Effect of different processing methods on anti-nutritional factors and *in vitro* protein quality of yellow peas and their related products

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Introduction

- Yellow peas (YP) contain excellent amounts of protein (23-25%), carbohydrates (53%), dietary fibres (14-26%), and micronutrients.
- Yellow peas are a valuable source of plant-based proteins due to their low-fat content, low pricing, and low allergenic risk, making them a viable alternative to soy, wheat, and animal-based proteins
- However, yellow peas have a lower protein quality when compared with animal-based protein sources mainly due to the presence of anti-nutritional factors (ANFs).
- Trypsin inhibitors, phytic acids, and polyphenols are examples of naturally occurring ANFs that have a negative impact on nutritional value, protein digestibility and protein quality.
- Food processing treatments such as boiling, autoclaving, and baking contribute to inactivating or eliminating ANFs.

Objective

- Evaluate the effect of boiling, autoclaving, micronization, and baking on the ANFs (i.e., trypsin inhibitors and phytic acids) in yellow peas.
- Compare the effect of baking on the tested ANFs in yellow pea protein concentrate and isolates to those in the unprocessed forms.
- Determine the effects of the processing treatments on amino acid profile, *in vitro* protein digestibility, and *in vitro* protein quality in yellow pea and its derivatives.

Materials and Methods

1. Sample Receiving and Preparation

1) YP seeds and YP protein isolates were offered by local pea protein isolate manufacturer.
2) One-third of the YP seeds were boiled, autoclaved, micronized, and baked.
3) Two-thirds of YP seeds were air-classified at the Richardson Centre (Winnipeg, MB, Canada) to produce protein concentrate (also known as fine fraction).
4) Baking was also applied to YP protein concentrate and isolates.
5) Unprocessed and processed samples were prepared in triplicate.
6) All ingredients came from the same lot of YP seeds.

2. Chemical Analysis:

1) **Trypsin inhibitor activity (TIA):** spectrophotometrically determined at 410nm, as described in Liu et al. (2021).
2) **Phytic acids (PA):** spectrophotometrically determined at 519nm by Haug & Lantzsch (1983) and the use of sodium salt of phytic acid to generate a standard curve.
3) **Dry matter (DM):** AOAC method 925.10 (AOAC, 1995).

Materials and Methods (Cont.)

2. Chemical Analysis (Cont.):

4) **Crude protein (CP; %N × 6.25):** measured at the Central Testing Laboratory (CTL; Winnipeg, MB) using the Dumas combustion method and a protein-nitrogen coefficient of 6.25.
5) **Amino acid composition:** determined using regular (AOAC 982.30), performic acid oxidized (AOAC 985.28), and alkaline (ISO 13904:2005) amino acid hydrolysis methods.
6) **Amino acid score (AAS):**

$$\text{AAS} = \frac{\text{mg of amino acid per gram of protein (test protein)}}{\text{mg of corresponding amino acid per gram of protein (reference pattern)}}$$

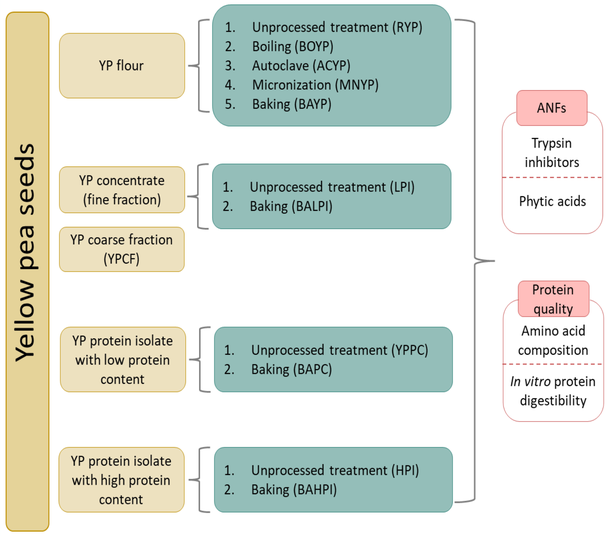
Note: The reference pattern is based on the amino acid requirement for pre-school (2-5 years) children (FAO/WHO).

7) ***In vitro* protein digestibility (IVPD):** determined using the pH-drop method described in Tinus et al. (2012).
IVPD (%) = 65.66 + 18.10×ΔpH10min
8) ***In vitro* protein digestibility corrected amino acid score (IVPDCAAS):**
IV-PDCAAS (%) = AAS × IVPD%

Note: The AAS of the samples is determined by selecting the essential amino acid with the lowest value, also known as the limiting amino acid (LAA).

3. Statistics:

1) Measurements were carried out at least twice, with the exception of the amino acid composition and crude protein analyses, which were done in singlet.
2) Microsoft Excel, GraphPad Prism, and R were used for the calculation of mean, standard deviation (STD), and one-way analysis of variance (ANOVA) followed by a Tukey's multiple comparison test. Significance was accepted if $p < 0.05$.



Results

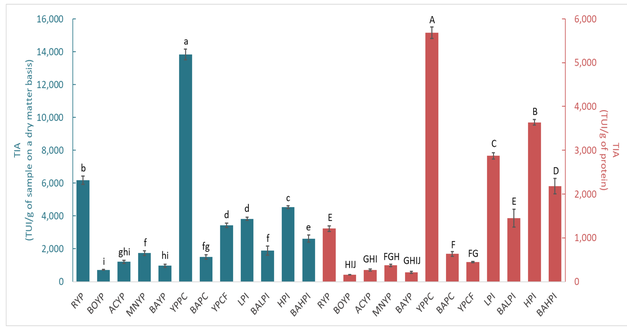


Fig. 1. Trypsin inhibitor activity (TIA) of unprocessed and processed YP and its derivatives. The TIA are expressed as trypsin units inhibited (TUI)/g of sample on a dry matter basis or TUI/g of protein. Mean values followed by different letters indicate statistically significant.

Findings: Processing treatments resulted in a significant decrease ($p<0.05$) in TIA of YP flour, protein concentrate, and isolates.

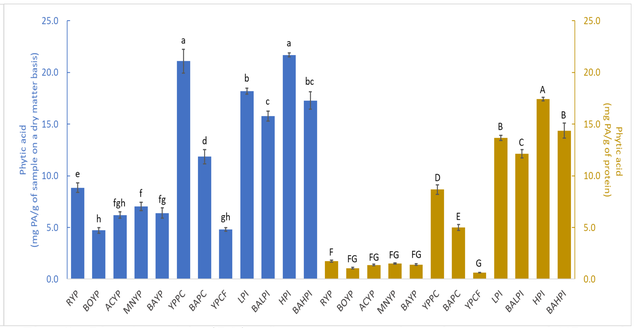


Fig. 2. Phytic acids (PA) of unprocessed and processed YP and its derivatives. The PA content was expressed as mg equivalents of PA/g of sample on a dry matter basis or mg PA/g of protein. Mean values followed by different letters indicate statistically significant.

Findings: There is a significant decrease ($p<0.05$) in the phytic acids of baked protein concentrate and isolates when compared to unprocessed forms on both basis units.

Table 1. Summary data for proximate analysis, essential amino acid profile, *in vitro* protein digestibility, and *in vitro* protein digestibility-corrected amino acid score of unprocessed and processed YP flour, protein concentrate, and protein isolates.

Sample	DM (%)	CP (%)	Essential amino acid profile (mg/g of protein, as-is basis)									AAS	LAA	IV-PD (%)	IV-PDCAAS (%)
			THR	VAL	MET +CYS	ILE	LEU	PHE +TYR	HIS	LYS	TRP				
RYP	91.67	21.43	36.36	46.39	25.58	41.40	71.27	77.47	23.52	73.37	10.45	0.95	TRP	82.21	78.13
BOYP	96.48	23.27	37.26	47.74	24.41	43.53	75.03	79.03	24.06	74.26	10.66	0.97	TRP	87.54	84.81
ACYP	95.51	23.17	36.69	46.45	24.09	42.17	71.91	77.61	23.96	73.55	10.36	0.94	TRP	86.35	81.32
MNYP	95.02	22.42	36.58	46.48	24.71	41.62	71.82	78.96	23.38	72.31	10.08	0.92	TRP	85.51	78.37
BAYP	97.54	22.65	36.47	46.19	23.58	41.55	71.18	75.95	24.02	70.87	10.24	0.93	TRP	84.42	78.62
YPPC	91.76	44.76	37.69	47.77	24.53	43.32	74.49	82.65	24.96	77.06	9.96	0.91	TRP	83.78	75.90
BAPC	90.45	46.65	35.22	45.26	22.40	41.03	69.72	77.50	24.08	68.04	10.27	0.90	M+C	84.26	75.51
YPCF	97.16	13.44	35.87	45.17	27.39	39.67	67.65	74.64	23.96	71.52	10.05	0.91	TRP	80.78	73.78
LPI	95.59	78.53	33.82	46.54	21.46	43.62	76.57	83.04	23.53	70.56	9.07	0.82	M+C	89.37	73.67
BALPI	96.89	79.33	34.30	47.05	19.85	44.32	77.16	83.56	22.70	71.50	10.29	0.79	M+C	88.93	70.62
HPI	95.01	84.47	40.49	49.92	22.43	44.89	76.23	88.60	24.98	80.38	10.48	0.90	M+C	90.38	81.10
BAHPI	97.06	85.65	39.89	49.11	20.90	44.16	75.24	86.86	23.98	79.09	10.43	0.84	M+C	89.83	75.10

Abbreviations: THR, threonine; VAL, valine; MET, methionine; CYS, cysteine; ILE, isoleucine; LEU, leucine; PHE, phenylalanine; TYR, tyrosine; HIS, histidine; LYS, lysine; TRP, tryptophan.

Findings: All processed treatments improved the protein quality for YP flour, especially boiling. However, the protein quality of YP protein concentrate and protein isolates decreased after baking.

Significance

- This research is expected to overcome the limitations of ANFs to increase the utilization of pea proteins and promote the use of sustainable plant-based protein alternatives.

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