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Extraction of Sericin from cocoon shell and raw silk of bivoltine and multivoltine breeds of the Silkworm, *Bombyx mori* L.

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Abstract
Silk is produced by large-scale rearing of silkworms and popularly known as Sericulture. Silk is made up of two proteins namely sericin and fibroin. Silk proteins are accumulated in the silk glands of the silkworm larva which consumes and ingests the mulberry leaf. In the present study, crude sericin was extracted from the cocoon shell and raw-silk of popular bivoltine and multivoltine breeds of the mulberry silkworm, *Bombyx mori* by high temperature autoclaving method. Two bivoltine breeds *viz*. NB_4D_2, and CSR_2; two multivoltine breeds MU_{303}, and PM were selected for the present study. Along with extraction the crude sericin powder by using acetic acid; quantitative characters like cocoon weight, shell %, silk filament length of these breeds were also analysed and correlated based on the results obtained. The experimental results revealed that the sericin extracted from silk and cocoon shell of CSR_2 was higher than NB_4D_2 among bivoltines and PM higher than MU_{303} among multivoltines.

Keywords: Bivoltine, Multivoltine, Raw-silk, Cocoon shell, Sericin

Introduction
Silk is the outcome of various activities of Sericulture. The word sericulture is derived from the Greek word 'Sericos' meaning silk and the English word ''culture'' means rearing. Sericulture can be practised by farmers even with small land holdings and it can also be a subsidiary occupation using waste land which is unsuitable for other agriculture crop cultivation. Sericulture is broadly classified based on their feeding habitat. Those which feed on mulberry and those which are reared in wild habitats feeding non-mulberry plants commonly known as Vanya silks. India has the unique distinction of being the only country producing all the five known commercial silks namely, mulberry, Tropical Tasar, Oak Tasar, Eri and Muga. Silk is the most elegant textile fibre in the world with unparalleled grandeur, natural sheen, and inherent affinity for dyes, high absorbance, light weight, soft touch and high durability popularly known as the "Queen of Textiles" the world over. Silk is produced by the silkworm by spinning cocoon using silk fibre and it is a commercially exploited commodity in India. The quality and quantity characters of cocoons and raw-silk vary from breed to breed [2], quality of cocoons are expressed in terms of cocoon colour, cocoon shape, grains, size, compactness [3] and quantitative characters expressed in terms of cocoon weight, shell weight, shell percentage, filament length, filament denier, reelability, renditta. In the present study, more prominence is given on the quantitative characters like cocoon weight, shell percentage and filament length because sericin content mainly depends on these above properties.
Silk comprises of two types of proteins sericin and fibroin which varies around 70-80% of fibroin and 20-30% of sericin depending on races/breeds of silkworm. Sericin contains 18 amino acids including essential amino acids and is characterized by the presence of 32% serine, total amount of hydroxyl amino acid in sericin is 45.89% [4]. Due to these properties sericin has a lot of applications in the fields of medical and cosmetics industry. At present it is used as bio-material in surgeries as it is a natural protein. In view of the above, extraction of sericin from cocoon shell and raw silk throws light for further investigations. Hence two bivoltine and two multivoltine breeds were taken up along with quantification in the present study. The results obtained were correlated with the sericin content present in cocoons and raw silk along with the quantitative traits.

Materials and methods

Two breeds each of multivoltines (PM and MU_{303}) and bivoltines (CSR_2 and NB_4D_2) were selected for the present study. The experiments were conducted at Post Graduate Department of Studies in Sericulture Science, University of Mysore, Mysuru. Sericin content depends on the qualitative and quantitative characters of raw-silk and cocoons used and quantitative characters are analysed below.

Ten good and healthy cocoons were selected from the cocoon lot, followed by average cocoon weight was taken for selected cocoons of all four breeds and weight of cocoon approximately indicates the quantity of raw silk that can be reeled from it. Cocoon shell percentage is the ratio between the shell weight and the cocoon weight, it is expressed in percentage as given below.

Filament length is the total length of the reelable silk of the cocoons, expressed in meters. All these characters have direct effect on sericin extraction from cocoon shell and raw-silk. For the present experiment 11 g. of cocoon shell and silk of both bivoltine and multivoltine breeds were taken as sample. Cocoon shell and raw-silk were boiled in 300 ml of water with 5 ml of acetic acid, for 20-30 minutes followed by cooling for about 24 hours, after cooling sericin solution was removed and centrifuged at 500 rpm for 18-20 minutes. Then, the supernatant was taken in the petri-plates and those petri-plates were placed in the hot air oven at 90°C till the solution get dried. After complete drying, the plates are scraped to get the crude sericin powder which is white to off-white in colour in colour.

Results and discussion

Sericin can be extracted from different sources namely cocoon shell, raw-silk, woven fabric [6][8] quality and quantity of crude sericin powder depends on the cocoon characters. The parameters of both multivoltine and bivoltine silkworm breeds selected for the present study namely bivoltine breeds CSR_2 and NB_4D_2 are good with respect to the yield, cocoon weight, shell % and filament length. The single cocoon weight (g.) was 1.88±0.11 and 1.61 ±0.13, shell percentage (%) was 22.34±0.38 and 19.43±0.57, single cocoon filament length (m) was 1043±39.77 and 1029±85.46 respectively. Multivoltine breeds like PM and MU_{303} were used and traits recorded like cocoon weight (g.) of these breeds was 0.99±0.06 and 0.91±0.08, shell percentage (%) was 12.37±0.52 and 11.40±0.40, single cocoon filament length (m.) was 410.0±36.88 and 455.1±109.0 respectively (Table-1). Highest sericin extraction was recorded in Pure Mysore followed by CSR_2, MU_{303} and NB_4D_2 in both raw-silk and cocoon shell for every 10 grams of material taken in the present study.

Quantity of sericin content present in cocoon shell and raw silk of bivoltine and multivoltine races/ breeds

Quantity of sericin content depends on the above listed parameters of cocoon and the sericin content is more in cocoon shell compared to silk because some quantity was lost during cocoon cooking process. The extracted crude sericin powder of CSR_2, NB_4D_2, PM and MU_{303} breeds in cocoon shell and raw-silk was 1.79g and 1.59g, 1.62g and 1.4g, 1.81g and 1.59g, 1.75g and 1.50g respectively (Table-2). Correlation of the sericin content present in cocoons and raw silk in bivoltine and multivoltine breeds from the present study, the multivoltine breeds contain 1% more sericin compared to the bivoltine breeds, cocoon shell contained 2% more sericin compared to the silk and finally the quality of sericin is good in raw-silk compared to the cocoon shells used (Table-3).

Table 1: Quality cocoon parameters of bivoltine and multivoltine silkworm races/breeds.

Race	Breeds	Cocoon parameters (Mean ± S.D.)		
		Cocoon Weight (g.)	Cocoon shell Percentage	Filament length (meters)
Bivoltine	NB_4D_2	1.61 ± 0.13	19.43 ± 0.57	1029 ± 85.46
	CSR_2	1.88 ± 0.11	22.34 ± 0.38	1043 ± 39.77
Multivoltine	PM	0.99 ± 0.06	12.37 ± 0.52	455.1 ± 109.0
	MU_{303}	0.91 ± 0.08	11.40 ± 0.40	410.0 ± 36.88

Sample size (n) =10

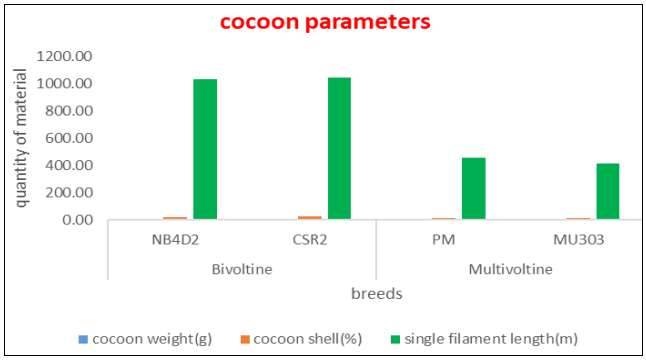

Fig 1: Cocoon parameters of bivoltine and multivoltine silkworm races/breeds

Table 2: Variation of sericin content in silk and cocoon shell materials of Bivoltine and Multivoltine silkworm races/breeds.

Material used	Breeds	Weight of material (g.)	Sericin powder extracted (g.)	Weight of material : Sericin powder
Raw Silk	NB_4D_2	11	1.40	10 :1.27
	CSR_2	11	1.52	10 :1.38
	PM	11	1.59	10 :1.44
	MU_{303}	11	1.50	10 :1.36
Cocoon shell	NB_4D_2	11	1.62	10 :1.47
	CSR_2	11	1.79	10 :1.62
	PM	11	1.81	10 :1.64
	MU_{303}	11	1.75	10 :1.59

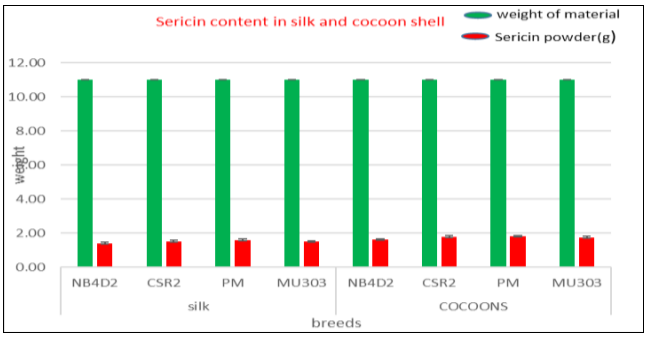

Fig 2: Variation of sericin content in cocoon shell and raw-silk in bivoltine and multivoltine races/breeds

Table 3: Sericin content in relation to certain quality cocoon parameters of bivoltine and multivoltine silkworm breeds.

Parameters	Silk				Correlation coefficient (r)	Cocoons				Correlation coefficient (r)
	NB_4D_2	CSR_2	PM	MU_{303}		NB_4D_2	CSR_2	PM	MU_{303}	
Sericin Powder (g.)	1.40	1.52	1.59	1.50	X +	1.62	1.79	1.81	1.75	X +
Cocoon weight (g.)	1.61	1.88	0.99	0.91	-0.429*	1.61	1.88	0.99	0.91	-0.283*
Shell (%)	19.43	22.34	12.37	11.40	-0.435*	19.43	22.34	12.37	11.40	-0.291*
Single filament length (m.)	1029	1043	455.1	410.0	-0.589*	1029	1043	455.1	410.0	-0.478*

* Non significant correlation coefficient values

Fig 3: Cocoons used for extraction of Sericin

Fig 4: Extracted crude sericin powder

Conclusion

Silk is a luxurious textile and only the rich and affluent can afford to buy. Silk is composed of two proteins namely sericin and fibroin, sericin is a gum coated on fibroin, adhesive in nature and helps in formation of the fibre by binding fibroin strands together into a single entity. It is a globular protein with high content of aspartic acid and sericin has high moisture absorption and retaining capacity. However, many may not be aware that it is also used as material for suture in surgeries since a long time. Recently it is used as a biomaterial and also for medical applications due to its excellent properties including remarkable mechanical properties, comparative biocompatibility, tuneable degradation rates in-vitro and in-vivo. Biodegradable materials can minimize the pain of patients from surgeries; there is no need of repeated surgery in order to remove the scaffold implanted covering the tissues and muscles [9], and it also used as an ingredient in cosmetics as face creams and talcs and also in food industry [1]. Mainly it is a biodegradable protein, because of all these properties sericin has lot of demand in a present situation so there is a need to extract the sericin from silk as well as degummed solution, which can be a value addition for sustainable sericulture industry.

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