The Haryana Veterinarian (September, 2023) **62(SI-2),** 50-54 ISSN 0033-4359 (P) **Research Article**

STANDARDIZATION OF METHOD OF MANUFACTURE OF WHEY PROTEIN ENRICHED YOGHURT POWDER

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ABSTRACT

Yoghurt has a low shelf life of a week under refrigeration due to high water activity (aw). To enhance the product's shelf life, the aw needs to be reduced by converting yoghurt to powder form. Additional SMP and cream were added to prepare concentrated yoghurt having 30% total solids, which is suitable for spray drying. To enhance the whey protein content in the yoghurt powder, the additionally added SMP in the preparation of concentrated yoghurt was replaced with whey protein concentrate (WPC_{70}) at different levels like 0, 30, 40 and 50% to produce whey protein enriched yoghurt. As the level of replacement of SMP with WPC_{70} increased from 0 to 40%; physico-chemical properties like pH, syneresis, penetration value and time of setting also increased. This study also attempted to optimize the effect of spray drying outlet temperature on the quality of Whey Protein Enriched Yoghurt Powder (WPEYP). As the outlet temperature increased insolubility index and hydroxyl- methyl-furfural (HMF) content increased; whereas, moisture, loose bulk density, dispersibility and survivability of starter cultures decreased significantly ($P \leq 0.05$) in WPEYP. Good quality WPEYP was produced at spray drying outlet temperature of 60 ºC.

Keywords: Whey protein concentrate, Outlet temperature, Maillard reaction, Spray drying, Yoghurt Powder

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Yoghurt is the most consumed fermented milk product worldwide due to its utritional and therapeutic values (Nikola *et al.*, 2020). Different varieties of yoghurt incorporated with fruits or milk proteins are available in the market (Arora *et al.*, 2021 and Chaudhary *et al.*, 2022). Traditionally, skim milk powder (SMP) was used to increase total solids in the manufacture of yoghurt. However, the availability and better functional properties of whey protein concentrate (WPC) make it an alternative for SMP. Replacing SMP with WPC in yoghurt improves buffering capacity of yoghurt with increased viability of the starter cultures (Camilla *et al.*, 2019). Also, whey, a byproduct of cheese, paneer and chhana contains significant amount of valuable nutrients and disposal of whey is a problem due to its high biological and chemical oxygen demand during treatment. As yoghurt has a short shelf life of a week, even under refrigeration; converting yoghurt into powder will result in better shelf life, low transportation and storage cost. Spray drying is a well-known practice suitable for drying of milk and milk products (Sunitha *et al.*, 2016), because of the high rate of evaporation, very short time of heat contact with low production cost. The biggest challenge for the spray drying of yoghurt is the viability of live starter culture organisms due to high inlet/outlet temperatures encountered during the spray drying. The outlet temperature of spray drying plays an major role in controlling the physico-chemical quality and survivability of starter organisms in the spray dried products. There is limited literature available on production of whey protein-enriched yoghurt powder. Hence, the present study was carried out to standardize the method of production of whey protein-enriched yoghurt powder and different factors affecting the standardization process.

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MATERIALS AND METHODS

Sharanagouda *et al.* (2013) procedure was followed for the preparation of concentrated yogurt with 30% total solids (TS). Whey protein-enriched concentrated yoghurt was prepared by using WPC_{70} to replace additionally added SMP (to increase SNF from 11 to 23.6% in concentrated yoghurt) at three different levels viz., 30%, 40% or 50%. Final heating temperature was reduced to 85 ºC for 5 minutes to avoid thickening the WPC enriched yogurt mix. The yoghurt prepared was subjected to various physico-chemical, and starter growth studies.

To prepare whey protein enriched yoghurt powder, Spray drier (MilkTek® Engineers, Bangalore) having five kg/h water evaporation capacity was used for spray drying. The protein enriched concentrated yoghurt was stirred well at a RPM of 600, using M/s. Sujata mixer grinder (New Delhi) and filtered in muslin cloth to make it lump free. The preliminary trials indicated that the feed rate of 25 g/min; 25,000 rpm of atomizer; inlet hot air temperature 160 ºC with air speed of 0.05 m^3/s were optimum for spray drying of yoghurt. To study the effect of outlet temperature of spray drying on the quality of yoghurt powder three outlet temperatures *viz*., 60, 65 and 70 ºC were selected. Thus obtained yoghurt powder was further subjected to various physico-chemical properties and starter culture survival studies.

Total solids, pH and the titratable acidity of the samples were determined as per the methods described in IS:SP:18 (Part XI) 1981. Fat content of the mix was determined as per the procedure of AOAC (1980), degree of syneresis (expressed as proportion of free whey) was measured as per Yeganehzad *et al.* (2007). The product's sensory evaluation was done using 9 point hedonic scale of Peryam and Pilgrim (1957). The lactic counts were done as per the procedure of Suk *et al.* (1997). The Physico-chemical properties like moisture and loose bulk density (Chevanan *et al.*, 2011), insolubility index and dispersibility [IS: SP: 18 (Part XI, 1981)], and hydroxy methyl furfural (HMF) contents (Mistry and Pulgar, 1996) were analyzed as per the standard procedures. The yoghurt powder containing mixed cultures was examined for the viability of *S. thermophilus* and *L. delbrueckii* subsps. bulgaricus using *S. salivarius* ssp. thermophilus agar (M 948) and *L. bulgaricus* agar (M 927) procured from Hi Media Laboratories Limited, Mumbai by following pour plate technique (Suk *et al.*, 1997). The final yoghurt powder was reconstituted into 14% TS yoghurt (that of control) in 10 mM$CaCl_2$ solution with 2% sodium alginate at 30±1 ºC and kept for 30 min for proper setting and then cooled to 4±1 ºC by keeping it in refrigerator. Such reconstituted yoghurt was subjected to sensory evaluation using 9-point hedonic scale (Peryam and Pilgrim, 1957).

The statistical significance was assessed using analysis of variance (ANOVA) at 5% level of significance to find the correlation between process variables and experimental results using SAS 9.2 Version. To study the effect of time on the results of the experiment, multiple linear regression analysis was carried out with time as one of the independent variables. The experiments were performed in triplicate and reported as mean values ± standard deviation.

RESULTS AND DISCUSSION

Effect of replacement of SMP with WPC_{70} on physico-chemical properties

It observed from the Table 1 that the pH of whey protein-enriched concentrated (WPEC) yoghurt increased non-significantly (P≤0.05). The small increase in the pH may be due to the buffering capacity of the WPC (Kailasapathy *et al.*, 1996). In contrast, the acidity increased significantly (P≤0.05) from 1.44 to 1.72% LA, possibly due to higher amounts of whey proteins and lactose. It is a well-known fact that whey protein containing lactose helps in the growth of starters by readily utilizing lactose; which might have produced higher acidity in the concentrated whey protein enriched yoghurt and increased total solids in concentrated yoghurt might have required significantly (P≤0.05) more time (120 minutes more time) for setting of WPEC yoghurt as reported by Sady *et al.* (2009). This increase in setting time could be attributed to the decreased moisture and water activity with increased total solids (Yeganehzad *et al.*, 2007). There was a significant (P≤0.05) increase in syneresis value from 0.65 to 2.25 ml as the SMP replacement level with WPC from 0 to 50%. This could be attributed to the release of bound water by the weaker gel formed due to decreased casein content (Neslihan and Ihsan, 2019).

Effect of replacement on the growth of starter culture

Whey proteins in the form of WPC_{70} were used to replace SMP in manufacturing whey protein enriched concentrated yoghurt. Due to the incorporation of WPC_{70}, viable counts ($\log_{10}$ cfu/g) of *S. thermophilus* and *L. bulgaricus* increased non-significantly from 9.85 and 9.96 to 10.16 and 10.26, respectively, as the level of replacement, increased from 0 to 50% (Table 2). Investigated products met demands of international standard (FAO/WHO, 2018), where the number of viable cells of typical microflora in yoghurts must be not less than 10^7 cfu/g. The components responsible for the increased growth of the starter might be due to α-nucleotides, non-protein nitrogen, or some specific peptides and sulphur containing amino acids (cysteine and methionine) from WPC (Sady *et al.*, 2009).

Effect Spray dying outlet temperatures on physico-chemical properties

The manufacturers consider physico-chemical properties like moisture content, loose bulk density, insolubility solubility index (SI), dispersibility and HMF content as quality parameters of milk powders. The solubility of spray-dried powders depends majorly on outlet air temperature apart from product feed rate and atomized droplet size (Sunitha *et al.*, 2016). It is observed from the results in Table 2 that as the outlet temperature decreased from 70 to 60ºC, the moisture content increased, and HMF content decreased significantly (P ≤ 0.05) from 4.16% to 4.95% and 51 to 36 μ mol/100 g, respectively. This may be due to the formation of larger powder particles with increased moisture content in the powder. The decrease in the HMF content may be due to the decrease in the outlet drying air temperature. The dissolution of soluble components of milk powders like lactose, salts, un-denatured whey proteins in addition to the disseminated components like caseins represents the solubility of any

Table 1. Effect of different levels of replacement of SMP with WPC_{70} on the physico chemical properties of protein enriched concentrated yoghurt

Physico-chemical attributes	% replacement of SMP with WPC (%)				CD (P≤0.05)
	0	30	40	50	
pH	4.75 ± 0.02[a]	4.81 ± 0.10[a]	4.85 ± 0.08[a]	4.88 ±0.11[a]	0.336
Acidity (% LA)	1.44 ± 0.01[a]	1.47 ± 0.03[b]	1.69 ± 0.04[c]	1.72±0.06[d]	0.001
Syneresis (ml)	0.65±0.01[a]	1.10±0.04[b]	1.55±0.05[c]	2.25±0.10[d]	0.446
Fat (%)	6.4 ± 0.20[a]	6.4 ± 0.20[a]	6.4 ± 0.10[a]	6.4 ± 0.20[a]	1.000
Total solids (%)	30.0 ± 1.43[a]	30.0 ± 1.51[a]	30.0 ± 1.35[a]	30.0 ± 1.39[a]	1.000
Time for setting (min) at 40 ºC	300 ± 15[a]	315 ± 15[b]	360 ± 15[c]	420 ± 15[d]	0.000

Note: 1. Data are presented as means ±SD (n=3).
2. Treatments bearing different superscripts in row are statistically different (p≤0.05).
3. All treatments have 30% TS; CD: critical difference.

Table 2. Effect of spray drying outlet temperature on physico-chemical and microbiological attributes of the Whey Protein Enriched Yoghurt Powder

Physico-chemical attributes		Spray drying outlet temperature (ºC)			CD (P ≤ 0.05)
		60	65	70	
Moisture (%)		4.95±0.4[a]	4.58±0.32[b]	4.16±0.26[c]	0.024
Loose bulk density (g/100ml)		47.60±1.0[a]	44.10±0.5[b]	40.20±0.5[c]	0.386
Insolubility Index (ml sediment)		0.15±0.02[a]	0.20±0.01[b]	0.23±0.01[c]	0.001
Dispersability (%)		96.5±1.5[a]	92.3±2.0[b]	88.0±1.5[c]	0.956
HMF (µ mol/100g)		36.0±0.90[a]	43.0±1.00[b]	51.0±1.25[c]	0.031
		Starter cultures			
S. thermophilus	*9.95 ± 0.15[a]	7.95±0.21[b]	8.20±0.25[b]	8.95±0.19[c]	0.563
L. bulgaricus	*9.85 ± 0.13[a]	7.56±0.17[b]	7.83±0.22[c]	8.34±0.20[d]	0.234

Note: 1. * Count in fresh yoghurt expressed as log10 cfu/g
2. Data are presented as means ±SD (n=3).
3. Treatments bearing different superscripts in row are statistically different

Table 3. Sensory evaluation of reconstituted whey protein enriched yoghurt

Sensory attributes	Fresh yoghurt	Reconstituted yoghurt	CD (P≤0.05)
Colour & Appearance	8.15 ± 0.30[a]	7.95 ± 0.20[a]	0.405
Flavour	8.25 ± 0.38[a]	8.20 ± 0.50[a]	0.332
Body & Texture	8.25 ± 0.25[a]	7.85 ± 0.25[a]	0.512
Overall acceptability	8.25 ± 0.25[a]	7.95 ± 0.50[a]	0.421

Note: 1. Data are presented as means ±SD (n=3)
2. Treatments bearing different superscripts in row are statistically different

milk powder, which relies on the heat treatment during processing and spray drying of the milk product (Dibyakanta *et al.*, 2017). It was also observed that insolubility index decreased from 0.23 to 0.15 ml as the outlet temperature decreased from 70 to 60 ºC may be due to the increased solubility of the undenatured whey protein during spray drying (Geanderson *et al.*, 2018).

In the preliminary trials of the present study it was observed that the total solids of yoghurt could not go beyond 25% due to the high viscosity formed by the whey proteins which were used to replace SMP to produce WPEC yoghurt (Sharanagouda *et al.*, 2013). Due to the whey proteins, the distribution of particles size is more or less uniform, which lead to the normal bulk density of yoghurt powder like other milk powders at higher outlet drying temperatures indicating the effect of outlet temperature on powder quality. It was noticed that as the outlet temperature decreased, bulk density of yoghurt powder increased significantly (P≤ 0.05) from 40.2 to 47.6 g/ml (Table 2). It may be due to the formation of more bigger particles with narrow particle size distribution and increased moisture content in the powder. It is also observed that as the outlet temperature decreased, the dispersibility of yoghurt powder increased significantly (P≤0.05) could be due to increased moisture content that might have increased size of particles which in turn improved the dispersibility (Junfu *et al.*, 2016; Haohan *et al.*, 2020).

Cow milk

↓

Standardization (6.4% fat, 30% TS)

(SMP replaced with WPC at 30, 40 and 50%)

↓

Pre-heating (60–65 ºC/5min.)

↓

Homogenization (Two stage, 2000 psi and 500 psi)

↓

Heat treatment (90 ºC/1 min)

↓

Cooling to 40 ±1 ºC

↓

Addition of yoghurt cultures (3% level)

[*S. thermophilus* and *L. bulgaricus* (1:1)]

↓

Incubation (40±1 ºC)

↓

Breaking coagulum (to make uniform slurry)

↓

Microwave preheating (40, 50 and 60 ºC)

↓

Spray drying

(Inlet temp. 165±5 ºC, outlet temp. 60, 65 and 70 ºC)

↓

Packaging

↓

Storage

Fig. 1. Flow diagram for the preparation of protein enriched probiotic yoghurt powder

Starter culture survivability

The initial counts of starter cultures in concentrated yoghurt just before drying were 9.95 and 9.86 log cfu/g for *S. thermophilus* and *L. bulgaricus*, respectively. As the spray drying outlet temperature decreased (Table 2) from 70 to 60 ºC, the survivability of starter cultures increased significantly (P ≥0.05), which may be due to a decrease in the severity of thermal effect and increase in moisture content of powder (Geanderson *et al.*, 2018). Stage of growth phase of starters decides their survivability during drying (Fang and Bhandari, 2012). Stationary and log phase starters will have more thermal resistance than other growth phases. Due to longer incubation period required to achieve the desired acidity in WPEC yoghurt, starters might have reached the stationary phase (Sharanagouda *et al.*, 2013). It was also observed that the survivability of *S. thermophilus* was more than that of *L. bulgaricus* due to the thermophilic nature of *S. thermophilus* (Kim and Bhowmik, 1990 and Geanderson *et al.*, 2018).

Sensory evaluation of reconstituted whey protein-enriched yoghurt

It was noticed that there was no statistical difference (P ≥ 0.05) in sensory scores of fresh and reconstituted yoghurts (Table 3). But, there was a slight decrease in body and texture scores of reconstituted yoghurt than control which may be attributed to loss of protein gel network during stirring and drying. Even though it was tried to regain original protein gel network by use of the divalent cationic solution of $CaCl_2$ and sodium alginate as a stabilizer, it was not possible to regain the original network. Similar study was done by Sunitha *et al.* (2016) who reported that as the total solids in yoghurt increased the degree of acceptance of reconstituted yoghurt also increased.

CONCLUSION

Results showed that, the whey protein-enriched concentrated yoghurt (30% TS) could be prepared by 40% replacement of SMP (additionally added to increase TS) with WPC_{70}. Higher levels of replacement have a significant effect on physico-chemical and textural properties WPEC yoghurt. The results of spray drying in the present investigation demonstrated that 60 ºC is the best suitable outlet temperature for spray drying of WPEC yoghurt concerning survivability of starters, physico-chemical and sensory properties keeping other spray drying parameters constant. Reconstituted yoghurt having 14% total solids was very much comparable with the control. WPEYP may be used to prepare soups, drink yogurt, reconstitute butter milk, etc. Further some aspects of rehydration kinetic needs to be further studied for marketing yoghurt powder.

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