



High Protein Powders Fortification of Nonfat Yoghurt: Impact of Protein Source, Protein to Total Solids Ratio, Storage, and Seasonality on the Functionality of Nonfat Yoghurt Made Using Glucono- δ -Lactone (GDL)

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ABSTRACT

Nonfat yoghurt is a popular fermented product across the world. High protein powders such as Skim Milk Powder (SMP), Nonfat Dry Milk (NDM), and Milk Protein Concentrate (MPC) can be utilized in yoghurt formulations. The final Total Solids (TS), source and amount of protein in the formulation, and the physicochemical changes during storage may impact the functionality of yoghurt-type products. The objective of the study was to evaluate the effects of storage of SMP, NDM, MPC40, and MPC70 on the functionality of nonfat yoghurts at three different protein/TS levels. Additionally, the impact of SMP and NDM manufactured in different seasons (summer and winter) on yoghurt functionality was also studied. Three different lots of each powder were collected and divided into 3 portions and were stored for 3, 9, and 15 months. At each storage time, yoghurt with %protein/%TS of 4/12.5, 4.5/13.5, and 5/15.5 were produced from each lot. A Rapid Visco Analyzer (RVA) method was utilized to produce yoghurt using glucono- δ -lactone (GDL). Storage time did not have a significant effect ($p>0.05$) on the functional properties of yoghurts fortified with NDM, MPC40, and MPC70 at all protein/TS ratios. In conclusion, the storage of milk powders has a minimal influence on the functional properties of nonfat yoghurt, whereas the use of MPC had a substantial impact on the functionality of nonfat yoghurt.

Keywords: Nonfat yoghurt; Storage; Functionality; Milk Protein Concentrate (MPC); Total Solids (TS); Nonfat Dry Milk (NDM); Seasonal variation; Rapid visco analyzer; Acid gels

HIGHLIGHTS

- Storage time of high protein powders did not have a significant effect on the functional properties of yoghurts fortified at all protein/Total solids ratios.
- Viscosity and syneresis of nonfat yoghurt depend based on fortification protein: TS in the yoghurt formulation.
- Selection of milk protein source plays an important role in determining the functional properties of yoghurt such as viscosity and syneresis.
- Storage of milk protein concentrate results in extensive fusion of casein micelles resulting in a heterogeneous casein matrix.

INTRODUCTION

The purpose of fermentation was to transform milk into milk

products with an extended shelf life [1]. In general, milk is transformed into fermented products through lactic fermentation by bacteria [2]. Among all fermented products, yoghurt represents a significant portion of consumer's diets. In the USA, yoghurt must contain a minimum of both 3.25% milk fat level and 8.25% Milk Solids Not Fat (MSNF) before the addition of any bulky flavours. Nonfat yoghurt must not contain less than 0.5% milk fat before the addition of bulky flavours (21CFR131.200, 131.203, and 131.206).

The composition of milk varies and parameters such as the source of milk, the season of its collection, and its handling procedures affect the composition of milk [1]. Therefore, standardizing milk to the required fat, protein, MSNF, or Total Solids (TS) content has become a common practice in yoghurt manufacturing industries. In international markets, high protein powders such as Skim Milk Powder (SMP), Nonfat Dry Milk (NDM), and Milk Protein Concentrate (MPC) are used for fortifying and standardizing

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the milk for yoghurt manufacture. Fortifying milk is a common procedure followed by yoghurt manufacturers. The process is accomplished by adding milk powders, whey protein concentrates, or blends of these powders to achieve the desired protein, TS or MSNF level in yoghurt [3]. Fortification (or increasing the TS) of yoghurt milk has been studied extensively by several researchers [1,4-12]. When fortifying yoghurt milk, the types of protein (casein (CN): Whey Protein (WP) ratio) and protein: TS ratio (protein: TS) in the yoghurt formulation influence the textural properties of yoghurt [4,7,9,10,13-15]. However, it is difficult to quantify the combined effects of both the CN:WP ratio and the protein: TS on the yoghurt textural properties, because, in most studies, these ratios are not considered experimental factors in combination [7].

Several approaches have been adopted to fortify (or increase the TS of) yoghurt milk which can be achieved by (1) Concentration processes such as Vacuum Evaporation (VE) and Reverse Osmosis (RO) as well as fortification of SMP or NDM that maintains CN:WP and protein: TS as in unfortified milk; (2) Fortification of Whey Protein Concentrates (WPC) (34% Protein) that alters CN:WP while maintaining the same protein: TS as in unfortified milk; (3) Fortification of WPC (40 to 70% protein) that alters both CN: WP and protein: TS of yoghurt milk as compared to unfortified milk, and (4) Fortification with MPC (40 to 70% protein) that maintains CN:WP with altered protein: TS of yoghurt milk compared to unfortified milk. While the use of VE and RO can incur additional costs, the use of WPC alters both CN: WP and protein: TS of yoghurt milk.

The MPC is classified based on the protein contents; therefore, products may contain various amounts of protein. Fortification methods using MPC include fortification to standardize % protein in the yoghurt formulation as compared to SMP, NDM, and WPC. Fortification of yoghurt milk with MPC allows manufacturers to further reduce the amount of powder required because of an increase in the protein content of MPC as compared to SMP or NDM. As a result of improved functional properties (i.e. water absorption and acid gel strength) of MPC, it can be considered a suitable alternative to other dried ingredients for yoghurt manufacture. However, some researchers note that the solubility of MPC is affected negatively due to various factors, such as the quality of the raw material, the degree of protein concentration, and the conditions of the final product's storage [16-19].

The high-protein powders can be used as food ingredients and can either be freshly made or stored at room temperature until needed. However, storing these high-protein powders may lead to several physicochemical and biochemical changes (lactose crystallization, Maillard reaction, and oxidation) that can adversely affect their functional properties and therefore, the product in which they are used. Several researchers have studied using NDM in yoghurt manufacture [4,9,14]. However, only limited information exists on the use of SMP or MPC as an ingredient in yoghurt formulations as compared to NDM. The use of MPC as an ingredient in a yoghurt formulation requires its complete dissolution in water typically at room temperature with moderate agitation. However, stored MPC can exhibit poor solubility [16,17,19,20-22]. The reduced solubility of powders may also affect functional properties such as foaming and hydrophobicity [23]. Similarly, this reduction in solubility of stored MPC powder may also affect the characteristics of the product in which it is used. However, limited information is available regarding the effects of such reduced solubilities of MPC in terms of either the functional properties or the textural

properties of a product in which it is used. Similarly, only limited information is available on the effects of storage on the functional properties of the products in which they are used.

The textural properties of the stirred yoghurt manufactured from bacterial culture can often vary with respect to parameters such as the type and activity of culture, incubation time-temperature, and pH development. "Acid milk gel" or Glucono- δ -Lactone (GDL) based yoghurt provides an alternative to studying the textural properties of yoghurt. The mechanism of GDL-based yoghurt includes a reduction of yoghurt milk pH because of hydrolysis of GDL to gluconic acid. Because of the rapid acidification at high temperatures, acid milk gels have become both a popular and reliable medium with which to evaluate the textural properties of yoghurt. Several authors have studied the textural properties of GDL yoghurt [24-29]. For example, Van Marle and Zoon studied the formation, textural properties, and microstructure of skim milk gels made with bacterial culture and GDL, concluding that GDL-based yoghurt can be studied for determining the textural parameters of yoghurt [30]. A study by Bennett et al., characterized the change in viscosity of nonfat yoghurt manufactured from low, medium, and high heat SMP [31]. In another study, Pollard et al., standardized the method for yoghurt manufacture in a Rapid Visco Analyzer (RVA) to study the impact of TS on both viscosity and syneresis of nonfat yoghurt [32]. Consequently, Juhász et al., stated that the RVA apparent viscosity curves contain both physical and chemical information that can be interpreted as physicochemical spectra [33]. The manufacture and analysis of GDL-based yoghurt through controlled heating and cooling profile in RVA can provide a suitable alternative that delineates differences in the textural properties of yoghurt manufactured.

Recently, Shah et al., reported that the storage of high protein milk powders has an impact on some functional properties, and a proper selection of powders is necessary [23]. Based on the rationale discussed above, the overall objective of this study was to evaluate the effect of the source of protein in yoghurt formulations (SMP, NDM, and MPC), the season of powder production, and the storage of powders on the textural properties of nonfat yoghurt. The first objective of the study was to evaluate the functional properties of nonfat stirred yoghurt manufactured standardized at 8.5% Milk Solids Not Fat (MSNF) using various high protein powders. The second objective of the study was to evaluate the effect of yoghurt milk fortification at various %protein/%total solids in the final yoghurt formulation with SMP, NDM, MPC40, and MPC70 stored for 3, 9, and 15 months (from the date of manufacture) on yoghurt viscosity and syneresis parameters. The third objective of the study was to evaluate the effects of the season of production of both SMP and NDM and storage of powders (3,9, and 15 months) on the functional properties (viscosity and syneresis) of nonfat stirred yoghurt.

MATERIALS AND METHODS

Experimental design and storage of powders

Three replicates of four different milk powders (low heat SMP, NDM, MPC40, and MPC70) manufactured in the summer season (May–September) were procured from commercial manufacturers based in the USA. Additionally, three replicates of two different milk powders (low heat SMP, and NDM) manufactured in the winter season (November–February) were procured from US manufacturers. Each replicate of milk powder was divided into three portions. The powder samples were sealed and stored in

specimen containers Ziplock bags (SC Johnson, City, MI, USA) at 25°C for 3, 9, and 15 months (from the date of manufacture) for analysis.

To study the effect of various high protein powders on yoghurt functionality, fortification of yoghurt milk with high protein powders (SMP, NDM, MPC40 and MPC70) was carried out to achieve 8.5% MSNF in the final yoghurt formulation. To study the effects of protein: TS of the formulation and storage of powders on nonfat yoghurt functionality, fortification of yoghurt milk with high protein powders (SMP, NDM, MPC40 and MPC70) was conducted at each time point. The fortification was accomplished to achieve 4% protein and 12.5% TS (Low Protein Low Solids-LPLS), 4.5% protein and 13.5% TS (Medium Protein Medium Solids - MPMS), and 5% protein and 15.5% TS (High Protein High Solids -HPHS), respectively in the final yoghurt formulation. Additionally, the SMP and NDM manufactured in the summer and winter seasons were compared for the effect on the yoghurt functionality. The evaluation was conducted at all three proteins: TS levels (LPLS, MPMS, and HPHS) and at all three-time point (3, 9, and 15 months of powder storage).

Chemical analyses of powders

The moisture content of the powder was determined as described in the American Dairy Products Institute (ADPI) method using a vacuum oven [34]. The total protein content of each powder sample was analyzed by the Kjeldahl block digester method as described by Hooi et al., [35]. The ash content of powders was obtained from the certificate of analysis provided by the manufacturers.

Yoghurt formulation using Glucono-δ-Lactone

Standardization to 8.5% MSNF: Each powder sample was formulated to achieve 8.5% MSNF in the final nonfat yoghurt formulation. Based on the chemical analysis of powders, the formulation for each powder sample was developed in an Excel-based formulation software program (Techwizard™, Owl software, Columbia, MO, USA). It is important to note as each powder sample contains a different amount of protein/g of TS. Therefore, the yoghurt formulation (standardized based on MSNF) using each powder sample will have different amounts of protein.

Titrateable acidity Standardization of three different % protein/% TS: Three different %protein/%TS were selected for nonfat yoghurt manufacture from each powder replicate and included LPLS (4.0/12.5), MPMS (4.5/13.5), and HPHS (5.0/15.5) %protein/%TS. Varying amounts of De-Proteinized Whey (DPW) and water were included to standardize the TS in the nonfat yoghurt formulation. Detailed ingredient blends and formulations of each %protein/%TS are shown in Table 1. The experiment was repeated at 3, 9 and 15 months of storage.

Yoghurt manufacture: Yoghurt was manufactured using a small-scale RVA-based methodology [32]. The RVA is a computer-integrated instrument developed by Newport Scientific (Warriewood, Australia) to determine the viscous properties of food products. A dry blend of milk powders and DPW were reconstituted in water and stirred for 20-25 minutes followed by overnight hydration at 4°C. The solution pH (Accumet® - gel-filled glass electrode with 41 spear tip, Fisher Scientific, USA) was measured and adjusted to 6.70 ± 0.05 using 0.1 N NaOH. Thirty grams of reconstituted milk were weighed into the RVA canister. The solution was subjected to heat treatment in the RVA consisting of a profile where it was heated from 45°C to 93°C over a period of 10 minutes, held for 6 minutes

at 93°C, and cooled to 45°C in 10 minutes. The stirring speed was maintained at 150 rpm for the duration of the heat treatment. After cooling to 45°C, the sample was acidified using Glucono-δ-Lactone (GDL, PMP Fermentation Products, Inc, Peoria, IL, USA) and was added to the RVA canister and mixed thoroughly at 500 rpm for 1 minute. The quantity of GDL required was previously determined for each powder replicate to achieve final yoghurt pH of 4.50 ± 0.05. The RVA canister was kept at 45°C in the water bath for 2.5 hours and stored at 4°C overnight. For each powder replicate three yoghurts were manufactured. Yoghurt viscosity and syneresis of yoghurt manufactured were evaluated as mentioned below.

Functional properties of yoghurt

Yoghurt viscosity: Nonfat yoghurt (30 g) was stirred using the RVA at 750 rpm for 3 minutes, followed by 10 minutes at 150 rpm. The temperature of the sample was kept at 10°C during the test. The final yoghurt viscosity at the end of the test was reported as yoghurt viscosity (centipoise).

Syneresis: The nonfat yoghurt was allowed to reach 25°C after analysis of yoghurt viscosity. Syneresis of nonfat yoghurt was recorded by weighing the amount of whey collected from 15 g of yoghurt placed in a funnel containing filter paper (Whatman 4, Whatman International Ltd., Maidstone, England) for 2.5 hours at 25°C.

Statistical analyses: A 4 × 3 factorial design or split-plot design consisting of four different powder types (SMP, NDM, MPC40, and MPC70) and three different storage times (3, 9 and 15 months) with 3 replications was used for statistical analysis, and changes in functional properties of yoghurt (viscosity, syneresis) were analyzed using a split-plot design. The PROC Mixed procedure of SAS, which involved 4 factors (powder type, replicate, season, and storage time) as class variables, was used for the data analysis [36].

RESULTS AND DISCUSSION

Composition of powders

Only proteins and total solids were analyzed for the study. The average protein of SMP, NDM, MPC40, and MPC70 was 32.3%, 33.5%, 39.8%, and 68.6% respectively [23]. Based on the moisture content, the average calculated %TS of SMP, NDM, MPC40, and MPC70 were 96.3, 96.2, 96.1, and 95.4 respectively.

Effects of standardization of yoghurt to 8.5% MSNF

The yoghurt standardized and manufactured at 8.5% MSNF using SMP, NDM, MPC40, and MPC70 contained 2.86%, 2.96%, 3.68%, and 6.27% protein in the yoghurt formulations respectively. This was expected because the MSNF was standardized to 8.5%, and different powders contributed to different protein content in the yoghurt formulation. The mean yoghurt viscosity and syneresis of the yoghurt manufactured from SMP, NDM, MPC40, and MPC70 at 8.5% MSNF are shown in Table 2. The mean viscosity of MPC70 yoghurt was significantly higher ($p < 0.05$) compared to SMP, NDM, and MPC40 yoghurts whereas, the mean syneresis of MPC70 yoghurt (2.8 g) was significantly lower ($p < 0.05$) as compared to SMP, NDM, and MPC40 yoghurt. However, there was no significant difference ($p > 0.05$) observed between yoghurt viscosity manufactured from MPC40 and NDM as well as NDM and SMP, but SMP yoghurt viscosity was significantly lower ($p < 0.05$) than that made using MPC40. The similar mean viscosity results of SMP and NDM or NDM and MPC40 may have resulted as a result of

a relatively higher mean viscosity value of MPC70 yoghurt (1884.3 cp) in the statistical model. All the powders were significantly different in syneresis values. These results were consistent with the results reported by previous studies [9,37]. Where the authors reported an increase in the viscosity and a decrease in the syneresis values as the protein content of the yoghurt increased. The yoghurt with increased protein content contains smaller interstitial spaces with a dense protein matrix which can hold a more liquid phase as compared to yoghurt with lower protein content [37]. In summary, an increase in the protein content of the yoghurt formulation results in an increase in yoghurt viscosity and a decrease in the syneresis of yoghurt.

Effects of %protein and %TS

Yoghurt viscosity: The overall mean viscosity of yoghurts manufactured from SMP, NDM, MPC40, and MPC70 at HPHS was significantly higher ($p<0.05$) as compared to yoghurts manufactured at MPMS and LPLS (results not shown). Similarly, the overall mean viscosity of yoghurts at MPMS was significantly higher ($p<0.05$) as compared to yoghurts manufactured at LPLS. These results were in agreement with previous studies [9,14,37]. Where the authors reported an increase in the viscosity as the protein content of the

yoghurt increased. The mean squares (MS) and probabilities (in parentheses) of yoghurt viscosity and syneresis (LPLS, MPMS, and HPHS) are shown in Table 3. The powder type and the interaction effect of powder type and replicates had a significant effect ($p<0.05$) on the yoghurt viscosity at each %protein/%TS.

The mean viscosity of yoghurt manufactured from SMP, NDM, MPC40, and MPC70 at LPLS, MPMS, and HPHS is shown in Table 4. The mean viscosity of yoghurts (LPLS) manufactured from MPC40 ranged from 336 cp to 343 cp and were significantly lower ($p<0.05$) as compared to SMP (382 cp to 397 cp) and NDM yoghurt (380 cp to 405 cp) at each time point. However, as compared to MPC40 yoghurts, the mean viscosity of MPC70 yoghurts was significantly ($p<0.05$) lower than SMP and NDM yoghurts. Similar results were noted at other %protein/%TS as well where the mean viscosity of yoghurts (MPMS) manufactured from MPC70 (370 cp to 389 cp) were significantly lower ($p<0.05$) as compared to MPC40 (459 cp to 474 cp), NDM (516 cp to 536 cp), and SMP yoghurts (495 cp to 524 cp) at each time point (Table 4). Also, as shown in Table 4, the mean viscosity of yoghurts HPHS manufactured from MPC70 ranged from 435 cp to 449 cp as compared to MPC40 (533 cp to 541 cp), NDM (593 cp to 608 cp), and SMP yoghurts (574 cp to 589 cp) at each time point.

Table 1: Yoghurt formulation manufactured from SMP¹, NDM², MPC40³, and MPC70⁴ at three different proteins to TS ratio.

%Protein / %TS	SMP Formulation (%)		NDM formulation (%)		MPC40 formulation (%)		MPC70 formulation (%)	
	SMP	DPW	NDM	DPW	MPC40	DPW	MPC70	DPW
LPLS ⁵	12.23	0.74	11.7	1.28	9.49	3.46	5.09	7.83
MPMS ⁶	13.9	0.12	13.3	0.73	10.79	3.2	5.79	8.17
HPHS ⁷	15.32	0.77	14.76	1.44	11.89	4.17	6.38	9.65

Note: ¹SMP=Skimmed Milk Powder; ²NDM=Non-Fat Milk Powder; ³MPC40=Milk Protein Concentrate Powder with 40% protein; ⁴MPC70=Milk Protein Concentrate powder with 70% protein; ⁵LPLS=Low Protein Low Solids (4% Protein/ 12.5% Total solids); ⁶MPMS=Medium Protein Medium Solids (4.5% Protein/13.5% Total solids); ⁷HPHS=High Protein High Solids (5% Protein/ 15.5% Total solids).

Table 2: Mean(n=3) RVA¹-viscosity and syneresis results of yogurt formulated at 8.5% MSNF from SMP², NDM³, MPC40⁴, and MPC70⁵.

Powder Types	RVA-viscosity	Syneresis
SMP	174.5 ± 23 ^c	8.1 ± 0.07 ^a
NDM	226.8 ± 8 ^{bc}	7.5 ± 0.10 ^b
MPC40	370.8 ± 7 ^b	6.2 ± 0.03 ^c
MPC70	1884.3 ± 153 ^a	2.8 ± 0.16 ^d

Note: ^{a-d} Means within the same column not sharing common subscripts are significantly different ($p<0.05$)

¹RVA= Rapid Visco Analyzer; ²SMP= Skimmed Milk Powder; ³NDM= Non-Fat Milk Powder; ⁴MPC40: Milk Protein Concentrate powder with 40% protein; ⁵MPC70= Milk Protein Concentrate powder with 70% protein.

Table 3: Mean squares and probabilities (in parentheses) of yogurt RVA¹-viscosity and syneresis manufactured at three different %protein/%TS.

Factors	df	LPLS ²		MPMS ³		HPHS ⁴	
		Viscosity	Syneresis	Viscosity	Syneresis	Viscosity	Syneresis
Powder Type	3	22663* (<0.0001)	0.413* (<0.0001)	39626* (<0.0001)	0.562* (<0.0001)	44593* (<0.0001)	0.544* (<0.0001)

Replicates	2	334 (0.10)	0.162* (0.0003)	348 (0.1886)	0.167* (0.0001)	1754* (0.002)	0.107* (0.0002)
Storage Time	2	373 (0.08)	0.007 (0.59)	972* (0.02)	0.003 (0.74)	229 (0.34)	0.003 (0.61)
Powder Type* Replicates	6	1198* (0.0002)	0.129* (<0.0001)	793* (0.0098)	0.097* (0.0001)	1450* (0.0006)	0.1714* (<0.0001)
Time* Powder Type	6	141 (0.40)	0.004 (0.90)	144 (0.60)	0.010 (0.43)	136 (0.65)	0.0453* (0.0011)
Error	16	126	0.011	187	0.009	195	0.0067

Note: *Statistically significant ($p < 0.05$)

¹RVA=Rapid Visco Analyzer; ²LPLS=Low Protein Low Solids (4% Protein/12.5% Total solids); ³MPMS=Medium Protein Medium Solids (4.5% Protein/13.5% Total solids); ⁴HPHS=High Protein High Solids (5% Protein/15.5% Total solids).

Table 4: Mean($n=3$) RVA¹-viscosity (cP) results of yogurt manufactured (LPLS², MPMS³, and HPHS⁴) from SMP⁵, NDM⁶, MPC40⁷ and MPC70⁸ at each storage time.

Storage Time	SMP	NDM	MPC40	MPC70
LPLS				
3 months	385 \pm 9.50 ^b	405 \pm 32.39 ^a	343 \pm 11.68 ^c	291 \pm 17.62 ^d
9 months	397 \pm 18.19 ^a	396 \pm 8.33 ^a	343 \pm 19.66 ^b	281 \pm 17.21 ^c
15 months	382 \pm 23.03 ^a	380 \pm 31.22 ^a	336 \pm 21.73 ^b	284 \pm 17.21 ^c
MPMS				
3 months	505 \pm 3.79 ^{bAB}	536 \pm 30.79 ^{aA}	474 \pm 9.54 ^{cA}	389 \pm 17.21 ^{dA}
9 months	524 \pm 20.55 ^{aA}	532 \pm 5.20 ^{aA}	469 \pm 2.65 ^{bA}	376 \pm 10.97 ^{cA}
15 months	495 \pm 26.58 ^{aB}	516 \pm 35.36 ^{aA}	459 \pm 13.86 ^{bA}	370 \pm 14.18 ^{cA}
HPHS				
3 months	574 \pm 12.06 ^b	608 \pm 7.55 ^a	533 \pm 19.97 ^c	449 \pm 19.67 ^d
9 months	589 \pm 19.14 ^a	604 \pm 19.67 ^a	541 \pm 27.75 ^b	442 \pm 20.43 ^c
15 months	574 \pm 14.57 ^a	593 \pm 53.27 ^a	541 \pm 32.13 ^b	435 \pm 25.70 ^c

Note: ^{a-d} Means within the same row not sharing common subscripts are significantly different ($p < 0.05$); ^{A-B} Means within the same column not sharing common subscripts are significantly different ($p < 0.05$)¹RVA=Rapid Visco Analyzer; ²LPLS=Low Protein Low Solids (4% Protein/12.5% Total solids); ³MPMS=Medium Protein Medium Solids (4.5% Protein/13.5% Total solids); ⁴HPHS=High Protein High Solids (5% Protein/15.5% Total solids); ⁵SMP: Skimmed Milk Powder; ⁶NDM=Non-Fat Milk Powder; ⁷MPC40=Milk Protein Concentrate powder with 40% protein; ⁸MPC70=Milk Protein Concentrate powder with 70% protein.

Textural properties of the yoghurt gel, such as viscosity depend on the density of the protein matrix consisting of casein aggregates. At the isoelectric point of caseins, they aggregate together via strand formation to form a homogeneous matrix and uniform pores containing the aqueous phase. Thus, both the controlled aggregation and strand formation during gel formation determines the viscosity of yoghurt. Harwalkar, and Kalab, studied the microstructure of NDM yoghurt (4.5% protein) and reported a uniform and homogeneous yoghurt gel [38] whereas, Modler and Kalab, compared yoghurt manufactured from MPC40 and NDM to achieve 4.5% protein in yoghurt [39] and reported the presence of tightly “fused” casein aggregates in the yoghurt gel manufactured from MPC40 as compared to NDM at the same protein (4.5%) level. Several researchers have reported the presence of “fused” casein micelle in MPC as a result of increased protein content in the powder [16,17,22,40]. Thus, it can be theorized that because of

the fusion of casein micelles in MPC, the yoghurt gel manufactured from MPC in this study may have resulted in uncontrolled casein aggregates (or clusters) and longer strand formation resulting in a heterogeneous casein matrix. Contrary to this argument, Modler et al., reported no change in the viscosity of yoghurt (4.5% protein) manufactured from NDM and MPC40 [39]. The possible deviation in the result of this study as compared to the study conducted by Modler et al., can be explained by using MPC40 for yoghurt manufacture [39]. Although, the viscosity of yoghurts manufactured from MPC40 was significantly ($p < 0.05$) different as compared to NDM yoghurts in this study, their values were in proximity at each protein: TS. Additionally, the significant ($p < 0.05$) difference between the viscosity of MPC40 and NDM yoghurts may have resulted because of relatively lower viscosity values of MPC70 yoghurts in the statistical model. Yogurts produced from MPC70 as compared to SMP, NDM, and MPC40 may contain increased

amounts of “fused” casein micelle. McKenna reported an increase in “fused” casein micelles as the protein content of MPC increases [16].

In summary, an increase in “fused” casein micelles in MPC may have resulted in uncontrolled casein aggregates in MPC fortified yoghurts resulting in heterogeneous casein matrix and significantly lower viscosity ($p < 0.05$) as compared to SMP and NDM yoghurts. The significant ($p < 0.05$) interaction effect of powder type and replicates on yoghurt viscosity can be attributed to changes in processing conditions during powder manufacture. Some of the processing conditions during powder manufacture include denaturation of whey proteins, change in mineral equilibrium during concentration, storage of concentrates before drying, and drying conditions [18]. Hence, yoghurt manufactured from a different source of milk solids at a given protein and TS contents may result in variation in the viscosity of yoghurts.

Yoghurt was manufactured from SMP, NDM, MPC40 and MPC70 that was stored for 3, 9, and 15 months at each %protein/%TS to evaluate the effects of storage time of powders on yoghurt viscosity. As shown in Table 3, storage time did not have a significant effect ($p > 0.05$) on the viscosity of the yoghurts manufactured from NDM, MPC40, and MPC70 at each %protein/%TS. In contrast, storage time had a significant effect ($p < 0.05$) on the viscosity of the yoghurt manufactured from SMP at MPMS (Table 4). The viscosity of yoghurt manufactured from 15-month stored SMP (495 cp) was significantly lower ($p < 0.05$) as compared to that manufactured from 9 months of stored SMP. However, this effect was not significant ($p > 0.05$) in yoghurts manufactured from SMP at LPLS and HPHS. Although the exact reason for variation was not understood, it can be argued that the irregularity in storage induced lactose crystallization and collapsing of the particle structure as a result of storage [40] may have resulted in the variation in the viscosity of SMP yoghurts at MPMS.

It is important to note that storage of NDM, MPC40, and MPC70 for 3, 9, and 15 months did not have a significant effect ($p > 0.05$) on the viscosity of yoghurts at each protein: TS. Although several researchers have reported a progressive loss of solubility of MPC with an increase in the storage time [16,17,22], the effects of reduced solubility of MPC (MPC40 and MPC70) on the yoghurt viscosity were not significant ($p > 0.05$) when used for yoghurt manufacture in this study. The overnight hydration of powders and the application of heat treatment at 95°C for 10 min during yoghurt manufacture may have ensured the complete dissolution of MPC in the yoghurt milk.

The yoghurt manufactured from NDM results in similar or improved viscosity as compared to SMP and improved viscosity as compared to MPC40 and MPC70 at a given protein: TS. Furthermore, the overnight hydration of powders and high heat treatment (95°C for 10 min) during yoghurt manufacture may negate the effects of reduced solubility of MPC during storage.

Yoghurt syneresis: The separation of the liquid phase from a gel is termed syneresis. Unlike rennet-induced gels, syneresis is considered an undesirable attribute in yoghurt gels. In yoghurt gels, casein micelles aggregate and form a protein matrix with relatively uniform pores or interstitial spaces. These pores or interstitial spaces hold the liquid phase known as “whey”. The amounts of liquid phase held by yoghurt gels are directly related to the dimensions of the pores and the density of the protein matrix [4].

The overall mean syneresis of yoghurts at HPHS was significantly lower ($p < 0.05$) as compared to yoghurts manufactured at MPMS and LPLS. Similarly, the overall mean syneresis of yoghurts at MPMS was significantly lower ($p < 0.05$) as compared to yoghurts manufactured at LPLS (results not shown). These results are in agreement with a study conducted by Harwalkar and Kalab, where the author reported an increase in the protein matrix density and a decrease in the pore size or interstitial space as %protein and %TS content increases [38].

The MS and probabilities (in parentheses) of yoghurt syneresis (at each protein: TS) are shown in Table 3. As shown in Table 3, the powder type, replicates, and the interaction effect of powder type and replicates had a significant effect ($p < 0.05$) on the yoghurt syneresis at each protein: TS. Additionally, the interaction effect of powder type and storage time was significant ($p < 0.05$) for the mean syneresis of yoghurt at HPHS. The mean syneresis of yoghurt manufactured from SMP, NDM, MPC40, and MPC70 at LPLS, MPMS, and HPHS are shown in Table 5. At LPLS, some of the mean syneresis of NDM, MPC40, and MPC70 yoghurts were significantly different ($p < 0.05$). However, the values were in close proximity ranging from 5.58 g to 6.17 g irrespective of storage time (Table 5). Similar results were found at MPMS and HPHS, where the mean syneresis of yoghurt (manufactured from NDM, MPC40, and MPC70) ranged from 4.70 g to 5.05 g and 3.85 g to 4.31 g respectively. In contrast, the mean syneresis of yoghurts manufactured from SMP at LPLS (6.06 g to 6.11 g), MPMS (5.30 g to 5.35 g), and HPHS (4.51 g to 4.60 g) was significantly higher ($p < 0.05$) as compared to NDM, MPC40, and MPC70 yoghurts. Although the exact reason for increased syneresis of SMP yoghurt as compared to NDM yoghurt was not understood, the former may have resulted because of the difference in the microstructure as compared to NDM. Powders containing high amounts of lactose (30%-40%) may induce collapsing of powder particles because of lactose crystallization [40]. Thus, SMP may contain a higher amount of crystallized lactose as compared to NDM. The presence of additional crystallized lactose in the SMP fortified yoghurt microstructure may have resulted in reduced water holding capacity or increased syneresis as compared to NDM yoghurts. Further investigation in this regard is required to identify microstructural differences.

Despite the lower viscosity of MPC40 and MPC70 yoghurts, their

Table 5: Mean(n=3) syneresis results of yogurt manufactured (LPLS¹, MPMS², and HPHS³) from SMP⁴, NDM⁵, MPC40⁶, and MPC70⁷ at each storage time.

Storage Time	SMP	NDM	MPC40	MPC70
		LPLS		
3 months	6.06 ± 0.07 ^a	5.77 ± 0.15 ^b	5.59 ± 0.16 ^b	5.77 ± 0.33 ^b
9 months	6.17 ± 0.07 ^a	5.86 ± 0.28 ^b	5.58 ± 0.17 ^c	5.76 ± 0.38 ^{bc}
15 months	6.11 ± 0.16 ^a	5.77 ± 0.06 ^b	5.62 ± 0.22 ^b	5.78 ± 0.40 ^b

MPMS				
3 months	5.30 ± 0.03 ^a	4.85 ± 0.16 ^b	4.77 ± 0.20 ^b	4.89 ± 0.38 ^b
9 months	5.35 ± 0.12 ^a	4.93 ± 0.13 ^b	4.72 ± 0.24 ^c	4.94 ± 0.36 ^{bd}
15 months	5.30 ± 0.10 ^a	4.85 ± 0.02 ^c	4.70 ± 0.19 ^c	5.05 ± 0.23 ^b
HPHS				
3 months	4.51 ± 0.10 ^a	4.14 ± 0.23 ^b	4.04 ± 0.19 ^b	4.02 ± 0.39 ^b
9 months	4.60 ± 0.15 ^a	4.03 ± 0.14 ^b	4.01 ± 0.19 ^b	4.20 ± 0.39 ^b
15 months	4.53 ± 0.08 ^a	3.85 ± 0.16 ^d	4.08 ± 0.13 ^c	4.31 ± 0.38 ^b

Note: ^{a-d} Means within the same row not sharing common subscripts are significantly different (p<0.05)

¹LPLS=Low Protein Low Solids (4% Protein/12.5% Total solids); ²MPMS=Medium Protein Medium Solids (4.5% Protein/13.5% Total solids); ³HPHS=High Protein High Solids (5% Protein/ 15.5% Total solids); ⁴SMP=Skimmed Milk Powder; ⁵NDM= Non-Fat Milk Powder; ⁶MPC40= Milk Protein Concentrate powder with 40% protein; ⁷MPC70= Milk Protein Concentrate powder with 70% protein.

improved syneresis as compared to NDM and SMP yoghurts may have resulted because of the difference in the microstructure of the MPC as compared to NDM and SMP. Kalab et al., studied the microstructure of SMP and MPC powder particles and reported the presence of smooth surface, shallow dimple, and well aerated aggregated powder particles in MPC powder as compared to the presence of wrinkles, cracked shell-like structure, and broken aggregates in SMP powder particle [41]. Although limited information is available regarding the water-holding properties of SMP and NDM powder particles as compared to MPC powder particles, it can be theorized that both the presence of well-aerated powder particle (of MPC) and fused casein aggregates in MPC yoghurt may have resulted in reduced syneresis rate as compared to SMP and NDM yoghurt. McKenna showed the presence of fused casein aggregates in 5% (w/w) protein solution (MPC85) after application of shear (20000 rev/min) and different homogenization pressure [16]. However, limited information is available regarding the presence of fused casein aggregates after overnight hydration followed and heat treatment (95 °C/10 min) in the milk fortified with MPC for yoghurt manufacture. Therefore, further investigation is required to study the microstructural changes in SMP and NDM yoghurts as compared to MPC yoghurts during the syneresis period (2.5 hours in the present study).

The yoghurts manufactured from SMP result in increased syneresis values as compared to NDM, MPC40, and MPC70 yoghurts. It is also interesting to note that the solubility of MPC70 decreased as a result of storage in a study conducted by Shah et al., [23]. However, the reduced solubility of MPC70 did not have a significant effect on the functional properties of yoghurt manufactured from MPC70 at each storage time. Shah et al., reported that the lower surface hydrophobicity index of MPC70 as compared to SMP, NDM, and MPC40 may have resulted because of the presence of fused casein aggregates [23]. These aggregates may have affected the functional properties of MPC70 fortified yoghurt at each protein: TS. Additionally, it was also noted that despite the significantly higher EAI of MPC70 powder, the viscosity of yoghurt manufactured from MPC70 was significantly lower as compared to SMP, NDM, and MPC40 yoghurts. It can be extrapolated that the improved EAI of MPC70 may assist in emulsifying fat globules which may act as structure-building components and thus improve the functional properties of full-fat varieties of yoghurt as compared to nonfat varieties [23].

Effects of the season of powder manufacture on functional properties of yoghurt

Seasonal effects of SMP and NDM on yoghurt functionality were studied. Table 6 shows the mean squares and probabilities (in parentheses) of viscosity and syneresis of yoghurts produced with powders manufactured in the summer and winter seasons. As shown in Table 6, the season of powder manufactured had a significant effect (p<0.05) on yoghurt viscosity and syneresis manufactured at each protein: TS. In contrast, the storage time of powders did not have a significant effect (p>0.05) on the viscosity and syneresis of yoghurts manufactured at each protein: TS. Additionally, syneresis of yoghurt was significantly affected (p<0.05) by powder type at each protein: TS. In contrast, powder type had a significant effect (p<0.05) only on the viscosity of yoghurts manufactured at MPMS. The interaction effects of powder type and replicates had a significant effect (p<0.05) on the viscosity of yoghurts manufactured at LPLS, MPMS as well as syneresis of yoghurt manufactured at HPHS. The interaction effect of powder type and season had a significant effect (p<0.05) on the syneresis of yoghurts manufactured at MPMS and HPHS.

As shown in Table 7, at MPMS and HPHS viscosity of yoghurts manufactured from winter season SMP (SMP-W) was significantly higher (p<0.05) as compared to the viscosity of yoghurts manufactured from summer season SMP (SMP-S). At MPMS, SMP-S fortified yoghurt viscosity ranged from 495 cp to 524 cp as compared to the viscosity of SMP-W yoghurts ranging from 549 cp to 560 cp (Table 7). Similarly, at HPHS, SMP-S fortified yoghurt viscosity ranged from 573 cp to 589 cp as compared to the viscosity of SMP-W yoghurts ranging from 671 cp to 675 cp. Also, the viscosity of yoghurts fortified with winter season NDM-winter (NDM-W) was significantly higher (p<0.05) as compared to the viscosity of yoghurts manufactured from summer season NDM-summer (NDM-S) at MPMS and HPHS (%protein/%TS). At MPMS, NDM-S fortified yoghurt viscosity ranged from 516 cp to 536 cp as compared to the viscosity of NDM-W yoghurts ranging from 559 cp to 563 cp (Table 7). Similarly, at HPHS, NDM-S fortified yoghurt viscosity ranged from 593 cp to 608 cp as compared to the viscosity of NDM-W yoghurts ranging from 668 cp to 672 cp (Table 7). Although, there was some significant difference (p<0.05) between the viscosity of yoghurts manufactured from SMP-W and NDM-W as compared to SMP-S and NDM-S at LPLS (Table 7), the effects were not consistent at each time point. It can be argued that the lower protein: TS of yoghurts may be responsible for these inconsistencies as compared to yoghurts

at MPMS and HPHS. As shown in Table 8, syneresis of SMP-S fortified yoghurts was significantly higher ($p<0.05$) as compared to syneresis of SMP-W fortified yoghurts at each protein: TS. Syneresis of yoghurts from SMP-S ranged from 6.06 g to 6.17 g, 5.30 g to 5.35 g, and 4.51 g to 4.60 g as compared to syneresis of yoghurts manufactured from SMP-W ranging from 5.60 g to 5.76 g, 4.74 g to

4.87 g, and 4.00 g to 4.14 g at LPLS, MPMS and HPHS respectively (Table 8). Similarly, syneresis of NDM-S fortified yoghurts was significantly higher ($p<0.05$) as compared to syneresis of NDM-W fortified yoghurts at LPLS and MPMS. In contrast, the significant ($p<0.05$) difference between syneresis of yoghurts at HPHS was not consistent throughout the time point (Table 8).

Table 6: Mean squares and probabilities (in parentheses) of yogurt RVA¹-viscosity and syneresis manufactured from SMP and NDM (summer and winter season) at three different %protein/%TS.

Factors	df	LPLS ²		MPMS ³		HPHS ⁴	
		Viscosity	Syneresis	Viscosity	Syneresis	Viscosity	Syneresis
Powder Type	1	761 (0.10)	0.46* (0.0001)	1502* (0.02)	0.74* (<0.0001)	860 (0.19)	0.77* (<0.0001)
Replicates	2	429 (0.21)	0.032 (0.23)	696 (0.06)	0.006 (0.49)	849 (0.18)	0.0008 (0.92)
Storage Time	2	199 (0.47)	0.027 (0.28)	643 (0.07)	0.006 (0.51)	127 (0.76)	0.002 (0.79)
Season	1	7555* (<0.0001)	1.14* (<0.0001)	14661* (<0.0001)	1.24* (<0.0001)	60434* (<0.0001)	0.54* (<0.0001)
Powder Type * Replicates	2	1226* (0.02)	0.005 (0.79)	1146* (0.01)	0.004 (0.59)	369 (0.47)	0.067* (<0.007)
Powder Type* Season	1	119 (0.50)	0.07 (0.09)	466 (0.16)	0.20* (<0.0001)	1547 (0.08)	0.55* (<0.0001)

Note: *Statistically significant ($p<0.05$).

¹RVA= Rapid Visco Analyzer; ²LPLS=Low Protein Low Solids (4% Protein/ 12.5% Total solids); ³MPMS=Medium Protein Medium Solids (4.5% Protein/ 13.5% Total solids); ⁴HPHS= High Protein High Solids (5% Protein/ 15.5% Total solids).

Table 7: Mean(n=3) viscosity results of yogurt manufactured (LPLS¹, MPMS² and HPHS³) from SMP⁴, NDM⁵, SMP (Winter)⁶, and NDM (Winter)⁷ at each storage time.

Storage Time	Summer(SMP)	Winter(SMP)	Summer(NDM)	Winter(NDM)
LPLS				
3 months	385 ± 9.50 ^b	419 ± 12.29 ^a	405 ± 32.29 ^{ab}	418 ± 9.87 ^a
9 months	397 ± 18.19 ^b	409 ± 1.89 ^{ab}	396 ± 8.33 ^b	432 ± 5.05 ^a
15 months	382 ± 23.03 ^b	412 ± 16.43 ^{ab}	380 ± 31.22 ^b	428 ± 24.72 ^a
MPMS				
3 months	505 ± 3.79 ^c	556 ± 12.29 ^{ab}	536 ± 30.79 ^b	563 ± 8.66 ^a
9 months	524 ± 20.55 ^b	560 ± 12.99 ^a	532 ± 5.20 ^b	561 ± 2.30 ^a
15 months	495 ± 26.58 ^b	549 ± 13.47 ^a	516 ± 35.36 ^b	559 ± 11.54 ^a
HPHS				
3 months	574 ± 12.06 ^b	675 ± 19.00 ^a	608 ± 7.55 ^b	672 ± 21.65 ^a
9 months	589 ± 19.14 ^b	671 ± 10.10 ^a	604 ± 19.67 ^b	671 ± 12.75 ^a
15 months	573 ± 14.57 ^b	675 ± 23.11 ^a	593 ± 53.27 ^b	668 ± 13.89 ^a

Note: ^{a-c} Means within the same row not sharing common subscripts are significantly different ($p<0.05$); ¹LPLS=Low Protein Low Solids (4%Protein/12.5% Total solids); ²MPMS= Medium Protein Medium Solids (4.5% Protein/13.5% Total solids); ³HPHS=High Protein High Solids (5% Protein/15.5% Total solids); ⁴SMP= Skimmed Milk Powder; ⁵NDM=Non-Fat Milk Powder; ⁶SMP (Winter)=Skimmed Milk Powder produced in winter season; ⁷NDM (Winter)= Non-Fat Milk powder produced in winter season.

Table 8: Mean(n=3) Syneresis results of yogurt manufactured (LPLS¹, MPMS², and HPHS³) from SMP⁴, NDM⁵, SMP (Winter)⁶, and NDM (Winter)⁷ at each storage time.

Storage Time	Summer(SMP)	Winter(SMP)	Summer(NDM)	Winter(NDM)
LPLS				
3 months	6.06 ± 0.07 ^a	5.60 ± 0.18 ^b	5.77 ± 0.15 ^b	5.61 ± 0.08 ^c
9 months	6.17 ± 0.07 ^a	5.76 ± 0.12 ^{bc}	5.86 ± 0.28 ^b	5.54 ± 0.13 ^c
15 months	6.11 ± 0.16 ^a	5.64 ± 0.10 ^{bc}	5.77 ± 0.06 ^b	5.52 ± 0.15 ^c
MPMS				
3 months	5.30 ± 0.03 ^a	4.74 ± 0.04 ^{bc}	4.85 ± 0.16 ^b	4.65 ± 0.08 ^c
9 months	5.35 ± 0.12 ^a	4.78 ± 0.06 ^{bc}	4.93 ± 0.13 ^b	4.65 ± 0.05 ^c
15 months	5.30 ± 0.10 ^a	4.87 ± 0.04 ^b	4.85 ± 0.02 ^b	4.67 ± 0.11 ^c
HPHS				
3 months	4.51 ± 0.10 ^a	4.00 ± 0.11 ^b	4.14 ± 0.23 ^b	3.98 ± 0.05 ^b
9 months	4.60 ± 0.15 ^a	4.02 ± 0.11 ^b	4.03 ± 0.14 ^b	4.00 ± 0.02 ^b
15 months	4.53 ± 0.08 ^a	4.14 ± 0.04 ^b	3.85 ± 0.16 ^c	4.03 ± 0.04 ^b

Note: ^{a-c} Means within the same row not sharing common subscripts are significantly different (p<0.05); ¹LPLS= Low Protein Low Solids (4% Protein/12.5% Total solids); ²MPMS= Medium Protein Medium Solids (4.5% Protein/13.5% Total solids); ³HPHS=High Protein High Solids (5% Protein/15.5% Total solids); ⁴SMP=Skimmed Milk Powder; ⁵NDM=Non-Fat Milk Powder; ⁶SMP (Winter)= Skimmed Milk Powder produced in winter season; ⁷NDM (Winter)= Non-Fat Milk Powder produced in winter season.

In summary, yoghurts fortified with powder manufactured in the winter season showed improved functional properties as compared to those manufactured in the summer season. The improved functional properties may have resulted because of the parameters such as the seasonal change in calcium activity of reconstituted skim milk and/or the seasonal variation in denaturation of whey proteins. Underwood and Augustin, reported a significant correlation (p<0.05) between calcium ion activity of reconstituted skim milk and rheological properties of acid milk gel where an increase in calcium ion activity in reconstituted milk led to a longer time for gelation and formation of weaker gels [42]. However, it is interesting to note that during acidification, the pH of the milk reduces which is accompanied by simultaneous dissolution of CCP from the casein micelle. As a result of dissolved CCP, the calcium ion in the reconstituted skim milk increases. Therefore, it can be theorized that because of the increased rate of CCP dissolution, an increase in calcium ions in reconstituted skim milk may have resulted in a shorter time for casein aggregation or time for gelation. Additionally, the time of gelation and the rheology of gels are also affected. Nevertheless, based on the study by Underwood & Augustin, it can be concluded that the season of milk production influences calcium activity in milk and therefore powders manufactured in different seasons [42]. Other parameters such as the presence of citrates in the milk [43], the stage of lactation [44], and mastitis infections [43] also affect the calcium activity in milk. As the calcium content of milk was not measured in this study, a direct comparison could not be established. The denaturation of whey proteins has also been studied to vary in milk collected from different seasons. Cheng et al., studied the effects of seasonal changes in proteins and minerals composition of milk powders and their effects on rheological properties of yoghurts standardized at different TS [45]. The authors reported variations in whey protein content and whey protein denaturation in powders with the highest whey protein denaturation observed in October-December. The seasonal variation in whey protein denaturation

has also been reported by a previous study [46]. The high heat treatment (90°C for 10 minutes) applied during nonfat yoghurt manufacture in this study ensures 80%-85% denaturation of whey proteins which improves its hydration and gelling properties and therefore textural properties of the milk gel. However, as reported by previous studies [45-46], the denaturation of whey proteins varies based on the season of powder manufacture despite applying the same heat treatment which may affect the functionality of whey proteins and therefore the nonfat yoghurt. Although the whey protein denaturation index of powders was not evaluated in this study, it may be theorized that the increased amounts of whey protein denaturation may have improved the functional properties of yoghurts in this study.

CONCLUSION

The functional properties of nonfat stirred yoghurt such as viscosity and syneresis depend based on fortification such as MSNF or protein: TS in the yoghurt formulation. Fortification based on MSNF using different protein sources results in varying amounts of protein content in the yoghurt formulation where an increase in the protein content can result in improved functional properties of yoghurts. Similar results were observed in yoghurts fortified with three different proteins: TS with different milk solids sources where an increase in the protein: TS resulted in improved functionality of nonfat yoghurts. However, at a given protein: TS in the yoghurt formulation, the selection of milk solids source plays an important role in determining the functional properties of yoghurt. At the same protein: TS, yoghurts produced from NDM showed improved functional properties as compared to SMP, MPC40, and MPC70 yoghurts. Functional properties such as viscosity and syneresis of yoghurts produced from MPC70 were significantly lower (p<0.05) as compared to SMP, NDM, and MPC40 yoghurts where the lower viscosity of MPC70 yoghurts may have resulted because of an extensive fusion of casein micelles resulting in a heterogeneous casein matrix.

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