

Optimisation of modification parameters for amaranth starch for the development of pudding and study of the quality traits of developed pudding

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ABSTRACT

Amaranth is considered to be a part of "superfood", however, due to multiple restricting properties, its functionality in the food industry is still not explored to its fullest. The present study investigated the effect of almond gum concentration (3–10 g), temperature (50–90 °C), and quantity of water (30–70 mL) on the functional properties of amaranth starch. A central composite rotatable design (CCRD) showed that the 6.9 g of almond gum, 64.43 mL of water, and temperature maintained at 90 °C, were the optimised conditions to attain 16.77 g g⁻¹ of swelling power, 12.97% of solubility index, and 20.13% freeze-thaw stability. Moreover, the modified amaranth starch was further employed to develop pudding as a value-added product. The findings concluded that the developed pudding using modified amaranth starch exhibited enhanced sensorial attributes due to an increase in cohesiveness, chewiness, and resilience of starch gel.

KEYWORDS

amaranth, central composite rotatable design (CCRD), swelling power, solubility index, freeze-thaw stability

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1. INTRODUCTION

Amaranth (*Amaranthus cruentus* Linn) is one of the important species of *Amaranthus* L. genus. It has enjoyed resurgence in recent years, primarily on the health food market. It is also considered "superfood" due to its balanced amino acid profile and high protein content (14–19%) (Brenner et al., 2000). Amaranth, a pseudo-cereal, is a rich source of nutritional compounds, especially proteins such as high-quality lysine, tryptophan, and squalene. Moreover, amaranth is gluten-free, making it more crucial around the world (Adhikary et al., 2020).

There are different kinds of native starches based on inherent properties like freeze-thaw stability, retrogradation, and viscosity. In addition to inherent properties, variations in physical attributes of grain, the particle size of granule, and hydrophobicity also result in different native starches, which often restrict the application of native starches in the food system. Thus, to promote and enhance specific functional properties, starches are frequently modified by physical, chemical, and enzymatic processes (Lee et al., 2002; Kierulf et al., 2020).

Starches are incorporated into the food system as hydrocolloids to improve and modify the textural properties of native starch (Pramodrao and Riar, 2014). Almond gum (*Prunus dulcis*) is a natural polymer, commercially utilised due to its good emulsification and matrix-forming properties. Heat-moisture treatment (HMT) is one of the hydrothermal modifications in low moisture content products, generally exposure to a temperature above the glass transition temperature but below the onset temperature of gelatinisation for a specified time period (Pramodrao and Riar, 2014).

Puddings are semisolid food composed mainly of milk protein-based starch pastes (Lim and Narsimhan, 2006). The commercial powders usually contain aroma, colourings, hydrocolloids, starch, and sugars, which can be easily dissolved in milk (Singh and David, 2017). Hence, the present study aimed to investigate the effect of gum concentration, amount of water, and temperature of treatment on starch modification based on swelling, solubility, and freeze-thaw stability of the starch for the development of pudding. Furthermore, sensory, surface characteristics, and textural properties of the developed pudding were examined.

2. MATERIALS AND METHODS

2.1. Materials

Almond gum (AG) was collected from the local market of Jammu and Kashmir, India, while amaranth starch (AS) powder and analytical grade chemicals were obtained from Merck Millipore and Himedia, India, respectively. Standardised pasteurised milk was procured from the local market of Tezpur, Assam, India.

2.2. Characterisation of AG

The procured AG was analysed for moisture content (AOAC 925.09), water activity using an electronic dew point water activity meter (Aqualab Series 4TE, Decagon Devices, Inc., Pullman, Washington, USA), and colour properties using Colour flex (Hunter Associates Laboratory Inc., Reston, VA, USA). The bulk and tapped density of AG was measured as per Bashir and Haripriya (2016).



2.3. Modification of starch

The modification of AS was carried out by the method of Lim et al. (2002). The chosen independent factors were gum concentration, amount of water, and temperature varied from 3 to 10 g, 30–70 mL, and 50–90 °C, respectively. The modification of AS was conducted as per the condition of the experimental run. In general, the first AG was slowly added to distilled water with continuous stirring using a magnetic stirrer (Spinot 6040, Tarsons, India) followed by AS, and the dispersion was stirred continuously for 30 min at 50–90 °C. The prepared mixture was transferred to a beaker and dried at 45 °C in a hot air oven (BST/HAO-1123, Bionics Scientific, India) to a moisture content of 10 g/100 g based on starch.

2.4. Experimental design for modification of AS

For modelling of variables, central composite rotatable design (CCRD) was used to design experiments that include 3 independent variables, 4 levels CCRD, and 20 tentative runs with six imitates at the centre point. A second-order polynomial model was fitted to predict the optimal point of correlation relationships between independent variables, including the amount of water, gum concentration, and temperature, and responses such as swelling power (SP), solubility index (SI), and freeze-thaw stability (FTS). Equation (1) presents the relationships between three factors:

$$Y_n = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 A B + \beta_2 A^2 + \beta_2 A^3$$
(1)

where Y_n is one of the three responses, A and B are the independent variables, β_0 is a constant, β_1 and β_2 are the linear term, β_4 and β_5 are the quadratic-term, and β_3 is interaction coefficient.

The obtained responses were SI (%), SP (g g⁻¹), and FTS (%). The obtained second-order quadratic equation was given as actual variables, used to direct the individual response as a function of independent factors. In the equations A, B, and C denote the amount of water, gum concentration, and temperature, respectively. A, B, C show the linear interaction and AB, AC, and BC show interaction among variables, while A^2 , B^2 , C^2 are quadratic regression coefficients.

The total error criteria were used to accomplish a 95% significance limit, and for analysing the significance level, analysis of variance (ANOVA) was used for each response. The R^2 value was used to analyse the efficiency of the model, whereas lack of fit test was helpful in the evaluation of accordance of models (Sablania and Bosco, 2018). The desirability function method helped in understanding the optimum levels of independent factors.

2.5. Physicochemical properties of MS

2.5.1. *Moisture content, water activity, and colour properties.* The moisture content, water activity, and colour properties of starch and pudding were analysed as per the methods discussed in section 1.2.

2.5.2. Swelling power and solubility index. SP and SI of the native starch (NS) and modified starch (MS) were estimated according to Subramanian et al. (1994) as per Eqs (2) and (3), respectively.



Swelling power
$$(gg^{-1}) = \frac{M_1}{M_0}$$
 (2)

Solubility Index(%) =
$$\frac{M_2}{M_0} \times 100$$
 (3)

2.5.3. *Freeze-thaw stability.* FTS of starch was estimated as per the method of Luo et al. (2006), where Eq. (4) was used for calculation of FTS.

$$FTS(\%) = \frac{\text{Weight of the supernatant}}{\text{Total weight of the gel before centrifugation}} \times 100$$
(4)

2.6. Development of pudding

The pudding was developed using the method described by Singh and David (2017), where standardised milk (4% fat and 8.5% SNF) was mixed with 30% starch. The mixture was stirred continuously with the addition of sugar (25%), and cooked further for 6 min at 90 °C. Then the mixture was cooled at 25 °C.

2.7. Texture profile analysis

The texture profile analysis of NS gel, MS gel, and pudding was carried out as per Domagała (2009).

2.8. Surface micrograph and sensory evaluation

Starch and pudding prepared from native (PNS) and modified starch (PMS) were studied for morphological structure by a scanning electron microscope (JSM-6060 JEOL, Tokyo, Japan). The sensory evaluation of pudding was performed using a hedonic scale of 1–9 measuring parameters such as appearance, colour, texture, taste, aroma, and overall acceptability by 20 semi-trained panel members.

2.9. Statistical analysis

IBM SPSS Statistics Version 20.0, Armonk, NY: IBM Corporation package was used for the statistical analysis of data, and the means were separated using Duncan's multiple range test (P < 0.05). All data are presented as the mean with the standard deviation. In the present study, Design-Expert software version 10.0.7.0 (Statease Inc., Minneapolis, USA) was used for the optimisation of modification parameters of amaranth starch.

3. RESULTS AND DISCUSSIONS

3.1. Model fitting

CCRD design was found suitable for significant regression with non-significant lack of fit and satisfactory determination coefficients (R^2) for the various responses. The R^2 value for



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Constraints	Goal	Lower limit	Upper limit	Predicted value	Measured value
Amount of water (mL)	In range	30	70	64.43	_
Gum content (g)	In range	3	10	6.90	-
Temperature (°C)	In range	50	90	90	-
SI (%)	Maximise	5.01	13.14	12.97	12.88
SP (g g^{-1})	Maximise	9.36	17.58	16.77	15.48
FTS (%)	Minimise	8	23	20.13	17.10

Table 1. Process parameters and responses obtained for HMT of amaranth starch

SP, SI, and FTS was found to be 0.80, 0.84, and 0.88, respectively. The desired goals for individual independent variables concerning each response are shown in Table 1. In contrast, 3D graphs for each response were established as a function of two independent factors (Fig. 1(i-iii)). At the optimised parameters, the predicted value for SP, SI, and FTS was found to be 12.97 g g⁻¹, 16.77, and 20.13%, respectively, at the desirability level of 0.89. These predicted values were quite closed to the experimental value 12.88 g g⁻¹, 15.48, and 17.10%, respectively, using the CCRD resulting in validation of the model. Therefore, the selected model can be used to optimise the modification of AS for the development of pudding.

3.2. Effect of process parameters on SI

Independent factors such as temperature and amount of water have shown a significant effect on SI (Table 2). SI of modified starch samples (12.88%) exhibited a significant difference from native starch (4.99%). The estimated SI was in the range of 5.01–13.14%. The regression coefficient, coefficient of variance (C.O.V), and adjusted R^2 for SI were found to be 0.84, 13.17%, and 0.71, respectively, showing that the model used for the optimisation of SI during modification was best-fit. The effect of independent variables and quadratic terms (A^2 and C^2) are significant (P < 0.05), while interaction among the variables was found insignificant (Fig. 1A). It was observed that as the gum concentration increased, SI decreased. An increase in SI was attributed to a temperature-dependent phenomenon (Chen et al., 2015). On the contrary, increasing concentration of gum affected the SI, which might be due to attractive interactions between AG (anionic gum) and AS (positively charged) as a result of restricted swelling of starch. Besides, hydrocolloids interact with the amylose outside the starch granules to produce a more complex matrix of amylose and hydrocolloid surrounding the gelatinised granules.

3.3. Effect of process parameters on SP

SP of native starch was 6.57 g g⁻¹, whereas modified starch registered 15.48 g g⁻¹. The regression coefficient, C.O.V, and adjusted R^2 for SP were found to be 0.80, 10.52%, and 0.62, respectively. The effect of independent variables and quadratic terms (A^2 and C^2) was significant, while interaction among the variables was found insignificant. SP of starch increased with an increase in temperature. However, an increase in gum concentration and amount of water results in decreased SP (Fig. 1B).



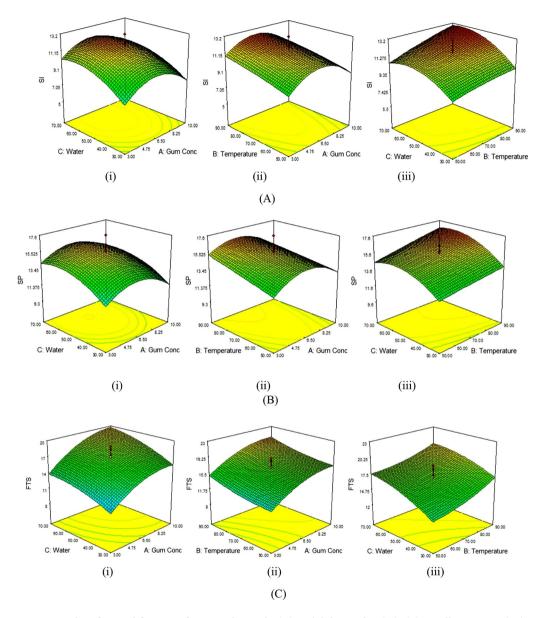


Fig. 1. 3D plots for modification of amaranth starch. (A): Solubility Index (SI); (B): Swelling power (SP); (C): Freeze-thaw stability (FTS)

3.4. Effect of process parameters on FTS

FTS of native and modified starch was 18.66 and 17.10%, respectively, and modification of AS with AG caused a decrease in FTS compared to native starch. The regression coefficient, C.O.V, and adjusted R^2 for FTS were found to be 0.885, 10.08%, and 0.78, showing that the model used



Parameter	NS	MS	PNS	PMS
Moisture content (%)	9.15 ± 0.74^{d}	$9.35 \pm 0.49^{\circ}$	85.05 ± 0.78^{a}	86.25 ± 0.78^{b}
Water activity	$0.37 \pm 0.00^{\circ}$	$0.34 \pm 0.00^{\rm d}$	0.86 ± 0.04^{a}	$0.87\pm0.09^{\rm b}$
L^*	96.22 ± 0.07^{a}	94.61 ± 0.31^{b}	86.11 ± 3.12^{d}	$90.83 \pm 5.17^{\circ}$
<i>a</i> *	$-0.26 \pm 0.02^{\circ}$	-0.03 ± 0.04^{d}	-4.36 ± 1.11^{a}	-2.47 ± 1.79^{b}
b^*	2.45 ± 0.07^{d}	$2.56 \pm 0.13^{\circ}$	8.44 ± 2.53^{b}	15.97 ± 2.19^{a}
Hue	-83.85 ± 0.75^{a}	30.11 ± 1.02^{d}	$-62.64 \pm 2.13^{\circ}$	-81.20 ± 1.23^{b}

Table 2. Physicochemical properties of starch and pudding

Values are means \pm standard deviation of three determinations (n = 5). Values followed by a different superscript letter in a row are significantly different ($P \le 0.05$).

for the optimisation of modification of starch was the best-fitted. The interactions (*AB*, *BC*, and *AC*) and quadratic models such as A^2 and C^2 had a negative effect on the FTS of starch. FTS of starch increased with an increase of temperature and amount of water; however, decreasing pattern was seen with an increase in gum concentration (Fig. 1C). This change might be due to an increase in the absorption efficiency, resulting in increased swelling and hydration capacity of granules. Also, increased concentration of gum inhibited syneresis of starch due to the high water holding capacity of the gum.

3.5. Characterisation of AG

The moisture content and water activity of AG were found to be 11.35% and 0.46, respectively. The bulk density of AG was 0.586 g mL⁻¹, while the tapped density was 0.816 g mL⁻¹. The colour properties such as L^* , a^* , b^* were recorded to be 81.65, 2.46, and 14.30, respectively.

3.6. Physiochemical properties of starch and pudding

3.6.1. *Moisture content and water activity.* Moisture contents of NS, MS, PNS, PMS were 9.15, 9.35, 85.05, and 86.25%, respectively. An increase in moisture content is attributed to the incorporation of gum in the starch, which has an impact on solute concentration; resulting in absorption of moisture from granules. Water activities of NS, MS, PNS, PMS were 0.37, 0.34, 0.86, and 0.87, respectively.

3.6.2. Colour properties. Modification of starch had a significant impact on the colour, significant decrease in the L^* values and increase in the a^* and b^* values were observed (Table 2). The changes correlated with the browning reaction and re-association of amylose at increased temperature (Pramodrao and Riar, 2014). There was a measurable difference observed in hue angle, where NS and MS had values –83.85 and 30.12, respectively.

Colour of pudding incorporated with AG was evaluated, where L^* , a^* , and b^* values were 90.83, -2.47, and 15.97, respectively, as a result of higher acceptability than PNS. Hue angles of the PNS and PMS were -62.64, and -81.20, respectively, corresponding to the second quadrant of hue angle, which refers to the yellow-green colour.

3.6.3. *Textural properties.* NS gel exhibited the highest hardness, while cohesiveness, chewiness, and resilience were higher in the MS gel (Table 3). The strength of starch gel mainly



Parameter	NS gel	MS gel	PNS	PMS
Hardness	298.40 ± 49.93^{a}	243.10 ± 21.23^{d}	268.10 ± 11.25^{b}	$260.42 \pm 21.25^{\circ}$
Adhesiveness	-14.05 ± 14.92^{a}	$-10.19 \pm 07.62^{\circ}$	-11.39 ± 05.22^{b}	$-10.51 \pm 3.72^{\circ}$
Springiness	$2.80 \pm 1.45^{\circ}$	2.50 ± 0.45^{d}	5.25 ± 0.25^{b}	5.69 ± 0.14^{a}
Cohesiveness	0.37 ± 0.02^{d}	$0.45 \pm 0.02^{\circ}$	0.55 ± 0.02^{a}	0.53 ± 0.02^{b}
Gumminess	110.80 ± 14.38^{d}	$140.18 \pm 7.48^{\circ}$	160.38 ± 5.48^{b}	168.72 ± 8.25^{a}
Chewiness	312.23 ± 175.31^{d}	$342.42 \pm 85.21^{\circ}$	362.32 ± 45.21^{a}	351.89 ± 21.63^{b}
Resilience	0.15 ± 0.07^{d}	0.25 ± 0.01^{a}	$0.22 \pm 0.01^{\circ}$	0.24 ± 0.01

Table 3. Texture properties of starch gel and pudding

Values are means \pm standard deviation of three determinations (n = 5). Values followed by a different superscript letter in a row are significantly different ($P \le 0.05$).

depends on the contributory factors such as the composition of flour and the interaction of water with molecules (Mir and Bosco, 2014). Values of springiness and gumminess were lower in MS gel in comparison to NS gel. However, cohesiveness increased. The gel formation is a result of interactions between protein, lipid, and non-starch polysaccharide, which have a positive or negative impact on the gel texture of the starch (Yu et al., 2012). Texture and consistency predominantly define the quality of pudding and affect its sensorial attributes. It was seen that the addition of AG did not affect the firmness of PMS when compared with PNS.

3.7. Sensory analysis

Figure 2 illustrates the puddings prepared from MS and NS. The addition of AG to the pudding significantly (P < 0.05) changed appearance, texture, and overall acceptability compared to control (Fig. 3). This change in sensory qualities may be due to increase in water holding capacity and total solid level with the addition of AG (Singh and David, 2017).

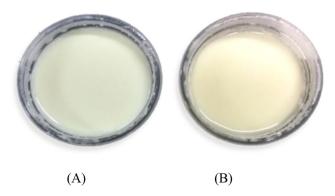


Fig. 2. (A): Pudding prepared from native starch; (B): Pudding prepared from modified starch



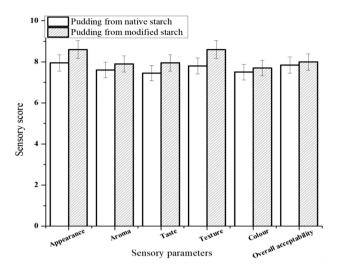


Fig. 3. Sensory score for pudding

3.8. Surface micrograph

Surface micrograms of NS and MS samples were analysed using SEM at $1,000\times$, which showed round and oval granules with the intended surface (Fig. 4). The fissures on the surface of modified starch granules were due to the disintegration of starch polymers caused by HMT. The surface micrograph of pudding revealed the compact and highly interspersed structure of starch.

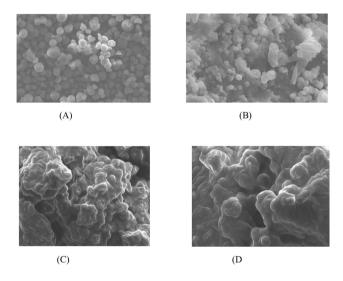


Fig. 4. Surface micrograph of (A): native starch; (B): modified starch; (C): pudding prepared from native starch; (D): pudding prepared from modified starch

Micrograms of puddings were taken at $500\times$, which revealed that HMT treatment of starch caused the formation of associative interaction between the casein micelles and AG. According to Corredig et al. (2011), electrostatic repulsion might be a possible reason for such interaction in the pudding as the AG and casein micelles are oppositely charged portions.

4. CONCLUSIONS

With the help of RSM, amaranth starch was successfully modified with AG using HMT. Upon modification, increased SP, SI, and decreased FTS of amaranth starch was noticed. SEM images displayed the round and oval shapes of starch granules, however, fissures were observed in MS. Also, the sensorial attributes of PMS were higher than the PNS. Modification of starch enhanced cohesiveness, chewiness, and resilience of starch gel, which agreed with the sensory evaluation, as the sensorial attributes of PMS were higher than the PNS. From the present study, it can be concluded that the AS was successfully modified and utilised for the development of pudding.

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