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## **Studies on Development of Protein Rich Germinated Green Gram Pickle and its Preservation by using Class One Preservatives**

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### **ABSTRACT**

Green gram on germination has high nutritive value in terms of protein, fibre and minerals yet it has low shelf life of two to three days at room temperature, hence to enhance the shelf life of germinated green gram, a protein rich value added product can be developed in the form of Pickle with vinegar, oil and salt as preservatives. To optimize the quantity of ingredients to be added, Response Surface Methodology was used. Statistical software Stat-Ease was used for statistical and graphical analysis of the experimental data. To consider all the responses simultaneously for optimization, the RSM was used to compromise optimum conditions and it was found that the sensory scores were 7.4, 7.6, 7.8 and 7.9 for colour, flavour, texture and overall acceptability corresponding to optimum conditions. Pickle having composition 6ml vinegar, 4 g salt, 16 mL oil and 7 g garlic per 50 g pickle was found optimum. Triplicate samples were prepared using the optimum conditions and were evaluated for all the responses. Corresponding values for colour, flavour, texture and overall acceptability were 7.6, 8.6, 7.8 and 8.1, respectively which were comparatively higher than the predicted values. Therefore the said formulation was recommended for pickle. From the storage study of optimized pickle at room temperature for shelf life prediction, it was found that the pickle can be stored effectively for 60 days at room temperature without any significant change in the quality attributes of the pickle. Germinated green gram pickle is found nutritious in terms of high protein and fiber when compare with the germinated green gram. The protein rich pickle can be preserved by using oil and salt as a preservative up to two months at ambient temperature without any significant change in the sensory attributes of the pickle. Further study of effect of storage conditions on storage stability of protein rich pickle is required.

**Key words:** Green gram, protein, germination, pickle, preservative, flatulence

### **INTRODUCTION**

Green gram (*Vigna radiata*) belongs to the family Leguminosae. Green gram is one of the important pulse crop in India. It has been reported that Green gram has been cultivated in India since ancient times. It is widely cultivated throughout the Asia, including India, Pakistan, Bangladesh, Sri Lanka (Chandrasekher and Ghosh, 2002). Green gram is a protein rich staple food. It contains about 23-25% protein (Khattak, 2007) which is almost three times that of cereals. It supplies protein requirement of vegetarian population of the country. It is consumed in the form of split pulse as well as whole pulse which is an essential supplement of cereal based diet. Green gram is a good source of vitamins, minerals, enzymes, complex carbohydrates and its protein quantity is better than others (Kataria *et al.*, 1989; Jood *et al.*, 1998) and are low in fat and have no cholesterol (Dostalova *et al.*, 2009).

Epidemiological observations and a number of human and animal studies have shown that the Non-Starch Polysaccharides (NSP) in pulses have a role in protecting against degenerative diseases such as diabetes, cardiovascular disease and cancer. NSP protects against chronic diseases such as diabetes by controlling blood glucose levels. Their hypo-cholesterolemic property protects against cardiovascular disease. They also help prevent bowel cancer by binding carcinogens and toxins (Sanchez-Castillo *et al.*, 1994; Cumming *et al.*, 2004).

Germinated green gram is free of flatulence-causing agents (Dostalova *et al.*, 2007). This makes it an acceptable food for convalescents and pleasant weaning food for babies (Sadana and Chabra, 2004). The protein is especially rich in the amino acid, lysine but it is somewhat deficient in sulphur-containing amino acids. The seeds are rich in calcium, phosphorous, magnesium, potassium, folate and other B Vitamins. They also contain appreciable amounts of Vitamin C. Raw seeds are rich in trypsin-inhibitors, that block the effects of protein digesting enzymes in the gut. Sprouted green gram has lower amounts of these inhibitors. It is reported that sprouting improved the protein/amino acid digestibility by decreasing anti-nutritional factors and increasing the true/apparent protein/amino acid digestibility (Bibi *et al.*, 2008). The green gram has good nutritive value and on germination its health benefits increase (Reddy *et al.*, 1978; Bau *et al.*, 1997). So, consumption of germinated green gram can be used to overcome many diseases. But, since due to potential microbial contamination of germinated legume seed, germinated green gram has very low shelf life, therefore there is a need to preserve it in a useful way. As pickle too have many functional properties. The pickles are good appetizers and add to the palatability of a meal and aid digestion by stimulating the flow of gastric juice (Girdhari Lal *et al.*, 2010). Pickles contain large amounts of lactobacilli bacteria which are important to the digestion of grains and vegetables which has usual beneficial probiotic properties (Panda *et al.*, 2007). Scientific research has shown that these "friendly" bacteria survive the trip through the acidic juices of the stomach to the small intestine. In the small intestine they aid pancreatic enzymes in the transformation of dextrin (a carbohydrate found in grains) into simple sugars that can be readily used by the body (Girdhari Lal *et al.*, 2010). In the view of above health benefit of pickles, present study was an effort to develop pickle from germinated gram which is protein rich and have more than two months of shelf life by the use of class one preservatives (oil, salt and vinegar).

## **MATERIALS AND METHODS**

Green gram (*Vigna radiata*) used for this investigation were purchased from the local market. All required ingredients like spices, vinegar, salt and oil etc were purchased from local market of Allahabad, India. The green gram was soaked in water for 6 h and was tied in muslin cloth for germination for six hours. Spices were roasted and were grinded in a laboratory mixer. All the chemicals used in analysis were of (analytical reagent) AR grade obtained from Centre of Food Technology, University of Allahabad. This work was carried out from March to June 2010 at Centre of Food Technology, University of Allahabad, Allahabad, U.P. and India.

### **Method**

**Development and optimization of germinated green gram pickle:** To optimize the quantity of oil, salt, vinegar, garlic to be added, Response Surface Methodology (RSM) was used while rest of the ingredient like amchur, black salt, black pepper, turmeric, red chilly powder, cumin powder, black mustard seed, kalaunji masala, green chili paste (Girdhari Lal *et al.*, 2010), level was kept constant on the basis of hit and trial method using 9-point hedonic scale. Response Surface

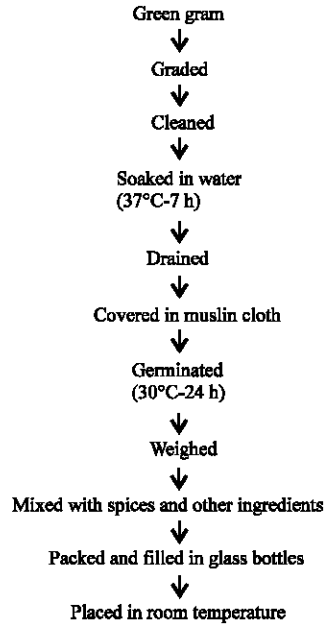


Fig. 1: Process flow chart in the preparation of germinated green gram pickle

Methodology (RSM) is a collection of statistical and mathematical technique useful for developing, improving and optimization process (Telesphore and He, 2009), for statistical and graphical analysis of the experimental data and also for monitoring the combined effects of pH and temperature (Kanu *et al.*, 2007). The oil (16 mL), salt (4 g), vinegar (6 mL), garlic (7 g) was repeated 5 times as central points. The lower and upper limits for oil, vinegar, salt, garlic were taken as 16-18 mL, 6-8 mL, 4-6 g, 4-7 g respectively. All 30 combinations were subjected for sensory quality evaluation by 15 trained panelists. Pickle having composition 6 mL vinegar, 4 g salt, 16 mL oil and 7 g garlic per 50 g pickle was found optimum. Triplicate samples were prepared using the optimum conditions and were evaluated for all the responses. Corresponding values for colour, flavour, texture and overall acceptability were 7.6, 8.6, 7.8 and 8.1, respectively The process flow chart is adopted for the preparation of germinated Green gram pickle by using various ingredients like vinegar, oil, salt, garlic, ginger, amchur, black salt, black pepper, turmeric, red chilly powder, cumin powder, black mustard seed, kalaunji masala and green chilly paste .The process flow chart for the preparation of pickle is given in Fig. 1.

**Vitamin C estimation:** Sample solution equivalent to 0.2 mg ascorbic acid mL<sup>-1</sup> was prepared in water containing 3% (w/v) metaphosphoric acid. It was titrated against standard 2, 6 dichlorophenol indophenol (2,6 DCIP) solution of 0.5 mg mL<sup>-1</sup> concentration until the pink color developed completely. The operation was repeated with a blank (Indian Pharmacopoeia, 1996).

**Moisture, crude fat, fibre, protein and mineral content:** The moisture, crude fat, fibre and protein content of the samples were determined as per AOAC (1997) procedures; whereas minerals (Ca, P and Fe) were estimated as per the AOAC (1990) procedures. The acidity of pickle was analysed by method given by Ranganna (1986).

**Sensory analysis:** To carry out initial optimization of the ingredients of RSM design, the 30 combinations were judged by a trained panel of 15-member using a 9 point hedonic scale (9-like extremely and 1-dislike extremely) (Murray *et al.*, 2001) for color, flavor, texture, overall acceptability.

**Storage study of germinated green gram pickle:** 50 gm germinated Green Gram Pickle (GGP) were packed in each of the glass bottle and were stored at ambient temperature for two months. The GGP was drawn at interval of 5 days and subjected to sensory analysis for color, flavor, mouthfeel, texture, overall acceptability using 9-point hedonic scale (9: like extremely and 1: dislike extremely). The Vitamin C, acidity was estimated at the interval of 5 days.

**Statistical analysis:** The data obtained were analyzed statistically for analysis of variance (ANOVA) using completely randomized design with Least Significant Difference (LSD) at  $p < 0.05$  using Co.Stat 6.303, CoHort software (USA).

## RESULTS AND DISCUSSION

For the optimization of the variables the response i.e., flavour, texture and overall acceptability were selected on the basis of that, these responses had direct effect on the quality of PRP (Sivakumar *et al.*, 2010). Garlic and vinegar (Fig. 2) had significant positive ( $p < 0.05$ ) effect on flavour and interaction between garlic paste and oil showed positive effect on the flavour (Fig. 3). The interaction between salt to vinegar show negative effect on flavour as we increase the concentration of vinegar (Fig. 4). Interaction between garlic paste and vinegar to green gram had significant positive effect on texture at ( $p < 0.05$ ) (Fig. 5). Interactive effect of vinegar with

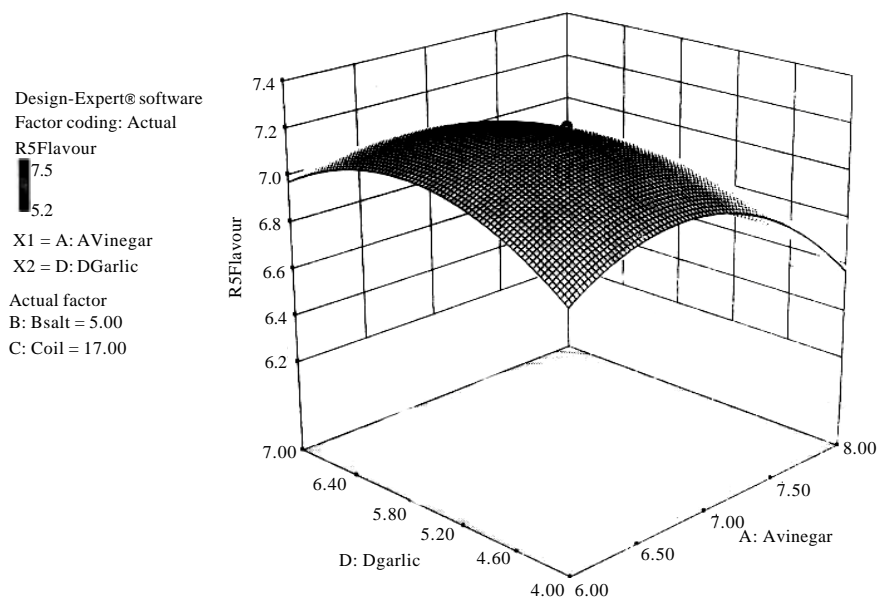


Fig. 2: Response surface and counter plots showing effects of variable on the flavor of Protein rich pickle a) garlic paste vs. Vinegar

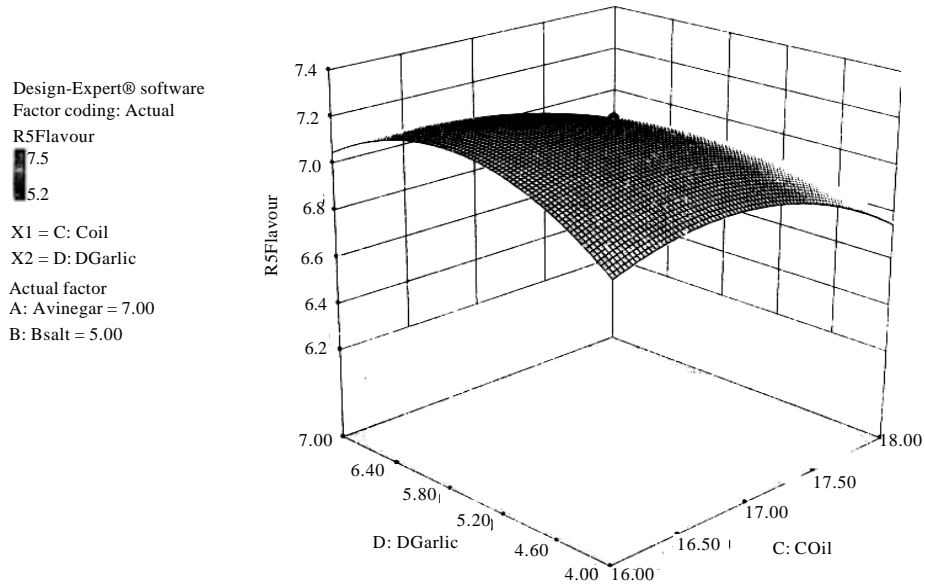


Fig. 3: Response surface and counter plots showing effects of variable on the flavor of Protein rich Pickle b) garlic paste vs. oil

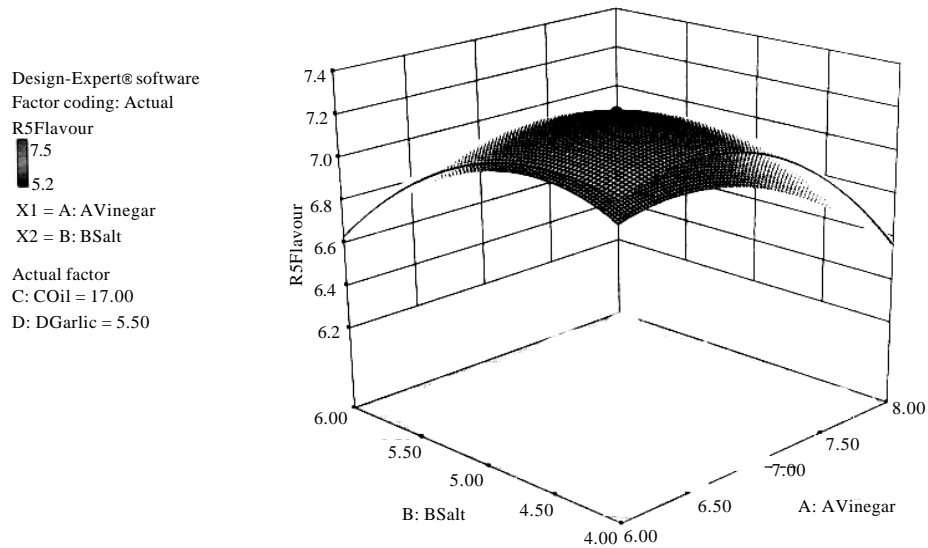


Fig. 4: Response surface and counter plots showing effects of variable on the flavor of Protein rich Pickle c) salt vs. vinegar

green gram showed significant negative effect at ( $p < 0.05$ ) on the overall acceptability of the product (Fig. 6) which can be minimized by decreasing the concentration of salt and vinegar. The interactive effect of garlic to vinegar and garlic to oil showed positive effect on overall acceptability

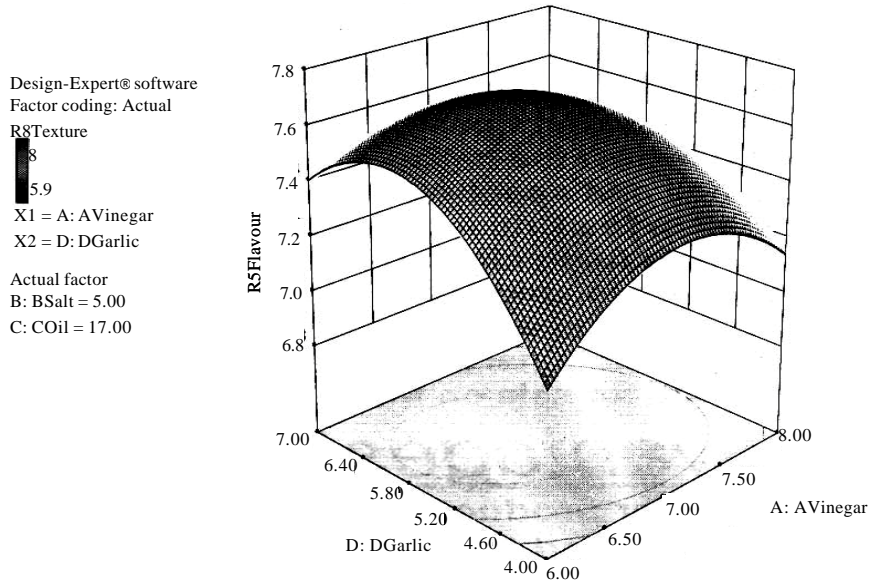


Fig. 5: Response surface and counter plots showing effects of variable on the Texture of Protein rich Pickle a) garlic paste vs. vinegar

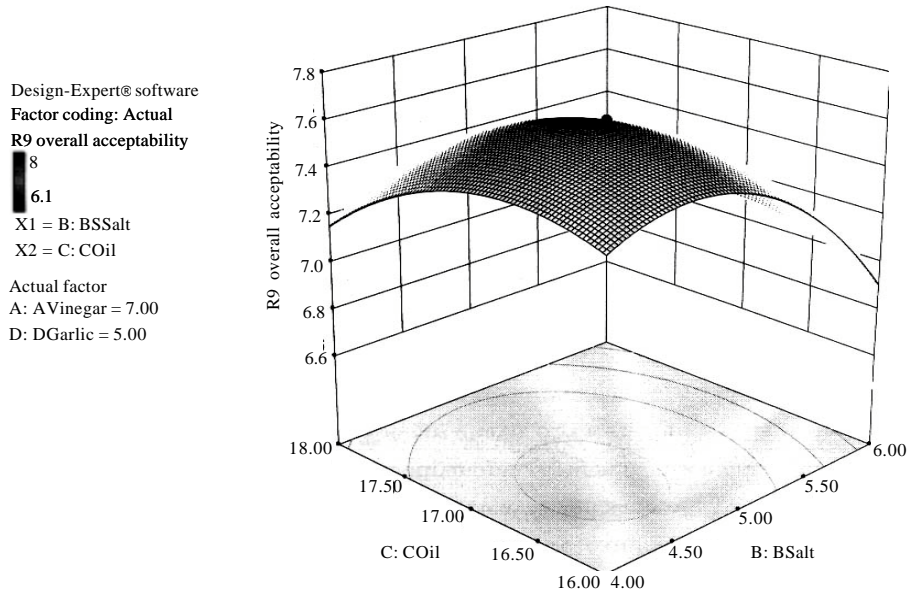


Fig. 6: Response surface and counter plots showing effects of variable on the Overall acceptability of Protein rich Pickle b) oil vs. salt

of PRP (Fig. 7, 8). The detail of each Figure with maximum and minimum values is given in Table 4. The overall effect of garlic was maximum on all sensory responses followed by oil. To

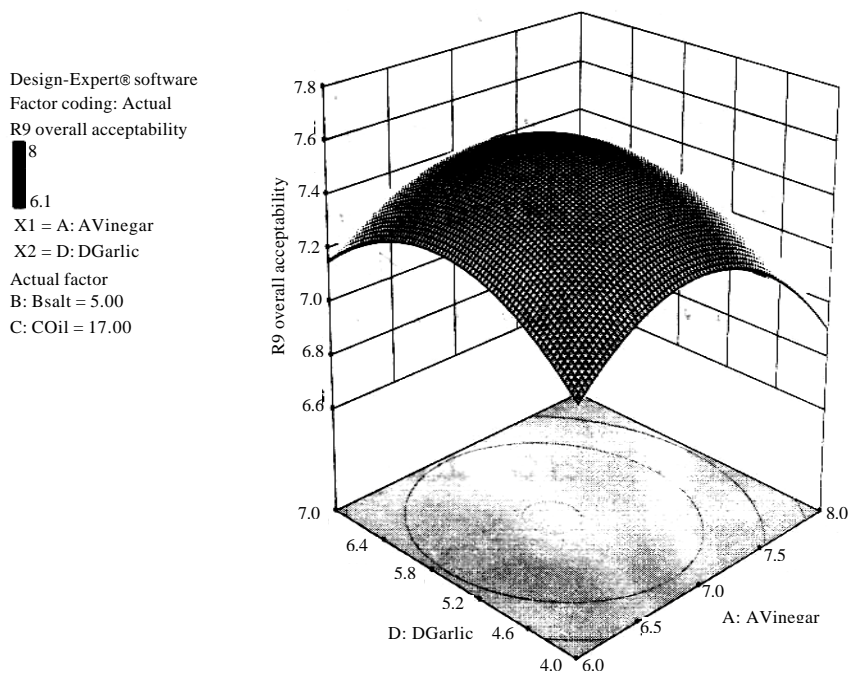


Fig. 7: Response surface and counter plots showing effects of variable on the Overall acceptability of Protein rich Pickle b) garlic paste vs. vinegar

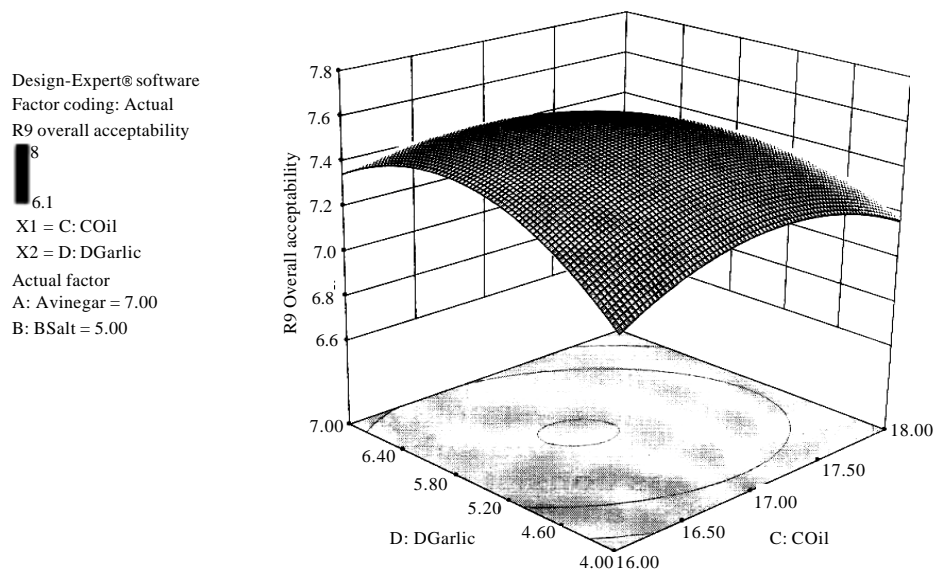


Fig. 8: Response surface and counter plots showing effects of variable on the Overall acceptability of Protein rich Pickle b) garlic paste vs. oil

consider all the responses simultaneously for optimization the RSM was used to compromise optimum conditions and it was found that the sensory scores were 7.6, 7.8, 7.9 for flavour , texture



and overall acceptability corresponding to optimum conditions .PRP having composition 6 mL vinegar, 4 g salt, 16 mL oil and 7 g garlic per 50 g of germinated green gram were found optimum. Triplicate samples were prepared using the optimum conditions and were evaluated for all the responses, corresponding values for flavour , texture and overall acceptability were 8.6 , 7.8 , 8.1 which were comparatively higher than the predicted value (Table 1). Therefore, the said formulation was recommended for germinated green gram pickle.

**Study of storage stability of germinated green gram pickle:** The optimized green gram pickle was served to the panelist to find out the acceptability of Protein Rich Pickle (PRP). The score for overall acceptability of pickle was 8.1 (very good) (Table 2). The germinated green gram pickle could be stored up to two months without any significant change in the quality attributes of the pickle (Table 2). In present investigation it was observed that the salt (Mishra *et al.*, 2010a), vinegar and oil concentration prevents microbial growth in the germinated pickle (Girdhari Lal *et al.*, 2010). During the storage of PRP it was analyzed for, Vitamin C and acidity content also. After the storage of 60 days PRP was analyzed for Proximate composition as compare to the germinated green gram (Table 3). It was found that there was decrease in the vitamin C content from (Mishra *et al.*, 2010b)

Table 1: Optimum levels of independent variables and response value

Response	Response optimum	Response predicted
Color	7.4	7.6
Texture	7.8	7.8
Flavor	7.6	8.6
OAA	7.9	8.1

Table 2: Study of effect of storage on changes in sensory quality attribute of protein rich green gram pickle during storage in room temperature

Particulars	0 day	10 day	20 day	30 day	40 day	50 day	60 day
Flavour	7.74	7.56	7.24	7.06	6.87	6.60	6.52
Color	7.80	7.72	7.60	7.54	7.30	7.07	7.00
Mouthfeel	7.96	7.80	7.60	7.50	7.47	7.34	7.25
Texture	8.00	7.91	7.80	7.70	7.70	7.60	7.45
OAA	8.10	8.00	7.90	7.84	7.70	7.60	7.32

Table 3: Proximate analysis of optimized green gram pickle

Particulars	Germinated green gram	Germinated pickle
Fat (%)	1.60	2.80
Moisture (%)	52.00	51.00
Protein (%)	24.20	26.30
Fibre (%)	4.00	5.30
Carbohydrate (%)	23.30	25.19
Ash (%)	2.10	3.20
Calcium (mg/100 g)	193.40	302.60
Iron (mg/100 g)	6.40	29.30
Phosphorus (mg/100 g)	465.70	496.80
Sodium (mg/100 g)	213.70	11342.00
Potassium (mg/100 g)	1524.00	2018.00
Energy (kcal/100 g)	204.40	231.16

Table 4: Effect of salt, vinegar, oil, garlic concentration on flavor, texture and overall acceptability of optimized product acceptability of optimized product

Parameter/variable	Minimum	Maximum
Flavor (score)	8.6	7.1
Texture (score)	7.6	6.8
Overall acceptability (score)	8.1	7.0
Vinegar (mL)	6.0	8.0
Salt (g)	4.0	6.0
Oil (mL)	16.0	18.0
Garlic (g)	4.0	7.0

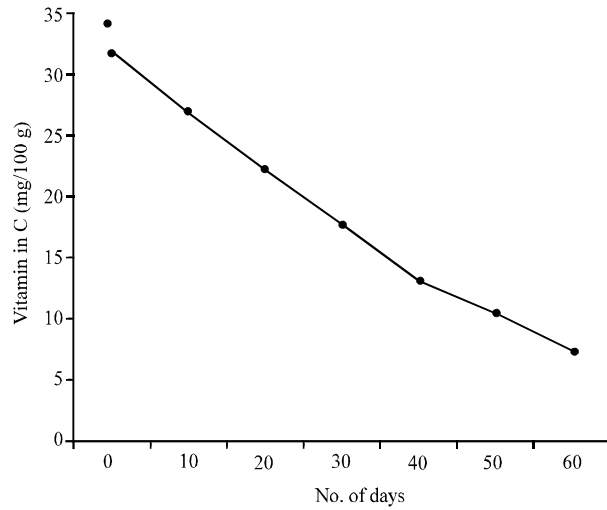


Fig. 9: Study of effect of storage on retention of Vitamin C in Protein rich Pickle (PRP)

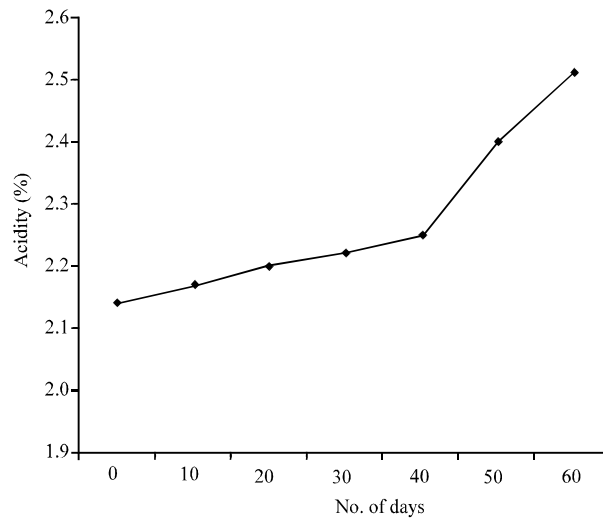


Fig. 10: Study of effect of storage on Acidity in Protein rich Pickle (PRP)

31.75 mg/100 g to 7.32 mg/100 g (Fig. 9) after 60 days of storage in room temperature while acidity of the pickle was increased from 2.14 to 2.51% (Fig. 10) which may be due to lactic acid fermentation during storage at room temperature (Girdhari Lal *et al.*, 2010).

## CONCLUSION

Green gram is said to be the good protein pulse due to its high net protein value and vitamin content. On germination, biological value of sprouts increases, flatulence decreases and complex compounds broken down into simpler ones, increasing digestibility. In spite of these advantages the shelf life of germinated grams is very low. So increasing of shelf life became essential. Present study was an effort to develop pickle from germinated gram which is protein rich and has higher shelf life. From the storage study at room temperature of optimized pickle for shelf life prediction, it was found that the pickle can be stored effectively for 60 days at room temperature without any significant change in the quality attributes of the pickle. Germinated green gram pickle is found nutritious in terms of high protein and fiber when compare with the germinated green gram. The protein rich pickle can be preserved by using oil and salt as a preservative up to two months at ambient temperature without any significant change in the sensory attributes of the pickle. Further study of effect of storage conditions on storage stability of protein rich pickle is required.

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