Purification and Characterisation of Soluble Rapeseed Protein for Food Application

Maria Kristjansson[1], Karin Loft Eybye[1] and Marie Bendix Hansen[2]

[1] Danish Technological Institute, Life Science Division, Kongsvang Allé 29, DK-8000 Aarhus
2 Upfront Chromatography A/S, Lersoe Parkallé 42, DK-2100 Copenhagen

ABSTRACT

Two protein fractions from rapeseed have been extracted and characterised with regard to application in the food industry. The functional properties such as gelling properties, water and oil holding capacity, foam capacity and stability properties have been investigated.

INTRODUCTION

Rapeseed residues from oil industry waste contain 22% protein that could be a valuable source of high quality proteins. The proteins have a well-balanced amino acid profile and could be an alternative to other protein sources such as milk proteins for human food. Furthermore, plant proteins are increasingly used as sources to perform functional roles in food formulations.

The rapeseed proteins of interest are mainly storage proteins, which can be divided into various fractions. The two major proteins are Cruciferin and Napin, which constitute 60% and 20%, respectively, of the total protein found in rapeseeds. Napin is a low molecular weight protein compared to Cruciferin with a higher molecular weight. The two types of protein exhibit different chemical, bioactive and functional properties.

The aim of the present work was to capture these two protein fractions using expanded bed adsorption (EBA) technology (chromatography). The EBA technology allows crude unclarified feedstock to pass unhindered through a column only retaining the target molecules. A subsequent wash removes the last traces of unwanted particles before the elution releases the target molecules efficiently.

The extraction process has been developed with focus on a large-scale production of these proteins. The process removes major anti-nutritional factors such as polyphenols and glucosinolates, which previously have been a hindrance for consumption.

Preliminary results of the produced rapeseed protein isolates show that the products contain up to 98% protein and the bitter tasting phenolic compounds have been reduced by 97%. The level of residue phenols is most likely below taste thresholds of individual phenolic acids. The results also suggest that the protein isolates can be useful as protein source with high nutritional value and a rich source of antioxidant activity for feed or food applications.

MATERIAL AND METHODS

Extraction and drying of protein fractions

The rapeseed cake (residual product from cold oil pressing) was supplied by Morsø Oliemølle and milled at Danish Technological Institute. 120 kg of milled

rapeseed cake was mixed with 600 L of water. Extraction time was 30 min. After extraction, the rapeseed residues were removed on a nylon filter.

The extract was passed through a two column setup. Column 1 mainly captures the specific low molecular weight protein (Napin) and the flow from column 1 was passed through a second column that captures a broader fraction of soluble rapeseed proteins present in the extract. The extract was loaded directly onto the columns without adjusting pH or any other pre-treatments than mentioned in the extraction section.

The two protein isolates from the chromatographic process (approximately 0.5% dry matter) were ultrafiltrated and diafiltrated (10 kD cut-off membrane) and freeze dried.

pH adjustments

Protein isolate samples were adjusted to pH 4 and 7 using 0.1M and 1M HCl and NaOH.

Water holding capacity

The water holding capacity was measured by mixing 2 g protein isolate with 16 mL demineralised water and pH adjusted to 4 and 7. The mixture was transferred to 50 mL centrifuge tubes. The tubes were vortexed for 30 s. every 10 min. for 1 hour and subsequently centrifuged at 2000xg for 15 min. After centrifugation, the tubes were drained for 30 min. by inversion. The final weight of each sample was measured and the water holding capacity was measured as the percentage increase in sample weight. Measurements were performed in duplicate.

Oil holding capacity

The oil holding capacity was measured by mixing 2 g of protein isolate with 12 mL rapeseed oil in 50 mL centrifuge tubes. The tubes were vortexed for 30 s. every 5 min. for 30 min. The samples were subsequently centrifuged at 1600xg for 25 min. After centrifugation the excess oil was decanted and the tubes inverted and drained for 1 hour. The final weight was measured and the oil holding capacity was calculated as the percentage increase in sample weight. Measurements were performed in duplicate.

Foam capacity and stability

The foam capacity and stability were determined by mixing 1.5 g protein isolate with 50 mL demineralised water in a 250 mL beaker and adjusting pH to 4 and 7. The mixture was homogenized for 5 min. using an Ultra Turrax at 20,000 rpm. Immediately after, the initial foam volume was noted and again after 5, 10, 20, 45, 60, 90 and 120 min. The percentage volume increase is used to express the foam capacity and stability. Measurements were performed in duplicate.

Gelling ability

The gelling ability was measured by mixing 2.4 g protein isolate with 20 mL demineralised water and adjusting pH to 4 and 7. The mixtures were measured in a cup and bob system on a rheometer (Anton Paar). The samples were heated and cooled over a temperature range from 25°C to 95°C at 2°C/min. with a 5 min. hold time at the final temperature for both the heating and cooling phase. After cooling, a frequency sweep from 0.1-20 Hz was performed and the gel strength was calculated at 1 Hz.

RESULTS AND DISCUSSION

The yield of the two isolated fractions was in the range of 40-50 g per kg milled rapeseed cake.

The functional properties of the two protein isolates were evaluated at pH 4 and 7. The water holding capacity (Fig. 1) of the protein isolates shows that only fraction 2 at pH 7 shows an increase in water holding capacity just above 200%, which is in agreement with the findings of Manamperi and Pryor[1]. Ghodsvali et al.[2] tested canola meal and found water holding capacities within the same magnitude. In the remaining

samples, the protein is almost totally dissolved and can only bind a very small amount of water.

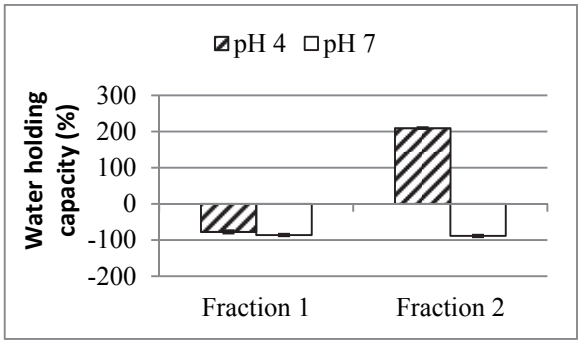

Figure 1. Water holding capacity of fraction 1 and 2 at pH 4 and 7.

The oil holding capacity (Fig. 2) shows that both isolates have oil holding capacities just above 500%, which is higher than the values reported in literature, which is about 100% for Manamperi and Pryor[1] and varies from 204-295% for canola meal for Ghodsvali et al.[2]

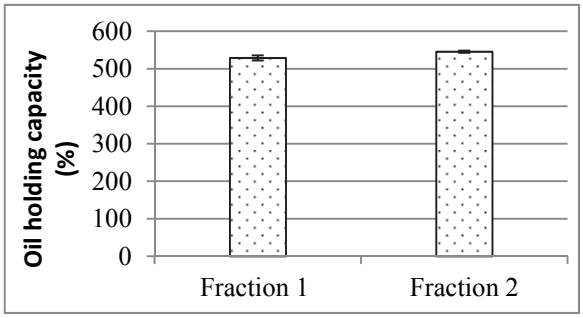

Figure 2. Oil holding capacity of fraction 1 and 2.

The foam capacity is quite similar for the two fractions at the two pHs. The foam stability is affected by pH and at pH 4 the foams are rather instable compared to those at pH 7 (Fig. 3). The foam capacity and stability are quite similar to the values found for canola meal by Ghodsvali et al.[2] but higher compared to the finding of Khattab and Arntfield[3], where foam capacities for canola meals range from 20-100%.

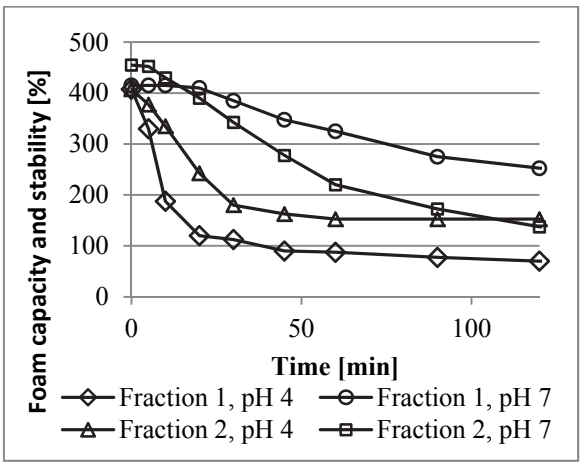

Figure 3. Foam capacity and stability of fraction 1 and 2 at pH 4 and 7.

The gel strength of 12.5% dispersions at 25°C at 1 Hz after heating to 95°C and subsequent cooling to 25°C were determined. The results shown in Fig. 4 indicate the high molecular fraction as expected form gels with higher gel strengths compared to the low molecular fraction, which is well known in literature[4]. Uruakpa and Arntfield[5] vary pH and NaCl content in their samples of protein isolate and find G´ values varying from 2.510-91.400 Pa.

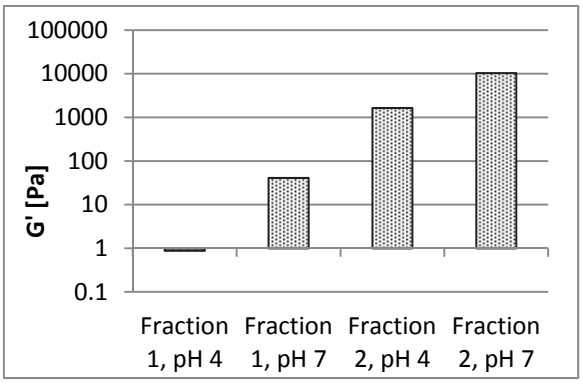

Figure 4. Gel strength at 1 Hz at 25°C of fraction 1 and 2 at pH 4 and 7.

CONCLUSION

The results of the present work demonstrate that the extracted and dried protein isolates from rapeseed residues from oil production show promising functional properties that are very relevant to the food industry. This includes all the tested

properties. Some of the properties are highly sensitive to pH and the influence of salt will also be of great importance, which means that there are a number of possibilities for manipulating the suspensions and solutions to achieve the desired functional property. Furthermore, the findings are generally in accordance with literature findings that indicate a successful gentle extraction process.

REFERENCES

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