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EFFECTS OF DIFFERENT METHODS OF DRYING ON ANTIOXIDANT AND MICROSCOPIC CHARACTERISTICS OF *Spirullina platensis* ENRICHED SOY YOGURT

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ABSTRACT

Spirulina platensis was discussed as an active compound with regard to the combined effects with soy yogurt in dried food formulation. Drying influenced the microscopic, and antioxidant properties of soy yogurts, and could be used to create new functionalities. The present investigation aimed to convert *Spirulina platensis* enriched soy yogurts to powder form by different drying methodologies such as air, vacuum, freeze, and microwave drying and evaluate the microstructure and antioxidant activity of the powders obtained. Antioxidant activities were assayed by using polyphenol assay, estimation of carotenoid content, DPPH radical scavenging activity and FRAP assay. Structural changes were analyzed using a scanning electron microscope and an X-ray diffraction pattern. Freeze-drying was produced significantly high quality dried *Spirulina platensis* enriched soy yogurt, i.e. better functional and antioxidant properties (Polyphenol 0.211 Gallic acid equivalent. g^{-1}, $p<0.001$ and DPPH activity 17.48±0.11%, $p<0.01$ of dried yogurt). In addition, *Spirulina platensis* enriched soy yogurt exhibited an amorphous type molecular structure in all four types of drying methods adopted. It could be concluded that the freeze-drying method could produce superior quality *Spirulina platensis* enriched dried soy yogurt powder compared to hot-air-oven and vacuum drying, while it is highly comparable to microwave drying.

Keywords: *Spirulina platensis,* soy yogurt, microwave-dried, vacuum-dried, freeze-dried, antioxidant

INTRODUCTION

Dairy products are highly perishable. Milk powder has increased shelf life and stored for long period without substantial loss of quality, even at ambient temperatures (**Sharma *et al.*, 2012**). The dairy-based powders are mainly employed for recombination or reconstitution purposes; whereas non-dairy based powder can be exploited for their distinct functional properties for application as a food ingredient in several "value-added foods" products. Like dairy-based powder, non- dairy based powdered ingredients are stable, produced in large amount, lactose free and convenient for storage. Nowadays one of the main areas of research in food area is the development of functional foods that provide health benefits beyond their basic nutrition. Yogurt a fermented food product has a high worldwide acceptance and considered as ideal matrices to deliver beneficial nutritional ingredients. In addition, probiotic bacteria present in yogurt decrease intestinal disorder and chronic diseases. Soy bean which is the most widely grown and utilized legumes in the world has good amino acid profile, contain higher levels of essential fatty acids, soluble fiber, vitamins, minerals, phytochemicals which include isoflavones, phytic acid and saponins which have strong antioxidant properties and have capability of lowering the cholesterol level. Development of new nondairy fermented food products from soybean will provide specific health benefits beyond the conventional dairy products, which have certain limitations from nutritional health aspects. Foods prepared from non-dairy based powdered ingredients like dried soy milk are usually considered to be of lower quality. Yogurts both dairy and non-dairy types are highly nutritious food products for having principally probiotic constituents and micronutrients that really help to reduce gastro intestinal problems and blood cholesterol level in human/our system and free radicals associated diseases such as cancer and osteoporosis (**Savini *et al.*, 2013**). The non-dairy yogurts particularly the soy yogurts are known for such nutritional properties. In recent years, soy yogurts have been developed with expectedly enhanced nutritional superiority by incorporating functional lipids such as Gamma linolenic acid (GLA), Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) and also providing specific antioxidants such as oryzanol and lignans through specific edible oils (**Sengupta *et al.*, 2013**). Soy yogurts enriched with *Spirulina platensis* to provide higher arginine content in protein part and antioxidant such as selenium, zinc, tocopherol etc (**Morsy *et al.*, 2014**). Studies showed that viability of *Spirulina platensis incorporated* yogurt was higher when compared to control yogurt for both *Streptococcus thermophilus* and *Lactobacillus bulgaricus* throughout the storage period (**Priyanka *et al.*, 2013**). *Spirulina platensis* is known to have beneficial influence on the survival of the starter bacterial culture owing to its high protein, essential fatty acids such as gamma linolenic acid, vitamins and minerals (**Kavimandan and Sharma**, **2015***)*. It may be pointed out that soy yogurts having probiotic and other micronutrients can be processed particularly by various drying methodologies to powder with the retention of various nutrients that in turn can find applications in various kinds of food products.

In fact, it has been stated by some researchers recently that dried yogurts in powder form have many applications such as confectionary, savory, biscuits and cakes in bakery and in soups, dips, ready meals baby foods etc. (**Jaya, 2009**). In recent days, there is great emphasis on adding value to powders, and therefore, an inclusive effort from non-dairy plant and powder processors, is requisite to identify the means to add more value. Consumers are willing to pay more for soy milk powders if they can perceive high functionality and quality, as well as multifunctional properties. The present investigation aims to convert *Spirulina platensis* enriched soy yogurts to powder form by different drying methodologies and evaluate the microstructure and composition of the powders obtained.

MATERIAL AND METHODS

All chemicals used were, purchased from MERCK, India. Soybean seeds were purchased from the local market (New Alipore Market, Kolkata West Bengal, India). Commercially available milk curd cultures were purchased from Microbial Type Culture Collection and Gene Bank, Chandigarh [*Lactobacillus delbrueckii subsp. bulgaricus* (MTCC 911) and *Streptococcus thermophilus* (MTCC 1938)]. *Spirulina platensis* was used as dry biomass having composition of protein 0.28 g, energy 1.74 Kcal, fat 0.0 g and carbohydrate 0.16 g per 500mg (SUNOVA SPIRULINA, Surya Herbal Ltd. Noida, India).

Preparation of soy yogurt and *Spirulina platensis* enriched soy yogurt

The soy milk was made according to the procedure described by **Sengupta *et al.*, (2013)**. The resultant soy milk was then homogenized in a homogenizer (REMI MOTORS-RQ-122) and pasteurized at 80° C for 15 min. Soy milk was then cooled down to 40°C for the addition of *Spirulina platensis*. After addition of *Spirulina platensis* to pasteurized soy milk (1g of dry biomass of *Spirulina platensis* 100 ml^{-1} of soy milk), the mixtures of soy milk and *Spirulina platensis* were homogenized again in a homogenizer prior to inoculation with starter culture until the *Spirulina platensis* was mixed properly throughout the soy milk. Soy milk mixtures were aseptically inoculated with 2% of starter (*Lactobacillus delbrueckii subsp. bulgaricus*, *Lactobacillus planterum*, *Lactobacillus casei* and *Streptococcus thermophilus*, 1:1:1:1 v/v). The inoculated soy milk containing the *Spirulina platensis* was then poured into 100 ml sterile transparent food grade plastic cups with lids and incubated at 37°C for 24 hours. Soy yogurts obtained were stored at 4°C in a refrigerator for further analyses. Control soy yogurt was made by following the above procedure only except incorporation of *Spirulina platensis* into soy milk.

Drying method of soy yogurt and *Spirulina platensis* enriched soy yogurt

A quantity of 200g of a fresh *Spirulina platensis* enriched soy yogurt was dried separately by using four different drying techniques.

Hot- air -oven drying

Spirulina platensis incorporated soy yogurt was dried in hot air oven at 60^0C for 24 hours till constant weight was obtained. The dried soy yogurt (control) and *Spirulina platensis* enriched soy yogurt were sealed airtight and stored at 5°C until analyses.

Vacuum tray drying

Spirulina platensis enriched soy yogurt was spread over a petri dish and placed on the rack of vacuum tray dryer unit (Vacuum Oven 8" dia-12" deep, Temperature up to 150°C, Model D- 50, India). Vacuum in the dryer was set at 758 mm Hg and the temperature kept between 45°C to 60°C. The drying was continued for 5 hours until the product became free flowing. After cooling, the soy yogurt (control) and *Spirulina platensis* enriched soy yogurt powder were sealed airtight and stored at 5°C until analyses.

Microwave drying

Spirulina platensis enriched soy yogurt was spread over a polymer plate in Microwave (SAMSUNG MW83H/XTL) and heated for 10 minutes at 60°C. The product was then collected, powdered, cooled and was sealed airtight and stored at 5°C until analyses.

Freeze drying

Soy yogurt (control) and *Spirulina platensis* enriched soy yogurt was frozen in refrigerator at 4^oC and then freeze-dried in a freeze dryer (Freezone plus 6, Labconco, USA) at -40^0C and 0.3 mPa until constant weight (72 hours). The dried soy yogurt (control) and *Spirulina platensis* enriched soy yogurt powders were sealed airtight and stored at 5°C until analyses.

Handling packaging and grinding of dried soy yogurt (control) and *Spirulina platensis* enriched soy yogurt

All the samples sealed in Ziploc bags were placed inside aluminum-coated polyethylene bags. To prevent oxidation, all the packaged samples were flushed with nitrogen gas, heat sealed and stored at 35°C until further analyses. Dried yogurt powders obtained from different drying processes were ground using mortar and pestle. The hot-air oven dried, vacuum dried, microwave dried and freeze dried or lyophilized powder was homogenized by a 12 mesh sized sieve.

Physical property of dried soy yogurt (control) and *Spirulina platensis* enriched soy yogurt

Water content

The water content of dried soy yogurt (control) and *Spirulina platensis* enriched soy yogurt obtained by different drying methods were determined separately using the standard oven method at 70°C for 24 h (**AOAC, 1998**). The drying, cooling and weighing of samples was continued until the difference between two successive weighing was less than 1 mg.

Flow ability evaluation: Carr's compressibility index (C) and Hausner ratio (H)

A quantity of 50g of dried soy yogurt (control) and *Spirulina platensis* enriched soy yogurt was filled separately into a graduated glass cylinder and repeatedly tapped on a shaker. The sample was tapped for 500 times and repeated at 3 turns. The volume of powder after tapping and Carr's index percent was measured as equation 1. Hausner ratio is related to inter-particle friction. In this test, values less than 1.25 indicate good flow (≈ 20% Carr), a value greater than 1.5 indicate poor flow (≈ 33% Carr).

$$\text{Carr's compressibility index} = \frac{\text{Bulked density}}{\text{Tapped density}} \quad (1)$$

$$\text{Hausner ratio} = \frac{\text{Tapped density}}{\text{Bulked density}} \quad (2)$$

Antioxidant property of dried soy yogurt (control) and *Spirulina platensis* enriched soy yogurt

Polyphenol assay

Estimation of polyphenols was determined using Folin–Ciocalteu reagent (**Singleton and Rossi, 1965**) with some modification. 0.1 ml dried soy yogurt (control) and *Spirulina platensis* enriched soy yogurt (10 mg ml^{-1} in distilled water) was extracted separately for 2 h at room temperature on a mechanical shaker. To them, 1 ml of Folin–Ciocalteu reagent (1:2 dilution) and 2 ml of 10% Na_2CO_3 was added. The mixture was centrifuged at 20,000×g for 20 min, and the supernatant was decanted and filtered through Whatman No.1 filter paper. The absorbance of the clear supernatant solution was measured at 765 nm (V-630 UV-VIS Spectrophotometer, JASCO). Gallic acid was used as a standard. Each sample was analyzed twice with duplicates. Results were expressed as mg GAE 100 g^{-1} dry weight.

1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay

DPPH radical scavenging activity was determined using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay (**Bansal *et al.*, 2014**) with some modification. 0.5 ml dried soy yogurt (control) and *Spirulina platensis* enriched soy yogurt (10 mg ml^{-1} in distilled water) was added separately to 2.5 ml DPPH reagent (0.2 mM). The reaction mixture was kept in dark at ambient temperature for 30 min. The absorbance was measured using a spectrophotometer at 517 nm (V-630 UV-VIS Spectrophotometer, JASCO). DPPH radical scavenging activity (%) was calculated using following formula:

$$\%\ \text{Inhibition} = \frac{(\text{Absorbance 517 control} - \text{Absorbance 517 extract})}{(\text{Absorbance 517 control})} \times 100 \quad (3)$$

Ferric reducing antioxidant power (FRAP) assay

Reducing power was determined using ferric reducing antioxidant power (FRAP) assay (**Barahona *et al.*, 2011**) with some modification. 0.5 ml dried soy yogurt (control) and *Spirulina platensis* enriched soy yogurt (10 mg ml^{-1} in distilled water) was added separately to 2.5 ml FRAP reagent The FRAP reagent consist of 300 mM acetate buffer (3.1 g sodium acetate + 16 ml glacial acetic acid, made up to 1 L with distilled water; pH = 3.6), 10 mM TPTZ in 40 mM HCl and 20 mM $FeCl_3.6H_2O$ in the ratio of 10:1:1. The reaction mixture was kept in dark at ambient temperature for 10 min. The absorbance was measured using a spectrophotometer at 593 nm (V-630 UV-VIS Spectrophotometer, JASCO). Reducing power was calculated using following formula:

$$Reducing\ power = Abs593\ sample - Abs\ 593\ FRAP\ reagent \quad (4)$$

Carotenoid estimation

0.5 ml dried soy yogurt (control) and *Spirulina platensis* enriched soy yogurt (10 mg ml^{-1} in distilled water) was centrifuged separately at 3000 rpm for 5 minutes. The pellet was washed with distilled water 2-3 times. To the pellet, 2-3 ml of acetone (85%) was added, which was then subjected to repeated freezing and thawing. The suspension was centrifuged and the supernatant containing pigment was collected. The extraction was repeated till the supernatant became colorless, for complete recovery of carotenoid. The pooled fractions of supernatants were made-up to a final known volume. The absorbance was taken at 450nm using 85% acetone as blank and the total amount of carotenoids was calculated in mg g^{-1} as follows (**Saleh et *al.*, 2011**).

$$C = \frac{D \times VF}{2500 \times 100} \quad (5)$$

D=OD at 450nm, V = Volume of the extract, F = Dilution factor (Assuming that average extinction coefficient is 2500)

Color parameter of dried soy yogurt (control) and *Spirulina platensis* enriched soy yogurt

Color parameters were determined using colorimeter (Minolta Chroma meter CR-300, USA). Dried soy yogurt (control) and *Spirulina platensis* enriched soy yogurt were poured into a clear glass petri dish and color coordinate values (lightness, L*, redness, a*, and yellowness, b*) were recorded separately.

X-Ray Diffraction pattern of dried soy yogurt (control) and *Spirulina platensis* enriched soy yogurt

X-ray diffraction patterns of dried soy yogurt (control) and *Spirulina platensis* enriched soy yogurt were obtained at room temperature using a Rigaku Multiflex powder diffractometer (CuKα radiation generator) operated at a voltage of 40 kV. Powdered, dried yogurt samples were analyzed separately at two theta (2θ) angle, range of 10–30.

Morphology analysis of dried soy yogurt (control) and *spirulina platensis* enriched soy yogurt using scanning electron microscopic analysis (SEM)

Dried soy yogurt (control) and *Spirulina platensis* enriched soy yogurt at micro level were examined separately by scanning electron microscope for desired structural properties. The dried Soy yogurt and *Spirulina platensis* incorporated soy yogurt was previously fixed on an iron stub and then made electrically conductive by coating in a vacuum chamber with a thin layer of gold for 40 s. The pictures were taken at an excitation voltage of 15 kv at different magnifications varying from 1500-1600.

Statistical analysis

Statistical analysis was performed by using analysis of variance (ANOVA) and the means were compared across groups by Tukey test. All analyses were carried out in triplicates repeated at least twice with the Origin Pro 8 and the significant differences were determined at $p \leq 0.05$.

RESULTS AND DISCUSSION

Soy yogurt is generally dried by freeze, spray, microwave and vacuum drying. **Fazaeli *et al.,* (2012)** observed that among the different drying methods freeze-drying is one of the most advanced methods for drying food products since it retains taste, aroma, flavor, color and the nutritional quality. In addition (**Rybka and Kailasapathy, 1997**) also observed that freeze-dried soy yogurt is the authentic product in comparison to yogurt obtained using other conventional drying methods. Another study revealed that there is no significant change on the final contents of total protein casein, serum and non-protein nitrogen obtained from freeze-drying at -40°C. **Radaeva *et al.,* (1975)** found that survival of lactic acid bacteria in yogurt was 50-60% during freeze-drying at -40°C. **Kitawaki *et al.,* (2009)** showed that freeze-dried soy yogurt is beneficial in preventing hepatic lipid accumulation in rats. From the above literature, it was revealed that that freeze drying best choice of the drying methods tested for evaluation of the soy yogurt. Other drying techniques also work well but should not be used for soy yogurts due to sensitivity to oxidation.

Physical property of dried soy yogurt (control) and *Spirulina platensis* enriched soy yogurt

The physical properties of yogurt powder are shown in **Tab 1.** No significant differences were observed in water content between Product-I and Product-V. Data showed that vacuum dried yogurt sample had the significantly highest (3.67±0.67%, $p<0.01$) and the microwave dried had the significantly lowest moisture content (1.89±0.55%, $p<0.001$) in comparison with Product-I. Hot air and freeze dried yogurt samples had moisture close to each other. During vacuum drying, the yogurt sample was expanded under the vacuum.

Table 1 Selected physical properties of dried soy yogurt (control) and *Spirulina platensis* enriched soy yogurt

Materials	Water content (%)	Bulk density (g cm^{-3})	Tapped density (g cm^{-3})	C	HR
Product-I (control)	2.55±0.61	0.22±0.08	0.41±0.49	53.65±0.21	1.86±0.48
Product-II	2.62±0.56	0.39±0.04^{a}	0.59±0.04^{a}	66.10±0.05^{a}	1.51±0.32^{a}
Product-III	3.67±0.67^{b}	0.32±0.04^{a}	0.58±0.03^{b}	55.17±0.06^{a}	1.81±0.11^{b}
Product-IV	1.89±0.55^{c}	0.29±0.07	0.44±0.09^{b}	65.90±0.03^{a}	1.51±0.23^{b}
Product-V	2.52±0.98	0.38±0.02^{a}	0.47±0.11^{a}	66.66±0.03^{a}	1.23±0.16^{c}

The data are mean ± S.D. and significantly different at $^{a}P < 0.05$, $^{b}P < 0.01$ and $^{c}P< 0.001$ vs. Product-I, SP- *Spirulina platensis*, C- Carr's Index, HR- Hausner ratio, SP- *Spirulina platensis*; Product-I: soy yogurt (freeze dried), Product-II: SP soy yogurt (hot air oven dried),Product-III: SP soy yogurt (vacuum dried), Product-IV: SP soy yogurt (microwave dried), Product-V: SP soy yogurt freeze dried.

Compressibility is the ability to reduce volume by tapping, developed by **Carr (1965)** as an average and indirect measure of cohesion forces. The flowability scale is "universal", as it has been constructed on previous measurements of 300 different powders, from [0–19] very poor, [20–39] poor, [40–59] not good, [60–69] normal, [70–79] good, [80–89] fairly good and [90–100] very good. All the yogurt powders that we had dried had been under the value of 60, except Product-I and Product-III corresponding to "normal flowability". Compressibility for the product was followed the same tendency as the Hausner ratio, declining when vacuum dried applied. Note that a Product-II, IV and V presented a normal flow, without special problems with a flowability index value of above 60. Only the Product-III presented significantly worse flowability (55.17±0.06, $p<0.05$) than Product-I. Product-I could not present good flowability because of its low content of carbohydrate and protein percentage. Product-IV presented extreme values for low Hausner ratio (1.23±0.16, $p<0.001$), together with the best flowability index (66.6±0.03 $p<0.05$). This product should therefore present high internal homogeneity due to *Spirulina platensis* incorporation and quite "exceptional properties" within the drying techniques studied. This flowability index value is quite unusual for non-dairy powder.

Antioxidant property of dried soy yogurt (control) and *Spirulina platensis* enriched soy yogurt

Antioxidant property of dried soy yogurt (control) and *Spirulina platensis* enriched soy yogurt affected by different drying methods is illustrated in **Fig 1**. Spirulina, are believed to be a rich source of polyphenol antioxidants. *Spirulina platensis* fortification was found to bring about a significant increase in the antioxidant capacity, but drying was found to bring about a reduction. Total poly phenolic content was significantly the highest in product-IV (0.185 GAE g^{-1}, $p<0.05$) and Product-V (0.211 GAE g^{-1}, $p<0.05$ of dried yogurt). Product-V showed significantly highest total phenolic content (0.211 GAE g^{-1}, $p<0.001$ of dried yogurt), in comparison with Product-II and Product-III. However, not a big difference in percentage increase of activity was observed for all four drying treatment, with *Spirulina platensis* fortification. In the FRAP assay, the antioxidant activity of Product-II and Product-III was not affected by respective drying treatments. On the contrary, in the DPPH assay, the antioxidant activity was significantly influenced for Product-II (14.58±0.04%, $p<0.05$ of dried yogurt) and Product-III (14.26±0.02%, $p<0.05$ of dried yogurt). DPPH radical scavenging activities were not significantly different between Product-IV (15.67±0.01%, $p<0.01$ of dried yogurt) and Product-V (17.48±0.11%, $p<0.01$ of dried yogurt), while both were significantly higher than Product-II and Product-III. The results showed (**Fig 1**) that the total carotenoid content of powders obtained by *Spirulina platensis* incorporated soy yogurt was significantly influenced by different drying method The highest carotenoid content (5.77 mg g^{-1} of dried yogurt) was for Product-III whilst the Product-II that was hot air dried had the lowest carotenoid content (3.27 mg g^{-1} of dried yogurt).

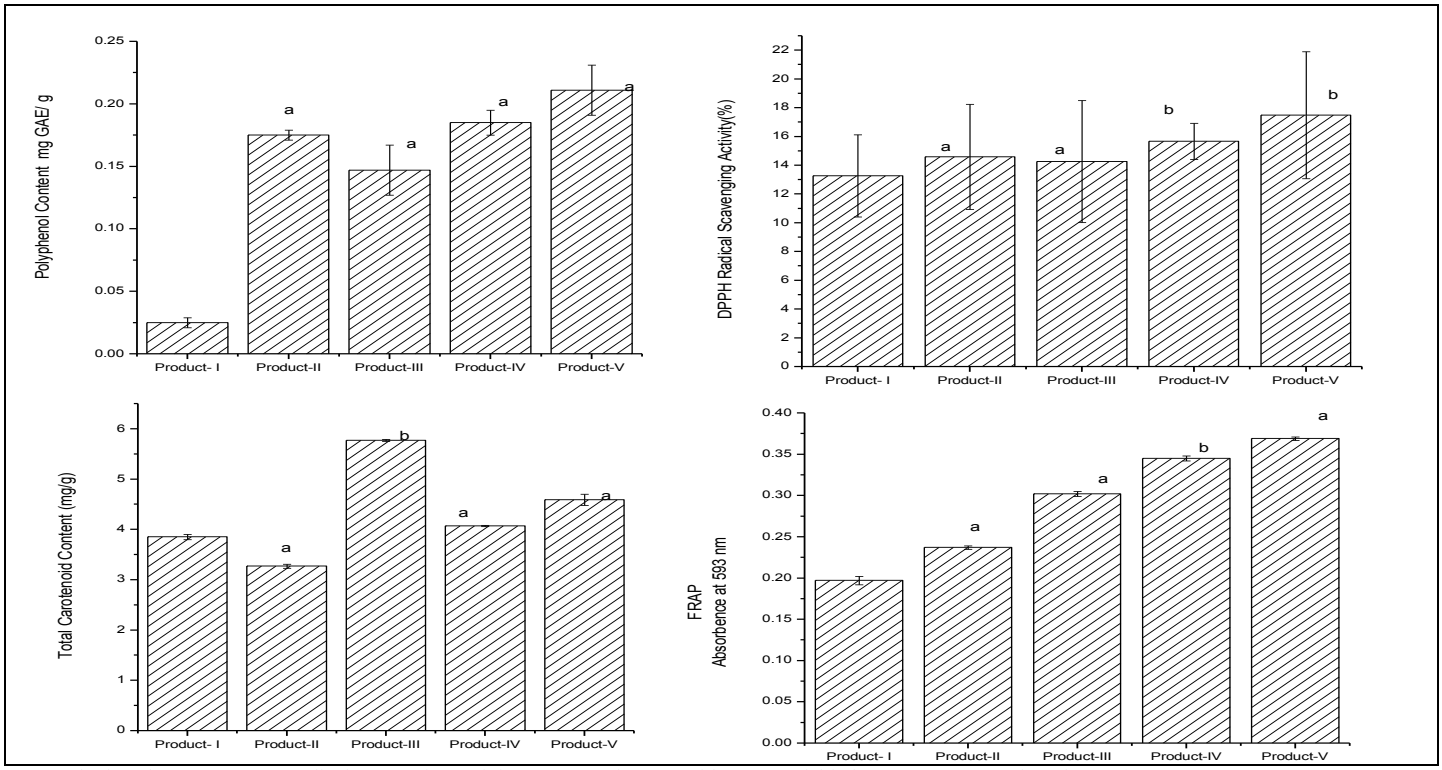

Fig 1 Polyphenol content, DPPH radical scavenging activity, FRAP and carotenoid content of dried soy yogurt (control) and *Spirulina platensis* enriched soy yogurt**.**SP- *Spirulina platensis*, C- Carr's Index, HR- Hausner ratio, SP- *Spirulina platensis*; Product-I: soy yogurt (freeze dried), Product-I: soy yogurt (freeze dried), Product-II: SP soy yogurt (hot air oven dried),Product-III: SP soy yogurt (vacuum dried), Product-IV: SP soy yogurt (microwave dried), Product-V: SP soy yogurt freeze dried. GAE: Gallic acid equivalent, DPPH: 1, 1-diphenyl-2-picrylhydrazyl, FRAP: Ferric reducing antioxidant power.

There was no significant difference between Product-IV and Product-V in terms of total carotenoid content. It is evident that vacuum drying was more effective in the retention of total carotenoid than air-drying. Therefore, it can be concluded that the main reason for carotenoid degradation is due to loss of anthocyanin. It is clear that the freeze-drying process can substantially preserve the nutritional value of *Spirulina platensis* in terms of total antioxidant activity. From the results of the various tests in the study, the quality of freeze-dried *Spirulina platensis* incorporated soy yogurt powder products is seen to be highest, followed by microwave-dried powder, vacuum-dried powder, air-dried powder, and then powders from soy yogurt as control.

Color parameter of dried soy yogurt (control) and *Spirulina platensis* enriched soy yogurt

It was observed from **Tab 2** that the Lightness (L*), redness (a*) yellowness (b*) and hue angle (h) were significantly different among the dried *Spirulina platensis* enriched soy yogurt. The highest L* value (59.34±2.65, $p<0.01$) and lowest a* value (-17.48±0.98, $p<0.01$) was observed in Product-V in comparison with Product-II, III and IV. The highest L* value could attribute to minimal color deterioration. Freeze-drying can retarded oxidation and other chemical reactions, and thus minimal color deterioration (**Ratti *et al.*, 2001)**. Meanwhile, the lowest L* value (40.57±4.67, $p<0.05$) and highest a* value (-14.58±5.58, $p<0.05$) was observed in Product-II. The lowest L* value could attribute to color degradation. Oven drying can cause oxidative degradation, and thus lead to color change (turn into darker color). No significant difference was observed in b* value (yellowness) between Product-IV and Product-V while there was a highly significant difference between Product-II and Product-III (**Tab 2).** Product-IV obtained a higher h value (150.77±7.64, $p<0.05$) compared to Product-II, II and V suggesting that microwave dried Product-IV is more vivid in its dark green color implying that it will be more attractive and appealing to consumers. The overall distinct vivid dark green color of the Product-IV may be indicative of high chlorophyll retention. The minimal color change of product produced by microwave dried Product-IV and freeze-dried Product-V suggests the appropriateness of these processes to produce high quality products.

Table 2 Color measurements of soy yogurt and *Spirulina platensis* incorporated soy yogurt powder obtained by different drying methods

Materials	L*	a*	b*	h
Product- I (control)	75.64±6.27	0.26±2.85	12.25±0.87	83.85±9.25
Product-II	40.57±4.67^{a}	-14.58±5.58^{a}	6.81±0.54^{a}	130.11±8.31^{c}
Product-III	51.74±6.25^{c}	-14.26±3.54^{a}	6.35±0.34^{c}	143.79±8.46^{b}
Product-IV	46.27±3.84^{a}	-15.67±1.26^{c}	7.65±0.87^{b}	150.77±7.64^{a}
Product-V	59.34±2.65^{b}	-17.48±0.98^{b}	7.29±0.69^{b}	144.07±6.47^{b}

The data are mean ± S.D. and significantly different at $^{a}P < 0.05$, $^{b}P < 0.01$ and $^{c}P < 0.001$ vs. Product-I, SP- *Spirulina platensis*, C- Carr's Index, HR- Hausner ratio, SP- *Spirulina platensis*; Product-I: soy yogurt (freeze dried), Product-II: SP soy yogurt (hot air oven dried),Product-III: SP soy yogurt (vacuum dried), Product-IV: SP soy yogurt (microwave dried), Product-V: SP soy yogurt freeze dried.

X-ray diffraction patterns of dried soy yogurt (control) and *Spirulina platensis* enriched soy yogurt

The X-ray diffraction patterns of the soy yogurt (control) and *Spirulina platensis* enriched soy yogurt gave valuable information about structural aspects of the *Spirulina platensis* (**Fig 2**). In this study, Product-II, III, IV and V had a significant influence on the degree of dispersion and aggregation in soy yogurt. XRD is a very common technique used to confirm the crystalline-amorphous state of dried yogurt powder. Dried yogurt obtained from all the form of drying methods exhibited amorphous nature. Amorphous products produce a broad background pattern while crystalline material exhibits sharp peaks while. Crystalline nature obtained from XRD is expected due to presence of raffinose and stachyose sugar. Rapid drying of low molecular weight sugar present in yogurt tends to produce amorphous metastable state of dried products due to insufficient time to crystallize. Drying methods of yogurt showed no crystalline peaks formation. In case of freeze drying the temperature of yogurt was less than 50°C. Crystal formation generally occurs above 50°C. Thus in case of freeze-drying, less crystalline is preferred. These figures exhibit essentially similar diffraction patterns (2θ values) for all samples suggesting that dried yogurts did not undergo any structural modifications (**Fig 2**). However, a major reduction in relative intensities of their peaks particularly for Product-II and Product-IV might be due to reduction in crystallinity and presence of amorphous state in the samples. Therefore, it was expected that in the case of drying by freeze drier, *Spirulina platensis* incorporated dried soy yogurt particles with less crystallinity were produced. However, it was interesting to note that the intensity count for Product-II as shown in the diffractograms was significantly lower compared to the other three powder products.

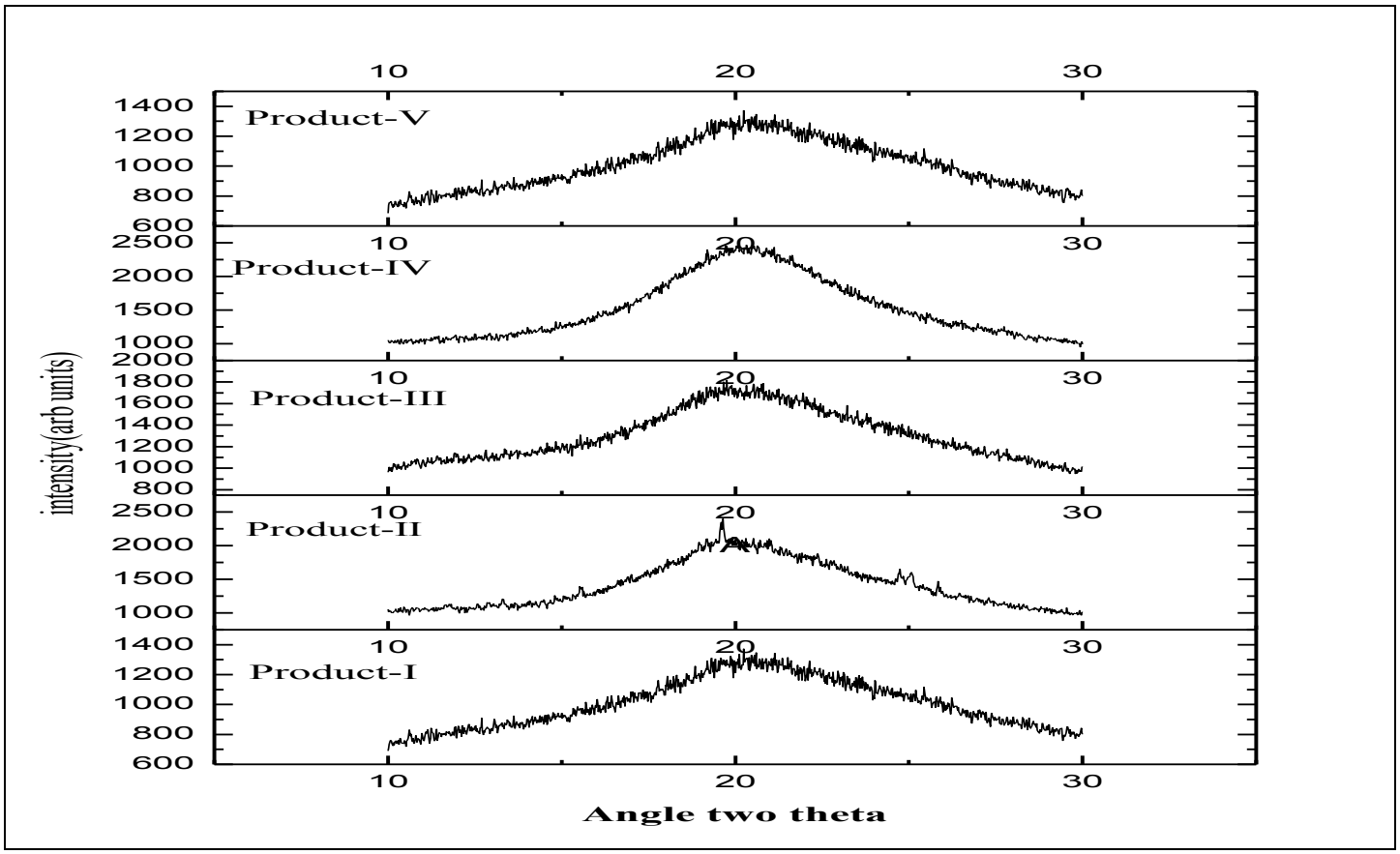

Fig 2 X-ray diffraction patterns of dried soy yogurt (control) and *Spirulina platensis* enriched soy yogurt
SP- *Spirulina platensis*, C- Carr's Index, HR- Hausner ratio, SP- *Spirulina platensis*; Product-I: soy yogurt (freeze dried), Product-II: SP soy yogurt (hot air oven dried), Product-III: SP soy yogurt (vacuum dried), Product-IV: SP soy yogurt (microwave dried), Product-V: SP soy yogurt freeze dried.

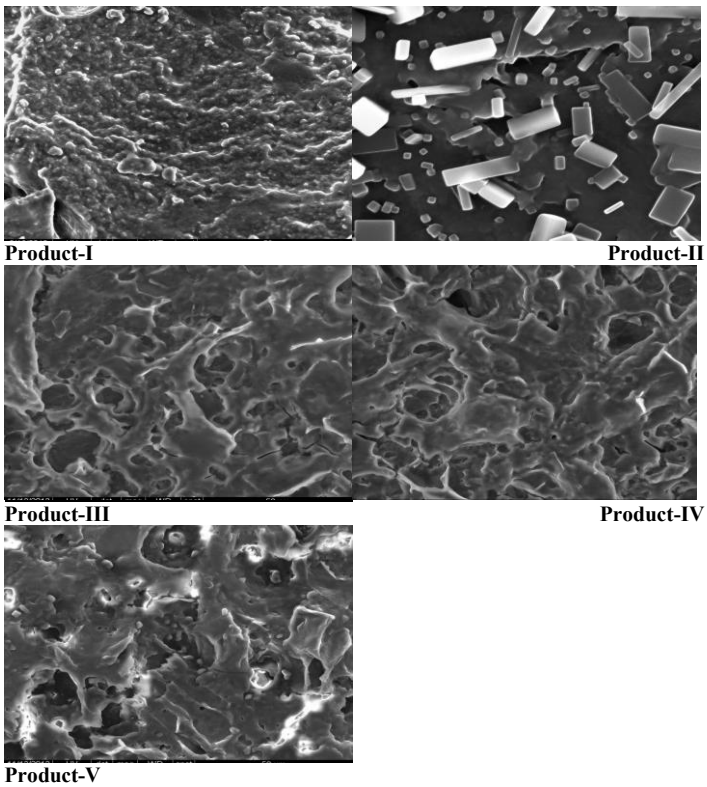

Fig 3 Scanning electron micrographs (SEM) of soy yogurt and *Spirulina platensis* enriched soy yogurt (magnification of 800×20 kV).
SP- *Spirulina platensis*; Product-I: soy yogurt (freeze dried), Product-II: SP soy yogurt (hot air oven dried),Product-III: SP soy yogurt (vacuum dried), Product-IV: SP soy yogurt (microwave dried), Product-V: SP soy yogurt freeze dried.Product- I: soy yogurt-freeze dried; Product-II: SP soy yogurt hot air oven Dried; Product-III: SP soy yogurt vacuum dried; Product-IV: SP soy yogurt microwave dried; Product-V: SP soy yogurt freeze dried.

Scanning electron micrographs (SEM) of dried soy yogurt (control) and *Spirulina platensis* enriched soy yogurt

Scanning electron micrographic studies of soy yogurt and *Spirulina platensis* enriched soy yogurt are shown in **Fig 3**. The microstructure of Product-II was compact and exhibited irregular particles with sharp edges and considerable indentation because of crushing into powder. The microstructure of Product-III and Product-IV was smooth, and flaky with uniform thickness. Product-V showed a skeletal like structure and was more porous than the other Product**.** This is due to the ice in the material during freeze drying helps prevent shrinkage and collapse of the structure and shape resulting in an insignificant change in volume.

CONCLUSION

The primary objective of manufacturing dried yogurt in powder form is to increase the shelf life of yogurt. Freeze-drying is a physical phenomenon of sublimation, which consists of first freezing the yogurt and then reducing the surrounding presence to allow the frozen yogurt to sublimate directly for solid phase to gaseous phase without passing through the liquid state. Freeze dried products have very good capacity to take up water again since have extremely large internal surface area and they. Thus, freeze-dried products can be stored from very long time without too much loss of nutritional quality. Thus in freeze drying, products ingredients are preserved with good nutritional value. Moreover, freely dried yogurt retained flavor, color, taste in comparison to yogurt obtained using other drying methods (**Fazaeli *et al.,* 2012***)* and also has better rehydration property. Absence of air causes very less deterioration of food product. Another study revealed that freeze-drying did not affect the final content of protein casein, serum and non-protein nitrogen. Again, it was observed that in yogurt 50- 60% of lactic acid bacteria were survived during freeze-drying (**Radaeva, 1975**). That is why freeze-drying method is best drying methods. Drying techniques were shown to exert significant effect on *Spirulina platensis* enriched dried soy yogurt. Freeze-drying was most suitable method to produce high quality dried *Spirulina platensis* enriched soy yogurt, i.e. better functional and antioxidant properties. Overall, our study concludes that the freeze drying method can produce superior quality *Spirulina platensis* enriched dried soy yogurt powder compared to hot-air-oven and vacuum drying, while it is highly comparable to microwave drying. The study provides an opportunity to the powder processing industry in selecting a

better drying technique that can be utilized for the production of high quality yogurt powder, a complementary formulated as a tablet from soy yogurt powder is possible and also may be therapeutically effective against lactose-intolerance syndrome and preventing antibiotic-associated diarrhea.

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INTERACTION OF SEMI-REFINED CARRAGEENAN (E407A) WITH NANO QUANTA OF SOME FOOD HYDROCOLLOIDS AND THEIR PHYSIOCHEMICAL, FUNCTIONAL AND RHEOLOGICAL PROPERTIES

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ABSTRACT

Interaction of semi-refined carrageenan with other food hydrocolloids in different ratio and their rheological, physicochemical and some functional characteristics were evaluated in the present study. Gel strength and viscous synergism index (I_v) were measured and used to analyze their interaction level. *Kappa* carrageenan had good synergistic effect with cassia, LBG, guar, HPMC and konjac and antagonistic effect with xanthan, alginate, CMC and agar. Only *Iota* carrageenan in the range of 60:40 with kappa showed neutral effect. A blend of SRC with konjac / LBG can be made for good gel strength whereas konjac / GG for good viscosity. Therefore, SRC can be used in place of refined carrageenan in some food applications.

Keywords: Semi-refined carrageenan, Food hydrocolloids, Synergism index, Gel strength, Viscosity, Water holding capacity, Emulsifying Activity

INTRODUCTION

Hydrocolloids are a heterogeneous group of long chain polysaccharides and proteins. When it fuses with water, it forms colloid which is between a true solution and suspension. The number of hydroxyl group indicate the increases their affinity towards water binding and acted as a hydrophilic compound. Hydrocolloids offer food formulators a strong value proposition, based on a unique synergy with other hydrocolloids and protein. Depending upon their origin, hydrocolloids can be classified as Natural, Semi-synthetic and Synthetic. The naturally (vegetable) derived hydrocolloids are mainly used as oil in water emulsifiers; whereas, animal derived hydrocolloids form water in oil emulsion. Most hydrocolloids derived from a plant source and remaining from microbial fermentation and others are the result of chemical modification of natural polysaccharide. A great number of synthetic and semi synthetic hydrocolloids along with natural using in food Industry (**Saha & Bhattacharya, 2010**).

Nanotechnology is an advanced technology that has the capacity to modify the food sector **(Luykx *et al*., 2008; Huang *et al*., 2010).** The application of nanotechnology to the food sector focus on color, taste, texture, sensory attributes and stability during shelf life. In addition, it improves the thermal stability, water solubility and oral bioavailability of functional compounds **(McClements *et al*., 2009)** Nano emulsions derived from the natural hydrocolloids act as colloidal drug carriers for pharmaceutical applications. Due to best internal absorption, the bioavailability of less water soluble drugs increases even though it passes through oral way **(Constantinides, 1995; Bates et al., 1975)**.Absorption in the gastrointestinal tract is improved by a small droplet size also been found **(Toguchi *et al*., 1990)**. Recently, more preferences have been given for the use of many natural hydrocolloids as drug delivery carriers in the bio-pharmaceutical sector **(Ogaji *et al*., 2012).**

In food industries, hydrocolloids play a dominant inevitable role especially as thickeners and stabilizing agent. Currently, carrageenans such as *kappa* and *iota* have an important share in the pudding and milk shakes **(Puvanenthiran *et al*., 2003).** As we known the Agar-Agar, oldest one plays the main role in the bakery products and jelly industries commercially **(Stanley, 2006).** Alginate used as the best ingredient in the restructured food and cold prepared bakery creams **(Roopa *et al*., 2009).** In fruit juice beverages and soft drinks, hydroxyl propyl methyl cellulose used extensively **(Williams & Philips, 2000)**. Konjac highly used in extruded product like noodles and also in jelly desserts **(Williams, 2006).** Due to high viscous and texture stabilizing nature Locust bean gum and Guar gum used in the various sectors such as ice cream, ketchup, fruit juice and pudding powder **(Koocheki *et al*., 2009).** Xanthan gum and carboxyl methyl cellulose play the main role in the soups, ketchup and gravies **(Sahin *et al*., 2004).**

Hydrocolloid market, which has been growing at compound annual growth rate (CAGR%) of 12% ± 3% in recent years, is valued at around $4.4 billion p.a. with a total volume of about 260,000 tons **(Kayacier *et al*., 2006).** In the food industry, combined hydrocolloids and their formulation have been proven successfully at the various industrial products during and after processing. Their properties mostly depend on individual hydrocolloid properties. Mixed hydrocolloids give strong synergistic effects than the individual one. For this reason, many researchers focused on the rheological characteristics of combined hydrocolloids **(Walkenstrom *et al*., 2003; Mandala *et al*., 2003).** The objective of present study was to investigate the interaction of Semi-Refined Carrageenan (E407a) with nano sized dosage of some mood hydrocolloids and their physio-chemical, functional and rheological properties.

MATERIAL AND METHODS

Materials

The ingredients of food grade carrageenan (E 407a) (*Kappa, Iota*) used were from the stock of AquAgri Processing Private Limited, Batch No-108/2015, Manamadurai, Tamil Nadu, India. Locust bean gum (LBG, E 410) (R.K Enterprises, India), Guar gum (GG, E 412) (Sarda Gums & Chemicals, India), Sodium carboxymethyl cellulose (CMC, E 466) (Wealthy Chemicals Industry, Suzhou, China), Konjac gum (E 425) (Shanghai Research Institute of Chemical Industry Testing Centre, China), Na-Alginate (E 401) (SNAP Alginate, India), Gelatine (E 441) (Nice Chemical Private Limited, Kerala, India), Hydroxy Propyl Methyl Cellulose (HPMC, E15) (LOBA Chemicals Private Limited, Mumbai, India), Agar-Agar (E 406) (Marine chemicals, Cochin, India), Cassia gum (E 427) (Swastik Gums, India) and Xanthan gum (E 415) were purchased from Ziboxan, Deosen Biochemical Limited, China. Equipments used were: Brookfield texture analyzer, Model CT3 4500, USA; Remi motor-RQ 122, Remi Elektrotechnik Limited, India; Water bath-250 W, Sigma Scientific Instrument Private Limited, Chennai, India; Samsung Refrigerator, Model RR 19 H1104RH/DL, India and Sony Cyber shot, GPS- DSC- HX 200.

Measurement of Gel strength and Viscosity

A 1.5 g hydrocolloid sample was weighed and incorporated slowly into 100 ml distilled water and the solution was stirred on a water bath at 80ºC till to get a

complete dissolution of hydrocolloids. After heat treatment, solution was analyzed immediately at 75 ºC for viscosity (Brookfield LVD-II+P viscometer, Brookfield Engineering Laboratories, Inc., MA, USA) and gel strength was measured after maintain 20 ± 2 ºC storage for overnight in Brookfield texture analyzer (CT3 Texture Analyzer, Brookfield Engineering Laboratories, Inc., MA, USA).

Physicochemical analysis

Moisture, Ash content of each hydrocolloid sample was determined by the gravimetric method. pH values of samples were measured with a pH meter (Eutech Instruments, Malaysia) in their solutions prepared with distilled water (1.5% w/v). Bulk density of hydrocolloids particle was measured by method of Okara et al. **(1977)**

Rheological analysis

Calculation of expected viscosity of binary gum mixtures at equal concentration in a solution was expressed as Eq.3 and Eq.4 **(Rao, 1999).**

$$_{mix} = X_A \eta_A + X_B \eta_B \quad (1)$$

$$\eta_{mix} = \eta A^{XA} * \eta_B^{XB} \quad (2)$$

Where *XA* and *XB* are the weight fractions of gum A and B, respectively, and *ηA* and *ηB* are the apparent viscosity of sole gum A and B solutions at the same concentration.Viscous synergism index (Iv) was calculated using the following expression in Eq.5 as described by Pellicer *et al*., **(2000)** as below

$$Iv = \eta i + j / \eta i + \eta j \quad (3)$$

Where i and j represent the two hydrocolloids present in the blend (i+j).

Functional property analysis

Water-holding Capacity (WHC) and Oil-holding Capacity (OHC)

WHC and OHC were determined according to methods described by Robertson *et al* (2000). Distilled water / commercial olive oil (25 mL) was added to 250 mg of dry sample, stirred and left at room temperature for 1 h. After centrifugation, the residue was weighed. The WHC was expressed as g of water held per g of sample, while the OHC was expressed as g of oil held per g of sample.

Emulsifying Activity (EA) and Emulsion Stability (ES)

A DIAX 900 Heidolph homogenizer (Schwabach, Germany) was used to homogenize a 2% (w/v) sample suspension in water at 11,000 rpm for 30 s. Sunflower oil (100 mL) was then added and homogenized for another 1 min. The emulsions were centrifuged in 15 mL graduated centrifuge tubes at 1,200 g for 5 min, and the volume of the emulsion left was measured. To determine the ES, emulsions prepared by the above procedures were heated at 80°C for 30 min and cooled to room temperature, and centrifuged at 1,200 g for 5 min. EA and ES were calculated using the following equations **(Chau *et al.*, 1997).**

$$EA = \frac{\text{Volume of emulsified layer (mL)}}{\text{Volume of whole layers in the centrifuge tube (mL)}} \times 100 \quad (4)$$

$$EA = \frac{\text{Volume of remaining emulsified layer (mL)}}{\text{Original emulsion volume (mL)}} \times 100 \quad (5)$$

RESULTS AND DISCUSSION

Physiochemical properties

The physiochemical parameters of hydrocolloids play an important role in the food and pharmaceutical application. The moisture, pH, bulk density, viscosity, gel strength and ash content of all the individual hydrocolloids shown in Table 1.

Table 1 Physiochemical properties of some food hydrocolloids

Hydrocolloids	Moisture	Ash	pH	Bulk density	Viscosity	Gel strength (g/cm^2)
K-Carrageenan	9.27	6.97	8.5	0.795	10	690
Ca-Iota	7.60	6.91	9.62	0.875	50	-
LBG	5.23	9.90	6.81	0.653	4700	-
Guar Gum	9.14	9.92	6.45	0.715	7250	-
Gelatin	9.60	9.93	5.47	0.601	5	-
Agar-Agar	9.68	8.90	6.12	0.712	2	485
Na-Alginate	11.84	7.13	7.42	0.947	60	-
Xanthan gum	9.56	9.09	6.21	0.729	4500	-
Cassia Gum	7.42	9.90	6.04	0.700	30	-
Na-CMC	11.20	8.22	7.72	0.666	6100	-
Konjac Gum	7.92	4.82	6.04	0.712	7800	-
HPMC	3.42	9.93	6.45	0.658	12	-

Properties of combined food hydrocolloids

pH

The pH value of different gums like Konjac gum Guar gum, LBG, Iota-carrageenan, Xanthan, Na-Alginate, Gelatine, Cassia gum, Na-CMC, HPMC and Agar-Agar with Kappa-carrageenan showed in Table 1. From a consideration of pH, along with the interaction of different food hydrocolloids were studied as shown in Figure 1. *kappa* carrageenan has been blended with gums with a ratio of 20:80, 40:60, 60:40 & 80:20. All from those blending the pH level of Iota decreases from 9.67 to 9.22; this is due to the fact that Iota carrageenan has higher pH value when compared to *kappa* carrageenan. So with the combination of this ratio's Iota-carrageenan has different property compared to other hydrocolloids. Whereas, viscous gums like Guar and LBG increases along with kappa blend ratio. Also, originality of gums changes from acidic to alkaline in nature. The pH value of LBG increases with the blend ratio of 20:80 as 7.31, 40:60 as 7.39, 60:40 as 7.65 and 80:20 as 7.92 which shows moderate increasing level. Similarly, Guar gum increases from 6.49 (20:80), 7.02 (40:60), 7.56 (60:40) and 7.87 (80:20) respectively. Likewise, Xanthan gum has 6.56, 7.07, 7.33 & 7.44 respectively. Also, Sodium alginate showed some narrow changes in the pH level. But in Gelatine, pH ranges from 5.65, 6.16, 6.6 and 7.23 as the level of kappa-carrageenan increases. They show quite high changes from the above gums.

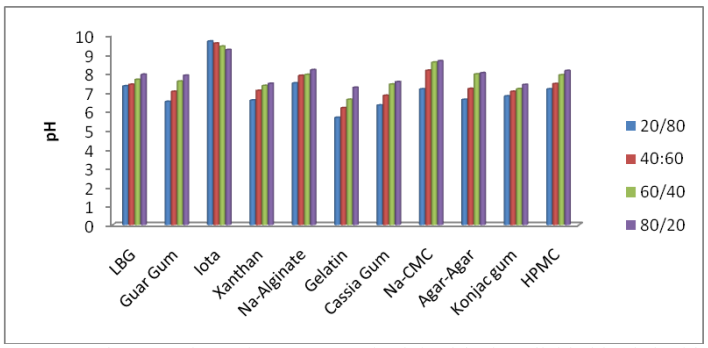

Figure 1 The pH values of some nano sized food hydrocolloids blended with kappa-carrageenan (1.5% w/v) at 60°C

The pH value for combined cassia and kappa carrageenan as follows 6.3, 6.81, 7.4 and 7.53 for 20:80 to 80:20 ratio blends. In the combination of both Na-CMC and HPMC with the kappa carrageenan started with bit alkaline and crossed pH 8 for the 80:20 ratio. The values such as 7.15, 7.43, 7.89 and 8.12 for the HPMC fusion with kappa carrageenan in the descending order of kappa and for Na-CMC, the values as 7.15, 8.13, 8.56 and 8.63 for descending order blend of kappa with the carboxyl methyl cellulose. Konjac gum and Agar-Agar come under the

bit acidic category when it's combined with the kappa; it doesn't have made a big change in the pH. The Agar-Agar with the kappa has the values of 6.6, 7.17, 7.94 and 8 for the 20:80 to 80:20 ratios and for the Konjac with the kappa has 6.78, 7.02, 7.16 and 7.38 for the same ratio. From the above discussion on food hydrocolloids with different blend ratio of Kappa carrageenan determines that the level of kappa increases in the blend ratio with gums, the range of pH decreases the only exception of Iota-carrageenan.

Gel strength

The gel strength and viscosity are the two important properties depict the quality of hydrocolloids. Only few food hydrocolloids having gel strength individually. As shown in Figure 2, Kappa carrageenan had found the result of gel strength (g/cm^2) individually 690 and Agar-Agar had 485. Remaining hydrocolloids couldn't able to form gel individually in a specific period of time. When the food hydrocolloids combined with kappa carrageenan, the results were found different. In the lower value of HPMC with kappa, 120 found in the 40/60 ratio and 350 in the 20/80. Higher the proportion in the kappa content led to form a gel. Likewise, it happened in the kappa combined sodium alginate and Na-CMC. The value 105 found in the 40/60 and 188 found in 20/80 of the ratio between Na-CMC and kappa, 105 found in the combination of sodium alginate with the kappa proportion of 40/60 and 201 for 20/80.Then, Xanthan gum with kappa carrageenan had the lesser effect of 100, 140, 410 for 60/40, 40/60 and 20/80.Next, it was followed by the Iota, Guar and cassia. The ratio of kappa leads to increase of gel strength 136, 263, 417 with Iota carrageenan, 143, 320, 512 with the guar gum in the 40/60, 60/40 and 80/20.

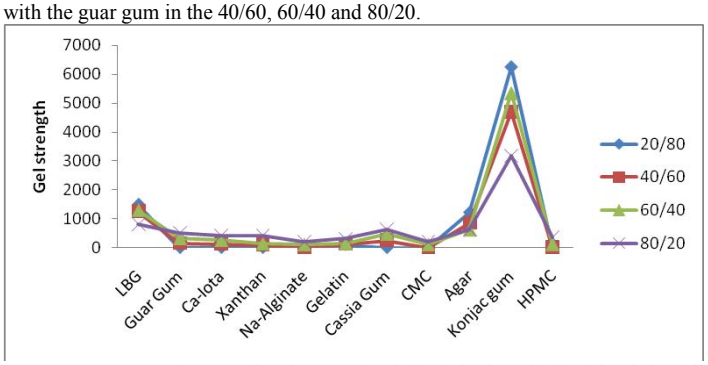

Figure 2 Water gel strength of nano sized food hydrocolloids blended with kappa-carrageenan

The cassia gum with the kappa carrageenan had also found better synergistic effect than the above said hydrocolloids, its values found as 245, 470 and 620 for 60/40, 40/60 and 20/80. The highly used hydrocolloid gelatine with the kappa carrageenan had found the value of 108 for 20/80, 113 for 40/60, 149 for 60/40 and 300 for 80/20.Agar-Agar and Locust bean gum with the kappa carrageenan showed the ascending value that it decreased with the increase of kappa carrageenan. The values such as 1210, 839, 619, 610 for kappa with Agar-Agar and 1481, 1253, 1302,805 for kappa with LBG in the ratios of 20/80, 40/60, 60/40 and 80/20 respectively. The combination of Konjac with kappa carrageenan had found the high gel strength value due to high synergistic effect. The ratio 40/60 had the value 4690, the lowest value was found in the 80/20 ratio as 3190 of kappa carrageenan with the Konjac respectively. While the ratio of kappa carrageenan with Konjac in 20/80 proportion showed the highest as 6240.

Viscosity

The viscosity of nano sized hydrocolloids plays an important role to improve the quality attributes for the food formulations and medicine formulation. It is mainly characterized by their property of forming viscous dispersions gels when dispersed in water. The viscosity values of all specific hydrocolloids and combination of hydrocolloids showed in Figure 3. Konjac gum have a highest viscous value (cps) of 7800 and followed by Guar gum (7250), Na-CMC (6100), LBG (4700), XG (4500), Na-Alginate (60), Iota-Carrageenan (50), Cassia gum (30), HPMC (12), Kappa-Carrageenan (10), Gelatine (5) and Agar-Agar of 2. Mostly hydrocolloids are bit acidic, so it had an impact on the viscous nature. The individual hydrocolloids viscosity is given heavy changes in the combination. It was related to the theory of synergistic effect. The kappa carrageenan viscosity value of 10 cps showed dominant force against the gelatine and Agar-Agar which have low viscous force. Likewise, it acted as recessive against rest of the gums because of the monstrous level of viscous nature.

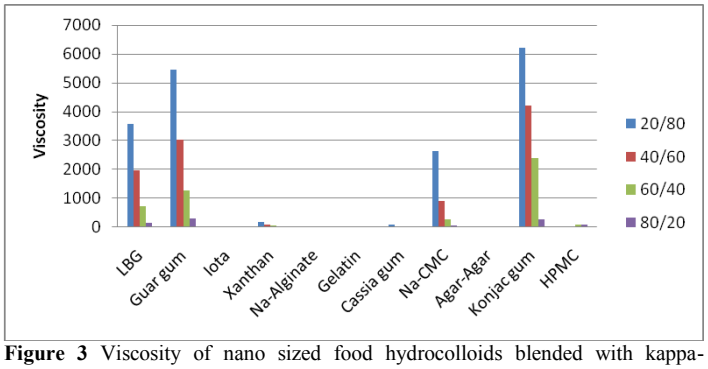

Figure 3 Viscosity of nano sized food hydrocolloids blended with kappa-carrageenan

The combination of kappa carrageenan with the other gums depends upon the specific viscosity. The viscous values increased in the ratios of 20:80, 40:60, 60:40, 80:20 for the combination of kappa carrageenan with the gelatine and Agar-Agar of 6, 7, 8, 10 and 3, 3, 4, 5 respectively. The sodium alginate was stood as odd value showed different as values got decreased even the sodium alginate viscosity of value 60.The viscous value of both Iota and cassia with the fusion of kappa carrageenan was maximum related to the individual viscosity of 40, 30, 20, 10 and 80, 40, 30, 10 for the ratio of 20:80, 40:60, 60:40, 80:20 kappa with Iota and cassia respectively.

The xanthan gum accounts the different zone in an antagonistic value of their individual viscous value "N" number higher than the interactions with the kappa carrageenan and the value depicted as 170, 96, 50 and 15 for the ratio 20:80 to 80:20. Due to the high viscous nature of Na-CMC, it accounts the high in the blend which has 80% of Na-CMC to the 20% of kappa carrageenan as 2630 and it simultaneously decreased as 900,280 and 60 for other combination. The Locust bean gum and Guar gum accounts best synergistic effect with the kappa carrageenan as 5470, 3019, 1280, 300 for guar and 3570, 1980, 740 and 160 for LBG combinations with kappa carrageenan in the ratios of 20:80, 40:60, 60:40 and 80:20 respectively. The synergistic effect occurs high in between kappa carrageenan and Konjac gum leads to the high viscous nature of combination. The ratio of kappa carrageenan increases with the decrease of viscosity in the Konjac gum combination as 6220 for 20:80, 4200 for 40:60, 2400 for 60:40 and 290 for 80:20 of kappa and Konjac gum respectively. In HPMC case, the result is totally opposite as ratio of kappa presence directly proportional to the viscous nature of the combination of gums. It has been increased as 20, 40,100 and 110 for 20:80 to 80:20.

Viscous Synergism Index

The synergistic and antagonistic effect of hydrocolloids spoke out the interaction or fusion range of hydrocolloids. If viscosity synergism index (I_v) value is between 0 and 0.5, the viscosity value of fusion of two hydrocolloids will be lower than the viscosity value of the sum of two hydrocolloids and also less than at least one of the hydrocolloid used in the interaction which means that this interaction is antagonism [22]. If $I_v = 0.5$, it means that there is nil interaction and if I_v is between 0.5 and 1.0, it means synergistic interaction happened between two hydrocolloids. Synergistic effect will occur when $\eta_{i+j} > \eta_i$ and $\eta i+j > \eta_j$. If I_v is higher than 1.0, the viscosity of interaction of two hydrocolloids will be higher than the sum of the viscosities of the two hydrocolloids and synergism will occur.

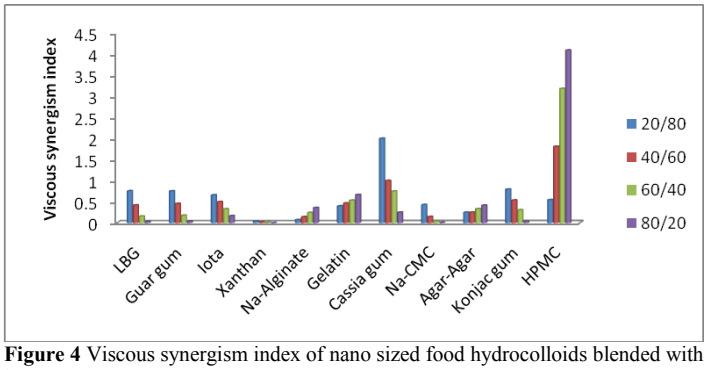

Figure 4 Viscous synergism index of nano sized food hydrocolloids blended with kappa-carrageenan

The synergistic effect combined hydrocolloids as shown in Figure 4. The blend ratio of kappa carrageenan (20) into the LBG, Guar gum, Iota carrageenan (80) individually had best synergistic effect in the rate of 0.757, 0.753 and 0.660. Among these three, LBG has possible similar characteristics with the kappa carrageenan than the other two hydrocolloids resulting to the high synergism index. The remaining ratio level between the kappa carrageenan with the LBG, guar gum and Iota carrageenan has shown the antagonistic effect. In the case of

xanthan, sodium alginate, Agar-Agar and carboxyl methyl cellulose found to have the full antagonist effect with the fusion of kappa carrageenan. In the different aspect, gelatine comes under the unique category that has shown synergistic effect as 0.666 in the ratio of 80:20 of kappa carrageenan and gelatine respectively and the other ratios blend showed the antagonist effect. It elaborates that concentration of kappa carrageenan increases in a blend with the gelatine, the synergism value increases. Finally, in the cassia and kappa carrageenan blend the maximum ratios such as 20:80,40:60 and 60:40 depicted the synergistic effect and last one 80:20 cassia and kappa carrageenan showed the antagonism index.

Incorporation of Kappa carrageenan with selected nano sized hydrocolloids provided an increment in their apparent viscosity. Eq.1 and Eq.2 were used for the prediction of apparent viscosity for the selected hydrocolloids mixture and compared with experimental values which shown in Table 2(a-d). Generally, it was found different values between measured and computed leads to conclude that mixture was not that much fit.

Table 2a Estimation of apparent viscosity of kappa carrageenan blended with some nano sized food hydrocolloid solution using binary mixture model

Blend	Measured	Guar Gum		Measured	LBG		Measured	CMC	
		Eq.1 Computed	Eq.2 Computed		Eq.1 Computed	Eq.2 Computed		Eq.1 Computed	Eq.2 Computed
20:80	5470	5802	1941	3570	3762	1372.2	2630	4882	1690.5
40:60	3019	4354	520	1980	2824	400.95	900	3664	468.86
60:40	1280	2906	138	740	1886	117.14	280	2446	130.02
80:20	300	1458	37	160	948	34.227	60	1228	36.06

Table 2b Estimation of apparent viscosity of kappa carrageenan blended with some nano sized food hydrocolloid solution using binary mixture model

Blend	Measured	Iota		Measured	Xanthan		Measured	Alginate	
		Eq.1 Computed	Eq.2 Computed		Eq.1 Computed	Eq.2 Computed		Eq.1 Computed	Eq.2 Computed
20:80	40	42	36.21	170	3602	1325.3	25	50	41.9
40:60	30	34	26.25	96	2704	390.63	17	40	2929
60:40	20	26	19.03	50	1806	115.12	10	30	20.47
80:20	10	18	13.79	15	908	33.93	5	20	14.3

Table 2c Estimation of apparent viscosity of kappa carrageenan blended with some nano sized food hydrocolloid solution using binary mixture model

Blend	Measured	Gelatin		Measured	Cassia Gum		Measured	Agar	
		Eq.1 Computed	Eq.2 Computed		Eq.1 Computed	Eq.2 Computed		Eq.1 Computed	Eq.2 Computed
20:80	6	6	5.74	80	26	24.06	3	3.6	2.757
40:60	7	7	6.59	40	22	19.32	3	1.6	3.805
60:40	8	8	7.57	30	18	15.51	4	6.8	5.251
80:20	10	9	8.70	10	14	12.45	5	8.4	7.247

Table 2d Estimation of apparent viscosity of kappa carrageenan blended with some nano sized food hydrocolloid solution using binary mixture model

Blend	Measured	Konjac gum		Measured	HPMC	
		Eq.1 Computed	Eq.2 Computed		Eq.1 Computed	Eq.2 Computed
20:80	6220	6242	2057	20	12	12
40:60	4200	4684	543	40	11	11
60:40	2400	3126	143	100	11	11
80:20	290	1568	38	110	10	10

Functional properties

Water-Holding Capacity (WHC) and Oil-Holding Capacity (OHC)

Water-Holding Capacity (WHC) and Oil-Holding Capacity (OHC) of selected nano sized hydrocolloids shown in Figure 5.WHC can be varied depends upon the types of gums and their nature. It was observed that Kappa carrageenan (46.31 g/g) had the highest WHC compared to all other hydrocolloids, while HPMC showed least value 2.20 g/g of WHC. For the case of OHC, Na-CMC marked the highest value of 4.39 and in the extent, Gelatine had the lowest value of 2.44 g/g.

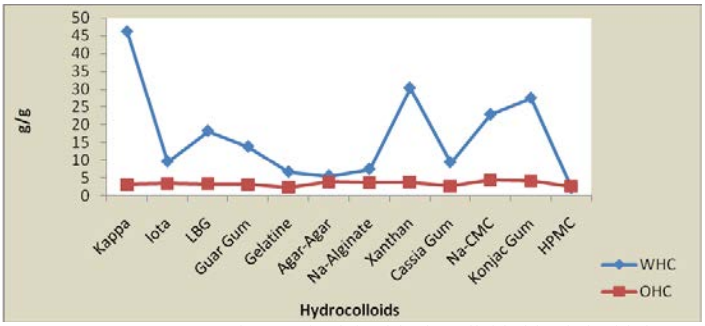

Figure 5 WHC & OHC of nano sized food hydrocolloids blended with kappa-carrageenan

Emulsifying activity (EA) and Emulsion stability (ES)

Figure 6 shows the EA and ES of selected nano sized hydrocolloids. Xanthan (62.12 % & 98.14 %), Guar gum (62.10 % & 76.16 %), Konjac (60.14 % & 93.14 %) were accounted best emulsion activity and emulsion stability respectively. While Iota carrageenan and Agar-Agar had the minimum value in both EA and ES.

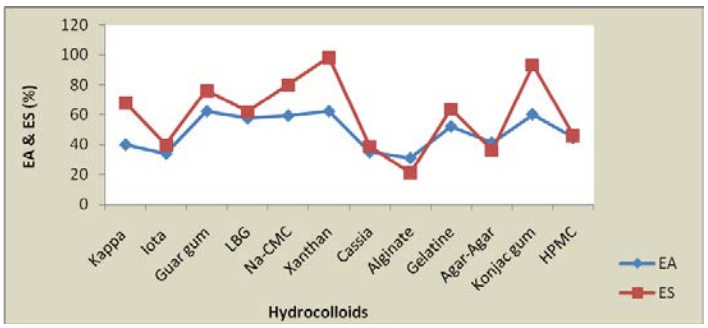

Figure 6 EA & ES of nano sized food hydrocolloids blended with kappa-carrageenan

CONCLUSION

As a nano sized food hydrocolloids are finding increasing applications in several foods, and pharmaceutical products as gelling agents, thickening and stabilizing agents. The thickening and gelling effects are mainly provided by sodium

alginate, carrageenan, hydroxypropylmethyl cellulose, locust bean gum, agar-agar and konjac gum. The frequently used thickening and stabilizing agent include xanthan gum, iota and guar gum. The physio-chemical, rheological and functional characteristics of each and combined food hydrocolloids were evaluated. This study suggests that nano sized food hydrocolloids have a lot of potential in nano bio-medicine, bio-nanotechnology, pharmaceuticals and Food industries.

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OPTIMIZATION OF INGREDIENT AND PROCESSING PARAMETER FOR THE PRODUCTION OF *Spirulina platensis* INCORPORATED SOY YOGURT USING RESPONSE SURFACE METHODOLOGY

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ABSTRACT

In this study, response surface methodology (RSM) was employed to optimize the ingredient formulation and processing parameters of *Spirulina platensis* incorporated soy yogurt production such as temperature, time, and amount of *Spirulina platensis* on the sensory evaluation responses on 9 point Hedonic rating. Besides, the physico–chemical properties such as pH, titratable acidity, viscosity and penetration of the *Spirulina platensis* incorporated soy yogurt were also analysed. The analyses show that the *Spirulina platensis* incorporated soy yogurts have a pH from 3.43 to 5.55, acidity from 0.64 to 2.32 (%), Brookfield viscosity from 752 to 903 Centipoise and penetration from 362 to 432 1/10th mm at 25°C during the optimization process. From the analysis of variance, the R^2 of all response variables is more than 0.77 that indicates that a high proportion of variability was explained by the model. Based on the response surface 3D plot of the sensory evaluation, the optimum acceptability of the *Spirulina platensis* incorporated soy yogurt processing parameter are at temperature of 40°C, 12 h of the fermentation duration and 0.80% (w/w) of the *Spirulina platensis*.

Keywords: *Spirulina platensis*, sensory, viscosity, penetration, response surface methodology, soy yogurt

INTRODUCTION

Soy–yogurts have some adventages to consumers due to their hypolipidemic, anticholesterolemic and antiatherogenic properties as well as due to reduced allergenicity (**Pourahmad and Ahanian**, **2015**). Protein content of soy yogurt and milk yogurt is similar (3.5 to 4.0%) and their amino acid pattern is fairly close except that soymilk is deficient in sulfur containing amino acids. Soy yogurt has about 60 to 90% nutritional value of milk yogurt and with adding small amounts of methionine to soy milk it has equivalent nutritional value to cow's milk (**Abdullah *et al.*, 2003**). The high content of indigestible raffinose and stachyose, the flatulence factors, limit the consumption of soy yogurt (**Tsai *et al.*, 2006**). Considerable research has been done to reduce off–flavors in soy yogurt by inhibiting formation of the isoflavones and lipid oxidation products responsible for them (**Izadil *et al*, 2015**).

Using *Spirulina platensis* can be desirable in producing beany odour free soy yogurt. Spirulina *platensis* has been shown to have many advantages, especially in terms of nutritional value and contains many valuable substances in the prevention and treatment of some diseases. *Spirulina platensis* has been used as a food source or supplement to provide nutrition and health improvements for people because it is rich in nutrients like protein, amino acid, essential fatty acids, vitamins and minerals. (**Deng and Chow, 2010**). In recent years, soy yogurts have been developed with expectedly enhanced nutritional superiority by incorporating functional lipids such as Gamma linolenic acid (GLA), Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) and also providing specific antioxidants such as oryzanol and lignans through specific edible oils (**Sengupta *et al.*, 2013; Sengupta *et al.*, 2016**). Soy yogurts enriched with *Spirulina platensis* to provide higher arginine and methionine content in protein part and antioxidant such as selenium, zinc, tocopherol etc. (**Morsy *et al.*, 2014**).

Soy yogurts have been used as the most popular carrier for incorporation of probiotic organisms (**Beheshtipour *et al.*, 2013**). Unfortunately, most of the commercial products contain less probiotic bacteria than the minimum required, because these microorganisms grow slowly in milk and often show loss of viability during storage. In addition, the probiotic bacteria are sensitive to pH, lactic acid, hydrogen peroxide, and dissolved oxygen in fermented milk (**Zhao *et al.*, 2006**). Some researchers have observed that growth of lactic acid bacteria in synthetic media was promoted by *Spirulina platensis* extracellular product (**De Caire *et al.*, 2000**). Therefore, it proved to be suitable for the cost–effective manufacture of novel functional fermented non–dairy yogurts.This addition will improve sensory characteristics of the final products.

To optimize process factors, an appropriate design of experiment was required, we were chosen response surface methodology (RSM) which was the key tool for reducing the number of experiments (**Yaakob *et al.*, 2012**). It can help to optimize manufacturing processes involving multiple factors with different numbers of levels, especially fermentation processes and new *Spirulina platensis* incorporated soy yogurt developments, simultaneously and economically (**Prabuthas *et al.*, 2011**).The application of RSM has not yet been adopted in studies related to soy yogurt supplemented with *Spirulina platensis*. Therefore, the aim of this work is to optimize the formulation ingredient and processing parameters of *Spirulina platensis* incorporated soy yogurt using RSM.

MATERIAL AND METHODS

All chemicals used were, purchased from MERCK, India. Soybean seeds were purchased from the local market (New Alipore Market, Kolkata West Bengal, India). Commercially available milk curd cultures were purchased from Microbial Type Culture Collection and Gene Bank, Chandigarh [*Lactobacillus delbrueckii subsp. bulgaricus* (MTCC 911) and *Streptococcus thermophilus* (MTCC 1938)]. *Spirulina platensis was* used as dry biomass having composition of protein 0.28 g, energy 1.74 Kcal, fat 0.0 g and carbohydrate 0.16 g per 500mg (SUNOVA SPIRULINA, Surya Herbal Ltd. Noida,India).

Preparation of *Spirulina platensis* enriched soy yogurt

The soy milk was made according to the procedure described by **Sengupta *et al.*, (2013)**. The resultant soymilk was then homogenized in a homogenizer (REMI MOTORS–RQ–122) and pasteurized at 80°C for 15 min. Soymilk was then cooled down to 40°C for the addition of *Spirulina platensis*. After addition of *Spirulina platensis* to pasteurized soy milk (1g of dry biomass of *Spirulina platensis* 100 ml^{-1} of soy milk), the mixtures of soy milk and *Spirulina platensis* were homogenized again in a homogenizer prior to inoculation with starter culture until the *Spirulina platensis* was mixed properly throughout the soy milk. Soy milk mixtures were aseptically inoculated with 2% of starter (*Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus*, 1:1 v/v). The inoculated soy milk containing the *Spirulina platensis* was then poured into 100 ml sterile transparent food grade plastic cups with lids and incubated at 37°C for 24 h. Soy yogurts obtained were stored at 4°C in a refrigerator for further analyses. However the amount of *Spirulina platensis*, incubation temperature and duration were followed at certain level suggested by RSM.

Quantitative descriptive analysis of SP incorporated soy yogurt response variables

Sensory evaluation on the produced *Spirulina platensis* incorporated soy yogurt was conducted among 20–trained panelists in Indian Institute of Engineering Science and Technology, Shibpur. Samples of 5 ml were put and served in a plastic container and coded alphabetically. The trained panelists evaluated all samples by marking the scale of intensity. Panelists were also served with a glass of water to neutralize the taste before analyzing the next sample. They were assisted in developing a consensus evaluation for sensory attributes for *Spirulina platensis* incorporated soy yogurt (**Yaakob *et al.*, 2012**). Evaluation was done at Nine Point Hedonic Scale. The quality properties that were evaluated were appearance, aroma, texture r and overall acceptance. The information contained on the sensory performance was indicated as 9=like extremely, 8=like very much, 7=like moderately, 6=like slightly, 5= neither like or dislike, 4=dislike slightly, 3= dislike, 2=dislike very much, 1=dislike extremely.

Experimental design and statistical analysis

The statistical analysis of *Spirulina platensis* incorporated soy yogurt production was performed by using Design Expert (2000, V 6.0.8; Stat–Ease Inc., Minneapolis, MN, USA) software. Central composite design (CCD) was used to study the interaction of process variables by applying RSM (**Bezerra *et al.*, 2008**). There are 3 variables which are temperature of fermentation process (X1), duration of fermentation (X2), and amount of *Spirulina platensis* (X3). Each variable has 3 different coded levels,from low (−1), to medium (0), and high (+1) as well as the star points (−α and +α). The range of temperature is between 35 to 45ºC, 8 to 16 h for duration of fermentation and 0.1 to 1.5 %(w/w) for the amount of *Spirulina platensis*. The design matrix of Central Composite Design and also experimental results for the responses of *Spirulina platensis* incorporated soy yogurt are shown in **Table 1**. Taste (Y1), odor (Y2), color (Y3), appearance (Y4), and overall acceptability (Y5) were taken as the responses of the design experiment.

Table 1 Experimental results of *Spirulina platensis* incorporated soy yogurt response variables

Run	Manipulated variables[1)]			Responses[2)]								
	X1	X2	X3	pH	Acidity	Brookfield Viscosity (Cp at 25ºC)	Penetration at 25 ºC (1/10th mm)	Y1	Y2	Y3	Y4	Y5
1	0	0	0	5.20	0.97	765	388	7.33	7.29	9.25	7.42	8.01
2	+1	+1	–1	4.03	1.96	849	415	5.33	4.84	8.36	4.26	3.22
3	+1	+1	+1	3.57	2.19	888	424	5.04	4.99	8.54	7.29	7.21
4	–1	+1	–1	4.68	1.51	816	401	5.28	4.21	8.26	5.59	3.22
5	–1	–1	+1	4.99	1.16	785	392	5.56	4.08	8.12	4.59	4.29
6	0	0	0	5.23	0.84	763	385	7.34	7.54	8.16	7.45	6.45
7	+1	–1	–1	4.22	1.72	826	405	5.64	4.29	8.14	4.36	4.29
8	–1	–1	–1	5.55	0.64	752	362	5.48	4.17	8.29	7.21	6.41
9	–1	+1	+1	4.85	1.22	812	395	5.74	4.27	8.27	6.34	5.26
10	0	0	0	5.11	1.04	769	385	5.48	7.28	9.21	7.46	6.46
11	0	0	0	5.12	1.03	769	385	7.34	7.22	7.36	7.44	6.26
12	+1	–1	+1	3.67	2.10	883	821	5.29	8.74	7.15	5.28	4.29
13	–α	0	0	4.72	1.43	814	398	5.28	5.02	7.26	4.26	4.45
14	+α	0	0	3.46	2.24	901	429	5.33	5.26	3.64	3.29	3.21
15	0	0	+α	4.12	1.86	843	411	6.48	7.24	8.19	5.28	4.25
16	0	–α	0	3.99	2.08	867	419	6.74	5.04	8.88	3.22	3.96
17	0	+α	0	5.01	1.46	779	390	6.93	7.37	8.33	5.26	5.21
18	0	0	–α	3.43	2.32	903	432	5.12	5.21	8.45	3.25	5.26
19	0	0	0	5.34	0.79	762	377	7.34	6.57	9.23	7.45	6.42
20	0	0	0	5.16	0.96	768	382	7.34	8.55	9.25	7.45	6.42

[1)] α=1.682; (X1) temperature, (X2) time, (X3) *Spirulina platensis*, (Y1) taste, (Y2) odor, (Y3) color, (Y4) appearance, (Y5) overall acceptability

[2)] Using 9–point Hedonic scale

Physicochemical analysis of *Spirulina platensis* incorporated soy yogurt

The pH of the *Spirulina platensis* incorporated soy yogurt samples was measured directly using pH meter (Hanna pH meter No. 211). The *Spirulina platensis* incorporated soy yogurt samples were stirred with a 2.5 ml of distilled water before pH measurement. The acid content of *Spirulina platensis* incorporated soy yogurt samples was determined according to **AOAC, (2005)** technique. Twenty grams of well homogenized sample was placed in a beaker and was titrated against 0.1N NaOH with phenolphthalein as indicator. Total Titratable acidity (TTA) was expressed as g equivalent lactic acid 100 g^{-1}.

Viscosity was based on measuring resistance to a rotating spindle (Spindle No 3 at 20 RPM) Brookfield Model DV–E Viscometer at constant temperature (25°C) for 5 min. The sample was taken out from freezer (–40ºC) and was left at room temperature for 5 min. Then, the sample was placed in a 10–mL beaker and the spindle was dipped into the sample before the viscometer was switched on.Viscosity measurements were expressed in centipoises (cP) performed in Triplicate. The Viscosity determined with the Brookfield viscometer is known as the Brookfield Viscosity **(Ozer *et al.*, 1997).**

Penetrometer Study The firmness of *Spirulina platensis* incorporated soy yogurts was determined by a Penetrometer (Stanhope–Seta Surrey, England) using the cone–form penetration body with an apical angle of 45°C, and a weight of 72.5 g. The depth of penetration was measured at 5 s at a product temperature of 25°C.

RESULTS AND DISCUSSION

Model fitting for RSM

The experimental results on the effect of temperature, time of fermentation, and amount of *Spirulina platensis* on the development of *Spirulina platensis* incorporated soy yogurt were shown in **Table 1**. The sensorial quality of the yogurt was evaluated as the responses for the factors studied. The quality of yogurt based on sensory evaluation was rated based on the taste, odor, color, appearance, and also the overall acceptability of the yogurt as responded by the panelists.

The results obtained from the ANOVA analysis are shown in **Table 2**. The F and p–values of ≤0.05 indicate that the quadratic model of the development *of Spirulina platensis* incorporated soy yogurt is statistically significant at 95% confidence interval. The lacks of fit for each response were insignificant. The R^2 values for the appearance exceeding 90%, which indicated that a high proportion of variability was well explained by the model while taste, aroma color and and overall acceptability show high values of R^2 was around 77.81%, 77.48%, 79.39% and 82.56% respectively indicating that only 22.19% for taste, 22.52% for aroma, 20.61% for color and 17.44% for overall acceptability of the data could not be interpreted by the model.

Table 2 Analysis of variance of the response variables*

	Source	Sum of square	DF	Mean square	F value	Prob>F	R^2 (%)
Taste	Model	11.46	9	1.27	3.51	0.0378	77.81
	Lack of fit	*0.68*	5	0.14	0.21	0.9407	
	Pure error	*2.59*	4	0.65			
	Total	15.56	19				
Aroma	Model	34.91	9	3.88	3.44	0.0399	77.48
	Lack of fit	8.16	5	1.63	3.22	0.1403	
	Pure error	2.02	4	0.51			
	Total	46.45	19				
Colour	Model	21.87	9	2.43	3.85	0.0286	79.39
	Lack of fit	4.83	5	0.97	4.55	0.0836	
	Pure error	0.85	4	0.21			
	Total	28.80	19				
Appearance	Model	48.41	9	5.38	13.06	0.0004	92.89
	Lack of fit	2.92	5	0.59	3.18	0.1428	
	Pure error	0.75	4	0.19			
	Total	63.53	19				
Overall acceptability	Model	28.20	9	3.13	4.73	0.0150	82.56
	Lack of fit	3.96	5	0.79	1.59	0.3369	
	Pure error	1.99	4	0.50			
	Total	34.59	19				

*significant at ≤0.05

Physico–chemical properties of *Spirulina platensis* incorporated soy yogurt

The value of pH, acidity, viscosity and penetration of freshly prepared *Spirulina platensis* incorporated soy yogurt were measured and the results were illustrated in **Table 1**. Generally, the changes of physico–chemical properties of *Spirulina platensis* incorporated soy yogurt were affected by the level of processing condition. The pH of the *Spirulina platensis* incorporated soy yogurt ranged from 3.43 to 5.55 (average pH of 4.57). Run 18 produced the lowest value of pH at 3.43. Meanwhile, run 8 with a coded condition of (–1, –1, –1) showed the highest value at 5.55. The different values of pH might due to the metabolic activities of the *Spirulina platensis* in the soy yogurt with lactic acid bacteria. Researchers had stated that the value of pH is inversely proportional to the lactic acid content in *Spirulina platensis* incorporated soy yogurt (**Kavimandan and Sharma, 2015**). Total acidity of the *Spirulina platensis* incorporated soy yogurt ranged from 0.64 to 2.32 (%). **Osundahunsi *et al.,* (2007)** reported that minimum value of acidity in soy yogurt is 1.00 to 1.99% and maximum formulations tested were successfully achieving the minimum value. From the result, the acidity of the yogurt was also controlled by the processing factors. The acid content of *Spirulina platensis* incorporated soy yogurt will be higher if the fermentation was carried out at 40°C temperature, for 12 h using 0.8% (w/w) starter culture.

In order to prevent over–acidification, the amount of *Spirulina platensis* needed to be controlled (**Champagne and Mollgaard, 2008**). Over acidification was probably caused from the addition of powdered *Spirulina platensis* which promoted the growth of lactic acid bacteria **(Mocanu *et al.*,2013)**.Selecting amount of *Spirulina platensis* was the most important aspect to consider in overcoming excessive acid production. The appropriate pH and temperature during processing need to be identified because both parameters will affect the activity of lactic acid bacteria. The rheological analysis of product did not show any significant effect on *Spirulina platensis* added products. No difference was observed in the shear stress and dynamic viscosity of fluid. Another way to prevent excess acidification was to reduce the refrigeration temperature to slow down the growth of the *Spirulina platensis*. However, **Guldas and Irkin (2010)** showed the positive effect of *Spirulina platensis* powder on the survival of the lactic acid bacteria during storage of yogurt. At refrigerated temperature activity of lactic acid bacteria was also reduced. Therefore, as indicated through this study, the combination level of processing parameter was really important to obtain appropriate acidity of *Spirulina platensis* incorporated soy yogurt.

The results showed that again different level of processing parameter gave different level of viscosity and penetration. For example, the viscosity and the penetration of *Spirulina platensis* incorporated soy yogurt showed that soy yogurt with the temperature higher than 37°C, incubation longer than 12 h, and amount of *Spirulina platensis* 0.8% (w/w) was more viscous compared to the *Spirulina platensis* incorporated soy yogurt with other processing level combination. The acidity of this yogurt was high and it seems that the viscosity and penetration of the *Spirulina platensis* incorporated soy yogurt depends on the acid production. This was because, when the acidity increased, the protein present in *Spirulina platensis* incorporated soy milk will form more gel resulting soy yogurt with high viscosity and penetration (**Lordan *et al.,* 2011**).

Effect of processing parameter on the sensory evaluations

The effect of temperature (X1), time of fermentation (X2), and amount of *Spirulina platensis* (X3) on the sensorial quality of the *Spirulina platensis* incorporated soy yogurt was represented by the quadratic model and is also aided by the response surface plots for better visualization shown in **Fig 1–5**. The sensory scores for taste, odor, color, appearance, and overall acceptability of the *Spirulina platensis* incorporated soy yogurt were shown in **Table 1**. **Fig 1–5** clearly visualizes that temperature, time of fermentation, and amount of *Spirulina platensis* affected the quality of the *Spirulina platensis* incorporated soy yogurt.The response surface plot for the taste of the *Spirulina platensis* incorporated soy yogurt was shown in **Fig 1**. For the overall acceptability of the yogurt taste, it was shown that the response was more towards 'like slightly' and 'like very much' which was numerically in the range of 5.82 to 8.52. As is evident in **Fig 1** the temp, time and amount of Spirulina platensis induced an increase of taste score at low levels, whereas at higher levels, the taste score decreased with higher the temp, time and amount of *Spirulina platensis* .Such a trend might be explained due to increased overrun and intense green colour. Though the growth of probiotic bacteria populations in the fermented functional foods were much higher in the presence of the *Spirulina platensis* than when the probiotics were grown alone, addition of *Spirulina platensis* higher than 0.8% might be the causes for decreased overall acceptability of the product.

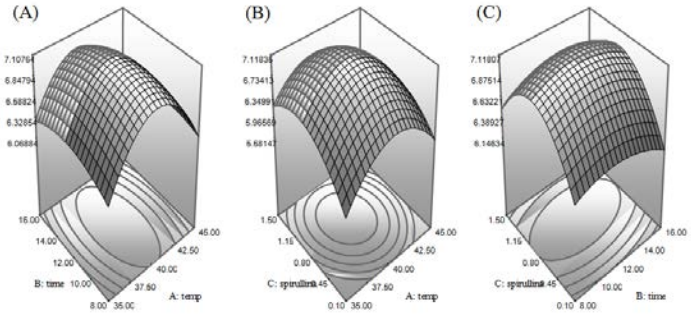

Fig 1 Response surface plot of the effects of temperature and time (A), temperature and amount of *Spirulina platensis* (B), and time and amount of *Spirulina platensis* (C) on the taste of *Spirulina platensis* incorporated soy yogurt

Soy yogurt smell was taken into consideration in the development of *Spirulina platensis* incorporated soy yogurt. The results had shown in **Fig 2** exhibited that panelists like the smell of these *Spirulina platensis* incorporated soy yogurts. The aroma was found to be most dependent on *Spirulina platensis* content rather than time and temp. Higher *Spirulina platensis* and lower time and temp resulted higher aroma in the studied experimental range. The steady increase in aroma with increasing *Spirulina platensis* could be due to the addition of increasing amounts of protein to the blend which may affect the extent of lactic acid fermentation.

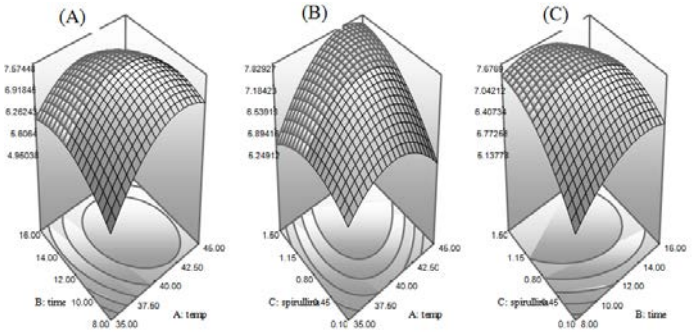

Fig 2 Response surface plot of the effects of temperature and time (A), temperature and amount of *Spirulina platensis* (B), and time and amount of *Spirulina platensis* (C) on the aroma of *Spirulina platensis* incorporated soy yogurt

The response surface plot for the color of *Spirulina platensis* incorporated soy yogurt was shown in **Fig 3**. With regard to the color data, all assays garnered mean hedonic scores ranging from 'moderately liked' to 'extremely liked'. Formulation 1 and 20 were given the best score for this attribute. A non–dairy soy yogurt containing *Spirulina platensis* received 82.56% of acceptance indicating that consumers were beginning to overcome the negative opinions they once held regarding soy–containing products not only due to the health benefits they may confer, but also because of their good taste, color and aroma.

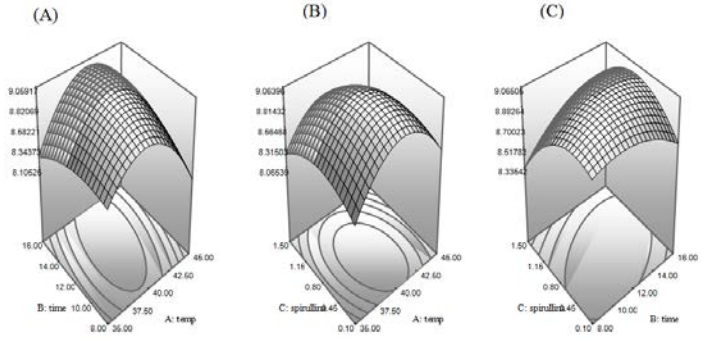

Fig 3 Response surface plot of the effects of temperature and time (A), temperature and amount of *Spirulina platensis* (B), and time and amount of *Spirulina platensis* (C) on the color of *Spirulina platensis* incorporated soy yogurt

The scopes of analysing the appearance characteristics of the *Spirulina platensis* incorporated soy yogurt prioritiesed its texture was shown in **Fig 4**. In order to produce *Spirulina platensis* incorporated soy yogurt with good appearance, the temperature range required is between 38.94 to 40.08°C, and time of between 8 to 13.51 h. This was observed for formulation 10 with the highest response of 7.46 (**Table 1**). Furthermore, the medium amount of starter culture (1.04 to 1.34 %) in this formulation could contribute to a better texture. The increase in appearance with increase in level of incorporation at 1.04 to 1.34% level is mainly due to beneficial influence of *Spirulina platensis* on the survival of lactic acid bacteria owing to its high protein, esential fatty acids such as gamma linolenic acid, vitamins and minerals (**Perez *et al.*, 2007***)* lower whey separation due to lower acidity and intense green colour.

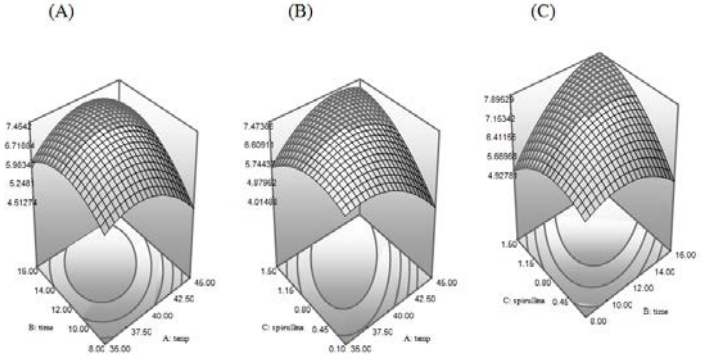

Fig 4 Response surface plot of the effects of temperature and time (A), temperature and amount of *Spirulina platensis* (B), and time and amount of *Spirulina platensis* (C) on the appearance of *Spirulina platensis* incorporated soy yogurt

The overall acceptability of the of *Spirulina platensis* incorporated soy yogurt priorities as affected by the temperature, time, and amount of *Spirulina platensis* with positive and negative quadratic effects at $p \leq 0.05$ were shown in **Fig 5**. Productions at the temperature of 39.62°C, time of 12.38 h and of *Spirulina platensis* of 0.95 %(w/w), indicated the optimum overall acceptability of 6.59. This showed that temp, time and starter culture are important parameters for *Spirulina platensis* incorporated soy yogurt acceptability. Hence, it was proven that the optimized region for overall acceptability of this of *Spirulina platensis* incorporated soy yogurt by the panelists was between the ranges of 'like slightly' and 'like moderately'.

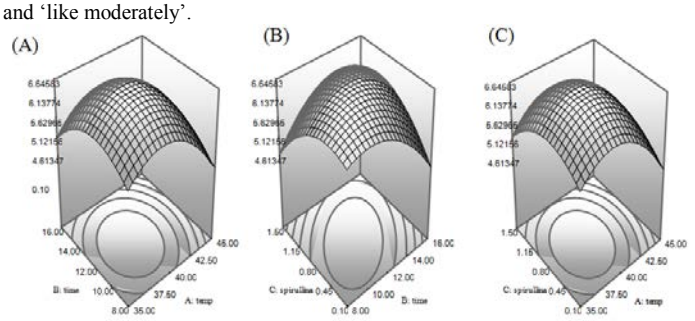

Fig 5 Response surface plot of the effects of of temperature and time (A), temperature and amount of *Spirulina platensis* (B), and time and amount of *Spirulina platensis* (C)on the overall acceptability of *Spirulina platensis* incorporated soy yogurt

CONCLUSION

RSM was successfully optimized for the ingredient formulation and processing parameter of *Spirulina platensis* incorporated soy yogurt. In general, the optimized value obtained from the RSM is different from the data calculated at **Table 1**. This is because the optimization has been carried out by the software and the variables in the range have been selected to obtain the optimum response. Based on the response surface 3D plot of the sensory evaluation, the optimum acceptability of the *Spirulina platensis* incorporated soy yogurt processing parameter are at temperature of 40°C, 12 h of the fermentation time, and at 0.80%(w/w) of the *Spirulina platensis*. Future studies to identify the active ingredients in *Spirulina platensis* incorporated soy yogurt and uncover the mechanistic insights into *Spirulina platensis* incorporated soy yogurt's medicinal effects will provide the bases for developing new non–dairy food products for preventing or treating hypercholesterolemia and cardiovascular diseases.

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EFFECT OF DIFFERENT TYPES OF COAGULATING AGENT ON PHYSICO-CHEMICAL AND ORGANOLEPTIC PROPERTIES OF NON DAIRY RASGULLA (CHEESE BALL)

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ABSTRACT

Non-dairy rasgulla NDR (cheese ball) were manufactured from soybean milk using different types ofcoagulants such as citric acid, lactic acid, tartaric acid and calcium lactate (2% each). Dairy rasgulla procured from local market was used as control (DR). This study investigated the effects of these coagulants on the physico-chemical, color, in vitro multienzyme protein digestibility and the sensory properties of DR and NDRs.Citric acid coagulated NDR (CNDR) recorded high fat value (4.95±0.18; $p<0.05$) among all the NDRs. Protein value of DR (8.24±0.05; $p<0.01$) was higher than lactic acid coagulated rasgulla (LNDR) (7.89±0.22; $p<0.01$), but was lower than the values obtained for other three types of NDRs.The moisture content of LNDR (51.20±0.56; $p<0.01$) was significantly higher than that of other NDRs. Among NDRs, the LNDR recorded highest carbohydrate content (34.37±0.49; $p<0.01$). The energy value of LNDR (208.55±6.88; $p<0.01$) was significantly lower than that of all other NDRs. Penetration values of all the five types of rasgulla sample were gradually decreased during the storage period.A similar trend of L^* values (lightness) was observed in case of CNDR and DR. The mean scores of DR and NDRs for color, aroma, texture and overall acceptability were gradually decreased during storage. It was observed that CNDR, tartaric acid coagulated rasgulla (TNDR) and calcium lactate coagulatedrasgulla (CLNDR) had higher overall acceptability than DR. LNDR, CNDR and CLNDR had lower protein digestibility values than DR. On the basis of analysis of different physicochemical and sensory parameters, tartaric acid proved to be optimum in the preparation of NDR.

Keywords: Rasgulla, Soymilk, Channa, Penetration, Digestibility

INTRODUCTION

Rasgulla, the sweet syrupy cheese ball is one of the most popular and charming sweets of India **(Bandyopadhyay *et al.*, 2008).**Rasgulla is made from heat and acid coagulant milk protein mass traditionally known as chhana which is kneaded into small balls that are boiled into 40-60% sugar syrup. Rasgulla is generally made from cow milk (**Rao *et al.*, 1989**) and very few reports are there regarding the manufacture of rasgulla from buffalo milk (**Kanwal *et al.*, 1980**). This dairy product is easily digested and has high food value due to its fairly high protein content, calcium, phosphorus, vitamin A and D content (**Tarafdar *et al.*, 2002**). Rasgulla are extensively consumed due to its good nutritional and health benefit to human (***Chavan et al., 2011;* Sahu and Das, 2009**). Production of non-dairy food products from non-conventional edible seed flour such as soybean has emerged as popular alternative to traditional dairy products due to ongoing trends of vegetarianism, milk cholesterol, saturated milk fat and lactose intolerance. Soybean which is the most widely grown and utilized legumes in the world has good amino acid profile, contain higher levels of essential fatty acids, soluble fiber, vitamins, minerals, phytochemicals which include isoflavones, phytic acid and saponins which have strong antioxidant properties and have capability of lowering the cholesterol level (**Barrett,2006**). Soy based food products have attained significant consideration for their potential role in improving health hazards such as risk factors for coronary heart disease. Soy foods such as soymilk, tofu, natto, miso, tempeh, textured vegetable protein like soy burgers, soy nuts and whole soybeans may offer various health benefits (**Jooyandeh, 2011; Sengupta *et al.*, 2016**). As a suitable alternative for probiotic dairy products, soy beverages and yogurts are another food category for which the healthy bacteria has played an important role in preventing health related disease outcome.Traditional soy foods, both fermented and non-fermentedproducts, are part of the daily diet in many areas of the world. Products such as soy sauce tofu, tempeh and others are richer in aglycone, and isoflavones than unfermented soy products and are becoming more popular in our country (**Wang and Murphy, 1994**). Because of this development, rasgulla from soy milk is very challenging and people have started to take an interest in soy product consumption.

Food value of rasgulla largely depends upon the quality of chhana. The type of coagulant used for coagulation of vegetable milk has prominent role in maintaining quality of *chhana* as it regulates the moisture content in *chhana*. Generally organic acids like citric, lactic acid, tartaric acid, calcium lactate, lemon juice and sour whey are used as coagulant. Effects of different coagulating agents in the production of dairy rasgulla (DR) have been reported (**Soni *et al.*, 1980; Ahmed *et al.*, 1981; Bandyopadhyay *et al.*, 2005**) but, there is no information regarding the effects of different coagulants in the preparation of chhana to manufacture rasgulla from soy milk.

The objective of this study was to prepare soy based rasgulla coagulated with different coagulating agents. Effects of different coagulants on the physicochemical, sensory and general acceptability of non-dairyrasgulla(NDR) were studied and compared with those of dairy rasgulla.

MATERIAL AND METHODS

Chemicals and reagents

Soybean seeds were purchased from the local market (New Alipore Market, Kolkata West Bengal, India). Polyethylene cups and aluminum foil were procured from the local market. Citric acid, lactic acid, tartaric acid and calcium lactate were obtained from MERCK (Emerck India Ltd. Mumbai, India). All other chemicals were of analytical grade. Dairy rasgulla was brought from sweet shop (Hindusthan sweets, Newalipore, Kolkata, W.B., India). Double refined cane sugar and rose water were obtained from local shop of New Alipore, Kolkata.

Preparation of soy milk

The preparation of soy milk from whole soy seeds was described by adopting the procedure of **Sengupta *et al.*, (2013).**

Preparation of coagulant solution

Citric, lactic acid, tartaric acid and calcium lactate were used as coagulant and each of them (2%) was dissolved in distilled water separately to prepare coagulant solution (**Aneja *et al.*, 2002**).

Preparation of Chhana and Rasgulla (Cheese ball) from soy milk

Chhana and rasgulla were prepared from soy milk using the method suggested by **Aneja *et al.*, (2002)** but with a slight modification. Schematic diagram for the preparation of chhana and rasgulla was given in **Fig 1**.

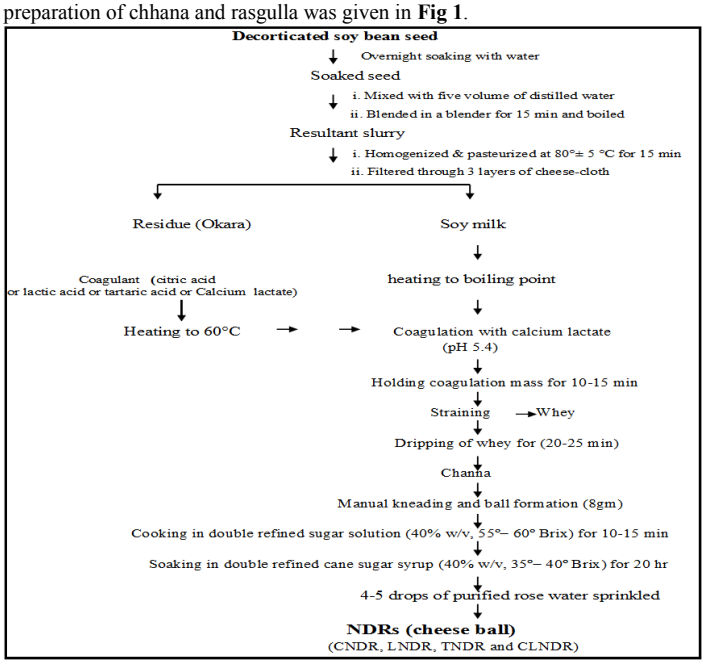

Fig 1 Flow diagram for manufacturing NDR from soymilk
NDRs: non-dairy rasgullas; CNDR: citric acid coagulated non-dairy rasgulla; LNDR: lactic acid coagulated non-dairy rasgulla; TNDR: tartaric acid coagulated non-dairy rasgulla; CLNDR: calcium lactate coagulated non-dairy rasgulla.

After completion of precipitation of soya milk (pH 5.4) with coagulating agent, chaana was collected in a cheese cloth and the whey was drained off by squeezing the lump (milk solid) as much as possible (20-25 min). The lumps of chhana were softened uniformly by messing. Chhana was kneaded thoroughly until visible separation of fat was occurred on the palms to form dough. This dough was converted to chhana balls by rolling between hand palms for 1 min without forming any crack on the balls surface **(Yadav *et al.*, 2012)** otherwise these balls usually lost their integrity during cooking. For cooking and soaking of rasgulla cooking syrup (40% w/v, 55°− 60 °Brix) and soaking syrup (35°− 40 °Brix) were prepared separately. The chhana balls (8gm) made by different coagulants were cooked in the boiled cooking syrup solution for 10-15 minutes. The cooked balls were then collected from the deep pan and placed in the freshly prepared soaking syrup solution for 20 hr in which 4-5 drops of purified rose water was sprinkled. Dairy rasgulla purchased from local shop was used as control. Four different types of NDRs were manufactured and designated as CNDR: citric acid coagulated non-dairy rasgulla; LNDR: lactic acid coagulated non-dairy rasgulla; TNDR: tartaric acid coagulated non-dairy rasgulla; CLNDR: calcium lactate coagulated non-dairy rasgulla. The control dairy rasgulla (DR) and non-dairy rasgullas (NDRs) were stored in closed container at 4 °C for 30 days.

Proximate composition of DR and NDRs

The proximate composition (protein, fat, moisture, total solids and ash content) of DR and NDRs from different coagulants were carried out in triplicate using the standard methods of **AOAC, (2005)**. Fat was determined according to Bligh and Dyer (1959) method by some process modification. Carbohydrate content was calculated by difference [100─ (moisture + crude protein + lipid +ash)]. Energy values were obtained using the Atwater formula (**Merrill and Watt, 1973**).

Penetration property of DR and NDRs

The penetration property of DR and NDRs were determined by a Penetrometer (Stanhope-Seta Surrey, England) using the cone-form penetration body with an apical angle of 45° and a weight of 72.5 g (**Sanli *et al.*, 2013**). The depth of penetration was measured at 5 s at a product temperature of 25°C.

Colour property of DR and NDRs

Color intensities in DR and NDRs were measured by use of the colorimeter (Konica Minolta CR 10) which gave the Hunter parameter (L^*, a^*, b^*) and also $c^*$and h^* values directly (**Morales and Boeckel, 1999**). Rasgulla samples were homogenized in a homogenizer and 5g of homogenized samples were placed in Petridishes with a cover. Colour was measured within 5 min of the sample preparation. L^* indicated lightness which describes the light reflecting or transmitting capacity of an object. Color analysis was also performed by determination of a^* (− green to + red component), b^* (−blue to yellow), c^* (chroma) and h^*(hueangle) values in triplicates.

Sensory evaluation of DR and NDRs

Freshly prepared DR and NDRs were kept at 37°C for 24 h for sensory evaluation. 20 members were chosen from School of Community Science and Technology, IIEST, Shibpur, Howrah, West Bengal**.** They developed a consensus evaluation for flavor attributes for DR and NDRs and the evaluation was carried out at Nine Point Hedonic Scale. The quality properties that were evaluated were color, taste, flavor and overall acceptance. The quality information contained on the sensory performance was indicated as 9=like extremely, 8=like very much, 7=like moderately, 6=like slightly, 5= neither like or dislike, 4=dislike slightly, 3= dislike, 2=dislike very much, 1=dislike extremely (**Sengupta *et al.*, 2013).**

In vitro multienzyme protein digestibility of DR and NDRs

The in vitro protein digestibility of DR and NDRs was carried out using the method of **Hsu *et al.*, (1977).** A suspension of the rasgulla from each coagulant was prepared by dissolving 1.75 gm in 50 ml distilled water. The pH of the suspension was adjusted to 8.0 with 0.1 M NaOH, while stirring in a water bath at 37^0C. A multienzyme solution consisting of 1.6 mg mL^{-1} trypsin, 3.1mg mL^{-1} chymotrypsin and 1.3 mg mL^{-1} peptidase was kept in an ice bath and adjusted to pH 8 with 0.1 M HCl. 0.05 mL of the multienzyme solution was added to each rasgulla sample suspension and was constantly stirred at 37^0C. The pH of the suspension was recorded 15 min after the addition of the multienzyme solution and the in vitro digestibility was calculated using the regression equation of **Hsu *et al.*, (1977).**

$$Y=210.46-18.10X$$

Where,Y= in vitro digestibility (%) and X= pH of the sample suspension after 15 min digestion with the multienzyme solution.

Statistical analysis

Statistical analysis of data collected from different parameters was performed by using analysis of variance (ANOVA) and the means were compared across groups by Tukey test. All analyses were carried out in triplicates with the OriginPro 8 and the significant differences were determined at $p \leq 0.05$.

RESULTS AND DISCUSSION

Proximate composition of DR and NDRs on 0 day at 4 °C in a refrigerator

Table 1 represented the proximate composition of DR and NDRs and it was found that there were significant differences in the proximate composition of NDRs ($p \leq 0.05$) in comparison with DR. CNDR recorded high fat value (4.95±0.18; $p<0.05$) followed by LNDR (4.39±0.17; $p<0.05$), TNDR (4.23±0.16; $p<0.05$) and CLNDR (3.56±0.15; $p<0.01$). Control DR had higher fat value (7.86±0.29; $p<0.05$) than those of all the NDRs. These values of fat are higher than the values (1–1.2%) obtained by **Garg *et al.*, (2014).** The same value about fat content of soy rasgulla was observed by **Nande *et al.*, (2008).** They also showed that fat content in freshly prepared soy based rasgulla was low as compared to dairy rasgulla. **Bhattacharya and Raj (1980)** also reported lesser fat content in non-dairy rasgulla which was due to lesser fat content in non-dairy channa.

TNDR recorded high value of protein (16.28±0.38; $p<0.01$) followed by CLNDR (15.24±0.36; $p<0.05$), CNDR (13.67±0.35; $p>0.05$) and LNDR (7.89±0.22; $p<0.01$). Protein value of DR (8.24±0.05; $p<0.01$) was higher than LNDR (7.89±0.22; $p<0.01$), but was lower than the values obtained for other three types of NDRs.

The moisture content of LNDR (51.20±0.56; $p<0.01$) was significantly higher than that of other NDRs. The variation in the moisture content of NDR prepared with different coagulants was probably due to the differences in gel network within the non-dairy rasgulla particles that was influenced by different coagulating agents towards the water holding capacity of soy protein gels. It may

also be due to the unique coagulating properties of different coagulating agents (**Yakubu, *et al.*, 2013**).

Carbohydrate content of DR (36.57±0.51; p<0.01) was highest among all the five types of rasgulla samples. Among NDRs, the LNDR recorded highest carbohydrate content (34.37±0.49; p<0.01) followed by CLNDR (32.27±0.43; p<0.01), CNDR (30.72±0.45; p>0.05) and TNDR (29.65±0.43; p>0.05). **Table 1** also showed the result of energy content of NDRs prepared using different coagulants. The energy value of LNDR (208.55±6.88; p<0.01) was significantly lower than that of CNDR (222.11±9.00; p<0.05), TNDR (221.79±7.50; p<0.05) and CLNDR (222.08±7.40; p<0.05). The energy value of DR was relatively higher than all NDRs. It was reported that rasgulla containing low-fat and high protein was helpful in lowering body weight **(Kolanowski, 1977).** Dairy rasgulla when stored at refrigerated condition had a shelf life of more than 40 days and not more than 6 days at room temperature.

Table 1 Proximate composition of DR and NDRs using different coagulating agentson 0 day at 4 ºC in a refrigerator

Proximate Composition (%w/w)	DR	NDRs			
		CNDR	LNDR	TNDR	CLNDR
Moisture	46.29±0.55	47.24±0.53	51.20±0.56[b]	46.25±0.53	45.29±0.52[a]
Total solids	53.71±0.60	52.76±0.60	48.80±0.55[b]	53.75±0.61	54.71±0.62
Protein	8.24±0.05	13.67±0.35	7.89±0.22[b]	16.28±0.38[b]	15.24±0.36[a]
Fat	7.86±0.29	4.95±0.18[a]	4.39±0.17[a]	4.23±0.16[a]	3.56±0.15[b]
Carbohydrate	36.57±0.51	30.72±0.45	34.37±0.49[b]	29.65±0.43	32.27±0.43
Ash	1.04±0.03	3.42±0.09	2.15±0.06	3.59±0.06	3.64±0.07
Energy (Kcal g^{-1})	249.98±7.72	222.11±9.00[a]	208.55±6.88[b]	221.79±7.50[a]	222.08±7.40[a]

Results are expressed as mean ±SD (n=3) and significantly different at [a]*p*<0.05, [b]*p*<0.01 and [c]p< 0.001 vs DR (control). DR: Dairy rasgulla; NDRs: non-dairy rasgullas; CNDR: citric acid coagulated non-dairy rasgulla; LNDR: lactic acid coagulated non-dairy rasgulla; TNDR: tartaric acid coagulated non-dairy rasgulla; CLNDR: calcium lactate coagulated non-dairy rasgulla.

Penetration property of DR and NDRs during storage at 4 ºC in a refrigerator

Table 2 revealed the results of the penetration property of DR and NDRs during 30 days of storage at 4ºC. In case of CNDR, penetration value was increased from 0 day of storage (255.98±12.48; p<0.05) to 10 days of storage (675.96±124.96; p<0.05). Penetration values then gradually decreased upto 20th (525.59±20.59; p>0.05) and 30thday of storage (316.18±12.48; p>0.05). Thus CNDR can be consumed up to 10 days of storage. In case of CLNDR the penetration value was increased from 0 day of storage (145.96±10.29; p>0.05) up to 20 day of storage (487.49±20.59; p<0.01) and then decreased at 30 day of storage (389.85±14.89; p<0.01). Thus CLNDR can be consumed up to 20 days of storage. For LNDR penetration values were gradually decreased from 0 day (565.59±20.89; p<0.05) of storage to 30 days of storage (201.48±10.59; p>0.05). In case of TNDR penetration values remained constant up to 20 days of storage and then decreased up to 30 day of storage. These results revealed that penetration values of all the five types of rasgulla sample were gradually decreased during the storage period and thus the quality of NDRs made by different coagulants was gradually decreased during storage period. The penetration values of different NDRs were higher than that of control. It can be concluded that penetration properties of NDRs were significantly affected by types of coagulants. The penetration properties of NDR varied significantly with the level of fat and moisture content as well as difference in coagulants. From the present study it can be concluded that among four types of coagulants used for the preparation of NDR from soy milk, tartaric acid is the best coagulant for providing best penetration properties. **Nande *et al.*, (2008)** showed that texture wise NDRs were rated better than that of DR however, differences were not significant. Fat played a dramatic role in the rheological factors of rasgulla. On the other hand **Haque *et al.*, (2003)** observed that the chhana produced from cow milk had a soft body and smooth texture, more suitable for rasgulla preparation than soy milk chhana, which had coarse and granular body. Our studies overcome the problem of making NDRs in respect of textural characteristics and tartaric acid coagulant nondairy rasgulla (TNDRs) was most promising among other NDRs in this context.

Table 2 Penetration properties of DR and NDRs using different coagulating agentsduring storage at 4 ºC in a refrigerator

Property	Day of storage	DR	NDRs			
			CNDR	LNDR	TNDR	CLNDR
Penetration at 25 ° C (1/10th mm)	0	230.29±12.01	255.98±12.48[a]	565.59±20.89[a]	432.96±18.59[a]	145.96±10.29[a]
	10	232.15±26.58	675.96±24.96[a]	306.96±20.47[a]	436.74±22.59[b]	346.74±18.89
	20	236.48±35.41	525.59±20.59	307.78±18.69[a]	433.19±19.78[a]	487.49±20.59[b]
	30	251.26±39.64	316.18±12.48	201.48±10.59	312.78±12.69[a]	389.85±14.89[c]

Results are expressed as mean ±SD (n=3) and significantly different at [a]*p*<0.05, [b]*p*<0.01 and [c]p< 0.001 vs DR (control). DR: Dairy rasgulla; NDRs: non-dairy rasgullas; CNDR: citric acid coagulated non-dairy rasgulla; LNDR: lactic acid coagulated non-dairy rasgulla; TNDR: tartaric acid coagulated non-dairy rasgulla; CLNDR: calcium lactate coagulated non-dairy rasgulla.

Colour properties of DR and NDRs using different coagulating agentsduring storage at 4 ºC in a refrigerator

Change in color in rasgulla samples was an important parameter in assessing the quality of this sweet product. The change of color for all the rasgulla samples was measured over the total storage period from 0 days up to 30 days and was compared. The results were represented in the **Table 3**.

A similar trend of L^* values was observed in case of CNDR and DR. L^* values of DR and CNDR were increased from 0 day (70.50±0.60; p<0.01 for DR and 73.60±0.21; p>0.05 for CNDR respectively) to 20 days (72.96±0.22; p<0.01 for DR and 77.25±0.22; p<0.01 for DR and CNDR respectively) were increased and then decreased at 30th day (71.59±0.85; p<0.01 for DR and 72.55±0.21; p<0.001 for CNDR respectively). Decrease in L^* values indicated lowering of lightness of rasgulla samples. For LNDR, L^* value initially decreased from 0 day (73.89±0.05; p>0.05) up to 10 days (67.52±0.09; p>0.05) of storage. Then it was increased up to 30 days of storage (70.60±0.60; p<0.001). For TNDR, L^* value gradually decreased from 0 day (73.89±0.03; p>0.05) up to 30 days (64.12±0.02; p<0.001) of storage. For CLNDR, L^* value initially decreased and then remained constant for 20 (69.53±0.01; p<0.01) and 30 days of storage (69.52±0.60; p<0.001). Hue-angle values fluctuated in a narrow range of 80-110 during storage. However, chroma had higher values after 20days of storage and then it decreased after 20 days.

Table 3 Colour properties of DR and NDRs using different coagulating agents

Sample	Day	Color properties				
		L	a*	b*	c	h
DR	0	70.50±0.60	-1.80±0.05	9.89±0.30	10.23±0.40	100.4±8.03
	10	71.25±0.69	-1.56±0.01	11.54±0.96	11.14±0.33	96.58±0.59
	20	72.96±0.39	-1.73±0.02	10.57±0.39	10.37±0.26	101.26±0.93
	30	71.59±0.85	-0.99±0.03	9.85±0.24	9.86±0.29	103.29±1.06
NDRs						
CNDR	0	73.60±0.21	-0.91±0.04	10.93±0.12[c]	11.11±0.12[c]	89.10±4.23
	10	74.05±0.20	-0.82±0.03[a]	12.10±0.14	12.10±0.14	80.25±0.75[b]
	20	77.25±0.22[b]	-1.00±0.01	11.99±0.03	11.92±0.03	87.43±0.59
	30	72.55±0.21[c]	-0.72±0.05[b]	9.83±0.07[b]	9.83±0.07[b]	87.36±2.80
LNDR	0	73.89±0.05	-0.76±0.05	10.84±0.23[c]	10.86±0.23[c]	93.76±3.12

	10	67.52±0.09	-1.20±0.01[a]	9.59±0.08	9.53±0.08	113.53±0.75[b]
	20	69.51±0.01[b]	-1.05±0.00	13.23±0.09	13.23±0.09	83.96±6.80
	30	70.60±0.60[c]	-0.63±0.05[b]	11.17±0.06[b]	11.11±0.06[b]	90.23±5.23
TNDR	0	73.89±0.03	+0.76±0.06	13.97±0.10[c]	13.92±0.10[c]	87.16±5.31
	10	67.23±0.06	1.01±0.07[a]	15.26±0.09	15.23±0.09	84.29±4.34[b]
	20	69.21±0.04[b]	0.63±0.05	13.89±0.14	13.89±0.14	85.76±4.61
	30	64.12±0.02[c]	0.83±0.07[b]	15.33±0.03[b]	15.33±0.03[b]	82.33±5.30
CLNDR	0	73.89±0.05	-0.19±0.00	11.09±0.23[c]	11.06±0.23[c]	90.53±3.12
	10	67.52±0.09	-1.28±0.03[a]	9.69±0.08	9.63±0.08	113.43±0.75[b]
	20	69.53±0.01[b]	-1.04±0.01	13.36±0.09	13.36±0.09	83.33±5.89
	30	69.52±0.60[c]	-0.53±0.01[b]	11.23±0.06[b]	11.23±0.06[b]	90.19±5.23

Results are expressed as mean ±SD (n=3) and significantly different at [a]$p<0.05$, [b]$p<0.01$ and [c]$p< 0.001$ vs DR (control). L* value represents lightness and darkness with a range from black (0) to white (100), a*value represents the green-red spectrum with a range from green (–100) to red (+100), while b* value represents blue-yellow spectrum with a range from blue (–100) to yellow (+100). c value represents chroma and h value represents hue angle. DR: Dairy rasgulla, NDR: Non-dairy rasgulla, NDR: CNDR: citric acid coagulated non-dairy rasgulla; LNDR: lactic acid coagulated non-dairy rasgulla; TNDR: tartaric acid coagulated non-dairy rasgulla; CLNDR: calcium lactate coagulated non-dairy rasgulla

Sensory evaluation of DR and NDRs during storage at 4 °C in a refrigerator

The sensory evaluation of DR and NDRs produced using various coagulants and stored at 4^0C for 30 days was shown in **Table 4.** The results revealed that general acceptability of DR and NDRs was gradually decreased during storage as exemplified by color, aroma and texture of rasgulla samples.

The mean scores of DR and NDRs for color gradually decreased on storage. LNDR showed a significantly lower score (5.59±0.15; $p<0.05$)for color than DR (6.29±0.85; $p>0.05$) other NDRs prepared using CNDR (7.08±0.69; $p>0.05$), TNDR (7.06±0.19; $p<0.05$) and CLNDR (6.75±0.18; $p<0.001$) on 30^{th} day of storage.

The mean aroma score of DR and NDRs produced using different coagulants was also decreased during storage. Results showed that this decrease was more significant in case of DR (6.33±0.59; $p>0.05$), CNDR (6.43±0.69; $p>0.05$) and LNDR (6.40±0.18; $p>0.05$) at 30^{th} day of storage.

A decreasing trend of mean texture score was observed for all the types of NDRs including DR during the storage period. A significant lower texture score was observed for LNDR at 30^{th} day of storage (6.28±0.18;$p>0.05$).

In general over all acceptability for NDR and DR were gradually decreased from 0 day of storage and it was lowest for 30 days of storage. Over all acceptability scores of NDR prepared from LNDR was lower (6.11±0.17; $p>0.05$) than that of other NDRs and DR for the storage period. Relatively lower overall acceptability scores for LNDR were due to lower scores for other sensory attributes. Overall acceptability scores for TNDR (7.63±0.22; $p<0.01$) and CLNDR (7.65±0.22; $p<0.001$) were significantly higher among all treatments throughout the period of storage. Thus, on the basis of analysis of different physicochemical and sensory parameters, tartaric acid proved to be optimum in the preparation of NDR.

The result revealed that overall acceptability of LNDR was lower than that of DR. On the other hand it was observed that CNDR, TNDR and CLNDR had higher overall acceptability than DR. These findings were similar with the observations of **Nande *et al.*, (2008), Katara and Bhargava, (1990)** who showed that slightly lower score in overall acceptability was obtained when the attribute of appearance of NDRs was compared with that of DR.

Table 4 Sensory properties of DR and NDRs using different coagulating agents during storage at 4 °C in a refrigerator

Sample	Day	Colour	Texture	Aroma	Overall acceptability
DR	0	8.12±0.29	8.95±0.29	8.83±0.26	8.95±0.28
	10	7.25±0.65	7.14±0.58	6.52±0.67	7.42±0.98
	20	6.59±0.39	7.26±0.48	6.41±0.48	6.69±0.69
	30	6.29±0.85	7.00±0.69	6.33±0.59	6.28±0.47
CNDR	0	7.35±0.29[a]	8.54±0.35[b]	6.93±0.20[c]	7.69±0.33[c]
	10	7.29±0.59	8.47±0.59[a]	6.84±0.69	7.68±0.85[a]
	20	7.11±0.89[a]	7.89±0.47	6.57±0.57	7.53±0.96[a]
	30	7.08±0.69	7.87±0.69	6.43±0.69	7.40±0.59
LNDR	0	6.25±0.23[b]	7.53±0.28[b]	6.98±0.21[c]	6.59±0.28[c]
	10	6.11±0.16[b]	7.40±0.20[a]	6.77±0.17	6.43±0.16
	20	6.02±0.15	7.33±0.18[a]	6.47±0.16[a]	6.20±0.46
	30	5.59±0.15[a]	6.28±0.18	6.40±0.18	6.11±0.17
TNDR	0	8.12±0.28[a]	8.16±0.30[b]	7.68±0.32[c]	8.20±0.46[c]
	10	8.02±0.30[b]	8.01±0.29	7.63±0.27[a]	8.11±0.31[b]
	20	7.12±0.28[a]	7.89±0.29[b]	7.64±0.28[a]	7.65±0.28
	30	7.06±0.19[a]	7.88±0.20[a]	7.50±0.28[a]	7.63±0.22[b]
CLNDR	0	7.34±0.28	8.29±0.32[b]	8.12±0.22[c]	8.24±0.55[c]
	10	7.29±0.22	8.18±0.25	7.98±0.23[c]	7.96±0.24[b]
	20	6.89±0.17	8.01±0.22	7.63±0.20[a]	7.77±0.21[b]
	30	6.75±0.18[c]	7.96±0.20[a]	7.51±0.21	7.65±0.22[c]

Results are expressed as mean ±SD (n=3) and significantly different at [a]$p<0.05$, [b]$p<0.01$ and [c]$p< 0.001$ vs DR (control). DR: Dairy rasgulla; NDRs: non-dairy rasgullas; CNDR: citric acid coagulated non-dairy rasgulla; LNDR: lactic acid coagulated non-dairy rasgulla; TNDR: tartaric acid coagulated non-dairy rasgulla; CLNDR: calcium lactate coagulated non-dairy rasgulla.

In vitro multienzyme protein digestibility of DR and NDRson 0 day at 4 °C in a refrigerator

Fig 2 showed the result of i*n vitro* multienzyme protein digestibility of DR and NDRs produced using various coagulants. The result revealed that there was significant difference in the digestibility of TNDR (121.77±1.55; $p<0.01$) when compared to CNDR (88.12±1.05; $p<0.05$), LNDR (83.76±1.06; $p<0.05$) and CLNDR (87.38±1.04; $p<0.05$). *In vitro* multienzyme protein digestibility of TNDR (121.77±1.55; $p<0.01$) was significantly higher than that of DR (100.25±2.54). LNDR, CNDR and CLNDR had lower protein digestibility values than control DR. LNDR had the lowest (83.76±1.06; $p<0$) value. The differences in the i*n vitro* protein digestibility of NDRs may be due to differences in the coagulating ability of each of the coagulant with regard to the different type of proteins in the presence of the various protease inhibitors (**Hwang *et al.*, 1978**).

Differences in the digestibility of rasgulla may be due to interaction of Tannin, trypsin and chymotrypsin inhibitors with the protein.

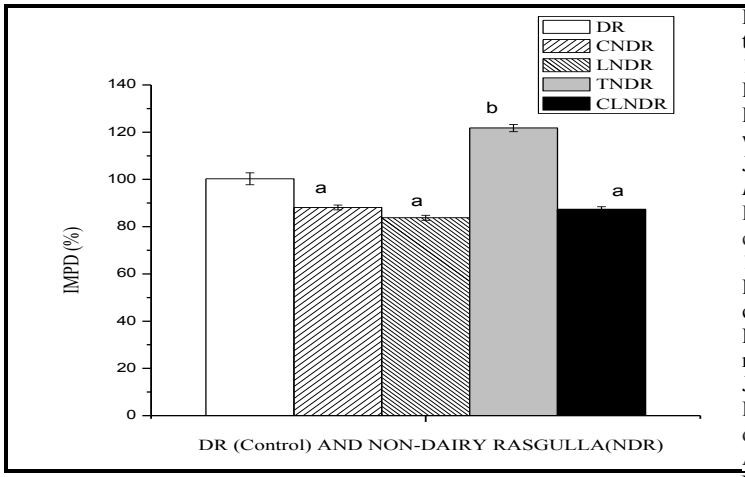

Fig 2 *In Vitro* Mutienzyme Protein Digestibility (IMPD) of DR and NDRs using different coagulating agents
DR: dairy rasgulla, NDR: Non-dairy rasgulla; DR: dairy rasgulla; CNDR: citric acid coagulated non-dairy rasgulla; LNDR: lactic acid coagulated non-dairy rasgulla; TNDR: tartaric acid coagulated non-dairy rasgulla; CLNDR: calcium lactate coagulated non-dairy rasgulla

CONCLUSION

Suitability of different coagulants (citric acid, tartaric acid, lactic acid and calcium lactate) for the manufacture of rasgulla was evaluated on the basis of proximate composition, hardness, color values and sensorial attributesand were compared with the respective values of most preferred of dairy rasgulla procured from market. Rasgulla manufactured using lactic acid, lack sensorial attributes, hardness and color values and accordingly was found unsuitable for better quality product. Tartaric acid coagulated NDR had significantly higher protein, ash, total solids, energy content and protein digestibility content, as well as the best sensory quality evaluated. Thus tartaric acid gave higher overall acceptability of rasgulla and was found the most suitable coagulant for manufacturing non-dairy rasgulla.

CONFLICT OF INTEREST

The authors are unanimous in publishing this paper. There is also no body to contradict this manuscript.

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EFFECT OF NUTRITIONAL COMPOSITION ON SHELF LIFE OF CEREALS-LEGUMES BLENDED FLOURS DURING STORAGE

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ABSTRACT

The ubiquitous indulging of cereals and legumes all over the world gives them a momentous abode in international nutrition. The exploration of food component plays dominating ole in the nutritional assessment, shelf life and the inclusive acceptance by the end user. Maize, chickpea and soybean flours were blended uniformly @ 10, 20, 30, 40 and 50% levels with wheat flour to prepared different compositions and stored at ambient temperature in polyethylene bags. During the fortnightly storage period of 45 days, each treatment was assessed for moisture, crude protein, crude fat, crude fiber, ash, nitrogen free extract, acidity, peroxide value and mold count. Mean squares of chemical analysis revealed significant differences due to different flour compositions and storage intervals. There was a slight increase in moisture content, total acidity, peroxide value and mold count of the composite flours whereas a decrease in protein, fiber, fat and nitrogen free extract during storage. Ash showed non-significant effect during storage. It is concluded that vacillations in temperature, relative humidity, storage circumstances, length of storage time, storage material as well as enzymatic activity made considerable nutrient losses in cereals-legumes blended flours.

Keywords: Proximate analysis, Nutrition, Storage, Cereals, Legumes, Flour

INTRODUCTION

The quality of food is centered on the natural composition and the balance between the nutrients. Chemical examination of a food sample determines the moisture, fiber, protein, fat, ash and carbohydrate reported as the percentage composition of the product. Hygroscopic nature of flour applies a strong influence on its quality, technological properties and physical characteristics. Moisture content of the flour is significant regarding shelf life, lesser the flour moisture, well its storage stability. Wheat is the indispensable food of Pakistani population and largest grain source because of its use for the preparation of various baked products. Chemical characteristics of wheat flours exhibited wide variations. In general, Pakistani wheat varieties contain on average 7.02-11.08% moisture, 9.57-14.3% crude protein, 1.47-2.93% crude fat, 1.48-2.03% ash, 0.98-2.68% crude fiber and 72.62-81.92% NFE (**Khan *et al*.,1987; Huma, 2004; Shahzadi*et al*.,2005; Akhtar*et al*., 2008**). Maize/Corn is world's 3rd leading cereal crop. Good amino acid profile of maize supports its use for supplementation to expand the protein quality of wheat based products. Proximate composition of maize flours vary significantly among different varieties. Established levels of protein content ranges from 5.18%-8.14%, fat content from 1.56-3.15%, ash level 0.19-2.1%, dietary fiber content 0.42-0.62% and carbohydrates 87.6-92.5% (**Shukla and Cheryan 2001; Sefa-Dedeh *et al*., 2004; Sandhu *et al*., 2007; Wu *et al*., 2010**). Protein, fiber and fat content range of 5.2%, 0.5% and 2% for corn flour have been reported earlier by (**Alexander, 1987**).

Legumes with their therapeutic agents, occupy an important place in human nutrition whilst are important sources of food proteins, (2-3 times more than cereal grain) and are a rich source of dietary fiber (**Siddiq *et al*., 2010**). Chickpea is the 5th most important crop worldwide. Chickpea is a good and cheap source of protein and carbohydrate. Its protein quality is better than other legumes (**Kaur *et al*., 2005**).In world, chickpea varieties have been found to contain, on an average 7.4-7.6%moisture, 17-29% protein,4.5-7.1%fat, 3-9% fiber, 2.6-3.5% ash and 59.5-65.45 carbohydrate (**Saleh and Tarek 2006; Chung *et al*., 2008; Boye *et al*. 2010**). Among different white and whole chickpea cultivars the moisture, crude protein, crude fat, crude fiber and ash are 7.26-8.47%, 23.06-25.18%, 3.81-7.22%, 1.14-2.78%, 3.27-3.46% respectively (**Gomez *et al*., 2008**). Soybean proteins, due to their good nutritional profile and excellent functionality, have become an ingredient of choice in many diverse food applications and a variety of nutritional foods and supplements. Yellow soybean contains 38.9-41.8% protein, 18.5-21.6% fat, 7.9-9.82% moisture, 4.81-5.28% ash and 8.92-13.3% carbohydrates. In green soybean moisture, protein, fat, available carbohydrates and ash are 10.19-10.81%, 36.8-37.1%, 0.93-0.98%, 38.5-38.8% and 3.08-3.39% respectively (**Cuenca *et al*., 2006**). Furthermore, soybean contain 36.54%, protein, 22.7% lipid, 7.36% moisture, 0.78% ash and 32.62% carbohydrates (**Pednekar *et al*., 2010**).

Molds are extensively present in nature and require minute moisture for growth than that of bacteria. Among different microorganisms molds were the most frequently detected microbes. Microorganism decreased during the milling process. Molds decreased by 88% compared with wheat before milling. In the end, products wheat flour contained molds from 10^2-10^3cfu/g. The acceptable quality limit (cfu/g) for molds is < 10^3 (**Berghofer *et al*., 2003**). Respiration and activity of microorganisms retarded at lower moisture content (**Staudt and Zeidler, 1973**). The mold contamination is also important in view of the possible mycotoxin production by a great number of mold species (**Hussein &Brasel, 2001**). It has been established that molds not only cause spoilage but sometime if favorable environmental conditions exist, production of toxin occurs. The occurrence of these toxins in grains and on other food products is influenced by environmental factors during the pre-harvesting, harvesting and post-harvesting periods. Therefore, the storage conditions, length of storage time and storage materials are the key factors contributing to the stability of the flours (**Akhtar *et al*., 2008**). Mold count in different wheat varieties during 45 days of storage ranged from 2.73-20.05 × 10^2. Mold count of flour in tin container was higher than polypropylene bags (Huma, 2004). Mold count in composite flour samples

at different level of storage intervals showed that mold counted at 0, 15, 30, 45 and 60 days were increased from 0.26×10^2 to 5.08×10^2 /g (**Shahzadi *et al*., 2005**). In other studies a higher degree of mold growth was observed under ambient storage condition (6.08×10^2 colonies/g) as compared to controlled storage condition (4.18×10^2/g). The length of storage time, irrespective of storage conditions demonstrated a concurrent increase in mold population. Whole wheat flour samples were analyzed for mold population under two storage conditions at 0, 15, 30, 45, and 60 days. Higher growth (31.44%) was observed in whole wheat flour under ambient storage condition in comparison with controlled storage condition at the termination of the storage period (**Akhtar *et al*., 2008**).

The present study was conceded to determine the effect on chemical composition and stability during storage of blended flours as well as extend the shelf life of flour by determining the proper value suitable for safe storage by analyzing the moisture, crude protein, crude fat, crude fiber, ash, nitrogen free extract, acidity, peroxide value and mold contents.

MATERIAL AND METHODS

Procurement of materials

Wheat (*Triticumaestivum L.*), maize/corn (*Zea mays L.*) soybean (*Glycine max L.*) and chickpea (*Cicerarietinum L.*) were purchased from the local market (Faisalabad, Pakistan) to make cereal-legumes blended flours.All reagents and standards were supplied by Merck (Merck KGaA, Darmstadt, Germany) and Sigma-Aldrich (Sigma-Aldrich Tokyo, Japan).

Preparation of raw materials

The raw materials were cleaned manually, to remove dirt, dust, damaged seeds, seeds of other crops and foreign matter. The particle size of wheat, maize, soybean and chickpea was reduced to fine flour through Quadrumat experimental mills.

Preparation of flour blends

Maize, chickpea and soybean flours were blended with wheat flour in different combinations to prepare composite flours, Table 1. Each treatment of composite flour was thoroughly mixed and sieved in order to achieve uniform mixing of legume flours with wheat flour and was stored at room temperature in polythene bags during a period of 45 days.

Chemical analysis

The moisture content was examined by using oven dry method, fiber using fiber tech apparatus Model: Labconco corporation, Kansas, USA), protein content was determined by using Kjeldahl's apparatus Model: Technik GmbH D-40599, Behr Labor, Germany), fat using soxhlet apparatus (Model: HT2 1045 Extraction unit, Hoganas, Sweden), ash using muffle furnace (Model: MF-1/02, PCSIR, Pakistan), total acidity, POV and mold count were determined by AACC Method No. 44-01, 32-10, 46-10, 30-10, 08-01, 02-31, 58-16 and 42-50, respectively (AACC, 2000).The nitrogen free extract was calculated according to the below expression:

NFE % = 100 - (% moisture+ % crude fiber + % crude protein + % crude fat + % Ash)

Table 1 Treatments used to prepare composite flours.

Treatments	Wheat Flour (%)	Maize Flour (%)	Chickpea Flour (%)	Soybean Flour (%)
T1	100	-	-	-
T2	90	10	-	-
T3	80	20	-	-
T4	70	30	-	-
T5	60	40	-	-
T6	50	50	-	-
T7	90	-	10	-
T8	80	-	20	-
T9	70	-	30	-
T10	60	-	40	-
T11	50	-	50	-
T12	90	-	-	10
T13	80	-	-	20
T14	70	-	-	30
T15	60	-	-	40
T16	50	-	-	50
T17	90	3.33	3.33	3.33
T18	80	6.66	6.66	6.66
T19	70	10	10	10
T20	60	13.33	13.33	13.33
T21	50	16.66	16.66	16.66

Statistical analysis

The data obtained for each parameter was subjected to statistical analysis to determine the level of significance (Analysis of variance technique) in completely randomized design as described by (Steel et al., 1997). Means were further compared through Duncan's multiple range test to determine the significant differences.

RESULTS AND DISCUSSION

It is obvious from the results that the mean squares (Table 2) for moisture, fiber, protein, fat, NFE, POV, acidity, mold content of different flour samples were significantly affected within treatments except ash that is non-significant and with storage intervals (Table 3) whereas non significantly difference were observed in the interaction between treatments and storage except protein that is significant.

Table 2 Mean squares for chemical composition of different composite flour samples

SOV	df	Moisture	Fiber	Protein	Fat	Ash	NFE	Acidity	POV	Mold count
Treatments	20	7.0418**	0.345311**	110.688**	0.79555**	0.65414NS	193.436**	0.036703**	0.097571**	0.4545**
Days	3	10.1151**	0.045515**	0.846**	0.06236**	0.00210**	3.218**	0.114670**	0.726507**	38.7022**
T×D	60	0.0152NS	0.003502NS	0.058**	0.00089NS	0.00000NS	0.072NS	0.002732**	0.000963**	0.0385**
Error	168	0.0142	0.002691	0.008	0.00108	0.00075	0.058	0.000590	0.000116	0.0052

NS = Non-significant (P>0.05) ** = Highly significant (P<0.01)

Table 3 Means for the effect of storage on chemical compositions of composite flour samples

Days	Moisture (%)	Fiber (%)	Protein (%)	Fat (%)	Ash (%)	NFE (%)	Acidity (%)	POV (mEq/Kg)	Mold count (x102/g)
0	11.33 d	2.096 a	13.68 a	2.678 a	0.983 a	69.24 a	0.181 d	0.484 d	0.271 d
15	11.56 c	2.073 b	13.60 b	2.658 b	0.983 a	69.14 b	0.211 c	0.540 c	0.553 c
30	11.91 b	2.053 c	13.46 c	2.631 c	0.973 a	68.97 c	0.248 b	0.640 b	1.029 b
45	12.24 a	2.033 d	13.43 d	2.606 d	0.973 a	68.72 d	0.279 a	0.726 a	2.055 a

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05).

Moisture

Results from various treatments showed in Table 4 significantly effect on the moisture content of composite flours. The higher moisture content 12.88% was noted in T_{16}while lowest value10.38% was shown by T_1.Higher increasing trend was found in wheat-maize flour blends from$T_2$to T_6followed by wheat-chickpea flour blends that are from T_7 to T_{11} whereas the lower were found in wheat-soybean flour blends from $T_{12}$to T_{16}. All flour blends having corn, chickpea and soybean flour also showed increasing trends. It is clear from result that lower increasing trend was found in legume flour. There was an increase in moisture

content of various flour samples because the stored sample absorbs moisture from surrounding that might be due to their hygroscopic nature and change in relative humidity during storage. The results regarding the moisture content increased during the storage period supported the findings of (**Huma 2004; Sharif 2009; Shahzadi *et al*., 2005**).

Crude Fiber

Means for crude fiber in Table 4 exhibited that T_{10} (2.355%) showed the highest value while T_2 showed the minimum value (1. 825%).These treatments were found to be non-significant with respect to each other. Combinations in which chickpea flours are use showed the highest values of fiber followed by soybean flour blends while the minimum value was noted in corn flour blends. (**Boye *et al*., 2010**) observed 3-9% fiber in different chickpea varieties. Corn fiber content as narrated by (**Sandhu *et al*., 2007**) that corn fiber in different varieties varied from 0.42-0.62%, whereas in corn germ the crude fiber was ranged from 2.63-3.97% noted by (**Nasir, 2009**). It was noted from the results that the addition of legume four increase the value of crude fiber. Storage has a significant effect on various treatments of composite flour. Means for fiber content showed significant differences, i.e. 2.096, 2.073, 2.053 and 2.033 in 0, 15, 30, 45 days of storage intervals, respectively.

Crude Protein

Means for crude protein content of different composite flour in Table 4 demonstrated that T_{16} contained highest value 18.41%, while the minimum value showed by T_5 (9.62%). In the present study the value of protein content in different flour samples ranged from 9.62%-18.41%. Combinations of wheat-soybean flour blends showed high values for protein content followed by wheat-chickpea flour blends. Wheat-corn flour blends presented low content of protein. All flour combinations showed, as the quantity of flour increased, protein content increased. Previous results showed 35-40% protein in soybean (**Pednekar *et al*., 2010; Olaoye *et al*., 2006**), whereas in chickpea it varied from 16.1% to 21.3% (**Kaur *et al*. 2005**). Protein content of corn ranged from 5.18% to 7.82% (**Sandhu et al., 2007**). Storage has significant effect Table 3 on protein content in various composite flours. Crude protein in present study decreased during 45 days of storage with accordance with (**Funami *et al*., 2005**) that with the passage of time crude protein decreased. This is due to absorption of moisture from the surrounding that accelerated the proteolytic activity of the enzymes. The enzymes are responsible for the degradation of protein during storage. It is observed by the means that there is significantly decrease in protein content during storage intervals. At 0, 15, 30 and 45 days the mean value of protein content decreased to 13.68%, 13.60%, 13.46% and 13.43% respectively.

Table 4 Means for the effect of various treatments on chemical compositions of composite flour samples

Treatments	Moisture (%)	Fiber (%)	Protein (%)	Fat (%)	Ash (%)	NFE (%)	Acidity (%)	POV (mEq/Kg)	Mold count (x 102/g)
T1	10.38 o	1.993 f	9.85 l	2.118 n	0.605 o	75.06 a	0.190 j	0.499 i	0.798 jk
T2	10.45 o	1.825 j	9.65 no	2.570 h	0.625 no	74.89 a	0.238 def	0.456 l	0.728 l
T3	10.81 n	1.855 ij	9.69 mno	2.695 ef	0.645 n	74.31 b	0.192 ij	0.464 l	0.753 kl
T4	11.13 l	1.895 hi	9.71 mn	2.808 d	0.675 m	73.78 c	0.258 cd	0.473 k	1.015 de
T5	11.32 k	1.915 gh	9.62 o	2.928 b	0.705 l	73.51 d	0.265 c	0.482 j	0.815 ijk
T6	11.48 j	1.945 g	9.75 m	2.975 a	0.715 l	73.14 e	0.348 a	0.489 j	0.934 fg
T7	11.03 m	2.025 ef	13.04 k	2.190 m	1.195 c	70.53 f	0.165 k	0.544 h	0.723 l
T8	11.23 kl	2.135 c	13.45 j	2.288 l	1.215 c	69.69 g	0.228 efg	0.566 g	1.028 de
T9	11.30 k	2.235 b	13.93 h	2.353 k	1.245 b	68.94 i	0.298 b	0.609 f	1.010 de
T10	11.80 hi	2.355 a	14.57 f	2.423 j	1.255 b	67.60 k	0.270 c	0.626 e	1.168 c
T11	11.88 gh	2.350 a	14.35 g	2.538 i	1.295 a	67.59 k	0.335 a	0.629 e	0.885 gh
T12	12.50 d	2.090 d	17.16 e	2.515 i	0.875 k	64.86 m	0.133 l	0.606 f	0.868 hi
T13	12.58 cd	2.145 c	17.68 d	2.640 g	0.895 k	64.07 n	0.213 ghi	0.614 f	0.820 ij
T14	12.65 bc	2.215 b	17.76 c	2.703 e	0.945 j	63.73 o	0.216 fgh	0.635 e	1.058 d
T15	12.73 b	2.158 c	18.16 b	2.850 c	0.995 i	63.12 p	0.234 efg	0.670 d	1.320 a
T16	12.88 a	2.335 a	18.41 a	2.968 a	1.095 fg	62.32 q	0.243 de	0.693 c	1.050 d
T17	11.75 i	1.865 ij	12.99 k	2.645 g	1.055 h	69.70 g	0.228 efg	0.671 d	0.763 jkl
T18	11.93 g	1.884 hi	13.06 k	2.673 f	1.085 g	69.38 h	0.131 l	0.679 d	0.983 ef
T19	12.15 f	1.945 g	13.41 j	2.783 d	1.115 ef	68.60 j	0.225 efg	0.701 b	1.205 bc
T20	12.40 e	2.045 e	13.79 i	2.913 b	1.135 e	67.72 k	0.202 hij	0.709 b	1.253 b
T21	12.60 cd	2.125 cd	14.35 g	2.938b	1.165d	66.82 l	0.214 gh	0.730 a	1.338 a

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05).

Crude Fat

Means of crude fat showed in Table 4 that T_6 (2.975%) contained highest value for fat content while the minimum value observed in T_1 (2.118%). Fat content in different composite flours ranged from 2.118-2.975%. In different flour blends, corn flour blended with wheat flour was ranked at the top in fat content followed by soybean flour blends while the minimum fat was observed in chickpea flour blends. Fat content was decreased during storage from beginning to end (**Shahzadi*et al*., 2005**). The mean Table 3 for fat content was 2.678% at 0 day, which decreased to 2.658%, 2.631% and 2.606% after 15, 30 and 45 days of storage. The gradual decrease in fat content may be due to the development of rancidity. A moisture content higher than 12%, there is a risk of fat oxidation and other chemical changes take place. Fat deterioration during storage might be due to activation of lipase enzyme which might split off the fat into free fatty acids and glycerol by the factors such as moisture, light and heat. Free fatty acid content increase during storage due to high activity of lipase.

Ash

The means for ash content of composite flour in Table 4 illustrated that ash content varies from 0.605 to 1.295%. The highest amount of ash in T_{11} followed by T_{10}, T_9, T_8 and T_7 with ash contents of 1.295%, 1.255%, 1.245%, 1.215% and 1.195%, respectively. Soybean was second to chickpea in term of ash content. It contained ash from 3.08%-3.39% revealed by (**Cuencee*t al*., 2006**). Ash content in chickpea ranged from 3.27-3.46% among different varieties (**Gomez *et al*., 2008**). Minimum ash contents were found in corn flour combinations followed by wheat flour. Ash content of corn reported 1.24-1.26% by (**Sefa-Dedeh*et al*., 2004**).T_1 (100% wheat) had minimum ash content because it is not fortified with corn, chickpea and soybean and same results by (**Senthil*et al*., 2002**) who noted 0.60% ash. It is clear from the results that legumes have higher ash content.

Nitrogen free extracts (NFE)

The means for NFE content of composite flour in Table 4 described that T_1 contained highest value (75.06%) while lowest value was found in T_{16} (62.32%). The overall range varies from 62.32 to 75.06%. The results regarding to all flour blends and wheat flour showed the highest value of NFE. Findings are in conformity with the work of (**Akhtar*et al*., 2008**) followed by corn and chickpea blends while soybean blends represented the lowest value of NFE content. With the passage of time NFE decreased significantly. At 0 days it was 69.24% followed by 69.14%, 68.97% and 68.72% after 15, 30 and 45 days of storage, respectively. This is due to decrease in fat and protein content. The decreasing trend in flours during storage was also observed by (**Huma 2004; Sharif, 2009**).

Acidity

The maximum acidity was observed in T_6 (0.348%) while the minimum in T_{18} (1.31%). T_1 showed minimum acidity (0.130%) while maximum showed by wheat-chickpea flour blends followed by corn and soybean flour blends. Between different treatments as the concentration of different flour blends increased, increased in acidity was observed. The acidity (0.181%) observed at 0 day, whereas increasing trend was noted at 15 days, it was (0.211%) that increased to (0.248%) to (0.279%) at 30 and 45 days, respectively. The present study coincides with the results of (**Anjum *et al*., 2003; Rehman, 2006**). Increased in acidity during storage is due to increase in moisture content of the flours sample with storage, may be one of the reasons for the increase in acidity. Increase in acidity of flour during storage may be due to accumulation of linoleic and linoleic acids (**Haruska and Machova 2002; Anjum *et al*., 2003; Shahzadi *et al*., 2005; Funami *et al*., 2005; Rehman, 2006**).

Peroxide value (POV)

Highest value of peroxide value Table 4 was observed in T_{21} (0.730mEq/Kg) while the lowest in T_3 (0.464mEq/Kg). Highest peroxide value values were found in all flour blends combinations from T_{17} to T_{21} followed by soybean and chickpea flour blends while the lowest value in wheat-corn flour blends. The means for peroxide value in Table 3 showed that peroxide value increased from 0.484 to 0.726 mEq/Kg during 45 days of storage. Increasing trend in peroxide value was also reported by (**Misfa *et al*., 2000; Shahzadi *et al*., 2005; Funami *et al*., 2005**) this may also be due to increasing moisture content during storage.

Mold count

Mean squares for mold count of different flour samples (Table 2) showed significant variations within treatments, during storage and among interactions. The means for mold count in flour samples at different storage intervals are shown in Table 3. At initiation of the study it was found to be 0.271×10^2 /g followed by 0.553×10^2, 1.029×10^2 and 2.055×10^2 /g after 15, 30 and 45 days of storage respectively. It is obvious from the result that at 0 day minimum mold count (0.110×10^2 /g) in T_{17} (90% wheat + 10% soybean flour) and maximum (0.420×10^2 /g) in T_{21} (50% wheat + 16.66% maize + 16.66% chickpea + 16.66% soybean) was found. There were increasing trend in the mold count up to 45 days. However, minimum mold count was observed 1.61×10^2 /g in T_1 (100% wheat) and maximum 2.6×10^2 /g in T_{21} (50% wheat + 16.66% corn + 16.66% chickpea + 16.66% soybean) at 45 days. During storage the growth of mold ranged from 0.271×10^2 to 2.055×10^2 /g. Finding of (**Huma, 2004; Shahzadi *et al*., 2005; Akhtar *et al*., 2008**) showed increased the growth of mold during storage.

Mold count was minimum at the beginning because the pH of the flour does not favor their growth. During milling, mold count decreased concluded by (**Berghofer *et al*., 2003**) but in the later stage high temperature, relative humidity and hygroscopic nature of flour support mold growth which also leads to toxin development. (**Akhtar *et al*., 2008**) observed that under controlled conditions of storage mold growth can be minimized. Thus, from results if mold count increases day by day than declines in overall quality of composite flour samples.

CONCLUSIONS

The addition of legumes with cereals significantly exaggerated the chemical features of composite flour. Results exposed as the concentration of legumes flour increased with cereals flour there was increased in protein, fat, fiber and ash content but decreased in NFE. Stability of flour was also affected during storage. The moisture content was increased during storage because flour absorbs moisture from surrounding due to their hygroscopic nature. Protein, fat and fiber were decreased. The decreased in protein is due to absorption of moisture from the surroundings that accelerated the proteolytic activity of the enzymes. The enzymes are responsible for the degradation of protein during storage. The gradual decrease in fat content may be due to the development of rancidity. Fat deterioration during storage might be due to activation of lipase enzyme which might split off the fat into free fatty acids and glycerol by the factors such as moisture, light and heat. Free fatty acid content increase in storage due to high activity of lipase. NFE also decreased that is due to decrease in fat and protein content. Acidity and POV increased because the decrease in fat might be due to increase in fatty acids that are due to higher lipase activity that leads to rancidity. The increased in peroxide value during present study was due to the development of rancidity. Moisture level below 12% is seemly for safe storage and minus detrimental deviations in the composition f blended flour. Cereal grains should not be stored above 25 ^{0}C in order to minimize nutrient losses during storage.

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IMPACT OF CHEESE WHEY PROTEIN ON GROWTH PERFORMANCE OF BROILER: AN APPROACH OF CHEESE WHEY UTILIZATION IN POULTRY FEED

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ABSTRACT

Cheese whey is greenish yellow liquid separated during cheese processing. It accounts 80-90% of milk after cheese processing. It is usually wasted by cheese industries, particularly in developing countries like Pakistan that increases BOD and COD of dairy effluent. Various useful components like lactose, proteins, minerals etc. are present in whey. Among proteins, whey proteins are very effective in body muscle anabolism along with other health benefits. Present research utilized whey proteins in the form of protein supplement. Whey protein was precipitated by combination of pH, temperature and salt treatment followed by filtration. Two types of whey protein concentrates (WPCs) were formed. One was creamy textured while the other was in powder form. WPCs were added in broiler feed at the rate of 0.2% in powder form and 2% in the creamy texture form. Growth parameters like feed consumption, body weight and weight gain increased with whey protein supplement while had no effect on feed conversion ratio (FCR). Carcass traits like carcass, breast, thigh, wings, drumstick weight had significantly increased with the incorporation of whey protein while having non-significant effect on liver weight, GIT weight and GIT/carcass ratio. Whey protein supplementation exhibit no significant influence on packed cell volume (PCV), hemoglobin, lymphocytes and polymorphnuclear leukocyte (PMN) while exhibit significant impact on leukocytes and platelets. It is concluded that only 0.2% WPC (powder) exhibit significant impact on carcass growth while 2% WPC (creamy texture) supplementation improved the growth parameters but statistical analysis revealed it non-significant.

Keywords: Whey, Whey Protein Concentrates (WPCs), Muscle anabolism, Essential amino acid, Fast protein, Hematology

INTRODUCTION

The dairy industry is divided into various sectors and each sector produces different kinds of products. Milk, Yoghurt, cheese, butter and ice-cream are common products of dairy industries. During processing of these products different effluents are produced. Each effluent has different characteristics depending upon the process and the product. All these effluents increase biological oxygen demand (BOD) and chemical oxygen demand (COD) of water, when discharged untreated.

Whey is one of the dairy effluents formed during cheese processing. It accounts 80-90% of total milk volume. Apart from being valued as a medicinal agent in the 17th and 18th centuries, whey has primarily been considered a waste by the dairy industry. In the late 20th century, regulations prevented the disposal of untreated whey. At the same time, recognition of the value of whey components accelerated. Modern science has unraveled the secrets of whey proteins and other whey components, and established a sound basis for their nutritional and functional value. Now it is possible to conserve valuable whey components, including lactose, proteins and minerals that results a variety of products available in the market (**Smither, 2008**).

Whey protein is one of the important components of whey. It is one of the two major proteins of milk that accounts 20% of total milk protein while the rest 80% is casein. Most of casein protein becomes the part of the cheese during cheese production while whey proteins left in the whey (the liquid left after cheese production). Whey protein is a protein complex which contains many kinds of proteins and enzymes like beta-lactoglobulin, alpha-lactalbumin, bovine serum albumin (BSA), lactoferrin, immunoglobulins, lactoperoxidase enzymes and glycomacropeptides. These proteins perform many functions. Whey proteins provide all essential and branch chain amino acids, improves body composition, immune modulation and have antimicrobial activity. In addition, whey protein has the ability to act as an antioxidant, antihypertensive, antitumor, hypolipidemic, antiviral, antibacterial and chelating agent. It also enhances strength of muscles; prevent osteoporosis and cardiovascular disease (**Bjorkman*et al.*, 2012**).

Whey proteins have strong position in sport nutrition. Active people take advantage of whey supplements. Whey proteins are considered as “fast protein”, have capability of muscle development during exercise training. Whey protein is also used in enriching some baking products (**Rostamie*t al.*; 2013**). The amino acid composition of these proteins is similar to that of skeletal muscle so they are directly involved in muscle anabolism along with growth and repair. Amino acids provided by whey proteins are efficiently utilized and absorbed. Supplementation of whey proteins provides a higher lean body mass gain and favorable effect on protein metabolism as compared to an iso-nitrogenous casein protein (**Cribb, 2006**).

Whey and whey proteins also found application in poultry feeding. Whey and its products have been reported to contain unidentified growth factor(s) when added to the diet of chickens. Different researches reported that supplementation of liquid whey, up to a certain level, improves broiler growth. Broiler became lactose intolerant at higher concentration of liquid whey (because of the presence of lactose in whey). This condition causes osmotic diarrhea that lower broiler weight (**Al-sadi*et al.*, 2008**). **Shariatmadari and Forbes. (2005)** concluded through various experiments that at least 1.8 parts of whey added to dry food (wet feeding), or whey offered as drinking liquid by diluted with an equal volume of water or used undiluted whey at alternative days with water, were all possibilities for taking advantage of this by-product. Supplementation of commercial whey protein concentrates at two levels i.e. 8g Kg $^{-1}$ and 32g Kg^{-1} increased carcass yield as compared to control group. However, higher level showed better growth performance (**Szczurek*et al.*,2013**).

Whey proteins can be concentrated and isolated through various techniques including ionic selection (including ion-exchange chromatography, gel filtration) and membrane filtration (ultrafiltration, reverse osmosis, gel permeation), polarity base separation (high performance liquid chromatography). Many of these techniques are not applicable in all cases due to the high cost of equipment, poor yield, less productivity and less selectivity during processing. It cannot be affordable for small industries (Jimenez *et al.*, 2012). Combination of pH, heat and chemical treatment is a technique of protein precipitation to avoid the cost of advance technologies. A number of studies reported that chemicals like NaCl, $CaCl_2$, heat treatment up to 100°C and pH adjustment usually 4 to7 were used for

the precipitation of whey proteins (**Bordenave-Juchereau*et* al., 2005; O'Kennedy and Mounsey, 2009; Stanciuc*et al*., 2012**).
The present research was designed to utilize futile whey by forming whey protein concentrates (WPCs) through a combination of pH, heat and chemical treatment. Keeping in view the lactose intolerance of broiler, implemented method reduce lactose content in WPCs as compare to whey powder that had higher lactose content. The amino acid profile of these proteins compelled to study the impact of these proteins on broiler growth performance by supplementation at minimum level.

MATERIAL AND METHODS

Research was conducted according to the following steps. 1) Preparation of whey protein concentrates (WPCs) from cheese whey 2) Bird housing and WPC supplementation in feed 3) Data recording regarding growth performance 4) Statistical analysis

1. Whey was collected during cheese processing from Technology Transfer Center (Processing Hall) National Institute of Food Science and Technology, University of Agriculture, Faisalabad (Pakistan). Whey was chemically treated by the addition of 6mM $CaCl_2$ @ 6mL per liter of whey and pH was adjusted to 7 by 1N NaOH. After chemical addition, it was heated to 90°C for 20 min proteins were denatured and precipitated. Muslin cloth was used for the filtration of precipitates. Most of soluble components, especially lactose was removed as filtrate and whey proteins left as retentate. These retentates were WPC (creamy texture), stored in freezer for supplementation in treatment C. For treatment B, WPC (creamy texture) was dried at 43°C by spreading on aluminum foil. After drying, hard crumbles were ground to fine powder. This was WPC (powder); stored in polythene bag for supplementation in treatment B. Both WPCs were analyzed for its protein content by using Kjeldhal's method (**AOAC, 2000**).
2. Forty five broiler birds (Hubbard strain), day old, of mixed sexes were purchased from a local hatchery. The chicks were weighed and randomly divided into three experimental units A, B and C with three replications each. The birds were placed and reared in deep litter pens each dimension 5 x 3 x 2.5 feet, which were disinfect and white washed before the start of the experiment. A layer of two inches saw dust was used as litter material in each pen which was stirred regularly during experiment to keep it in dry condition. Birds were vaccinated against ND and IBD disease. Commercially available starter (1-3 week) and finisher (4-5 week) rations were used. Feed of each experimental unit was manually supplemented with WPCs on a weekly basis. Birds had free access to feed and water throughout the experimental period. A treatment plan is elaborated in Table 1.Some growth performance parameters like feed consumption, birds' weight, weight gain and feed conversion ratio were recorded on a weekly basis. Performance of fifth week was recorded after five days instead of seven. After 35 days, one bird from each pen was slaughtered according to Islamic Halaal Principles and weights of carcasses and its different cuts were recorded. Blood samples were collected from wing vein two days before slaughtering and sent to Rehmat Laboratory, Faisalabad (Pakistan) for analysis.
3. Weekly data was analyzed LSD under two factor factorial by using Statistic 8.0 software while after slaughter parameters and hematological parameters were analyzed by using CRD with LSD.

Table 1 Treatment plan for supplementation

Treatment	Feed	Water
A (control group)	Normal feed No protein supplementation	Fresh clean water
B	Supplementation of WPC (powder) 0.2%	Fresh clean water
C	Supplementation of WPC (creamy) 2%	Fresh clean water

RESULTS AND DISCUSSION

Protein content in raw whey varied from 0.5-0.9%. Amount of protein in WPC (creamy texture) increased to 21% after filtration while it was further increased to 40% in WPC (powder). Amount of protein in WPC (creamy) was compared to ricotta cheese because of its processing and appearance in accordance with ricotta cheese (**El-Sheikh *et al*., 2010**) while WPC (powder) had a wide range having a protein content i.e.35-80% (**Bylund, 1995**). Protein content of present finding was in the prescribed range.
Weekly growth performance parameters live bird weight, weight gain, feed intake and FCR depicted in Figure 1, 2, 3 and 4 respectively. In figures mean values sharing different subscript differ significantly. It was observed that supplementation of WPCs did not exhibit any significant effect on body weight, weight gain and feed intake from 1-3 week but at 4^{th} and 5^{th} week statistical analysis showed significant difference. It was evident that treatment A and B were significantly differing from each other while mean values of treatment C was in between the mean values of treatment A and B. Amount of protein in treatment C was less than treatment B that is why mean values of treatment C were higher than treatment A but less than treatment B. Supplementation of whey proteins did not exhibit any significant difference on FCR. A little variation had been observed in different treatment groups, but the overall impact was non-significant. Findings of the present study showed an agreement with **Torki and Molanapour (2005), Karimi (2006), Omara (2012) and Abro*et al.* (2012)**. But the results of FCR were different from **Szczurek*et al.* (2013).** who reported that incorporation of whey protein concentrates at the rate of 32g per Kg had a significant effect on FCR. It might be due to higher level of WPCs that exhibit significant impact on FCR.
Parameters regarding different carcass traits were presented in Table 2. Maximum increase in carcass, breast, and heart, wings, thigh and drumstick weight were observed in treatment B. Treatment C also showed an increase in above mentioned parameters than treatment A (controlled group) but less than treatment B. Non-significant difference had been observed in GIT, GIT/Carcass ratio, body fat and liver weight. Amino acid profile of whey proteins is similar to that of skeletal muscles that is why they are directly involved in muscle anabolism (**Cribb*et al.* 2006**). Present research also showed that it was an amino acid profile of whey protein, which exhibited significant impact on wings, thigh, and breast and drumstick muscles. **Majewska*et al.* (2009)**. conveyed that supplementation of liquid whey increase carcass and thigh weight. Similar findings were shown by **Salahuddin*et al.* (2012)**. where 20% additional protein increase dressed, leg and breast weight. In the study of **Huwaida*et al.* (2013).** only 2% difference of protein level in feed resulted an increase in the live bird, carcass, drumstick, thigh, chest, back and wing weight. Supplement WPC @ 8g and 32g per kilogram of feed that increase breast meat yield while liver weight was not affected (**Szczurek*et al.*, 2013**). **Abro*et al.* (2012)**. stated that replacement of plant protein with animal protein had no significant effect on heart weight while **Huwaida*et al.* (2013)**. indicated that heart weight increased with a high proportion of protein.
Blood image is good signal of health status of animals. It helps to identify the severity of infection and the indirect signal of immune status of the birds. Present research reported (Table 3) that supplementation did not distress hemoglobin, polymorphnuclear leukocytes (PMN), lymphocytes and pack cell volume (PCV) while it increased number of white blood cells and platelets. Increase in the number of platelets is a healthy sign. It was due to bioactive components of whey proteins but a higher number of white blood cells indicated sign of any disease. **Ahmed *et al*. (1994), Donkoh*et al*. (1999), Odunsi*et al*. (1999).** reported that hematological parameters were unchanged in protein treatment. Reason behind increase in WBC might be temperature fluctuation during storage. WPC is a sensitive product, especially creamy textured WPC. It was stored in the freezer, but due to unavoidable load shedding it undergoes temperature fluctuation that might cause undesirable changes in it and highest number of white blood cells in treatment B so it is recommended that try to use fresh creamy texture WPC to avoid any harmful effect on hematology. WPC (powder) was preferred to dry in constant temperature to avoid any undesirable changes.

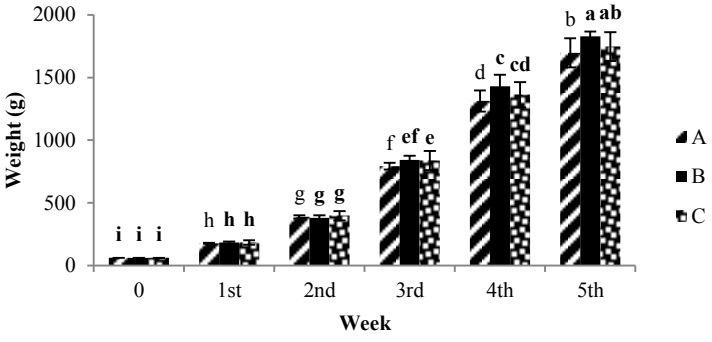

Figure 1 Comparison of means of weekly live bird weight of broiler
A= Normal feed (controlled)
B= Feed supplement with 0.2% whey protein (powder)
C= Feed supplement with 2% whey protein concentrate (creamy)

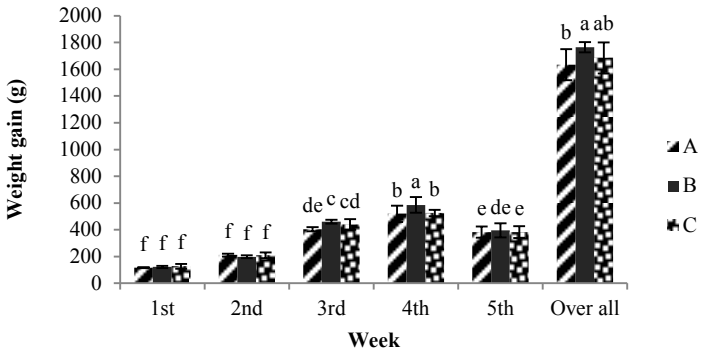

Figure 2 Comparison of means of weekly weight gain of broiler
A= Normal feed (controlled)
B= Feed supplement with 0.2% whey protein (powder)
C= Feed supplement with 2% whey protein concentrate (creamy)

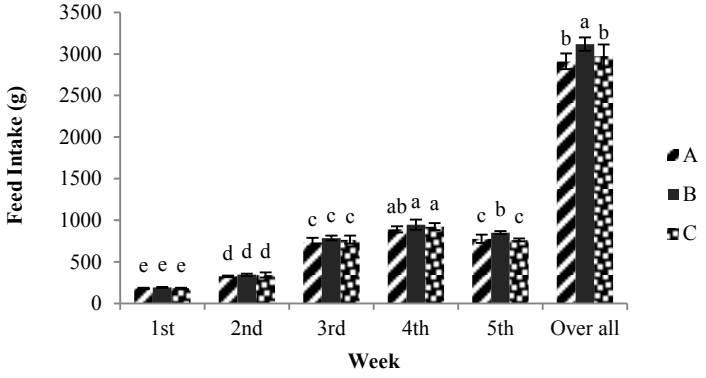

Figure 3 Comparison of means of weekly feed intake of broiler
A= Normal feed (controlled)
B= Feed supplement with 0.2% whey protein (powder)
C= Feed supplement with 2% whey protein concentrate (creamy)

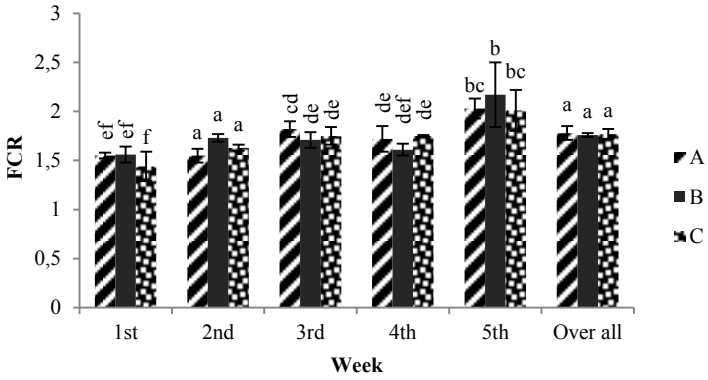

Figure 4 Comparison of means of weekly FCR of broiler
A= Normal feed (controlled)
B= Feed supplement with 0.2% whey protein (powder)
C= Feed supplement with 2% whey protein concentrate (creamy)

Table 2 Comparison of means ±SD of carcass traits

Parameters	A	B	C
Carcass weight	1018±0.04^{b}	1235±0.029^{a}	1083±0.06^{b}
GIT weight	89.16±5.20	101.66±2.88	86.6±12.58
Ratio GIT/carcass	0.087±0.006	0.082±0.002	0.08±0.01
Breast weight	320±12.21^{b}	396±12.219^{a}	348±7.49^{b}
Heart weight	7.06±0.23^{b}	9.63±1.2^{a}	7.33±0.73^{b}
Wings weight	43.60±0.79^{b}	51.76±0.83^{a}	45.2±3.04^{b}
Thigh weight	73.53±3.37^{b}	923±4.40^{a}	81.33±5.50^{b}
Drum stick weight	72.78±3.57^{b}	85.25±3.48^{a}	75.43±8.83$^{a\,b}$
Body fat weight	28.267±4.86	37.03±7.07	33.23±6.54
Liver weight	35±5.96	45±4.86	43±3.18

Table 3 Comparison of means ±SD of hematology

Parameters	A	B	C
Hemoglobin (g/dL)	8.9±1.29	7.9±1.49	10.08±1.19
Polymorphnuclear leukocytes (PMN)	7±3.27	5.6±2.02	4.17±2.02
White Blood Cells (thousands/mm^{3})	8133±2931^{b}	14866±3695^{a}	15833±2010^{ab}
Platelets	9833±1892^{b}	15500±1527^{a}	12400±2291^{ab}
Lymphocytes (%)	91.16±2.84	90.33±0.28	93.33±2.25
Pack Cell Volume (PCV)	28.72±4.73	26.08±3.79	28.72±4.83

CONCLUSION

To date, whey protein concentration (WPC) and its derivatives have not only considered as a good source of essential amino acids but they are a good source of protein substitution in many feeing and food products as well. In case of feeing livestock with a nutrient source like mentioned whey products, it is also effecting on different parameters like, protein amounts of carcasses. WPC is sensitive product especially creamy textured WPC. It was stored in freezer but due to unavoidable load shedding it undergoes temperature fluctuation that might cause undesirable changes in it and highest number of white blood cells in treatment B so it is recommended that try to use fresh creamy texture WPC to avoid any harmful effect on hematology. WPC (powder) was preferred to dry in consistent temperature to avoid any undesirable changes.

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