# Common beans cooked at high altitudes have higher trypsin inhibitor activity and lower protein digestibility than beans cooked at sea level

Honors Thesis

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## Abstract

Legumes are high in protein but also contain heat labile trypsin inhibitors (TI's) that reduce protein digestibility. To test if cooking at high altitudes is less effective at inactivating TI than cooking at sea level, common beans and fava beans were cooked in water at temperatures chosen to simulate boiling points at different altitudes. In addition, each variety was autoclaved in water at 121°C to simulate a pressure cooker. Trypsin inhibitor activity (TIA) was measured with N-α-Benzoyl-L-arginine 4-nitroanilide hydrochloride, and *in vitro* protein digestibility was determined by digestion of samples with 3 peptidases. Raw common and raw fava beans inhibited 100% and 36% of trypsin activity, respectively. Cooking common beans at 100°C inactivated 88.6% (SD±4.8) of TI, while cooking at temperatures between 87-97°C inactivated 73.2% ($p<0.05$). In fava beans, TI inactivation was 75% at all cooking temperatures. TI inactivation in an autoclave was positively correlated with time for common beans ($p<0.05$) but not fava beans. Protein digestibility in common beans was 79.1% in raw beans, 85.0% in beans cooked at 87°C and 89.7% in beans cooked at 100°C. Fava bean digestibility was greater than that of common beans when raw (86.0%) and when cooked at 100°C (95.6%). Regression analysis showed a negative correlation between TIA and protein digestibility ($r^2=0.86$). Residual TIA of common beans cooked at high altitudes in boiling water is higher than when cooked at sea level, lowering protein digestibility. In populations where protein intakes are marginal, this could negatively impact protein nutritional status.


## Practical Application

This study has implications for how consumers at high altitudes may choose to cook legumes for maximal protein digestibility. Common beans have a higher concentration of protease inhibitors and a lower protein digestibility than fava beans. Therefore, cooking

common beans in a pressure cooker may be the best method for maximizing nutritional quality of these legumes at higher altitudes.

## Introduction

The nutrient composition of legumes (plants of the *Fabaceae* family*)* offers multiple benefits, being high in protein (21-25%) and a significant source of iron (Geil and Anderson 1994). Legumes are dietary staples in most developing countries because of their affordability, long shelf-life, and low moisture content. However, legumes also contain substantial amounts of protease inhibitors, most notably trypsin and chymotrypsin inhibitors, which are unaffected by human gastric juices and reduce the digestibility of bean proteins. Because of the reduced efficiency of trypsin and chymotrypsin, growth retardation and pancreatic hypertrophy are observed in animals fed uncooked bean flour (Geil and others 1994). The two major trypsin inhibitors, the Kunitz and Bowman-Birk inhibitors, bind to the protease's active site and are then not easily released from the complex (Song and others 1998, Werner and others 1992). Using a wet heat treatment, purified Kunitz inhibitor extracts of soy protein can be almost completely inactivated within three hours, while purified Bowman-Birk inhibitor extracts continue to show high activity after six hours of wet heat treatment (DiPietro and others 1989). The thermal stability of the Bowman-Birk inhibitor is likely due to its tertiary structure with seven disulfide bonds and an extensive network of hydrogen bonds. As the Bowman-Birk inhibitor is able to inhibit two protease equivalents per one inhibitor molecule (Werner and others 1998), it has greater potential to inhibit protein digestion compared to other inhibitors. Among the different legume varieties, raw common beans (*Phaseolus vulgaris*) demonstrate one of the highest levels of trypsin inhibitor activity while raw fava beans (*Vicia faba*) have markedly lower levels of such activity (Guillamón and others 2008). Differences in inhibitory activity across different

legume varieties may be due to differences in the amino acid residues in the trypsin binding loop of the Bowman-Birk inhibitor or different concentrations of the inhibitors. Yet TI from all bean varieties contain seven disulfide bonds, as the number of cysteine residues remains highly conserved (Pergiovanni and others 2004).

Extensive research on effective methods of inactivation of trypsin inhibitors in raw beans has been reported (Barampama and others 1993, Yuan and others 2008, Martín-Cabrejas and others 2009), with a strong focus on soybeans. The general consensus is that higher processing temperatures and longer processing times result in the greatest inactivation of soybean trypsin inhibitors. The effect of processing conditions on trypsin inhibitor inactivation varies among bean varieties. With fava beans, pre-soaking showed a favorable increased inactivation of TI (Vidal-Valverde and others 1997), while pre-soaking had no effect on TI inactivation in common beans (Alonso and others 2000). Both common and fava beans have shown TI inactivation with heat treatment (Tsukamoto and others 1983, Vidal-Valverde and others 1997), but the range of temperatures and times researched are more limited compared to soybeans. Undercooked beans with residual trypsin inhibitor activity may contribute to lower protein digestibility for the entire meal, not just for the beans themselves. *In vitro* protein digestibility assessments of several legumes obtained protein digestibility values of 79-81% for the raw beans, which increased to 90% after cooking (Martín-Cabrejas and others 2009). The *in vitro* protein digestibility method has been correlated to *in-vivo* rat digestibility studies ($r^2 = 0.85$) by Petersen and others (1983) and found to be reproducible with different protein sources (McDonough and others 1990).

Recent efforts to improve the nutrition of communities in the rural Andes Mountains recommend including more beans in the diet, as beans are often recommended as a good source of protein, especially in diets low in meat and dairy (USDA 2012). It is hoped that introducing

new bean species into developing countries will not only improve the community's health but also advance their economic independence (International Center for Tropical Agriculture 2008). However, at high altitudes where the boiling point of water is depressed due to lower atmospheric pressure, there is concern that beans would not be cooked at sufficient temperatures to adequately inactivate the trypsin inhibitors present, potentially decreasing the nutritional benefits. Although past studies have investigated cooking beans at high altitudes (Bressani and Chon 1996), the study did not assess trypsin inhibitor activity and its impact on protein quality.

The present study was conducted to determine the effects of lower cooking temperatures, corresponding to higher altitudes, on the inactivation of trypsin inhibitors in common beans and fava beans commonly eaten in the Andean region. The effect of cooking time on the inactivation of trypsin inhibitors was also investigated, and a comparison of cooking effects in an autoclave was completed to simulate the effect of cooking beans in a pressure cooker. *In vitro* protein digestibility assessments were done for the beans with the highest and lowest trypsin inhibitor activity. It was hypothesized that cooking beans at lower temperatures (simulating higher altitudes) would require longer cooking times to achieve inactivation of trypsin inhibitors comparable to that at sea level, and beans cooked at lower temperatures would have lower *in vitro* protein digestibility values than beans cooked at sea level.

## Materials and Methods

All chemicals and digestive enzymes were obtained from Sigma Chemicals (St. Louis, MO, USA) or Fisher Scientific (Fair Lawn, NJ, USA). Common beans (*Phaseolus vulgaris L.*, NUA 35) were obtained from the International Center for Tropical Agriculture in Cali, Colombia. Fava beans (*Vicia faba L.*, Goya Foods Inc., Secaucus, NJ, USA) were purchased at a local supermarket. All water used was deionized.

Preparation of Beans: Cooking at Temperatures Corresponding to Selected Altitudes

Common beans were divided into 25.0 g samples and were cooked in triplicates in a 600 mL beaker with deionized water (1:10 w/v) in a covered water bath at a temperature corresponding to the boiling point of water at altitudes between 0-4000 m above sea level (only temperatures in **Table 1** were tested). Cooking times varied between 80-120 min, with 80 min representing the standard cooking time as reported by Bressani and Chon (1996). Samples were drained of cooking water and dried in a convection oven at 65 °C for 1 hr, then ground to fine flour using a coffee grinder (Krups, Fast Touch, Group SEB Canada Inc. Scarborough, Ontario, Canada) and stored in a freezer (-18 °C) until analysis.

Fava beans were divided into 13.0 g samples and pre-soaked in deionized water (1:3 w/v) for 18 hr. Soaked beans were then cooked in deionized water (1:6.7 w/v) identically to the common beans at the same temperature points, in triplicate trials. Cooking times were between 35-55 min, with 35 min representing the standard cooking time according to package directions. Fava beans were drained, dried, ground to fine flour, and stored in a freezer (-18 °C) until analysis.

**Table 1. Cooking temperatures used to simulate higher altitude cooking by boiling beans***

| Altitude above Sea Level (m) | Boiling Point (°C) |
|---|---|
| 0 | 100. |
| 1000 | 96.6 |
| 2000 | 93.4 |
| 3000 | 90.0 |
| 4000 | 87.0 |

*As calculated from the following formulas: $P = e^{(-ay)}$ where P = pressure (atm), $a = 1.16 \times 10^{-4}$ $m^{-1}$ and y = altitude (m). With the calculated pressure, the temperature was determined using the Clausius-Clapeyron Equation: $\log(P_0/P) = (40{,}700 \text{ J/mol})/(R) * [(1/T) - (1/T_0)]$ where $P_0 = 1$ atm and $T_0 = 373$ K and R = gas constant.

Autoclave Treatment

Common beans were divided into 13.0 g samples and autoclaved (American Sterilizer, Erie, PA, USA) in beakers of deionized water (1:10 w/v) at 121 °C and 198.5 kPa for 15-35 min. Beans were drained, dried, ground to fine flour, and stored in a freezer. Fava beans were divided into 13.0 g samples and autoclaved in beakers of deionized water (1:6.7 w/v) following the same procedure. All trials were performed in triplicate.

Chemical Analysis – Trypsin Inhibitors

Trypsin inhibitor activity was determined according to the method of Kakade and others (1974) using N-α-Benzoyl-L-arginine 4-nitroanilide hydrochloride (BAPNA) as a trypsin substrate. To extract the trypsin inhibitors, 1 g of bean flour was mixed with 50 mL of 0.01N NaOH solution and stirred on a magnetic stir plate for 65 minutes. Then 0.5 mL of bean suspension was mixed with 1 mL of trypsin enzyme solution (Trypsin IX-S from porcine pancreas, Sigma-Aldrich) and 0.5 mL water, and the mixture was incubated in a 37 °C water bath for 5 minutes. Three mL of BAPNA solution was then added, and the reaction proceeded for 20 minutes at 37 °C when it was terminated with the addition of 0.5 mL 30% acetic acid. Each mixture was filtered (Whatman 41) and the absorbance was read at 410 nm using a UV/Vis spectrophotometer (Beckman-Coulter DU 520, Brea, CA, USA) against a reagent blank. The reagent blank was prepared by adding 0.5 mL of 30% acetic acid to 1.0 mL trypsin solution and 1.0 mL water before addition of 3.0 mL BAPNA solution. A control tube without bean TI suspension (replaced with 0.5 mL water) was included with each assay. Sample blanks were prepared by adding 3.0 mL of BAPNA solution to 0.5 mL of bean suspension, incubating the mixture at 37°C for 20 minutes, and then adding 0.5 mL 30% acetic acid, followed by the addition of 1.0 mL trypsin solution. Any absorbance of the sample blanks at 410 nm was

attributed to the color of the bean suspension, and the reported absorbance values were corrected by subtracting the values for the blanks. One trypsin unit (TU) is defined as an increase of 0.01 absorbance units at 410 nm. Trypsin inhibitor activity (TIA) is expressed in terms of trypsin units inhibited (TUI), or the decrease in TU of the sample compared to the TU in the control tube in the absence of bean suspension. Inactivation of TIA is expressed as the percent decrease of TIA from that present in the raw bean.

*In vitro* Protein Digestibility

*In vitro* protein digestibility (IVPD) was assessed by the method of McDonough and others (1990). From among the cooked bean samples, only the beans with statistically significant differences in trypsin inhibitor activity ($p < 0.05$) were evaluated for IVPD. The bean flour samples, 4 mg N $mL^{-1}$ (determined by Kjeldahl analysis, AOAC method 928.08), were first mixed with 12.5 mL distilled water and 12.5 mL 0.2N NaOH. The suspensions were incubated at 37°C for 30 minutes, after which 25.0 mL 0.075N HCl was added and the pH of the suspensions were adjusted to 8.0. The reaction was initiated by adding 1 mL of a multi-enzyme system containing, per milliliter, 23100 units of trypsin, 186 units of chymotrypsin, and 0.052 unit of leucine aminopeptidase. The amount of 0.1N NaOH required to maintain the pH at 7.98 for exactly 5 minutes by hand titration while monitoring with a pH electrode (Ross SureFlow® electrode, ThermoScientific) was recorded. The percent IVPD was calculated using the following equation (McDonough and others 1990):

$$IVPD = 79.28 + 40.74B$$

where B = mL 0.1N NaOH used during the 5-minute reaction time. Activity of the multi-enzyme system was calibrated each day by measuring the IVPD of a control sodium caseinate

solution of 10 mg N $mL^{-1}$, and digestibility scores were corrected with the IVPD value of the casein, by multiplying with the following correction factor:

$$Correction\ factor = \frac{100}{IVPD\ of\ sodium\ caseinate}$$

Protein digestibility-corrected amino acid scores (PDCAAS) were calculated using literature values for amino acid composition of the two bean varieties and measured digestibility values (Schaafsma 2000). The PDCAAS of the food is determined to be the same value as the amino acid with the lowest PDCAAS. The amino acid pattern referenced was the FAO/WHO/UN amino acid requirements for children 2-5 years old, as this age group has the highest requirement for essential amino acids other than infants (Henley and others 1994).

Statistical Analysis

Results were analyzed by independent t-tests using Excel and regression analyses with regression models using JMP 9.0.0 software. Differences were considered significant at $p < 0.05$.

## Results and Discussion

TIA inactivation by conventional methods

Inactivation of TIA for common beans cooked at temperatures between 87-96.6 °C ranged from 72.1% to 74.7% (**Figure 1**). Variation among these data points was not statistically significant ($p>0.05$). For common beans cooked at 100 °C, 88.6% (SD±4.8) of TIA was inactivated, which was significantly greater compared to the TIA inactivation at the lower temperatures ($p<0.05$). However, a portion of the trypsin inhibitors appear to be very heat-resistant and were not inactivated in the selected temperature range. The increase in inactivation at 100 °C may be attributed to denaturation of inhibitors that were not inactivated at the lower temperatures, such as possibly the more heat-stable Bowman-Birk inhibitor.

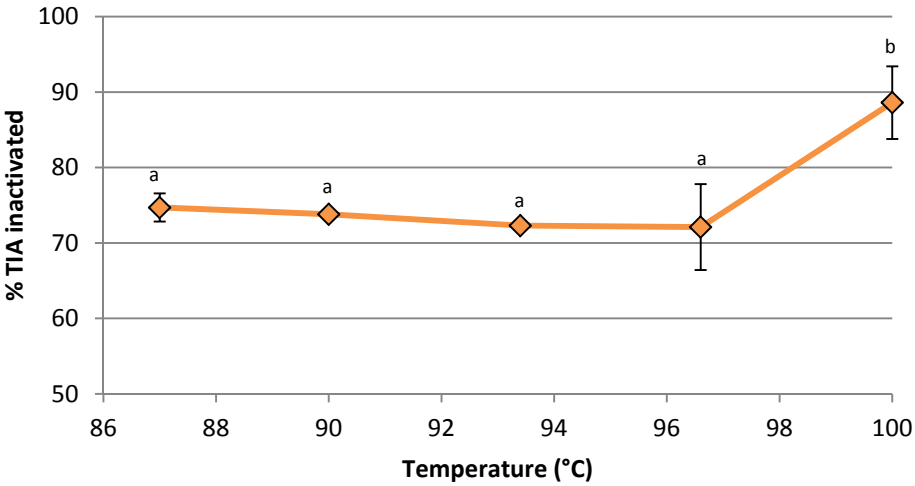


**Figure 1. Inactivation of trypsin inhibitor activity in common beans cooked for 80 minutes at varying temperatures**

Error bars represent mean ± SD (n = 4). Where errors bars are not visible, SD is indiscernible.
[a,b] Means with different letters are statistically significant.

Cooking time was positively correlated with inactivation of TIA at 87 °C but not at 100°C (**Figure 2**). There was an increased inactivation of TIA with increasing time up to 100 minutes in samples cooked at 87 °C, after which the TIA was constant with cooking time up to 120 minutes. DiPietro and Liener (1989) reported a similar plateau of inactivation with purified Bowman-Birk inhibitor extracted from soy flour, in which the extract was heated at 100 °C for up to 6 hr and 75% of the activity remained. It may be that a different ratio of trypsin inhibitor proteins in common beans causes the total TIA to be more resistant to heat inactivation, which might be due to greater concentration of Bowman-Birk inhibitor in comparison to Kunitz inhibitor, as the Bowman-Birk inhibitor can withstand greater heat treatment. The common beans cooked at 100°C had a constant 86% inactivation (SD±1.4%) of TIA up to 120 minutes, which further supports the possibility that the composition of TIs in common beans is less affected by heat treatment. As shown in Figure 2, even with the increased inactivation of TI with increasing time at 87°C, the maximum inactivation at 87°C (at 100 min) is still significantly lower than the TIA inactivation at 100°C ($p < 0.01$). These results show that the longer cooking

time could not completely compensate for the lower temperature used in the cooking process, indicating that common beans cooked at higher altitudes would contain significantly greater TIA than common beans cooked at sea level.

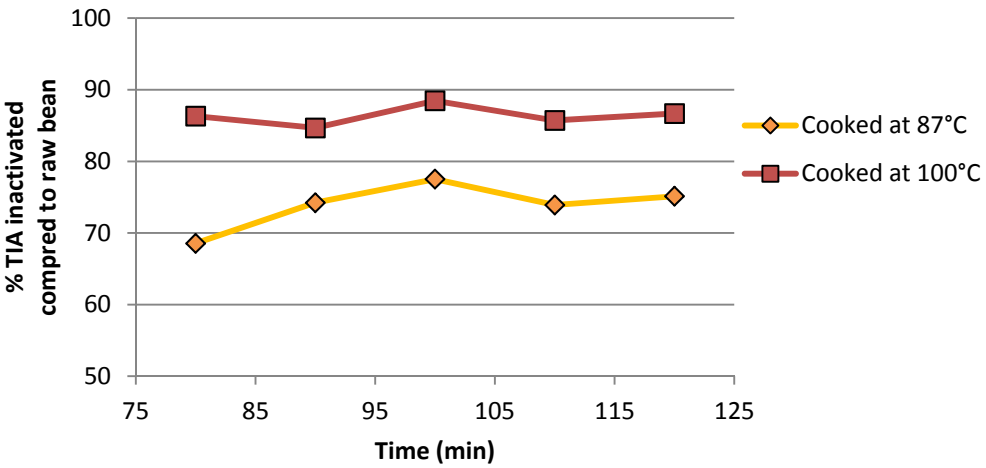


**Figure 2. Observed inactivation of TIA in common beans with increased cooking time at 100 °C and 87 °C**

Error bars represent mean ± SD (n = 4). Where errors bars are not visible, SD is indiscernible.

Fava beans displayed much lower TIA than common beans (**Figure 4**). The raw fava beans had 64.4% less TIA than the raw common beans, and these results are consistent with results from other researchers (Guillamón and others 2008, Gupta and others 2000). One explanation for the lower TI activity, beyond different TI concentrations, may be that differences in the primary structure of the protease inhibitor in fava beans may lower its binding affinity for trypsin, as genomic studies have reported a different amino acid in the binding loop of the fava bean TI. The fava bean also exhibited constant 75% (SD±1.65%) inactivation of TIA regardless of heating time or temperature (**Figure 3**) when cooked in distilled water ($p > 0.05$).

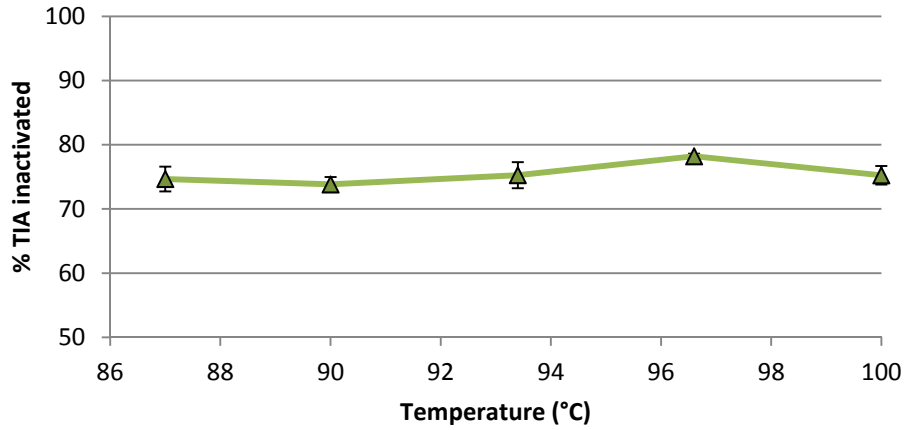


**Figure 3. Inactivation of trypsin inhibitor activity in fava beans cooked for 35 minutes at varying temperatures**
Error bars represent mean ± SD (n = 4). Where errors bars are not visible, SD is indiscernible.

However, the TIA of fava beans was significantly less than the TIA of the common beans at every cooking temperature (**Figure 4**). It may be concluded that the TIs in fava beans are either lower in total concentration or less effective at inhibiting trypsin compared to those in common beans. It is unknown if either of these reasons may explain why the fava bean TIs are also less susceptible to heat treatment, as indicated by the lower percentages of TIA inactivation. Further studies may look to isolate the TIs from both fava and common beans and directly compare the kinetic analyses of the protease inhibitors.

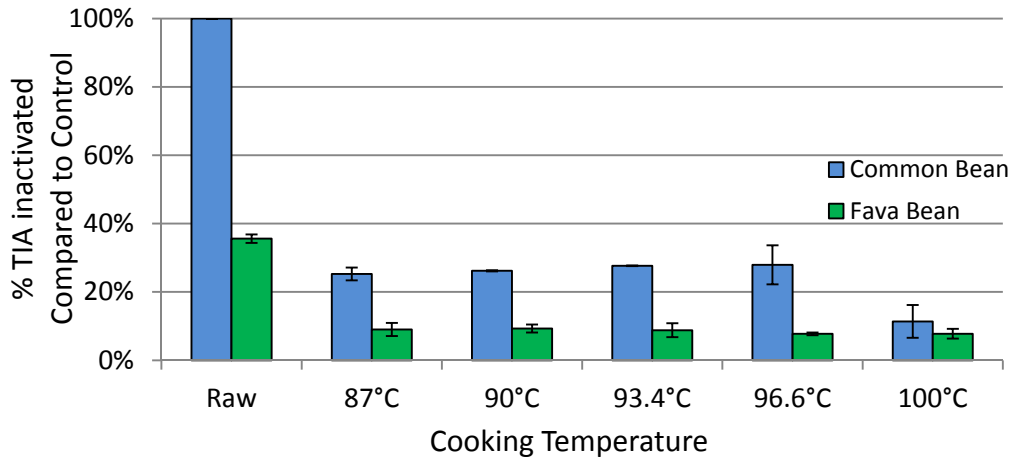


**Figure 4. TIA of common beans and fava beans, raw and cooked for 80 minutes at varying temperatures**
Error bars represent mean ± SD (n = 4). Where errors bars are not visible, SD is indiscernible.

TIA inactivation by autoclave treatment

With the autoclave treatments of common beans, there was a positive correlation between time and TIA inactivation (**Figure 5**). The percentage of TIA inactivation increased from 81.0% to 88.9% with an increase of autoclave time from 15 minutes to 35 minutes ($p < 0.05$). The highest inactivation of TIA in common beans was observed at 35 minutes of autoclave treatment (88.9%, SD±2.0), which was greater than the inactivation when the common beans were cooked at 100°C. As both an autoclave and pressure cooker may be configured to produce a cooking temperature of 121°C, cooking common beans in a pressure cooker for 35 minutes should inactivate more TIA than boiling the beans at sea level. We conclude that the best method for cooking common beans to inactivate the greatest amount of trypsin inhibitors would be pressure cooking, especially at higher altitudes, although potential obstacles are discussed later.

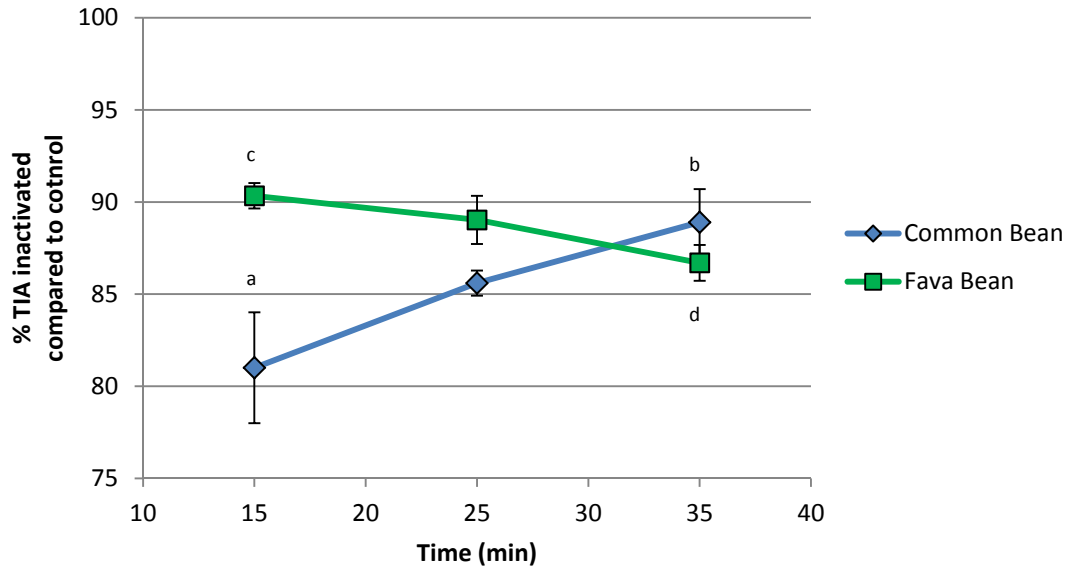


**Figure 5. Effect of autoclave treatment on inactivation of TIA in common and fava beans with increasing autoclave time**

Error bars represent mean ± SD (n = 3). Where errors bars are not visible, SD is indiscernible. [a,b] Means with different letters are statistically significant. [c,d] Means with different letters are statistically significant.

Unlike with common beans, there was a negative correlation between TIA inactivation and cooking time in an autoclave with fava beans. Inactivation at 35 minutes was 86.7% while

inactivation at 15 minutes was 90.3%. The difference in percent inactivated between 15 minutes and 35 minutes was statistically significant ($p < 0.01$). This negative correlation between time and inactivation of TIA was unexpected. It is possible that trypsin activity was depressed not because of the trypsin inhibitors, but rather that the heat treatment altered the protein in a way that reduced digestibility. Fontaine and others (2007) and Antunes and others (1980) reported decreased lysine availability after autoclaving soybeans and common beans. They suggested that Maillard browning likely occurred during the heat treatment and this reduced the digestibility of the protein. During Maillard browning, amine groups in lysine react with reducing sugars to form a Schiff base. This would alter the binding of the protein to the trypsin active site, thereby reducing trypsin digestibility. A study of protein bioavailability completed by Bressani and Chon (1996) corroborates this possibility. They showed that overcooking beans did result in decreased net protein ratio (NPR) and protein efficiency ratio (PER) values, which indicate a decrease in protein quality. Therefore, higher heat processes may produce negative effects on protein quality, although high heat does demonstrate greater TI inactivation with some bean types.

*In vitro* protein digestibility and protein quality

Since only beans cooked at 87 °C and 100 °C and those autoclaved were found to have statistically different inactivation levels of TIA, these three cooking methods were the only ones assessed for *in vitro* protein digestibility (IVPD). The IVPD of raw common beans was approximately 79.1% (SD ± 0.86), concurring with previous studies (Martín-Cabrejas and others 2009, Gilani and others 2005). IVPD of raw fava beans was higher than raw common beans at 86.0% (SD ± 1.47), a trend that was also reported by other researchers, though the difference in digestibility between bean types was not as great in previous studies (Alonso and others 2000).

Comparison of IVPD of both bean types cooked at 87 ºC and 100 ºC showed a general increase in IVPD with higher cooking temperatures, with fava bean protein digestibility higher in all cases than the common bean and a peak of 96% digestibility at 100 °C (**Figure 6**). Although the increase in IVPD of common beans correlates inversely with TIA, the IVPD increase with fava beans does not correlate with constant TIA observed in the fava beans with all temperatures tested. This lack of correlation in the case of fava beans may be due to factors unrelated to trypsin inhibitors, such as structural changes in proteins that affect protein accessibility to the digestive proteases (Martín-Cabrejas and others 2009), and thus increased protein digestibility is observed even though TIA inactivation does not change. The IVPD of autoclaved common beans were identical to the IVPD of common beans cooked at 100 ºC, which matches the nearly identical TIA values as well. Similarly, IVPD values of autoclaved fava bean samples were lower than IVPD values of fava beans cooked at 100 ºC, also corresponding to the increase of TIA observed in the autoclaved fava beans.

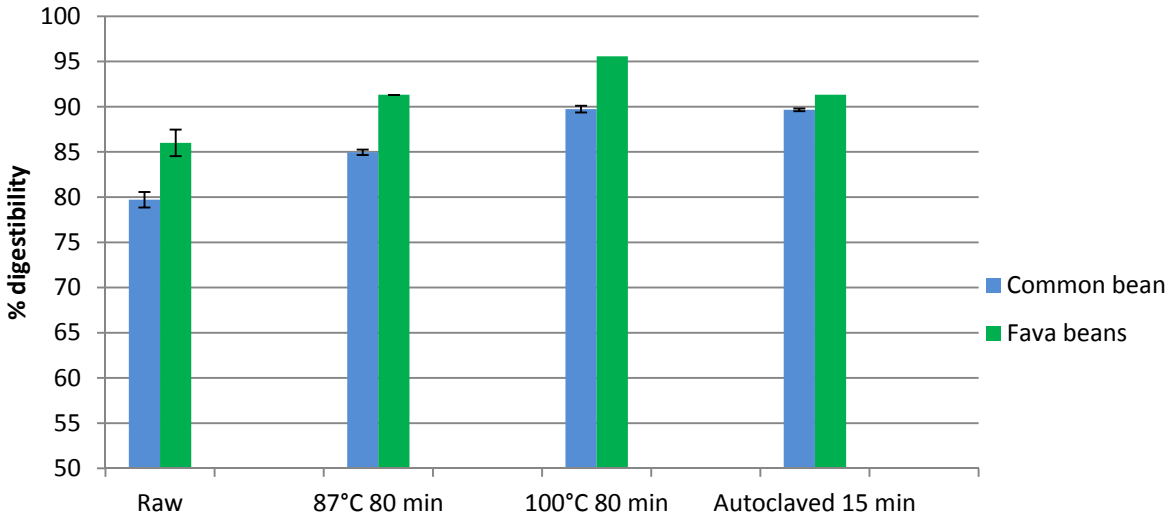


**Figure 6. In-vitro Protein Digestibility of Common and Fava Beans prepared under different cooking parameters**

Error bars represent mean ± SD (n = 3). Where errors bars are not visible, SD is indiscernible.

A regression plot of trypsin inhibitor activity on protein digestibility showed a strong negative correlation for both common bean ($r^2 = 0.8594$) and fava bean ($r^2 = 0.8115$) (**Figure 7)**. The correlation was statistically significant ($p < 0.001$), regardless of whether raw bean data points were included or not in the regression analysis.

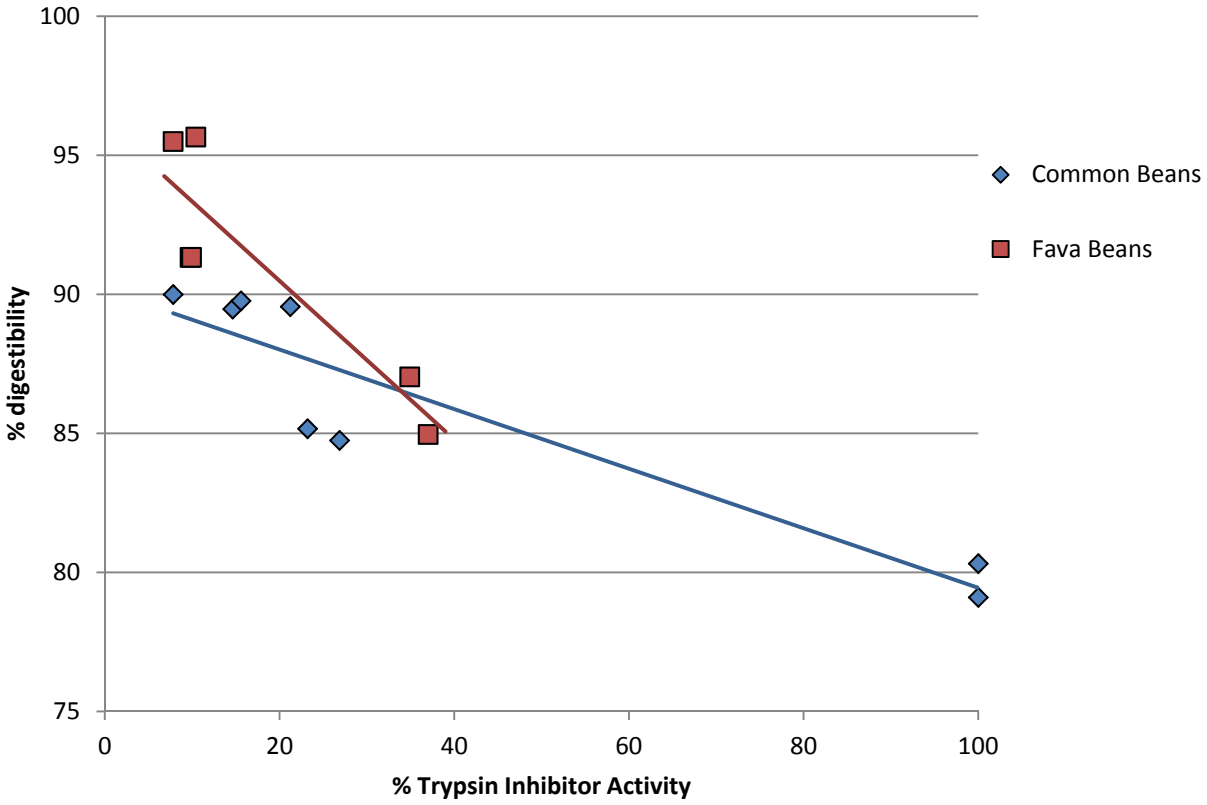


**Figure 7. Correlation of Trypsin Inhibitor Activity with Protein Digestibility**

Protein Digestibility-Corrected Amino Acid Scores (PDCAAS) were calculated for both bean varieties when cooked at the various simulated altitudes (**Table 2**). For common beans, the PDCAAS when cooked at sea level or pressure-cooked was 0.969, while PDCAAS for common beans cooked at 87 °C (simulated to be cooked at 4000 meters) was 0.917. The observed decrease indicates that the protein quality of beans available for human nutrition cooked at high altitudes may be lower than the protein quality of beans cooked at sea level. The PDCAAS values of fava beans ranged from 0.877 when cooked at 87 °C or autoclaved to 0.91 when

cooked at 100 °C (**Table 3**). Both bean varieties were limiting in the sulfur amino acids. Although fava beans were determined to have better protein digestibility compared to common beans, the lower PDCAAS values indicate that fava beans have lower overall protein quality than common beans. As the protein in diets of Andean communities is already low, lower protein digestibility due to trypsin inhibitors may have serious consequences, especially for children who have greater amino acid requirements.

**Table 2. PDCAAS Calculations for Common Beans Cooked at Different Temperatures**

| | | | | PDCAAS by cooking method | | |
|---|---|---|---|---|---|---|
| | mg/g protein* | FAO/WHO pattern (mg/g protein) | Uncorrected AA score | 87 °C (4000 m) | 100 °C (sea level) | Autoclaved |
| Lys | 77 | 58 | 1.33 | 1.128 | 1.191 | 1.190 |
| Thr | 52 | 34 | 1.53 | 1.299 | 1.372 | 1.371 |
| Val | 43 | 35 | 1.23 | 1.044 | 1.102 | 1.102 |
| **Met + Cys** | 27 | 25 | 1.08 | **0.917** | **0.969** | **0.968** |
| Ile | 42 | 28 | 1.50 | 1.274 | 1.346 | 1.345 |
| Leu | 97 | 66 | 1.47 | 1.249 | 1.319 | 1.318 |
| Phe +Tyr | 87 | 63 | 1.38 | 1.173 | 1.239 | 1.238 |
| Trp | 13 | 11 | 1.18 | 1.004 | 1.060 | 1.060 |
| His | 26 | 19 | 1.37 | 1.162 | 1.228 | 1.227 |

*Amino acids profile for common bean referenced from Antunes and others 1980

**Table 3. PDCAAS Calculations for Fava Beans Cooked at Different Temperatures**

| | | | | PDCAAS by cooking method | | |
|---|---|---|---|---|---|---|
| | mg/g protein* | FAO/WHO pattern (mg/g protein) | Uncorrected AA score | 87 °C (4000 m) | 100 °C (sea level) | Autoclaved |
| Lys | 73 | 58 | 1.26 | 1.149 | 1.203 | 1.149 |
| Thr | 41 | 34 | 1.21 | 1.101 | 1.153 | 1.101 |
| Val | 37 | 35 | 1.06 | 0.965 | 1.010 | 0.965 |
| **Met + Cys** | 24 | 25 | 0.96 | **0.877** | **0.918** | **0.877** |
| Ile | 33 | 28 | 1.18 | 1.076 | 1.126 | 1.076 |
| Leu | 72 | 66 | 1.09 | 0.996 | 1.043 | 0.996 |
| Phe +Tyr | 78 | 63 | 1.24 | 1.131 | 1.183 | 1.131 |
| Trp | 11 | 11 | 1.00 | 0.913 | 0.956 | 0.913 |
| His | 32 | 19 | 1.68 | 1.538 | 1.610 | 1.538 |

*Amino acids profile for fava bean referenced from Khalil and others 1995

Social Implications in the Andean region

Although this study reports that pressure cookers achieve the greatest degree of TI inactivation and best level of protein quality, there are additional issues to consider for rural Andean communities. Pressure cookers are relatively expensive, ranging from $50 to $110, depending on quality and size. Stovetop pressure cookers are generally cheaper but do require greater attention than electric options. Yet limited availability of electricity and conventional stovetops may present further obstacles to the Andean consumers with realizing the full potential of this kitchen appliance. One solution could be for the community to share one pressure cooker among several households, thereby defraying the costs but allowing many families to receive the nutritional benefits of cooking beans in this manner. Education about how to use a pressure cooker and why it is important may also be necessary to encourage the Andean community to view the technology as feasible and adopt it within their traditional cooking methods.

It is acknowledged that there would be obstacles with this message reaching the most remote communities, which are those most likely in need of dietary improvements. For these communities, efforts may be better focused on education about cooking times and bean variety selection. As cooking common beans at higher altitudes for longer times was found to make some improvements in greater TI inactivation, informing communities of increasing the cooking time could aid the protein quality. Furthermore, regardless of cooking time or temperature, fava beans exhibited the same degree of inactivation of TIA, which would beneficial for households that want to minimize fuel consumption while still optimizing nutritional quality. Future research investigating the most efficient cooking times for different bean varieties at higher altitudes would be valuable for these communities, particularly by conducting the cooking trials with the cooking methods currently employed by the Andean heads of household.

## Conclusions

The results show that boiling common beans at high altitudes is less effective at inactivating TI activity than boiling at sea level. Increasing cooking times at higher altitudes can partially, but not completely, overcome the reduced inactivation of common bean TIA resulting from lower cooking temperatures. None of the cooking treatments completely inactivated TIA in either common or fava beans, suggesting that some fraction of trypsin inhibitor is highly resistant to heat treatment. The TIA of raw fava beans were markedly lower than that of common beans, although heat inactivation (expressed as a percentage of the raw TIA) was less pronounced. *In vitro* protein digestibility is highly correlated with trypsin inhibitor activity, indicating that beans cooked at higher altitudes with higher residual TIA have decreased protein availability. Pressure-cooking is the best method for cooking common beans at high altitudes as it inactivates the greatest percentage of TIA and maintains high protein digestibility, though the practicality of such an adoption has multiple challenges. These results may have implications for communities living at high altitudes, especially where intake of digestible protein is low and there are large populations of children.

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