



QUALITY CHARACTERISTICS OF SET CURD SUBSTITUTED *PENNISETUM TYPHOIDEUM* DEHYDRATED POWDER

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Abstract:

The aim of the present study was to formulate a pearl millet dehydrated powder incorporated in set-curd to act as an enhanced immuno nutritive functional rich food by sustainable technological method with efficiency quality characteristics and better sensory attributes. The influence of pearl millet dehydrated powder concentration (0.5 – 1.5 %) and incubation time (5 - 8 h) was investigated on water holding capacity, reducing power and Overall acceptability of the prepared curd using Response Surface Methodology. The models were found to be highly significant ($p < 0.05$). The high value of R² for WHC (0.76), reducing power (0.90) and overall accessibility (0.96), indicated that response surface second order quadratic models were adequate and applicable. The optimum levels of pearl millet dehydrated powder concentration and incubation time were respectively predicted to be 1.45 (mg/g) and 6.7 for the maximum WHC of 15.4, reducing the power of 0.70 and overall acceptability score of 6.4 with the desirability of 0.852 in curd with the incorporation of pearl millet dehydrated powder. The CCRD design was used to investigate the influence of two independent factors on the quality characteristics of the prepared functional curd. It was beneficial in optimizing the factors for formulating

of pearl millet dehydrated powder incorporated curd based on the desired goals of the maximum antioxidant activity, water holding capacity and overall accessibility. Hence the incorporation of pearl millet dehydrated powder could be the nutritional and functional approach to enrich immuno nutritive food product and antioxidant potential of curd.

Key words: Pearl millet dehydrated powder, Response Surface Methodology, Functional Curd, Antioxidant, Water Holding Capacity and Immuno nutritive food product

1. Introduction:

Immunonutrition is one of the major important weapons to manage for the certain viral and communicable diseases (Clancy, 2003). There are several macronutrients and micro nutrients such as vitamins and minerals which are essential for the common functioning of the immune system. Besides, researcher reported that supplementation of Vitamins and minerals especially Zinc has enhancing immunity in viral infections (Gombart et al., 2020). In addition, various nutraceuticals and probiotics products have also found a supportive role in enhancing immune responses. Millet is one of the indigenous foods known to human and has been widely used in India as a staple food for thousands of years. Millets are common and readily available food sources in arid and semiarid regions, they play an important role in the food and nutritional security of the low income peoples (Amadou . et al., 2013).

Pearl Millet grains are usually processed in various technologies that are used in manufacturing of food products. Several traditional household food processing and preparation methods can be used to enhance the bioavailability of micronutrients in plant-based diets (Akinola et al., (2017). These comprise thermal processing, mechanical processing, soaking, fermentation, and germination/malting. These methods help to enhance the physic-chemical accessibility of micronutrients, reduce the content of anti-nutrients, such as phytates and increase the content of compounds that improve bioavailability (Saleh et al., 2013).

Pearl millet is a rich source of protein and moreover it contains bioactive components enriched with the health-promoting activities. However, their presence in the Indian food basket has been declining over the years (Jukanti et al., 2016). The lack of technologies for their effective processing and utilization is an important

reason for their refuse. Hence, we are in need to a technique which can aid the people to intake the bioactive compounds available in millets. Here novel techniques will be used for the dehydration to be given as food supplement.

Fermented dairy products are termed as functional foods (Chandan, 1999). Nutritionally, the presence of phosphor peptides released by the hydrolysis of short chain proteins accelerated the absorption of calcium, phosphorous and iron in fermented dairy products (Chandan, 1999). Functionally, the beneficial cultures in curd enhance the beneficial gut microflora. The curd is through lactic acid fermentation by using a mixed culture of *Streptococcus thermophilus*, *Streptococcus lactis*, *Streptococcus diacetylactis* and *Lactobacillus bulgaricus* (Mogra and Maya, 2008). Lactic acid fermentation in curd has been stated to release the bioactive peptides entrapped in native proteins. Sour milk and yogurt have been reported for the release of antihypertensive peptides and ACE inhibitory peptides (Nakamura et al., 1995; Meisel et al., 1997; Ashar and Chand, 2004). These studies emerged out the notion to release the antioxidative components entrapped in pearl millet dehydrated powder in curd through lactic acid fermentation over microbial and enzymatic hydrolysis of protein in a food-based approach manner.

Protein/ immuno-modulatory substance from dehydrated millet powder will be optimized for the formulation and incorporated in set-curd to act as functional food and the formulation can impact viral infections through improved immune status, cognitive and behavioral changes of peoples. The objectives of the work could be developing a function diet/immunonutrition based development product from the indigenous foods available from the specific geographical trait.

2. Materials and methods

2.1.Raw Materials

Pearl millet grains (*Pennisetum typhoideum*) for this study were procured at a local market in thanjavur, Tamilnadu, India. The grains were sorted, cleaned and screened to remove unwanted other irrelevant material. The pearl millet garins were well dried and cleaned manually from damaged grains and other foreign particles prior to their use. The grains were packed in screw-capped plastic containers and stored at 4° C for further analysis. For preparation of nutritive product development to purchased fresh- pasteurized and homogenized double toned milk, i.e., 3 per cent of fat and 8.5 S.N.F would be obtained from PONLAIT (Pondicherry

cooperative milk supply society), puducherry, India. Skim milk powder with 1 per cent milk fat would be obtained from super market. Starter cultures for preparing curd, i.e., *Lactobacillus bulgaricus* and *Streptococcus thermophilus* would collect from Starter cultures for preparing curd, i.e., *Lactobacillus bulgaricus* and *Streptococcus thermophilus* was collected Ponlait. All reagents and solvents used in the study were of analytical grade.

2.2. Preparation of pearl millet powder (PMP) and pearl millet dehydrated powder (PMDH)

Stored grains divided into two parts for preparation of pearl millet powder (PMP) and pearl millet dehydrated powder (PMDH). Soaking, preparation of slurry and dehydration processed would carry out for the development of PMD and PMDH. Soaking was a simple prolongation of the obligatory washing of the seeds and also has other advantages, such as facilitating dehulling or swelling of seeds. After pre processing, the two parts of selected millets would be soaked in water for 8 in ambient temperature. After completed soaking process, the water would be drained from millets. Preparation of PMP, drained millets kept into tray drier at 60°C 15-16 hours for drying, then it would be milled into fine powder by using a laboratory mill (A11B, IKA Inc, India) and preparation of PMDH, drained millets would ground to fine slurry by using wet grinding techniques. After grinding, the slurry would be filtered using muslin cloth and the filtrate would be dehydrated. The obtained millet slurry would be subjected to forced convection tray drying at 60°C 15-16 hours. The PMP and PMDH would be packed in aluminium foil laminated LDPE pouches and stored at refrigerator temperature (4°C) for further analysis and nutritive product development.

2.3. Proximate composition, Bulk density and Water activity of Pearl millet dehydrate powder (PMDH) and Pearl millet powder (PMP)

The proximate composition, including moisture, ash, protein, total fiber, energy, and fat contents, of flours, was analyzed using standard (AOAC 2005). The value of carbohydrate was calculated by difference. The bulk density of samples was analyzed by recording the volume occupied by powder in a pre-weighed 10-mL graduated cylinder up to the 10 mL mark. During flour filling, the cylinder was tapped 20 times and was weighed again, and the bulk density of flour is expressed as g/ml. Water activity was determined by using water activity meter (Aqualab Series 4TE, Decagon Devices, Inc., USA) at room temperature until the values were concurrent.

2.4. Preparation of set-curd (Nutritive product development)

Standard curd was prepared by heating the pasteurized and homogenized double toned milk in a milk boiler at about 90°C for 10 minutes. Milk was then cooled to 37°C for inoculating with 1 ml of starter culture along with through mixing for one minute for uniform distribution of culture into the whole milk. Cultured milk was transferred into 120 ml sterile cups and inoculation temperature was maintained for incubation until the semi-solid mass of curd was formed reaching the pH 4.5 ± 0.1 . Curds set in cups were stored in the refrigerator.

Experimental curd was prepared with the incorporation of pearl millet dehydrated powder (PMDH) following the protocol of standard curd. The pasteurized and homogenized double toned milk was heated in a milk boiler at about 90°C for 10 minutes. Milk was cooled to 37°C and PMDH was added at a concentration range of 0.5 – 1.5 mg/g and completely mixed into milk followed by the immediate inoculation with 1 ml of starter culture. PMDH incorporated cultured milk was transferred into 120 ml sterile cups and inoculation temperature was maintained for incubation until the semi-solid mass of curd was formed reaching the pH 4.5 ± 0.1 . Curds set in cups were stored in the refrigerator.

2.5. Physicochemical analysis of Nutritive product development (NPD)

2.5.1. Protein

Protein content was estimated using the micro Kjeldahl method. 3 g of standard and experimental curd were determined for nitrogen content. The total protein content was calculated from $N \times 6.38$ (AOAC 2005).

2.5.2. pH

pH values were measured using a pH meter model 34 (Backman Instruments, Inc., Fullerton, CA, USA), previously calibrated with buffers pH 4 and 7.

2.5.3. Total solids

The total solids content of standard and experimental curd was determined following the procedure of Hooi et al. (2004). Curd was stirred using magnetic stirrer at room temperature for one minute and accurately weighed (1.0g) curd was placed in an initially weighed aluminum dish, closed with aluminum dish lid. Curd was kept in a Hot air oven at 105° C for 24 hours. Curd samples were desiccated and weighed. Total solids were determined using the following formula

$$\text{Total solids (\%)} = \frac{\text{Dried sample with dish weight} - \text{Dish weight}}{\text{Initial curd weight}} \times 100$$

2.5.4. Water holding capacity

The water-holding capacity (WHC) of standard and experimental curd was analyzed following the procedure of Parnell et al. (1986). In this assay, curd sample incubated in the auto clave centrifuge tubes stirred for 20 min at 30°C and centrifuged at 3000×g for 15 min. The WHC is expressed as percent precipitate weight over initial curd weight

$$\text{Total solids (\%)} = \frac{\text{Drained tube weight} - \text{Dish weight}}{\text{Initial curd weight}} \times 100$$

2.5.5. Syneresis

Syneresis of standard and experimental curd was determined following the procedure of Amatayakul et al. (2006). The volumes of whey on the surface of the container of standard and experimental curd sample were taken and weighed. The syneresis was expressed as percent weight over initial curd weight.

$$\text{Syneresis(\%)} = \frac{\text{Siphoned whey weight}}{\text{Initial curd weight}} \times 100$$

2.5.6. Colors properties

The color was expressed by CIE color scales L*, a* and b* using Hunter digital colorimeter (Model D25M, Hunter Associates Laboratory, Reston, VA). L* represents the lightness of the sample extended from 0(black) to 100 (white). a* and b* represent redness (+a) to greenness (-a) and yellowness (+b) to blueness (-b) respectively.

2.5.7. Antioxidant properties

Antioxidant property of curd was evaluated regarding reducing power (RP) through following in vitro methods. Standard curd and experimental curd were taken in different vials and were dissolved in 1 ml of distilled water. 100µl of sodium phosphate buffer (100mM, pH 7.2) and 100µl of Potassium hexacyanoferrate (III) (1%) were added to each sterile vial and incubated at shaking water bath at 50°C for 20 min. After incubation 100µl of TCA (10%) was added to each sterile vial and centrifuged at 3000×g for 15 min. 100 µl ml

of the upper layer was added with 100 µl ml of sterile distilled water and 10 µl of ferric chloride (0.1%). The color developed was read at 700 nm absorbance.

2.5.8. Sensory evaluation

Curd was evaluated for overall acceptability with the effect of incorporation of PMDH as compared to standard curd and yogurt. A sensory panel of fifteen semi-trained members was involved from the School of Agriculture, PRIST Deemed to be University and a nine-point hedonic scale rating from dislike extremely to like extremely be used for evaluating the overall acceptability of samples. Mean ± standard deviation is out of overall acceptability scores by fifteen-panel members.

2.6. Response surface optimization

2.6.1. Experimental design

Response surface methodology was adopted using central composite rotatable design (CCRD) to optimize the incorporation of PMDH in curd and design of experiments are designed for a variable at five levels ($-\alpha$, -1 , 0 , $+1$, $+\alpha$). The total number of experiments runs was 13 with a different combination of PMDH concentration (0.5- 1.5 %, w/v) and incubation time (5- 8 Hrs) was carried out evaluating optimum values. Multiple responses such as water holding capacity (Y1), reducing power (Y2) and overall acceptability (Y3) were taken. CCRD matrix of two different factors was reported in Table 5.1. CCRD design matrix with PMDH factor and the response were analyzed through multiple regression analysis and the following second-order model

$$y = \beta_0 + \sum_{i=1}^3 \beta_i x_i + \sum_{i=1}^3 \beta_{ii} x_i^2 + \sum_{i=1}^2 \sum_{j=i+1}^3 \beta_{ij} x_i x_j$$

“Where y is the dependent variable (Measured response); β_0 , β_i , β_{ii} and β_{ij} are coefficients estimated by the model, x_i , x_j are levels of the independent variables. They represent the linear, quadratic and cross product effects of the X_1 , X_2 and X_3 factors on the response, respectively.”

2.6.2. Analysis of the model

The adequacy of the model for predictability was analyzed through multivariate statistical analysis. Analysis of variance of (ANOVA) of the model was performed to determine the significance of the model and significance of linear, interactive and quadratic coefficients. Model reduction was performed for eliminating the non-significant independent factors to improve the accuracy of the model closer to the true system (Myers & Montgomery, 2002). The coefficient of determination (R^2) explains the measure of variation in responses that can be explained by the model within the domain of experiments. R^2 value closer to 1.00 confirms the better the model to explain the variability in the response (Khuri & Cornell, 1996). Adequacy of the model was further confirmed by diagnostic plots such as normal probability plot for residuals to the normal distribution of residuals, the plot for predicted versus experimental values for the level of difference between them and plot for predicted values versus run order. The model was graphically represented into three-dimensional response surface graphs and two-dimensional contour graphs to predict the linear, interactive and quadratic effects on the response so that optimum response was also readable. Response surface plots predict the responses for the experimental values of independent factors while the shape of contour plots is used to interpret the interaction between independent factors (Montgomery, 1997). A circularly shaped contour plot designates the insignificant relationship between independent factors and elliptical and saddle-shaped contour represents that the significant interaction between the corresponding independent factors (Liu and Chiou, 2005). Numerical optimization method was used, wherein, PMDH concentration and incubation time were set to be in the range and the response, WHC, reducing power and overall accessibility maximum. Experimental solutions with the highest desirability closer to one were taken as adequately predicted optimum hydrolytic factors for the maximum response.

2.6.3. Validation of the model

The predictability of the model was validated by conducting experiments at the predicted experimental combination of PMDH for maximum response, and experimental response value was observed. The significance difference between the experimental and predicted responses was analyzed by independent 't' test, and the validity of the model was confirmed from the comparable difference between experimental and predicted response.

3. Result and Discussion

3.1. Proximate composition, Bulk density and Water activity of PMP and PMDH

Table 1 illustrates the proximate composition, water activity and bulk density of PMP and PMDH. Nutritional quality is eventually important in considering processed flour as a food ingredient and its successful performance depends principally on functional characteristics imparted to the final products. Generally, protein, fat, ash and moisture are significant substantial basic to measure the processing and nutritional quality of millet flour. The moisture content of the PMP and PMDH was found to be 5.98 ± 0.02 g/100g and 5.27 ± 0.03 g/100g. Moisture content of powder could be affected by handling method and immediate atmospheric humidity (Vasanthakumari and Jaganmohan, 2018). PMDH contained higher amount of protein and ash content as compared to PMP. Protein and ash content of PMP (10.08 ± 0.03 g/100g and 4.7 ± 0.01 g/100g) and PMDH (11.05 ± 0.02 g/100g and 5.3 ± 0.01 g/100g) were respectively. The crude fat content of PMDH observed 2.1 ± 0.02 g/100g and was lowest in the PMP sample (2.8 ± 0.02 g/100g). Low fat content of the PMDH will result in less energy value, but with increase in the shelf life due to reduced probability of rancidity. The Carbohydrate and energy values of PMDH were significantly ($p \leq 0.05$) higher than the corresponding PMP samples. The total crude fibre value of the PMP and PMDH obtained from 1.5 ± 0.01 g/100g and 1.8 ± 0.01 g/100g respectively. PMDH had the highest crude fibre content which might be due to enzymatic degradation of the fibre during the dehydration.

Commonly, bulk density is used to analyze the sample mass, handling requisite, and the type of packaging materials suitable for the storage and transportation of food materials. Also, bulk density indicates the behavior of product and the influence of numerous factors including the preparation method, drying procedure, fineness of particles, and moisture content (Shaviko et al., 2010). As shown in Table 1, PMP and PMDH had varying bulk densities of 0.95 ± 0.02 , and 0.92 ± 0.02 respectively, with a significant difference ($p < 0.05$). From the results, PMP had the highest bulk density. The variance in the bulk density of sample is possible because the bulk density is mainly influenced by the structure of proteins. Furthermore, the bulk density of dehydration process is influenced by the hydrophobicity, solubility, and hydrodynamic properties of proteins. High bulk density is inconvenient for the formulation of weaning foods, which are required to be of low density.

In general, the shelf life of a precise food system is calculated based on its water activity, which is an intrinsic property that denotes the availability of free water in the food system. In the food system, lower water activity

provides protection from microbial growth and contamination and delays deterioration through biochemical reactions (Primo-martin et al., 2010). As shown in Table 1, PMP and PMDH had varying water activities of 0.250 and 0.215 respectively, with a significant difference ($p < 0.05$). In a food system, moisture migration between domains can be avoided by adding an edible layer between the domains, resulting in a change in the water activity of the food ingredients (Kumarakuru et al., 2017). However significant difference was observed in proximate composition, bulk density and Water activity between PMP and PMDH for the production of nutritive product development, PMDH sample founded be suitable ingredient therefore PMDH sample selected for product development.

3.2. Response surface optimization

Response surface optimization was carried out using central composite rotatable design (CCRD). Experimental factors including the amount of PMDH (mg/g) from 0.5 to 1.5 and incubation time 5- 8Hrs were fixed based on the results were shown in Table 2.

3.3. Optimization for incorporation of PMDH in curd

CCRD design matrix consisted of four factorial points, five center points and four-star points resulted totally in 13 experimental runs for two experimental factors at five experimental levels at low (-1) and high (+1) as well as ($-\alpha$) and ($+\alpha$) as shown in Table 3. Curd incorporated with PMDH was observed with experimental responses of WHC (%), RP (absorbance 700nm) and overall acceptability ranged between 8.4 and 18.9, 0.45 and 0.85, 3.4 and 6.9 respectively. Experimental level of factors and corresponding responses WHC (%), RH and overall acceptability were fixed to the second order regression equation.

Curd with PMDH was obtained with the following predictable second order models as shown in equations 1, 2 and 3 respectively.

$$\text{WHC} = +15.60 + 1.06 * A + 0.0430 * B + 0.1175 * AB - 1.27 * A^2 - 2.62 B^2$$

$$\text{RP} = +0.6900 + 0.1379 * A - 0.0178 * B - 0.0100 * AB - 0.0087 * A^2 - 0.0788 * B^2$$

$$\text{Overall acceptability} = +6.72 + 0.1250 * A + 0.6523 * B + 0.6000 * AB - 1.27 * A^2 - 1.35 * B^2$$

Analysis of variance of the above regression model for the responses in curd with PMDH was performed, and the results are evidencing the predictability and reliability of models are depicted in Table 4. The second-order model

was quadratic for WHC, RP, and overall acceptability. As shown in Table 4, models for WHC, RP and overall acceptability were significant along with significant linear (A, B), interactive (A X B) and quadratic (A^2 , B^2) terms indicating the significance of the models for prediction. Co-efficient of determination (R^2) values stood for the capacity of the model to explain variability in responses and observed to be about 0.76 for WHC, 0.90 for RP and 0.96 for overall accessibility. Lack of fitness of models was found to be non-significant evidencing the fitness of models in the prediction ($P>0.05$). Adjusted and predicted R^2 values were observed with difference less than 2 between each of them and adequate precision was greater than 4 indicating the signal noise ratio indicating the model that can be used to navigate the design space.

Hence ANOVA table proved the reliability and predictability of models in prediction of responses such as WHC, RP and overall acceptability in curd incorporated. Second order models were visualized through two-dimensional contour graphs and three-dimensional response surface design graphs to read the liner, interactive and quadratic effect of independent factors on responses for the effect of incorporation of PMDH in curd as shown in Figure 1.

WHC was observed to increase with the significant effect of increasing PMDH concentration and incubation time. Reducing power was increasing with the increase in the amount of PMDH concentration and about the center point of incubation time. Overall acceptability score was maximum about the center points of the experimental range of the amount of PMDH incorporation an incubation time.

Numerical optimization method was performed, and overlay plot was obtained as shown in Figure 2. The optimum levels of PMDH concentration and incubation time were respectively predicted to be 1.45 (mg/g) and 6.7 for the maximum WHC of 15.4, reducing the power of 0.80 and overall acceptability score of 5.9, with the desirability of 0.852 in curd with the incorporation of PMDH.

3.3 Physicochemical properties

3.3.1. Protein, Ph, Total solids and Titrable acidity

The effect of incorporation of optimized PMDH on the protein, pH, total solids and titrable acidity content of curd was illustrated in Table 5. The total protein content of curd was increased from 4.2 g/100 g to 7.62 g/100g corresponding to the amount of incorporation of optimized PMDH percent in the curd. pH is played important role in the curd quality. pH was significantly affected by incorporation of PMDH. The considerable increase in the pH of experimental curd (4.58 ± 0.08) while compared to standard curd (4.52 ± 0.13). A total solid of curd was

increased from 12.5 per cent to 13.4 per cent with the consequent increase in PMDH content. While compared to standard curd, titrable acidity value slightly increased upon the incorporation of optimized of PMDH. It could be due to the proteolysis of PMDH and release of terminal COOH and acidic amino acid residues upon fermentation in the curd.

3.3.2. Water holding capacity (WHC) and Syneresis

Table 5 shows the comparative WHC and syneresis analysis of curd incorporated with optimized PMDH as compared to standard curd. Water holding capacity (WHC) and syneresis are physical parameters depicting the separation of whey which is the undesirable characteristic for the quality of curd. Incorporation of optimized PMDH increased WHC and decreased syneresis comparable to standard curd respectively. Hence the effect of incorporation of optimized PMDH on WHC and syneresis in curd was concentration dependant and could be attributed to the increase in total solids content especially the protein content at PMDH (Lee and Lucey, 2010).

3.3.3. Firmness

The texture of curd was an important quality parameter in the stir of its consumer acceptance. The texture of the prepared curd was determined regarding firmness. Experimental curd was found from the decrease in the firmness of curd with the increase in the incorporation of optimized PMDH as compared to the firmness of standard curd (Table 5). Standard curd had the firmness requiring the newton force of 152.4 ± 5.68 g for the structural breakdown whereas experimental curd had required the decreased newton force of 110.5 ± 4.08 g respectively. The results showed that soluble PMDH did not contribute to the gel matrix and thus did not support the structure.

3.3.4. Color properties

The effect of incorporation of optimized PMDH on changing the color of curd was studied regarding L^* , a^* and b^* values of Commission on Illumination (CIE) color scale. L^* represents lightness (0) to the darkness of the product (100). Chroma a^* value represents redness ($+a^*$) and greenness ($-a^*$) and chroma b^* value represents yellowness ($+b^*$) and blueness ($-b$) of the product. The results showed that L^* value was 85.4 ± 2.80 and found significantly lower ($P < 0.05$) than that of 92.56 ± 1.6 in the standard curd. While standard curd had chroma a^* value of 0.56 ± 0.025 and b^* value of 1.28 ± 0.06 , those were -1.58 ± 0.18 and 15.5 ± 1.65 in PMDH respectively. It could be interpreted that the incorporation of PMDH with the increase in concentration was

found increasing the yellowness and decreasing the whiteness of curd and yogurt as compared to standard curd ($P < 0.05$) as illustrated in Table 5. It could be solely attributed to the addition of PMDH in the curd.

3.3.5. Antioxidant properties

The antioxidant activity of the prepared curs was determined in terms of reducing power. Reducing power of optimized PMDH incorporated curd was illustrated as compared to standard curd in Table 5. The effect of increasing the antioxidant potential of curd through the incorporation of optimized PMDH was observed in the increase in activity of curd incorporated with PMDH as compared to the activity found in standard curd. Standard curd showed the absorbance of 0.32 ± 0.04 whereas optimized PMDH exhibited the absorbance of 0.70 ± 0.04 respectively. Results revealed that the effect of incorporation of PMDH was found to improve the antioxidant activity of curd as compared to standard curd.

3.3.6. Sensory Evaluation

Overall acceptability of optimized PMDH incorporated curd was illustrated as compared to standard curd in Table 5. Experimental curd were scored overall acceptability significantly lesser than their respective standard curd with a sensory report. Results showed that increased acidity and lack of fermentation flavor than those of standard curd. This effect of increasing acidity by the incorporation of PMDH incorporated in curd.

3.4 Conclusion

The CCRD design was used to investigate the influence of two independent factors on the quality characteristics of the prepared functional curd. It was observed effective in optimizing the factors for formulating of PMDH incorporated curd based on the desired goals of the maximum antioxidant activity, water holding capacity and overall accessibility. Curd prepared with optimum condition of PMDH concentration (1.45 mg/g) and incubation time (6.7) was better regarding physicochemical characteristics in comparison to the standard curd. Hence the incorporation of PMDH could be the nutritional and functional approach to enrich the protein content and antioxidant activity of curd.

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Conflict of Interest

The authors declare no conflict of interest.

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Table-1 Proximate composition, Bulk density and Water activity of PMP and PMDH

Parameters	PMP	PMDH
Moisture (g/100g)	5.98 ± 0.02 ^a	5.27 ± 0.03 ^b
Protein (g/100g)	10.08 ± 0.05 ^b	11.05 ± 0.06 ^a
Ash(g/100g)	4.7 ± 0.01 ^b	5.3 ± 0.01 ^a
Fat(g/100g)	2.8 ± 0.02 ^a	2.1 ± 0.02 ^b
Carbohydrate (g/100g)	74.82 ± 0.12 ^b	75.08 ± 0.10 ^a
Crude Fibre (g/100g)	1.5 ± 0.01 ^b	1.8 ± 0.01 ^a
Total Energy (Kcal)	352 ± 12.3 ^b	385 ± 14.5 ^a
Bilk density (g/ml)	0.95 ± 0.02 ^a	0.92 ± 0.02 ^b
Water activity (aW)	0.250 ± 0.01 ^a	0.215 ± 0.01 ^b

Values were Means ± S.D of triplicates. Values with the same superscripts in a row did not differ significantly ($p < 0.05$) by DMRT, PMP-Pearl millet powder, PMDH-Pearl millet dehydrated powder

Table 2 CCRD design space for incorporation of PMDH in curd

Experimental factors	Experimental Level				
	$-\alpha$	Low (-1)	Center (0)	High +1	$+\alpha$
PMDH Concentrate (%)	0.29	0.5	1	1.5	1.70
Incubation time	4.3	5	6.5	8	8.6

Table 3 CCRD design matrix for optimization of incorporation of PMDH in curd

S.No	Independent facto		Response for curd with PMDH		
	FPHF (%)	Incubation time	WHC	RP	OA
1	0.5	8	13.9	0.45	3.8
2	1	8.6	18.9	0.51	5.3
3	0.29	6.5	10.4	0.48	4.1
4	1	6.5	14.2	0.60	6.7
5	0.5	5	10.4	0.48	4.1
6	1.70	6.5	11.9	0.85	4.1
7	1	6.5	12.9	0.69	6.3
8	1.5	5	9.4	0.79	3.4
9	1	4.37	8.4	0.54	3.6
10	1	6.5	10.4	0.79	6.6
11	1.5	8	15.4	0.72	5.3
12	1	6.5	11.2	0.65	6.9
13	1	6.5	13.9	0.72	6.6

WHC: Water holding capacity, RP-Reducing power, OA: Overall acceptability and PMDH: Pearl millet dehydrtaed powder

Table 4. ANOVAs variance for the predictability of RSM of curd with PMDH

Response surface quadratic model						
Source	WHC		RP		OA	
	F-Value	P>F value	F-Value	P>F value	F-Value	P>F value
Model	5.15	0.0267	12.69	0.0021	32.73	0.0001
A-PMDH	3.70	0.0957	48.71	0.0002	0.78	0.4052
B-Incubation time	6.07	0.9401	0.81	0.3976	21.36	0.0024
AB	0.023	0.8846	0.13	0.7310	9.04	0.0198
A²	4.59	0.0695	0.17	0.6920	70.56	< 0.0001
B²	19.5	0.0031	13.81	0.0075	79.13	< 0.0001
Lack of fit	2.93	0.1633	0.082	0.9664	4.66	0.0855
R²	0.7655		0.9007		0.9612	
Adj R² Value	0.718		0.8297		0.9277	
Pred R² Value	0.600		0.8128		0.7589	
Adeq precision	11.82		10.817		13.809	

WHC: Water holding capacity, RP-Reducing power, OA: Overall acceptability and PMDH: Pearl millet dehydrated powder

Table 5 Physicochemical properties of standard and experimental curd

Characterization	Standard curd	Optimized curd
Protein g/100g	4.20 ± 0.06 ^b	7.62 ± 0.36 ^a
pH	4.52 ± 0.13 ^b	4.58 ± 0.08 ^a
Total solids	12.5 ± 0.25 ^b	13.4 ± 0.10 ^a
Titration acidity	0.75 ± 0.05 ^b	0.86 ± 0.18 ^a
WHC	13.6 ± 0.56 ^b	15.4 ± 0.17 ^a
Synersis	10.56 ± 0.98 ^a	8.62 ± 0.57 ^b
Firmness	152.4 ± 5.68 ^a	110.5 ± 4.08 ^b
L*	92.56 ± 1.6 ^a	85.4 ± 2.80 ^b
a*	0.56 ± 0.02 ^b	-1.58 ± 0.18 ^a
b*	1.28 ± 0.06 ^b	15.5 ± 1.65 ^a
Reducing power	0.32 ± 0.04 ^b	0.70 ± 0.12 ^a
Overall acceptability	7.2 ± 0.02 ^a	6.4 ± 0.05 ^b

Values were Means ± S.D of triplicates. Values with the same superscripts in a row did not differ significantly ($p < 0.05$) by DMRT
 WHC: Water holding capacity, L*: light or dark, a*: redness or greenness and b*: yellowness or blueness

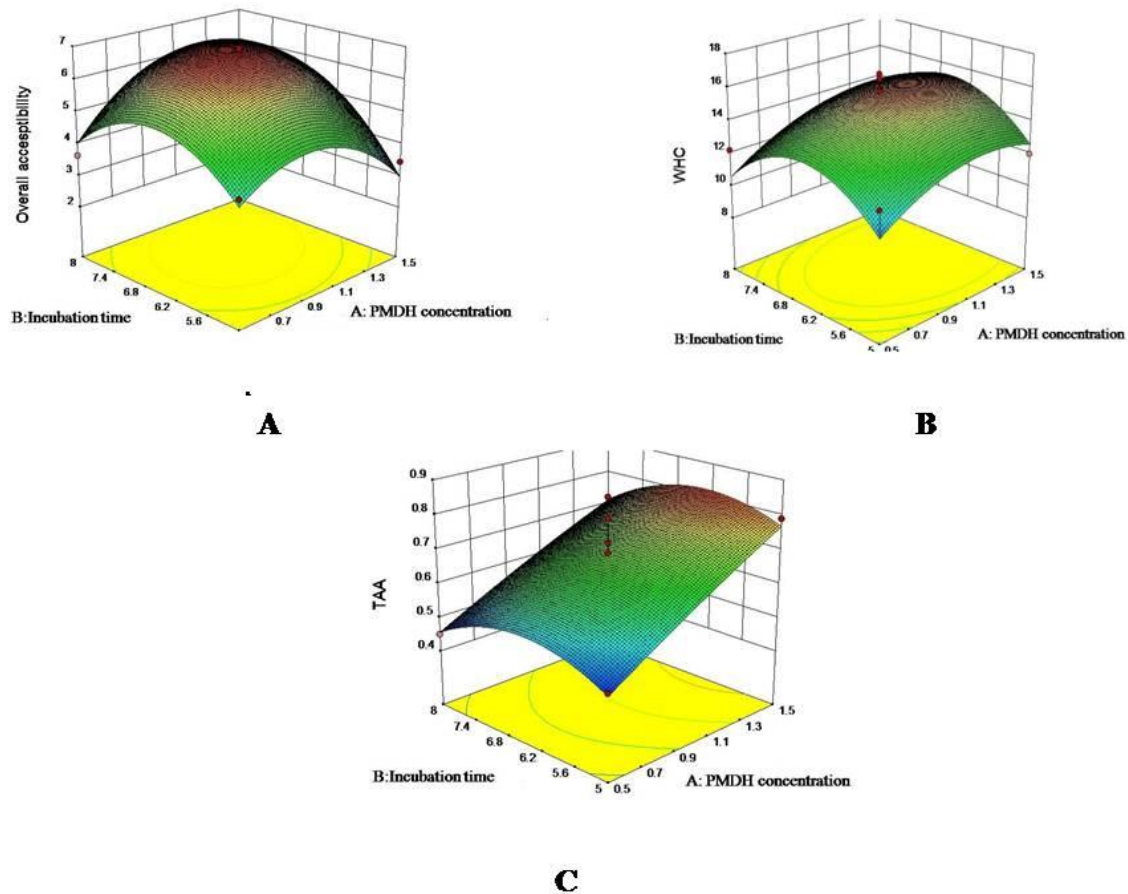


Figure 1 3D surface plot for different response variable; A- Overall acceptability, B- Water holding capacity, and C- Reducing Power

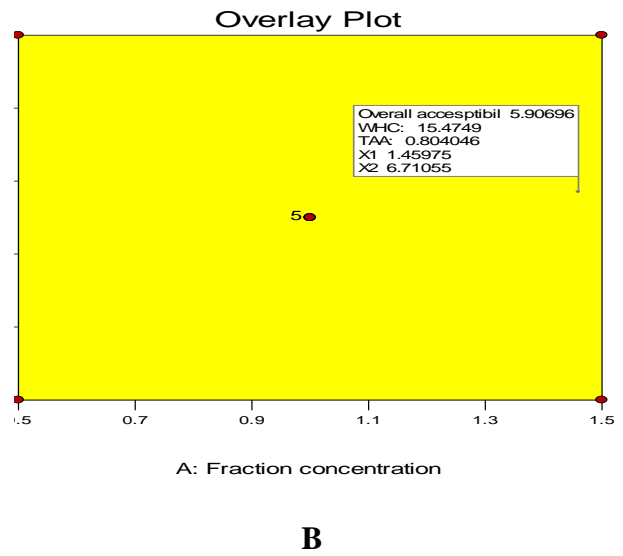
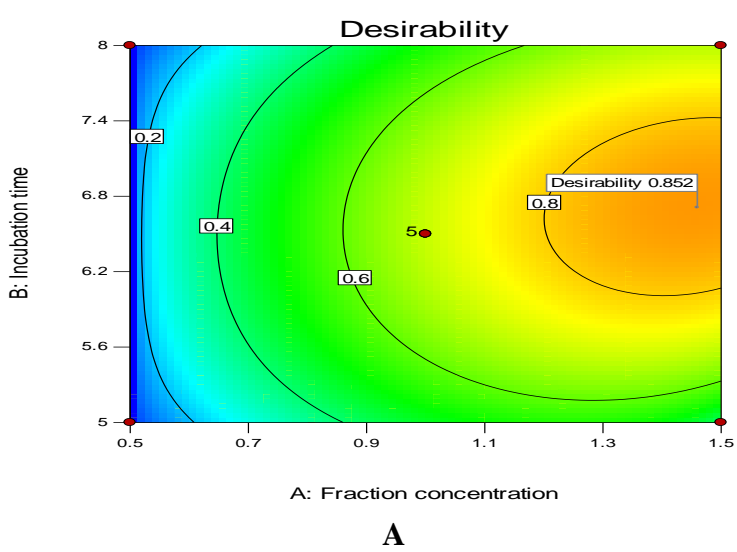


Figure 2 Numerical optimization of the PMDH : A- Desirability and B-Overlay plot optimum level

