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Effect of fluorescence on the technological characteristics of cocoons at different cooking temperatures

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Abstract. *The subject of research are Bombyx mori L. cocoons, differentiated by the nature of their fluorescent radiation. In each fluorescent group 2 subgroups were formed prepared for unreeling through cooking at 80 and 90°C for 5 min. To account for the effect of fluorescence at various cooking temperature levels the basic technological traits signs of the silk filament and cocoons have been defined and analysed: total length of the silk filament (m), nonbroken filament length (m, %), raw silk pesentage (%) and reelability (%). It was found that fluorescence of cocoons has statistically significant (p≤ 0.01-0.001) effect on the phenotypic manifestation of the technological traits. Within the two cooking temperature levels, the yellow fluorescent cocoons demonstrate higher values at 80 °C, and violet ones at 90°C. The better technological qualities and the lower cooking temperature in the yellow fluorescent cocoons give reason to believe that their use is economically more profitable for the silk reeling industry compared with violet fluorescent cocoons.*

Keywords: *Bombyx mori*, fluorescence, temperature, cooking

Introduction

The main parameters defining the physico-mechanical and technological properties of the silk filament depend primarily on preserving the natural qualities of the silk sheath in the process of preliminary preparation and drawing of cocoons. The existing technologies for processing cocoons into silk are based on the ability of sericin to swell and dissolve in water. Crucial for the effectiveness of the unreeling process incl. silk quality is the extent, dissolution time of sericin and the factors influencing them. The conditions of cocoon brewing affect significantly the process of water absorption by the silk shell and cocoon, which reflects on the effect of drawing (Naik et al., 2010).

Cocoon quality affects the productivity of the unreeling process and the quality of raw silk. Which in turn depends on the hybrid, technologies of cultivation and processing of cocoons (Somashekar, 2003). The interaction between the factors breed affiliation of cocoons and conditions for their unreeling affects the physical and mechanical properties of raw silk (Radhalakshmi et al., 2013; Bandyopadhyay et al., 2014).

One of the main factors influencing the properties and behavior of sericin during the unreeling process is the temperature at which it is carried out. The effect of temperature depends on both its values and the duration of the processes (Hu and Kobayashi, 1992; Takabayashi et al., 1998; Hariraj and Somshekar, 2004; Mwasiagi et al., 2012). According to Takasu et al. (2002) the high temperature and its prolonged impact destroy the sericin molecules. At elevated temperatures from 40 to 80°C, the dissolution of sericin initially decreases, then increases to reach 100% at 80°C. The low and high cooking temperature cause uneven dissolution of sericin.

To achieve optimum results in unreeling it is necessary sericin swelling to reach a certain degree. This makes the continuous cooking of cocoons at high temperature economically inefficient for the unreeling process. Both overexposure and insufficient cooking of cocoons has a negative impact on the quality of the silk filament and raw silk yield at the expense of more waste products (Kar et al., 2016).

The optimum temperature for the preparation of cocoons depends on their quality. Ramesh et al. (2005) tested the effect of two levels of cooking (86 and 90°C) on some technological parameters of normal and defective cocoons. The highest values in normal cocoons by mass of the silk skein, total and continuous unreeling length, the lowest average number of breaking and the lowest percentage of waste products from drawing have been obtained from normal cocoons cooking at 86°C, followed by those cooking at 90°C. As a result of the conducted study the conclusion is that the most suitable temperature for reeling normal cocoons is 86°C, and for the defective ones - 90°C.

Kinoshita et al. (1980) and Shimazaki (1983) considered it necessary to standardize the conditions for the preparation of cocoon cooking depending on their qualities. Standardization and improvement of conditions leads to lower prime cost of the raw silk. The need for differentiation of the conditions under which the optimal reeling preparation process takes place (temperature, duration, pressure) is accounted for by the different solubility of sericin in the shells of different hybrids. Kar et al. (2016) also believe that by manipulating the set of conditions for reeling cocoons the quantitative and qualitative results of the unreeling process can be improved. As an alternative, they propose replacing the traditional method of cooking for 3 min and drawing at 100°C by brewing under vacuum for 2-3 min and drawing at 50-60°C. An effective means for manipulating the conditions of unreeling is brewing of cocoons in strongly alkaline water with Ph 11.50 at temperature of 70°C for 15 min and obtaining the same or better results compared to the conventional conditions (Cao et al., 2014).

The review of available literature shows that for optimizing the conditions of cocoon preparation for reeling and enhancing the economic efficiency of the reeling process it is necessary to develop

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and implement different treatment based on criteria that objectively reflect their technological properties.

In this regard, we aim to determine the effect of fluorescence on the phenotypic expression of major technological characteristics in Bombyn mori L. cocoons, depending on the cooking temperatures.

Material and methods

This study was conducted in the period 2014 – 2015 at the Training experimental base of "Silk-worm breeding"section at the Faculty of Agriculture, Trakia University, Stara Zagora.

The material used comprised top quality cocoons of the tetrahybrid (19x1013) X (20x1014). The latter has been created at the base of the section by crossing populations of univoltine breeds of *Bombyx mori* L. The cocoons produced from the hybrid cocoons were divided into three factions – with yellow, intermediate and violet fluorescence. For this purpose we used a quartz lamp with a filter permeable to ultraviolet rays within the range of 330-400 nm. The preparation of cocoons for reeling was conducted in two temperature modes - 80 and 90°C with duration of 5 min. For this purpose in each fluorescent fraction we formed two subsets. The reeling of cocoons from all subsets was made in distilled water at 60°C. To account for the impact of various cooking modes, using standard methods in sericulture, we identified and analyzed the key technological features of cocoons and silk filaments. The primary information needed to identify them was obtained by individual reeling of cocoons and weighing the components extracted from them and dried to constant weight by using electronic scales (KERN PCB250-3) with an accuracy of 0.001g.

By analysis of variance (ANOVA) for each model were derived by classes of fixed factors the least square mean (LSM) and least square estimate (LSE), representing sums of squares calculated as deviation from the mean value of the parameter derived from the model.

Results and discussion

Table 1 presents the results of analysis of variances for the effect of fluorescence on the phenotypic manifestation of technological traits of cocoons and silk filament: total length of silk filament (m), nonbroken filament length (m, %), reelability (%) and raw silk percentage. It is obvious that the factor fluorescence has a statistically significant impact on the controlled technological traits ($p \leqslant 0.01$-0.001).

The longest silk filament (1165m) has been drawn from cocoons with yellow fluorescence at cooking temperature of 80°C and the shortest in cocoons with intermediate one (1034 m) at 90°C (Table 2).

The analysis of the data in the table indicates that cooking temperature affects differently the total length of silk filament drawn from cocoons with different fluorescence. The increase of temperature from 80 to 90°C is followed by a decrease in the values of the trait by 39 and 66 m, respectively, for the intermediate and yellow fluorescent cocoons and increase by 42 m for the violet fluorescent ones. It is evident from the stated data that the most significant change occurs in cocoons with yellow fluorescence (6%). In support of the findings made are the Ls-estimates of the analysed fluorescent groups, depending on the temperature regime. It is evident that the factors fluorescence and temperature affect both the extent and the nature of differences compared to the average for the model. At 80°C the violet and intermediate fluorescent groups have silk filament 38 and 21 m shorter than the average for the model, while the yellow ones - 71 m longer. By increasing temperature to 90°C, change occurs only in the violet group, which from lower at 80°C demonstrated albeit slightly higher than the average value for the model. In the other two groups the type is preserved, but the level of difference compared to the average for the model changes. In the intermediate group the difference increases from -21 to -60 m, while in the violet one it declines from +71 to +5 meters. The combined analysis of the data in Table 2 shows that for the yellow fluorescent cocoons more effective is cooking of cocoons before drawing at 80, while for the violet fluorescent ones at 90°C.

Ls-means and Ls-estimates about the effect of fluorescence on the nonbroken filament length in m and in % of the total unreeling length are presented in Table 3. The highest mean values in both temperature modes have cocoons with yellow fluorescence (1037 m), and the lowest ones – those with violet at cooking mode 80°C (835 m). The increase of temperature results in an improvement of the trait values in the cocoons with violet and yellow fluorescence. This is better manifested in the violet fluorescent ones, the mean values of which are 117 m less at 80°C and 4 m more than the average for the model. With increase of temperature the yellow fluorescent cocoons improve their characteristics by 25 m, while the intermediate ones deteriorate it by 40 m compared to the average for the model.

The mean values and estimates presented in Table 3 show that in both modes with the highest % compared to the total silk filament length are cocoons of the yellow, followed by the intermediate and the lowest ones are those of the violet fluorescent group. Increase of temperature results in decrease of the average percentage of the nonbroken silk filament by 5.74 and 1.38% in the intermediate and yellow fluorescent groups and increase in the violet one by 13.7%. Ununiform effect of the temperature mode on the percentage of nonbroken length of the silk filament depending on fluorescence is confirmed by the changes in Ls-estimates, too. In the intermediate and yellow fluorescent groups, the increase of temperature deteriorates the values of the trait by 5.74 and 1.35%, respectively. An opposite trend is observed in the violet group. In it, increase of

Table 1. Analysis of variance of effects of fluorescence on studied technological traits

Sources of variation	Degrees of freedom (n-1)	F	P
Total filament length (m)	2	9.01	***
Nonbroken filament length (m)	2	5.32	**
Nonbroken filament length (%)	2	13.08	***
Reelability (%)	2	23.4	***
Raw silk percentage (%)	2	12.07	***

NS: **: P<0.01; ***: P<0.001

Table 2. LS- mean and LS- estimate for influence of the fluorescence on the total filament length (m) at different temperatures of cooking

Source of variation	n	LS-mean±SE	LS-estimate
Mean of the model	264	1094±8.81	
80°C			
violet	56	1056±28.53	-38
intermediate	30	1073±21.93	-21
yellow	46	1165±14.34	+71
90°C			
violet	45	1098±23.98	+4
intermediate	39	1034±14.66	-60
yellow	48	1099±22.24	+5

temperature results in improvement of the trait values. Despite the fact that they are lower than the average for the model, the difference of -14.9% reduced to -1.28%. According to Takabayashi et al. (1996), the conditions of preparation of cocoons influence the process of drawing the silk filament by changes in the binding forces between the filament and the silk sheath as a whole. Optimizing the conditions in the preparation of cocoons reduces resistance and breaking of the silk filament. The values about the continuous unreeling length in m and % give us reason to assume that the swelling of sericin and reducing the resistance forces in the yellow fluorescent cocoons is achieved at lower (80°C) cooking temperature, and for the violet-fluorescent ones - 90°C.

The highest reelability rate (89.12%) is exhibited by cocoons with yellow fluorescent characteristics at mode of 80°C, and the lowest one (85.24) with violet at the same temperature mode (Table 4). The data from the table show that the change in temperature mode has an ununiform effect on the phenotypic manifestation of the unreeling trait depending on the fluorescent characteristics of silk sheaths. Intermediate and yellow fluorescent ones deteriorate their reeling qualities by 2.75 and 0.64%, respectively, by increasing temperature from 80 to 90 °C, more pronounced, as evidenced in the group with intermediate fluorescence. Unlike the above groups, the higher temperature improved by 2.59% the reeling qualities of cocoons with violet fluorescence. The reported impact of the temperature mode depending on fluorescence is confirmed by the comparison between the average unreeling rate values of the fluorescent groups and the average for the model.

The reeling capacity of the silk sheath affects directly the weight characteristics of components the ration of which determines one of the basic technological features, with major contribution to the economic efficiency of the unreeling process. This explains the uniformity of the results reported above with the data about the raw silk percentage.

The highest production of raw silk (42.58%) have the yellow fluorescent cocoon mode at mode of 80°C, and the lowest, the

Table 3. LS- mean and LS - estimate for influence of the fluorescence on the nonbroken filament length at different temperatures of cooking

Source of variation	n	Nonbroken filament length, m		Nonbroken filament length, %	
		LS-mean±SE	LS-estimate	LS-mean±SE	LS-estimate
Mean of the model	264	952±17.09		87.44±1.44	
80°C					
violet	56	835±38.97	-117	72.46±3.55	-14.98
intermediate	30	996±39.32	+44	93.39±3.20	+5.95
yellow	46	1012±25.56	+60	96.17±1.00	+8.73
90°C					
violet	45	956±49.07	+4	86.16±3.55	-1.28
intermediate	39	912±47.27	-40	87.65±4.29	+0.21
yellow	48	1037±32.51	+85	94.79±2.37	+7.35

Table 4. LS- mean and LS- estimate for influence of the fluorescence on the raw silk percentage and reelability (%) at different temperatures of cooking

Source of variation	n	Reliability, %		Raw silk percentage, %	
		LS-mean±SE	LS-estimate	LS-mean±SE	LS-estimate
Mean of the model	264	87.53±0.28		40.70±0.28	
80°C					
violet	56	85.24±0.55	-2.29	39.37±0.76	-1.33
intermediate	30	88.77±0.62	+1.24	40.33±0.64	-0.37
yellow	46	89.12±0.56	+1.59	42.58±0.50	+1.88
90°C					
violet	45	87.83±0.69	+0.3	40.96±0.66	+0.26
intermediate	39	84.02±0.73	-3.51	38.37±0.86	-2.33
yellow	48	88.48±0.60	+0.95	41.80±0.53	+1.1

intermediate fluorescent ones at 90°C (Table 3). The increase of temperature results in decrease of yield of the above groups by 0.78 and 1.96%, respectively, and increase in the violet one by 1.59%. Cocoons with yellow fluorescence are characterized by the highest Ls-estimates. They exceed the average yield for the model by 1.24 and 0.3%, respectively, at 80 and 90°C.

The Ls-estimates presented in Table 4 show that in mode 80°C, the yield from violet fluorescent cocoons is 1.33% lower, while in mode 90°C is 0.26% higher than the average for the model (40.70%). The results in Table 4 show that the two end fluorescent fractions differ in temperature modes, determining higher degree of phenotype manifestation of the traits analyzed in the table. Higher values of cocoons with yellow fluorescence produced at a lower cooking temperature mode compared with violet fluorescent ones can be explained by the lower sericin content in yellow fluorescent cocoons (Panayotov, 2014) and the established relationship between the nature of ultraviolet radiation from cocoons and the behavior of sericin in its dissolution (Aoki et al., 1986; Chang and Nahm, 1988). The higher solubility of sericin from the cocoons with yellow fluorescence is related to the weaker adhesive properties of sericin in them, as compared with the violet fluorescent ones (Ajisava, 1998).

Drawing of cocoons at a lower temperature is important for the fluorescent cocoons, which as a result of the denaturing of the proteins at temperature of 100 °C lose their fluorescent radiation. In this respect, Park et al. (2013) developed a new method of cooking, wherein the fluorescent cocoons are soaked in a solution of 0.2% (Na_2CO_3), and 0.1% Triton X100 at 60°C, then placed under vacuum. The results show that low-temperature processing of cocoons allows to obtain colored fluorescent silk, which does not change its color in the process of drawing.

Conclusion

The analysis of the data from the present study showed that fluorescence of cocoons has statistically significant ($p \leqslant 0.01$-0.001) effect on the phenotypic manifestation of the technological traits of cocoons and silk filament: total filament length (m), nonbroken filament (m, %), raw silk persentage and reelability). Cocoons differentiated by the nature of the fluorescent radiation react differently to the conditions under which the cooking process is performed (80 and 90°C) prior to their unreeling. In general, yellow fluorescent cocoons demonstrate higher values in the analysed traits at 80°C, and violet ones at 90°C. Having in mind that the optimum level of brewing cocoons is determined by the results of unreeling (Lee, 1999), it can be said that for the yellow fluorescent cocoons lower temperature is needed for preparing the cocoons for unreeling and for the violet ones - higher. The better technological qualities and the lower cooking temperature in the yellow fluorescent cocoons give reason to believe that their use is economically more profitable for the silk reeling industry compared with violet fluorescent cocoons.

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