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Utilization of silkworm (*Bombyx mori*) pupal residue powder in masala cookies

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The results of the experiment carried out on the development of silkworm pupal Masala Cookies (SPMC) with incorporation of silkworm pupal residue powder (SPRP) revealed that,7 per cent SPRP incorporated was found to be best accepted with scores for appearance (7.8), colour (7.6), texture (8.0), aroma (7.4), taste (7.6) and overall acceptability (7.6). Silkworm pupal masala cookies had better nutrient content as compared with the control masala cookies (0 % SPRP).The 7 per cent SPRP incorporated SPMC contained 16.6 g protein, 79.3 g carbohydrate, 51.3 g fat, 854 kcal energy, 114.5 mg calcium and 6.6 mg iron. Microbial population of SPMC in initial day was found to be nil in case of all groups of microorganisms like bacterial, *E. coli* and molds. Bacteria of 6.10×10^4 CFU and molds of 3.50×10^2 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 silkworm pupal masala cookies were recorded from initial day to end of storage study period for the best accepted SPMC (7 %). There was decrease in the overall acceptability from initial (7.4) to 90th day of storage (6.6). Microbial population of SPRP and protein in initial day was found to be nil in case of all groups of microorganisms like bacterial, *E. coli* and molds. Bacteria of 6.97×10^4 CFU and 7.63×10^4 CFU and molds of 4.73×10^2 CFU and 5.07×10^2 CFU was noticed on 90th day of storage. Interestingly, no *E. coli* was observed from initial to 90th day of storage. The SPRP contained 140 mg of phytic acid and 0.2 mg of tannin/ 100g of powder. The production cost of control and SPMC was Rs. 20 /100g.

Key Words : Silkworm, Pupa, Cookies, Nutrient analysis, Storage

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INTRODUCTION

Silk –is considered as queen of textiles and which is secreted by silkworm (Chandra, 1997). In the world raw silk production, India ranks second next to China. In India, Karnataka, Tamil nadu, Andhra Pradesh contributes for major portion of raw silk production. In India, the 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 a commercially exploited lepidopteron insect, producing large quantity of natural silk. In silk reeling industry, huge quantity of pupal residue is obtained during the process of silk reeling. Silkworm pupae contain nutrients which are of great value as feed for animals, human beings, medicine and manure for crops. The by-products generated presently felt as wastes, can put to better use in producing the value added products and thereby catapult the sericulture 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 disliked pupae have not been fully utilized. Indeed, disposed off disliked silkworm pupae is a serious problem because the putrilage of the waste are toxic. Recently, chemical composition of disliked pupae have attracted considerable attention in the world and disliked silkworm pupae are considered to be a good source of a large number of bioactive substances (Dandin and Rajan, 2005).

The silkworm pupa has vast applications in a variety of field (Velayudhan *et al.*, 2008). Effective use of sericulture by-products not only increases the silkworm rearer's income, but also helps in fuller utilization of by-products. This vital aspect so far seems to have not been taken proper care in sericulture sector. Pupae can be utilized in a better way by producing value added products with the adoption of improved technology (Majumder, 1997). The sericulture by-products presently felt as wastes can put to better use in generating the value based products and there by catapult the industry to a more profitable and economically viable spot. The full utilization of silkworm pupae as different marketable products and such an integrated operation can certainly make the sericulture more practical (Dandin and Kumar, 2007). The cost of final 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.

In view of the above, the studies was planned and conducted in order utilize the silkworm pupal residue in a better way for preparation of silkworm pupal White Chocolate as part of value addition.

METHODOLOGY

The investigation on the development of SPMC 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 masala cookies:

Ingredients required:

Wheat flour, chilli, carrot, curry leaves, coriander leaves, baking powder, sugar, salt, fat and silkworm pupal residue powder (concentration 2.5, 5 and 7%).

Procedure:

Wheat flour, baking powder, cumin powder and pupal powder were sieved three times. Creaming of fat was done with sugar and flour was prepared by mixing with creamed fat and sugar into dough then rolled the dough and gave the shape to the biscuits using biscuit cutter and baked for 15 min at 160 ± ^{0}C.

Nutrient and biochemical composition was estimated for protein, fat, calcium, iron and computed for energy and CHO by using standard procedure.

Sensory evaluation:

Criteria for selection of the panel members was their familiarity with the developed products selected for value addition. The panel members (twenty one numbers) were exposed to the product as preliminary evaluation before the final evaluation.

SPTB was developed by incorporating silkworm pupal powder, was coded and subjected to sensory evaluation by twenty one panel members of Department of Food Science and Nutrition. The panelists were asked to rate each sensory attribute using the control products as the basis for evaluation. The product was evaluated for appearance, texture, colour, aroma, taste and overall acceptability on 9-point hedonic scale.

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 upto 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 ± 1^0C 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.

OBSERVATIONS AND ASSESSMENT

Results on sensory evaluation of silkworm pupal masala cookies (SPMC) recorded that, control masala cookies scored higher values for sensory attributes for appearance, colour, texture, aroma, taste and overall acceptability of 8.0, 8.1, 8.3, 8.1, 8.2 and 8.4, respectively. Among three variations of SPMC, 7 per cent SPRP incorporated was found to be best accepted with scores for appearance (7.8), colour (7.6), texture (8.0), aroma (7.4), taste (7.6) and overall acceptability (7.6). However, least scores were observed for SPMC prepared with 2.5 per cent of SPRP *viz.,* appearance (7.0), colour (6.9), texture (7.1), aroma (6.8), taste (6.5) and overall acceptability (6.4), respectively. Significant differences were recorded for all the attributes (Table 1).

The sensory scores of SPMC was found to be best for 7 per cent SPRP incorporated masala cookies for all the sensory attributes *viz.,* appearance, colour, texture, aroma, taste and overall acceptability. The SPRP might also have added the better taste for the masala cookies. This could be due to the taste of the masala cookies which contained SPRP along with wheat flour, coriander, curry and cumin added better taste to the SPMC. Similar results were also observed for the masala cookies prepared using barn yard millet in the work of Yenagi (2003).

Nutrient compositions per 100 g of control (0 % SPRP incorporation) masala cookies *i.e.* protein, carbohydrate, fat and energy was 12.8 g, 77.7 g, 50.7 gand 827 kcal/100 g, respectively. The calcium and iron were 107.4 and 6.4 mg. SPMC at 7 per cent SPRP incorporated level contained 16.6 g protein,79.3 g carbohydrate, 51.3 g fat, 854 kcal energy, 114.5 mg calcium and 6.6 mg iron (Table 2).

SPMC shad better nutrient content as compared with the control masala cookies as the formulations included SPRP along with wheat flour, coriander leaves, curry leaves, cumin powder and carrot. The nutritional and biochemical analysis revealed (Kanika *et al.,* 2008) that;

Table 1 : Mean sensory scores of silkworm pupal masala cookies

Level of silkworm pupal residue powder incorporation (%)	Mean sensory scores (n=21)					
	Sensory attributes					
	Appearance	Colour	Texture	Aroma	Taste	Overall acceptability
Control	8.0	8.1	8.3	8.1	8.2	8.4
2.5	7.0	6.9	7.1	6.8	6.5	6.4
5	7.6	7.4	7.5	7.1	7.1	7.0
7	7.8	7.6	8.0	7.4	7.6	7.6
Mean	7.6	7.5	7.7	7.3	7.3	7.3
F -Test	*	*	*	*	*	*
S.E.±	0.16	0.13	0.17	0.23	0.24	0.21
C.D. (P=0.05)	0.47	0.37	0.49	0.65	0.68	0.13

SPRP is very rich in the nutritional and biochemical compositions. So, this has added the extra nutrient status compared to that of the control. Thus, SPMC can prove to be nutrient dense attribute for the plain masala cookies available in market. This is in relation to the work conducted by (Gopalan *et al.,* 2012) stated that due to higher percentage of protein in green gram, cookies prepared using green gram contained highest protein and other nutrient compositions.

Microbial population of control masala cookies at initial day was found to be nil in case of all groups of microorganisms like bacterial, *E. coli* and molds. But at 10^{th}, 30^{th}, 60^{th} and 90^{th} day the total bacterial count was 1.1×10^4 CFU, 1.7×10^4 CFU, 2.7×10^4 CFU and 5.0×10^4 CFU, respectively. However, from initial to 90^{th} day no *E. coli* population was observed. Whereas, there were no mold population from initial to 60^{th} day, but on 90^{th} day there was 1×10^2 CFU molds were recorded. Microbial population of SPMC at initial day was found to be nil in case of all groups of microorganisms like bacterial, *E. coli* and molds. But at10^{th}, 30^{th}, 60^{th} and 90^{th} day the total bacterial count was 1.38×10^4 CFU, 2.00×10^4 CFU, 2.73×10^4 CFU and 5.07×10^4 CFU, respectively. However, there was no presence of *E. coli* from initial day to 90^{th} day. Whereas, from initial day to 60^{th} day there were no molds noticed. Later, the molds were found to be 2×10^2 CFU at 90^{th}day, respectively. There was a significant difference between total bacterial counts from

Table 2 : Nutritional composition of silkworm pupal masala cookies per 100 g

Nutrients	Control (0 % SPRP)	Best accepted product (7% SPRP incorporated)
Protein(g)	12.8	16.6
Carbohydrates (g)	77.7	79.3
Fat (g)	50.7	51.3
Energy (Kcal)	827	854
Calcium (mg)	107.4	114.5
Iron (mg)	6.4	6.6
Phyto-nutrients		
Phytic acid (mg)	286	140
Tannin (mg)	0.74	0.2

Table 3: Microbial load of best accepted silkworm pupal masala cookies on storage (7 % SPRP incorporated)

Samples	Duration (Days)	Group of microorganisms		
		Total Bacterial count ($\times 10^4$ CFU)	*E. coli* ($x10^2$CFU)	Molds ($x10^2$ CFU/g)
Control	Initial (0 Day)	Nil	Nil	Nil
	10^{th}day	1.1	Nil	Nil
	30^{th} day	1.7	Nil	Nil
	60^{th} day	2.7	Nil	Nil
	90^{th}day	5.0	Nil	1
	Mean	2.8	-	-
	F-test	*	-	-
	S.E.±	0.07	-	-
	C.D. (P=0.05)	0.23	-	-
7 % SPRP incorporated	Initial (0 Day)	Nil	Nil	Nil
	10^{th}day	1.38	Nil	Nil
	30^{th} day	2.00	Nil	Nil
	60^{th} day	2.73	Nil	Nil
	90^{th}day	5.07	Nil	2
	Mean	2.24	-	-
	F-test	*	-	-
	S.E.±	0.10	-	-
	C.D. (P=0.05)	0.32	-	-

initial day to 90th day (Table 3).

There was no *E. coli* from initial day to 90th day and no molds were seen till 50th day of storage of SPMC. Banusha and Vasantharuba (2014) reported that, malted flour blend incorporated cookies were evaluated for sensory attributes and storage study. The bacterial count of 30 per cent malted flour blend incorporated biscuit was 7.6×10^4 CFU/g after two months of storage and there was no yeast and mold growth observed during two months of storage. The results of present investigation are comparable to above reported results.

The control masala cookies and SPMC (7 % level SPRP incorporated masala cookies) 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 masala cookies from initial day to end of shelf life study period are presented. The control masala cookies showed sensory scores of 7.0, 7.5, 6.9, 7.2, 7.2 and 7.1 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 masala cookies had low sensory scores (6.2, 6.4, 6.0, 6.2, 6.1 and 6.5) for appearance, colour, texture, aroma, taste and overall acceptability. And by 100th day there were unfit for sensory evaluation.

The results of the mean sensory score evaluation of SPMC from initial day to end of storage study period are presented (Table 4). The 7 per cent SPRP incorporated SPMC showed scores of 7.8, 7.5, 7.5, 7.4, 7.8 and 7.4 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 7 per cent level of incorporation SPMC showed lower scores (6.3, 6.2, 6.2, 6.4, 6.0 and 6.6) 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, taste and overall acceptability from initial to 90th day (Table 4).

A decrease in the scores from the initial day upto 90th day was shown. Score for all parameters decreased as the storage days increased. The acceptability of silkworm pupal masala cookies was affected by the storage days. The product can be successfully stored at ambient condition with good acceptability upto 90 days. The present findings are in corroboration with the findings of Selvaraj *et al.* (2002) who reported that masala biscuits can be stored for more than 90 days.

Table 4 : Mean sensory scores for shelf life study of best accepted silkworm pupal masala cookies (7 % SPRP incorporated) (n=21)

Products	Duration (days)	Mean sensory scores					
		Sensory attributes					
		Appearance	Colour	Texture	Aroma	Taste	Overall acceptability
Control	Initial	7.0	7.5	6.9	7.2	7.2	7.1
	30th	6.4	7.0	6.5	6.9	6.8	6.9
	60th	6.4	6.7	6.5	6.8	6.3	6.9
	90th	6.2	6.4	6.0	6.2	6.1	6.5
	Mean	6.5	6.9	6.4	6.7	6.6	6.8
	F-test	*	*	*	*	*	NS
	S.E.±	0.18	0.17	0.20	0.16	0.17	0.16
	C.D. (P=0.05)	0.51	0.48	0.55	0.44	0.46	0.45
7 % SPRP incorporated	Initial	7.8	7.5	7.5	7.4	7.8	7.4
	30th	7.2	7.3	7.3	7.3	7.2	7.2
	60th	6.6	7.0	6.9	6.8	6.5	6.8
	90th	6.3	6.2	6.2	6.4	6.0	6.6
	Mean	6.9	7	6.9	6.9	6.8	7
	F-test	*	*	*	*	*	*
	S.E.±	0.17	0.25	0.18	0.20	0.17	0.20
	C.D. (P=0.05)	0.47	0.70	0.50	0.54	0.48	0.54

NS = Non-significant

Table 5 : Microbial load of silkworm pupal powder on storage

Samples	Duration (Days)	Group of microorganisms		
		Total Bacterial count (x10^4 CFU)	*E. coli* (x10^2CFU)	Molds (x10^2 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.E.±	0.48	-	-
	C.D. (P=0.05)	1.52	-	-

* indicate significance of value at P=0.05

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 10^{th}, 30^{th}, 60^{th} and 90^{th} day 1.28×10^4 CFU, 2.23×10^4 CFU, 3.63×10^4 CFU and 4.03×10^4 CFU of total bacteria was recorded, respectively. However, there was no presence of *E. coli* from initial day to 90^{th} day. Whereas, from initial day to 60^{th} day there were no molds noticed. Later, the molds were found to be 3×10^2 CFU at 90^{th}day, respectively. There was a significant difference between total bacterial population counts from initial day to 90^{th} 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 were compared with phytic acid and tannin content of finger millet which was in the range of 225-347 mg/100 g and 0.28-1.20 mg/100g (Hiremath, 2011).

The production cost of control and SPMC was Rs. 20 /100g (Table 6). 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 over head 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.

Conclusion:

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

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