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STUDY OF DIFFERENT TECHNIQUES TO REDUCE MICROBIAL LOAD IN NUTS

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Abstract

Nuts are very nutritive generally used in food and beverages preparation. In modern perspective, the shelf life extension and its quality is important parameter to be taken in consideration for its processing in food industry. Microbiological and chemical studies have been carried on different batches of received raw nuts stored in woven sack bag contained LDPE bag. For reducing microbial load in nuts four method of treatment was chosen. They are autoclave sterilization, Hot air oven pasteurization, Vacuum oven pasteurization, Microwave oven pasteurization. All four methods temperature kept constant and time is varying. In autoclave sterilization there will be nil microbial count and increasing in all physic-chemical parameters. In vacuum oven pasteurization there will be decreasing in microbial load as the time increasing. And all physic-chemical parameters are coming within specification. In hot air oven pasteurization there will be decreasing in microbial load as the time increasing but sensory and physic-chemical properties of almonds got affected. In micro wave oven sensory parameters of nuts got affected as the time increasing there will be development of roasting flavour and texture of nuts became crunchy.

Keywords: Autoclave Sterilization; Physico-Chemical Analysis; Hot Air Pasteurization; Vacuum Oven Pasteurization Acid Value; Peroxide Value.

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1. Introduction

Nuts are used in various raw materials in many industries as well as for direct consumption. The relatively high cost of animal protein as well, compared with plant protein in suggest an increasing market for the latter and nuts have attracted interest as a potential source of supplementary protein for human food. There are several products available from the nuts and by its use. They contain an important amount of protein and fat. Due to the extremely high fat,

protein and low water content it can be easily spoilage by bacteria. Moulds can grow upon them if they stored in improper conditions that can permit sufficient moisture for their growth and propagation. Therefore, this topic was found to be interest, in this study to investigate the microbial contamination of different treated nuts compared with raw nuts. Nuts having longer shelf life (up to 2 years), nutritional parameter of nuts will not change but there will be major changes in flavor, texture, and appearance. The reactions causing these undesirable changes can be very complex. Nuts with kernels are very less prune to microbial contamination because it contains protective layer outside, whereas seeds of nuts are more prone to chances of getting contamination especially yeast and mould. Yeast and moulds is spore forming and can grow at the temperature of 10-50˚C, and growing at lower moisture foods having water activity of <0.85. In view of the above discussion and found research gap from literature study following objectives has been decided for this study [6].

2. Materials and Methods

2.1. Autoclave Sterilization

Autoclave sterilization also called as steam heat sterilization is usually done at 121˚C for 20min. But higher the temperature for longer time will affect the texture and sensory attributes of nuts. Reduce the microbial count as well as to retain the sensory attributes four different temperature and time combination have been selected. Keeping temperature constant and varying the time for different minutes like: 121˚C for 20 min, 15min, 10 min and 5min respectively.

Process flow chart of autoclave sterilization

Weigh the nut sample and transfer into retort pouch seal tightly

⇩

Place the sample inside the autoclave and close lid

⇩

Autoclave at 121℃ for 5 min.

⇩

Take out the sample from autoclave after completion of the cycle

2.2. Vacuum Oven Pasteurization

Vacuum oven method also called as dry heat pasteurization under vacuum where modified atmosphere will be created inside the oven sterilizing in the absence of air and reducing the microbial activity by 90% retaining the sensory attributes of the nuts. We choose four time and temperature combination here temperature is constant with varying time like: 90˚C for 45 min, 30 min, 25 min and 1 hr respectively.

Process flow chart of vacuum oven pasteurization

Clean the inside part of oven by using 70% of IPA solution

⇩

Close the oven properly and tight the valve

⇩

Set the air pressure to 100 psi

⇩

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Set the temperature up to 90˚C

⇩

Simultaneously other sides prepare the sample for keeping

⇩

Clean the glass Petri plate by using 70% IPA solution

⇩

Place the sample in Petri plate

⇩

Once temperature reaches to 90˚C open the door of oven place the sample inside and Close the door tightly

⇩

Create the vacuum at 100psi and note the time

⇩

Once treatment get over remove the sample from oven, transfer it in to aluminium pouches seal it tightly

2.3. Hot Air Oven Pasteurization

Hot air treatment also called as dry air pasteurization method. In this method samples are treated at different time keeping the temperature constant. The general temperature for hot air oven is 105˚C for 15 min, 25 min, 35 min and 45 min respectively.

Process flow chart of Hot air oven pasteurization

Switch on oven set the temperature at 105˚C

⇩

Take glass Petri plates wipe it with 70% alcohol

⇩

Place the sample in each Petri plate

⇩

Place the Petri plates inside the oven once it reaches 105˚C

⇩

Close the door of oven note the exact time

⇩

Remove the sample from oven transfer it in to Aluminium pouches seal it tightly

2.4 Microwave oven method

Microwave oven method also called as roasting. Roasting will impart flavour development in nuts and also microbial growth reduction. Nuts were treated at different time like 1 min, 2:30 min, 3:30 min and 5 min.

Process Flow Chart of Microwave oven method

Clean the inside microwave oven by using 70% IPA solution

⇩

Select four well cleaned Petri plates clean again with 70% IPA solution

⇩

Place nut samples in each Petri plates and transfer the Petri plates inside oven

⇩

Set the time for different minutes

⇩

Remove the sample from oven transfer it in to aluminium pouches seal it tightly

2.4. Microbial Analysis

Total Plate Count

Serial Dilution:
Weigh 10 gm of nut sample grind the nut sample by using pestle and motor. Prepare decimal dilutions of the grinded by transferring 10gm into 90ml of pre-sterilized diluents to get the stock solution of 10^{-1} dilution. From this test tube, transfer 1ml of solution into a test tube containing 9ml of diluents to obtain dilution of 10^{-2}. Similarly, prepare further dilutions as per requirement.

Pour Plate Technique:
Raise the petridish lid and add 1 ml of sample to the dish with a micropipette. Touch only the upper half of the sterile pipette when withdrawing it from its wrapper. Draw enough of the sample into the pipette so it is above the volumetric line to be measured. Lower the petridish lid and mix the plate count agar and sample with a gentle circular motion. Do not splash the mixture over the edge of the Petri dish. Allow the media to solidify, then invert the dish and incubate at the 37℃ temperature.

Yeast and Mould:
Serial Dilution:
Weigh 10 gm of nut sample grind the nut sample by using pestle and motor. Prepare decimal dilutions of the grinded by transferring 10gm into 90ml of pre-sterilized diluents to get the stock solution of 10^{-1} dilution. From this test tube, transfer 1ml of solution into a test tube containing 9ml of diluents to obtain dilution of 10^{-2}. Similarly, prepare further dilutions as per requirement.

Pour Plate Technique:
Raise the petridish lid and add 1 ml of sample to the dish with a micropipette. Touch only the upper half of the sterile pipette when withdrawing it from its wrapper. Draw enough of the sample into the pipette so it is above the volumetric line to be measured. Lower the petridish lid and mix the CYGA (chlromphenical yeast glucose agar) agar and sample with a gentle circular motion. Do not splash the mixture over the edge of the Petri dish. Allow the media to solidify, then do not invert the dish and incubate at the 25℃ temperature.

2.5. Physic-Chemical Analysis

Moisture
Procedure
Initially dry the nickel dish beside its lid in the oven for 30minutes at 95^{O}C. Cool it in a desiccators for about 45minutes and weigh the dish with its lid to the nearest 0.1mg (M1).

Transfer the homogenized nut sample into the dish and record the weight with the lid to the nearest 0.1mg (M2).Place the dish beside its lid into the oven without draught, at ordinary pressure Dry the whole sample for 5hours at 105±2^{O}C. Then place the lid onto the dish and let cool to room temperature in desiccators for 45 minutes. Weigh the closed dish to the nearest 0.1mg (M3).

Calculation:

Moisture content% by mass = $\frac{(M_2-M_3)\ x100}{M_2-M_1}$

$M_{1:}$ Mass in grams of the empty dish.

$M_{2:}$ Mass in grams of the empty dish + Test portion.

$M_{3:}$ Mass in grams of the empty dish + Test portion after

Water Activity

Procedure

Use the Aqua lab duo on an even level surface under controlled environment temperature. Connect the main power supply & switch-on the instrument. For best results of measuring high a_w (>0.9 a_w), let Aqua lab duo to warm-up for 15minutes. Fill the grinded nut sample cup half (do not overfill), Minimum sample amount should cover bottom of the sample cup. Ensure that the rim and outside of sample cup are clean. Ensure that the sample temperature is not more than 4^{o}C above the chamber temperature. Place the sample cup in sample chamber. Close lid carefully to avoid spillage. Move latch to the left side to start analyzing the samples. Aqua lab duo gives a beep sound once while starting a_w measurements. The first a_w reading will display in a minute or two. Aqua lab duo beeps after the analysis is over. Samples final a_w reading, temperature and moisture content will be displayed in the provided digital screen.

Acid Value

Procedure

Weigh the 50 gm of nuts grind it in to fine powder by using mixer or pestle and motor. Soak the sample in 150 ml of petroleum ether in 250 ml of amber conical flask. Keep it for 24 hours in dark condition Mix the oil or melted fat thoroughly before weighing. Weigh accurately a suitable quantity (Refer below table) of the cooled oil or fat in a 250ml conical flask. Add 50ml of freshly neutralized hot ethyl alcohol and 1ml of phenolphthalein indicator solution. Boil the mixture for about five minutes (till the solution starts boiling) and titrate while as hot as possible against standard aqueous alkali (0.1N sodium hydroxide) solution, shaking vigorously during titration, till the appearance of pale pink color. The pink color must persist for 30sec. Note down the titer value, when pale pink color appears.

Calculation:

Acid Value = $\frac{\text{Titre value x Normality of alkali x 56.1}}{\text{Sample weight}}$

FFA % as Oleic acid = $\frac{\text{Titre value x Normality of alkali x 28.2}}{\text{Sample weight}}$

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Peroxide Value

Procedure

Weigh the 50 gm of nuts make in to fine powder by using mixer. Soak the powdered sample in 150 ml of petroleum ether and mix well. Keep aside for 24 hours in darker condition. Mix the oil or melted fat thoroughly before weighing. Weigh accurately 5g of the cooled oil or fat in a 250 ml iodine flask. Add 30 ml of Solvent mixture & mix well. Add 1 ml of freshly prepared, saturated potassium iodide solution. Stopper the flask immediately and swirl to dissolve. Keep the flask in dark for 2 minutes with occasional shaking. After that, add 50 ml of distilled water to the flask. [Part of the water is used for the rinsing of the stopper and the washings are also transferred into the flask]. Using Starch indicator solution, titrate the contents of the flask against 0.02N sodium thiosulphate [hypo] with vigorous shaking solution till the disappearance of blue colour. Carry out the experiment in duplicates for better interpretation of results and Note down the titre value. Carry out/conduct a blank determination and Note down the titre value.

Calculation:

$$\text{Peroxide value (meq/kg)} = \frac{(T-B) \times N \times 1000}{W}$$

Where,

T=Sample Titre Value (Volume in millilitres of 0.02 N sodium thiosulphate consumed by sample)

B=Black Titre value.

W= weight in gram of sample taken.

N = normality of sodium thiosulfate solution.

3. Result and Discussion

The main motto is to reduce microbial load as well as retain sensory attributes and quality parameters. Firstly we did steam sterilization by autoclave in different timings here temperature is constant i.e. 5 min, 10 min,15 min, 20 min, for doing sterilization we packed almonds in retort pouches and sealed tightly. Retort pouch which is act as good barrier against heat and steam. After sterilization we did microbial analysis we kept in observation for five days for results, after five days of results we concluded that there is no growth in all four trails, but there is some changes in quality attributes and also in sensory attributes. In quality parameter water activity, moisture and non-volatile ether extract got increased this is due to steam absorption by almonds during sterilization , due to that almonds absorbed some extra moisture during sterilization this leads to increase in water activity, moisture, and non-volatile ether extract. Among four trails we selected one trail which is having less microbial growth and less water activity, moisture and non-volatile ether extract for sensory evaluation. Secondly we have chosen vacuum oven method where vacuum is applied at different pressure that is at 100 psi and 200 psi at same temperature for different timings i.e. 45 min, 30 min, 25 min, 1 hour. In that we did microbial analysis for all four samples, we kept observation for 5 days. After five days we took microbial count for all samples. We chose one sample from all four then conducted sensory for selected sample.

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Table 1: Various process parameters and autoclave sterilization

Methods	**Parameters**	**1**				
		Autoclave Sterilization				
Time and Temperature		Control	121˚C for 5 min	121˚C for 10 min	121˚C for 15 min	121˚C for 20 min
Microbiology Parameters	TPC	380	40	10	<10	<10
	Yeast and Mould count	200	<10	<10	<10	<10
Sensory Parameters	Colour	5	2	2	2	2
	Appearance	5	2	1	1	1
	Taste	5	2	1	1	1
	Aroma	5	2	1	1	1
	Mouth feel/Texture	5	2	1	1	1
Physico-chemical Parameters	Moisture(% by mass)	4.16	4.2	4.52	5.32	5.83
	Water activity	0.4657	0.592	0.629	0.693	0.712
	Non-volatile ether extract(%by mass in DB)	50.83	42.18	41.23	40.29	39.38
	Acid value (% as oleic acid)	0.19	0.23	0.26	0.28	0.29
	Peroxide value(meq/kg)	4.38	7.12	7.03	6.8	6.58

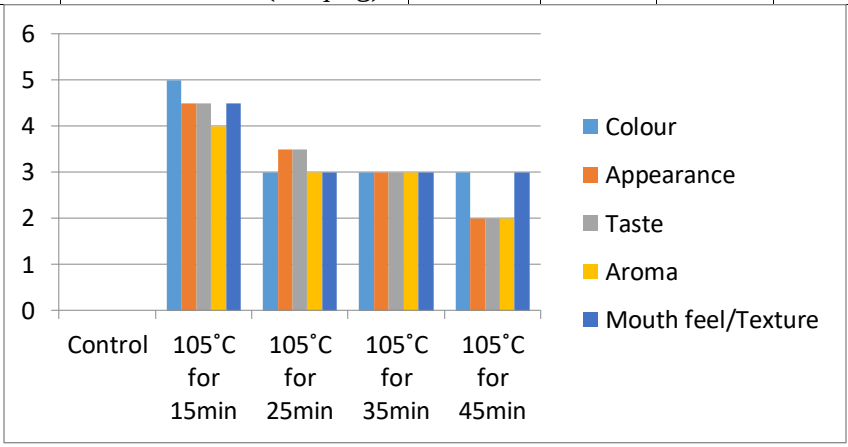

Table 2: Various process parameters and vacuum oven sterilization

Methods	**Parameters**	**2**				
		Vacuum oven sterilization				
Time and Temperature		Control	90˚C for 25 min	90˚C for 30 min	90˚C for 45 min	90˚C for 1 hour
Microbiology	TPC	450	150	100	60	30

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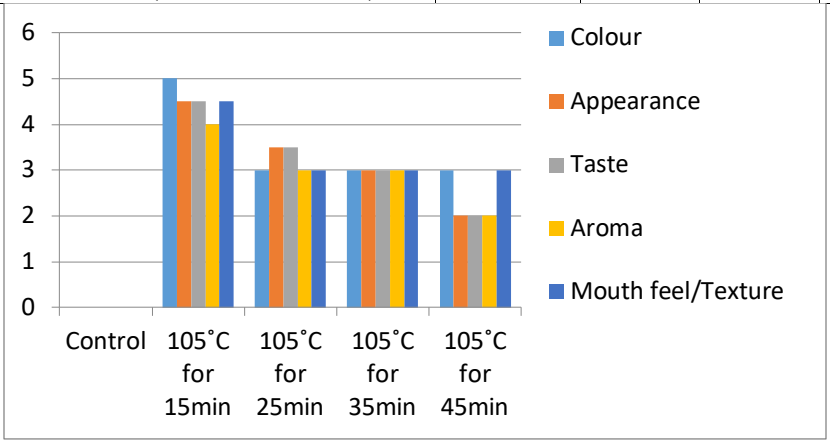
Parameters	YM	230	90	70	30	10
Sensory Parameters	Colour	5	4.5	4	4	3
	Appearance	4.5	4	4	4	3.5
	Taste	4	4	4	4	3
	Aroma	4	4	4	4	3
	Mouth feel/Texture	4	4	4	4	3
Physico-chemical Parameters	Moisture(% by mass)	4.2	3.25	3.39	2.84	2.35
	Water activity	0.4645	0.4362	0.4385	0.4232	0.4201
	Non-volatile etherextract(%by mass in DB)	53.23	36.48	37.12	39.1	40.08
	Acid value (% as oleic acid)	0.18	0.16	0.16	0.17	0.19



In vacuum oven method we observe that moisture got decreased slightly compared to the control. And also water activity got decreased slightly. Peroxide value and acid value is not varied much the readings are within specification. Moisture and water activity decreased due to moisture loss during heat treatment 90˚c is the higher temperature where moisture can evaporate up to certain level. After treatment the sample took for moisture analysis already some of the moisture loss during heat treatment again for moisture sample kept in oven for 5 hours so again sample dried to bone dry condition.

After microbial analysis and sensory evaluation we observed gradual difference in microbial load in each sample after counting colonies of each different trail we have selected one trail from each method which is having less microbial growth as well as more score in sensory evaluation. We did analysis for choose sample again and conducted the sensory analysis.

Third method we have chosen is hot air oven method in which almonds are treated at same temperature for different timings. i.e. 105˚C for 15, 25, 35, 45 min. After treatment all four samples are did microbial analysis. After that all four samples are kept under 5 day's observation.

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Table 3: Various process parameters and hot air oven sterilization

Methods	**Parameters**	**3**				
		Hot air oven sterilization				
Time and Temperature		Control	105˚C for 15min	105˚C for 25min	105˚C for 35min	105˚C for 45min
Microbiology Parameters	TPC	360	170	80	40	10
	YM	150	140	30	20	10
Sensory Parameters	Colour	5	3	3	3	3
	Appearance	4.5	3.5	3	2	3
	Taste	4.5	3.5	3	2	3
	Aroma	4	3	3	2	3
	Mouth feel/Texture	4.5	3	3	3	3
Physico-chemical Parameters	Moisture(% by mass)	4.23	3.06	2.93	2.5	2.18
	Water activity	0.4628	0.4598	0.4581	0.4523	0.435
	Non-volatile ether extract(%by mass in DB)	52.13	30.03	29.39	28.26	29.06
	Acid value (% as oleic acid)	0.18	0.17	0.17	0.16	0.16
	Peroxide value(meq/kg)	4.45	3.65	3.63	3.59	3.47

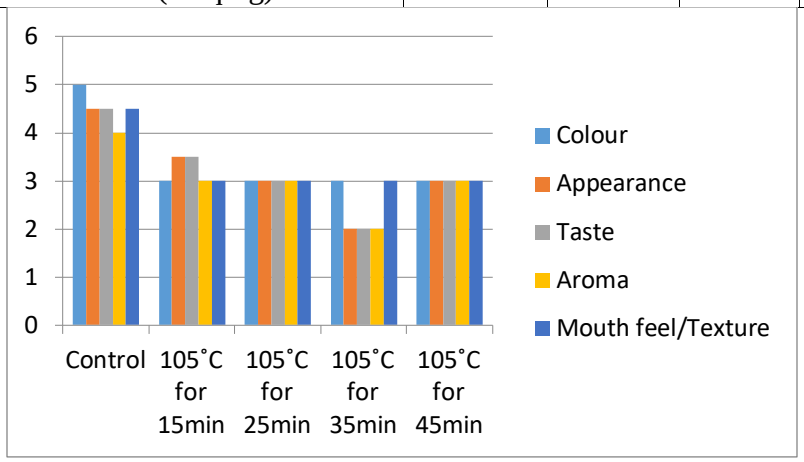

Table 4: Various process parameters and microwave oven sterilization

Methods	**parameters**	**4**				
		Microwave oven				
Time and Temperature		control	1 min	2 min	3:30 min	5 min
Microbiology Parameters	TPC	480	300	200	70	50
	YM	250	150	90	40	20
Sensory	Colour	4.5	3	2	2	2

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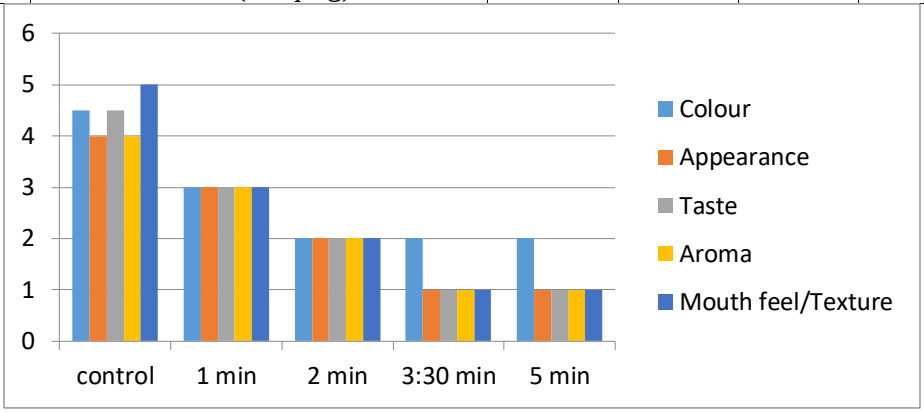
Parameters	Appearance	4	3	2	1	1
	Taste	4.5	3	2	1	1
	Aroma	4	3	2	1	1
	Mouth feel/Texture	5	3	2	1	1
Physic-chemical Parameters	Moisture(% by mass)	4.19	3.72	3.5	3.1	1.52
	Water activity	0.4638	0.4529	0.4028	0.3824	0.3986
	Non-volatile ether extract(%by mass in DB)	52.36	51.32	51.93	50.36	49.96
	Acid value (% as oleic acid)	0.2	0.13	0.15	0.16	0.18
	Peroxide value(meq/kg)	3.09	3.09	3.18	3.28	3.43



In hot air oven method we observe that there is decrease in moisture content and water activity due to moisture loss during heat treatment whereas non-volatile ether extract and peroxide value, acid value meeting specification. Fourth method we have chosen is micro wave oven treatment in that method samples are treated to different timings in heat treatment mode respective timings are 1 min, 2 min, 3:30 min, and 5 min. After heat treatment all four samples are analysed for microbial analysis. All four samples kept under observation for 5 days then we have chosen one sample which is having low microbial count and good sensory attributes. The five min treated sample is having low microbial count but in sensory wise it get roasted more. Loses all sensory parameters. In physic chemical parameters moisture and water activity got decreased all other parameters are meeting specification.

In the part of sensory analysis one product is accepted based on the rating. Sensory analysis is conducted by 5 semi trained panels. Duo trio test was conducted it means four selected samples kept for sensory analysis including control among three samples the sample which is having similar taste to control was to be identified and ratings has to give based on that. Based on the ratings mean should be calculated for each sample and the sample which is having higher mean should be finalised. In the final result of sensory analysis we finalised vacuum oven for 90˚c for 45 min. This is meeting all sensory attributes of control sample. The rating given by all 5 sensory panels for this sample is 4 which mean sample is good. There will be five ratings given for all four samples, ratings given by number each number is having different description i.e. 5 means very good 4 means good 3 means 2 means 1 means poor. Based on considering different ratings

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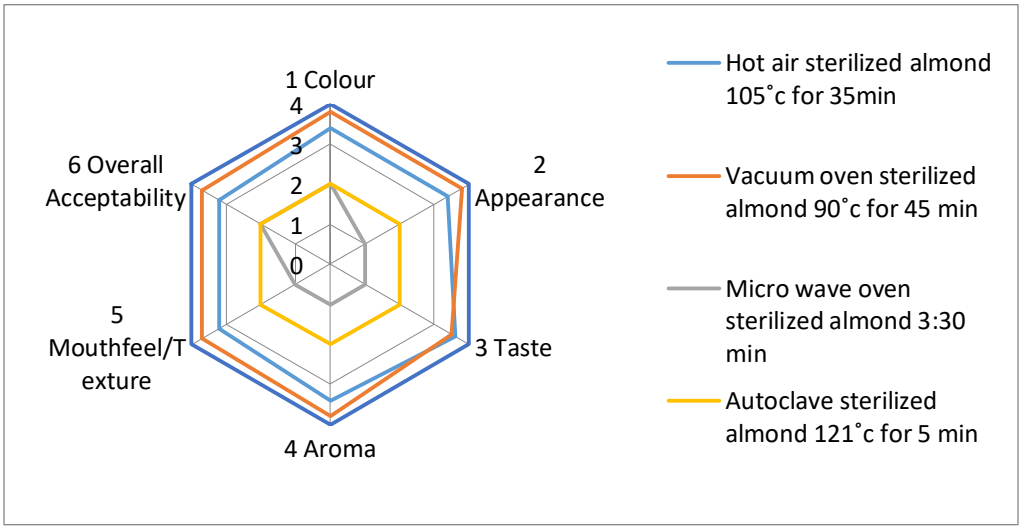
given by 5 semi trained panel, vacuum oven (90˚C for 45min) has been finalised. This is meeting all quality attributes and also having similar sensory parameters of control.

Sensory Analysis

Table 5: Sensory evaluation parameters and panels score

S.No.	Product Parameter	Hot air sterilized almond 105˚C for 35min	Vacuum oven sterilized almond 90˚C for 45 min	Micro wave oven sterilized almond 3:30 min	Autoclave sterilized almond 121˚C for 5 min	Control
1	Colour	3.4	3.8	2	2	4
2	Appearance	3.4	3.8	1	2	4
3	Taste	3.6	3.5	1	2	4
4	Aroma	3.4	3.8	1	2	4
5	Mouthfeel/Texture	3.2	3.7	1	2	4
6	Overall Acceptability	3.2	3.7	2	2	4



4. Conclusions

Vacuum oven method is best for controlling microbial load and to minimise sensory attributes of nuts. From all four methods we finalised vacuum oven method (90˚c for 45 min). In this method colour of sample and texture of sample is same as like control sample. Microbial result also came within specification. Finally vacuum oven sterilization suggested for preventing microbial load in nuts. After sterilization proper storage and packaging is necessary to prevent contamination from surrounding. If proper package is not done again re contamination of nuts may happen.

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