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PROCESSING CONDITIONS FOR MICRONIZATION OF PEAS (*PISUM SATIVUM*) AND AN *IN-VITRO* EVALUATION OF THE PRODUCT

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Abstract

Tempering and storage conditions were investigated for the processing of peas using infrared heat (micronization). The criteria for evaluation of processing conditions included the extent to which starch was gelatinized, the extract viscosity of the peas and the availability of lysine in the peas. In the initial study using the pea (*Pisum sativum*) cultivar Croma, a tempering level of 24% moisture was selected for the micronization treatment as it resulted in significant increases ($p \leq 0.05$) in starch gelatinization, while maintaining available lysine levels and reducing extract viscosity. With minor exceptions, which included a decrease in available lysine for the cultivar Carneval, similar results were obtained when these conditions were applied to four other pea cultivars (two yellow and two green) in a second study. Storage at room temperature (22ºC) was able to preserve these characteristics, and there was no benefit to storing at a lower temperature of 4ºC for up to 6 weeks. Differences in available lysine for the yellow Carneval and unknown cultivars, seen immediately after processing, were not a factor following storage. The production of micronized peas suitable for incorporation into animal feed is possible if the appropriate moisture content during tempering is selected.

WARUNKI MIKRONIZACJI DLA GROCHU (*PISUM SATIVUM*) ORAZ OCENA PRODUKCJI *IN VITRO*

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S ł o w a k l u c z o w e: mikronizacja, pasza, kiełkowanie skrobi, lepkość, dostępność lizyny.

S t r e s z c z e n i e

Badano wpływ nawilżania oraz warunków przechowywania grochu, który został poddany obróbce cieplnej w zakresie promieniowania podczerwonego (mikronizacji). Kryteria do oceny warunków przetwarzania obejmowały ilość skleikowanego krochmalu, lepkość ekstraktu z grochu oraz dostępność (dyspozycyjność) lizyny w grochu. W początkowych badaniach, używając grochu (*Pisum sativum*) odmiany Croma, wybrano poziom nawilżenia 24%, ponieważ wynikiem tego był znaczny ($p \leq 0.05$) wzrost skleikowania krochmalu przy jednoczesnym utrzymaniu dyspozycyjności poziomu lizyny i obniżonej lepkości ekstraktu z grochu. Podobne rezultaty otrzymano, gdy takie same warunki przetwarzania były zastosowane w drugiej części eksperymentów dla czterech innych odmian grochu (dwie żółte odmiany i dwie zielone), z małym jednak wyjątkiem, gdy zanotowano obniżenie lizyny w odmianie Carneval. Przechowywanie grochu po mikronizacji w pokojowej temperaturze (22°C) zachowało otrzymane charakterystyki materiału, nie dając żadnych różnic w mierzonych właściwościach podczas przechowywania grochu w temperaturze 4°C przez 6 tygodni. Różnice w dostępności lizyny w żółtej odmianie Carneval i nieznanych odmianach, a oceniane zaraz po mikronizacji, zatarły się po kilkutygodniowym okresie przechowywania. Wyniki badań potwierdzają, że groch poddany mikronizacji może być użyty z powodzeniem w celach paszowych, jeżeli zostanie on nawilżony do właściwego poziomu przed mikronizacją.

Introduction

Modification of dietary feed components by processing is one way to improve animal performance. Modification of cereals has often involved some type of heat processing where steam cooking and flaking (MEDEL et al. 2002), extrusion (MEDEL et al. 1999) and micronization or high intensity infrared heat (MEDEL et al. 1999, THACKER 1999, YIN et al. 2001) have been used. While much work has been done with barley (MEDEL et al. 2002, MEDEL et al. 1999, THACKER 1999, YIN et al. 2001), heat treatments have also been used for maize (MEDEL et al. 1999) and wheat (ZARKADAS, WISEMAN 2002).

The effect of these heat treatments has primarily resulted in an increase in starch digestibility (MEDEL et al 2002) due to an increase in the level of gelatinized starch because of the heating effect (THACKER 1999). For some studies, this resulted in an improvement in pig performance (MEDEL et al. 2002, MEDEL et al. 1999) whereas in other studies there was no improve-

ment in pig performance (YIN et al. 2001, ZARKADAS, WISEMAN 2002) or a reduction in growth rate (THACKER 1999). One explanation for the poor performances in some studies was an increase in diet viscosity and accompanying decrease in feed intake (THACKER 1999), although this explanation was not supported in the work of ZARKADAS, WISEMAN (2002). Processing conditions also play a role in determining the effectiveness of the heat treatment. During the micronization of wheat, higher heat exposure resulted in higher levels of starch gelatinization and extract viscosity (ZARKADAS, WISEMAN 2002). In work with lentils, it was shown that higher moisture levels prior to micronization resulted in increased levels of starch gelatinization (ARNTFIELD et al. 1997).

There has been increased interest in using peas as a component in feed for animals such as pigs. It has been shown that the inclusion of micronized peas, with and without enzyme supplementation, resulted in a reduction in manure volume as well as reduced fecal phosphorus and total nitrogen excretion (ZHANG et al. 2003). In this investigation, the moisture content during micronization required to produce an appropriate feed ingredient is being investigated using a single pea cultivar. Using the appropriate moisture level, four other cultivars are micronized and the effects of two storage conditions evaluated.

Materials and Methods

Two studies were conducted to assess the effect of micronization on peas using in vitro lysine availability, starch gelatinization, and water extract viscosity as indicators of quality. In the first study, a single pea (*Pisum sativum*) cultivar (Croma) was examined at four tempering moistures to determine the optimal moisture level for micronization. Samples of raw, tempered, or tempered plus micronized peas were used for in vitro lysine availability, starch gelatinization, and water extract viscosity analysis. In the second study, four cultivars of peas, AC Advantage, Radley, Carneval, and an unknown cultivar, were used to confirm the effects of the first study using different cultivars and to assess the effect of storage conditions on the nutritive value of micronized peas. In this second study, the samples were stored at either 4°C or room temperature (22°C) for up to 6 wk. Samples were analyzed for starch gelatinization and extract viscosity at 0, 2, 4, and 6 weeks and for available lysine at 0 and 2 weeks only. In practical feeding situations, it is expected that the micronized materials would be fed within a two week period.

In both studies, 5 kg of each sample were tempered to a designated moisture level (i.e. 21, 24, 27 or 30% in the first study or 24% in the second study). The peas were micronized in a gas-fired micronizer unit (Micronizer Ltd. Co., UK) to an average final external surface temperature in the range of 110 to 115°C determined using an infrared thermometer gun (Cole-Palm-

er Instruments Co., Niles, NJ). Once the peas attained a steady flow, samples were collect at two different time intervals during the run for further analyses.

Water extract viscosity was determined using a 0.25 g sample of each ground pea, following an established method (BOROS et al. 1993). To each sample, 3 mL of distilled water was added and the mixture was shaken at 40°C in a water bath for 2 h. The mixture was then centrifuged at 13 000 g and 4°C for 10 min. The viscosity of the supernatant was measured at 25°C using the Wells Brookfield Cone and Plate Digital Viscometer (Middleboro, MA. 02346 USA). Determination of available lysine followed a published protocol (HALL et al. 1973). In brief, a sample was ground to a fine powder, suspended in a solution of agar and mixed with sodium hydrogen carbonate solution. A solution of trinitrobenzenesulphonic acid was added, which reacted with the free ε-amino groups of lysine in the intact protein. Following hydrochloric acid hydrolysis, ε-trinitrophenyllysine was released and measured spectrophotometrically at 425 nm. Pure DL-lysine monohydrochloride was used as a standard. Starch gelatinzation was evaluated using Differential Scanning Calorimetry (DSC) as described previously (WANG, SASTRY 1997). A 10 mg sample containing 20% ground peas in water was heated at a rate of 10°C/min using a Dupont 990 Thermal Analyzer with a 910 DSC cell base. The starch gelatinization temperature was taken as the point of maximum heat flow on the resulting thermogram. The enthalpy (ΔH) of the peak corresponding to the starch gelatinization transition was used to calculate the % starch gelatinized as follows:

$$\% \quad \text{gelatinized} \quad \text{starch} = \left(\frac{\Delta H_{\text{raw}} - \Delta H_{\text{sample}}}{\Delta H_{\text{raw}}} \right) \cdot 100.$$

All analyses were duplicated and statistical analysis involved an Analysis of Variance using Duncan's test to identify significantly different treatments ($p \leq 0.05$) (SAS 1989). This approach shows the relative change in % starch gelatinized assuming a zero value for raw starch. It should be noted that in previous work where starch gelatinization was based on enzyme susceptibility, % gelatinizated starch values of approximately 7% have been reported for raw peas (TOEWS 2001).

Results and Discussion

Effect of Tempering Level

It has been shown that the moisture content during micronization can have a major influelnce on the effectiveness of the infrared heat treatment (ARNTFIELD et al. 1997). The effect of tempering moisture levels between 21

and 30% are shown for Croma peas in Table 1. To determine if the presence of water alone was sufficient to change the properties of the peas, tempered samples that had not been micronization were included for comparison. Although there is a lot of variability in the data obtained for the percent starch gelatinized due to the technique used, the amount of starch gelatinized was significantly higher ($p \leq 0.05$) for the micronized samples tempered at 24 and 30% moisture, with the sample at 30% having the highest value. There was no significant difference ($p > 0.05$) between the samples that had not been micronized. It appeared that the infrared heat treatment was necessary to produce the desired difference in the level of gelatinized starch.

The fact that the micronization treatment affected the starch component of the pea was also evident when comparing the starch gelatinization temperatures (Tg) for the raw, tempered and micronized peas. The Tg for all micronized samples were higher ($p \leq 0.05$) than those for the raw and tempered peas. The latter two were not significantly different ($p > 0.05$). If starch gelatinization were the only criterion for evaluation of the micronization conditions, a 30% tempering regime would be appropriate.

Changes in extract viscosity and available lysine are also shown in Table 1. For all tempered plus micronized peas, extract viscosity was significantly reduced compared to the raw and tempered peas, although there were no significant differences ($p > 0.05$) among the different tempering levels for either the tempered or tempered plus micronized peas. Increases in diet viscosity have previously been reported for pig diets formulated using micronized barley (THACKER 1999) and wheat (ZARKADAS, WISEMAN 2002). For barley, this increase in viscosity was believed to contribute to the poor feed intake and growth rate (THACKER 1999). This is clearly not an issue with peas as extract viscosity actually decreased with micronization. Available lysine levels were unaffected by the micronization treatment regardless of tempering level (Table 1) suggesting that the Maillard reaction had not occurred. Similar results for reactive lysine of heat treated peas were reported previously (RUTHERFURD, MOUGHAN 1997), where heating peas at 110°C for 15 min had no effect.

Practical considerations also have to be factored into the selection of the preferred processing conditions. Although micronization is a heat treatment, it is of short duration, and for samples with high initial moisture, we have observed that this process does not remove a sufficient amount of water to ensure safe storage (i.e. prevent mold growth). As a result, peas had to be air-dried following micronization. To minimize this drying step, it is desirable to keep the initial moisture as low as possible. With this in mind, a tempering level of 24% was chosen for further study, as this represented a significantly higher level of gelatinized starch, without a loss of available lysine. The peas tempered at 30% moisture, which had a higher level of starch gelatinization were not selected because of the requirement for additional drying.

Table 1

Effect of tempering moisture level on the extract viscosity, lysine availability and starch gelatinization of tempered and tempered plus micronized peas (*Pisum savitum v. Croma*)[1]

Tempering level (%)	Treatment	Extract viscosity (cP)[2]	Available lysine (mg/g)[2]	Starch gelatinization temperature (^{0}C)[2]	Starch gelatinized (%)[2,3]
	Raw peas	1.67±0.03 ^{a}	20.1±0.2^a	67.9±0.2 ^{b}	*nv*[3]
21	Tempered	1.64±0.07 ^{a}	19.0±0.9 ^{a}	67.7±0.1 b	14.3±8.8 ^{de}
	Tempered + Micronized	1.31±0.04 ^{b}	21.0±0.2 ^{a}	72.0±1.2 ^{a}	23.5±3.6 ^{cd}
24	Tempered	1.64±0.08 ^{a}	20.5±0.4 ^{a}	68.1±0.1 ^{b}	9.6±1.2 ^{e}
	Tempered + Micronized	1.28±0.07 ^{b}	22.4±1.2 ^{a}	71.5±0.0 ^{a}	48.2±6.1 ^{b}
27	Tempered	1.61±0.09 ^{a}	20.4±0.1 ^{a}	68.1±0.1 ^{b}	19.7±7.7 ^{cde}
	Tempered + Micronized	1.32±0.12 ^{b}	22.0±0.4 ^{a}	73.1±0.0 ^{a}	31.3±1.3 ^{c}
30	Tempered	1.61±0.09 ^{a}	20.8±1.8 ^{a}	68.9±0.0 ^{b}	20.4±0.7 ^{cde}
	Tempered + Micronized	1.27±0.10 ^{b}	19.8±0.5 ^{a}	72.4±0.9 ^{a}	59.2±6.3 ^{a}

[1] Values are means ± standard deviation.

[2] Column values followed by the same superscript are not significantly different ($p \leq 0.05$).

[3] The percent starch gelatinized has been calculated based on assumption of zero gelatinized starch in the raw pea as follows:

$$\% \quad \text{gelatinized} \quad \text{starch} = \left(\frac{\Delta H_{\text{raw}} - \Delta H_{\text{sample}}}{\Delta H_{\text{raw}}} \right) \cdot 100.$$

As a result, no value (*nv*) was determined for raw peas.

Pea Cultivar and Storage in Relation to the Value of Micronization of Peas

The second study was initiated to evaluate the effects of micronization on other pea cultivars and to see if refrigeration conditions were required to maintain the benefits of micronization during a short storage period between processing and diet formulation. The effects of micronization and storage conditions over a 2 week period on available lysine in two cultivars of green peas (AC Advantage and Radley) and two cultivars of yellow peas (Carneval and Unknown) are shown in Table 2. For AC Advantage peas, micronization itself had no effect ($p>0.05$) on the available lysine, but there

Table 2

Effect of micronization of tempered Western Canadian peas on the available lysine content at two different storage conditions

Treatment	Storage time and temperature	Available lysine (mg/g)[1]			
		AC Advantage (green)[2]	Radley (green)[2]	Carneval (yellow)[2]	Unknown (yellow)[2]
Raw	time 0	11.4±1.2 ^{c}	19.6±0.5 ^{a}	23.2±0.2 ^{a}	19.4±0.8 ^{b}
	2 weeks at 4°C	15.8±0.2 ^{ab}	15.8±0.4 ^{bc}	15.2±0.5 ^{c}	15.9±0.4 ^{c}
	2 weeks at 22°C	14.8±0.5 ^{ab}	17.5±1.4 ^{ab}	16.0±1.3 ^{c}	18.9±1.5 ^{b}
Tempered and micronized	time 0	11.8±1.0 ^{c}	19.6±0.2 ^{a}	19.4±0.8 ^{b}	22.6±0.2 ^{a}
	2 weeks at 4°C	16.1±0.4 ^{a}	13.7±2.9 ^{c}	16.2±0.4 ^{c}	17.1±0.5 ^{c}
	2 weeks at 22°C	14.2±0.5 ^{b}	17.7±1.5 ^{ab}	15.0±1.1 ^{c}	18.8±0.6 ^{b}

[1] Values are means ± standard deviation.
[2] Column values followed by the same superscript are not significantly different ($p \leq 0.05$).

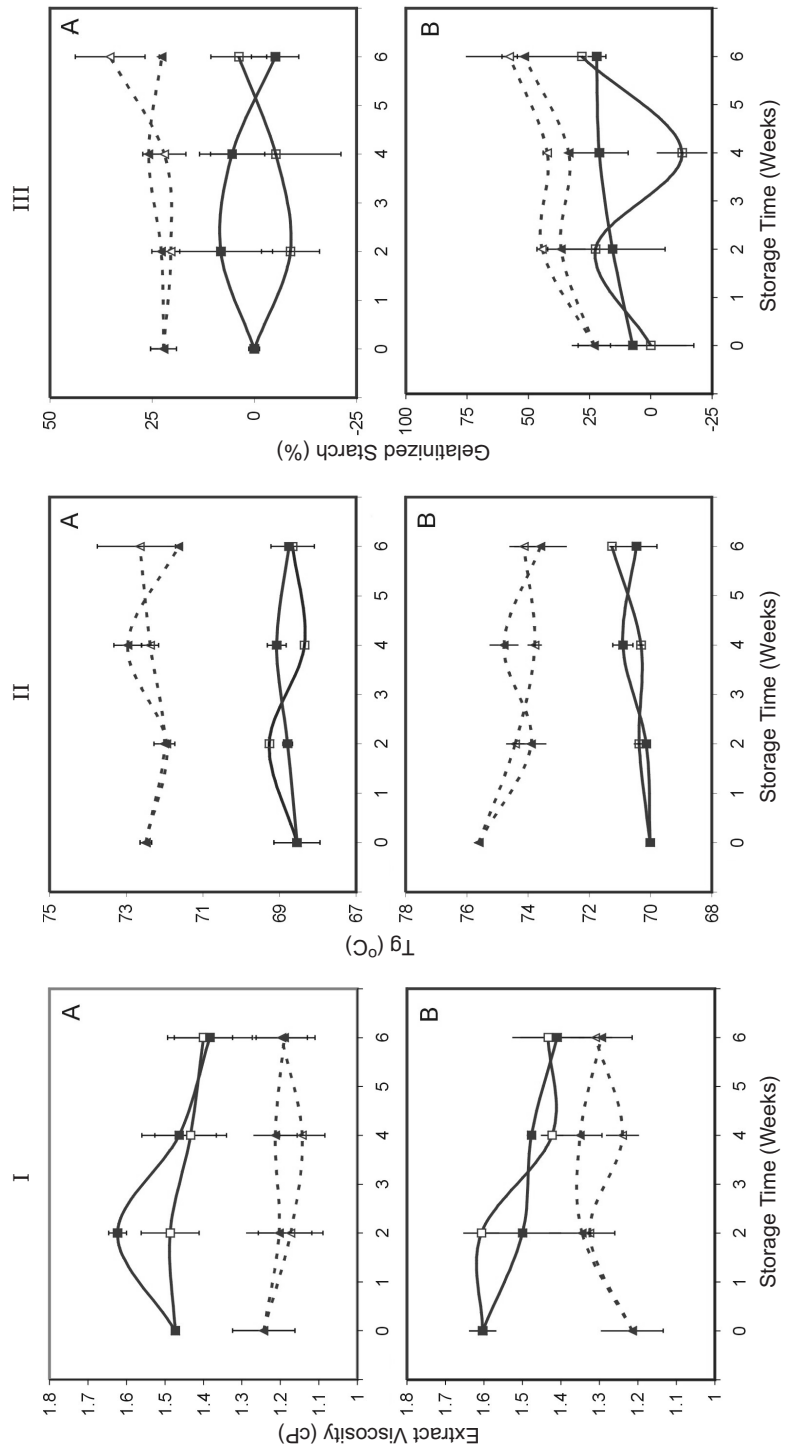
did appear to be an increase in the level of available lysine after two weeks of storage at both room temperature (22°C) and refrigeration temperature (4°C) in micronized and non micronized samples. The reason for this increase is unclear, but may be associated with moisture losses during storage. For the micronized peas, this increase was greater under refrigeration conditions. With the Radley peas, available lysine was again unaffected by micronization itself, but did change with storage. Unlike AC Advantage, however, there was a decrease in available lysine for Radley with time; the greatest loss occurred for the micronized peas stored at 4°C. For the two yellow cultivars, both the initial micronization and storage condition affected the available lysine levels. For the Carneval peas, micronization resulted in a decrease in available lysine and for the unknown cultivar, available lysine levels were significantly higher ($p \leq 0.05$) right after micronization. These differences, however, were not sustained during storage as available lysine levels significantly decreased during storage such that there were no differences between the raw and micronized peas after two weeks of storage at either 4°C or 22°C. For Carneval peas, there were no differences due to storage conditions and for the unknown cultivar, the decrease in available lysine was greater during storage at 4°C. For all four cultivars, it is interesting to note that changes during storage of the micronized peas were similar to those noted for raw peas and that there were no significant differences in available lysine values for micronized and raw peas of a given cultivar stored under the same conditions. This was true for both the green cultivars where available lysine was unaffected by micronization and the yellow cultivars which had different levels of available lysine immediately after processing.

Although some of these results, particularly the increases in available lysine over time, were difficult to explain, it is clear that for all four cultivars, the available lysine levels after 2 weeks of storage were not different ($p>0.05$) from those found in raw peas stored under similar conditions. There was no benefit from storing the peas at refrigeration temperatures.

Extract viscosities for these four cultivars were monitored every two weeks over a 6 week period (Figure 1-I). For the two green cultivars (AC Advantage and Radley; Figures 1-IA and 1- IB, respectively), the decrease in extract viscosity right after micronization noted for Croma peas in the first study, was again evident. This difference was maintained during storage with storage temperature having no effect on the extract viscosity. With the yellow cultivars (Carneval and unknown; Figures 1-IC and 1-ID, respectively), the extract viscosity following micronization was essentially the same as that for the raw peas. The effect of storage was such that after 6 weeks the extract viscosities for the micronized peas were lower than for the raw peas. As was the case for the green cultivars, the storage temperature did not influence the extract viscosity.

It was noted for the Croma peas in the first study that the Tg for starch significantly increased ($p\leq0.05$) as a result of the micronization process. A similar increase was observed for all four cultivars examined in the second study (Figure 1-II). For most cultivars, the Tg was unaffected by storage and the difference between the micronized and raw peas was still evident after six weeks of storage. For the unknown variety (Figure 1-IID), which had the highest Tg values following micronization, there was a decrease in the Tg values during the first two weeks storage so that they were in the same range as the Tg values for other cultivars. There were no further changes during storage, and a difference between the raw and micronized was clear after 6 weeks of storage. For storage of both raw and micronized peas for all four cultivars, Tg was unaffected by the storage temperature.

The data for the percent gelatinized starch after micronization and during 6 weeks of storage is presented in Figure 1-III. For all four cultivars, an increase in the level of gelatinized starch as a result of the micronization process was evident. This is expected as heat is often used to produce gelatinized starch such that the starch is more accessible to digestive enzymes (HARALAMPU 2000) during cooling and storage, however, there is a slow reassociation of the starch components and as retrogradation proceeds and levels of gelatinized starch decreases, the starch becomes more resistant to enzymatic hydrolysis (HARALAMPU 2000). The variability in the assay used in this study, however, has resulted in some interesting changes during storage. For the AC Advantage (Figure 1-IIIA), the level of gelatinized starch for the micronized peas remained the same for the first four weeks and a slight increase was observed at week 6 for the peas stored at 22°C. While there was variability in the % starch gelatinized for the raw AC Advantage peas, the numbers remained close to zero (the assumed value for raw peas) and did not change during the 6 week period. With Radley peas (Figure 1-IIIB),


I
II
III
A
B
Extract Viscosity (cP)
Tg (°C)
Gelatinized Starch (%)
Storage Time (Weeks)

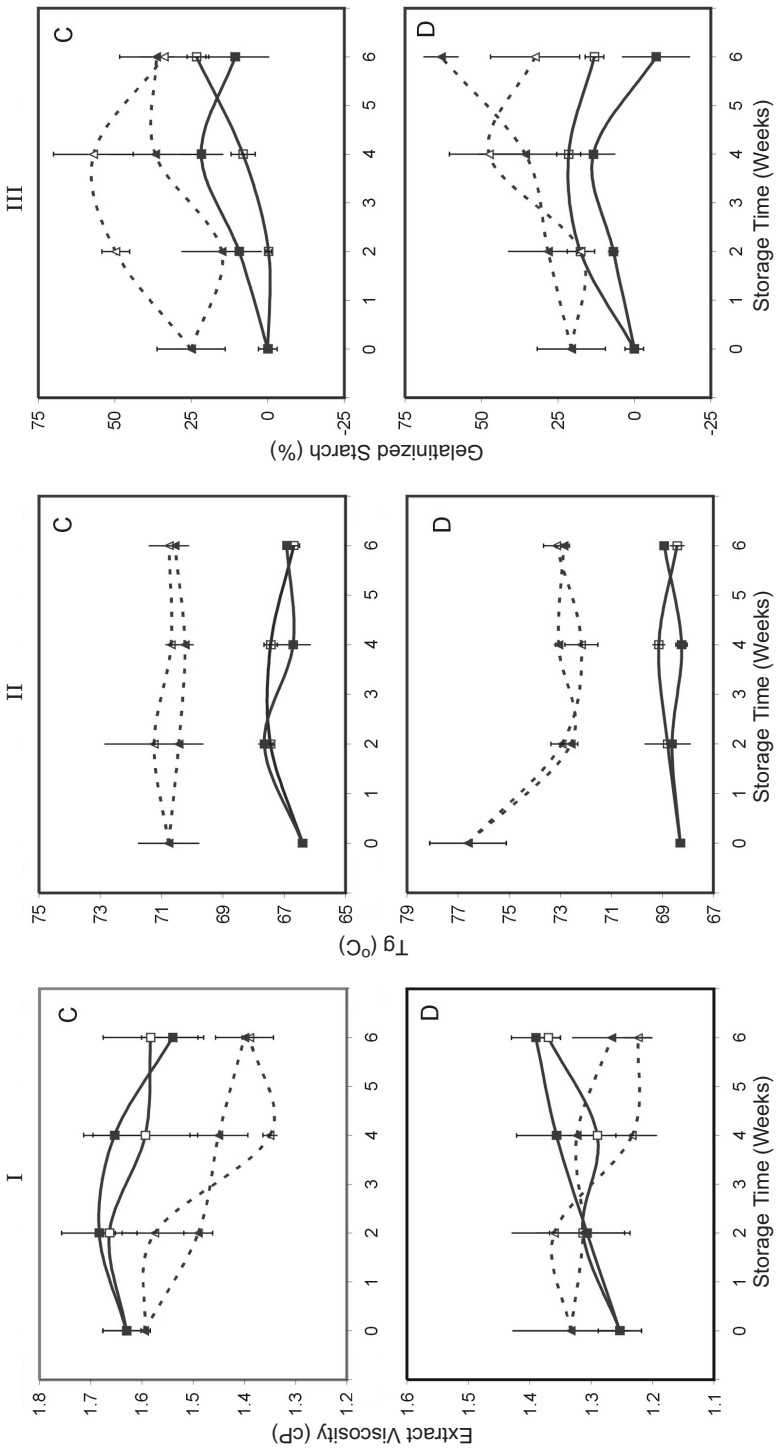


Fig. 1. Effect of storage time and temperature on the extract viscosity (I), starch gelatinization temperature (Tg) (II) and percent gelatinized starch (III) for four pea cultivars. A – AC Advantage, B – Radley, C. – Carneval and D – Unknown. Lines and symbols used within the graphs represent: □ (solid line) – Raw sample at 22°C, ˇ (solid line) – Raw sample at 4°C, Δ (broken line) – Micronized sample at 22°C, p (broken line) – Micronized sample at 4°C. Bars represent standard deviation

the level of gelatinized starch in the micronized peas appeared to increase during storage. Expect for the value for the raw peas at 4ºC at week 4 which may be an anomaly the level of gelatinized starch did not change with time. For raw and micronized peas, differences due to storage temperature were minor. The level of gelatinized starch in Carneval peas (Figrue 1-IIIC) may have been influenced by storage temperature with higher levels of gelatinized starch at weeks 2 and 4 for the peas at 22ºC. This difference was not seen at week 6. For the unknown cultivar (Figure 1-IIID) the difference in the % starch gelatinized following micronization was maintained during storage except for the values obtained after 2 weeks where the micronized sample stored at 4ºC was similar to the raw sample at 22ºC. Otherwise, differences due to temperature were minor.

Concerns that the increase in the level of gelatinized starch due to the micronization process may be reversed due to starch retrogradation as has been suggested in the literature (HARALAMPU 2000) were not seen during this 6-week storage study. Changes in the starch conformation, as reflected in the Tg values for the different cultivars, were maintained during storage. Despite variability in the % gelatinized starch data obtained using DSC, the improved level of gelatinized starch was generally maintained during storage.

Conclusions

It is clear from this study that for all five pea cultivars examined micronization resulted in a change in the overall conformation of the constituent starch and an increase in the level of starch gelatinzation. Extract viscosity was either unaffected (yellow cultivars) or decreased (green cultivars) as a result of this heat treatment. The level of available lysine in the raw and micronized green pea cultivars were the same. The differences in the starch and extract viscosity were maintained or accentuated during storage. There was no evidence to suggest that changes in the peas that resulted from the use of infrared heat would be better maintained by storage at 4ºC. Based on this study, peas micronized to a temperature of 110-115ºC following tempering at 24% moisture, will have improved levels of gelatinized starch without losses in available lysine or increases in extract viscosity. These peas can be stored for two to six weeks without adversely affecting these properties.

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