RECOVERY AND FUNCTIONAL PROPERTIES OF RUBISCO PROTEIN FROM CONVENTIONAL AND ENZYMATIC EXTRACTIONS

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Abstract
Protein isolates extracted by conventional and enzymatic protocols from pumpkin leaves were evaluated. Pumpkin leaves represent waste material that can be used for extraction of RuBisCO protein. Results showed that usage of Viscozyme for enzyme-assisted extraction enhanced recovery of protein by 30% compared to conventional extraction protocol. Moreover, protein extracted by enzymatic treatment showed improved solubility and oil holding capacity by 71% and 13%, respectively. Our findings might indicate a possibility of usage of enzyme treatment that would enable production of protein isolate with properties and/or in quantities tailored to their particular application in food systems.

Introduction
Globally, there is a constant search for protein sources that should provide healthier and lower-cost alternatives without compromising product quality and safety. Green leaves, that present waste material, are an alternative source of proteins for human consumption. RuBisCO (ribulose-1,5-bisphosphate carboxylase/oxygenase) is the main protein in green leaves and is the most abundant protein in nature [1]. Moreover, RuBisCO presents a source of protein with good functional and nutritional properties [2].
The basic principle of enzyme-assisted extraction is a disruption of plant cell wall by hydrolyzing its polysaccharides by enzymes in order to enhance the release of intracellular components. Degradation of structural polysaccharides usually is achieved with different carbohydrases [3].

Experimental
Pumpkin leaves were selectively harvested at suitable maturity from the fields where pumpkins were grown (JS&O, d.o.o. Novo Miloševo, Serbia). After harvest, leaves free from decay or damage were washed and stored at -18 °C in the freezer for couple days prior to analysis.
Enzymatic extraction was performed using enzyme preparation Viscozyme (Novozymes) in previously determined dosages. Pumpkin leaves were pressed and suspension containing both solid and liquid streams was enzymatically treated (45 ℃ and pH 5.5 for 1 hour). After extraction, suspension was centrifuged and the contaminant proteins from green protein fraction were removed by thermal denaturation at 55 ℃ for 30 minutes. Subsequently, supernatant from centrifugation was subjected to isoelectric precipitation at pH 4.5. After precipitation, protein curd was collected by centrifugation and freeze dried. Conventional protocol was performed in equal way but without enzymatic treatment.
Oil holding capacity (OHC) of protein isolates was determined according to Tan et al. [4] while solubility was measured by the method described by Perović et al. [5].

Results and discussion

Results showed that protocol with usage of enzyme enabled enhanced recovery of RuBisCO proteins from pumpkin leaves. Improvement of recovery with Viscozyme was approximately 30% compared to conventional extraction process. Additionally, protein extracted with the assistance of enzyme preparation improved solubility and oil holding capacity by 71% and 13%, respectively.

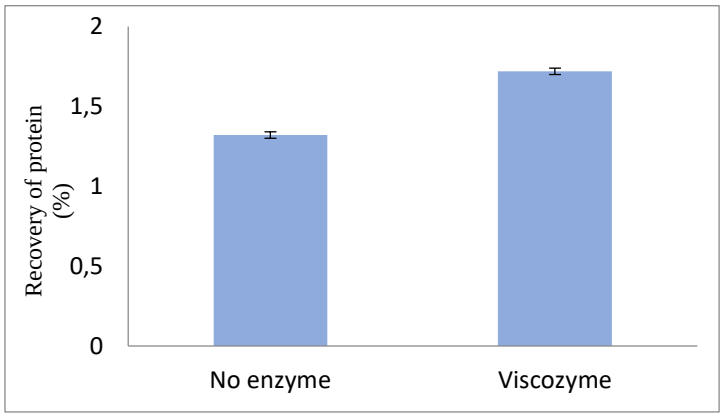

Figure 1. Recovery of RuBisCO protein extracted from pumpkin leaves with and without treatment with enzyme

Table 2. Functional properties of RuBisCO protein extracted from pumpkin leaves with and without treatment with enzyme

	No enzyme	Viscozyme
Solubility (%)	9.40±0.02	16.10±0.07
OHC (g/g)	6.62±0.01	7.49±0.41

Conclusion

Enzymatic treatment of pumpkin leaves with commercional enzyme preparation Viscozyme greatly affected recovery of protein as well as functional properties. Our findings showed that enzymatic extraction enable production of protein isolate in higher amounts and with improved functional properties in comparison to protein isolate from conventional extraction process.

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