

Original Research Article

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Formulation of Silkworm (*Bombyx mori* L.) Pupal Tamarind Ball and its Shelf Life Study

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ABSTRACT

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The results of the experiment carried out on the development of silkworm pupal tamarind ball (SPTB) with incorporation of silkworm pupal residue powder (SPRP) revealed that, 5 per cent SPRP incorporated SPTB was found to be best accepted with scores for appearance (7.5), colour (7.6), texture (7.8), aroma (7.8), taste (7.0) and overall acceptability (7.7). Silkworm pupal tamarind ball had better nutrient content as compared with the control tamarind ball. 5 per cent SPRP incorporated SPTB contained 4.6 g protein, 37.8 g carbohydrate, 0.6 g fat, 172 kcal energy, 17.6 mg calcium and 9.0 mg iron. Microbial population of SPTB in initial day was found to be nil in case of all groups of microorganisms like bacterial, *E. coli* and molds. Bacteria of 5.37×10^4 CFU and molds of 3.00×10^2 CFU was noticed on 90th day of storage. Interestingly, no *E. coli* was observed from initial to 90th day of storage. The mean sensory evaluation scores of SPTB were recorded from initial day to end of storage study period for the best accepted SPTB (5 %). There was decrease in the overall acceptability from initial (7.7) to 90th day of storage (6.0).

Introduction

Silk – The queen of textiles is the natural fiber, spells luxury, elegance, class and comfort, secreted by silkworm (Chandra, 1997). India is the second largest producer and also the largest consumer of silk in the world. In India, sericulture provide means of livelihood to a large section of population like mulberry, silkworm seed producer and cocoon producers (56.8%), traders (16.6%), weavers (10.7%), twistors (9.1%) and reelers (6.8%) (www.Indiansilk.kar.nic.in).

Traditionally sericulture in India is practiced in tropical environmental regions such as Karnataka, Tamil nadu, Andhra Pradesh and West Bengal and to a limited extent in temperate regions of Jammu and Kashmir. In India mulberry is cultivated in 2, 16,810 hectares. The total raw silk production accounts for 30,348 MT of which mulberry silk is 21,273 MT and Vanya silk is 9,075 MT (Anonymous, 2017).

The silkworm (*Bombyx mori* L) is an economically important insect, which

produces large quantity of silk. In silk reeling process, large quantity of waste accumulates in the form of pupae. Silkworm pupae contain numerous biological constituents which are of great value as feed for animals, human beings, medicine and manure for crops. The by-products presently felt as wastes, can put to better use in generating the value based products and thereby catapult the industry to a more profitable and economically viable spot (Manohar Reddy, 2008).

Annually India produces about 40,000 MT of silkworm pupae on dry weight. Lots of desilked pupae have not been fully utilized. Indeed, disposed off desilked silkworm pupae is a serious problem because the putrilage of the waste are toxic. Recently, chemical composition of desilked pupae have attracted considerable attention in the world and desilked silkworm pupae are considered to be a good source of a large number of bioactive substances (Dandin and Rajan, 2005).

A silkworm pupae has long been part of human food in Asian silk producing countries and considered as delicacy. The vitamins like pyridoxal, riboflavin, thiamine, ascorbic acid, folic acid and minerals like calcium, iron and phosphorus make the pupae more nutritive (Koundinya and Thangavelu, 2005).

The pupa of silkworm has vast applications in a variety of field (Velayudhan *et al.*, 2008). Effective use of by-products not only increases the rearer's and reeler's income but also helps in fuller utilization of natural resources. This vital aspect so far seems to have not been taken proper care in sericulture industry. The full utilization of silkworm pupae as different marketable products and such an integrated operation can certainly make the sericulture more practical.

The cost of end product *i.e.* the silk can be proportionately brought down by the

combination of regulating the processing methods and converting the wastes as useful by-products. The optimal by-product utility concept can be highly useful to sericulture industry, which can help in elevating the socio economic status of the rural poor rearer's.

Profitable conversion of wastes/ by-products to high value utilities through phyto and post-harvest technologies (Majumdar, 1997), the collaboration of seri scientists with related industries, to locate functional activities for potential applications can reduce the production cost, pollution, recycles resources to cater the ever growing population and their demanding wants.

At present the multipurpose use of silkworm pupae on a grand scale is yet to become a reality. The silkworm pupae serves as an alternative source to the soil, plants and humans to maintain their health with an idea of best from waste the experiment was planned and conducted in order to make fully utilization of silkworm pupae for preparation of silkworm pupal Tamarind Ball as part of value addition.

Materials and Methods

The investigation on the development of SPTB with incorporation of SPRP was conducted at Department of Food Science and Nutrition, University of Agricultural Sciences, Gandhi Krishi Vighyan Kendra, Bengaluru. The materials used and the methods adopted during the study are detailed below.

Preparation of tamarind ball

Ingredients required

Tamarind, garlic, coriander powder, cumin powder, salt, sugar, chilli powder and silkworm pupal residue powder (concentration 2.5, 5 and 7%).

Procedure

Tamarind, garlic, coriander powder, cumin powder, salt, sugar, chili powder were mixed well in a grinder and made in to bite size balls later balls were rolled on sugar to coat evenly.

Nutrient and biochemical composition was estimated by adopting the standard procedures for protein, fat, calcium, Iron, energy and CHO by using AOAC (1980) method.

Organoleptic evaluation

The Value added product was standardized in laboratory and organoleptic evaluation was carried out. A nine point Hedonic Scale was adopted for the evaluation by 21 semi trained panelists.

Estimation of anti-nutritional factors

The anti-nutritional factors such as phytic acid and tannins were estimated.

Microbial load of silkworm pupal residue powder and product developed out of it

Ten grams of SPRP and products sample was mixed in 90 ml sterile water blank to give 10^{-1} dilution. Subsequent dilutions up to 10^{-4} were made by transferring serially 1 ml of the dilution to 9 ml of sterile water blanks. The populations of bacteria, molds and yeasts were estimated by transferring 1 ml of 10^{-2} , 10^{-3} and 10^{-4} dilutions respectively to a sterile Petridish and approximately 20 ml of media *viz.*, Nutrient Agar, Martins Rose Bengal Agar and Davis Yeast Extract Agar for bacteria, molds and *E. coli* respectively was poured into plates. The plates were rotated twice in clockwise and anticlockwise direction for uniform distribution of the inoculums. After solidifications of the media, plates were kept for incubation in an invert position at $30 \pm 1^{\circ}\text{C}$ for 2-4 days and emerged colonies were

counted. The cost of the product was calculated per 100 g of the product. The cost of the product was calculated by taking into consideration the cost of ingredients and overhead charges. Though the overhead charges are same for all the products the cost of production varied according to the cost of ingredients.

Statistical analysis

The data of the experiment was subjected to appropriate statistical analysis. One way analysis of variance was applied to sensory scores. The analysis of variance and interpretation of data were done as per procedures given by Fisher and Yates (1963) and Panse and Sukhatme (1985). Level of significance used in 'F' test was $P = 0.05$.

Results and Discussion

Results on sensory evaluation of SPTB revealed that, three variations of SPTB were prepared by incorporating SPRP at different levels (2.5, 5 and 7 per cent). Control tamarind balls were prepared without the addition of SPRP. Control tamarind balls scored higher values for sensory attributes for appearance, colour, texture, aroma, taste and overall acceptability of 8.1, 7.8, 8.1, 8.0, 8.4 and 8.0 respectively. Among different levels of SPRP incorporation, 5 per cent found to be best accepted with scores for appearance (7.5), colour (7.6), texture (7.8), aroma (7.8), taste (7.0) and overall acceptability (7.7). However, least scores were observed for SPTB prepared by incorporating 7 per cent of SPRP with scores for appearance (7.3), colour (7.5), texture (7.6), aroma (6.5), taste (6.2) and overall acceptability (7.0) respectively (Table 1).

The sensory scores of SPTB was found to be best for 5 per cent SPRP incorporated tamarind ball for all the sensory attributes

viz., appearance, colour, texture, aroma, taste and overall acceptability. The silkworm pupal residue powder along with garlic, cumin powder and coriander powder might also have added the better taste for the tamarind ball. This was in accordance with Padmashree *et al.*, (2012) reported similar values for cereal chocolate nutria bars.

Nutrient compositions per 100 g of control (0 % SPRP incorporation) tamarind ball i.e. protein, carbohydrate, fat and energy was 1.8 g, 36.6 g, 0.1 g and 153 kcal respectively. The calcium and iron were 12.4 and 8.6 mg. SPTB at 5 per cent SPRP incorporated level contained 4.6 g protein, 37.8 g carbohydrate, 0.6 g fat, 172 kcal energy, 17.6 mg calcium and 9.0 mg iron (Table 2).

SPTB had better nutrient content as compared with the control tamarind ball as the formulation included silkworm pupal powder. The nutritional and biochemical analysis revealed by Tomotake *et al.*, (2010) showed that, silkworm pupal powder is very rich in the nutritional and biochemical compositions. So, this has added the extra nutrient status compared to that of the control.

Microbial load of SPTB on storage

Microbial population of control tamarind ball at initial day was found to be nil in case of all groups of microorganisms like bacterial, *E. coli* and molds. But at 10th, 30th, 60th and 90th day the total bacterial count was 1.3×10^4 CFU, 1.7×10^4 CFU, 2.8×10^4 CFU and 5.7×10^4 CFU respectively. However, from initial to 90th day no *E. coli* population was observed. Whereas, there were no mold population from initial to 60th day, but on 90th day there was 1×10^2 CFU molds were recorded (Table 3).

Microbial population of SPTB at initial day was found to be nil in case of all groups of microorganisms like bacterial, *E. coli* and

molds. But at 10th, 30th, 60th and 90th day the total bacterial count was 1.41×10^4 CFU, 1.87×10^4 CFU, 2.73×10^4 CFU and 5.37×10^4 CFU respectively. However, there was no presence of *E. coli* from initial day to 90th day. Whereas, from initial day to 60th day there were no molds noticed. Later, the molds were found to be 3×10^2 CFU on 90th day respectively. There was a significant difference between total bacterial population counts from initial day to 90th day (Table 3).

In the storage conditions, the microbial load has been increased as the days of storage increased. As the silkworm tamarind ball contained the formulations of garlic might have showed the immune power against different microbes. Hence, there were no *E. coli* populations and molds noticed till 60th day of storage. The results are on par with work of Selvaraj *et al.*, (2002) reported that masala biscuits had increased the microbial load with increase in the storage days.

Shelf life study of SPTB

The control and silkworm pupal tamarind balls (5 per cent level SPRP incorporated tamarind balls) which were best accepted by the sensory panel members kept for shelf life study. The samples were observed daily for visual changes and were subjected to sensory evaluation on 30th, 60th and 90th days respectively. The results of the mean sensory score evaluation of control tamarind ball from initial day to end of shelf life study period are presented. The control tamarind ball showed sensory scores of 7.4, 7.3, 7.3, 7.4, 7.5 and 7.4 for all the sensory attributes *viz.*, appearance, colour, texture, aroma, taste and overall acceptability for the initial day. However, at the end of 90th day, the control tamarind ball had low sensory scores (7.2, 6.7, 6.3, 6.6, 6.9 and 7.1) for appearance, colour, texture, aroma, taste and overall acceptability (Table 4).

Table.1 Mean sensory scores of silkworm pupal tamarind ball

Level of silkworm pupal residue powder incorporation (%)	Mean sensory scores (n=21)					
	Sensory attributes					
	Appearance	Colour	Texture	Aroma	Taste	Overall acceptability
Control	8.1	7.8	8.1	8.0	8.4	8.0
2.5	7.3	7.5	7.8	7.2	6.7	7.5
5	7.5	7.6	7.8	7.8	7.0	7.7
7	7.3	7.5	7.6	6.5	6.2	7.0
Mean	7.5	7.6	7.8	7.3	7.0	7.5
F- Test	*	*	*	*	*	*
S. Em±	0.17	0.29	0.13	0.13	0.19	0.25
CD at 5%	0.4	0.8	0.38	0.37	0.56	0.7

*- Significant at 5 %

Table.2 Nutritional composition of silkworm pupal tamarind ball per 100 g

Nutrients	Control (0 % SPRP)	Best accepted product (5 % SPRP incorporated)
Protein(g)	1.8	4.6
Carbohydrates (g)	36.6	37.8
Fat (g)	0.1	0.6
Energy (Kcal)	153	172
Calcium (mg)	12.4	17.6
Iron(mg)	8.6	9.0

SPRP- Silkworm pupal residue powder

Table.3 Microbial load of best accepted silkworm pupal tamarind ball on storage (5 % SPRP incorporated)

Samples	Duration (Days)	Group of microorganisms		
		Total Bacterial count ($\times 10^4$ CFU)	E. coli ($\times 10^2$ CFU)	Molds ($\times 10^2$ CFU/g)
Control	Initial	Nil	Nil	Nil
	10 th day	1.3	Nil	Nil
	30 th day	1.7	Nil	Nil
	60 th day	2.8	Nil	Nil
	90 th day	5.7	Nil	1
	Mean	2.8	-	-
	F-test	*	-	-
	S. Em\pm	0.10	-	-
	CD at 5 %	0.35	-	-
	5 % SPRP incorporated	Initial	Nil	Nil
10 th day		1.41	Nil	Nil
30 th day		1.87	Nil	Nil
60 th day		2.73	Nil	Nil
90 th day		5.37	Nil	3
Mean		2.28	-	-
F-test		*	-	-
S. Em\pm		0.33	-	-
CD at 5 %		1.05	-	-

*- Significant at 5 %

Table.4 Mean sensory scores for shelf life study of best accepted silkworm pupal tamarind ball (5 % SPRP incorporated)

Products	Duration (Days)	Mean sensory scores (n=21)					
		Sensory attributes					
		Appearance	Color	Texture	Aroma	Taste	Overall acceptability
Control	Initial	7.4	7.3	7.3	7.4	7.5	7.4
	30th	7.3	7.0	7.1	7.2	7.5	7.1
	60th	7.3	6.7	7.0	6.9	7.4	7.1
	90th	7.2	6.7	6.3	6.6	6.9	7.1
	Mean	6.5	6.9	6.4	6.7	6.6	6.8
	F-test	NS	*	*	*	NS	NS
	S. Em\pm	0.18	0.13	0.16	0.14	0.19	0.13
	CD at 5%	0.48	0.35	0.45	0.40	0.53	0.36
5 % SPRP incorporated	Initial	7.9	7.8	7.9	7.7	7.7	7.7
	30th	7.7	7.3	7.6	7.5	7.4	7.3
	60th	7.3	7.0	7.2	7.1	6.9	6.8
	90th	6.9	6.6	6.8	6.6	6.4	6.0
	Mean	7.4	7.1	7.3	7.2	7.1	6.9
	F-test	*	*	*	*	*	NS
	S. Em\pm	0.23	0.19	0.2	0.27	0.21	0.17
	CD at 5%	0.67	0.54	0.56	0.78	0.6	0.48

*- Significant at 5 %; NS- Non-significant

Table.5 Microbial load of silkworm pupal powder on storage

Samples	Duration (Days)	Group of microorganisms		
		Total Bacterial count ($\times 10^4$ CFU)	<i>E.coli</i> ($\times 10^2$ CFU)	Molds ($\times 10^2$ CFU/g)
	Initial	Nil	Nil	Nil
	10 th day	1.28	Nil	Nil
	30 th day	2.23	Nil	Nil
	60 th day	3.63	Nil	Nil
	90 th day	4.03	Nil	3
	Mean	2.24	-	-
	F-test	*	-	-
	S. Em\pm	0.48	-	-
	CD at 5 %	1.52	-	-

*- Significant at 5 %

Table.6 Production cost of best accepted silkworm pupal tamarind ball (5 % SPRP incorporated) (Round off to Rs.10)

Ingredients	Price /kg (Rs)	Quantity (g)	Price (Rs)
Tamarind	80	50	4
Garlic	150	3	0.45
Cumin powder	210	0.5	0.11
Coriander powder	170	0.5	0.09
Sugar	45	2	0.09
Chilli	60	2	0.12
Salt	20	1	0.02
Pupal powder	20	2.5	0.05
Total		61.5	4.93
Overhead charges (20% of the cost)			0.99
Cost of the product/100g		61.5	5.92
		100	9.63

Overhead charges- Cooking, processing and utilities

Rs.10 per 100gm of the product both for control and silkworm pupal tamarind ball

The 5 per cent level incorporated SPTB showed scores of 7.9, 7.8, 7.9, 7.7, 7.7 and 7.7 for all sensory parameters viz., appearance, colour, texture, aroma, taste and overall acceptability for the initial day. However, at the end of 90th day the 5 per cent level of incorporation of SPTB showed lower scores (6.9, 6.6, 6.8, 6.6, 6.4 and 6.0) for appearance, colour, texture, aroma, taste and overall acceptability. By the 100th day they were unfit for sensory evaluation due to increase in the

peroxide and free fatty acid value. When analyzed statistically there was significant difference among appearance, colour, texture, aroma and taste except for overall acceptability from initial to 90th day (Table 4).

The sensory evaluation of SPTB at storage conditions showed that, there was decrease in the scores for all the sensory attributes at 90th day of storage. Due to increase of microbial load in the SPTB also adds up for the

decrease in the sensory attributes. These findings are in relation with the findings of Bhardwaj *et al.*, (2016) who prepared guava milk chocolate and a shelf life study for same was reported.

Microbial load of SPRP on storage

Microbial population of SPRP in initial day was found to be nil in case of all groups of microorganisms like bacterial, *E. coli* and molds. But at 10th, 30th, 60th and 90th day 1.28×10⁴ CFU, 2.23×10⁴ CFU, 3.63×10⁴ CFU and 4.03×10⁴ CFU of total bacteria was recorded respectively. However, there was no presence of *E. coli* from initial day to 90th day. Whereas, from initial day to 60th day there were no molds noticed. Later, the molds were found to be 3×10² CFU at 90th day respectively. There was a significant difference between total bacterial population counts from initial day to 90th day (Table 5).

As the silkworm pupal powder is rich in biochemical compositions and nutrient compositions (Mishra *et al.*, 2003) and also the ability of silkworm pupal powder to absorb moisture have attracted the microbes. This is the first kind of study and literature pertaining to it is not available and hence it was not compared.

Anti-nutritional content of SPRP

The SPRP contained 140 mg of phytic acid and 0.2 mg of tannin/ 100g of powder. The results of anti-nutritional content of SPRP was compared with phytic acid and tannin content of finger millet which was in the range of 225-347 mg/100 gm and 0.28-1.20 mg/100gm (Hiremath, 2011).

The production cost of control and silkworm pupal tamarind balls was Rs. 10 /100g (Table 5). The production cost was estimated by taking the prevailing cost of all the

ingredients in the market and the overhead cost including the labour cost, power cost, machinery cost and packaging cost. Though the same overhead charges were taken for all the products the cost of product varied, because of varied cost of ingredients. The estimated costs were within the reach of Indian costumers.

Three variations of SPRP of 2.5, 5 and 7 per cent were incorporated for preparation of SPTB. However, 5 per cent incorporated SPTB was best accepted. The present study can be extended to study the consumer acceptability and popularization of SPTB prepared using silkworm pupal residue powder.

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