

# Hydrophilic Isolates of rye flour as a potential matrix for the use as a vehicle of active compounds

Cristián A. Ferretti\*, Mariana Celman, Pablo Noriega and María N. Kneeteman

*IQAL (UNL-CONICET), Laboratorio Fester – QUÍMICA ORGANICA (FIQ), Universidad Nacional del Litoral, Santa Fe, Argentina.*

\* Correspondence: [cferretti@fiq.unl.edu.ar](mailto:cferretti@fiq.unl.edu.ar); Tel.: +543424571164

---

**Abstract:** The isolation of water-soluble polysaccharides and proteins solubilized during the treatment of rye flour in an isotonic aqueous medium is described. Protein and polysaccharide assays of the aqueous fractions showed the high extraction performance of these compounds. Then, nanoparticles were synthesized from these hydrophilic isolates using fluorescein as a model drug and sodium hexapolyphosphate as a cross-linker. The preparation process consisted of dispersion, desolvation, drug incorporation, crosslinking and separation of nanoparticles. The physicochemical analysis revealed that nanoparticles were successfully synthesized with rye hydrophilic isolates using a desolvation method. These studies demonstrated that rye nanoparticles are a promising candidate as an active compound vehicle system.

**Keywords:** rye hydrophilic isolates, organic matrix, nanoparticles.

---

## 1. Introduction

Biomass is a complex natural renewable material with enormous chemical variability and great potential application. Biomass is defined as matter originating from living plants, including tree stems, branches, leaves as well as residues from agricultural harvesting and processing of seeds or fruits [1]. The valorization of natural compounds obtained from biomass is a topic of growing study in many areas of chemical sciences.

Rye flour is a product of biomass and it is used extensively for making a range of bread, as a filler for sauces, soups, and custard powder, but it is also used in the glue, match and plastics industries.

The rye protein, when hydrated, does not form gluten because the proportion of the protein that is soluble is much larger in rye than in wheat (up to 80% soluble in rye sourdough as compared with 10% soluble protein in wheat dough), and because the high content of pentosans inhibits the formation of gluten [2]. Pentosans are hemicellulose, the soluble fraction of polysaccharides. Conversely, the pentosans and starch in rye are much more important than in wheat. The pentosans, which comprise 4%–7% of rye flour, and the starch have an important water-binding function. [3]

In this research work, the extraction of proteins and polysaccharides from rye flour using an isotonic aqueous solution was performed. Then, with these isolates were evaluated the synthesis of nanoparticles using fluorescein as a model drug. These biopolymer nanoparticles have shown to be interesting as an active compound release system with different applications.

## 2. Materials and methods

### 2.1. Materials

Rye (*Secale cereale*) flour was obtained from commercial source. All reagents were of analytical grade and used without purification.

### 2.2. Polysaccharide and protein extractions

Polysaccharides and proteins (PP) were extracted from 40 g of rye flour with 250 mL of 0.9 w % NaCl aqueous solution a pH 7.0 by stirring at 500 RPM for 12 hours at ambient temperature. Then, the mixture was centrifuged at 3500 RPM for 20 min. The supernatant solution was separated and maintained at 4 °C overnight. As a result of the maceration of solution at 4 °C, two liquid phases were obtained: an upper clear phase and a lower viscous phase.

For this study, the upper phase was used and characterized.

### 2.3. Determination of total dry residue

A sample of 10 ml of the upper phase was accurately weighed on a previously dry and heavy container. The sample was subsequently evaporated in an oven at 105 °C until being completely dry. Finally, the container was weighed and the total dry residue of the sample was determined by weight difference based on the initial weight of the sample.

### 2.4. Determination of proteins

For the quantification of proteins, the Biuret assay was used [4]. The Biuret reagent was prepared to dissolve 2.25 g of potassium sodium tartrate, 0.75 g of cupric sulfate x 5 H<sub>2</sub>O and 1.25 g of potassium iodide with 100 ml of 0.2 M NaOH in a volumetric flask of 250 mL. Then, the solution was diluted at 250 ml with distilled water. A sample of 1.25 ml of the upper phase was transferred quantitatively in a volumetric flask of 10 ml and diluted at volume with an aqueous solution of 0.9 % wt. NaCl. Then, 1 mL of this solution was taken and mixed with 4 ml of Biuret reagent and the mixture was stirred. Samples were incubated for 30 minutes at room temperature and after this time the absorbance of samples was tested. Measurement of absorbance was done with UV - VIS Perkin Elmer Lambda 20 spectrophotometer, at wavelength  $\lambda = 550$  nm. The calibration curve was made from a solution of ovalbumin in an aqueous solution of 0.9% wt. NaCl in the concentration range of 0.20-0.10 mg/ml and the equation describing it was:  $y = 0.044x + 0.095$  with  $R^2 = 0.9986$ . The study was performed in duplicate.

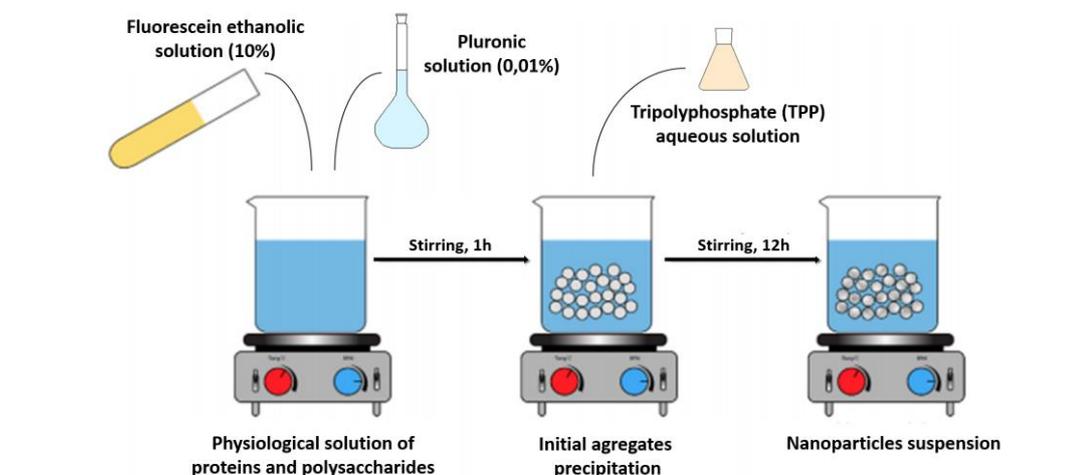
### 2.5. Determination of polysaccharides

For the quantification of polysaccharides, the DuBois assay was used [5]. A sample of 50  $\mu$ L of the upper phase was taken and volume was brought to 5 ml with distilled water. Then 1 ml of this solution was taken (in duplicate), and 500  $\mu$ L of a solution of 0.5 % wt. phenol and 2.5 ml of concentrated sulfuric acid were added. It was homogenized and allowed to stand for 30 minutes in an ice bath. The absorbance of the samples so prepared was measured at wavelength  $\lambda = 490$  nm against the zero test. Calibration curve was made from sucrose solution in the concentration range 10-100 mg/L and the equation describing was:  $y = 0.0115x + 0.0678$  at  $R^2 = 0.9955$ . The study was performed in duplicate.

### 2.6. Preparation of fluorescein loaded PP nanoparticles

The isolation of water-soluble polysaccharides and proteins (PP) corresponding to upper phase was used as matrix for preparation of nanoparticles. The PP nanoparticles were prepared by a desolvation method [6] with modification following the scheme of Figure 1. Typically, over 10 ml of PP solution was added dropwise 10 mL of a solution of 10 wt. % fluorescein in ethanol, followed by 10 mL of a solution of 0.1 wt. % Pluronic in ethanol. After 1 h of equilibration, a solution of 0.5 wt. sodium hexapolyphosphate (trypolyphosphate) in water was added as a cross-linker. After 12 h of cross-linking reaction at room temperature, the organic solvent was evaporated and the resulting

suspension was centrifuged at 3500 RPM for 20 min. The supernatant obtained after centrifugation was dried in oven under reduced pressure and stored at 4 °C.



**Figure 1.** Schemes of preparation of PP nanoparticles.

### 3. Results and discussion

#### 3.1. Characterization of extract of water-soluble polysaccharides and proteins of rye flour

The main properties obtained in the characterization of the upper phase of the isolate of water-soluble polysaccharides and proteins of rye flour are summarized in Table 1. Similar values were reported in the literature [7].

**Table 1.** Properties of extract of water-soluble polysaccharides and proteins of rye flour.

Property	value
pH	7.1 ± 0.1
Total dry residue (wt. %)	3.6 ± 0.5
Total proteins (wt. %)	1.09 ± 0.34
Total polysaccharides (wt. %)	1.26 ± 0.05

#### 3.2. Characterization of fluorescent nanoparticles

The PP fraction, water-soluble polysaccharides and proteins of rye flour, precipitated into nanoparticles by the desolvation method as nanoprecipitates. The preparation process consisted of dispersion, desolvation, drug incorporation, crosslinking and separation of nanoparticles.

The nanoparticles were synthesized by a rapid diffusion of polysaccharides and proteins solution with fluorescent alcohol solution, which results in surface tension at the interface between two liquids causing an increase in surface area, and leading to precipitation of PP nanoparticles. The process can take place in both, the presence and the absence of surfactants, but the incorporation of Pluronic increase the interaction of fluorescein with proteins and polysaccharides reducing the surface tension [8]. The process is simple, rapid, and reproducible. The fluorescein nanoparticles are spherical, with sizes between 20 and 100 nm (determined by dynamic light scattering (DLS) technique).

#### 4. Conclusions

The study of isolation of proteins and polysaccharides of rye flour using an isotonic solution at pH 7 and room temperature, with a relation solid:liquid of 6.25 g/ml was adequate to obtain a liquid phase rich in polymers with concentrations of  $1.09 \pm 0.34$  wt. % and  $1.26 \pm 0.05$  wt. % in proteins and polysaccharides, respectively.

The aqueous extract rich in proteins and polysaccharides of rye, was subjected to a process of desolvation with sodium hexamorphosphate (TTP) to encapsulate fluorescein, obtaining spherical nanoparticles with sizes between 20 and 100 nm. These nanoparticles proved to be a suitable system to vehicle active molecules of interest.

**Acknowledgments:** This research was supported by the Agencia Nacional de Ciencia y Tecnología (ANCyT) of Argentina -PICT 2014 No. 1587 and by the Universidad Nacional del Litoral, Santa Fe, Argentina.

**Conflicts of Interest:** The authors declare no conflict of interest.

#### References

1. *Fuel Flexible Energy Generation, Solid, Liquid and Gaseous Fuels*, Publisher: Elsevier, 2016, pp 241-269.
2. Drews E., Seibel, W., 1976. *Bread-making and other uses around the world (Chapter 6)*. In: Bushuk, W. (Ed.), *Rye: Production, Chemistry and Technology*. Amer. Assoc. of Cereal Chemists Inc., St. Paul, MN, USA.
3. Cauvain S. P., Young L. S., *Technology of Breadmaking, Second Edition*. Publisher: Springer-Verlag US, 2007, pp m., d567-622.
4. Gornall A.G., Bardwill C.J., David M.M., "Determination of serum proteins by means of the biuret reaction", *J. Biol. Chem.* 1949, vol. 177, pp 751-766.
5. Dubois M., Gilles K., Hamilton J.K., Rebers P., Smit F., "Colorimetric tric Method Determination of Sugar and Related Substances", *Anal. Chem.* 1956, vol. 28, no. 3, pp. 350-356.
6. Duclairoir C., Orecchioni A.M., Depraetere P., Osterstock F., Nakache E., "Evaluation of gliadins nanoparticles as drug delivery systems: a study of three different drugs", *Int. J. Pharm.* 2003, vol. 253, pp. 133-144.
7. Cyran M., Cygankiewicz A., "Variability in the content of water-extractable and water-unextractable non-starch polysaccharides in rye flour and their relationship to baking quality parameters", *Cereal Research Comm.* 2003, vol. 32, pp. 143-150.
8. Betancourt T., Brown B., Brannon-Peppas L., "Doxorubicin-loaded PLGA nanoparticles by nanoprecipitation: preparation, characterization and in vitro evaluation", *Nanomedicine.* 2007; vol. 2, pp. 219-232.



© 2019 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).