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PACKAGING SOLUTIONS TO PROLONG SHELF LIFE OF GREEN PIGEON PEA KERNELS UNDER REFRIGERATED CONDITION

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ABSTRACT

Green pigeon pea kernels (GPPK's) are protein rich vegetable. GPPK of BDN-2001-2009 at 70 days of crop maturity was tested to improve shelf-life and reduced browning. 17 treatments of blanching temperature 75, 85, 95°C in combination with 1, 4, 7 min treatment duration and two packaging films polypropylene (PP) and low density polyethylene (LDPE) of 100, 200, 300 gauge were applied. Packets were stored at refrigerated condition (7-8°C; 80-85% RH). Observations were recorded at 10 days interval till 40th day for physiological loss of weight (PLW), firmness, color deviation, moisture content and sensory status. Experiment was conducted in Box-Behnken model and 17 treatments with 6 categoric factors giving 102 treatment combinations were studied. Optimized independent parameters for modified atmosphere packaging of GPPK by response surface methodology were 95°C blanching temperature, 6.70 min blanching time and 235 gauge packets. Predicted responses were 1.45% PLW, 4.41 N firmness, 55.69% color deviation, 58.67% moisture content and sensory status of 8.17. Blanching for 7 min at 95°C and packaging in 200 gauges PP, nearest to optimized treatment was found acceptable by HACCP standards at level-2 for microbial load with headspace composition of 12.6% O_2 and 5.6% CO_2 on 40th day under refrigerated storage.

INTRODUCTION

Pulses are the major source of protein for vegetarians among Indian population. The per capita availability of protein in the country is 28 g/day, while WHO recommended it should be 80 g/day (Saroj *et al.*, 2013). Demand of proteins in daily diet is met out from pulses which are low in quantity. As far as productivity of pulses is concerned in agricultural production system we have almost reached to maximum level. India produced 17.38 million tonnes of pulses in 2014 - 2015 (Agricultural Statistics at a Glance, 2015). Pulses are chief and economical source of proteins particularly for vegetarians and for poor because animal proteins are beyond their reach. It is mainly consumed as split pulse as 'dal' or fresh vegetable (de-hulled split peas) in some parts of India such as Gujrat, Maharashtra and Karnataka or as green vegetable in some tribal areas of various states (Balai *et al.*, 2013). Like green peas there is a large scope for processing other pulses like green pigeon pea kernels (GPPK), green gram etc. as green vegetables to improve per capita availability of protein. Like green peas (*Pisum sativum*) GPPK is a source of vitamins (A, B complex and C), minerals (Ca, Fe, Zn and Cu), carbohydrates and dietary fibre (Singh *et al.*, 2015). In comparison to green peas (*Pisum sativum*), the GPPK has five times more beta carotene content, three times more thiamine, riboflavin and niacin content and double vitamin C content. This indicates that GPPK is nutritionally rich vegetable for use in daily cuisine. (Saxena *et al.,* 2010).

Food packaging technologies maintain freshness, quality and help a lot in extending shelf life (Day, 1998; Thompson, 1998). The physical shelf life of the packaged product taking into account their fitness for human consumption along with their essential nutrients and bioactive compounds etc. can be enhanced by means of suitable packaging technologies such as Modified Atmosphere Packaging (MAP) that has become a widely used food preservation technique, which minimally affects fresh produce characteristics and hence fit in the recent consumer preference for fresh and additives free food (Mangaraj and Goswami, 2009; Mohammed *et al.*, 2015). MAP is being used increasingly for extending shelf life, improving the product image and reducing the wastage of a wide range of whole fresh prepared fruits and vegetables (Day *et al.*, 2001).

Therefore, this experiment was formulated with an objective to develop a MAP system for GPPK's to increase shelf life and to study changes in physico-chemical and textural properties of GPPK's under MAP to identify the extent for its suitability of consumption after storage under refrigerated condition; with an hypothesis that "Minimal processing followed by modified atmospheric packaging will improve the shelf life of green pigeon pea kernels and thereby will increase the possibility of their availability in the other areas than production catchment"

MATERIALS AND METHODS

Selection of variety

Fresh green pigeon pea (*Cajanus cajan*) of promising entry no. BDN-2001-2009 was found superior for vegetable purpose over four years under study at pigeon pea research experiment at Pulses Research Unit, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India (Annual Report on Pulse Breeding, Pulses Research Unit, Dr. PDKV, Akola, Maharashtra 2012-2015). It was procured from University fields of Dr. PDKV, Akola. The fresh green pigeon pea was shelled manually. Damaged, under matured and over matured pigeon pea kernels and foreign matter was discarded from lot of shelled kernels and the remaining uniform size kernels were taken for the experimental purpose (Mali, 2016).

Pre-treatment

The cleaned and graded fresh GPPK's were blanched with solution containing citric acid and potassium meta-bisulphite (KMS) at the rate of 0.24% each. Three blanching temperatures used were 75°C, 85°C and 95°C and its combination with 1, 4 and 7 minutes of exposure time. Thereafter treatment wise the blanched GPPK's were kept in colander to remove excess water (Nicholas, 1974).

Packaging material and storage condition

The fresh GPPK's were packed in polypropylene (PP) and low density polyethylene (LDPE) pouches of size 127×178 mm of various thicknesses viz. 100, 200, 300 gauge each (Ben-Yehoshua, Fishman and Rodov, 1994), heat sealed and placed in refrigerated condition i.e. 7-8°C temperature and 80-85 % RH (Masniyom *et al.*, 2002 and Keditsu *et al.*, 2003). The sample size was 100 g in each packet (Mali, 2016).

Experimental design & treatment of the data

The experiment was conducted by Box-behnken design and data was subjected to analysis of variance by Response Surface Methodology (RSM). Three variables and three levels including 17 treatments formed by 5 central points were used for analysis. The dependent variables viz. physiological loss in weight (PLW), firmness, chlorophyll, color deviation (Ä E), moisture content (% Wb), sensory status & the shelf life of green pigeon pea kernels was assumed to be affected by three independent variables (factors) *viz.*, blanching temperature (A), blanching duration (B) and thickness of packaging material (C) as well as categoric factors viz., type of packaging material (PP & LDPE) and refrigerated storage condition. Using statistical design of Box-Behnken model, 17 treatments multiplied by six categoric factors giving total one hundred and two (102) treatment combinations were conducted and RSM was employed to study the effect of factors (process parameters) on responses and accordingly process parameters were optimized (Mali, 2016).

Optimization

RSM was applied to the experimental data using the package, Design-Expert version 9.0.6.2 (Stat Ease Inc, Minneapolis, MA Trial version, 2016). Numerical optimization of input parameters was carried out for storage study under refrigerated condition (Mali, 2016).

Quality parameters

Physiological loss of weight (PLW)

The weight loss of GPPK's packed in PP and LDPE polymeric package under MAP was determined by weighing the individual package initially and on day of observation using a laboratory level weighing balance (Model CT-300, Contech Make, India) having least count of 0.001g (Ranganna, 1995).

$$PLW\,(\%) = \frac{(\text{Initial Weight - Final Weight})}{\text{Initial Weight}} \times 100 \quad \ldots\ldots\ldots\ldots(1)$$

Firmness

Texture was determined as firmness / hardness. The fresh GPPK's are tender and, as they dry out they become hard. As the MAP of the kernels have been done it is expected the kernels to store fresh and maintain tenderness characteristic. Texture analysis was done on 10 fresh GPPK's from each treatment stored under refrigerated condition (7-8°C temperature and 80-85% RH). The force necessary to compress the GPPK's was measured using Texture Analyzer-Stable Micro System Model TA-HD Plus, Hamilton, MA (Szisesnaik, 1963; Brown, 1967 and Ranganna, 1995).

Color measurement

The change in color also serves as a parameter for judging the freshness and quality of fresh GPPK's while storage. Colour of the fresh GPPK's was measured using a Colorimeter (Model CR-410, Konika Minolta Make, USA). On 40th day of storage, the 'L', 'a' and 'b' values were noted. Colour of GPPK's was measured to calculate the change in colour after storage under refrigerated condition. Colour deviation is calculated by using formula (Ranganna, 1995) -

$$\Delta E = \text{Colour Deviation} = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2} \quad (2)$$

Here, L = lightness ($\Delta L = L_2 - L_1$ = Final value – initial value); a = red-green axis ($\Delta a = a_2 - a_1$ = Final value – initial value); and b = yellow-blue axis ($\Delta b = b_2 - b_1$ = Final value – initial value)

Moisture content

The moisture content of fresh GPPK's was determined by using the hot air oven (Swastik, Scientific Instruments Pvt. Ltd. make, India). It's operating temperature range between 0°C to 100°C. The flat metallic plate of known weight was taken. Nearly 15 g sample was spread on the dish. The weight of sample plus dish was noted. The hot air oven was maintained at 100°C. The samples were heated at this temperature for 16-18 hr. The sample was reweighed and the initial moisture content was determined by the following formula (Ranganna, 1995).

$$\text{Moisture Content}\,(\%) = \left(\frac{\text{Initial Weight of sample - Final Weight of sample}}{\text{Initial Weight of sample}}\right) \times 100 \ldots(3)$$

Sensory evaluation

To ascertain sensory status, samples were evaluated for overall visual quality, color, odor, texture, taste and overall acceptance using a 9-point hedonic scale with 10 trained panelists. For product attributes and acceptance, scores above 5 were considered acceptable (Ranganna, 1995).

Headspace gaseous composition

The in-pack gaseous composition measurement was done as per the method adopted by Singh *et al.* (2014). A single hole covered with silicon septum was made in polymeric package for measurement of gas concentrations directly with gas analyzer (Model 902 D DualTrak O_2/CO_2 Analyzer, USA) at each day and gaseous composition O_2/CO_2 was recorded.

Microbial analysis

The microbial profile of GPPK's on 40th day of storage was studied by standard plate count method in completely randomized design. One gram of sample was taken for microbial analysis. Serially diluted samples (1.0 ml) were spread plated on Potato Dextrose Agar and Nutrient agar to determine fungi and bacteria, respectively. The plates were incubated, observations recorded and results are expressed as colony forming units (CFU) per gram of sample (Charpe *et al*., 2016). The data was subjected to statistical analysis using software Web Agri Stat Package - 1 (WASP-1) (http://www.ccari.res.in).

RESULTS AND DISCUSSION

This is a unique work as for the first time any effort for minimal processing and passive modified atmospheric processing of GPPK's has been made and therefore discussion is done logically and appropriate references are provided where they are available.

Physiological loss of weight (PLW)

The variation in PLW in different treatments kept for storage study under refrigerated condition at the end of 40th day was found to be minimum (0.94%) for treatment having the blanching of 4 min at 75°C temperature and packaging in PP pouches of 300 gauge thickness. It was found to be maximum (3.02 %) in case of treatment having the blanching of 4 min at 85°C temperature and packaging in PP pouches of 200 gauge thickness.

From Fig.1 (A&B) it is evident that PLW varies with change in duration. At low blanching duration PLW was the least. As duration of blanching increased the PLW also increased upto certain point and again it decreased with increase in duration and decrease in blanching temperature. It also revealed that PLW decreased with increase in thickness of packaging material. This may be due to the fact that at refrigerated condition there is slow rate of respiration as compared to ambient and also high thickness allows minimum permeability for gases, so due to slow respiration the weight loss was observed to be the least, hence the independent parameter thickness of packaging material shows indirect correlation with PLW. The results confirm the findings of Drake *et al*. (1981), Jowitt *et al*. (1987) and Jayas and Jeyamkondan (2002) those reported loss in physiological weight under storage.

The variation in firmness in different treatments kept for storage study under refrigerated condition at the end of 40th day was found to be minimum (3.5 N) for treatment having the blanching for 4min at 75°C temperature and packaging in 100 gauge thick PP pouches and it was found to be maximum (4.5 N) in case of treatment having the blanching for 7 min at 85°C temperature and packaging in 300 gauge thick PP pouches.

From Fig. 2 (A&B) it is evident that firmness decreased with increase in blanching temperature and duration and increased with increase in thickness of packaging material. Thus, from Fig. 2 (A&B) it is clear that thickness of packaging material show significant difference as compared to blanching temperature and duration. Though, minimum blanching temperature and duration and high thickness of packaging material that slows the rate of respiration by preventing the transpiration of gases, maintains the tenderness of GPPK's. Loss of firmness has been earlier also observed by Gangwar (1972) in ripe fruits and by Singh *et al*. (2014) in chick pea sprouts.

Color deviation

The color deviation in different treatments kept for storage study under refrigerated condition at the end of 40th day was found to be minimum (48.10%) for treatment having the blanching at 85°C temperature for 1 min and packaging in 300 gauge thick PP pouches and it was found to be maximum (60.12%) in treatment having the blanching at 85°C temperature for 1 min and packaging in 300 gauge thick LDPE pouches.

From Fig.3 (A&B) it is evident that color deviation decreased with increase in temperature and increase in duration and increased with increase in thickness of packaging material. These results are supported by the findings that the citric acid curtails activity of enzyme polyphenol oxidase, thus, reducing the browning of shelled GPPK's due to phenol synthesis (Macheix *et al*., 1990).

Moisture content

The variation in moisture content in different treatments kept for storage study under refrigerated condition at the end of 40th day was found to be minimum (48.66%) for treatment having the blanching at 85°C temperature for 4 min and packaging in 200 gauge thick PP pouches and it was found to

Table 1: Optimization constraints for different variables and responses for GPPK's stored under refrigerated condition

Name	Goal	Lower Limit	Upper Limit
Blanching temperature (°C)	is in range	75	95
Blanching duration (min)	is in range	1	7
Thickness of packaging material (gauge)	is in range	100	300
Type of bag	is in range	PP of D	LDPE of D
PLW (%)	Minimize	0.939	3.125
Firmness (N)	Maximize	3.5	4.5
Chlorophyll (%)	Maximize	16.12	21.66
Color deviation (delta E)	Minimize	48.10	60.12
Moisture content (%)	Maximize	48.66	63.2
Sensory evaluation	Maximize	7.6	8.2

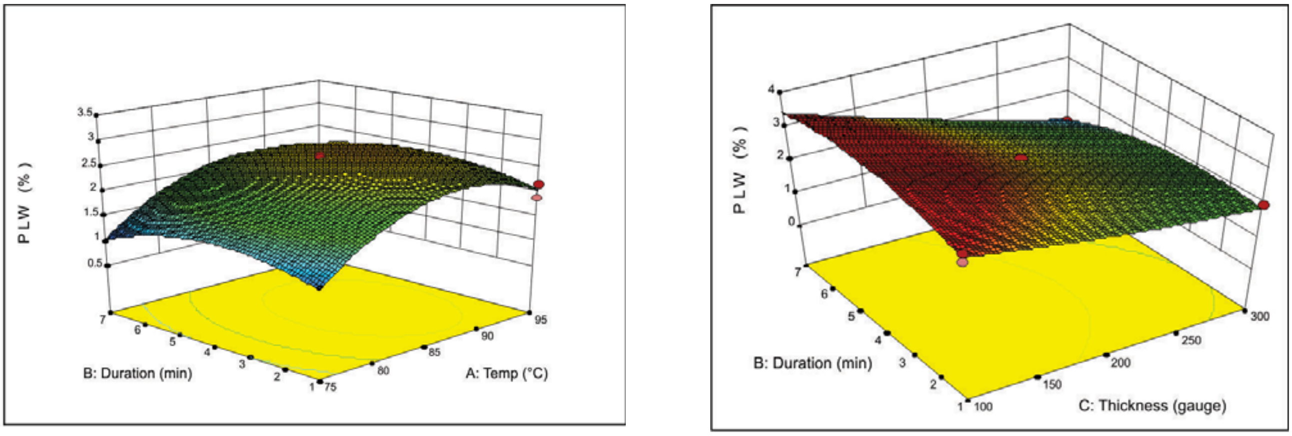

Figure 1 (A&B): Effect of blanching temperature, blanching duration and thickness of packaging material on PLW of GPPK's kept under refrigerated condition Firmness

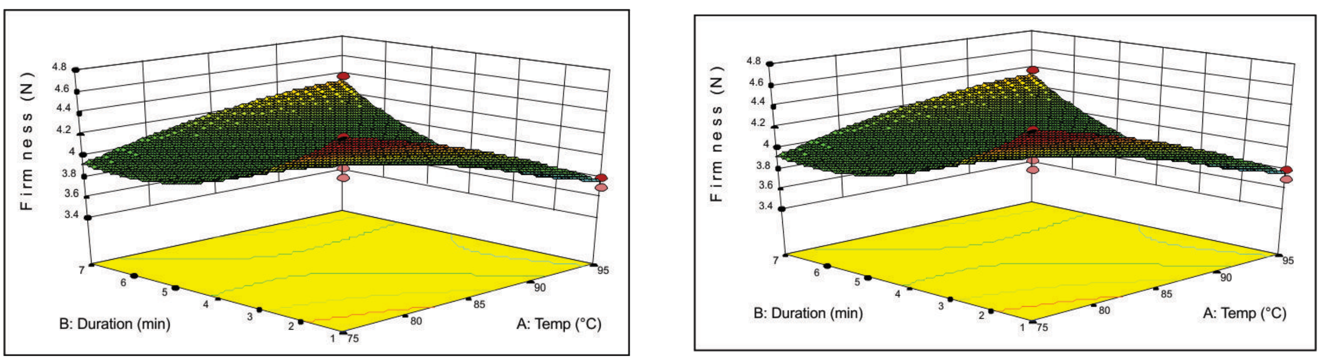

Figure 2(A&B): Effect of blanching temperature, blanching duration and thickness of packaging material on firmness of GPPK's kept under refrigerated condition

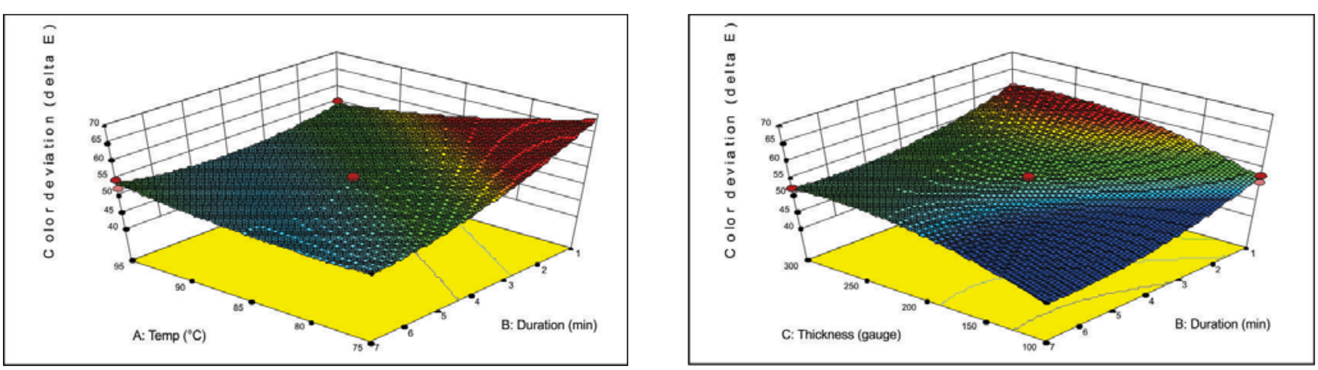

Figure 3 (A&B): Effect of blanching temperature, blanching duration and thickness of packaging material on color deviation of GPPK's kept under refrigerated condition

be maximum (63.20 %) in case of treatment having the blanching at 95°C temperature for 7 min and packaging in 200 gauge thick LDPE pouches.

From Fig. 4 (A&B) it is evident that initially moisture content was the maximum at low temperature and duration of blanching. As temperature and duration of blanching increased upto certain level, moisture content decreased and then again moisture content increased as temperature and duration of blanching increased. This may be due to the fact that at low temperature with less exposure time evaporation losses are less; evaporation losses increases due to increase in temperature and exposure to certain extent (Jowitt *et al.*, 1987). Also, it is revealed that moisture content increased with increase in thickness and increase in duration of blanching. This may be due to the fact that at ambient condition there is high rate of respiration and hence GPPK's packed in higher thickness of packaging material shows maximum moisture content as high thickness allows minimum permeability for transmission of gases as explained by Rai *et al*. (2002).

Sensory evaluation

The variation in sensory status of GPPK's in different treatments kept for storage study under refrigerated condition at the end of 40th day was found to be minimum (7.6) for treatment having

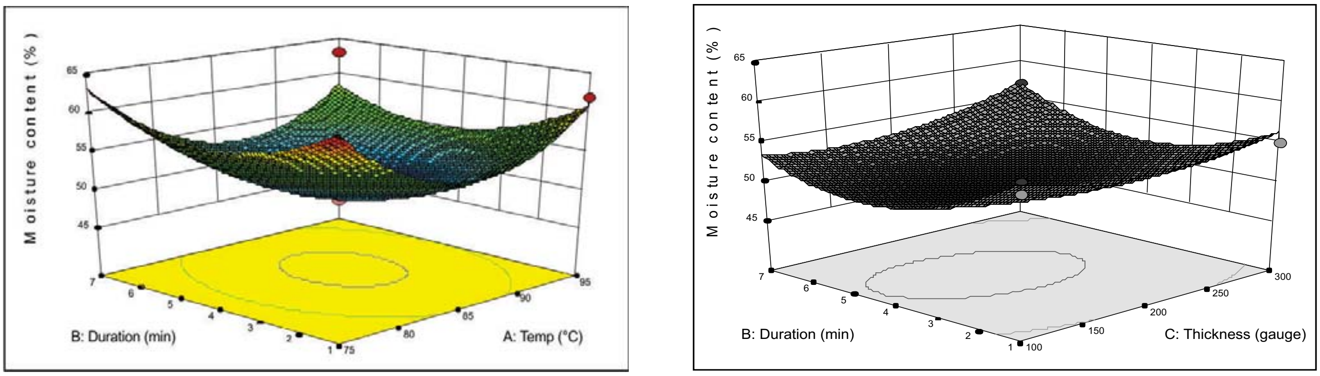

Figure 4 (A&B): Effect of blanching temperature, blanching duration and thickness of packaging material on moisture content of GPPK's kept under refrigerated condition

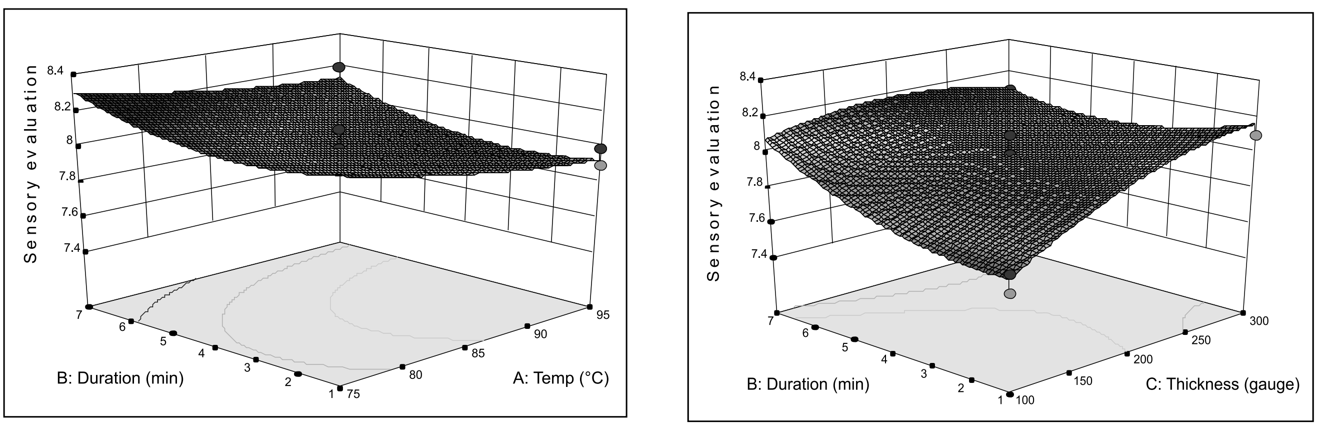

Figure 5(A&B): Effect of blanching temperature, blanching duration and thickness of packaging material on sensory status of GPPK's kept under refrigerated condition

the blanching at 85°C temperature for 1 min and packaging in 100 gauge thick PP pouches and it was found to be maximum (8.2) in case of treatment having the blanching at 75°C temperature for 7 min and packaging in 200 gauge thick PP pouches.

From Fig.5 (A&B), it is evident that sensory status deteriorated with increase in temperature and improved with increase in duration of blanching. It may be due to the fact that higher temperature causes loss of aroma (Kader *et al*., 1989 and Carla Barbosa *et al*., 2015) whereas prolonged exposure at low temperature results into partial cooking of grains thus increasing aroma. Also, it is revealed that sensory status improved with increase in thickness of packaging material. Similar results were obtained by Kenawi *et al*. (1992) when they studied the effect of packaging materials and storage conditions on vitamin C retention in green pepper, spinach and guava.

Optimization of independent variables for GPPK's stored under refrigerated condition

For numerical optimization the desired goals for each factor and responses were chosen as shown in Table 1. On the basis of numerical optimization the combination having the maximum desirability value i.e. 1.0 selected as the optimum condition for MAP of GPPK's under refrigerated condition was blanching temperature 95°C, blanching duration 6.70 min and thickness of packaging material 235 gauge thick PP pouches in which 1.45% physiological loss in weight, 4.41N firmness, 55.69% color deviation, 58.67% moisture content and sensory status of 8.17 was obtained.

Thus, the optimized packaging conditions for best shelf life of GPPK's under refrigerated condition are -

Blanching temperature (ºC)	:	95
Blanching duration (min)	:	7
Thickness of packaging material (gauge)	:	200 (PP)

These results indicate that the shelf life of green pigeon pea kernels may be improved greatly by minimal processing and storage under cold condition and are in confirmation with the findings of Keditsu *et al*. (2003) those have recorded improvement in shelf life of mandarins by minimal processing and storage under cold conditions.

Headspace gaseous composition of GPPK's stored under refrigerated condition

The variation in headspace gaseous composition, (O_2 and CO_2 %) of GPPK packets from the first day to 40th day of the treatment (95°C for 7min and 200 gauge of PP material) nearest to optimized treatment (95°C for 6.70 min and 235 gauge of PP material) kept for storage study under refrigerated condition is presented in Fig 6.

The headspace gaseous composition of the sample blanched at 95°C for 7min and packed in 200 gauge polypropylene

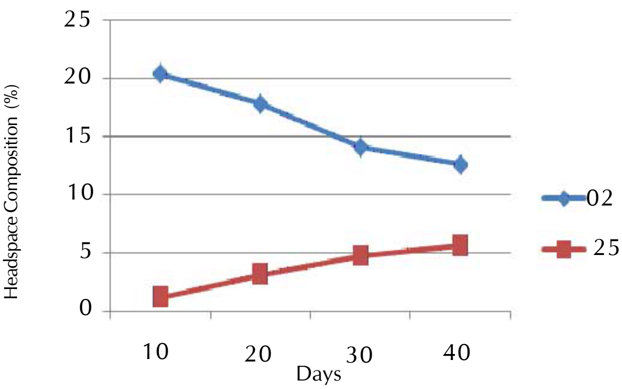

Figure 6: Variation in headspace gaseous composition of GPPK packets stored under refrigerated condition

which was the treatment nearest to the optimized treatment (95°C for 6.70 min and 235 gauge of PP material) shows that, O_2 was found to be decreased from 20.95 % to 12.6 % and CO_2 was found to be increased from 0.003 % to 5.6 % as storage period increased up to 40 days under refrigerated condition. These results are in accordance with the findings of Kader (1987), Fonseca *et al.* (2002), Ho and Young (2004) and Finnegan *et al.* (2013) where they recorded increase in CO_2 level with decrease in O_2 level under storage.

Microbial load

The data generated through microbial analysis of GPPK's by standard plate count technique was subjected to statistical analysis using software Web Agri Stat Package-1 (WASP-1). The bacterial load of GPPK's stored under refrigerated condition was observed to be minimum (0.67 x 10^4 cfu/gm) and significantly superior over other treatments in the treatment 95°C for 4 min and packed in 300 gauge of PP material on 40th day of packaging. Optimized treatment 95°C for 7 min and packed in 200 gauge of PP material was at par with this treatment with a bacterial load of 1.00 x 10^4 cfu/gm. The fungal load of GPPK's stored under refrigerated condition was observed to be minimum (0.67 x 10^4 cfu/gm) and significantly superior over other treatments in the optimized treatment 95°C for 7 min and packed in 200 gauge of PP material on 40th day of packaging.

On the basis of quality parameters as well as microbial count blanching of the GPPK's in 0.24% citric acid with 0.24% KMS solution at 95°C for 7 minutes and packaging in PP bags of 200 gauge thickness was the most suitable for preserving GPPK's under refrigerated condition. Being a commodity it is necessary that the microbial load in packaged GPPK's should be less than the threshold level as per the safety standards (Snyder, 2003). In current findings, microbial load of packaged GPPK's in optimized conditions are found to be less than the threshold level set for the microbial safety of minimally processed vegetables (Rajkowski and Baldwin, 2003)

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