1. Objective of Analysis

The scientific quantitative and qualitative evaluation of Sericin and its partial thermal structural stability characterization in the analyzed community products, Silk protein bar soap and Silk protein liquid soap, with supplemented silk protein extract prepared from silkworm cocoon.

2. Introduction

The silkworm cocoons are made mainly from silk fibers ensuring the protein envelope that is cohesively glued by the Sericin that constitutes 25 to 30 % of silk proteins weight in silkworm’s cocoons. The Sericin is a globular water-soluble protein and during raw silk production it is separated from silk fibers. The Sericin molecular weight ranges from 10 to 400 kDa and its structure and molecular weight depend on the method of extraction and purification. Usually the Sericin is discarded in waste water during raw silk production and the process of Sericin degradation is usually highly demanding in respect of biological oxygen consumption. Lower molecular weight Sericin peptides/oligopeptides are mainly used in cosmetic products, whereas higher molecular ones are utilized for many other applications (e.g. medical biomaterials).

3. Goals

3.1. The development and optimization of advanced laboratory techniques for quantitative and qualitative analysis of Sericin in two products: Silk protein bar soap and Silk protein liquid soap.

3.2. The Sericin quantification and statistical analysis of two closely related batches (in time) of each product (hard Silk protein bar soap, Silk protein liquid soap):

- the hard Silk protein bar soap: five pieces per each batch for statistical analysis (in total 10 pieces from two closely related batches in time),
• the Silk protein liquid soap: two pieces per each batch for statistical analysis (in total 10 pieces from two closely related batches in time),

• the same raw protein silk extracts for each batch used for manufacturing of each product (the minimal volume of 200 ml of each raw extract, in total 4 raw protein extracts).

3.3. The thermal stability of protein components: e.g. simulation of thermal-dependend structural changes during the product preparation from raw silk protein extract (e.g. comparison of the protein secondary structure changes in samples of Sericin standard and raw silk extracts).

3.4. The recommendations, based on results of analytical and structural methods that could accent e.g. the Sericin content standardization during the product preparation by traditional community procedure.

3.5. The preparation of scientific report with result discussion that could be potentially used for BEDO marketing promotion:

• the report on sample preparation (enrichment from product) and its analysis (method development, detailed description of individual steps and approach optimization),

• the summary analysis result - presentation and discussion of all qualitative and quantitative data,

• the suggestion for marketing promotion (e.g. "The Department of Biochemistry, Faculty of Science, Charles University in Prague analyzed the product, batch number .AAA. produced at XX/YY/2015, and determined .BBB. mg of Sericin per gram of analyzed product.")

• the report of thermal dependent structural changes of Sericin components - structural characterization,

• the result discussion and recommendations for approaches used during traditional community procedure,

• the design of graphical symbol (e.g. logo of Department or Laboratory) that could be presented together with analysis result by BEDO for marketing promotion or publicity campaign presentations of both analyzed products.

4. Suggested methodological approaches

4.1 Design of extraction (e.g. LLE, SPE), precipitation (e.g. by acidification, by organic solvent, or by combination of both previously described methods of precipitation) or additional purification procedures specifically concentrating the protein/oligopeptide content and eliminating the interfering impurities (e.g. additive or fragrance materials).

4.2 Development and optimization of the Qualitative analysis determining the potential presence of the protein impurities in raw silk extracts of Sericin.

4.3 Performance of quantitative analysis with accent to Sericin (employing one of selected and optimized methodology approaches e.g. amino acid analysis, polyclonal antibody dependent approaches, densitometric methods, UV-VIS protein determination).

4.4 Design and preparation of methods for structural characterization of thermal stability in Sericin-containing protein extracts (simulation of thermal conditions used during product preparation) by employing e.g. thermal stability diagrams of the protein secondary structure determination by circular dichroism.
5. **Contract conditions**

5.1. The suggested quantitative and qualitative analysis can be completed within 4 months from date of signing the acceptance of the purchase order.

5.2. The offered prize for described analysis is 10 000 EURO.

5.3. The proposed budget – in details

<table>
<thead>
<tr>
<th>Description</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>chemicals and consumables</td>
<td>1 550 EU</td>
</tr>
<tr>
<td>analysis price including equipment utilization</td>
<td>2 350 EU</td>
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<tr>
<td>personal salary (including social security and healthcare taxes)</td>
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<tr>
<td>overhead of Faculty of Science</td>
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<tr>
<td>taxes</td>
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<tr>
<td><strong>GRAND TOTAL</strong></td>
<td><strong>10 000 EU</strong></td>
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