DEVELOPMENT OF READY TO EAT MINT SAUCE AND EFFECT OF STORAGE ON ITS PHYSICOCHEMICAL, PHYTOCHEMICAL AND ORGANOLEPTIC CHARACTERISTICS

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Abstract

The investigation entitled studies on development of ready to eat mint sauce was undertaken with objectives tostandardize the method of preparation and preservation of mint sauce and to ascertain its shelf stability at room temperature. For this study, mint and other ingredients were analyzed for their physicochemical, phytochemical and organoleptic quality. Guar gum and CMC were added in the combination of 0.3%+0.3% and homogenization was carried out at 7400RPM for 7-8 minutes at 20°C to overcome the problem of separation in the final product. The samples prepared were stored in five batches i.e. control, preserved with KMS, preserved with sodium benzoate, preserved with combination of KMS+ sodium benzoate and thermal processing at room temperature and refrigeration for a storage interval of four months. The control sample could retain shelf life of 27 days at room temperature and 56 days under refrigeration. Samples preserved with sodium benzoate showed maximum nutrient retention and were found to be highly acceptable by the panel of semi trained judges as a result of organoleptic evaluation.

Keywords: physicochemical, phytochemical, organoleptic, mint, sauce, room temperature,

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Introduction

Sauce, a french word taken from the latin *salsa*,is generally used to add flavor, moisture and visual appeal to another dishes (Anonymous 2, 2016). It is one of the most important and versatile Indian condiments that can be used in many different ways.

It is commonly served with many appetizers like *Tikka, Tikki, Samosa, Pakora* and so on; and also works great as a spread for sandwiches, rolls or as a side to dishes like *Biryani* and other rice dishes. It is best used as a condiment with grilled or fried food, but may be used as a spread or dip too (Anonymous 1, 2008). These are popular as they offer a refreshing taste to both ready to eat foods and meals, and are also believed to be nutritious (Ghosh *et al*, 2004).

Mint sauce, also called as green sauce, can be commonly used as an accompaniment in many Indian meals. The sauce,appreciated for its unique flavor and convenience, also, due to its various health benefits, contributes significantly in maintaining the nutritional status of the population.

The present investigation was undertaken with the objectives tostandardize the method of preparation and preservation of ready to eat mint sauce and to ascertain its shelf stability at room temperature.

Materials and methods

The present study on the development of ready to eat mint sauce was carried out in the Department of Food Science and Technology, Punjab Agricultural University, Ludhiana.

Mint and other ingredients

Mint, onion, garlic, green chili, good quality crystal sugar, salt and spices and condiments such as black pepper powder, cumin powder, cloves powder, cinnamon powder, cardamom powder was procured from the local market for the preparation of ready to eat mint sauce. Synthetic vinegar (4% acetic acid) was added as a Class II preservative for saucepreparation

Optimization of levels of ingredients

The sauce was made with different concentrations of salt, sugar, spices and condiments. Salt was tried from 10.2-5 g, sugar from 8-18 g, onion from 40-25 g, garlic from 4.4-1 g, green chili from 4.2-1 g, cumin from 2.55 to 0.25 g, cloves from 1.0 to 0.04 g, cinnamon from 2.0 to 0.025g, cardamom from 1.0 to 0.05 g, black pepper from 2.0 to 0.05 g and synthetic vinegar from 0.4 to 1.08%. The final recipe was standardized with TSS 23°B and acidity 1.04%

Optimization of hydrocolloid level and homogenization treatment

A problem of separation of serum from solids was observed during the preparation of sauce, to overcome which, hydrocolloid optimization was carried out. Guar gum and CMC were used to overcome the problem of separation in the final product. Different concentrations of guar gum and CMC i.e. 0.1% to 0.5% were used and combination of guargum + CMC in 0.3%+0.3% was selected on the basis of organoleptic evaluation. But, this couldn't solve the problem completely; hence, the process of homogenization was carried out. The homogenization was tried at 2400, 3600, 4800, 6000, 7200, 7400 and 7600 RPM for 2, 4, 6 and 8 minutes. The process solved the problem of suspension and gave the product a smooth texture and consistency. It was standardized at 7400RPM for 7-8 minutes at 20°C.

The addition of guar gum and CMC in combination of 0.3%+0.3% and homogenization at 7400RPM for 7-8 minutes showed maximum suspension and hence, were chosen for storage.

Optimization of levels of sodium benzoate and KMS for final storage

The sauce was prepared and stored in five different batches where first batch was control and second, third, fourth and fifth batches were treated with KMS, Sodium benzoate, KMS+ Sodium benzoate and thermal processing respectively. The KMS and sodium benzoate were added as per FSSA standards.

Storage studies

Saucewas packed in glass bottles and glass jars and stored at room temperature (16-32°C) and under refrigeration (5°C) for about four months. The effect of storage on physicochemical, phytochemical and organoleptic characteristics was analyzed at fixed interval of one month.

Analytical methods

Total solids

Moisture content was estimated by following the method of AOAC (2005). One gram of fresh mint leaves were weighed for analysis of fresh product and five grams of prepared product was weighed in pre-weighed aluminium dish, dried in an oven at 60-65°C to a constant weight. The weight of the samples was taken after cooling the moisture dishes in a dessicator.

$$\text{Total solids} = \frac{\text{Mass of dried sample}}{\text{Mass of initial sample}} \times 100$$

Total soluble solids

Total soluble solids content of raw material as well as the product was determined by using a hand refractometer (Erma, Japan) with scale ranging from 0 to 32°B for mint leaves, as well as mint sauce and readings were obtained. The observations were corrected to 20°C and the values were expressed as total soluble solids (AOAC 2005).

Titrable acidity

The titrable acidity was determined following the method of (Ranganna, 1997) by titrating a known quantity of sample solution against standard 0.1 N NaOH solution to a faint pink color in the presence of phenolphthalein indicator. One gram of fresh leaves and prepared ready to eat mint coriander sauce was taken for estimation. Volume was made up to 100ml with distilled water and filtered through Whatman filter paper no.4.Ten ml aliquot was taken and after adding 1-2 drops of phenolphthalein indicator, it was titrated against 0.1 N NaOH to faint pink end point. The % acidity was expressed as per the source of acid used.

$$\text{Acidity (as acetic acid) (\%)} = \frac{\text{Titre value x 60 x normality of NaOH x volume made up}}{\text{Weight of sample x volume of aliquot x 1000}} \times 100$$

Total phenols

Total phenols were determined by colorimetric method described by Swain and Hills (1959). Five gram of fresh mint leaves as well as prepared sauce was taken and refluxed with 80%

methanol for two hours in a round bottom flask and residue was then further refluxed for one hour. After filtration of the extract volume was made to 100 ml with 80% methanol. Filtrate (0.5 ml) was taken into a test tube containing 0.5 ml water. The Folin- Ciocalteau reagent (5 ml) then kept for 5 min, and saturated solution of sodium carbonate (1 ml) was mixed. Absorbance of the developed color after 60 minutes was measured at 765 nm using spectronic- 20 spectrophotometer. A standard curve was plotted by taking known amount of Gallic acid as reference standard.

Ascorbic acid

Ascorbic acid was extracted from the sample with 0.4 per cent oxalic acid and determined by titrimetric method using 2, 6-dichlorophenol indophenol dye solution (0.04 per cent) which was standardized against standard L-ascorbic acid (0.1 mg/ml of 0.4 per cent oxalic acid). Sample taken for estimation was 1g for fresh mint leaves and 5 g for mint sauce. In case of fresh samples, the leaves were crushed and then volume was made to 100 ml with oxalic acid, and in case of prepared sauce, 5 g of sample was taken and volume was made to 100 ml directly with oxalic acid. It was filtered through Whatman filter paper no.4. Ten ml aliquot was taken and was titrated with standardized dye and the end point was recorded as pink color, which persisted for atleast 15sec. The results were expressed as ascorbic acid mg % of sample (Ranganna 1997).

$$\text{Ascorbic acid (mg /100 g)} = \frac{\text{Titre value x dye factor x volume made up}}{\text{aliquot of extract taken x weight of sample}} \times 100$$

Total chlorophyll

Total chlorophyll of the fresh leaves as well as final product was determined following the method given by Nagata and Yamashita (1992). The fresh leaves were crushed and the sauce samples were taken as such. 1g sample was weighed and put into mortar and 0.1 g of Na_2CO_3 was added. The tissue was mascerated with pestle. All the pigments were extracted with acetone: hexane 4:6 at once and then the optical density of the supernatant was checked at 663 and 645 nm and the values of chlorophyll a, Chlorophyll b and Total Chlorophyll were estimated

Chlorophyll a $= 0.999\ A_{663} - 0.0989\ A_{645}$

Chlorophyll b $= 0.328\ A_{663} + 1.77\ A_{645}$

Total Chlorophyll = Chlorophyll a + Chlorophyll b

Antioxidant activity

Free radical scavenging activity was determined by DPPH (2, 2- di phenyl L picrylhydrazyl) method. A method according to Shimada *et al*(1992) was followed with some modifications. Five gram of mint leaves and mintsauce were taken and refluxed with 80% methanol for two hours in a round bottom flask and residue was then further refluxed for one hour. After filtration of the extract volume was made to 100 ml with 80% methanol. To 1ml of methanolic extract of sample, 2ml of 1mM freshly prepared DPPH and 1ml of 50 mM tris buffer was added and absorbance was determined at 517 nm (blank as 80 per cent methanol and tris buffer) after 30 minutes. The free radical scavenging activity was evaluated by comparing the absorbance of the sample solution with control solution to which distilled water was added instead of sample, 2ml of 1mM DPPH and 1ml of 50 mM tris buffer. DPPH was taken as standard.

$$\text{Radical scavenging activity (\%)} = \frac{\text{Control OD (0 min)} - \text{Sample OD (30 min)}}{\text{Control OD (0 min)}} \times 100$$

Viscosity

Viscosity of sauce was determined at 20°C by using Brookfield viscometer (model LVT) using spindle number 3.

Organoleptic evaluation

The organoleptic evaluation of the samples were conducted by a panel of eight semi-trained panellists for appearance, consistency, mouthfeel, flavor and overall acceptability using the 9-point Hedonic Rating Scale (Larmond, 1970) as scores 9, 8, 7, 6, 5, 4, 3, 2 and 1 represented liked extremely, liked very much, liked moderately, liked slightly, neither liked nor disliked, disliked slightly, disliked moderately, disliked very much and disliked extremely respectively.

Statistical Analysis

Results were analyzed statistically for their interpretation using completely randomized design experiment as discussed by Singh *et al* (1991). Each value presented is a mean of three observations.

Results and discussion

Physicochemical and phytochemical composition of mint leaves

The mint had 13.27 % total solids and 8.07% TSS, 0.3% acidity, 8.01% ascorbic acid, 207.97mg/100g total phenols, 16.22% antioxidant activity and 0.98 mg/g total chlorophyll. Singh *et al* (2001) also found mint having higher moisture i.e. 88.2 %. Kizhedath and Suneetha (2011) obtained 6.17mg/g total chlorophyll while estimating the chlorophyll content in mint leaves.

Physicochemical and phytochemical characteristics of spices and condiments

Acidity, total phenols, antioxidant activity and total chlorophyll content of onion, garlic, green chili, cumin, cloves, cinnamon, cardamom and black pepper were analyzed under physicochemical and phytochemical characteristics. Cumins had the highest acidity content of 2.22 % and onion and garlic had the lowest i.e. 0.04 %. Highest ascorbic acid content was found in green chili that is 45.62 mg/100g and lowest in garlic that is 9.11mg/100g. All the spices were found to be rich in total phenols and antioxidant activity. Cinnamon had the highest amount of total phenols that is 6.81g/100g where as black pepper had the lowest that is 0.77g/100g. About antioxidant activity, cloves had the highest antioxidant activity of 92.44 % and green chili had the lowest that is 0.34 % amongst all the tested spices. Similar results were obtained by Shobhana and Naidu (2000) and Nuutila *et al*(2002) while studying antioxidant activity of selected Indian spices and analyzing antioxidant activities of onion and garlic extracts respectively.

Analysis of physicochemical and phytochemical characteristics of control sample

Zero day analysis of physicochemical and phytochemical characteristics of control sample was done in the month of February. Amongst the physicochemical analysis, total solids, total soluble solids, acidity and viscosity were tested. Total solids were found to be 23.12 %, TSS 23°B, acidity 0.98 % and viscosity 746 P. Total phenols, antioxidant activity, ascorbic acid and total chlorophyll were tested amongst the phytochemical characteristics and the values came out to be 348.26mg/100g, 19.21%, 5.92mg/100g and 46.66 mg/100g respectively. The control sample retained shelf life of 28 days at room temperature, after which it became organoleptically unacceptable.

The samples treated with KMS, sodium benzoate, KMS+ sodium benzoate and thermal processing were stored at room temperature for four months and effect of storage and method of preservation on physicochemical, phytochemical and organoleptic quality of mint saucewas studied at a fixed interval of one month.

Effect of storage and method of preservation on physicochemical characteristics of ready to eat mint sauce

Total solids, total soluble solids, acidity and viscosity were observed under physicochemical characteristics and a non significant change was found in total solids, total soluble solids and acidity during storage.The results were in agreement with Ahmed *et al* (2003) who also found non-significant change in total solids while studying the storage characters of coriander leaf puree. Similar results were found in green chili puree by Ahmed *et al*(2002). Ahmed and Shivhare (2001) found non- significant change in total soluble solids while studying the storage characteristics of garlic puree/paste. Jasim Ahmed (2004) also found similar results while studying the storage characteristics of ginger paste.

Effectof storage and preservative on viscosity of ready to eat mint sauce samples was found to be significant ($p<0.05$).(Fig 1) The samples with sodium benzoate were found to be most viscous, as, sodium benzoate, being compatible with guar gum, has an interesting property of increasing the viscosity of the sample (R.J. Chudzikowski, 1971). These were followed by samples preserved with KMS+ sodium benzoate, KMS and the least viscosity was observed in thermally processed samples. The viscosity decreased significantly with storage time in all the samples andleast was found in thermally processed samples (Fig 1).The decrease in viscosity with increase in storage time was also observed by Ahmed *et al*(2004) while studying the rheology of coriander leaf puree and Jasim Ahmed (2004) while studying the rheological behavior and color changes of ginger puree during storage. Decrease in viscosity or consistency of the puree can be attributed to softening of tissues as a result of alteration in the structure of macromolecular polymeric substances forming the cell walls (Rudra *et al*, 2008)

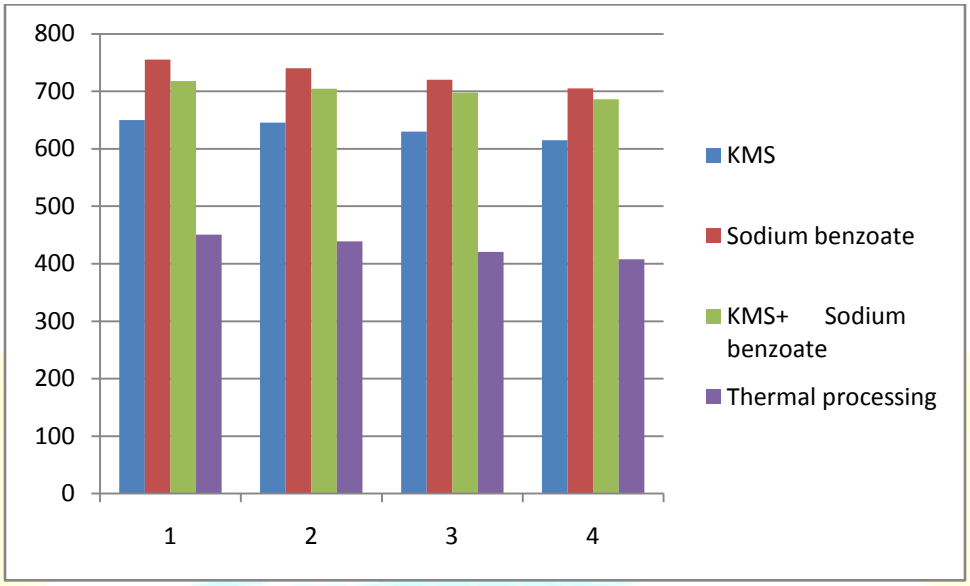

Fig 1: Effect of storage and method of preservation on viscosity (poise) of ready to eat mint *sauce* samples

Effect of storage and method of preservation on phytochemical characteristics of ready to eat mint sauce

Total phenols, ascorbic acid, total chlorophyll and antioxidant activity were observed under phytochemical characteristics and significant ($p<0.05$) reduction was observed during the storage period (Fig 2 and 3) Out of the four samples stored, maximum loss of phytochemical characteristics was observed in thermally processed samples which could be due to degradation of components by heat and light. Similar results were also observed by Burdurlu *et al* (2006) in citrus juice concentrates. Loss of chlorophyll in thermally processed samples is due to the fact that green vegetables contain chlorophyll, which breaks down on exposure to heat during thermal processing. (Gupte *et al*, 1964)(Buckle and Edwards, 1970). Shin and Bhowmik, (1995) also analyzed similar reasons for chlorophyll while studying the thermal kinetics of color changes in pea puree. Maximum decrease was found in the thermally processed samples which could be mainly due to its degradation with heat and light (Conesa *et al*, 2009.) Decrease in antioxidant activity in all the samples during storage could be due to decrease in bioactive compounds such as ascorbic acid, total chlorophyll and total phenols as a result of thermal degradation. Anese *et*

al (1999) also found decrease in antioxidant activity of preheated tomato juice for short heat treatments.

Highest phenolic content was found in the samples containing sodium benzoate which can be due to the reason that sodium benzoate combines with ascorbic acid to form benzene, which directly contributes in increasing the number of total phenols (Gardner and Lawrence 1993). The results were agreement with Conesa *et al*, 2009 while studying the changes in bioactive compounds and antioxidant activity during homogenization and thermal processing of tomato puree. Similar results were also obtained by Jacobo *et al*, 2011 while studying the influence of ultra-high pressure homogenization on antioxidant capacity, polyphenol and vitamin content of clear apple juice.

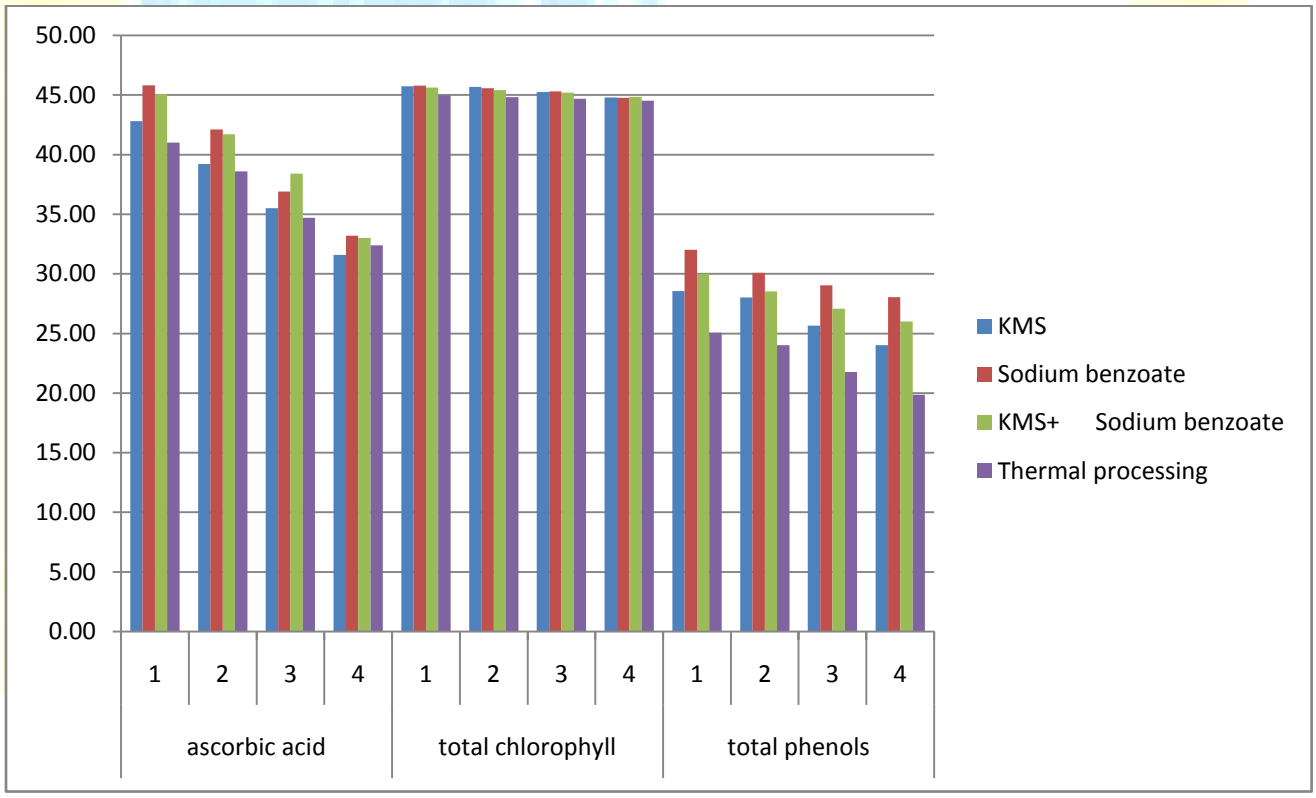

Fig 2: Effect of storage and method of preservation on *ascorbic acid(mg/100g), total chlorophyll (mg/100g) and **total phenols(mg/100g) of ready to eat mint *sauce* samples

* All values of ascorbic acid are multiplied by a factor of 10 to show them in same graph

**All values of total phenols are divided by a factor of 10 to show them in same graph.

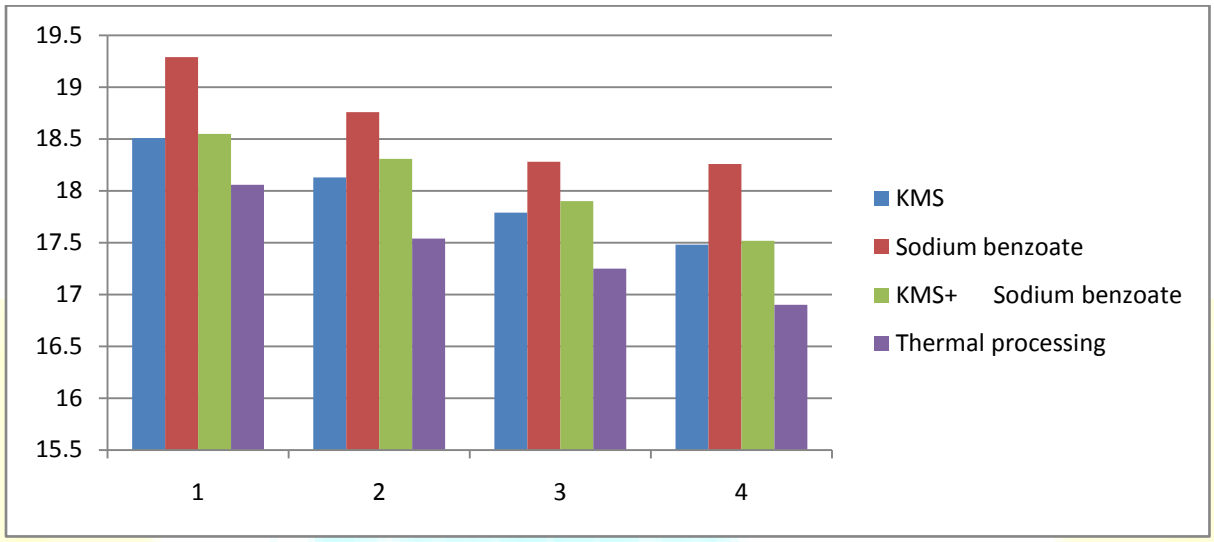

Fig 5: Effect of storage and method of preservation on antioxidant activity (%) of homogenized ready to eat mint *sauce* at room temperature

Effect of storage and method of preservation on organoleptic evaluation of ready to eat mint sauce during storage

Effect of storage on organoleptic evaluation of ready to eat shelf stable mint sauce is depicted in table 1. Appearance, consistency, mouth-feel, flavor and overall acceptability were the major parameters which were considered out of which, the effect of storage on appearance and flavor was found to be non-significant. Sallam *et al* (2007) also determined non-significant changes in appearance and flavor while studying the Chemical quality and sensory attributes of marinated Pacific saury (*Colola bissaira*) during vacuum-packaged storage at 4 °C. Similar results were found by Kezban Candogan (2002) while studying the effect of tomato paste on some quality characteristics of beef patties during refrigerated storage.

Consistency, mouthfeel and overall acceptability of sauce were found to be significant ($p<0.05$) during storage. The sauces preserved with sodium benzoate scored highest in terms of consistencyand mouthfeel followed by the sauces preserved with KMS+ sodium benzoate, followed by KMS and least scores were obtained by thermally processed samples.

In terms of overall acceptability, sauces preserved with sodium benzoate were most liked by the panelists followed by the samples preserved with KMS+ sodium benzoate, samples preserved with KMS and least scores were given to thermally processed samples. However, all the sauces were found to be under acceptable limits by the semi trained panelists. Celik *et al* (2006) observed consistent decrease in overall acceptability while analyzing the physicochemical and organoleptic properties of Yogurt with Cornelian Cherry past.

Table 1: Effect of storage and method of preservation on organoleptic quality (9 point hedonic scale) of homogenized ready to eat mint *sauce* at room temperature

Treatments	**Storages (months)**			
	1	**2**	**3**	**4**
Appearance				
KMS	8.63	8.63	8.50	8.63
Sodium Benzoate	8.75	8.75	8.88	8.75
KMS+Sodium Benzoate	8.63	8.63	8.75	8.63
Thermal Processing	8.50	8.50	8.56	8.50
Consistency				
KMS	8.25	8.25	8.25	8.25
Sodium Benzoate	8.63	8.63	8.63	8.50
KMS+Sodium Benzoate	8.38	8.38	8.31	8.31
Thermal Processing	8.13	8.13	8.00	8.00
Mouthfeel				
KMS	8.50	8.50	8.44	8.44
Sodium Benzoate	8.69	8.63	8.63	8.56
KMS+Sodium Benzoate	8.56	8.56	8.56	8.50
Thermal Processing	8.25	8.25	8.19	8.13
Flavor				
KMS	8.75	8.81	8.81	8.75
Sodium Benzoate	8.94	8.94	8.88	8.88
KMS+Sodium Benzoate	8.88	8.88	8.88	8.81

Thermal Processing	8.69	8.75	8.75	8.69
Overall Acceptability				
KMS	8.53	8.55	8.50	8.52
Sodium Benzoate	8.75	8.73	8.75	8.67
KMS+Sodium Benzoate	8.61	8.61	8.63	8.56
Thermal Processing	8.39	8.41	8.38	8.33

	ANOVA				
	MSS				
CD	**Appearance**	**Consistency**	**Mouth-feel**	**Flavor**	**Overall Acceptability**
Treatment (T)	NS	0.257	0.238	NS	0.138
Storage (S)	NS	NS	NS	NS	NS
T X S	NS	NS	NS	NS	NS

Conclusion

The present study was carried out with the objectives tostandardize the method of preparation and preservation of ready to eat mint sauce and to ascertain its shelf life stability at room temperature. Mint was taken along with other ingredients such as onion, garlic, green chilli, salt, sugar, cumin, cloves, cinnamon, cardamom, black pepper, synthetic vinegar and water were taken to formulate the recipe. The problem of separation of solids was observed to overcome which, guar gum and CMC were added in combination of 0.3%+0.3% and samples were homogenized at 7400 RPM for 7-8 minutes at 20°C. Once the recipe was formulated, the sauce was prepared in five batches i.e. control, samples preserved with KMS, sodium benzoate, KMS+ sodium benzoate and thermal processing. These samples were stored at room temperature and then tested for their physicochemical, phytochemical and organoleptic characteristics. The control sample could retain shelf life of around 28 days at room temperature. The samples preserved with KMS, sodium benzoate, KMS+ sodium benzoate and thermal processing were studied for four months for their physicochemical, phytochemical and organoleptic characteristics. Physicochemical characteristics studied were total solids, total soluble solids, acidity and viscosity. Non significant change was observed in total solids, total soluble solids and acidity of sauce but a significant reduction was seen in viscosity during storage. Also, the phytochemical

characteristics i.e. total phenols, antioxidant activity, total chlorophyll and ascorbic acid showed a significant ($p<0.05$) reduction. Out of all the four samples studied, maximum reduction was found in thermally processed samples which may be due to degradation of phytochemical characteristics by heat and light, and the highest were found in the samples preserved with sodium benzoate. As per organoleptic evaluation, the effect of storage on flavor and appearance was found to be non significant. The samples stored under refrigeration with sodium benzoate as preservative ranked best in terms of consistency, mouthfeel and overall acceptability.

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