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## Bread-making potential of pea protein isolate produced by a novel ultrafiltration/diafiltration process

Louis-Philippe Des Marchais, Mathieu Foisy, Samuel Mercier, Sébastien Villeneuve \*, Martin Mondor

*Food Research and Development Centre, Agriculture and Agri-Food Canada, 3600 Casavant Blvd West, Saint-Hyacinthe, Quebec, Canada, J2S 8E3 (sebastien.villeneuve@agr.gc.ca)*

### Abstract

The incorporation of ingredients like legume flour, concentrate or isolate in cereal-based matrices can lead to the production of nutritionally enhanced products like bread with high protein content. However, many ingredients currently available on the market have a large phytate to protein ratio resulting in reduced protein digestibility and minerals bioavailability. This work aimed to study the potential of supplementing bread with pea protein isolate having a low phytate to protein ratio produced by a novel ultrafiltration/diafiltration (UF/DF) process. Isolate was produced by extracting pea flour in water at room temperature and pH 7.5 followed by purification with UF/DF using 50 kDa hollow fibres membranes. Commercial wheat flour was then substituted at a 10% level (dry basis) with isolate in order to produce bread with protein content over 20%. Flour properties, dough mixing properties and bread characteristics were determined. Supplementing wheat flour with pea protein isolate did not affect damaged starch but slightly decreased falling number. Water absorption was increased for substituted flour while dough stability and development time were not affected. The addition of isolate caused a diminution of the minimum water content for dough formation under continuous water addition. Furthermore, higher maximum torque was observed for the substituted flour with corresponding lower water content at maximum torque. Substitution with pea protein isolate allowed to produce bread with protein content over 20% but induced a decrease in loaf specific volume compared to unsubstituted flour. However, the specific volume of bread substituted with pea protein was maintained over 4 g cm<sup>-3</sup>, which should be considered as satisfactory by the consumers. Flour substituted with low phytate pea protein isolate produced by UF/DF showed a good bread-making potential when compared to unsubstituted flour.

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\* Corresponding author. Tel.: +1-450-768-3335; fax: +1-450-773-8461.

*E-mail address:* [sebastien.villeneuve@agr.gc.ca](mailto:sebastien.villeneuve@agr.gc.ca).

## 1. Introduction

The incorporation of ingredients like legume flour, concentrate or isolate in cereal-based matrices can lead to the production of nutritionally enhanced products like bread with high protein content. Many studies have been made on the supplementation of wheat flour with legume-based ingredients like lupin flour [1, 5], pea flour [6, 7], fababean flour or concentrate [8, 9], navy bean flour [10] and soy protein flour or isolate [11, 13] to produce enriched bread. Substitution with legume-based products allows to improve bread protein content and to compensate wheat deficiencies in lysine and threonine, two essential amino acids [4, 14, 15].

However, many of the aforementioned ingredients currently available on the market have a large phytate to protein ratio resulting in reduced protein digestibility and minerals bioavailability. Moreover, substitution of wheat flour with legume-based ingredients at a 10% level or more is generally harmful to the processing of bread. It can lead to reduced dough stability [7], decreases in loaf volume [4, 12] and increase in crumb hardness [5].

This work aimed to study the potential of supplementing bread with pea protein isolate having a low phytate to protein ratio produced by a novel ultrafiltration/diafiltration (UF/DF) process.

## 2. Materials & Methods

### 2.1. Raw materials

Standard bread flour (Robin Hood KEYNOTE 80 Bleached Enriched Flour; Horizon Milling, Montreal, Quebec, Canada) was used. Pea protein isolate produced by membrane technologies were made from certified #1 Eclipse Yellow peas purchased from Wagon Wheel Seed Corporation (Churchbridge, Saskatchewan, Canada).

### 2.2. Pea protein isolate

Isolate was produced by extracting pea flour in water at room temperature (ratio 1:15 w/w) and pH 7.5 followed by purification with UF/DF using 50 kDa hollow fibres membranes. The UF/DF sequence was a UF step with a volume concentration ratio (VCR) 5 and a discontinuous DF step with a re-VCR 5. The resulting pea protein isolate was lyophilized and placed in aluminium pouches which were hermetically sealed and stored at 4°C until used [16].

### 2.3. Dough analysis

Bread with protein content over 20%. Moisture content of flour and pea protein isolate was determined according to AACC International method 44-15.02. Total protein content was obtained using an FP-428 LECO apparatus (LECO corp., Saint Joseph, MI) with EDTA as nitrogen standard. Conversion factors were  $N \times 5.7$  for flour and  $N \times 6.25$  for pea protein isolate. Starch damage (iodine absorption) was measured with a SDmatic (Chopin, Villeneuve-la-Garenne, France) according to AACC International method 76-33.01. Alpha-amylase enzyme activity was measured with the falling number system (model FN1500; Perten Instruments, Sweden) according to AACC International method 56-81.03. Farinograph analysis was performed according to AACC constant flour weight method 54-21.01 and characterised using a modified version of the continuous water addition method developed by Landillon *et al.* [17]. In addition to water absorption, dough development time and stability, three parameters were determined: minimum water content for dough formation, maximum torque and water content at maximum torque. A

Brabender-E Farinograph equipped with a 50-g bowl (Model FA-R/2; Brabender Co., South Hackensack, NJ, USA) was used and tests were conducted in triplicate.

#### 2.4. Bread preparation

Bread was prepared according to AACC International Method 10-10.03. The 100g-flour procedure was applied. Optimal mixing times were determined from Farinograph dough development time. Ingredients were mixed in a pin-type mixer (National Mfg. Co., Lincoln, Nebraska, U.S.A.). Dough was sheeted-molded with a sheeter-molder (Model FSM-24-A; L & M Co., Downsview, Ontario, Canada) and baked in a revolving oven (Equipement de Boulangerie L. P., Victoriaville, Quebec, Canada).

#### 2.5. Bread properties measurement

After a 1-hr cooling, loaf specific volume was measured by rapeseed displacement (National Mfg. Co. Lincoln, Nebraska, U.S.A.). Color measurements of crust and crumb were performed using a Minolta colorimeter model CR-300 (Konica Minolta Sensing Americas, Ramsey, NJ, U.S.A.). Measurements were done in a light testing box (Macbeth, The Judge II, Grand Rapids, MI, USA) under “day” light intensity. Results are expressed in terms of the Hunter color system L (blackness to whiteness), a\* (greenness to redness) and b\* (blueness to yellowness). Bread crumb was dried with a fluidized bed (Tornado, Sherwood scientific Ltd, Cambridge, UK) at 40°C for 30 minutes and protein content was obtained with an FP-428 LECO apparatus as previously described.

#### 2.6. Statistical analysis

Analysis of variance was performed a priori on each parameter using SAS software (version 8.2, SAS Institute Inc. Cary, NC, USA). Multiple comparison procedures (Least Significant Difference,  $P = 0.05$ ) were performed *a posteriori* to compare parameters whose variance was significantly different.

### 3. Results & Discussion

Dough mixing properties are presented in Table 1. The protein content of the pea protein isolate was  $96.1 \pm 0.2\%$  (dry basis) resulting in dough protein content of about 23% (dry basis). Supplementing wheat flour with pea protein isolate did not affect damaged starch but slightly decreased falling number. Supplementing wheat flour with pea protein isolate at a 10% level induced an increase in dough water absorption, which is typically observed when wheat flour or semolina is enriched with a supplement of high protein content [7; 18-20]. This result has been attributed to the ability of proteins to absorb high quantity of water, thus limiting the water available for the development of the gluten network when in competition with wheat proteins [2-3]. Dough stability and development time were not affected by substitution by pea protein isolate produced by the novel ultrafiltration/diafiltration process [16]. This observation is of interest since decreases in stability have been observed for the enrichment of wheat flour with soya flour [3], lupin flour [2] or pea flour [7]. The maximum torque obtained during the continuous water addition Farinograph method was higher for dough substituted with pea protein isolate. This suggests that dough substituted with pea protein isolate is stronger at the water content of maximum resistance to mixing than dough obtained from unsubstituted flour. The addition of isolate caused a diminution of the minimum water content for dough formation and of the water content at maximum torque. This might be because the proteins of the pea protein isolate, which are highly soluble [16], are able to interact directly with wheat proteins. It would thus influence the development of the gluten

network in a different manner than through water competition, similarly to what was observed in the case of soy proteins [21].

Table 1. Protein content, absorption of Iodine, falling number and mixing properties of dough

Parameters	Dough with 100% flour (control)	Dough enriched with 10% pea protein isolate
Moisture content (% dry matter)	13.5 ± 0.1	12.7 ± 0.1 ***
Protein content (% dry matter)	15.2 ± 0.2	23.3 ± 0.2 ***
Absorption of Iodine (%) *	94.85 ± 0.01a	94.82 ± 0.03a
Falling number (s) **	358 ± 4a	344 ± 3b
Water absorption (%) **	59.3 ± 0.1a	62.5 ± 0.1b
Dough development time (min) **	6 ± 1a	7 ± 1a
Stability (min) **	6.8 ± 0.9a	8.0 ± 0.7a
Maximum torque (FU) *	867 ± 75a	965 ± 18b
Minimum water content for dough formation (mL g-solid <sup>-1</sup> ) **	0.46 ± 0.01a	0.41 ± 0.00b
Water content at maximum torque (mL g-solid <sup>-1</sup> ) **	0.61 ± 0.02a	0.54 ± 0.01b

\* Values not sharing a common letter are significantly different (P < 0.05)

\*\* Values not sharing a common letter are significantly different (P < 0.01)

\*\*\* Calculated values

Substitution with pea protein isolate allowed to produce bread with protein content over 20% (20.9±0.1% compared to 14.5±0.0% dry basis) but induced a decrease in loaf specific volume (4.3±0.0 cm<sup>3</sup> g<sup>-1</sup> compared to 5.4±0.3 cm<sup>3</sup> g<sup>-1</sup>) compared to unsubstituted flour (Figure 1). A protein content of this magnitude is relatively high considering that reports in the literature conclude that high protein breads contain up to 15-20% protein [13]. A decrease in loaf volume is usually what is observed when wheat flour is substituted with legume-based ingredients [2; 4; 13]. This effect has been partly attributed to the dilution of the gluten network, causing weakening of the dough [12]. However, the specific volume of bread substituted with pea protein was maintained over 4 g cm<sup>-3</sup>, which should be considered as satisfactory by the consumers.



Fig. 1. 1) Bread with 100% flour (control); 2) Bread enriched with 10% pea protein isolate

Color characteristics of bread are presented in Table 2. The substitution of wheat flour with isolate induced a decrease in the whiteness and an increase in the yellowness of the crumb, while inducing an increase in the blackness and a decrease in the yellowness of the crust.

Table 2. Color characteristics of the crust and the crumb of bread

Parameters		Bread with 100% flour (control)	Bread enriched with 10% pea protein isolate
Color of the crust **	L value	49 ± 2a	37 ± 1b
	a value	15.2 ± 0.4a	15.1 ± 0.5a
	b value	29 ± 1a	18 ± 1b
Color of the crumb *	L value	86 ± 1a	81.4 ± 0.6b
	a value	-0.9 ± 0.1a	0.1 ± 0.3b
	b value	13.7 ± 0.6a	19.1 ± 0.3b

\*Values not sharing a common letter are significantly different ( $P < 0.05$ )

\*\*Values not sharing a common letter are significantly different ( $P < 0.01$ )

#### 4. Conclusion

Flour substituted with low phytate pea protein isolate produced by UF/DF showed a good bread-making potential when compared to unsubstituted flour. Next step will be to assess the potential improvement in protein digestibility and mineral bioavailability for the bread substituted with this new pea protein isolate compared to other alternative ingredients. Ultimately, this could lead to the marketing of high protein breads with improved health properties.

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