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# The impact of adding oat proteins on the physicochemical properties of yogurt

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## Abstract

This research investigates the influence of incorporating oat protein concentrate (OPC) into yogurt on its physicochemical, sensory and structural properties during 21 days of storage at 4°C. The yield and percentage of OPC were assessed at different pH. The highest yield (77%) and protein content (79%) were observed at pH 10, while lower percentages were noted at pH 5 (59% yield, 74% protein content). Samples of yogurt with different OPC concentrations were made: 0% (control), 0.3%, 0.6%, and 1%. The results indicated that both OPC levels and storage periods significantly influenced ($p \leq 0.05$) parameters. The pH value of all samples decreased during storage, while acidity increased. The 1% OPC yogurt maintained higher pH and showed lower acidity (0.96%) compared to the control (1.06%). Water holding capacity (WHC) decreased in all treatments, with the 0.6% OPC group having the lowest WHC (45%) after 21 days. Syneresis increased over time but remained lower in the OPC-treated especially in the 1% OPC (14%) compared to control (22%). Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM) showed that OPC enhanced structural integrity and functional group stability in yogurt. Of the formulations, the 1% OPC sample demonstrated the most stable texture during storage, whereas the 0.6% OPC sample scored the highest sensory evaluation, especially for flavor, texture, and overall acceptability. In conclusion, adding OPC, especially at 0.6%–1%, can improve yogurt's quality, stability, and attractiveness to consumers during cold storage.

***Keywords: Chemical functional properties, Lactic acid fermentation, Milk fortification, Oat protein.***

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## I. INTRODUCTION

The use of natural protein materials, which have high nutritional value and the ability to improve a variety of physical and chemical functions, has advanced significantly in the world. This is because these materials can replace chemical additives, some of which are toxic or carcinogenic when used over an extended period, raising concerns about their safety for human health. Over time, the benefits of consuming foods high in protein, particularly functional foods, have garnered significant attention due to their potential to avoid a host of health issues.(Huang *et al*., 2022; Qasim *et al*., 2021). Around the world yogurt is one of the most widely consumed food items. As a type of functional food product yoghurt has always been well-received by consumers and is made by fermenting milk with lactic acid bacteria. The most commonly used starters are *Streptococcus thermophiles* and *Lactobacillus bulgaricus delbruckii* subsp (Lee and Lucey, 2010).Yoghurt is seen by health-conscious consumers as a special kind of baby food since it helps prevent diarrhea and lowers the risk of cancer by acting as an antioxidant. It improves health, aids with digestion, and increases calcium absorption, which can help avoid Osteoporosis. In order to meet the increasing demand from consumers and to enhance the nutritional value, quality attributes, and health benefits of the products, various varieties of yogurt are now being manufactured. The qualities of many goods we use daily basis are impacted by the presence of proteins, which are an important part of our diet. Due to the linked health benefits and rising consumer knowledge of health issues, consumers are becoming more interested in switching from eating animal protein to plant protein. Furthermore, in order to maximize resources, maximize efficiency, and protect biodiversity, plant proteins that can replace conventional animal ingredients with plant-based alternatives are required (Loken and DeClark, 2020).Texture, color, smell, and other sensory characteristics of the product influence how the consumer feels about it and how much they like it (Pramudya and Han-Seok, 2019). It is possible to add some ingredients to food to enhance its flavor, color, and aroma. Since it was recently discovered that adding more than 6% causes unfavorable sensory impressions, the texture, especially yogurt, is typically improved by adding some solids, such as skim milk, by 3-4% (Karam *et al*.,2013). It is possible to keep the texture and sensory qualities of yogurt by utilizing proteins from different sources, such as oat proteins.

The significance of oat grains (*Avena sativa* L.) to consumers has drawn the attention of numerous studies. Compared to other grains, grains are ingested in the form of baked foods or liquids. The high protein and essential amino acid content of oat grains contributes to their great nutritional value. Enhancing oats with milk, which is a source of lysine and increases the nutritional content of dairy products, is preferred as oats, like other cereals, lack this amino acid (M€akinen *et al*., 2017). Via hydrophobic contacts, hydrogen bonds, ionic interactions, and covalent bonds, proteins associate with one another to form complex structures that affect texture, viscoelasticity, stability, and microstructure. According to Mäkinen *et al*., (2016), these characteristics forecast an element that will function effectively from a technological, nutritional, and sensory standpoint, all of which will shape its attractiveness.

## II. MATERIAL AND METHOD

**Material**

Pasteurized cow milk (3.1% protein, 3.2% fat, 3.54% lactose, and pH 6.97) and oat (*Avena sativa*) seeds were collected from the local market in Basrah, Iraq. *Streptococcus thermophilus* (*S. thermophilus*) and *Lactobacillus delbrueckii subsp. bulgaricus* (*L. bulgaricus*) were obtained from the Department of Food Science, College of Agriculture, Iraq. Chemicals utilized in this study were purchased from Merck (Sigma Aldrich).

**Preparation of oat protein concentrate**

The oat protein concentrate was made following the method described by Li and Huang (2020), beginning, 100 g of oat flour was mixed with 600 ml of distilled water. The pH of the mixture was adjusted to10 by adding 2 M

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sodium hydroxide. The solution was mixed for 150 min at 40°C using a magnetic homogenizer. It was centrifuged for 15 min at 3000 rpm. The supernatant was collected, and the pH was adjusted to5 by adding 0.5 M hydrochloric acid. The mixture was allowed to stand for 120 min for protein precipitation. The precipitated protein was washed three times and centrifuged again for 15 min at 3000 rpm . The pH was adjusted to 7 and the protein was dried in an oven at 40°C (Figure 1).

**Figure 1**. Stages of oat protein extraction

**Estimation of protein percentage**

The protein percentage was determined by, using the Micro Kjeldahl method as described by AOAC (2010).

**Measurement of oat protein concentrate yield**

The protein concentrate yield from oat seeds was determined using the method described by Kim *et al*. (2020). The percentage of protein concentrate was calculated using the following equation:

Yield (%) = (weight of concentrated protein (g) / weight of oat flour(g)) x 100

**Manufacture of yogurt**

Oat protein concentrated was suspended in 100 g milk at three enrichment levels used, 0%, 0.3%, 0.6%, and 1%, resulting in four formulation YC (control,0%), YO1(0.3%), YO2(0.6%), and YO3(1%). Due to the poor solubility of oat protein concentrated, all mixtures were stirred for 30 min at 37 °C using a magnetic stirrer. The temperature was then increased to 80 °C and held for 20 min. The mixture was then cooled to 40 °C in a water bath and 0.2U yogurt culture containing *Lactobacillus bulgaricus* and *Streptococcus thermophiles* were added. The inoculated milk was transferred to 100 g plastic containers, and incubated at 42°C until the pH reached to 4.6. All of the samples were stored in a refrigerated at 4 ± 1°C. for a period of 21 day (Brückner-Gühmann *et al*., 2019).

**Physico-Chemical properties of Yogurt**

The pH value of the yogurt was measured using a pH meter (Crisongl p22, Japan). Titratable acidity was determined by titration with 0.1N NaOH, following the AOAC method (2010), and the percentage of total acidity was then calculated using the following equation:

$$Titration\ acidity\ \% = \frac{(0.09\ \times (0.1\text{N}) \times \text{ml NaOH})}{weight\ sample} \times 100$$

**Water-Holding Capacity (WHC)**

The water holding capacity(WHC) was estimated using the method described by Raikos *et al*. (2020), A10 ml of the sample was weighed and then centrifuged at 4000 rpm. After centrifugation, the suspension was then separated, weighed, and the water holding capacity was determined using the following equation:

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$$\text{WHC \%} = \frac{\text{weight of samples} - \text{weight of separated liquid}}{\text{weight of samples}} \times 100$$

**Syneresis**

Five grams of each sample were placed on Whatman No. 1 filter paper over a glass container and left for 120 min at 4°C (Kailasapathy, 2006) . The weight of the liquid collected in the glass container was measured and syneresis was calculated using the following equation:

Syneresis (%) = A/B ×100

A= total weight of separated liquid

B =weight of the sample (g)

**Study of the Compositional Characteristics of Yogurt Formulas**

**Fourier Transform Infrared Spectroscopy (FTIR)**

The method described by Zhou *et al*. (2022) was followed for identifying active functional groups. For analysis, 20 mg of each lyophilized yogurt sample was mixed with 10 mg of anhydrous potassium bromide (KBr). The spectral range was between 400–4000 $cm^{-1}$ .using Fourier transform infrared (FTIR) spectroscopy (Japanese Jasco), located at the Polymer Research , University of Basrah.

**Microstructure analysis Using Scanning Electron Microscopy (SEM)**

The morphological characteristics were examined using a scanning electron microscope(SEM), model Balzers, type DEMg60A, at the Faculty of Pharmacy .The analysis was conducted following the methodology described by Brückner-Gühmann *et al*. (2018).

**Sensory Evaluation**

Eleven trained panelists evaluated the sensory attributes of yogurt from the Department of Food Sciences, College of Agriculture, University of Basrah. The evaluation was based on that assessed five attributes related to color and appearance, body and texture, flavor and aroma, mouthfeel, general acceptance , as outlined in Table 1. According to Zhao *et al*. (2023) with some modifications.

**Table (1).** Sensory evaluation for yogurt samples

| Samples | Color And Appearance 15 | Body and Texture 35 | Flavor and aroma 15 | Mouthfeel 20 | General Acceptance 15 | Total |
|---|---|---|---|---|---|---|
| YC | | | | | | |
| YO1 | | | | | | |
| YO2 | | | | | | |
| YO3 | | | | | | |

**Statistical analysis**

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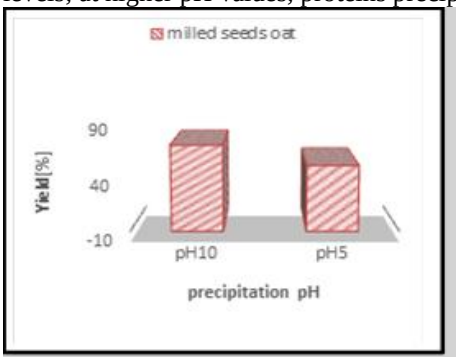
A completely randomized design (One –way ANOVA) was used to analyze experimental data using SPSS software (2018) .The differences among means were assessed using the Least Significant Difference (L.S.D.) test at P≤ 0.05.

## III. RESULTS AND DISCUSSION

**Yield and Content of Oat Protein Concentrate.**

The percentage of the yield and protein content of oat protein concentrate at different pH values are showed in Figures 1 and 2. The results demonstrate significant differences in both yield and protein content depending on the pH, determinate that the production of a high percentage of yields and protein content may be affected by the pH values. At pH 10 the yield and protein content were 77% and 79%, respectively. While, when the pH was reached to 5, the yield decreased to 59%, and the protein content fell to 74%. Oat proteins exhibit low solubility at low pH levels; at higher pH values, proteins precipitated and display higher solubility compared to those



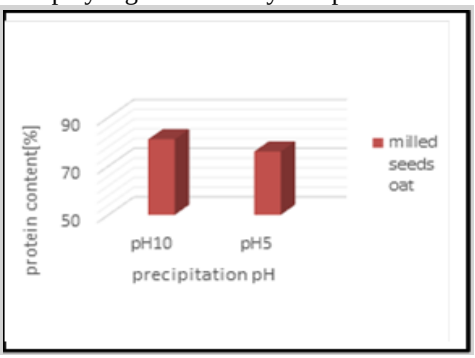




Figure 1: Percentage yield of oat protein extraction at different pH values

Figure 2: Percentage of protein content concentrate in oat protein concentrates at different

**Physico-Chemical Properties of Yogurt**

**pH and Titration Acidity**

Figure 3, illustrates the variation in pH values of the yogurt enriched with oat protein concentrate at different concentrations, as well as the control treatment during over cold storage periods (1, 7, 14, and 21 days) The statistical analysis show significant differences at (p≤0.05). On the first day of storage, the pH values of the YO1, YO2, and YO3 treatments were 4.65, 4.76, and 4.8 for, respectively. After 21 days. the pH values decreased to 4.20, 4.37, and 4.54, for three treatments, respectively. In comparison with the YC had pH values of 4.57 on day 1and 3.92 on day 21 storage. The sample containing the concentrated oat protein showed a decrease in pH during storage. This decrease might be explained by post-acidification caused

by lactic acid bacteria, which convert lactose to lactic acid to continue metabolic activity. In this study, the average pH values decreased after storage but remained within the range of 4 to4.4 when compared to the control (Donkor *et al*., 2006 ; Deshwal *et al*., 2021). According to Bulut *et al*. (2023), the proteins in oats, peas, and peanuts act as

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buffer solutions to prevent pH value fluctuations. This buffering effect helps maintain the acid function and prevents the pH from dropping below 4.6.

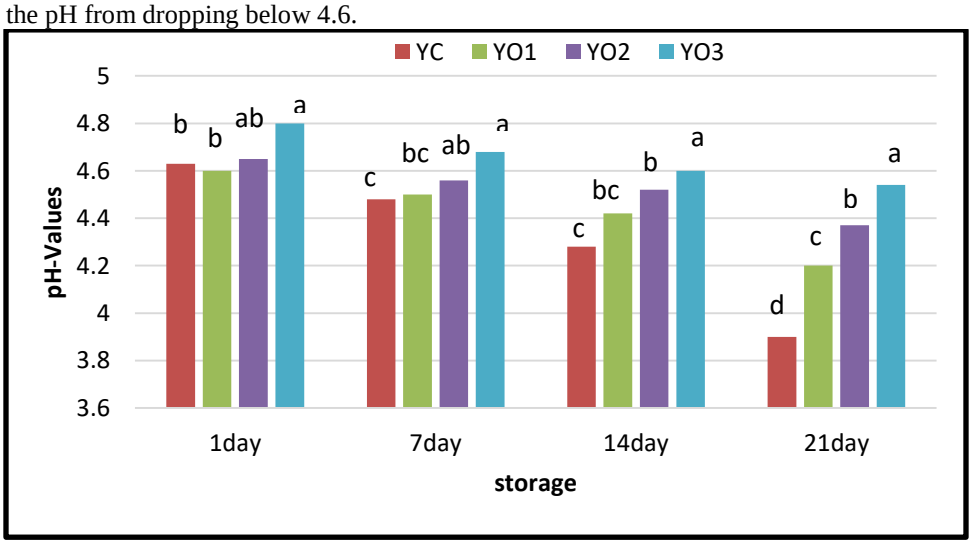


**Figure 3**. pH of yogurt enriched with oat protein concentrate at various concentrations during cold storage**.**

Figure 4. shows that the acidity level. of all yogurt samples increased over time, from the first day to the last, during storage .The statistical analysis revealed significant differences ($p \leq 0.05$) for the product treatments enriched with oat protein concentrate. The acidity values ranging from 0.74% to 1.06% in the YC treatment and from 0.68 to 0.96% for the YO3 treatments at 1 and 21 days respectively. The increase in acidity levels can be attributed to the continuous hydrolysis of lactose by the lactic acid bacteria's, which also increases the bacterial population over time (Bulut *et al*., 2023).

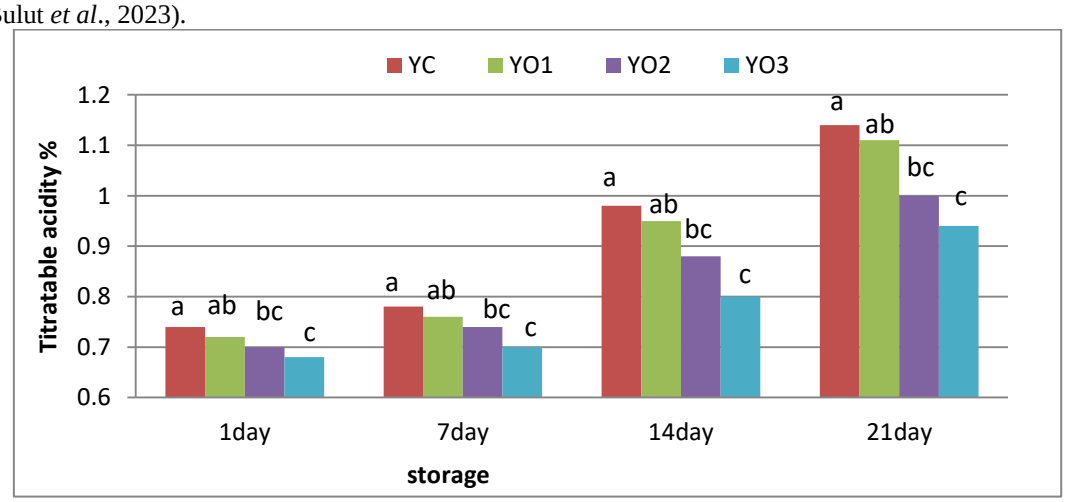


**Figure 4.** Titratable acidity of yogurt enriched with oat protein concentrate at various concentrations**.**

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**Water holding capacity (WHO)**

One of the important and practical factors that determine the quality of fermented dairy products is their water holding capacity. This property helps to extend the product's the shelf life and is evaluated depending on its quality. In this study the WHC levels of yogurt ranged from 84-75% on the first day of storage (Figure 5). After 21 days of storage, the WHC value decreased significantly to 75-45%, for the YC and YO1 samples, with the YO1 treatment containing 0.3% oat protein concentrate showing the lowest WHC value. Similar results were reported by Xu *et al*., (2022), who found that WHC decreased when yogurt was mixed with fruits or rice bran. The WHC of yogurt enriched with oat protein concentrate decreased during the storage periods ($p \leq 0.05$). These results indicate that, over time, there is an increase in the water holding capacity and a decrease in the whey Syneresis. This can be can be attributed several factors, including the use of a combination of seed proteins, such as oat protein concentrate, and animal proteins, such as milk proteins. The heat treatment of these proteins modifies their structural composition, leading to the formation of a three-dimensional gel network containing hydrophobic, hydrophilic, and hydrogen bonding groups. The most common types of bonds involved in this network are disulfide bridges and hydrogen bonds (Ali and Ahmed, 2018). Several studies have shown that the formation of WHC depends on multiple factors, including the metabolites and structural protein of lactic acid bacteria, as well as the type and concentration of protein added to the yogurt-curd mixture. This results in the formation of a three-dimensional gel network (Xu *et al*., 2022; Ziarno *et al*., 2023).

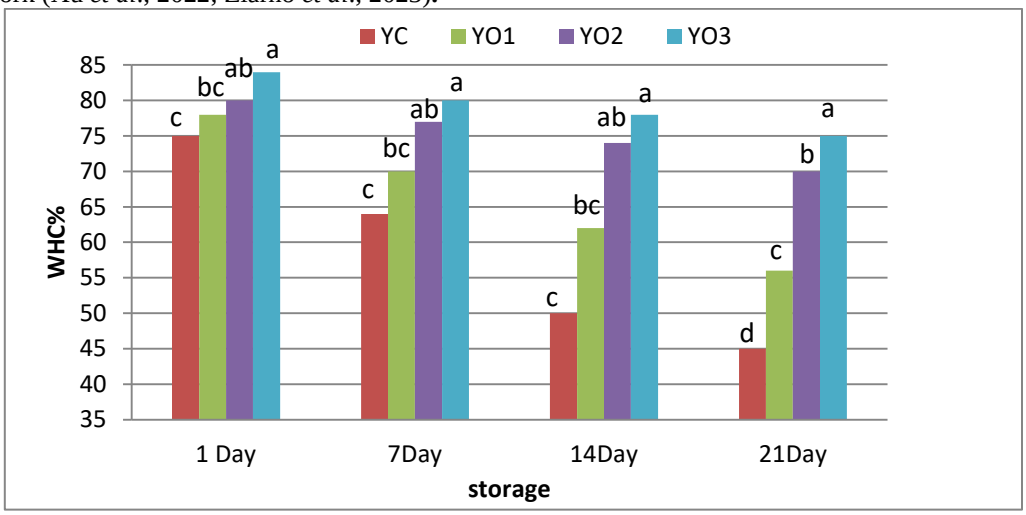


**Figure 5.** Water holding capacity of yogurt enriched with oat protein concentrate at various concentrations.

**Syneresis**

The syneresis results for yogurt are shown in Figure 6. On the first day of storage, the syneresis values were 4.10, 2.07, and 0.50% for treatments YO1, YO2, and YO3, respectively. While, the syneresis increased in YC treatment, reaching 5.17. After 21 days of storage, the syneresis values for the three treatments were 21.03, 18.03, and 14 respectively, compared to the syneresis values 22.00% for the YC. Statistical analysis showed significant differences between the treatments ($p \leq 0.05$). According to Mohammed *et al*. (2014) and Amaya-Liano *et al*. (2008), whey syneresis is a phenomenon that occurs in fermented dairy products and causes the gel to shrink. This shrinkage lead to the release of bound water (the gel's ability to bind water becomes lower), which may be

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influenced by the substance's content. Several factors affect syneresis, including the solids content, storage temperature, the duration of storage (especially with varying acidity levels), and movement during the transfer of the product from the incubator to the cooling rooms.

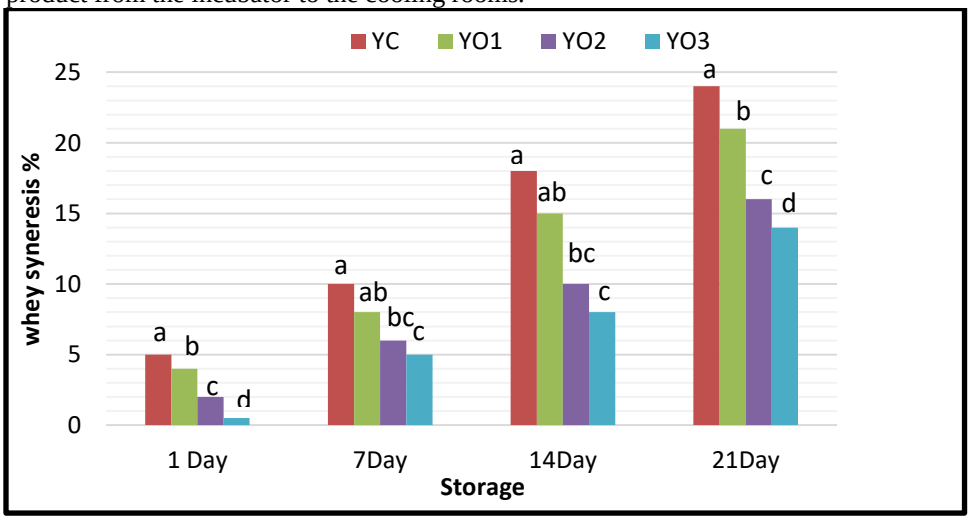


**Figure 6.** syneresis of yogurt enriched with oat protein concentrate at various concentrations**.**

**Study of the Compositional Characteristics of Yogurt**

**Fourier Transform Infrared Spectroscopy (FTIR)**

Fourier Transform Infrared (FT-IR) spectroscopy is a method employed to determine the chemical structure of a biological molecule by using the wavelength and intensity of infrared light absorbed by the sample. This method is commonly employed to characterization secondary structures of protein. FT-IR spectroscopy was used to examine the impact of various concentrations of concentrated oat protein on structural changes in yogurt (Figures 9–16). The vibration zones of yogurt treated with oat protein concentrate are displayed in the figures after one day of cold storage. The stretching vibrations of the O-H and N-H bonds are indicated by wave numbers between 3311–3906 $cm^{-1}$, while the stretching vibrations of the C-N and C-H bonds are shown by wave numbers 2934–2360 $cm^{-1}$. The C=O bond in amide I is identified by the wave numbers 1659–1744 $cm^{-1}$. Wave numbers from 1452 to 1074 $cm^{-1}$ demonstrate the C–O, C–C stretching, C–H bending, and N–H bending properties seen in Amide II and Amide III. After 21 days of storage, the wave numbers shifted. The stretching of the C-H and C=C bonds within Amide III was observed at wave numbers 2934–2361 $cm^{-1}$ and the stretching of the O-H, C-H, and N-H bonds within Amide was observed at I 3395–3941 $cm^{-1}$ .Additionally, the vibration wave numbers between 1656-1635 $cm^{-1}$ was associated with C = O stretching, while wave numbers from 1043 to 1424 $cm^{-1}$ were link to N-H bending, C-N stretching, and the stretching of C-O and C-N bonds. For the control treatment, the wave numbers were 3396 $cm^{-1}$, indicated the O-H and NH bond stretching vibrations, and 2928 $cm^{-1}$, indicated the C-N and C-H bond stretching. The C=O bond is represented by a wave number of 16501, with other bond types represented by the wave numbers between 1035 and 1257 $cm^{-1}$. Including N-H bending, C-N stretching, and C-N stretching. The two most prominent vibrational bands of the protein backbone are the amide I and II bands. Amide I, which is primarily relates with C=O stretching vibrations, is considered the most sensitive spectral region (1700–1600 $cm^{-1}$) in the secondary structural components of proteins. The main protein (control) amide and band were slightly shifted to 1659, 1653, and 1657

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$cm^{-1}$ in the YO1, YO2, and YO3 samples, respectively. This suggests that the oat protein concentrated influenced the C=O groups in the yogurt proteins. After 21 days of storage, the amide II band (1600–1500 $cm^{-1}$), associated with NH bending and CN stretching vibration, showed less conformational sensitivity than the amide I band (Kong and Yu, 2007). The amide II band observed in the control protein at 1653 $cm^{-1}$ were marginally moved in the YO1, YO2, and YO3 samples, respectively, to 1642, 1635, and 1656 $cm^{-1}$ respectively. This shift in wave numbers may be due to the interaction between the curd protein and the oat protein concentrate.. The presence of hydrogen bonds in the protein structures within the polymeric matrix may cause the hydrogen bonding sites to adjust ,favoring recognition.

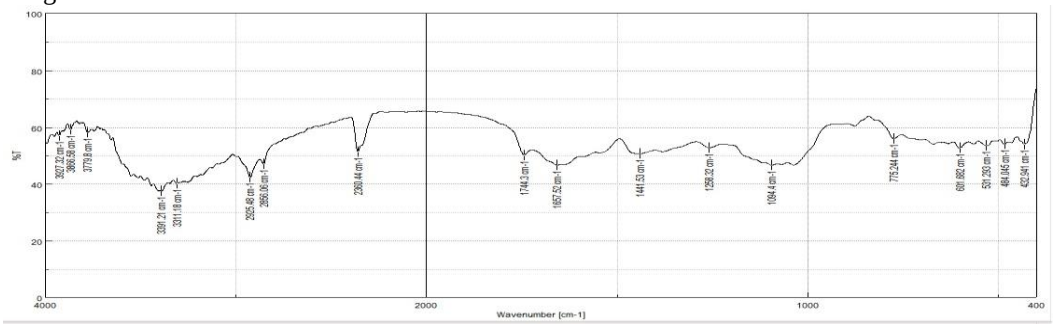


**Figure 7.** FTIR spectrum of yogurt treated with YO3 after 1 day storage

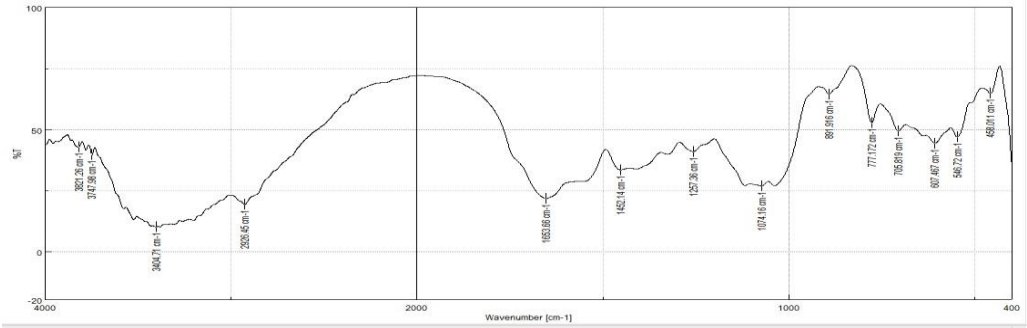


**Figure 8.** FTIR spectrum of yogurt treated with YO2 treatment after 1 day storage

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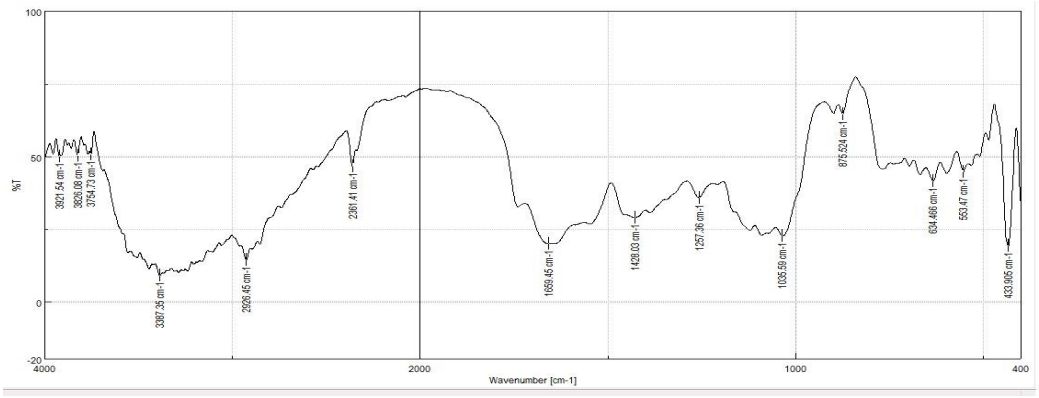


**Figure 9.** FTIR spectrum of yogurt treated with YO1 treatment after 1 day storage.

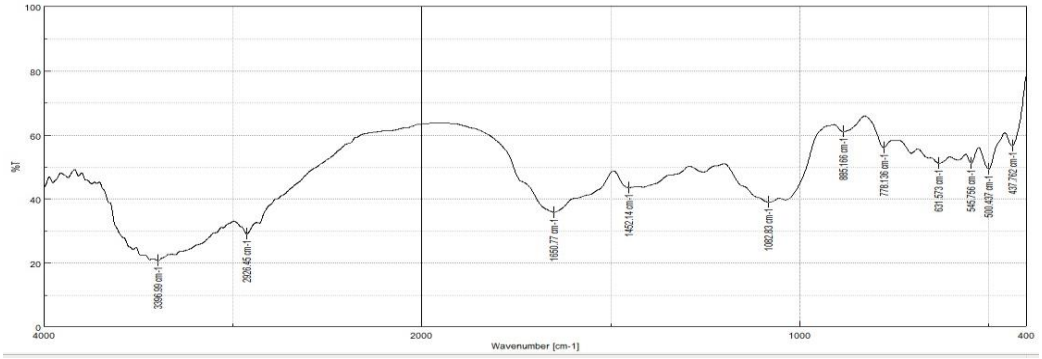


**Figure 10.** FTIR spectrum of yogurt YC treatment after 1 day storage.

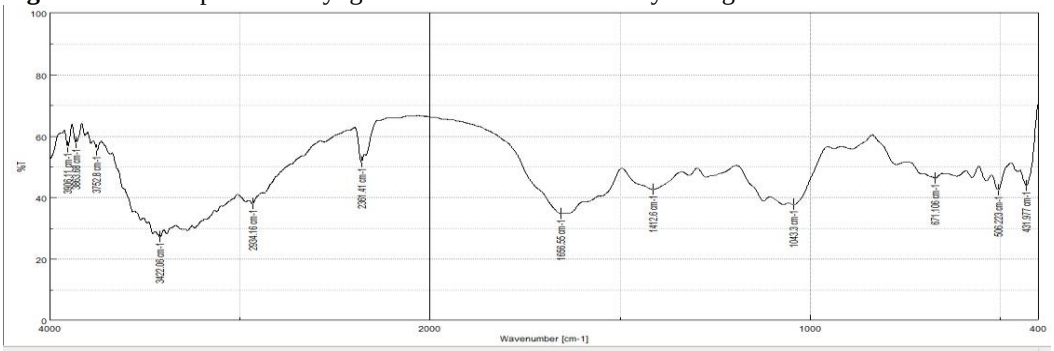


**Figure 11**. FTIR spectrum of yogurt treated with YO3 treatment after 21 day storage.

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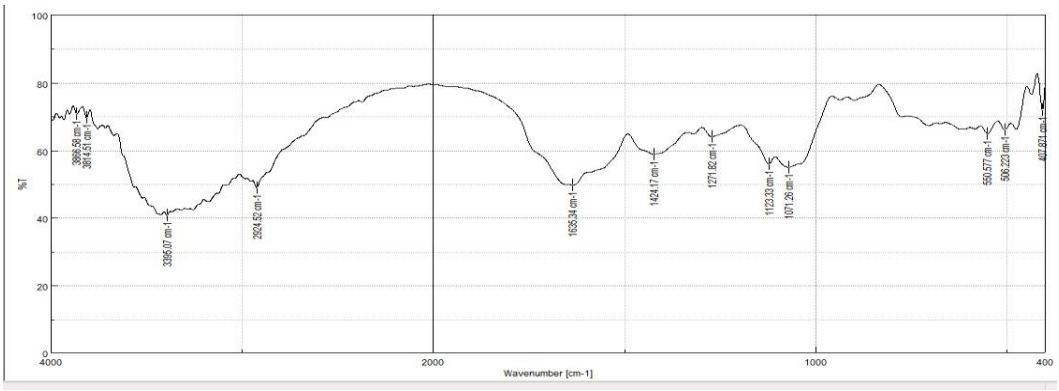


**Figure 12.** FTIR spectrum of yogurt treated with YO2 treatment after 21 day storage

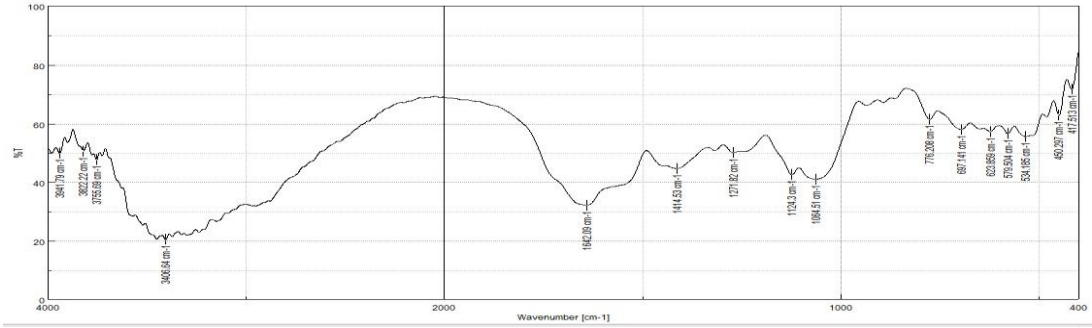


**Figure 13.** FTIR spectrum of yogurt treated with YO1 treatment after 21 day storage

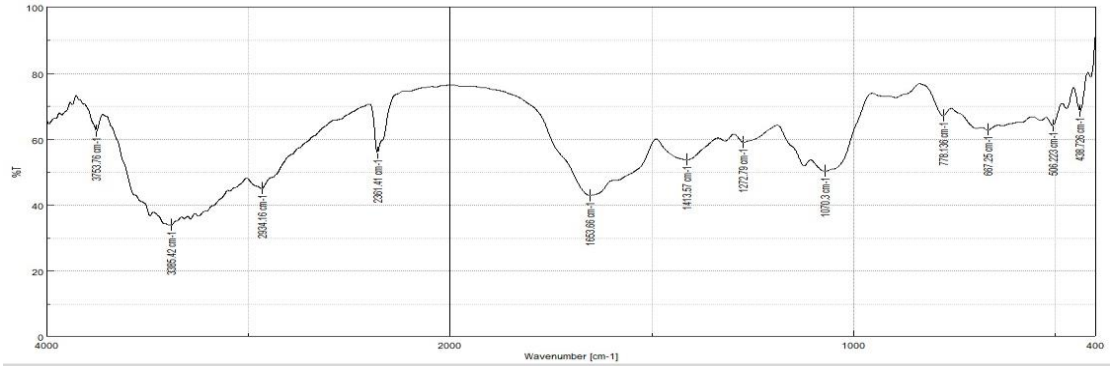


**Figure 14.** FTIR spectrum of YC treatment of yogurt after storage 21 days

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**Table (1).** The effective sites FTIR spectrum of Yogurt

| Concentration % | Storage /day | Frequency ($cm^{-1}$) | Designation | Description |
|---|---|---|---|---|
| 1 | 1 | 3311-3391 | Amide A and B | NH stretching |
| | | 3779-3927 | Amide A and B | NH stretching |
| | | 2360-2925 | Amide III | C-N and C-H stretching |
| | | 1744 | Amide I | C=O stretching |
| | | 1657 | Amide I | C=O stretching |
| | | 1094,1441 ,1258 | Amide II and Amide III | C-O ,C-C stretching and C-H bending |
| 0.6 | | 3404-3821 | Amide A and B | NH stretching |
| | | 2926 | | C-H stretching |
| | | 1653 | | |
| | | 1452 | Amide II | N-H bending and C-N stretching |
| | | 1074,1257 | | C-N and C-O stretching |
| 0.3 | | 3387-3921 | | NH stretching |
| | | 2926,2361 | | |
| | | 1659 | Amide I | C=O stretching |
| | | 1428 | Amide II | N-H bending and C-N stretching |
| | | 1035-1257 | | C-N stretching |
| Control | | 3396 | | NH stretching |
| | | 2928 | Amide III | C-H stretching |
| | | 1650 | Amide I | C=O stretching |
| | | 1452 | Amide II | N-H bending and C-N stretching |
| | | 1082 | Amide III band | C-O |
| 1 | 21 | 3422-3906 | Amide A and B | NH stretching |
| | | 2934, 2361 | Amide III | C-H and C=C stretching |
| | | 1656 | Amide I | C=O stretching |
| | | 1412 | | |

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| | | | | |
|---|---|---|---|---|
| | | 1043 | | |
| 0.6 | | 3395-3868 | Amide A and B | NH stretching |
| | | 2924 | Amide III | C-H stretching |
| | | 1635 | Amide I | C=O stretching |
| | | 1424 | | |
| | | 1271 | | |
| | | 1071-123 | Amide III band | C-O bending and C-N stretching |
| 0.3 | | 3408-3941 | | |
| | | 1642 | | |
| | | 1414 | Amide II | N-H bending and C-N stretching |
| | | 1271 | | |
| | | 1124 | | |
| | | 1064 | Amide III band | N-H bending and C-N stretching |
| Control | | 3385-3753 | Amide A and B | NH stretching |
| | | 2934,2361 | | |
| | | 1653 | | |
| | | 1413 | | |
| | | 1272 | | |
| | | 1070 | Amide III | |

**Microstructure analysis of Yogurt**

Scanning electron microscopy (SEM) was used to investigate the microstructure of yogurt. The results show the microstructure of yogurt differenced because of addition of oat protein concentrate, which increased the levels of total solids, particularly proteins. The increase of total solids and protein or carbohydrates caused the development of a gel network by cross-linking during fermentation of yogurt. Images of yogurts are shown in Figure15.The inclusion of oat protein concentrate resulted in distinct surface structures with some fine cross-linking among globular casein proteins, lead to a porous structure. These structural features, combined with the cross-linking capacity of oat protein concentrate, would enhance and reduce the syneresis by increasing the bridging degree between protein particles. Ismail *et al.* (2020)using a scanning electron microscope to comparing the yogurt formulation with the control formulations. The figure presents a microscopic analysis of yogurt enriched with oat protein concentrate at varying concentrations and highlights the differences in the fine composition of the yogurt mixtures. As shown in the SEM image, an increase in magnification (1.00–75.00 kx) and voltage by (10Kv) revealed that the control and treated formulas exhibited similar compositions, both undergoing a single stage of protein denaturation and the formation of a gel network between casein and whey proteins. followed by the blurring of the gelatinous network. This is dependent upon the curd's drying or lyophilization process, the kind and source of the protein, and the acidity function. These elements influence how the curd forms. The denaturation of the protein

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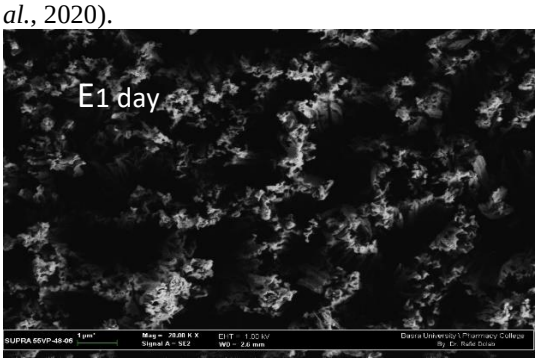
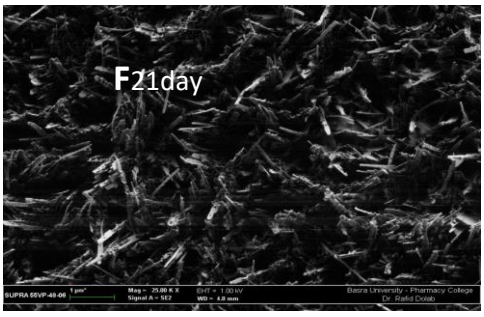
without its aggregation was facilitated by the gelatinous network and the heat, resulting in the creation of a weak gel with a smooth surface and no pores. This may be explained by the protein's denaturation alone, which prevents aggregation. The explanation is that the gel's composition is affected by variations in texture (Asaduzzaman *et al*., 2021). Along with the type, quantity, and composition of the fortified ingredients used to prepare the curd product, the interactions between the whey proteins and casein particles with porosity also contribute to the variation in composition structure observed under a scanning electron microscope. Food products with a biopolymer makeup are joined by bonding, hydrophobic interactions, and covalent bonds. Hydrogenation causes variations in the tiny structure's appearance when viewed with a scanning electron microscope (Hernández-Rodríguez *et al*., 2017). Microscopic analysis of the yogurt composition for the control formulas over two storage periods (1 and 21 days) is displayed in (Figures A-B). These two forms were noticeably different, which can be explained by the formation of cross-linking bonds that strengthen the gel's increase its porosity. This has to do with the size and its capacity to retain water. Pores: Water absorption capacity decreases with increasing pore size. It is therefore possible to conclude that cross-linking is the cause of the yoghurt gel of curd's increased hardness in forms and that more links result in a more entwined gel network and this indicates the formation of cross-linking bonds for proteins. The microscopic analysis of yogurt enriched with 0.3% oat protein 1 and 21 days of storage is presented in Figure C-D. The addition of oat protein, contributes to the formation of connection between casein proteins, which result in noticeable structural changes. Compared to the control formulas, whey syneresis is somewhat reduced during storage times. Similarly, the microscopic analysis of yogurt enriched with 0.6% oat protein over two storage periods of (1 and 21 days) is presented in Figure E-F. The presence of oat protein facilitates the formation of connections between casein proteins, resulting in observable structure differences between the two formulations. As storage progresses, whey exudation is reduced in comparison to the control formulas. When protein is added to the curd mixture, it increases the dimer because the protein molecules connect to casein through K-casein binding sites. This accumulation of extra proteins is what causes the curd mixtures' different forms. Through the use of a scanning electron microscope (Aziznia *et al*., 2008), it was found that aggregated proteins are linked to whey proteins via disulphide bridges (Patel and Velikov, 2011) . For yogurt enriched with 1% oat protein over 1 and 21 days of storage microscopic analysis (Figures G-H)reveals noticeable differences between the two formulations. The addition of 1% oat protein enhances the interaction between the casein and whey proteins lead to the formation of a robust gelatinous network. This network is the result of proteins to aggregate and the polymer formation under heat treatment in an acidc environment. Additionally, during storage, the amount of whey excreted in mixtures fortified with oat protein is lower than in the control formulas, However, as the pore size increases and acidity rises ,the water –holding capacity of the formulas decreases, affecting the composition and stability of the gel network. (Ismail *et al*., 2020).



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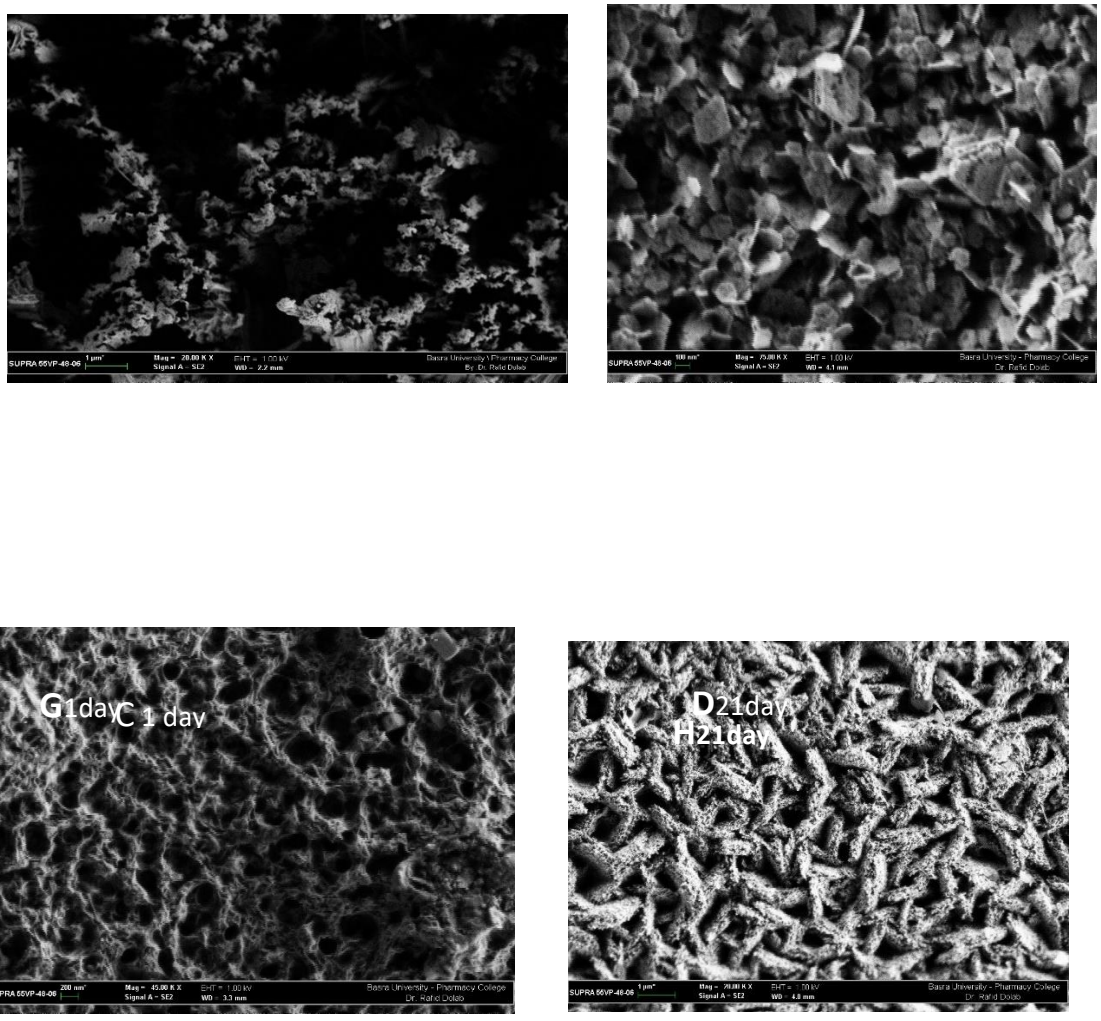


**Figure 15.** Scanning electron microscopic (SEM) images of freeze-dried yogurt formulas fortified with concentrated oat protein at different concentrations over a period of 1 day (represented by G, E, C, and A) and 21 days (represented by H, F, D, and B).

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**Sensory evaluation**

The sensory evaluation results of yogurt enriched with oat protein concentrate at different concentrations are shown in Figure 16, where statistical analyses show significant differences ($p \leq 0.05$). The product parameters were as follows: Yogurt enriched with oat protein concentrate at different concentrations maintained good texture after one day of cold storage, particularly at a concentration of 1%, (represented by YO3). The YO1 sample was found to be within acceptable limits, while the YO2 sample obtained the highest evaluation scores. On the other hand the control YC sample obtained the lowest scores, and the addition of oats notably affected the taste of yogurt. The difference in flavor and aroma could be attributed to the variation in the concentration of solids in yogurt formulations enriched with oat protein concentrate (Gomez-Betancur *et al*., 2020 ; Soukoulis *et al* ., 2007 ). Several studies have shown that increasing the cross-linking density enhances the curd texture by improving its ability to retain water. Polar, hydrophobic amino acids, which are essential for water transport, are responsible for hydrogen bonding (Moreno *et al*., 2020 , Martínez-Padilla *et al*., 2015). Appearance, flavor, and taste are some of the most important elements that determine a product's acceptance by consumer preferences and its success in the market. As a result, sensation is one of the most important variables, and the attributes that a product relies on must be chosen to maximize consumer acceptance, enhance the product's attributes for its intended use, and extend its shelf life.

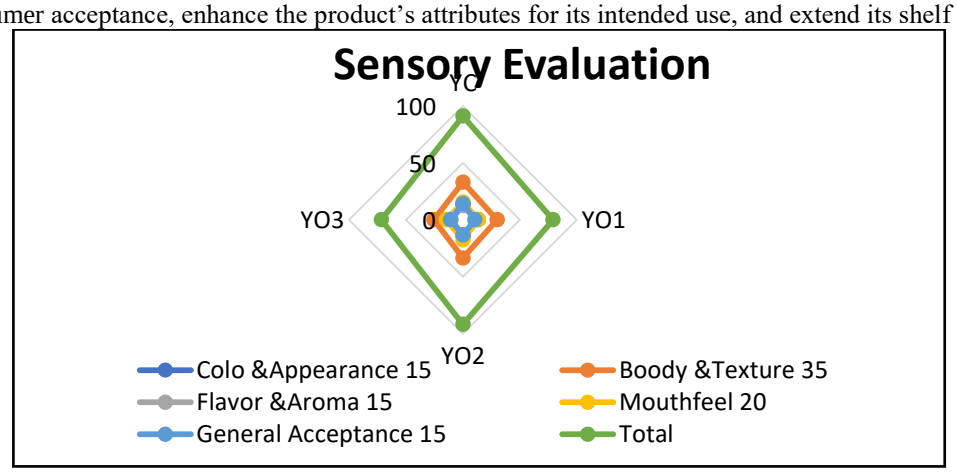


**Figure 16.** Sensory evaluation profiles of oat protein concentrate added yogurt Formulas stored at 4ºC on the first day of storage**.**

## IV. CONCLUSION

This study shows that the incorporation oat protein concentrate (OPC) to yogurt significantly influences its physicochemical, sensory, and structural characteristics during cold storage. The optimal extraction of OPC evaluated at pH 10, yield the highest protein content. Yogurts enhanced with OPC, especially at levels of 0.6% and 1%, showed improved pH stability, decreased acidity, and lower syneresis compared to the control. Structural analyses using FTIR and SEM conformed enhanced integrity and functional stability in OPC fortified samples. While the 1% OPC yogurt showed the most stable texture over time, the 0.6% OPC formulation received the highest sensory scores, especially regarding flavor and overall acceptability . These results suggest that adding OPC to yogurt at concentrations of 0.6% to 1% can enhance product quality, structural integrity, and attractiveness to consumers during cold storage.

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**COMPETING INTERESTS**

The authors declare no competing interests.

**HIGHLIGHTS**

- This study investigated the effect of incorporating varying concentrations of oat protein concentrate (OPC) as a natural stabilizer and thickener in yogurt.
- The addition of OPC significantly affected the physicochemical, structural, and sensory characteristics of yogurt during 21 days of cold storage.
- OPC enhanced the pH stability of yogurt , decreased acidity, and lowered syneresis , especially at 1% concentration.
- Structural analysis (FTIR and SEM) confirmed improved gel integrity and protein network stability in OPC-fortified samples.
- Yogurt enriched with 0.6% OPC achieved the highest sensory acceptability, whereas 1% OPC provided the greatest texture stability over time.
- The study highlights OPC as potential functional component for improving yogurt quality and extending shelf-life naturally.

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