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# FUNCTIONAL, RHEOLOGICAL AND MICROSTRUCTURAL PROPERTIES OF FREEZE-DRIED YOGHURT POWDER

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## ABSTRACT

In this study, functional, rheological, and microstructural properties of freeze-dried yoghurt powder produced at -49°C and 0.0035 mBar conditions from pre-concentrated non-fat yoghurt were investigated. The sample showed good reconstitutional properties with 204 s, 192 s for wettability and dispersibility respectively and 74% for solubility index. Bulk density value was lower, as 285.71 $kg/m^3$, which may be due to the deformation of casein micelles by freeze drying. The water activity of the yoghurt powder sample was 0.1125, much lower than the critical water activity value. The colour of the original yoghurt, yoghurt powder and reconstituted yoghurt were almost white. Reconstituted yoghurt showed better flow behaviour and consistency when compared to original yoghurt. Loss of soluble dry matter in pre-concentration of original yoghurt may have resulted in high hygroscopic powder. The microscopic imaging of original and reconstituted yoghurt demonstrated homogeneous structures for both, however the latter showed a coarser gel network.

**Keywords:** yoghurt powder, reconstitution, freeze drying, confocal Raman microscopy

# DONDURARAK KURUTMA İLE ELDE EDİLMİŞ YOĞURT TOZUNUN FONKSİYONEL, REOLOJİK VE MİKROYAPISAL ÖZELLİKLERİ

## ÖZ

Bu çalışmada, konsantre edilmiş yağsız yoğurttan -49°C ve 0.0035 mBar koşullarında dondurularak kurutma ile elde edilmiş yoğurt tozunun fonksiyonel, reolojik ve mikroyapısal özellikleri araştırılmıştır. Örneğin, 204 s ıslanabilirlik, 192 s dağılabilirlik ve %74 çözünürlük endeksi değerleriyle iyi rekonstitüsyon özelliklerine sahip olduğu görülmüştür. Ancak, kitle yoğunluğu 285.71 $kg/m^3$ olup, bu düşük değerin dondurarak kurutma işleminin kazein misellerini deforme edici özelliğinden kaynaklanmış olabileceği düşünülmüştür. Su aktivitesi 0.1125 olup kritik su aktivitesi değerinden oldukça düşük bulunmuştur. Orijinal yoğurdun, yoğurt tozunun ve rekonstitüe yoğurdun rengi beyaza çok yakındır. Rekonstitüe yoğurt orijinale göre daha iyi akış davranışı ve kıvam özellikleri göstermiştir. Ön konsantrasyon aşamasındaki çözünür kuru madde kaybının yüksek oranda higroskopik bir toz ürün eldesine yol açmış olabileceği düşünülmektedir. Orijinal ve rekonstitüe yoğurdun mikroskobik görüntüleri her ikisinin de homojen bir yapıya sahip olduğunu göstermiştir, ancak rekonstitüe yoğurdun daha kaba bir yapıya sahip olduğu gözlenmiştir.

**Anahtar sözcükler:** yoğurt tozu, rekonstitüsyon, dondurarak kurutma, konfokal Raman mikroskopisi

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## INTRODUCTION

Recently consumers' attitude towards the consumption of healthy and nutritious foods has increased (Jouki et al., 2021). Yoghurt is one of the most consumed fermented product, which is manufactured to decrease the milk volume to less than 50-75% to its original volume (Aryana and Olson, 2017). The shelf life of yoghurt is shorter than other dairy products and prone to be exposed by spoilage microorganisms due to its nutrient composition (Santos et al., 2018).

Water removal is applied to milk and dairy products to yield in powdered products with less than 5% moisture (Vasiljevic et al., 2021). Besides, packaging, handling and transportation costs are also reduced due to the reduced product weight (Song and Aryana, 2014). Powdered dairy products are consumed directly by reconstituting to its original composition or by using it as an additive in the food industry (Tamime et al, 2007).

There are several researches on yoghurt powder production by using different drying techniques, either applying more than one or only one technique, such as spray drying, freeze drying, refractance window drying and air drying (Koç et al., 2014; Zungur-Bastıoğlu et al., 2016; Carvalho et al., 2017; Fournaise et al., 2020; Ismail et al., 2020; Monsalve-Atencio et al., 2021). Freeze drying is applied to extend the shelf life in which the quality of the reconstituted product remains unchanged. Among the other drying techniques freeze drying is known to yield in products with superior quality where morphological, biochemical, structural and surface characteristics are maintained (Waghmare et al., 2021). This technique is regarded as the most convenient technique for the heat-sensitive materials and performed at low temperature and pressure (Ji et al.; Kovacı et al, 2021), where the solvent (generally water) or the suspension is first crystallized and then sublimated (Ciurzyńska and Lenart, 2011). It is possible to produce yoghurt powder maintaining its quality up to three months, in terms of microbiological and physical properties by freeze drying (Chutrtong, 2015; Tontul et al., 2018). Furthermore, freeze-dried yoghurt powder was reported to exhibit the best organoleptic characteristics (Gallardo-Rivera et al., 2021).

Functional properties of food powders are known to have crucial significance in the formulations and processing in the food industry and consumer's acceptance (Silva and O'Mahony, 2016; Fournaise et al., 2021). The cited literature mostly focuses on the processing conditions and quality attributes of yoghurt powders produced by other above-mentioned drying techniques, however published research on the functional and physical properties of freeze-dried yoghurt powder is scarce and yet there is no finding about the fractal parameters of reconstituted freeze-dried yoghurt powder. The objective of the study was to evaluate the reconstitution, rheological and microstructural properties of yoghurt powder produced by freeze drying.

## MATERIALS AND METHODS

### Yoghurt production

Skimmed milk (0.05% fat) and yoghurt starter culture (*Streptococcus salivarus* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) used in the manufacturing of non-fat yoghurt were supplied from Pinar Dairy Co. Milk was pasteurized at 90°C for 5 min and cooled to 42°C, then inoculated with 0.03 g/l starter culture and incubated until pH decreased to 4.70 then immediately cooled to 4°C. Before drying, total solids content was increased from 8.86% to 14% by draining through a cloth bag.

### Freeze drying of yoghurt

Yoghurt samples were placed in plastic beakers and frozen overnight at -80°C. The frozen samples were freeze-dried for 48 h by using FreeZone 6 litre Benchtop Freeze dryer (Labcocnco Co., Kansas City, USA) having a condenser temperature of -49°C at 0.0035 mBar.

### Methods

### Moisture content

Moisture content was determined in both yoghurt (dry solids) and yoghurt powder samples by gravimetric method (AOAC, 1990).

### Reconstitution properties

### Wettability and Dispersibility

Wettability and dispersibility analyses were performed according to the procedure described in FIL-IDF: 87 (IDF, 1979).

### Solubility index

The solubility index of yoghurt powder was measured after reconstituting 13.0 g powder in 100 mL water at 24 °C (IDF, 1988)

### Bulk density

The bulk density of the freeze-dried yoghurt sample was calculated by weighing approximately 20 g of sample into a 100 ml graduated cylinder. The mass of the powder was divided by the volume occupied by the powder into the cylinder (Jinapong et al., 2008).

### Water activity

The water activity (aw) values of yogurt powders were measured using a water activity measurement device (Testo AG 400, Lenzkirch, Germany), with a 0.001 sensitivity.

### Hygroscopity

The freeze-dried yoghurt powder samples were spread evenly on the moisture dishes and put in the closed environment of 75% relative humidity (RH) (provided by saturated NaCl solution) and 25 °C temperature. Although hygroscopicity is based on the equilibrium moisture content, to provide a fast evaluation of the sample's hygroscopicity, the weight increase of sample for 1 hour intervals up to 4 hours and lately at the 24th hour was determined (Al-Kahtani and Hassan 1990; Koc et. al., 2014; Li et al., 2020). The hygroscopicity of the powder sample was evaluated by the rate of hygroscopicity ($R_{hygroscopicity}$) (Eq. 1):

$$R_{hygroscopicity} = (m_t - m_o)/m_o \times 100 \quad \text{Eq. (1)}$$

Where, $m_t$ is the mass of the sample at time t, $m_0$ is the initial mass of the sample. This term can be expressed with a unit of $kg_{moisture}/100kg_{powder}$.

### Colour measurement

The colour of fresh, dried, and reconstituted yogurt powder samples (Lightness-L, redness-a, and yellowness- b values) was measured with a colorimeter (Colorflex, CFLX 45-2 Model Colorimeter, HunterLab, Reston, VA) and results were expressed in accordance with the CIE Lab system (Hunter, 1975).

### Rheological analysis

Apparent viscosity (Pa.s) and shear stress (Pa) against an increasing range of 1–1000 (1/s) shear rate (and then reducing from 1000-1(1/s)) were measured in twice in two parallels, for both the original and the reconstituted yoghurt samples. Then, the average values of results for the original yoghurt sample and the reconstituted yoghurt samples were calculated. The rotary viscometer (TA Ins., Hybrid Rheometer Discovery HR-2, USA) with 20 mm parallel plate, was used for the measurements and the temperature of the sample chamber was kept at 20°C. Before the measurement, the fresh yoghurt sample was needed to apply a mixing stage, in the same way as with the reconstituted samples, to achieve the same thermo-mechanical effect on the structure (Sakin-Yilmazer et. al., 2014a; Sakin-Yilmazer et. al., 2014b).

Power law (Eq.2) was applied to the averaged measurements of shear rate (1/s) against shear stress (Pa) to find out the constants and flow behaviour index.

$$\tau(Pa) = K \times \gamma^n \quad \text{Eq. (2)}$$

Where, K is the consistency index (Pa.sn) and n is flow behaviour index (Sakin-Yilmazer et. al., 2014b).

### Confocal Raman Microscopy

In determining the fractal parameters, dried yoghurt samples were reconstituted to its original dry matter. Both fresh yoghurt and reconstituted yoghurt were analysed for imaging at 570-725 nm spectral range by using Renishaw Confocal Raman Microscopy.

### Statistical analyses

All the analyses for determining the reconstitution properties and the colour were conducted at least in triple. Mean values and standard deviations were determined with descriptive statistical analysis. Data were processed with the univariate analysis of variance to determine the significance of individual differences of means on the level of *P*<0.01 and *P*<0.05. Significant means from the colour measurements were compared with the Duncan test. The correlation analyses were performed with 2-tailed Pearson correlation analysis to reveal the correlations between reconstitution and colour properties. For the rheological analyses, independent t-test was performed to results from two parallel measurements to understand whether the n and K values, i.e. the flow behaviour for both samples are different significantly. Statistical analyses were conducted using the SPSS (Version 25) commercial statistical package.

## RESULTS AND DISCUSSION

### Moisture content

The moisture content of freeze-dried yoghurt powder from drained yoghurt having 14% dry matter was found as 4.50%, which is lower than those reported for freeze-dried yoghurt samples. Carvalho and colleagues (2017) produced freeze-dried yoghurt foam with 5.6% moisture. Tontul et al (2018) found the moisture content of freeze-dried yoghurt as 8.3%. Ismail et al. (2020) could manufacture freeze- dried yoghurt powder with no additive having 4.25%.

It has been stated that moisture content of around 5% enables a prolonged shelf life for dried foods (Santos et al., 2018; Vasiljevic et al., 2021). Freeze drying seems to be an efficient method in terms of moisture reduction and therefore shelf life extension.

### Reconstitution properties

Results from dispersibility, wettability, solubility index, bulk density and water activity are summarized in Table 1.

Table 1. Some reconstitution properties of freeze-dried yoghurt powder

| Properties | Values (Mean ±SD) |
|---|---|
| Wettability (s) | 204±3.646 |
| Dispersibility (s) | 192±1.732 |
| Solubility index (%) | 74±1.732 |
| Bulk density (kg/m$^3$) | 285.71±1.816 |
| Water activity (aw) | 0.1125±0.029 |

### Wettability and Dispersibility

It is known that wettability, dispersibility and solubility are the three main and consecutive characteristics for reconstitution of powders, therefore the efficiency of rehydration strongly depends on the completion of each process (Ji et al., 2016). In the first step, named wettability, the powder absorbs water on the surface and wets (Sharma et al., 2012). The wetting time was determined as 374 s for spray dried yoghurt powder (Koç et al., 2014), which is higher than the wettability measured in this research, suggesting that the freeze-dried powder wets faster. It is known that fat content has a negative effect on wettability and flowability characteristics and when compared to particle size, milk fat has a greater effect on wettability and flow properties (Silva and O'Mahony, 2016). Milk fat is removed for the improvement of powder's wetting ability (Selomulya and Fang, 2013). In this research, non-fat yoghurt was used as raw material to yield a powder product with good functional characteristics and ease in drying steps.

The dispersibility was measured as 192 s. For spray dried yoghurt powder, Koç et al. (2014) calculated the dispersibility as 351 s, indicating that freeze-dried yoghurt powder disperses faster. A correlation between wettability and dispersibility (*P*<0.01, r=1.00) was determined in freeze-dried yoghurt powder.

### Solubility index (%)

High wettability and faster solubility are favourable in the reconstitution of powders (Koç et al., 2014). The solubility index (SI) was calculated as 74%. The wettability and solubility index values of freeze-dried yoghurt powder was reported as 71.0 s and 34.8% respectively by Tontul et al. (2018). In a research carried out by Ismail and et al. (2020), the solubility of non-treated yoghurt powder was reported to be 82.66%, where the addition of modified starch significantly increased the solubility**.** When compared to spray dried yoghurt powder, freeze-dried yoghurt powder showed better wettability, dispersibility (351 s) and solubility (68.7 %), as reported by Koç et al. (2014).

### Bulk density

High bulk density values indicate that the powder's volume is reduced for transport and hence less packaging material is consumed. Sharma et al. (2012) stated that the bulk density was subject to particle density, particle internal porosity and the particle arrangement in the container. The average bulk density value was calculated as 285.71 kgm$^{-3}$. In a research done by Ismail et al (2020), the bulk density of freeze-dried yoghurt powder was 1.3 g/ml. The bulk density value found in this research was lower but similar to that found by Tontul et al (2018), which was 0.20 g/ml. Authors attributed this low value to the porous structure of the powder. It was reported that the shape of casein micelles was subjected to deformation in the freeze drying process (Tamime et al., 2007).

### Water activity

According to Roos (2001), critical water activity value in dairy powders is 0.37 and the increase in this value result in a decrease in viscosity, and some problems like stickiness, caking, induced lactose crystallisation, and Maillard reaction, linked to the powder stability (Lin et al., 2020). The water activity of freeze-dried yoghurt powder sample was measured as 0.1125 which is much lower than this critical value. Similar result was reported by Tontul et al (2018) for freeze-dried yoghurt powder as 0.14.

### Hygroscopicity

Moisture adsorption of freeze-dried yoghurt powder at 25 °C and 75 % RH, during 24 h is shown in Fig. 1. As the figure illustrates 7.1 (±0.5) kg $_{\text{moisture}}$ was adsorbed per 100 kg of powder sample, on average of three replicates. Knowing the sample's initial moisture content as 4.50% (w/w); after 24 h at the given conditions, the powder sample in this research resulted in a moisture content of 0.13 kg/kg (dry basis).

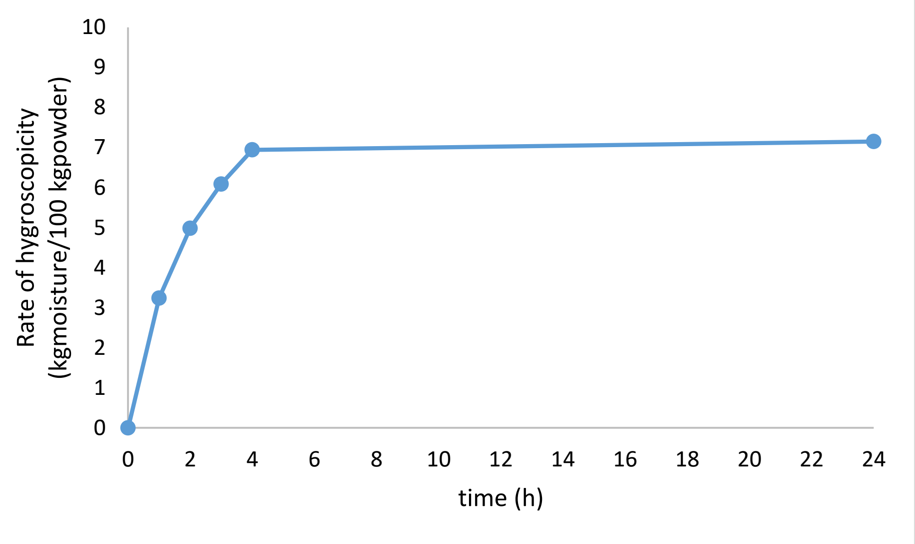


Figure 1. The hygroscopicity of freeze-dried yoghurt powder.

About the hygroscopicity of their spray-dried yoghurt powder sample, Koc et. al., (2014) reported an uptake of 4 kg moisture/100 kg solid, during 90 minutes of exposure to the 25 °C and 75% relative humidity conditions. Their sample's moisture content prior to hygroscopicity measurement was 6 % (w/w), on average.

Zungur-Bastioglu et al. (2016) declared an equilibrium moisture content of 0.24 kg/kg (dry basis) for the freeze-dried plain yoghurt, at 25 °C and 75% relative humidity. The big difference of this value with that found in this study, even the same drying method used, was attributed the preliminary straining process applied to yoghurt to fasten the long lasting and expensive drying stage, by doing so some water soluble dry matter leaved from the sample, possibly changing its moisture adsorption behaviour.

### Colour

Hunter L, a and b values for original and reconstituted yoghurt and yoghurt powder were given in Table 2.

Table 2. Colour properties of yoghurt and freeze-dried yoghurt powder

| | Values (Mean ±SD) | | |
|---|---|---|---|
| | L | a | b |
| Original yoghurt | 97.65 ±0.176$^{a}$ | -1.64±0.197$^{a}$ | 8.36±0.149$^{a}$ |
| Yoghurt Powder | 96.83±0.149$^{b}$ | -1.89±0.065$^{a}$ | 8.36±0.215$^{a}$ |
| Reconstituted yoghurt | 97.15±0.260$^{b}$ | -1.63±0.059$^{a}$ | 8.33±0.258$^{a}$ |

$^{a,b}$: Means with different lowercase letters in the same column are significantly different (p<0.05)

Colour is an important characteristic for a food product which has an influence on consumer's acceptance. As seen from the table, L values of original yoghurt, yoghurt powder and reconstituted yoghurt imply almost white colour. Similar results for L and a values were reported by Tontul et al (2018), as well. L and b values of all the samples are very close and a values are almost the same, on the other hand, there is still a significant difference between L values of samples, the original yoghurt is the whitest sample (*P*<0.01). In a research done by Monsalve-Atencio et al. (2021), freeze drying of yoghurt minimally altered the colour of the powdered product compared to convection and spray drying processes, where this difference was not found significant. Increase in the intensity of yellowness due to Maillard reactions occurring at high temperatures was reported in powdered milk (Stapelfeldt et al., 1997). In this research, no difference was detected between yellowness of the samples (*P*>0.05). Carvalho et al. (2017) stated that the freeze-dried yoghurt foams showed the higher L and lower b values compared to air-dried ones and attributed this the low processing temperature in freeze drying, avoiding the formation of brown pigments coming from the Maillard reaction. All the samples showed negative a values indicating the absence of a red colour.

### Rheological analysis

The average values of apparent viscosity against shear rate for both the original and reconstituted yoghurt samples are shown by Figure 2.

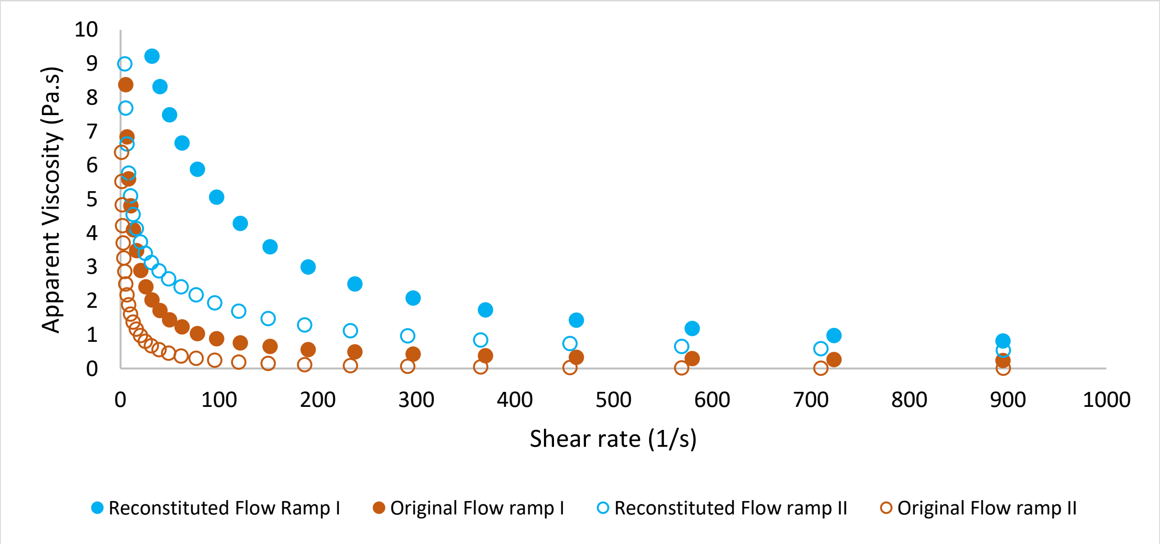


Figure 2. Apparent viscosity of yoghurt samples.

Shear stress values against shear rate, for both samples at increasing shear rates, are shown on Figure 3.

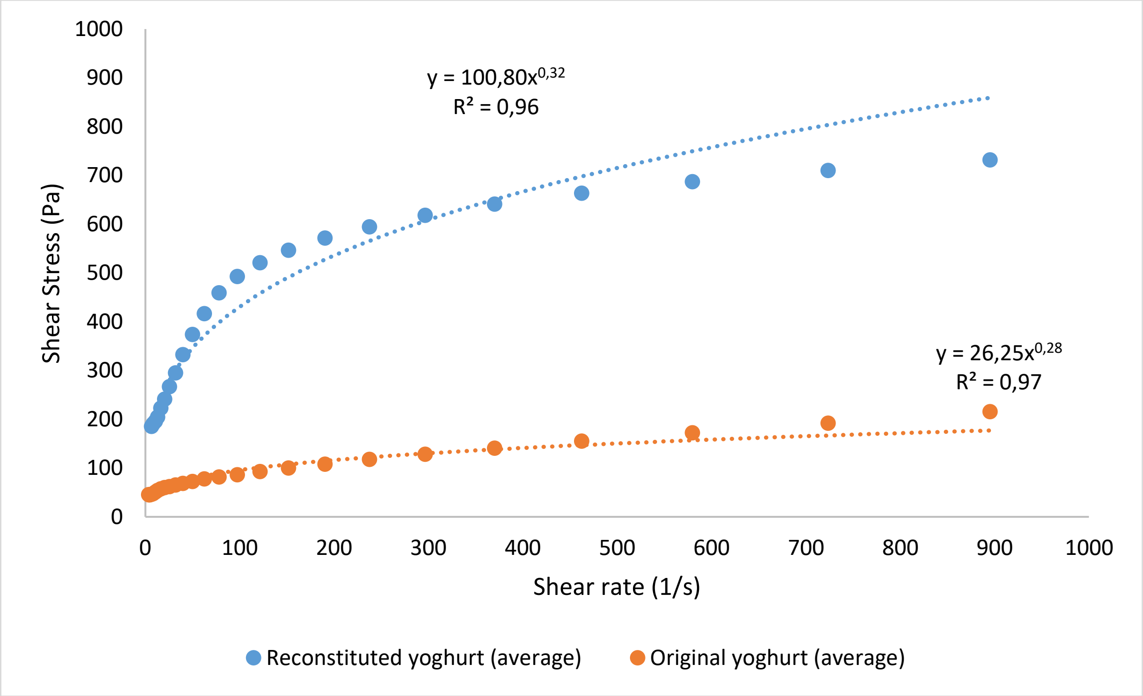


Figure 3. Shear stress vs shear rate

Flow behaviour values (n and K) for original and reconstituted samples were given by Table 3. It was seen that there was a difference between the original and reconstituted samples ($P<0.05$), in terms of flow behaviour. The reconstituted sample has significantly higher values of n and K; i.e. it is more consistent than the original sample. This result was attributed first to the removal of yoghurt serum as a pre-process, therefore concentrating the yoghurt to the dry matter content of 14%, before freeze drying. This pre-concentrating stage was applied to shorten the freeze drying time and to increase the efficiency of the process. The drainage (serum) can be further processed with a different path either to a powder or an alternative product, so the pre-concentration of yoghurt does not account for any loss of valuable protein. Author acknowledges the added value of freeze-dried yoghurt powder of which the sensory properties will be evaluated after larger scale production.

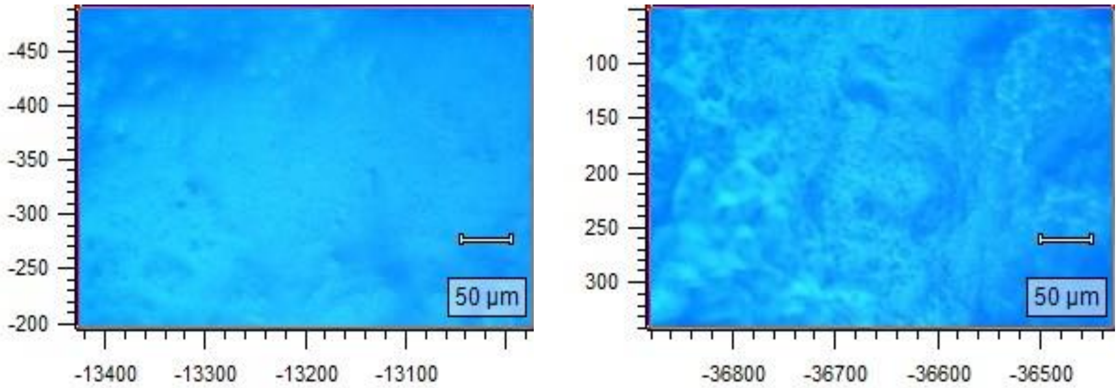
Table 3. Flow behaviour values.

| | N | K | $R^2$ |
|---|---|---|---|
| original yoghurt | 0.28 | 26.25 | 0.97 |
| reconstituted yoghurt | 0.32 | 100.80 | 0.96 |

**Microstructure**

Micrographs from original and reconstituted yoghurt samples were shown in Fig 4.

The microstructures of original and reconstituted yoghurt samples were determined by using Confocal Raman Microscopy technique in which the samples are directly scanned without any pre-treatment as in the other techniques. The fluorescent dyes used in other imaging techniques may be absorbed by the interface and thus influence the system's physical chemistry (Nair et al., 2021). The 3-D images acquired by Raman microscopy offer homogeneous structures for both samples. The original non-fat yoghurt sample has a fine network. Small pores in the micrographs indicate that more water is held in the matrix (Jose et al., 2016). The reconstituted yoghurt demonstrated a coarser gel network where the voids can be observed. According to Venir and co-workers (2007), while rheological properties of reconstituted yoghurt powder from non-fat yoghurt were not significantly affected by the freeze drying process, the network structure weakened.



(a) (b)

Fig 4. Confocal Raman micrographs of (a) nonfat yoghurt (b) reconstituted yoghurt powder obtained at x500 magnifications

## CONCLUSION

Good functional and reconstitution properties of powdered food products are significant in terms of formulation, handling, processing as well as consumption. The freeze-dried non-fat yoghurt powder showed favourable characteristics in terms of wettability, dispersibility and water activity. On the other hand, low bulk density value was obtained, which might be due to the porous structure of the powder induced by freeze drying. Solubility of yoghurt powder was 74%, further studies can be done by using some additives to improve solubility. The freeze-dried yoghurt powder showed good colour properties with almost white colour, indicating the superiority of the freeze-drying process when compared to other drying techniques. The water activity value was much lower than the critical value stated for dried food products, which would have a positive influence on powder stability.

Concentration of original yoghurt prior to the drying process had an impact on both hygroscopicity and rheology. Freeze-dried yoghurt powder sample was found to be more hygroscopic than those studied by others. However, reconstituted yoghurt demonstrated greater consistency than the original yoghurt. To conclude, pre-concentration step before freezing can be recommended in terms of improvement of rheological behaviour.

Reconstituted yoghurt demonstrated a coarser gel network in which larger voids can be observed. Nevertheless, it still showed a homogeneous structure. Along with functional properties and rheological behaviour, the results certify the efficiency of freeze drying in yoghurt powder production. Further research on the selection of packaging material and determination of powder stability throughout storage of freeze-dried yoghurt powder seem quite likely to offer important findings in the future.

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## CONFLICT OF INTEREST

The author declares no conflict of interest.

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