

Simultaneous Isolation of Oil, Proteins, Myrosinase, and Glucosinolates from Cruciferous Oilseeds



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Introduction

Cruciferous oilseeds are an important source of high erucic acid oils, proteins, and bioactive molecules such as glucosinolates (GLs) (see Table 1) [1]. GLs in particular are important secondary metabolites with a great potential in fine chemistry, food technology and crop protection. At present, however, most of these bioactive products are not commercially offered on an industrial scale, although this feature appears to be fundamental for their exploitation in agriculture [2]. The present study, aimed at evaluating an advantageous biorefinery process that makes it possible to extract oils, myrosinase (MYR) laden soluble proteins, and GLs. This fractionation technology is based on the use of reverse micelles (RM), a water in oil system able to solubilize hydrophobic and hydrophilic compounds, suitable for extraction from meals and in principle easy to scale up [3,4,5,6].

Methods

Reverse micelles extraction process:

Reverse micelles are micro-dispersions (10-100 Å) of aqueous solutions in apolar organic solvents, formed by suitable surfactants. When applied to cruciferous oilseed processing, this ternary system allows the solubilization of the oil in the bulk of organic solvent, but also the isolation, in the RM polar core, of small hydrophilic molecules, like GLs. This system also permits the isolation of larger macromolecules, such as proteins [5], according to predetermined extraction conditions, thus permitting the simultaneous extraction of different oilseed products [4,6] (see table 2). W_o ($H_2O/[surfactant]$) is an important parameter that determines most of the extraction properties of RM, affecting their dimensions [3] and, consequently, the capability of hosting molecules with different molecular weights (MW) (see Figure 1). In our study, the RM solution with the best extraction yield was: 100 mM Cetyl Trimethyl Ammonium Bromide (CTAB), as surfactant, in isooctane and 10% v/v butanol (ButOH), as co-surfactant, and different amounts of water or buffer solution, depending on the desired W_o .

The extraction process is based mainly on three steps:

- Seed conditioning: grinding and heat treatment for MYR deactivation, in the case of GL extraction;
- Solid-liquid phase extraction by RM solution (1: 50 w/v);
- Back-transfer of RM solution and recovery of extracted compounds.

Results and Discussion

Table 1. Oil, proteins, GLs, and MYR contents of some Brassica oilseed meals of industrial interest and their potential applications.

Species	Oil %	Total Proteins %	Total MYR U/g	GLs main type (%)	GLs $\mu\text{mol/g}$
<i>Crambe abyssinica</i> (dehulled)	50.4 ± 0.1	26.0 ± 0.1	45.7 ± 2.5	epi-Progoitrin (~90)	89.7 ± 0.4
<i>Brassica carinata</i>	31.9 ± 0.4	29.1 ± 0.1	6.7 ± 0.2	Sinigrin (~90)	101.1 ± 1.0
<i>Brassica juncea</i>	43.5 ± 0.2	24.4 ± 0.1	3.9 ± 0.1	Sinigrin (~97)	79.0 ± 1.2

- Spinning in the textile industry
- Steel casting and hardening
- Metal forming
- Emulsifying oils for machinery
- Offshore gas & oil drilling
- Sea engine lubrication

- Myrosinase(s)
- Thionins
- Proteinase Inhibitors
- Myrosinase Binding Proteins

MYR-GL formulations

- Fine chemistry
- Food technology
- Crop protection

Table 2. Extraction yields of oil, GLs, proteins, and MYR using RM technique in batch (CTAB 100 mM in isooctane and 10% ButOH), in some Brassica seed meals.

Species	OIL % (RM $W_o = 10$)	GLs % (RM $W_o = 10$)	Soluble PROTEINS % ^a (RM $W_o = 6 - 50$)	Soluble MYR % ^a (RM $W_o = 40 - 50$)
<i>Crambe abyssinica</i> (dehulled)	95.1 ± 0.7	86.3 ± 4.0	n.d.	n.d.
<i>Brassica carinata</i>	96.3 ± 0.5	83.2 ± 2.5	10.0 - 91.7	25.0 - 44.0
<i>Brassica juncea</i>	95.8 ± 0.5	83.0 ± 1.2	n.d.	n.d.

^aProteins and MYR expressed as % on their soluble fraction.
n.d., not determined

The **OIL** extraction yield from cruciferous seed meals with RM technology is comparable with current organic solvent oil extraction methods.

GLs are extracted in high percentages even at low W_o (low water content of the RM solution and low micelle dimension). The extraction yield rises to 94% at higher W_o as shown in Figure 1.

PROTEIN extraction is more affected by W_o than GLs, due to their different MW and solubility. Among proteins, **MYR** is an important enzyme with a great potential in biotechnology and, together with GLs, in special formulations, in crop protection. This technology makes it possible to extract up to 91.7 % of soluble proteins and 44.0% of soluble MYR with RM, at high W_o values.

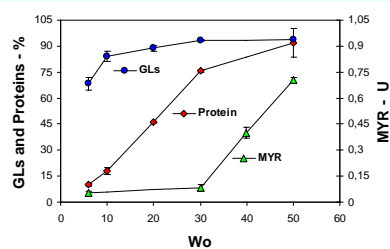


Figure 1. Extraction yields of GLs, proteins, and MYR from *B. carinata* meal, by RM (CTAB 100 mM in isooctane and 10% ButOH), as a function of W_o . Extracted soluble proteins % (♦ — ♦); extracted GLs % (● — ●); extracted soluble MYR U (▲ — ▲). Soluble proteins and GLs are expressed as % on the total content of soluble proteins and GLs in the starting meal; MYR is expressed as total units U in the final extract.

The good water solubility and the low MW of **GLs** makes the RM system an efficient extraction method for this class of compounds, in a wide range of W_o . **PROTEIN** extraction yield is directly proportional to W_o up to W_o 30, extracting 76% of soluble proteins. **MYR** is solubilized in RM only between W_o 40-50, due to its higher MW (~140 kD).

These results indicate that W_o affects GLs and protein extractability differently, thus permitting seed-meal component fractionation.

Conclusions

This fractionation technology notably increases the value of cruciferous oilseeds, and appears to be a promising alternative to the current oil extraction methods, as it makes it possible to diversify oilseed extraction products without strongly modifying the current solvent extraction plants.

References

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