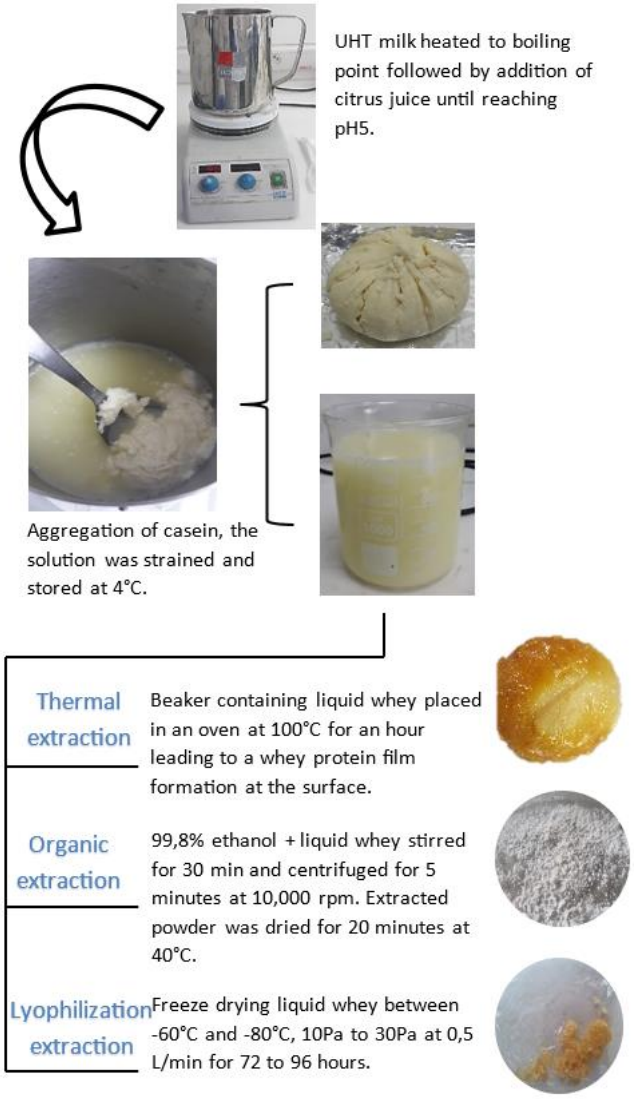

Fig. 1. Workflow.

Fig. 2. Collected films through thermal extraction via the use of a laboratory oven.

c. Organic extraction of the whey protein

Pre-processed whey was used for organic protein extraction to isolate and purify the protein component. The extraction process began by combining two-thirds whey protein with one-third 99.8% (v/v) ethanol in a centrifuge tube to obtain a homogeneous mixture. After thorough mixing, the solution was stirred at room temperature for 30 minutes to facilitate protein dissolution and interaction with ethanol molecules. The mixture was then left in suspension overnight to allow the proteins to precipitate. During this incubation period, a distinct white precipitate formed at the bottom of the tube, indicating that protein extraction was successful.

To collect the precipitate from the supernatant, the centrifuge tube was spun at 10,000 rpm for 5 minutes. The resulting whey protein powder, located at the bottom of the tube, was carefully extracted after centrifugation, to minimize contamination from the supernatant. The extracted protein powder was then dried in an oven for 20 minutes at 40°C to remove residual moisture and ethanol.

This extraction process was then repeated three times iteratively to ensure complete separation and collection of the protein precipitate, thereby refining the quality and purity of the extracted protein powder. By employing this rigorous extraction method, the study aimed to obtain a high-quality protein powder suitable for diverse applications in food, pharmaceuticals and biotechnology. Additionally, the iterative nature of the extraction process removed impurities and contaminants, thereby enhancing the overall purity and functionality of the extracted protein powder [26].

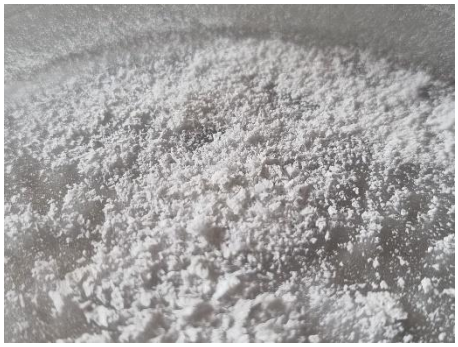

Fig. 3. Extracted powder of whey protein through organic extraction.

d. Lyophilization extraction of the whey protein

Freeze-drying is one of the techniques used to extract whey protein. [26], [27] Five liters of previously prepared whey underwent a lyophilization process during the recovery of whey protein, a method that includes freezing the liquid and eliminating the water content through sublimation under low pressure. The freeze-drying process was performed at a temperature of between -60 and -80°C, accompanied by a pressure gradient ranging from 10 Pa to 30 Pa and a flow rate of 0.5 L/min. The duration of the freeze-drying procedure varied, but was generally between 72 and 96 hours. Using a freeze-drying device, a vacuum was established to facilitate the controlled sublimation of frozen liquid into whey under precise conditions of temperature and pressure. As a result, the freeze-dried feed produced a yellowish substance, indicative of concentrated whey protein. This method not only concentrated the protein content, but also effectively preserved the structural and functional integrity of the whey protein. The controlled freeze-drying process ensured minimal denaturation and degradation of the proteins, thereby preserving their native properties and functionalities.

Fig. 4. Extracted whey protein through lyophilization process.

e. TGA Analysis

The Thermo-Gravimetric Analysis (TGA) was employed to assess the effect of process temperature on WPI-based films, specifying the thermal characteristics of the synthesized films and evaluating their thermal sensitivity. The test utilized TA Q500 equipment (TA Instruments, New Castle, DE, USA), with overall parameters defined according to [10], conducted in an environment under nitrogen and oxygen flow.

Samples weighing approximately 10 mg were heated in a platinum pan. The procedure began at a stabilized temperature of 22°C for 10 minutes, then increased to 900°C at a rate of 20°C/min, stabilizing for 2 minutes under a nitrogen flow of 40 ml/min. In the second cycle, oxygen gas was selected at 60 ml/min, and the temperature increased to 1000°C at 20°C/min. TGA was performed once per sample.

Fig. 5. Q500 Thermogravimetric analyser from TA Instrument.

f. DSC Characterization

The phase transition of the commercialized whey was determined using a Differential Scanning Calorimeter (DSC). This study used a type Q20 TA calorimeter and an RCS intercooler to assess the thermal behavior of the product sample over a temperature range of -20°C to 150°C. The sample used weighed 5,75 mg, and was subjected to a double cycle that began at -20°C for 2 minutes, then rose and held at 150°C for another two minutes at a ramp rate of 20°C per minute. The temperature plummeted to -20°C after the same ramp. The entire cycle was carried out in a nitrogen environment in the first cycle and oxygen in the second with a flow rate of 50 ml/min.

Fig. 6. Q50 DSC.

g. FTIR characterization of the samples

All samples were meticulously characterized by Fourier Transform Infrared Spectroscopy (FTIR) on the Perkin Elmer instrument (UTAR TWO), renowned for its precision and reliability in analytical measurements. FTIR spectra were systematically acquired in absorbance mode in the spectral range from 4000 cm^-1 to 450 cm^-1. This in-depth spectral analysis was aimed at discerning complex absorption patterns in the infrared wavelength range, thereby enabling a comprehensive examination of the chemical composition and molecular structure of the samples.

Leveraging the capabilities of FTIR, the research sought to delve deeper into the vibrational modes present in the samples, yielding invaluable insights into their chemical properties and underlying molecular interactions. Through the meticulous interpretation of FTIR spectra, the study aimed to unravel the complex molecular architecture of the samples, offering a deep understanding of their structural features and functional attributes. This detailed characterization, facilitated by FTIR analysis, laid the foundations for further exploration of the samples' behavior and their potential applications in a variety of fields, including materials science, biotechnology and environmental engineering.

Fig. 7. Fourier Transform Infrared Spectrometer.

4. Results and Interpretation

4.1. Thermogravimetric Analysis (TGA) of the Samples

The thermal stability of the formulations was analyzed using TGA. The prominent peaks in the weight percentage curves effectively identify distinct degradation stages, as shown in Table 1.

The first stage of weight loss was observed below 100°C where around 20 wt% was lost corresponding to the removal of the free and bond water. [28] The second stage of decomposition occurs between 274-325°C, reaching a maximum degradation of 89%. This stage is primarily due to the breakage of covalent peptide bonds in the amino acid residues, as confirmed by the literature [29], [30]. Only the commercialized whey protein exhibited a third decomposition stage, likely caused by the breakdown of partially degraded proteins resistant to the addition of conservative additives in their mineral forms. All samples show a variable percentage of residue, attributed to the added minerals in the commercialized sample and naturally occurring minerals in the whey.

4.2. DSC Characterization of the Samples

Differential Scanning Calorimetry (DSC) is a popular method for determining how temperature affects the structural changes and thermodynamic stability of proteins and their conjugates. The temperature at which a polymer transitions from crystalline to amorphous form determines the strength of its intra- and intermolecular linkages, which is directly proportional to the polymer's glass transition temperature (Tg). A greater Tg suggests stronger bonding between monomers, implying that the polymer is more likely to be crystalline. In contrast, a lower Tg indicates weaker monomer bonding, implying that the polymer is amorphous. This melting temperature is important for considering potential parameter choices, to

guarantee that the material is processed within the proper temperature range. Differential Scanning Calorimetry (DSC) analysis was used in this case to assess the purity of commercially procured whey protein, which was then compared to DSC profiles reported in the literature. All the samples showed a melting temperature which suggests the similarity in the extracted in the denaturation phase which involves the unfolding of the protein structure, leading to the loss of its native properties. The highest melting temperature was noticed in the commercialized sample which suggests that the protein structure in this sample is the most thermally stable. Compared to the extracted samples, they are slightly less stable in their native form which suggests a more brittle structure that can be caused by the purity or the pretreatment of the liquid whey beforehand. Other studies of untrated and treated whey proteins show similar results [31]. The sharpness and definition of the DSC temperatures give an overall insight into the purity of the proteins and based on these results, the extraction processes are promising and could use a slight optimization in the parameters and the pretreatment of the samples.

4.3. FTIR Characterization of the Samples

The stretching vibration of the O-H and N-H groups is attributed to a significant maximum band, prominently located around 3276 cm-1, [32] serves as a pivotal indicator of the structural integrity of whey protein samples. Across Figs. 8, 9, 10, and 11, this characteristic feature remains consistent, suggesting the preservation of essential molecular elements during the extraction process. However, the larger contour of the equivalent peak in Fig. 10 hints at potential compositional variations in the whey under scrutiny.

Furthermore, the spectral region spanning from 3000 cm-1 to 2500 cm-1, corresponding to secondary bonds and C-H stretching [25], offers valuable insights into the molecular composition of whey protein. Figures 8, 9, and 10 depict distinct peaks in this range, further affirming the presence of characteristic protein structures [33]. Additionally, the presence of peaks in the 1500-1650 cm-1 ranges, attributed to N-H (Amide II) and O-H bending hydroxyl or water bending groups [33], [34], underscores the protein quantity within the samples. While Figs. 8 and 9 exhibit prominent peaks in this region, Fig. 9 displays a reduced magnitude, and Fig. 10 deviates slightly from the established pattern at 1590 cm-1. The consistent spectral signature between Figures 8 and 9 suggests comparable protein compositions, with the thermal extraction method probably yielding the most precise and purest product. Conversely, the higher number of peaks visible in Fig. 8 may stem from the residual ethanol concentration after extraction [35], while the larger, less defined peaks in Fig. 10 indicate the presence of remaining fat molecules in the introduced whey sample [36], [37]. In essence, detailed analysis of the FT-IR spectra in these figures provides valuable insights into the structural variations and compositional complexities associated with different whey protein extraction methods, thereby offering crucial guidance for optimizing extraction processes and ensuring product quality.

Table 1. TGA results of the extracted and crude commercialized whey protein.

Sample	Residue	1st decomp. phase		2nd decomp. phase		3rd decomp. phase	
	%	°C	%	°C	%	°C	%
Therm. Extraction	0,2	40,15	10,396	274,12	89,404	-	-
Org. Extraction	0,35	52,68	15,749	288,92	83,901	-	-
Lyo. Extraction	0,24	56,89	17,92	291,44	81,84	-	-
Comm. Whey proteins	1,611	68,66	6,636	325,51	70,32	909,82	16,55

Table 2. DSC characterization results of the samples extracted and crude commercialized whey protein.

Samples	Tm (°C)	Reaction heat, ΔH (J/g)
Therm. Extraction	74,12	137,82
Org. Extraction	78,74	144,22
Lyo. Extraction	79,15	147,80
Comm. Whey protein	85,61	158,5

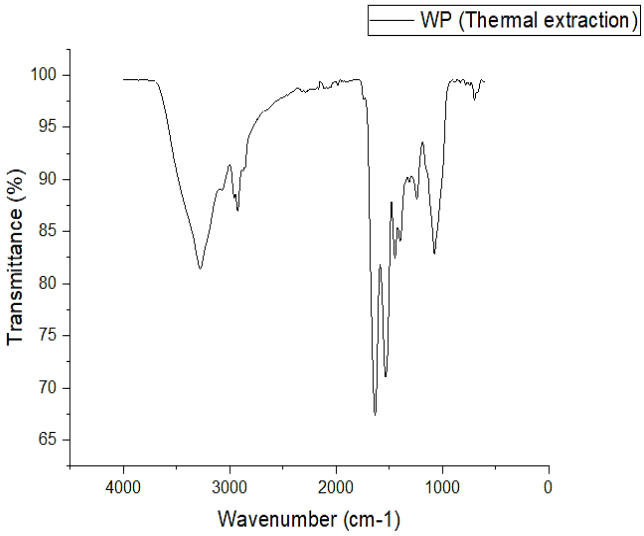

Fig. 8. FTIR spectrum of Thermally extracted whey protein.

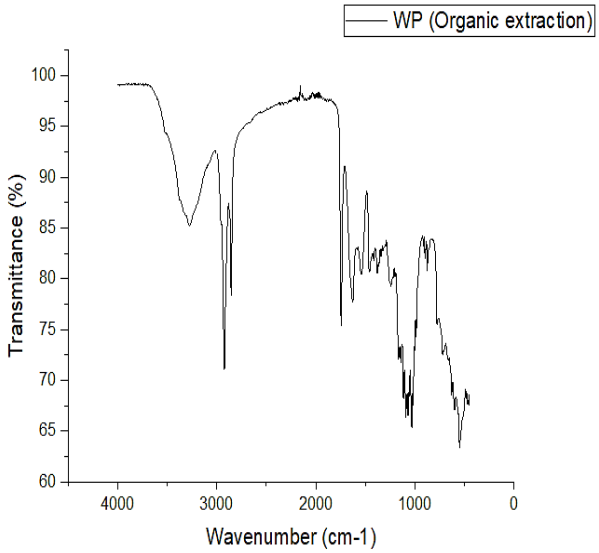

Fig. 9. FTIR spectrum of organically extracted whey protein.

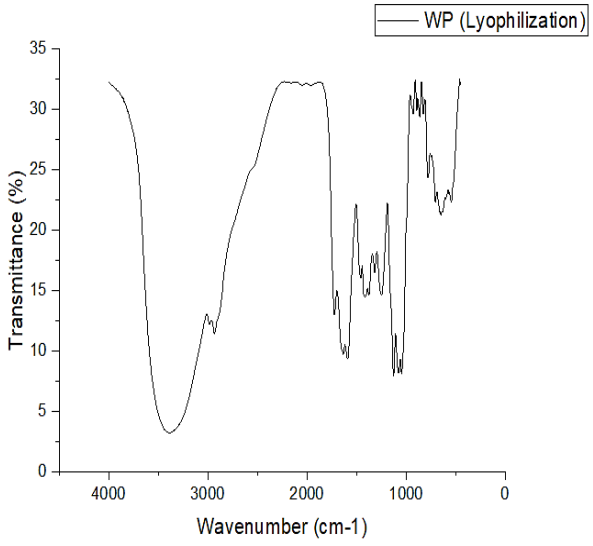

Fig. 10. FTIR sectrum of extracted whey protein through lyophilization process.

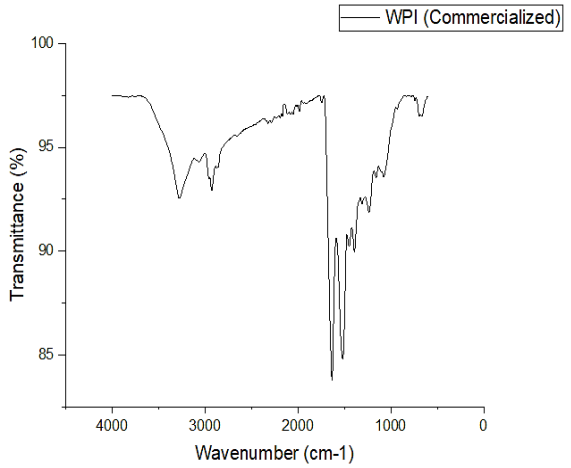

Fig. 11. FTIR stectrum of the commercialized crude whey protein.

5. Conclusion

This study presents a comprehensive analysis of whey protein isolates using Fourier Transform Infrared Spectroscopy (FT-IR), Thermogravimetric Analysis (TGA), and Differential Scanning Calorimetry (DSC) to evaluate structural, thermal, and compositional characteristics across various extraction methods. The findings highlight the significant influence of extraction techniques on the thermal stability, purity, and molecular composition of whey proteins. TGA results reveal distinct degradation stages, emphasizing the role of covalent peptide bonds and mineral residues, while DSC profiles underscore the thermal stability differences, with commercial whey demonstrating superior stability due to additives. FT-IR spectra provide detailed insights into molecular integrity, showcasing consistent structural features but also identifying compositional variations linked to extraction processes.

By combining these methodologies, this research offers guidance for optimizing extraction and pretreatment techniques to enhance protein quality and functionality. It underscores the critical importance of refining processing methods to address challenges in sustainability, resource efficiency, and innovation within the dairy and protein industries. The integration of advanced analytical techniques not only broadens the scientific understanding of whey protein structure but also supports advancements in production methods, fostering progress in sustainable protein utilization.

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Credit Authorship Contribution Statement

Goudali Lina: Writing – Orginal Draft, Writing – Review & Editing, Conceptualization, Methodology, Validation and Visualization. **Nagoor Basha Shaik:** Review & Editing, Methodology and Validation. **Belouaggadia Naoual:** Conceptualization, Review & Editing and Supervision. **Jammoukh Mustapha:** Conceptualization, Review & Editing and Supervision. **Elfarissi Latifla:** Resources, Supervision and Methodology.

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