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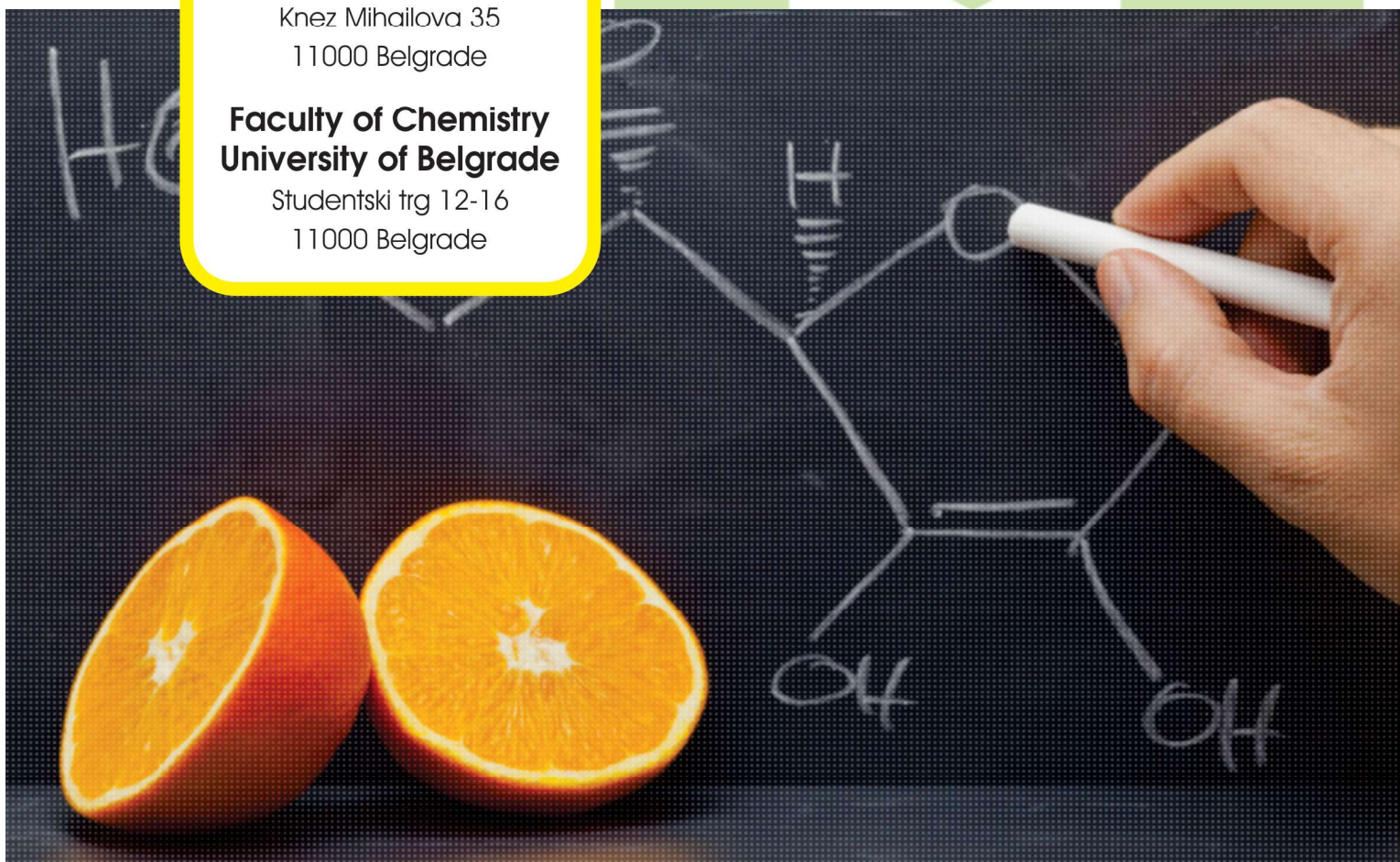
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Investigation of structural changes in ovalbumin induced by two types of MPs and its impact on protein digestibility

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Ovalbumin (OVA) is the most abundant protein in chicken egg white. It is one of the major allergens in eggs. Micro- and nanoplastic particles (MNPs) are a widespread contaminant and have been found in food and water. It is still unclear how MNPs might affect human health. However, due to their large surface area they have been found to bind various biopolymers, including proteins. These biopolymers can be bound more strongly or loosely, and are referred to as hard and soft corona, respectively [1]. MPs have been found in eggs, in the size range of 50-100 µm [2]. It is shown that these particles can interact with proteins and induce structural changes, but there is still not enough information on this topic [3]. These structural changes could lead to a decreased digestibility in the gastrointestinal tract, which could increase the immune response to known allergens.

The aim of this study was to determine whether there are structural changes present in the OVA after incubation with two types of MPs – 120 µm polyethylene terephthalate (PET) and 120 µm polystyrene (PS) and whether they could influence digestion of OVA with gastrointestinal enzymes. 20 mg of MPs were incubated with 1.3 mg/mL ovalbumin for 4 h at room temperature in a 20 mM phosphate buffer at pH 7. Bulk ovalbumin was separated from the MPs by centrifugation and by filtration through a 0.22 µm PVDF filter. Soft corona was obtained by washing the MPs with water, and the MPs were later removed as described with bulk ovalbumin. Formation of amyloids was monitored with a Thioflavin T (ThT) assay at room temperature and after thermal treatment, and additional structural analysis was performed by circular dichroism (CD) spectrometry in the far-UV region. Thermal stability was also determined by spectrofluorimetry. Digestion with two proteases (pepsin and trypsin) was performed to determine whether there is a change in the gastrointestinal digestibility of OVA.

Results from the ThT assay show that at room temperature there is no significant difference between the fluorescence emission obtained for all samples, with bulk OVA from both MPs showing a slight decrease. However, there is an increase of fluorescence after thermal treatment in all OVA samples, where OVA from the soft corona emits significantly less fluorescence than control and bulk samples for both types of MPs. Additionally, soft coronas have been shown to have more β-sheet content than other samples, which is more pronounced for OVA incubated with PET. For the heated samples there is a sharp change from α-helix to β-sheets in all the samples, but it is the most dramatic in the soft coronas. This could impose rigidity to the tertiary structure, which would explain why the ThT molecule does not bind as strongly. Despite differences in both the secondary and tertiary structure, the thermal stability is almost the same in all samples. Digestion of the samples shows that the soft corona incubated with PS tends to be more resistant to trypsin than other samples after 2 min, but it is not significant. For digestion with pepsin there is no difference between the samples. In conjunction with the previous results, which indicates a structural stabilisation of the soft corona at pH 7, it is not surprising that there is an increased resistance to trypsin, compared to pepsin which is a gastric enzyme and for which digestion is performed at an acidic pH.

In conclusion, there is a structural change present in samples upon contact with MPs, particularly in the soft corona, of which the most pronounced is a decrease of α-helix content and increase in β-sheet content as determined by far-UV CD. This leads to a structural stabilization which could further impact the digestibility of the OVA protein and impact its allergenicity. However, this must be confirmed with further experiments.

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