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Research Article

Characterization of Soy Cheese Spread Using Plant-based Rennet Obtained from Kiwi Fruit Extract: Morphological, Physico-chemical, Antioxidant and Antimicrobial Properties

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Abstract

This study aimed to evaluate the potential of plant-based rennet obtained from kiwi fruit extract for the production of soy cheese spread. The morphological, physico-chemical, antioxidant, and antimicrobial properties of the resulting product were characterized. The results indicated that the use of kiwi fruit extract as a source of rennet resulted in soy cheese spread with a desirable texture, flavor, and appearance. The product also exhibited good physico-chemical properties, such as low moisture content and high protein content. Additionally, the soy cheese spread showed good antioxidant activity, indicating its potential as a functional food. Moreover, the product exhibited significant antimicrobial activity against common foodborne pathogens, making it a safer option for consumption. Therefore, plant-based rennet obtained from kiwi fruit extract could be considered as a promising alternative to traditional animal-based rennet for the production of soy cheese spread.

Keywords: Soy Cheese Spread; SEM; EDS; Antioxidant Activity; Antimicrobial Activity; Physico-chemical; FTIR Spectroscopy

Graphical Abstract

Introduction

For many people, cheese is a favorite food item, in addition to being an important ingredient in favorite foods, such as pizza, lasagna, enchiladas, and cheeseburgers. Traditionally, cheese has been made with milk from a variety of animals, primarily from cows, but also from goats, sheep, water buffalo, and others [1]. As interest in reducing, one's consumption of animal-based products has grown due to concerns related to health, sustainability, and animal welfare [2]. In terms of conventional production of dairy, there are three major areas of concern: environment impact (emissions of greenhouse gases, pollution of soil and water, and land use), human health (exposure to zoonotic diseases and increased antibiotic resistance), and animal welfare (treatment of farmed animals, including disease, injury, and mental/emotional well-being). Therefore, plant-based products offer a more sustainable and ethical option to consumers that are rapidly increasing in

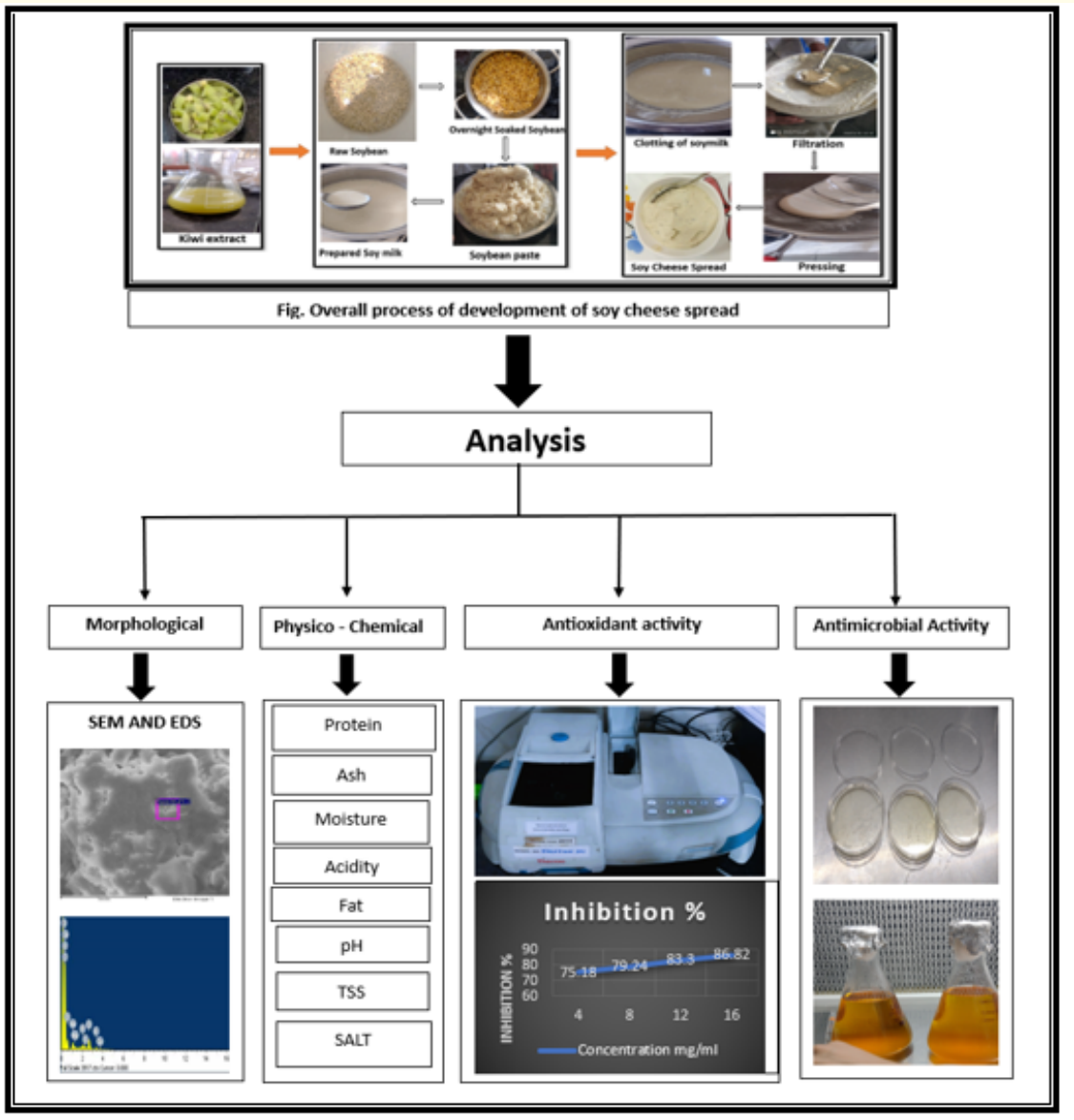

Figure 1

popularity among consumers. plant-based cheese alternatives based on nuts, oils, grains, soy, and other plant products have been developed [3].

Soybeans are excellent source of high-quality protein and has many uses in human nutrition [4]. Consumption of soy foods and the incorporation of soymilk and its by-products in human diets are increasing due to their reported beneficial effects on nutrition and health. These effects include lowering of plasma cholesterol, prevention of cancer, diabetes, osteoporosis, and obesity, and protection against bowel and kidney disease, and relief of menopausal problems [5]. Soybean proteins are used in human foods in a variety of forms, including infant formulas, flours, protein isolates and concentrates, and texturizing fibers. Soy foods include cheese, drinks, miso, tempeh, tofu, salami, and vegetarian

meat substitutes. New soy foods are continually being developed [6].

Milk and milk products are increasing around the world with the future has been expected. More than 6 billion people consumed milk and milk products in the world. Worldwide milk production has been estimated 843 million tons in 2018, with an increase of 2.2 percent from 2017 [7]. In India, about 66.8 million metric tons of milk was consumed in 2018. The demand for milk is increasing day by day and fulfilling the future need will be a big challenge [8]. About 55-58 percent of the human population has little or no ability to digest lactose after infancy. That's why there is need of a substitute which can replace the bovine milk. Soy milk has been considered as an excellent economic dairy substitute [9].

Soymilk is a cheaper source of high-quality protein with many other health-promoting properties. Currently, it is most popular non-dairy alternative for bovine milk [10]. It has the same profile of protein as cow milk even it gives fewer calories on consumption than bovine milk. Per cup consumption of soymilk provides about 7 grams of protein whereas per cup of cow milk gives 8 grams. It is one of the high-quality "complete" proteins of plant-based sources.

Cheese is a popular dairy product that has been consumed worldwide for centuries. However, traditional cheese production involves the use of animal-based rennet, which is not suitable for vegan or lactose-intolerant individuals. Therefore, there is a growing interest in the development of plant-based rennet as a sustainable and ethical alternative to animal-based rennet [11]. Various plant sources have been investigated for their ability to produce coagulant enzymes that can replace animal-based rennet, including kiwi fruit extract. Kiwi fruit is a rich source of proteolytic enzymes, including actinidin, which has been shown to have similar coagulating properties to animal rennet in cheese production. Moreover, kiwi fruit extract contains various bioactive compounds with potential health benefits, such as antioxidant and antimicrobial properties [12].

Cheese is a generic term for a diverse group of milk-based food products. Cheese is produced throughout the world in wide-ranging flavors, textures, and forms. Processed cheese is produced by blending natural cheese of different ages and degrees of maturity in the presence of emulsifying salts and other dairy and non-dairy ingredients followed by heating and continuous mixing to form a homogeneous product with an extended shelf life. The origin of processed cheese dates back to the early 20th century [13]. Contrary to the present status of processed cheese, the initial idea of processed cheese was to increase the shelf life of natural cheese and alternative uses for natural cheese that was difficult to sell. Cheese analogues are being used increasingly due to their cost-effectiveness, attributable to the simplicity of their manufacture and the replacement of selected milk ingredients by cheaper vegetable product [14]. Because of a recent expansion in the use of cheese spreaded bread as a meal or a main meal supplement into countries where such a food has not been customarily used (particularly the Asian countries), the need for such spreadable products has increased accordingly [15]. Most spreadable cheese products are made with dairy products derived from mammalian milk (primarily cow milk). Needless to say, such dairy products are presently in short supply in many of these countries and future supply will even become less because of the limited land area available for raising dairy animals and feeds therefor, particularly in view of the rapidly expanding population. In the world's context, cheese is highly demanding dairy product [16,17]. It is well known that soybeans have a very high protein content and represent a much more productive source of protein than dairy animals. Therefore, considerable effort has been directed toward making an acceptable spreadable cheese-like product using soybean derived products as a primary raw material [18].

Cheese is a wealthy supply of minerals, protein, vitamin, Fats and carbohydrate [19,20]. The physical appearance, texture, composition and the basic processing technology of soy cheese are quite similar to the milk cheese in many aspects. Unfortunately, unlike milk cheese, comprehensive scientific studies of soy cheese leading to further developments and improvements were not carried out in the past. Soy cheese spread is a popular non-dairy alternative to traditional cheese, but its production requires a suitable coagulant. Therefore, the present study aimed to investigate the potential of plant-based rennet obtained from kiwi fruit extract for the production of soy cheese spread. The morphological, physico-chemical, antioxidant, and antimicrobial properties of the resulting product were characterized. The use of plant-based rennet in cheese production has gained significant attention in

recent years due to ethical and environmental concerns related to animal-based rennet. Furthermore, the potential health benefits of plant-based coagulants, such as antioxidant and antimicrobial properties, make them an attractive option for functional food production.

Material and Methods

Material

The experiment was carried out at the food science and technology laboratory, Babasaheb Bhimrao Ambedkar University, Lucknow, U.P., India. In this analysis, the sample of the prepared soy cheese spread was taken as per needed.

Equipment

Weighing Machine, scanning electron microscope, Fourier transform infrared spectrometer, digital pH meter, UV Visible Spectrophotometer, laminar air flow, digital refractometer, muffle furnace, Soxhlet appratus, Kjeldahl apparatus, etc.

SEM technique

Scanning electron microscope (SEM) is a method for imaging the morphology and microstructure of the material and EDS (Energy dispersive X-ray spectroscopy) is used to provide element identification. The microstructure of soy cheese spread was analyzed by scanning electron microscopy using SEM (Model: JSM6490LV, JEOL, JAPAN). Samples were mounted on aluminium stub using double-sided carbon tape, then the sample was coated using a sputter coater (JEOL JFC-1600) auto fine coater.

Figure 2: Scanning Electron microscope.

FTIR analysis of Soy cheese spread

The functional group of cheese spread was determined by analysis using Fourier transform infrared (FTIR) spectrometer. FTIR analysis was performed at instrument research centre of Babasaheb Bhimrao Ambedkar University, Lucknow (UP). The FTIR with model no. Nicolet 6700, Thermo-scientific, USA. The spectral range is 4000-500 cm^{1}. Sample with practical size < 0.15 mm was employed for analysis.

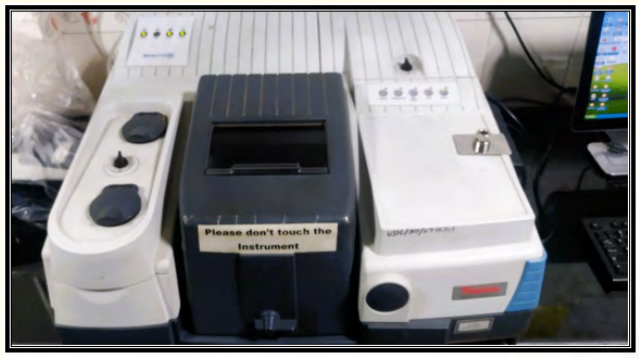

Figure 3: Fourier transform infrared spectrometer (FTIR).

Physico-chemical analysis

Determination of protein

The protein content of processed cheese spread determined by kjeldahl method described in AOAC (1980). The Kjeldhal method for protein analysis is based on nitrogen determination. The nitrogen content was determined by Kjeldhal distillation as described by IS: 7219-1973 RA 2005 methods and converted to total protein by multiplying with a factor 6.25 for nitrogen.

Total protein (%) = Titre value × Normality of acid × 0.014 × 100 Sample weight

Protein conversion factor = 6.38 for milk

Protein (%) = Percentage of Nitrogen × 6.38 Normality of Acid = 0.1 N

Determination of fat content

Fat percentage of processed cheese spread was determined by Soxhlet method as per adopting the procedure as laid down

in manual in Analytical Techniques for Scientists, 2012. Soxhlet extraction method is a continuous solvent that is usually used to remove fats from the sample. Other that that, the determination of crude fats in the food sample can also be determined by using the Soxhlet extraction. About 2 grams of cheese spread sample was weighed and put in thimbles using a dry paper and plugged with cotton wool. The thimbles were dried and inserted into a soxhlet system. The extraction round bottom flask were dried and weighed and then 50 ml solvent (petroleum ether) was added in each round bottom flask. The samples were extracted for 15 minutes in a boiling position. The extraction was carried out continuously for three hours. This was cooled and reweighed. The following formula for fat content was:

Fat content (%) = W_2 - W_1/W × 100

Where,

W = Weight of the sample.

W1 = Initial weight of round bottom flask.

W2 = Final weight of round bottom flask.

Determination of ash content

Ash percentage of processed cheese spread was determined from the procedure as laid down in manual in dairy chemistry I.C.A.R (1972). About 2 grams of the cheese spread sample was weighed into a porcelain crucible previously ignited and weighed. The material was ignited in the fume until no fume was seen charred of organic matter. This was then transferred into a muffle furnace at 550˚C using a pair of tongs and was ignited for 6 hours, cooled in a desiccator, and weighed immediately. The formula for ash content was –

Ash (%) = W_3- W_1/W_2-W_1× 100

Where,

W_1 = Weight of empty crucible

W_2 = Weight of sample before drying (crucible + sample)

W_3 = Weight of sample after ashing (crucible + sample)

Determination of moisture content

The moisture content of soy cheese spread was determined as per the procedure given manual in dairy chemistry, I.C.A.R (1972). Moisture content of cheese spread sample was determined by Hot Air-Oven method. The hot air ovens are electrically heated and the air within them is at atmospheric pressure and is circulated by convection with the help of fan or mechnical means. 10 g of the extracted samples were weighed accurately in the moisture dish. The samples were dried in the oven at 105˚C and weighed. The dish was placed in the oven and maintained at 105 ± 20˚C for 4 hours. After 4 hours, the samples were cooled in the desiccator and weighed. It was calculated as-

Moisture Content (%) = ÷ W × 100

Where,

W = Wet Weight

D = Wet after Drying

Determination of acidity

For determination of titrable acidity, the titermetric method was used according to the AOAC. 5 g of sample taken and dispersed in 100 ml of distilled water in 250 ml conical flask, after which six drops of phenolpthalein indicator have been brought. The sample turned into then titrated with 0.1 N sodium hydroxide until a stable pink color changed into fashioned. The titrable acidity changed into expressed as % lactic acid from the subsequent formula.

Titrable acidity (%) = $\frac{\text{ml of NaOH} \times T \times \text{Dilution Factor} \times 100}{\text{Weight of sample} \times 1000}$

Where,

T = amount of lactic acid reacted with 1.0 ml of 0.1 N sodium hydroxide.

Determination of pH

The pH of soy cheese spread sample was determined using digital pH meter (LT- 501). The pH meter was calibrated with buffers of pH 4 and 7. The cheese spread sample was stirred and the pH value was recorded according to AOAC.

Determination of TSS

The total solid content of the soy cheese spread sample was determined using digital refractometer. For determining the TSS, a drop of the sample is placed on the prism and the percentage of dry subtance in it is read directly. The reading was expressed as a percentage of concentration of total soluble solids as ˚Brix.

Determination of salt

The salt content of soy cheese spread sample was determined according to AOAC. 5 gm of sample have been weighed and placed into 250 ml conical flask, additionally 100 ml boiling water were brought. Then swired for 10 min and cooled to 50 - 55˚C. Titrated against to silver nitrate until the color of indicator potassium chromate changed from faded yellow to buffered color and the reading is noted (v). Calculation of salt % w/w as follows:

$$\text{Salt \% (w/w)} = 58.45 \times V \times N \div W \times 100$$

Determination of antioxidant activity (DPPH)

The antioxidant activity of 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) is calculated via spectrophotometer (Tekao., *et al*. 1994) [12] with small modifications (Kumarasamy., *et al*. 2007) [18]. In methanol, the color of DPPH is dark blue. In its reduced form, the antioxidant compound changes color from purple to yellow, allowing DPPH to gain electron. DPPH shows strong absorption at 517 nm, determined by 2,2-diphenyl-1-picrylhydrazine (DPPH). Briefly, 0.002 g DPPH was taken and made to 50 ml by adding methanol. 1g of food sample has been taken in a round bottom flask and in that 10 ml of methanol was added for extraction. After extraction, the food sample was filtrated by filter paper in a flask. From the sample different concentration has been taken and for control DPPH has taken and incubated in darkroom for 30 minutes at ambient temperature. After incubation, the absorbance of the sample was read at 517nm using a UV Visible spectrophotometer. Methanol was used as a blank. Reduction in the absorbance value, shows high activity in scavenging free radicals (Zubeyir et al. 2017)[19]. It was measured as a percentage of DPPH scavenging activity by using following formula given below.

$$\text{DPPH Scavenging activity} = (A_{control} - A_{sample}) / A_{control} \times 100$$

Microbiological analysis

Samples of soy cheese spread were prepared for microbiological analysis according to (Marshell. 1992) the method described in the standard methods. The samples were examined for the standard plate count method at 37 ± 1˚C for 24, 48 and 72 hrs interval. The result expressed as colony forming unit (CFU/g). The media used were in a dehydrated form and prepared according to the manufactures instruction. Total viable bacteria were enumerated by pour plate method using standard plate count agar method [9,10] and plate were incubated at 37˚C for 24 hours.

Result and Discussion

Analysis of the microstructure:

SEM (Scanning electron microscope) was created to determine the relationship between the microstructure of samples and their composition (Figure 4). Shows the picture obtained for the soy cheese spread. The SEM-image of soy cheese spread presents Agglomerated structure and a highly branched-like structure and spreadable cheese was compact supermolecule matrixes with little number of erractically spread fat globules were discovered on top of thi ngs cheese just like that privy by [16]. This type of structure is formed for common cheese spread from milk.

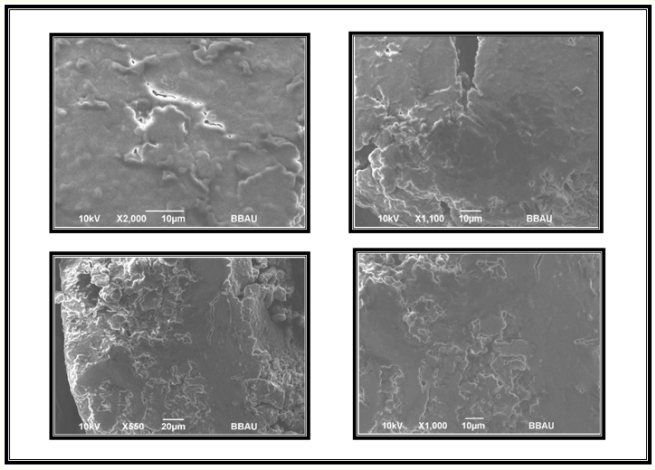

Figure 4: SEM images of soy cheese spread sample.

The EDS graph spectra exhibit the purity of the material and the complete element composition of soy cheese spread.

Energy dispersive x-ray spectroscopy (EDS)

The present EDS figures 5 shows different elements of composition present in soy cheese spread sample. Result shown in figure. Attained from the EDS characterization indicate that the soy cheese spread sample has an carbon content of 70.18%, oxygen content of 23.14%, calcium content 0.19% and Na, P, S, Cl, K are elements found in soy cheese spread sample.

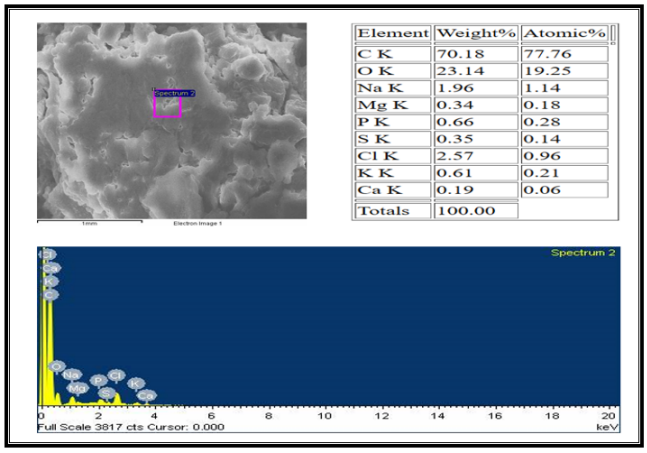

Figure 5: EDS image of soy cheese spread sample.

Proximate composition

The proximate compositions were as shown in Table 1 and figure 6. The protein content of soy cheese spread was 13.5% ± 0.11. It has highest protein composition because it is all soy and soy foods have high protein content and high protein utilization. The fat content of the soy cheese spread was observed from the table to be 16.5% which is less as compaired to dairy cheese spread. The moisture content was 53.23% which make it less vulnerable to microbial attack. Ash composition was 6.32%. The pH of soy cheese spread was 4.5 indicating that it is acidic in nature. The TSS of soy cheese spread was 4.7% and the salt percentage in soy cheese spread sample was found out to be 6.33%. The titrable acidity of the soy cheese spread sample was found out to be 1.944%.

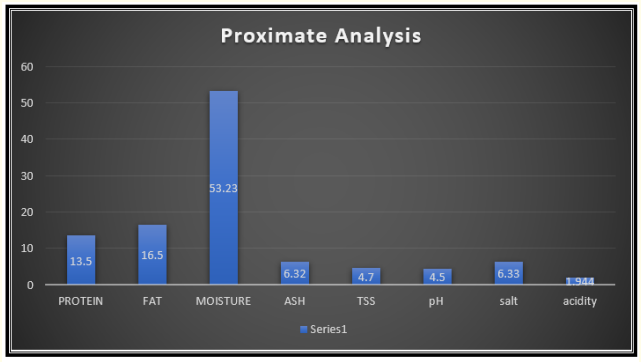

Figure 6: Proximate analysis of soy cheese spread sample.

Proximate Analysis	Soy cheese spread
Protein	13.5%
Fat	16.5%
Moisture	53.23%
Ash	6.32%
TSS	4.7%
pH	4.5
Salt	6.33%
Acidity	1.944%

Table 1: Result of Proximate analysis.

FTIR analysis of soy cheese spread

The infrared spectrum of soy cheese spread is proven in figure 7. consequently, the peak at 3515.0 cm^{-1} were represent O-H streching within the hydroxyl groups. This area in our result turned into the range of 3230-3550 cm^{-1}. The peak at 3008.8 cm^{-1} represent O-H (Acids). The interval of 3000-2800 cm^{-1} from FTIR sprectra offers the absorption bands which characteristic to symmetrical and asymmetrical vibrationnin fatty acids. The interval 1800-1600 cm^{-1} constitute C = O of acids and ester and exhibit versions within the wave number variety (1744.9 cm^{-1}, 1655.0 cm^{-1}) along with absorption from ester of fatty acid that can be attributed to differences in the degree rate of lipolysis at special ranges of ripening. The interval 1600-1400 cm^{-1} represents Amide I and Amide II of protein. This area in our end result became in the variety of 1529.8 cm^{-1}, 1456.3 cm^{-1} as shown in fig.5. The interval 1000-1300 cm^{-1} represent band feature to the C-F strech respresenting Alkyl and Aryl Halides.

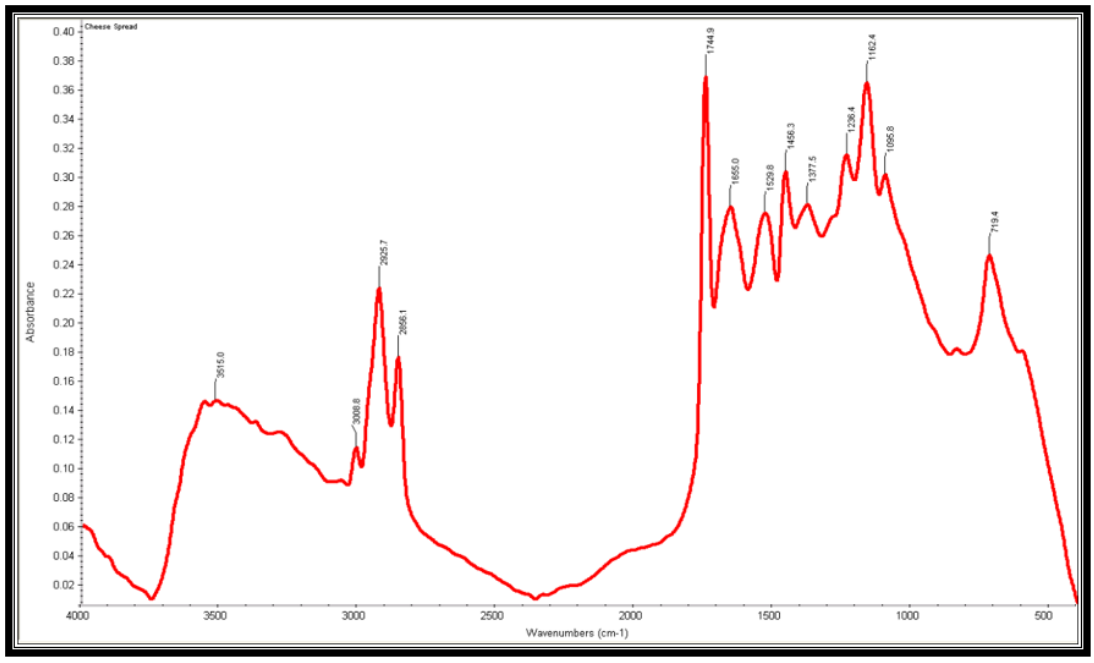

Figure 7: FTIR spectrum of soy cheese spread.

*peak at 3515.0 cm^{-1}, 3008.8 cm^{-1}, 2925.7cm^{-1}, 2856.1cm^{-1}, 1744.9 cm^{-1}, 1655.0 cm^{-1}, 1529.8 cm^{-1}, 1456.3 cm^{-1}, 1377.5 cm^{-1}, 1236.4 cm^{-1}, 1162.4 cm^{-1}, 1095.8 cm^{-1}, 719.4 cm^{-1}.

DPPH Activity of soy cheese spread

DPPH is the most suitable way to determine the antioxidant property of a sample. The colour of the sample changes from purple to yellow as DPPH free radicals are scavenged by antioxidant chemicals. Figure 4. Depicts the relationship between soy cheese spread sample concentration(mg) and antioxidant activity (%). By using a spectrophotometer, the optical density of a sample and optical density of the control can be calculated to determine DPPH value of a sample. According to (Fridianny., *et al*. 2014), if DPPH value is between 50 ug/ml then it has a very strong antioxidant property and if it is above 20.048 mg/ml, it has weak antioxidant property. The antioxidant activity of soy cheese spread sample at different concentration (4, 8, 12, 16u/ml) was evaluated and the result obtained were illustrated in table 2 and figure 8. According to these result, the antioxidant activity of the soy cheese spread can be increased.

Obtained extracted solution (ml)	Concentration mg/ml	Absorbance	Inhibition %	ic50
Control	0	0.408		
1	4	0.397	4.166	1.281134
2	8	0.359	12.009	3.647577
3	12	0.326	20.09	6.014021
4	16	0.310	24.01	8.380465

Table 2: Readings of DPPH.

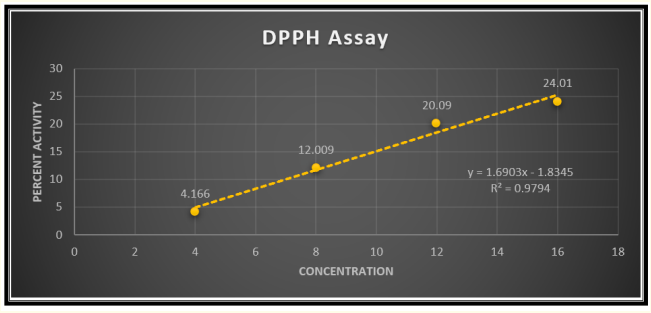

Figure 8: Antioxidant activity of soy cheese spread.

Microbial analysis of soy cheese spread

Changes in microbiological quality during storage of soy cheeese spread was observed. During examine shelf life, major components like protein, fat, tss, ash has major changes to microbial growth. The growth of the microbes was slow as compaired to other dairy products. As shown during the shelf life period treatement, sample with greater dilution show less microbial growth with the time period and can be stored for 1month. The shelf life of the soy cheese spread can also be increased by addition of preservatives.

Total bacterial count: 3.70 x 10^4 colony forming units (CFU)/g

Total yeast and mold count:3.19 x 10^3 CFU/g

The total bacterial count in the soy cheese spread sample was 3.70 x 10^4 CFU/g, indicating that the product may contain a moderate level of bacteria. The total yeast and mold count was 3.19 x 10^3 CFU/g, which falls within acceptable limits for this type of product.

(log10 cfu/gm)	Microbiological count				
	Total bacterial Count				
Sample	Stoarge period				
	24 hrs	48hrs	72hrs	15 days	21days
Plate 1	00	2.62	2.74	3.15	3.70
Plate 2	00	2. 14	2.47	2. 86	3.19

Table 3: The microbiological quality (log10cfu/gm) of soy cheese spread during storage period.

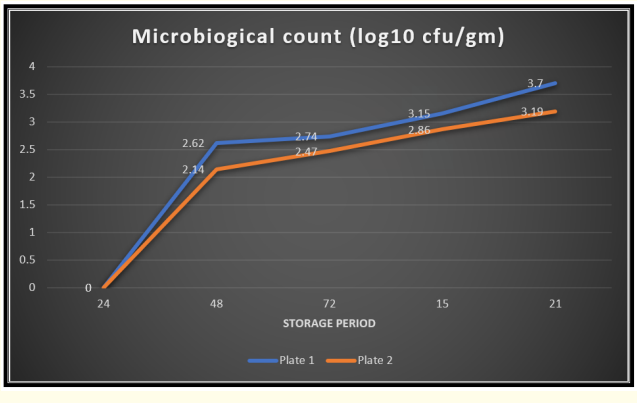

Figure 9: Microbial Analysis.

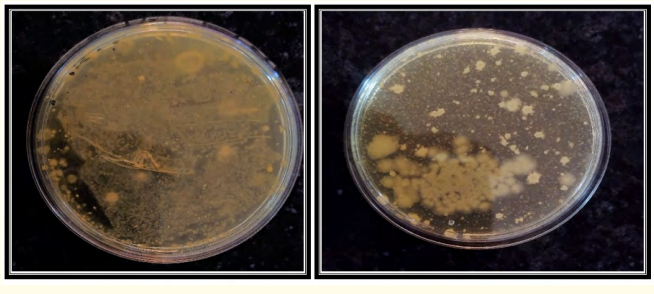

Figure 10: Microbial Growth in petri plate 1st and 2nd after 21 days.

Conclusion

The soy cheese spread was successfully developed by using kiwi fruit extract. The soy cheese spread sample has the good morphological structure. Many functional group was present in the sample. The soy cheese spread sample was found to have highest nutritional value as campaired to animal based product. The soy cheese spread was shown to have good antioxidant activity and the shelf life of the prepared soy cheese spread was 21 days without addition of any preservative. All the results indicate that the soy cheese spread has good antimicrobial as well as antioxidant properties. Thus, the use of soy cheese spread was beneficial from nutritional, microbial and health perspective.

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