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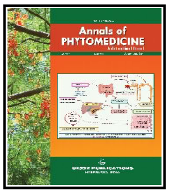

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Process standardization for development of nutritionally rich microwave-roasted germinated horsegram (*Macrotyloma uniflorum* Lam.) snack

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Abstract

Horsegram is an underutilized crop, grown in a wide range of adverse climatic conditions. It is a rich source of protein, minerals, vitamins and many phytochemicals. Use of horsegram is limited due to its poor cooking quality, low nutrient bioavailability and presence of antinutritional factors. Therefore, the present study was undertaken to decrease antinutritional components of horsegram and enhance its marketability in a profitable manner by developing germinated and microwave assisted ready-to-eat snacks (namkeen). For this purpose, three-level factorial design of Response Surface Methodology was employed to analyze the effect of germination temperature (30, 35, and 40°C) and germination time (12, 18, and 24 h) on germination percentage and antinutritional factors (oxalate and tannin). It was observed that germination of horsegram at 34.5°C for 24 h produced maximum germinated seeds (89%), with greater reduction in oxalate (66.84%) and tannin (71.69%) content. The optimized germinated horsegram seeds after mixing with flavoring agents were subjected to different microwave roasting conditions (11, 12, 13, 14, and 15 min for 350 Watt and 4, 5, 6, 7, and 8 min for 500 Watt). The observation revealed that roasting of germinated horsegram at 500 Watt for 5 min gave maximum rating (=8) for all the sensory attributes (appearance, odor, taste, texture, and overall acceptability) on 9-point Hedonic scale with desirable textural properties (103.20 N hardness). It was concluded from the study that germinated and roasted horsegram namkeen was more nutritious with fewer antinutritional components.

1. Introduction

Increasing pace of global population, has resulted in the rapid depletion of natural resources. Therefore, it becomes increasingly necessary to diversify today's agricultural production in order to fulfill our demands. After cereal, pulses are the second most important agricultural crop (Bhatt and Karim, 2009). Being an affordable and essential component of the regular human diet, pulses are also known as 'poor man's meat. Pulse crops are excellent sources of protein, resistant to change in climate and sustainable. A variety of pulses can account for 33% of dietary requirements as they comprise high levels of albumins and globulins (Sundarrajan, 2023). In comparison to commercial, traditional pulses such as sword bean, grass pea, horsegram, faba bean, velvet bean, winged bean, *etc*., are underutilized which are more agronomically suited to rainfed locations and even marginal soil (Gupta *et al.,* 2013). Species of these crops are often indigenous and are mostly used by local tribal communities in different countries. The nutritional value of some traditional and underutilized pulses is equivalent to or even superior to some commercial pulses, particularly in terms of protective components (Kaundal and Kumar, 2020).

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Horsegram (*Macrotyloma uniflorum* Lam.) locally known as 'Gaheth' or 'Kulthi' is a minor, under-exploited legume of tropics and subtropics. It is traditionally cultivated in various countries of the world including India, Sri Lanka, Orissa, Malaysia, West Indies, *etc.* In India, it is traditionally grown in Uttarakhand, Gujrat, Madhya Pradesh and Jharkhand, *etc.* Horsegram has good tolerance to drought, salinity and heavy metal stresses, hence can adapt wide range of temperature regimes where other crops invariably fail to survive, thereby, supporting the sustainability of agriculture in the future (Bhartiya *et al*., 2015). Horsegram is recognized as a potential source of protein (17.9-25.3%), carbohydrates (51.9-60.9%), dietary fibre (5.3%), essential amino acids, and important minerals like calcium (287 mg/100 g), phosphorous (311 mg/100 g) and iron (6.77 mg/100 g). It also contains an appreciable amount of vitamin B such as thiamine (0.4 mg), riboflavin (0.2 mg) and niacin (1.5 mg) per 100 g of dry matter (Bolbhat and Dhumal, 2012). The nutraceutical properties of horsegram are well-quoted in ancient Indian medicine. It serves as a remedy for various diseases like kidney stones, diabetes, joint pain, *etc.* (Prasad and Singh, 2014). Various phytochemicals such as flavonoids, urease, glycosides, linoleic acid, polyphenols, β-sitosterol, isoflavones, genistein, isoferririn, cumesterol, psoralidin, galactosidase, glucosides and streptogenin, *etc*., can be extracted from horsegram (Morris, 2008). Although, horsegram is being used as traditional medicine from long time to cure many diseases, but still the pulse is limited to household and is neglected for its remedial potential. Horsegram has been cited in Charak Samhita and Sushruta Samhita for curing piles, hiccups, abdominal lumps, bronchial asthma and regulating perspiration (Pati and Bhattacharjee, 2013). Traditional texts describe the use of horsegram for treating kidney stones, asthma, bronchitis, leucoderma, urinary discharges and heart diseases (Bhartiya *et al*., 2015). It also has anthelmintic activity which can be utilized as dietary food for infants to eradicate worms (Philip *et al*., 2009). Water soluble seed extract of horsegram has non-tannin and non-protein crystallization inhibitors and reported a marked decrease in anti-calcifying activity (Ranasinghe and Ediriweera, 2017). The extract of horsegram exerts hypolipidemic and hypoglycaemic actions (Senthil, 2009) and has also been found beneficial in urinary troubles, acid peptic disorder (gastritis), constipation, sun-burn, kidney stone, female diseases, rheumatism, hemorrhagic disease, intestinal worms, *etc.* (Pati and Bhattacharjee, 2013). Horsegram help in reducing intestinal diseases, diabetes, coronary heart disease, prevention of dental caries, *etc*., due to presence of bioactive compounds (Prasad and Singh, 2014). The seeds, sprouts or whole meal of horsegram are used by large populations in rural areas. They are considered as garam dal; and are therefore, preferred during the winter months by rural communities (Ramesh *et al*., 2011). Raw horsegram is a rich source of antioxidants and exhibits higher health benefits for hyperglycaemic individuals (Tiwari *et al*., 2013). The seeds and sprouts of horsegram are excellent examples of 'functional food' as it has role in lowering the risk of various diseases and exerting health promoting effects in addition to its nutritive value (Ramesh *et al*., 2011).

Horsegram is low-cost pulse with high protein and acceptable cooking quality and therefore by end-large, has the potential to be utilized for the development of value-added products (Bhartiya *et al*., 2015). Due to its high nutritional value, remedial properties and ability to with stand harsh climatic condition, horsegram has great opportunity to prove itself as poor man's meat in malnourished and drought-prone areas of the world (Morris, 2008). Today, the practice of horsegram consumption is restricted to the rural parts of the country and industrial commercialization is limited due to lack of agronomical practices, longer cooking procedures, low bioavailability of nutrients, and presence of antinutritional factors, mainly the high content of phytic acids, trypsin inhibitors, oxalates, and tannins (Pal *et al.,* 2016; Ojha *et al.,* 2020). These antinutritional factors interfere with the bioavailability of nutrients present in horsegram and can cause dangerous effects on health (Pagar *et al.*, 2021). They not only reduce mineral absorption and protein digestibility but also create a 'hard-to-cook' phenomenon in legumes which results in longer cooking times and more difficult preparatory procedures (Badola *et al*., 2023). Use of processing methods such as soaking, dehusking, germination, cooking, and roasting can eliminate or reduce these antinutritional factors. The above mentioned nutritional and medicinal advantage and processing drawbacks of horsegram, provides a driving force for encouraging its utilization in development of value-added products, especially 'convenience food'.

Convenience foods are those which are commercially designed to provide ease during preparation and consumption of the product. They can be further categorized as shelf-stable foods and frozen foods. Shelf-stable convenience foods are further classified as ready-to-eat foods and ready-to-cook foods. Shelf-stable ready-to-eat foods are invariably dry products that are pre-cleaned, pre-cooked, packaged, and ready for consumption without prior preparation or cooking. This includes chutneys, pre-cooked sausages, chicken products, various bakery products, and different types of snacks like extruded products and namkeen, *etc*. Processing methods such as roasting, frying, puffing, baking, *etc*. are used for the preparation of these products. Stability and quality of snacks are highly dependent on the processing methods, packaging, and composition of the food. Snacks consumption increases during celebratory social occasions and are found higher in consumers having desire for tempting food. They are considered as fourth meal accounting for 25% of complete day's meal (Mounika *et al*., 2021). In some countries, like France, Philippines and Mexico "fourth meal" or snacks is part of a traditional meal pattern (Hess *et al.,* 2016). Savoury snacks market in India accounted for USD 5.57 billion in the year 2020, which is expected to reach almost USD13 billion by 2026 (SRD, 2021). The increasing demand for protein-rich snacks has opened the doors for underutilized pulses. Puffed pulses and namkeen are most common ingredients in breakfast food and snack formulation. Till date no information has been reported about healthy snacks from horsegram. Therefore, the study was conducted on development of snack (namkeen) from germinated horsegram.

2. Materials and Methods

2.1 Raw materials procurement

The study was carried out in the Department of Food Science and Technology, College of Agriculture, GBPUAT, Pantnagar. The local variety of horsegram grown in Uttarakhand, was used for the preparation of namkeen. Horsegram, salt and spices, were purchased from the local shops. Seeds of horsegram were manually cleaned and stored at room temperature (25 ± 2°C) in airtight plastic containers to prevent moisture changes. All chemicals used in this study were of analytical grade and obtained from Himedia.

2.2 Preparation of germinated horsegram (GH) snack (namkeen)

Grains of horsegram were cleaned, washed and soaked in tap water at 30ºC until they attained maximum weight (Figure 1). Thereafter, the excess water was drained and the seeds were allowed to germinate at three different levels of temperatures, *i.e*., 30, 35, and 40ºC for three different time durations, *i.e*., 12, 18, and 24 h as suggested by the Three Level Factorial Design (Table 1). After germination (Figure 1), the horsegram seeds were washed and surface dried for 10 min. The data on germination percentage and antinutritional factors (oxalate and tannin) was recorded and then analysed by the software Design Expert-12. Based on the results, an ideal condition for horsegram germination was selected for further processing, *i.e.*, mixing salt and spices followed by microwave roasting.

2.3 Microwave roasting of germinated horsegram

For the development of horsegram namkeen, the germinated seeds of horsegram (germinated at optimized conditions) were air dried for 10 min to dry the surface and small amount of olive oil was applied followed by mixing of salt and spices (Figure 1). Spice-coated germinated horsegram seeds were subjected to microwave roasting at different power and time combinations (11, 12, 13, 14, and 15 min for 350 Watt; 4, 5, 6, 7, and 8 min for 500 Watt). Color, texture, and organoleptic evaluation were performed and analysed statistically by ANOVA.

Raw horsegram | Soaked horsegram | Germinated horsegram | Germinated microwave roasted horsegram namkeen

Figure 1: Unit operations for manufacturing of sprouted horsegram snack (namkeen).

Table 1: Effect of germination time and temperature on germination percentage, oxalate and tannin content of horsegram

Run Expt.	Independent variables				Responses		
	X_1 Germination time (h)		X_2 Germination temperature (ºC)		Germination (%)	Oxalate (mg/100 g)	Tannin (mg/100 g)
	Actual values	Coded values	Actual values	Coded values			
1	12	(-1)	30	(-1)	68	196.24	439.904
2	18	(0)	30	(-1)	73	187.41	431.654
3	24	(1)	30	(-1)	75	175.51	425.708
4	12	(-1)	35	(0)	81	159.76	394.721
5	18	(0)	35	(0)	83	148.04	380.575
6	24	(1)	35	(0)	87	139.41	372.97
7	12	(-1)	40	(1)	55	229.88	464.65
8	18	(0)	40	(1)	58	219.29	457.881
9	24	(1)	40	(1)	61	210.45	451.2
10	18	(0)	35	(0)	83	148.04	380.57
11	18	(0)	35	(0)	83	148.82	380.99
12	18	(0)	35	(0)	83	148.45	380.674
13	18	(0)	35	(0)	84	148.95	378.01

2.4 Experimental design and regression model

Three-level factorial design of Response Surface Methodology (RSM) was used for this study. The input variables considered in the experiments, their original values, and coded levels with various responses are presented in Table 1. The design consisted of 13 treatment combinations as suggested by the Software Design Expert 12.0, including 3 levels for each variable, which were fixed to allow the investigation of wide range of experimental conditions within practical limits. Independent variables were optimized for obtaining the ideal condition of germination of horsegram and the data was analyzed by creating response surfaces. The experimental data obtained based on the above design were fitted to a second-order polynomial equation (1) as follows:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 \quad (1)$$

where, Y is the response, X_1 and X_2, are coded values of independent variables, β_0 is model coefficient, β_1, and β_2 are linear regression coefficients, β_{11} and β_{22}, are quadratic regression coefficients and β_{12} is the interactive regression coefficient (Barfal *et al.*, 2017; Sangeeta *et al.*, 2016). These models were analyzed for their adequacy based on coefficient of multiple determinants (R^2), F-value, adequate precision ratio, lack of fit and associated statistics, *etc*. Contour plots and 3D graphs were developed using the fitted quadratic polynomial models using the Software Design Expert 12.0.

Coefficient of determination (R^2) represents the proportion of the variance for a dependent variable that is explained by independent variables in a regression model. Adjusted R-squared is a modified version of R-squared that has been adjusted for the number of predictors in the model. The predicted R-squared indicates how well a regression model predicts responses to new observations. Adjusted and predicted R-squared help in resisting the urge to add too many independent variables to the model. Adjusted R-squared compares models with different numbers of variables. Predicted R-squared can guard against models that are too complicated. Adequate precision measures the signal-to-noise ratio. It campers the range of the predicted values at the design point to the average prediction error. A ratio greater than 4 is desirable for acceptance of the model (Noordin *et al.,* 2004; Sangeeta *et al.,* 2023). *p*-value were used in hypothesis testing to decide whether to reject or accept the null hypothesis., smaller the *p*-value, higher the probability of its rejection. *p*-value less than 0.05 indicated the significance of model terms. Models having a *p*-value lower than 0.01 (indicating the lack of fit is insignificant at 90% confidence level) was accepted (Dogan and Okut, 2003).

Completely Randomized Design (CRD) was used for the analysis of color and hardness of microwave-roasted samples while Randomized Block Design (RBD) was used for sensory parameters (Cornell and Knapp, 2006).

2.5 Determination of physicochemical characteristics

2.5.1 Germination percentage

Germination percentage was calculated and evaluated by following formulae (Hou and Ma, 2022):

Per cent germination

$$= \frac{\text{Number of germinated seeds}}{\text{Number of total seeds taken for germination}} \times 100$$

2.5.2 Color

The color of microwave-roasted sample was analyzed in terms of CIE-L,*a,*b value using Color-Mine free library available online software.

2.5.3 Texture

Texture (hardness and fracturability) of microwave-roasted samples was measured by slightly modifying the method suggested by Bourne (2002) using a universal table texture analyzer TAXT2i (Stable Micro Systems) with a probe (SMS P/2).

2.5.4 Nutritional and chemical composition

The raw horsegram and GH namkeen were analysed for proximate composition as well as antinutrient components (oxalate and tannin) by standard procedures of AOAC (2012) while the total phenolic content was measured by the method described by Sadashivam and Manickam, (2004) using Eppendorf Bio spectrophotometer.

3. Results

3.1 Effect of germination conditions on germination percentage of horsegram

Regression analysis (Table 2) for full second order polynomial model for germination percentage showed that the model F-value (617.67) and coefficient of determination ($R^2 \geq 80\%$) was significant ($p \geq 0.01$). The predicted R^2 (98.46%) was in reasonable agreement with the adjusted R^2 (99.61%). The difference in both was less than 20% which indicates that model was desirable for the given response. Adequate precision is a measure of signal-to-noise ratio and was found to be greater (67.69) than 4, which specifies the desirability of response for the germination percentage. It can be observed from the data (Table 2) that the germination percentage of horsegram was greatly affected by the increase in germination time (X_1) and temperature (X_2). It can be inferred from the polynomial equation (2) that the time had a positive whereas temperature had a negative correlation with the germination percentage, which means that the germination percentage is enhanced with an increase in germination time while increase in temperature declined the germination percentage in horsegram.

Germination percentage

$$= 83.41 + 3.17X_1 - 7.00X_2 - 0.2500X_1X_2 + 0.0517X_1^2 - 18.45X_2^2 \quad (2)$$

A careful study of the data depicted in 3-D graph as well as contour plots (Figure 2), indicated that with the increase in time from 12 to 24 h, and a corresponding increase in temperature from 30 to 35ºC, the estimated germination percentage of horsegram seeds increased whereas after the increase in temperature from 35 to 40ºC a gradual decline in germination percentage was recorded.

3.2 Effect of germination conditions on antinutritional factors (oxalate and tannin) of horsegram

The effect of germination temperature and time on antinutritional factors of horsegram are given in Table 1. The full second-order polynomial model for oxalate and tannin showed that F-value for oxalate (3999.71) and tannin (604.43) were several times more than the table F-value of 7.46 as indicated by regression analysis in Table 2. The coefficient of determination (R^2) for oxalate and tannin content was 99.97% and 99.77%, respectively. These values showed that the models explained ≥99% variability. According to Joglekar and May (1991) and Sangeeta *et al*. (2016) a value of R^2 of more than 80% is adequate for explaining the variability in the model, while Prasad (2009) considered a minimum value of 85% for R^2 as excellent for explaining variance for data. Adequate Precision ratio for oxalate (173.68) and tannin (61.68) was greater than 4 which indicates an acceptable signal-to-noise ratio. The difference between predicted R^2 and Adjusted R^2 was less than 20% which also intimate that this model can be used to navigate the design space for oxalate and tannin as suggested by the software (Table 2). Thus, the model developed for predicting oxalate and tannin content for sprouted horsegram with varied germination conditions was adequate. The negative coefficient of time (X_1) shows the antagonistic effect whereas positive coefficient of temperature (X_2) depicts synergistic effect with the reduction of oxalate and tannin as inferred from the polynomial equations 3 and 4.

$$\text{Oxalate} = 148.66 - 10.09X_1 + 16.74X_2 + 0.3250X_1X_2 + 0.4226X_1^2 + 54.19X_2^2 \quad (3)$$

$$\text{Tannin} = 380.59 - 8.23X_1 + 12.74X_2 + 0.1865X_1X_2 + 2.19X_1^2 + 63.12X_2^2 \quad (4)$$

A minimum level of oxalate and tannin was considered essential to increase the bioavailability of nutrients like calcium, iron, protein, *etc*. The contour plot and 3D graph give effect of various levels of temperature and time on both the antinutritional components. Superimposition of oxalate data in contour plot and 3D graph between germination temperature (30 to 40ºC) and germination period (12 to 24 h) depicted that at a temperature near to 35ºC and longer time of germination significantly declined the oxalate in sprouted horsegram (Figure 3).

Figure 4 illustrates the 3D graph and contour plots concerning the effect of interaction of time and temperature on tannin content of germinated horsegram samples. Increasing the temperature from 30 to 40°C decreased the tannin content of the germinated samples irrespective of time.

Table 2: Regression analysis of various responses of germinated horsegram samples

Source		Germination percentage		Oxalate (mg/ 100 g)		Tannin (mg/ 100 g)	
		RC	*p*-value	RC	*p*-value	RC	*p*- value
Model		83.41	< 0.0001	148.66	< 0.0001	380.59	< 0.0001
Linear	X_1	< 0.0001	< 0.0001	-10.09	< 0.0001	-8.23	< 0.0001
	X_2	< 0.0001	< 0.0001	16.74	< 0.0001	12.74	< 0.0001
Interaction	X_1X_2	-0.2500	0.4895	0.3250	0.4261	0.1865	0.8701
	X_1^2	0.0517	0.9037	0.0426	0.3917	2.19	0.1413
Quadratic	X_2^2	-18.45	< 0.0001	54.12	< 0.0001	63.12	< 0.0001
R^2		99.77 %	99.97 %	99.77 %			
Adj. R^2		99.61 %	99.94 %	99.60 %			
Pred. R^2		98.56 %	99.74 %	98.15 %			
Adequate precision		67.69	173.68	61.68			
Model F-value		617.67	3999.71	604.43			
Lack of Fit		NS		NS		NS	

**,* significant at 1% and 5% levels of significance, respectively
RC stands for Regression coefficient and NS stands for Nonsignificant
$F_{(1,7)}$ = 12.24 (1%), 5.59 (5%)
$F_{(5,7)}$ = 7.46 (1%), 3.97 (5%)

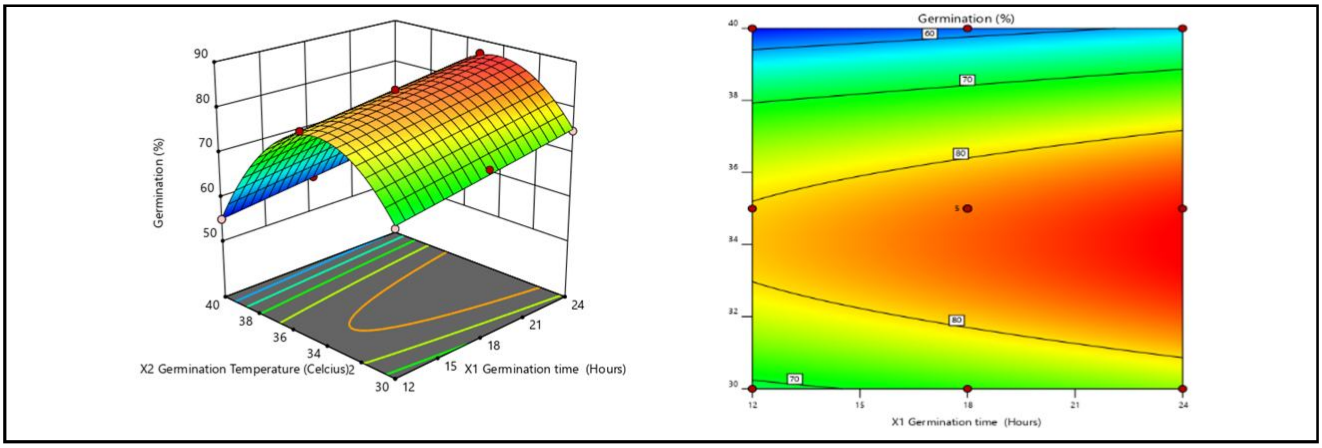

Figure 2: Effect of different times and temperatures combinations on germination percentage.

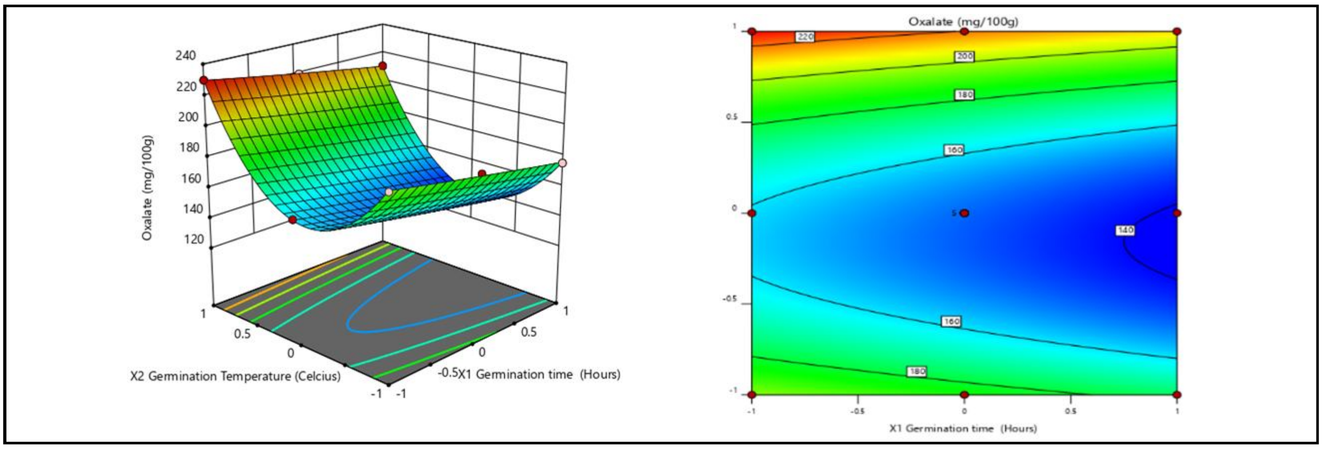

Figure 3: Effect of different times and temperatures combinations on oxalate content.

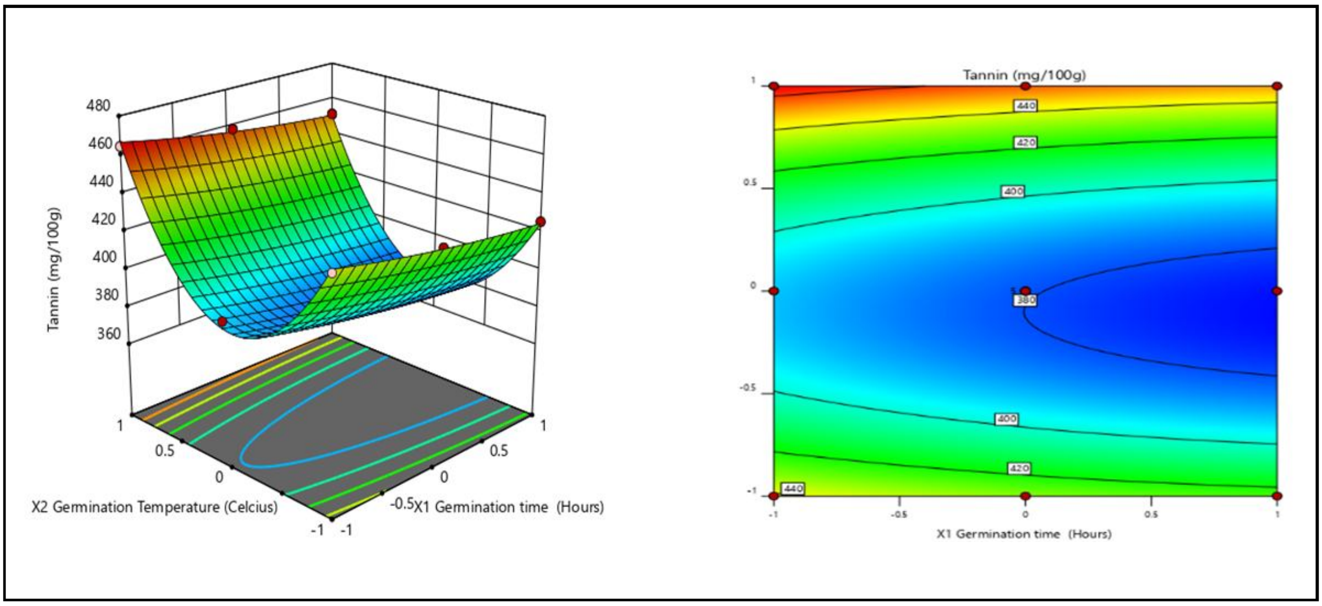

Figure 4: Effect of different times and temperatures combinations on tannin content.

3.3 Process optimization

Multiple response optimizations were done using the software Design Expert-12 to maximize the germination percentage and minimize the oxalate and tannin content individually and in combination of responses to get a compromised optimum point. Optimum germination temperature and time for horsegram were found to be 34.5°C and 24 h, respectively, which produce the best quality germinated horsegram having 87% germination, 137.816 mg/100 g of oxalate and 373.887 mg/100 g of tannin content with desirability of 99.97 (Figure 5).

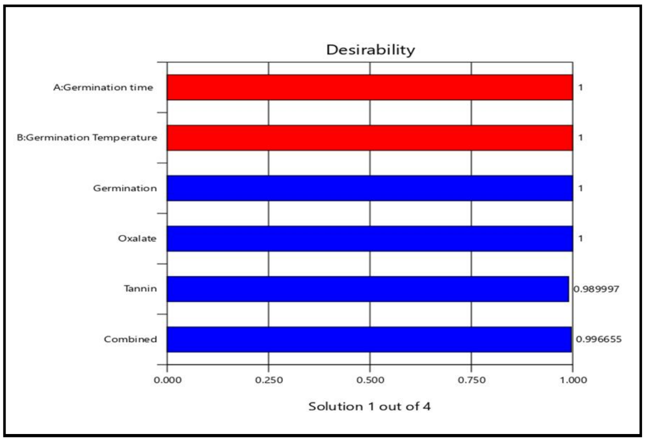

Figure 5: Contribution of optimized factors and predicted responses towards overall desirability.

The above results were confirmed by germinating horsegram by optimized condition and evaluation showed that 89% of horsegram were sprouted with reduced level of oxalate (134.07 mg/100 g) and tannin (369.91 mg/100 g) content (Table 3). Hence, germination at 34.5°C for 24 h was selected as an optimum condition based on highest percentage of germination and least level of oxalate and tannin content, and was therefore selected for the preparation of GH namkeen.

3.4 Effect of microwave roasting condition on sensory attributes and hardness of GH namkeen

The highest scores for different sensory parameters such as color (8.8), odour (8.9), taste (8.9), texture (8.8) and overall acceptability (8.85) were achieved by the roasting germinated horsegram at 500 Watt for 5 min among all the roasting treatments (Table 4). There was a significantly ($p \leq 0.05$) difference in the sensory attributes among all treatments. Roasting of germinated horsegram at 350 Watt took longer time which greatly affected the color of the namkeen due to burning while at 500 Watt took less time which maintained the color as well as the texture properties.

The effects of microwave roasting on the textural properties of GH namkeen have been presented in Table 4. It can be observed that the hardness of GH namkeen at 350 Watt, first increased from 108.29 N to 147.88 N with increase in time, thereafter it reduced. But in the case of roasting at 500 Watt hardness was first reduced and after that hardness gradually enhanced. As per the consumers' acceptance of sensory scores, 500 Watt for 5 min of roasting was selected to produce germinated horsegram namkeen. Hardness of the selected GH namkeen was higher than the market available namkeen such as Haldiram's roasted channa cracker (25.72 N) and Organic Nature roasted masala matar green peas (64.48 N).

3.5 Comparative study of raw horsegram and GH namkeen

It can be evident from Table 5 that the values for hardness (103.21 N) were less in GH namkeen as compared to raw horsegram. This difference in texture might be due to the enzymatic breakdown of complex protein and starch into simple form during germination. Germination also bursts the outer coat of the grain and the grain becomes soft which improves its texture (Srilakshmi, 2010). The available namkeen in the market like roasted channa cracker (Haldiram's) and roasted masala matar green peas (Organic Nature) had 25.72 and 62.48 N hardness, respectively.

The color of the food product is the first attribute that affects the decision of consumers for purchasing or consuming any food. The color values L*, a*, and b* of GH namkeen were 38.53, 19.85, and 10.26, respectively (Figure 1). Namkeen prepared from germinated horsegram was dark in color with respect to raw horsegram as indicated by the L* value (less than 50). The a* value of both the samples was positive indicating that both are near red color and the b* value of GH namkeen sample was in the range of blue color (Table 4).

As compared to raw horsegram (Table 5), GH namkeen had lower moisture content (4.8%). Protein and carbohydrate content of GH namkeen was 2 % and 5%, respectively higher than raw horsegram. Total minerals/ash content was slightly more in GH namkeen. Total phenolic content was found to be less in GH namkeen as compared to raw horsegram significantly (Table 5).

Oxalate and tannin content in GH namkeen were less as compared to raw horsegram as per the data presented in Table 5. Maximum reduction of oxalate and tannin content was recorded as 66.84 and and 71.69%, respectively.

Table 3: Predicted and observed values of various parameters of germinated horsegram seeds

Parameter	Predicted value	Observed Value
Germination time (h)	24	24
Germination temperature (^{o}C)	34.485	34.5
Germination percentage	87.183	89
Oxalate (mg/100 g)	137.816	134.07
Tannin (mg/100 g)	373.887	369.91

Table 4: Effect of microwave roasting conditions on GH namkeen

Treatment	Sensory characteristics					Texture*
	Appearance	Odour	Taste	Texture	Overall acceptability	hardness (N)
T_1 (350W, 11 min)	7.4	5.7	6.8	5.3	6.3	108.29
T_2 (350W, 12 min)	7.8	7.5	7.9	7.9	7.8	129.23
T_3 (350W, 13 min)	8.2	6.9	7.5	7.5	7.5	147.88
T_4 (350W, 14 min)	7.8	6.3	6.8	7	6.9	138.41
T_5 (350W, 15 min)	6.5	5.5	5.5	6.6	6.0	133.88
T_6 (500W, 4 min)	8	7.9	7.5	7.9	7.4	107.26
T_7 (500W, 5 min)	8.8	8.9	8.9	8.8	8.85	103.20
T_8 (500W, 6 min)	7.9	8.1	7.9	7.5	7.85	120.81
T_9 (500W, 7 min)	6.8	7.5	6.3	6.8	6.85	136.24
T_{10} (500W, 8 min)	6.2	5.9	5	6.5	5.9	141.81
C.D $_{5\%}$	0.405	0.268	0.260	0.239	0.327	6.07
SE(m)	0.135	0.089	0.087	0.080	0.109	2.046
SE(d)	0.191	0.126	0.123	0.113	0.155	2.894
C.V.	3.104	2.205	2.148	1.925	2.655	2.797

GN stands for Germinated Horsegram

*Hardness of roasted channa cracker (Haldiram's): 25.72 N

*Hardness of roasted masala matar green peas (Organic Nature): 62.48 N

Table 5: Physicochemical characteristics of raw horsegram vs. GH namkeen

Responses	Raw horsegram seed	GH namkeen	$CD_{(5\ \%)}$
Texture hardness (N)	301.32 ± 0.23	103.21± 0.15	12.55
Fracturability (mm)	0.92 ± 0.19	1.00 ± 0.17	20.047
Color			
L*	67.18 ± 0.24	38.53 ± 0.13	4.035
a*	19.52± 0.20	19.85 ± 0.31	NS
b*	37.85 ± 0.10	10.26 ± 0.14	0.858
Moisture (%)	10.82 ± 0.55	4.8 ± 0.45	0.383
Protein (%)	22.59 ± 0.25	24.31 ± 0.34	1.382
Fat (%)	0.59 ± 0.2	1.53 ± 0.23	0.091
Crude fibre (%)	4.55 ± 0.2	3.71 ± 0.37	0.296
Carbohydrate (%)	56.47	61.44	3.345
Total minerals/ash (mg/100 g)	4.09 ± 0.21	4.21 ±0.54	NS
Total phenols (mg/100 g)	2478.68 ± 0.19	1659.86 ± 0.14	151.747
Antinutritional factors			
Oxalate (mg/ 100 g)	384.05 ± 0.16	127.34 ± 0.13	11.837
Tannin (mg/ 100 g)	1185.18 ± 0.22	332.17 ± 0.23	20.895

GH stands for germinated horsegram and NS stands for Nonsignificant

4. Discussion

Germination percentage of horsegram seeds was increased as the time (12 to 24 h) and temperature enhanced from 12 to 24 h and from 30 to 35°C, respectively. However, after the increase in temperature from 35 to 40°C a gradual decline was observed in germination percentage. This change might be due to the fact that germination percentage normally rises linearly with temperature until it reaches an ideal temperature, beyond which it drops dramatically (Torres, 2016).

Oxalate content in sprouted horsegram seeds was reduced at 35°C temperature with longer period of germination. This might be due to activation of the oxalate oxidase enzyme during germination process which breaks oxalic acid during germination and triggers the enzymatic movement of seeds, which further breaks the starches, proteins, and fats into simpler structures. Further leaching of oxalate during soaking prior to germination is also responsible for the reduction of oxalate content in the pulses.

Germination temperature and time were inversely proportional to tannin content of the seed. Increasing the temperature of germination gradually decreased the tannin content of the seeds irrespective of time. Tannins are generally present in seed coat of the pulses and are water soluble. Loss of tannin may be due to leaching during soaking prior to germination (Shimelis and Rakshit, 2007; Pathak and Singh, 2022) as they are water soluble antinutrient factors (Nwosu, 2010). In addition, the further reduction in tannin content after germination process might be due to activity of polyphenol oxidase and other catabolic enzymes which are activated during germination and result in the hydrolysis of various components including carbohydrate, protein, fibre, and lipid, as well as phenolic compounds (Singh *et al*., 2000).

Roasting generally affects the sensory as well as textural properties of the products. Maximum organoleptic scores for all sensory parameters were scored by the samples reasted at 500 Watt for 5 min while roasting of germinated horsegram seeds at lower power (350 Watt) for longer time greatly affects the color. The variation in color may also be attributed to the production of brown pigments due to maillard reaction (Gujral *et al.,* 2011). Continuous removal of water during roasting cause case hardening of the product (Srilakshmi, 2010). Higher values of hardness in GH namkeen than conventional may be due to the higher protein content of horsegram which gives a harder texture to the product (Scanlon *et al.,* 2018).

The color of the prepared GH namkeen was darker than the original horsegram as indicated by lower L value. The reduction in L* value with increased roasting temperature and time was also reported by Mridula *et al*. (2007).

Lower moisture content of the GH namkeen as compared to raw horsegram is might be due to the opening of micropyle pores during germination, which promotes moisture loss during roasting treatment. Protein content in GH namkeen was higher because during sprouting, dormant enzymes get activated and the availability of nutrients is improved. Protein is converted into a simpler form as germination proceeds.

The reduction in phenolic compounds could be due to physical leaching of the phenolic compounds into the soaking water (Dicko *et al.,* 2005) and enzymatic activity during germination (Gujral *et al.,* 2011). During the germination process trans ferulic acid and trans p-coumaric acid are decreased in pulses as the length of the germination time increases which also contributes to the reduction in total phenols (Lopez-Amoros *et al.,* 2006; Radwan, *et al*., 2019)

Reduction in oxalate and tannin content in GH namkeen might be due to enzymatic breakdown of phenolic compounds during germination of horsegram seeds. More reduction in oxalate and tannin after germination may be due to the release of enzymes that metabolize the oligosaccharides which reduce the side effects of these antinutritional components (Srilakshmi, 2010). Soaking, roasting and cooking treatments also help in reducing in antinutritional components (Hefnawy, 2011).

5. Conclusion

Horsegram is one of the underexploited and neglected minor crop but it has special significance in subsistence farming and is a resource of nutritional security for poor masses in developing countries. Inference can be drawn that development of snack foods by utilizing the horsegram enhance the commercialization of this underutilized pulse. It is evident from the above study that the germinated horsegram namkeen presents us with an opportunity to cater the public with protein and fibre-rich ready-to-eat product which is low in antinutritional factors and therefore, can further be recommended for commercialization.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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