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Shelf-life Assessment of Tulsi and Stevia Incorporated Low Sugar Kadaknath Egg Jam

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ABSTRACT

The study was conducted during from September–January, 2020–2021 in the department of Livestock Products Technology, college of veterinary science and animal husbandry, Mhow, Madhya Pradesh, India. The Kadaknath egg jam was developed to increase nutritional content and decrease sugar content, jam with 1% tulsi and stevia was found suitable and most acceptable by sensory panelists. Hence, selected for further storage stability under refrigeration. TBA, FFA value and microbial count of values for control jam were non-significantly (p<0.05) lower as compared to developed tulsi incorporated low sugar kadaknath egg jam throughout the entire storage. A progressive and significant (p<0.05) increased in the TBA and FFA values of control as well as developed tulsi incorporated kadaknath egg jam observed with advancement of storage. The total plate count (TPC) followed a steadily significantly (p<0.05) increasing pattern from 0–15 day in control as well as developed tulsi incorporated low sugar kadaknath egg jam. Psychrotropic counts as well as Yeast and Mold count were not detected on 10 and 15 day of storage respectively in both control and as well as developed tulsi incorporated low sugar kadaknath egg jam. Coliform were not detected during the entire period of storage in both control as well as developed tulsi incorporated low sugar kadaknath egg jam. The observation indicated that microbial count and rancidity level as well as sensory attributes remained well below the permissible level and product was stable up to 15 days of storage under refrigeration (4±1°C).

KEYWORDS: Kadaknath egg jam, storage, tulsi

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1. INTRODUCTION

Kadaknath or kali masi (fowl having black flesh) is only greyish black meat breed of India. It is local breed of Jhabua and district Dhar of western Madhya Pradesh, reared by tribal communities of Jhabua. The bird is very popular in local region and nationally nowadays due to its adoptability, disease resistance and taste of the meat, texture, flavour and medicinal properties. Egg is rich source of protein, vitamin A, iron, vitamin B12, riboflavin, choline, and the second lowest-cost source for zinc and calcium (Sophie et al., 2019). Egg is also the rich source of fat which is considered as bad, due to the low-density cholesterol (LDL) which leads to cardio vascular diseases (CVD). However, recent studies have shown that regular egg consumption does not increase the risk of stroke and cardiovascular diseases (Jung and Wayne, 2018).

Jam is an example of fruit preserve usually made from pulp and juice of one fruit (whole fruit) with inclusion of more than 50% sugar in its composition. It can be defined as cooked and gelled fruit purses packaged for long term storage which is normally used as bread spread, fillings and food jellies. The preparation of fruit jam traditionally involves the use of pectin / sugar/ honey and citric acid as well. Good jam has a soft even consistency without distinct pieces of fruit, a bright colour, good flavour and a semi-jelled texture that is easy to spread but has no free liquid.

Sugar is the substance which is addictive (Wiss et al., 2018) and numerus studies show that it leads to weight gain and detrimental to cardiometabolic and diabetic patients (Tauseef and Sievenpiper, 2016). WHO guideline recommends adults and children reduce their daily intake of free sugars to less than 10% of their total energy intake. A further reduction to below 5% or roughly 25 g (6 teaspoons) day^{-1} would provide additional health benefits. (Anonymous, 2015). As the low sugar food helps in many nutritional benefits like diabetes, reduced cardio vascular diseases, decision making, and weight management. Thus, substitutions of the processed sugar with the sugar substitutes help in the acceptability of the products by the consumer. The fruit jam contains 50–60% sugar of its weight. Thus, reduction of sugar in jam and substitution with the natural sugar substitutes may help to reach the health goals of the consumer.

Stevia leaves powder which is a natural plant (*Stevia rebaudiana*) with active compound as srevioside and rebaudioside of 0 calories and have 150–400 times sweeter than table sugar (Margaret, 2015). It may be an excellent alternative for low sugar protein rich jam. It helps the patients to fight against obesity (Margaret, 2015) it is a good source of natural antioxidants and also helps in treating metabolic syndrome, diabetes, hypertension and dyslipidaemiaetc (Areli et al., 2017).

Basil (Ocimum basilicum) is one of the oldest spices, which is widely used in the flavouring of confectionary, baked goods and condiments. Basil oil was also used in perfumery as well as in dental and oral products with its antimicrobial activity (Suppakul et al., 2003). The methanol extracts of basil leaves show significant inhibitory zones against 10 bacteria and 4 yeast strains, which is observed under electron microscope (Kaya et al., 2008). Looking to the facts and current global demand of the consumer, the present study was undertaken.

2. MATERIALS AND METHODS

The study was conducted in department of Livestock Products Technology from September–January, 2020–2021, at college of veterinary science and animal husbandry, Mhow, Indore, Madhya Pradesh, India. Commercial kadaknath eggs for the experiment were procured from department of poultry science/nearby market place. The kadaknath egg was cracked by holding it between the thumb and fore finger and striking against hard platform. The albumen and yolk were separated and isolated by yolk separator. Ingredients used in the study like cream, corn starch, sugar, xanthan gum, were procured from standard firm and local market as per availability and requirement of the study. All the chemicals used in the study were of analytical grade and procured from Hi Media laboratories (P) Ltd, Mumbai and Tulip Diagnostics (P) Ltd, Goa, India. The food grade glass jars were sourced from local market for packaging and were pre-sterilized by exposing to U.V. light for 30 m before use.

2.1. Preparation of Kadaknath egg jam

Kadanath eggs were cracked by holding it between the thumb and fore finger and striking against hard platform. The albumen and yolk were separated and isolated by yolk separator. The dry ingredients i.e., sugar, corn flour, xanthan gum pre weighed were added to the measured quantity of cream, egg albumin and egg yolk and mixed to in a glass jar. The glass jar was kept in the water bath at 100°C 25 minutes. The jam was then cooled to room temperature and immediately packaged in the glass jar and stored at 4±1°C. Functional Kadaknath egg jam was prepared by incorporation of tulsi leaves by replacing stevia and added and abbreviated as: C-Kadaknath egg jam without tulsi leaves and stevia, T-Kadaknath egg jam with 1.0% tulsi leaves and stevia P* (Table 1).

2.2. Analysis of product

pH was determined by using digital pH meter (WTW, Germany, model pH 330i) by immersing the spear type combination electrode (Sentix®, Germany) directly into sample.Prior to measurement, pH meter was calibrated

Table 1: Formulation for the development of tulsi incorporated low sugar kadaknath egg jam

Ingredients	Control	Functional
Egg albumin+Yolk	48.17	48.17
Cream	21.5	21.5
Corn starch+Xanthan gum	4.5	4.5
Sugar	26.25	26.25
Stevia P*	P*	P*
Tulsi leaves	0.0	1.0

C (control): Kadaknath egg jam without tulsi leaves, T: Kadaknath egg jam with (1.0%) tulsi leaves; stevia P*: Patent

every time as per the manufacturer's instructions using known buffers of pH 7.0 and 4.0. Reading was taken twice for each sample and average of reading was taken as pH of sample.

TBA (Thiobarbituric Acid Value) value was estimated as per procedure given by Tarladgis et al. (1960). 10 grams of sample was taken and added to 49 ml of distilled water and 1 ml of sulphanilamide reagent (1 g of sulphanilamide dissolved in solution containing 40 ml of concentrated HCl and 160 ml of distilled water) and blended with the help of pestle and mortar. After this 48 ml of distilled water was used for washing the mortar and to it 2 ml HCl solution (diluted 1:2 with distilled water) was added. The contents were transferred to Kjeldhal flask after adding several glass beads. This was heated on high heat and 50 ml of distillate was collected into a graduated cylinder. After mixing the distillate well, a 5 ml portion was taken into a 50 ml glass stoppered flask and 5 ml of TBA reagent was added. The contents were mixed and the flask was immersed in boiling water bath for 35 m along with blank solution. The blank prepared consisted 5 ml distilled water and 5 ml TBA reagent. The flasks were then cooled under tap water for 10 m and the optical density (O.D.) was recorded at 538 nm against blank. The TBA value as mg of malonaldehyde per 1000 gram of sample was calculated using following formula:

TBA value (mg of malonaldehyde/1000 g of sample) = O.D. of sample × 7.8 .....(1)

Free fatty acid (FFA) value was determined by modified AOCS method (Koniecko, 1979). 5 g of sample of different treatments was blended with the help of pestle and mortar in 30ml chloroform in presence of anhydrous sodium sulphate. Then it was filtered through whatman's filter paper no.1 in to 150 ml conical flask. About 2–3 drops of phenolphthalein indicator (0.2%) was added to the filtrate, which was titrated against 0.1N alcoholic potassium hydroxide to get the pink color end point. The free fatty acid content of the sample was calculated as: % FFA as Oleic acid=(0.1 X ml of 0.1alcohalic KOH used in titration X 0.282/ Sample weight (g)×100 .....(2)

Microbiological analysis (Total Plate Count, lipolytic count, Coliform count and Yeast & Mould Count): Samples was prepared and analyzed as per A.P.H.A. (1992).

2.3. Sensory evaluation

The sensory quality of samples was evaluated by using 8 point descriptive scale (Keeton, 1983) where 8 denoted extremely desirable and 1 denoted extremely poor. A sensory panel (semi trained) of seven judges drawn from post-graduate students and staff of Veterinary College, Mhow after training/briefing will be requested to evaluate the product for different quality attributes viz., colour and general appearance, aroma, texture, sweetness, spread ability, mouth coating and overall acceptability.

2.4. *Statistical analysis*

The data obtained in the study on various parameters were statistically analyzed on 'SPSS-16.0' software package as per standard methods of Snedecor and Cochran (1994).

3. RESULTS AND DISCUSSION

3.1. Physico- chemical parameter

The mean value and ANOVA for pH, TBA, and FFA of control and tulsi incorporated low sugar kadaknath egg jam during storage at 5 days regular interval are presented in Table 2.

The pH of developed tulsi and incorporated kadaknath egg jam was lower as compared to control throughout the storage period. The pH value of control as well as developed tulsi incorporated kadaknath egg jam increased significantly (p<0.05) with the advancement of storage period. Although, developed tulsi incorporated kadaknath egg jam showed significant (p>0.05) difference between 0^{th} and 5^{th} days of storage period under refrigeration. subsequently, increased gradually and showed significantly (p<0.05) difference on 10^{th} day. However, on 15^{th} day of storage slight decrease in the pH value was observed. Such decline in pH might be due to the action of psychotrophic bacteria which ferment carbohydrate present in the ingredients used in the formulation of the product. The subsequent increment in the pH value was due to the liberation of metabolites from the bacterial activities as the microbial load enhance with the storage period. Tanwar et al. (2016) also observed increasing pH value during storage under refrigeration in ocimum sanctum L incorporated chicken nuggets.

The TBA value for control product was significantly (p>0.05) higher as compared to developed tulsi incorporated low sugar kadaknath egg jam throughout the storage. A progressive and significant (p<0.05) increment in the TBA values of control as well as developed tulsi incorporated

Table 2: Storage effect on the pH, TBA and FFA values (Mean±SE) of aerobically packaged low sugar tulsi incorporated kadaknath egg jam

Treatment	Storage days			
	0	5	10	15
PH				
Control	6.33±0.12[a]	6.52±0.01[b]	7.07±.0.01[d]	6.90±0.05[c]
Treatment	6.02±0.01[a]	6.23±0.01[b]	6.56±0.01[c]	6.62±0.01[b]
TBA (mg malonaldehyde/kg)				
Control	0.361±0.006[a]	0.645±0.012[b]	0.976±0.0.006[c]	1.560±0.007[d]
Treatment	0.235±0.010[a]	0.435±0.005[b]	0.863±0.004[c]	1.460±0.013[d]
FFA (% oleic acid)				
Control	0.242±0.001[a]	0.315±0.001[b]	0.563±0.001[c]	0.993±0.001[d]
Treatment	0.214±0.001[a]	0.295±0.0031[b]	0.545±0.001[c]	0.983±0.001[d]

Means bearing different superscripts (a, b, c, d…) In a row differs significantly (p<0.05)

low sugar kadaknath egg jam were observed with the advancement of storage. This could be due to increased lipid oxidation and production of volatile metabolites in the presence of oxygen during aerobic storage. Sriket and Senphan (2018) also reported 1% sweet basil powder had the lower formation of TBA values compared to other samples in 21 days storage period in basil incorporated pork emulsion sausage. Tanwar et al. (2016) also reported similar findings between treatment upto 1% tulsi incorporation as well as during storage.

FFA values are a measure of hydrolytic rancidity in food. FFA is the product of microbial or enzymatic degradation of lipids and determination of FFA gives information about stability of fat during storage (Das et al., 2008). FFA values of developed tulsi incorporated low sugar kadaknath egg jam were observed lower compared to control on each day of storage. However, during subsequent storage period the FFA value showed linear significantly (P<0.05) increasing trend from 0th to 15th day of refrigeration. It was due to the urolic acid, apignin and luteolin present in tulsi had antilopolytic activity due to which FFA was less. Smitzis et al. (2008) and Husain et al. (2017) also reported dietry natural antioxidents obtained from different herbs had positive effect on oxidative stability by producing fewer among of FFA during storage.

3.2. Microbiological analysis

The Total plate count, Psychotropic count, Coliform count and Yeast & Mold count for control and aerobically packaged tulsi incorporated low sugar kadaknath egg jam During storage at 5 days of regular interval are presented in

The total plate count (TPC) followed a significant (p<0.05) rise from 0th-15th day in control as well as developed tulsi incorporated low sugar kadaknath egg jam However, TPC did not differ significantly (p>0.05) between control and treatment with the progress of storage period. The urolic acid, apigenin, luteonin are the active principal content of tulsi was having broad spectrum antimicrobial activity Alo et al. (2012). Uikey et al. (2018) and Badole et al. (2019) also observed the similar trend in meat products.

Psychrotrophic count were not detected until 5th day of storage either in control and/or developed tulsi low sugar kadaknath egg jam. This could be due to destruction of psychrotrops during cooking. These counts were detected on 10th day of storage in both control and developed tulsi incorporated low sugar kadaknath egg jam. This might be due to recovery of injured organism and then multiplication during subsequent period of storage. Tulsi may defunct electron transport system and ion gradient and other enzymes dependent cellular mechanism of psychotropic bacteria Burt et al. (2007). Tanwar et al. (2016) also observed psychrophilic count in ocimum sanctum incorporated chicken nuggets from 14th day onwards during the storage.

Coliform were not detected during the entire period of storage in both control as well as developed tulsi incorporated low sugar kadaknath egg jam. The absence of coliform is due to their destruction during cooking above their death point of 57^{0} C. Further hygienic practices followed during and after preparation of product. Our present findings were supported by (Ben-sassi et al., 2008, Kuete et al., 2008, Windward et al., 2008) who concluded that the extract and essential oil obtained from herbs had significant (p<0.05) antimicrobial effect against almost all coliforms.

Yeast and Mould count were detected only on 15th day of storage in control and tulsi incorporated low sugar kadaknath egg jam. This might be due to absence of favourable condition (like humid climate for the growth

of yeast and mold during the experiment). The appearance of yeast and mould may be due to the fact that yeast and mould requires incubation period of approximately 10 days. Moreover, natural herbs may possess natural fungicide (Fisher and Phillips 2008, Majhenic et al., 2007).

Table 3: Microbial count (Mean±SE) of aerobically packaged aerobically packaged low sugar tulsi incorporated kadaknath egg jam under refrigeration temperature

Treatment	Storage days			
	0	5	10	15
Total plate count (cfu g^{-1})				
Control	2.84±0.01^{a}	3.46±0.10^{b}	4.14±0.10^{c}	4.92±0.09^{d}
Treatment	2.84±0.11^{a}	3.33±0.06^{b}	4.03±0.08^{c}	4.84±0.07^{d}
Psychotropic count (cfu g^{-1})				
Control	ND	ND	0.23±0.07^{b}	1.43±0.02^{c}
Treatment	ND	ND	0.17±0.03^{b}	1.68±0.03^{c}
Coliform count (cfu g^{-1})				
Control	ND	ND	ND	ND
Treatment	ND	ND	ND	ND
Yeast & mould (cfu g^{-1})				
Control	ND	ND	ND	1.34±0.04
Treatment	ND	ND	ND	1.47±0.05

*ND: Not Detected; Means bearing different superscripts (a, b, c, d…) in a row differ significantly (p<0.05)

3.3. Sensory evaluation

The mean score and ANOVA of colour & general appearance, aroma, texture, sweetness, spread ability, mouth coating and overall acceptability for control and tulsi and stevia incorporated low sugar kadaknath egg jam. during storage at 5 days of regular interval.

The score for colour and general appearance did not have any significant (p>0.05) difference between control and developed tulsi incorporated low sugar kadaknath egg jam on all storage days. The mean score for general appearance of both control and developed and tulsi incorporated low sugar kadaknath egg jam decreased gradually with increasing in storage period.

The mean aroma score between control and developed tulsi incorporated low sugar kadaknath egg jam did not differ significantly (p>0.05) in the storage period upto 5th day. However, on subsequent storage significant (p<0.05) difference was noticed from 10th day onwards. The pattern was similar for control as well as tulsi incorporated low sugar kadaknath egg jam. Husain and David (2018) in tulsi incorporated herbal Sandesh and Rai et al. (2018) reported decreased in flavour score with the advancement of storage in thulsi enriched herbal shrikand.

The mean texture score between control and developed tulsi incorporated low sugar kadaknath egg jam did not differ significantly (p>0.05) in the storage period upto 5th day. However, on subsequent storage significant (p<0.05) difference was noticed from 10th day onwards. The pattern was similar for control as well as and tulsi and stevia incorporated low sugar kadaknath egg jam. Husain and David (2018) in tulsi incorporated herbal Sandesh and Rai et al. (2018) reported decreased in texture score with the advancement of storage in tulsi enriched herbal shrikand

Mean sweetness score between control and developed tulsi and stevia incorporated low sugar kadaknath egg jam did not differ significantly (p>0.05) in the storage period upto 10th day. However, on further storage non-significant (p<0.05) decline was noticed on 15th day in developed tulsi incorporated low sugar kadaknath egg jam. The pattern was similar for control as well as and tulsi incorporated low sugar kadaknath egg jam

No significant (p>0.05) difference in the score of mouth coating between control and developed tulsi incorporated low sugar kadaknath egg jam was recorded during the storage till 5th day. However, the scores for developed tulsi incorporated low sugar kadaknath egg jam were recorded similar to previous storage days.

The mean overall acceptability score differs significantly (p>0.05) between control and developed tulsi incorporated low sugar kadaknath egg jam during the the storage. With the subsequent storage the scores were gradually decreased and showed significant (p<0.05) difference from 5th day of storage. Husain and David (2018) also observed a decreasing score for overall acceptability at room temperature with the advancement of storage in tulsi incorporated herbal Sandesh.

The developed product was fairly accepted up to 15th day under refrigeration (4±1^{o}C). However, By the action of microbes protein breakdown release ammonia and lipid oxidation lead to production of off odour sensory panelists refused acceptability of the product beyond 15 days. This observation indicated that microbial count and rancidity level as well as sensory attributes remained well below the permissible level and product was stable up to 15 days of storage under refrigeration (4±1^{o}C).

Table 4: Sensory attributes (mean±SE) of aerobically packaged low sugar tulsi incorporated kadaknath egg jam under refrigeration

	Storage days			
	0	5	10	15
Colour and general appearance				
Control	7.22±0.96[a]	7.14±0.14[a]	6.92±0.11[a]	6.22±0.53[b]
Treatment	7.08±0.05[a]	7.02±.12[a]	6.99±0.09[a]	6.09±0.50[b]
Aroma				
Control	7.09±0.07[a]	6.93±0.15[a]	6.54±0.18[b]	5.39±0.13[c]
Treatment	7.13±0.06[a]	7.03±0.12[a]	6.89±0.10[b]	5.86±0.06[c]
Texture				
Control	7.06±0.08[a]	7.01±0.10[a]	6.74±0.12[b]	6.02±0.80[c]
Treatment	6.98±0.08[a]	6.93±0.97[a]	6.59±0.99[b]	5.98±0.72[c]
Sweetness				
Control	7.08±0.89[a]	6.96±0.99[a]	6.81±0.95[a]	6.79±0.11[b]
Treatment	7.05±0.08[a]	6.84±0.79[a]	6.77±0.11[a]	6.65±0.11[b]
Spread ability				
Control	6.97±0.12[a]	6.92±0.09[a]	6.86±0.09[a]	6.59±0.10[b]
Treatment	6.81±0.10[a]	6.79±0.09[a]	6.66±0.94[a]	6.35±0.07[b]
Mouth coating				
Control	7.18±0.63[a]	7.02±0.93[a]	6.73±0.13[b]	5.79±0.13[c]
Treatment	7.12±0.50[a]	7.04±0.98[a]	6.75±0.13[a]	5.92±0.92[b]
Overall acceptability				
Control	7.14±0.14[a]	7.04±0.98[a]	6.49±0.17[b]	5.23±0.11[c]
Treatment	7.24±0.11[a]	7.08±0.08[a]	6.62±0.14[b]	5.35±0.94[c]

Means bearing different superscripts (a, b, c, d) In a row differ significantly (p<0.05)

4. CONCLUSION

Hence 25% reduced sugar with 1% tulsi leaves incorporated low sugar kadaknath egg jam was selected for storage study. This storage observation indicated that microbial count and rancidity level as well as sensory attributes remained well below the permissible level and tulsi leaves and stevia incorporated low sugar kadaknath egg jam was stable up to 15 days of storage under refrigeration (4±1°C).

5. FURTHER RESEARCH

Storage study under different packaging material and under different storage temperatures may also be undertaken.

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