Effect of Curing Conditions on Amino Acid Induction between FCV and BLY Leaves

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Background

- Amino acids are representative key components of cured tobacco leaves.
- The amounts of amino acids in cured tobacco leaves are different between leaf types, and are drastically altered during the curing process.
- Although amino acid behaviors during curing have already been studied, differences in the composition of amino acids between leaf types based on the effect of curing conditions, have not previously been interpreted.

Objective

To clarify the effect of differences in curing conditions and leaf type on amino acid behavior during the curing process

Contents

- Dynamic analysis of amino acids during the curing process
- Comparing amino acid behaviors between leaf types

Materials

Leaf type: Flue-Cured Virginia / Burley-type leaf cultivated in JPN

Position on stalk: mid-stalk position

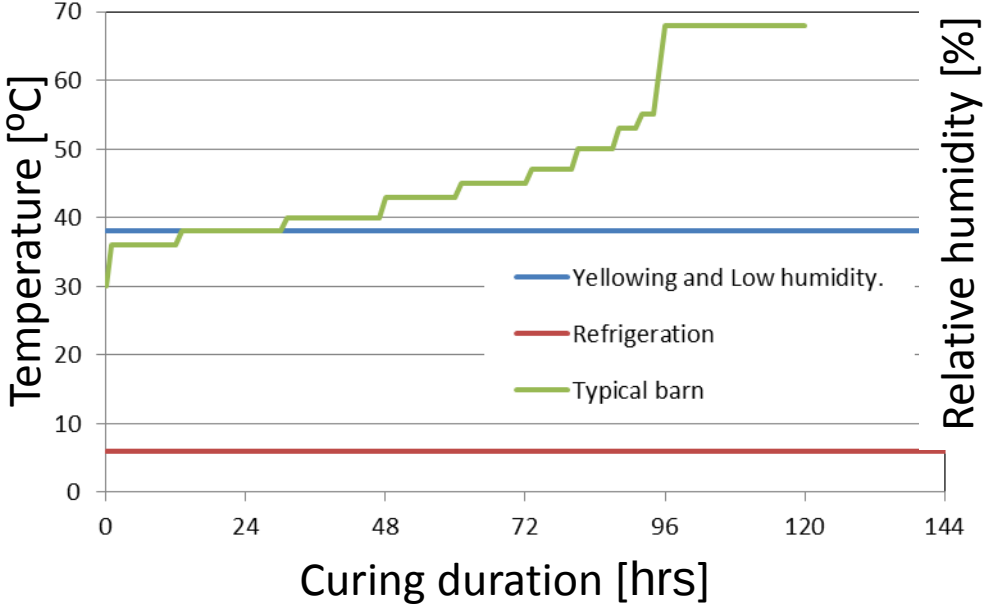
Harvest information: normal cultivating method

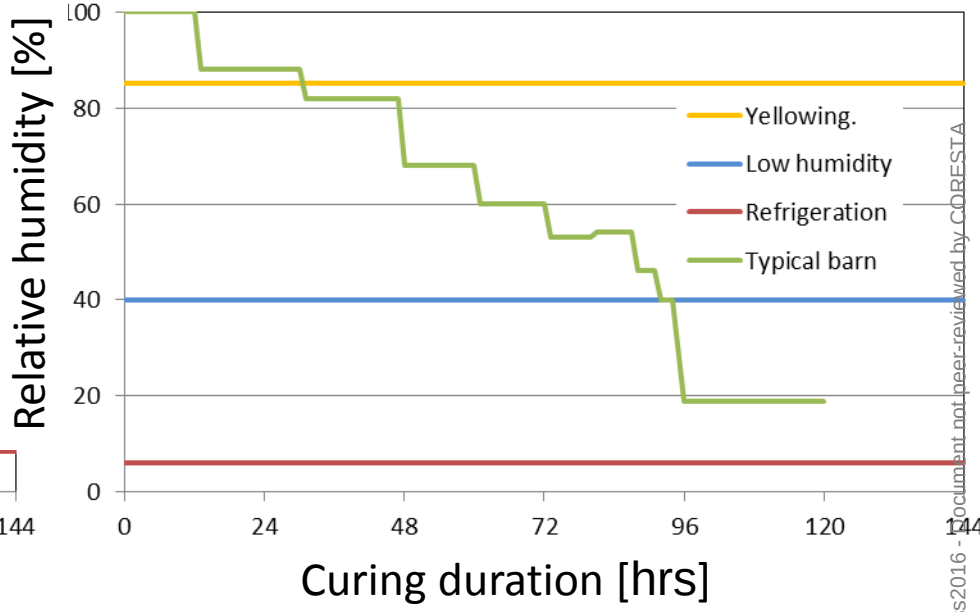
FCV

BLY

Curing conditions

・Conventional barn curing: 120 hrs
・Model test: under static conditions (temp./relative humidity): 144hrs
38ºC/85%R.H.(yellowing), 38ºC/40%R.H.(low humidity), 6ºC/80%R.H. (refrigeration).





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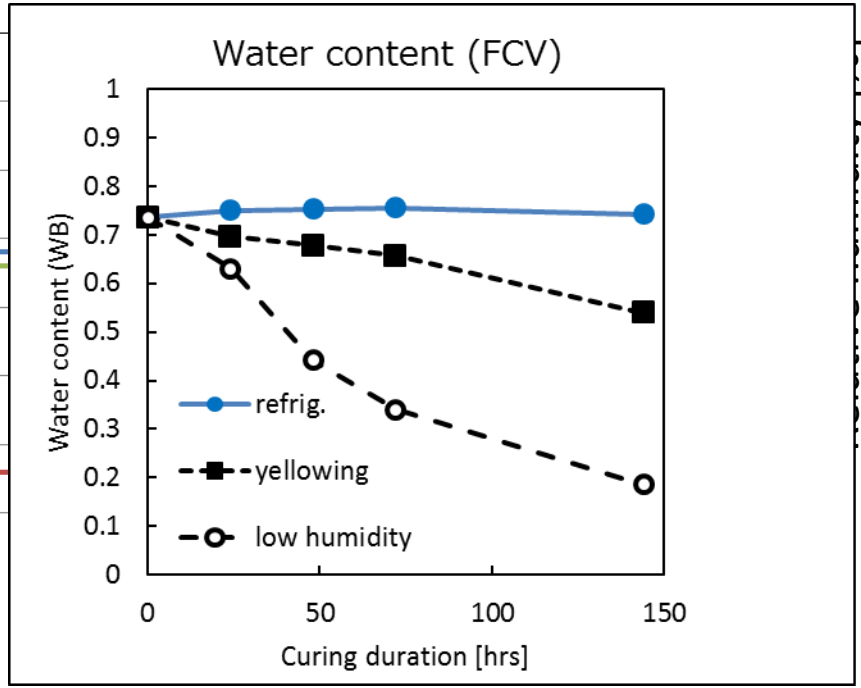
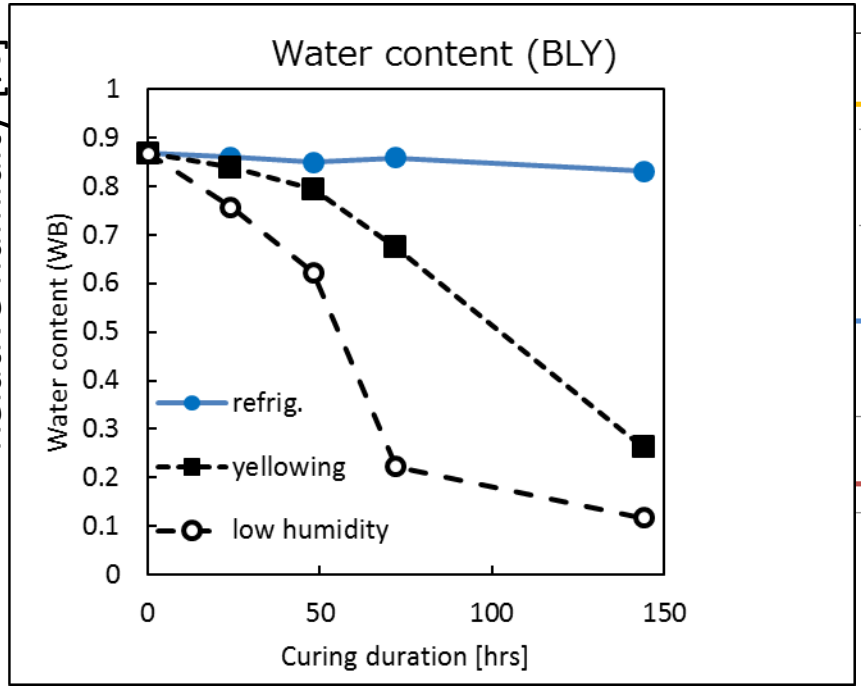




Temperature [ºC]

Experimental procedure

Fresh tobacco leaves

Curing

Freeze drying

Pulverizing

Extraction with 50%MeOH

Centrifugation

Filtration (0.45 μm PTFE)

Ultrafiltration (10kDa)

Injection into CE/MSD

Curing conditions

Temperature (6℃, 38˚C)

×

Relative humidity (40%, 85%)

×

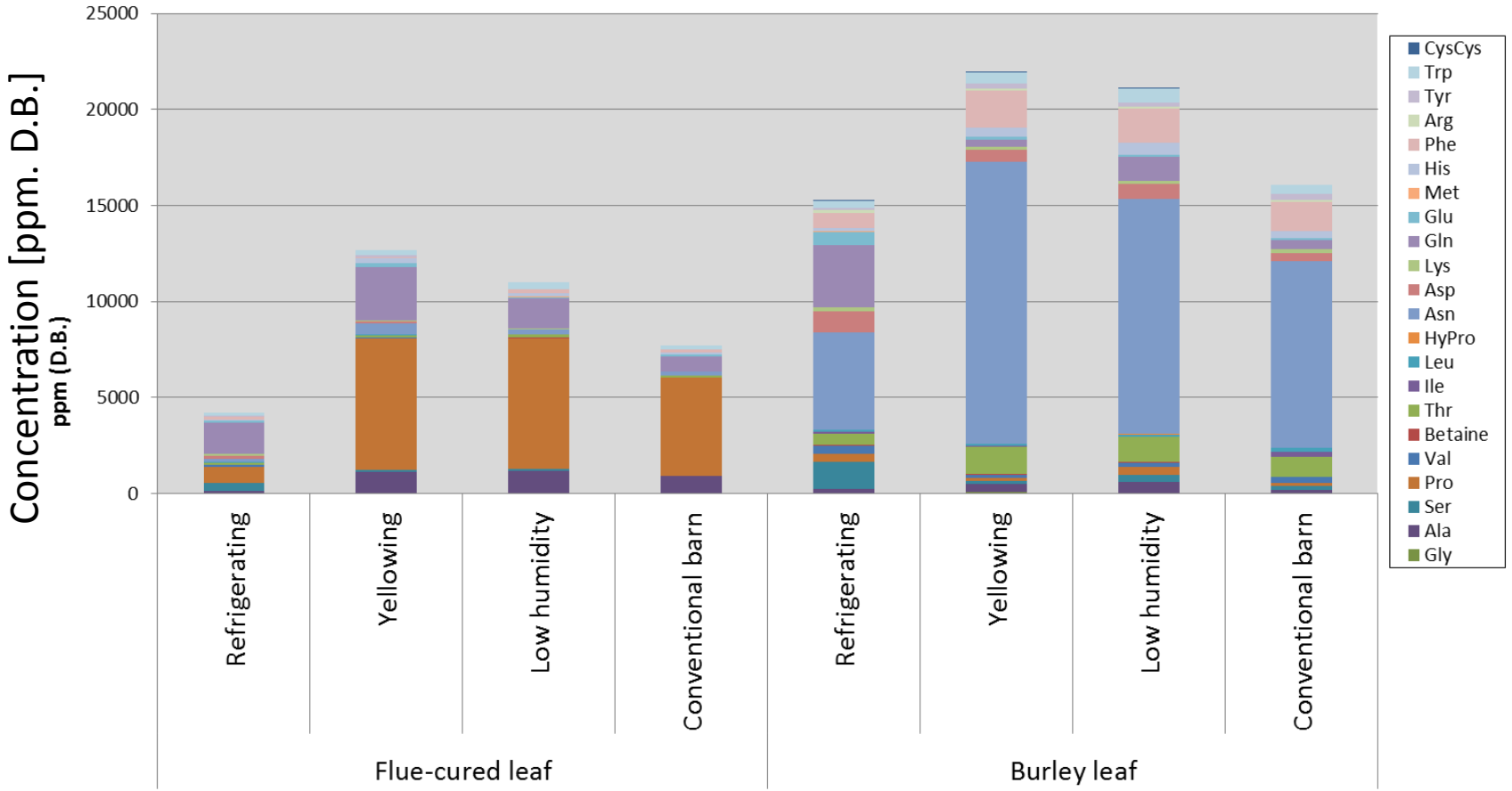
Sampling period (0, 24, 48, 76, 144 hrs.)

×

FCV and Burley Leaf

Agilent CE 1600, 6130 MSD with ESI chamber (Agilent Tech., CA, USA)

Results



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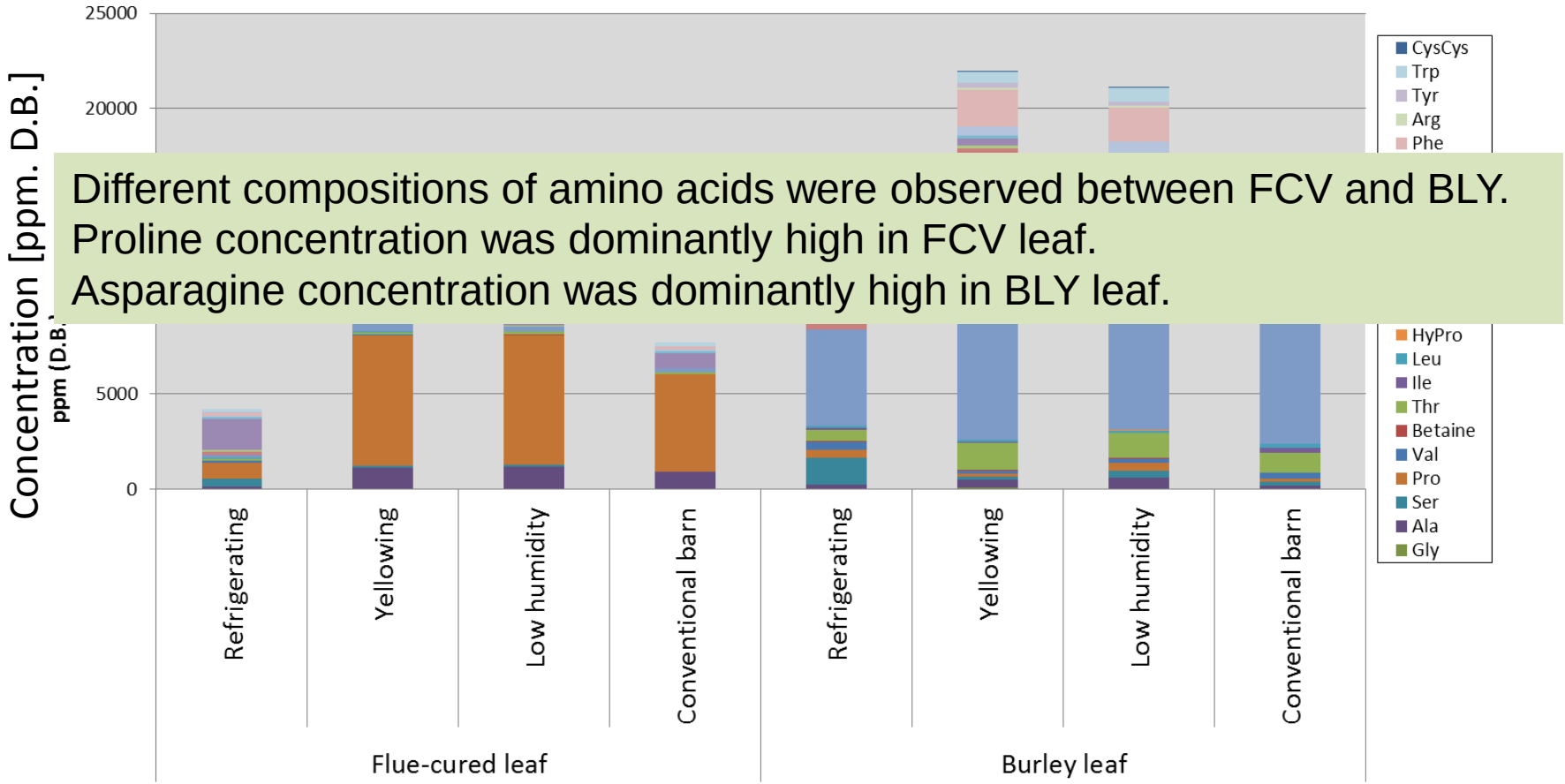
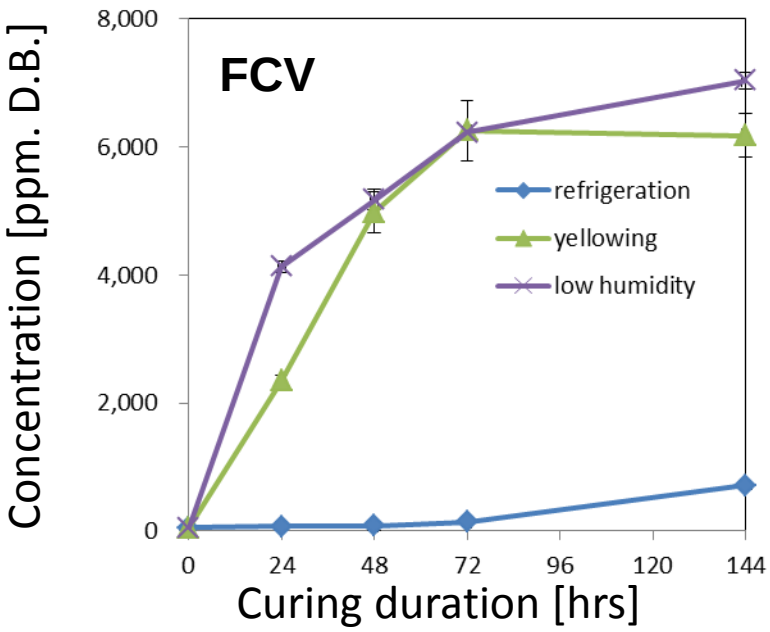
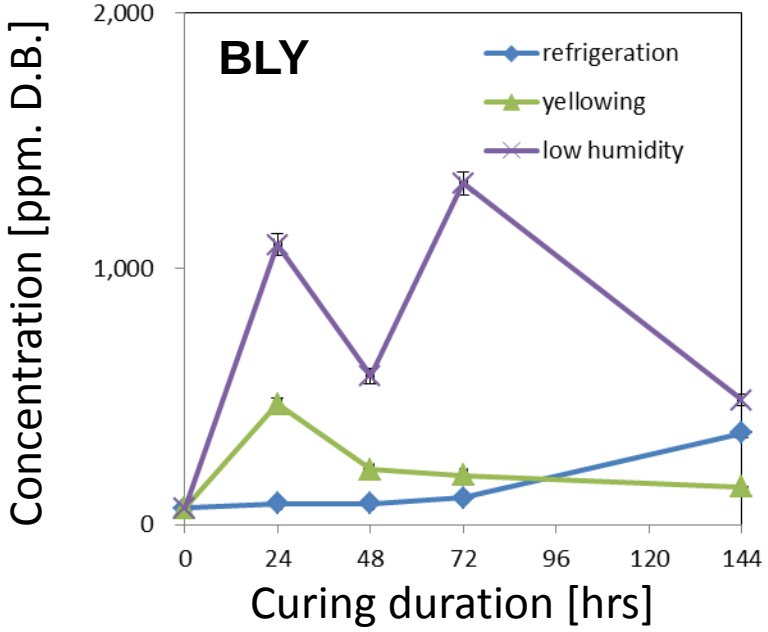


Different compositions of amino acids were observed between FCV and BLY.
Proline concentration was dominantly high in FCV leaf.
Asparagine concentration was dominantly high in BLY leaf.

Results

Proline induction





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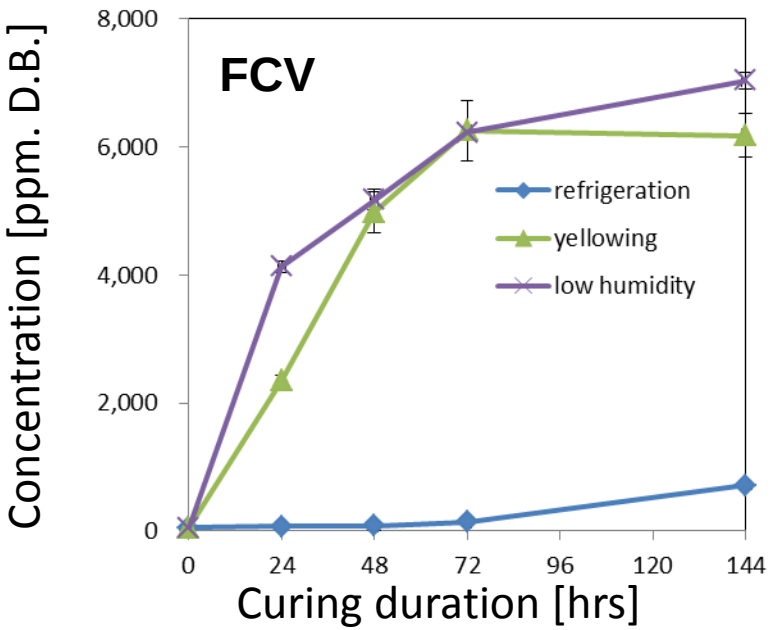
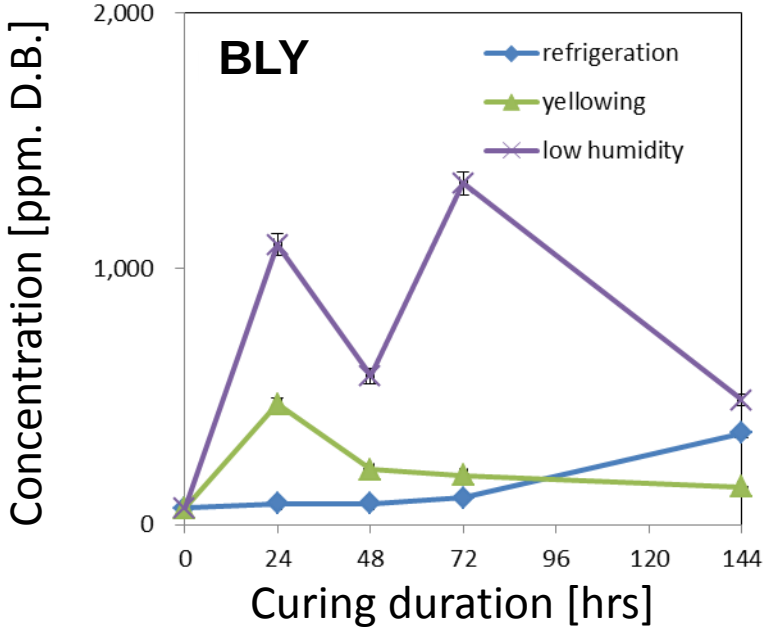
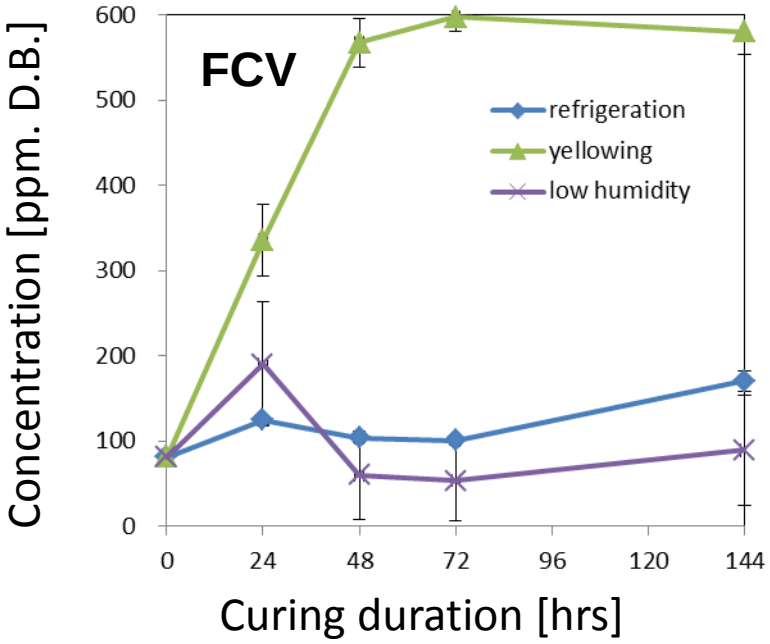


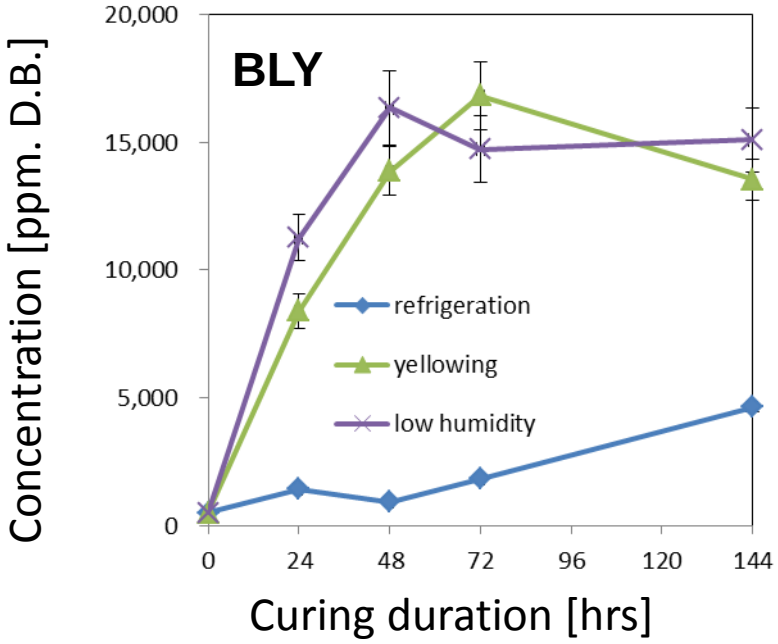


Different proline induction profiles were observed between FCV and BLY.
Proline concentration in FCV was significantly increased compared with that in BLY leaf.

Results

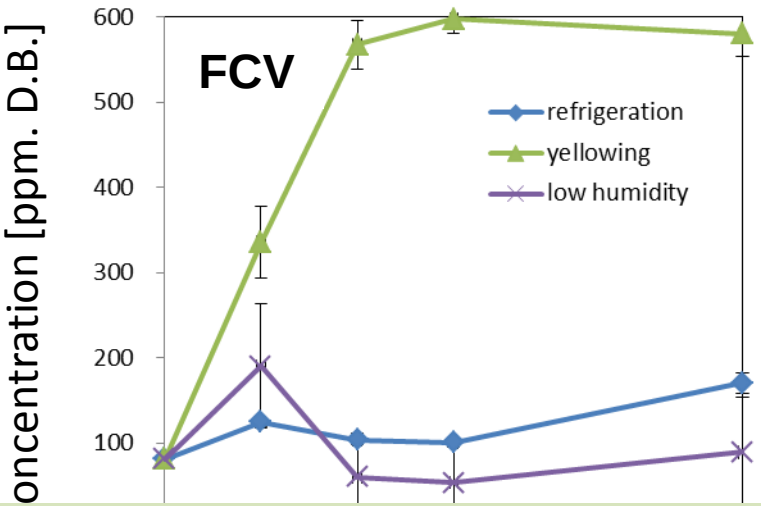
Asparagine induction

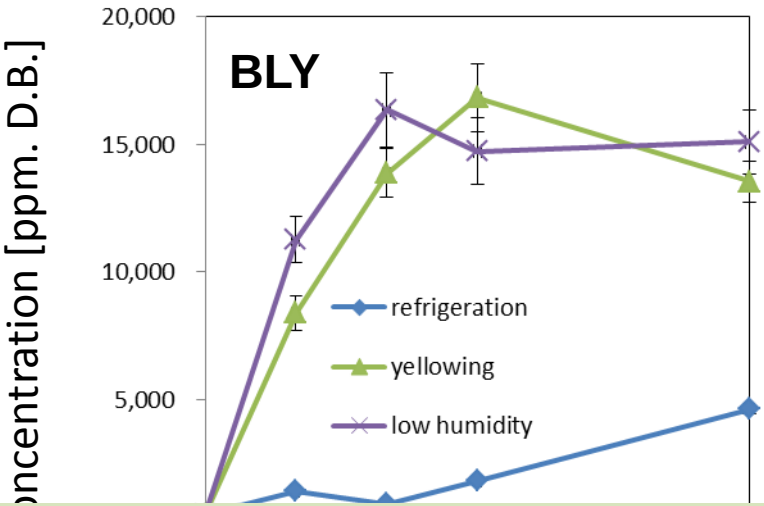




Results

Asparagine induction

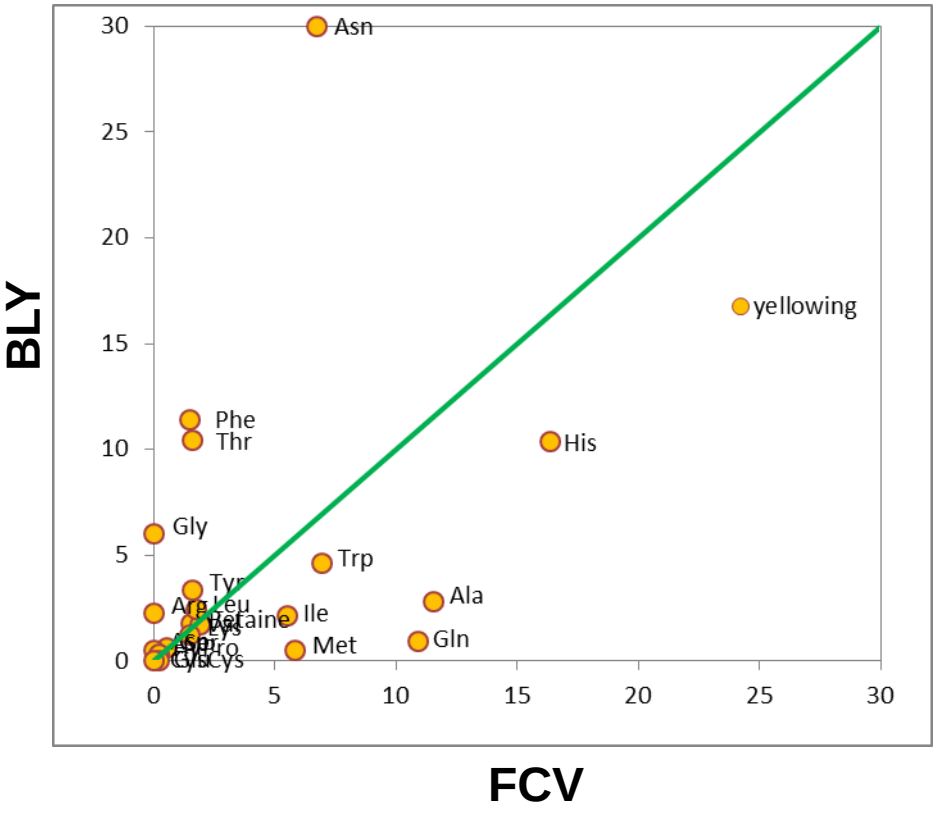


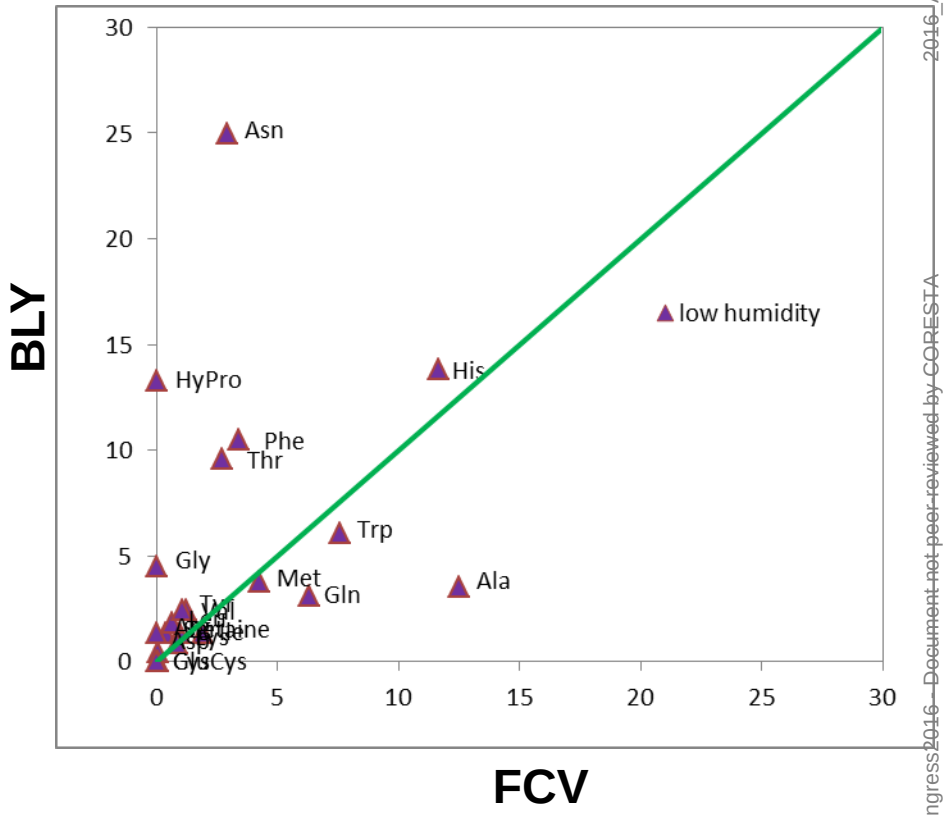


Asparagine concentration was significantly increased in BLY compared with that in FCV.
During the yellowing condition, asparagine induction followed a similar timescale in both FCV and BLY.

Results

Comparison of induction ratio between FCV and BLY under the same curing conditions





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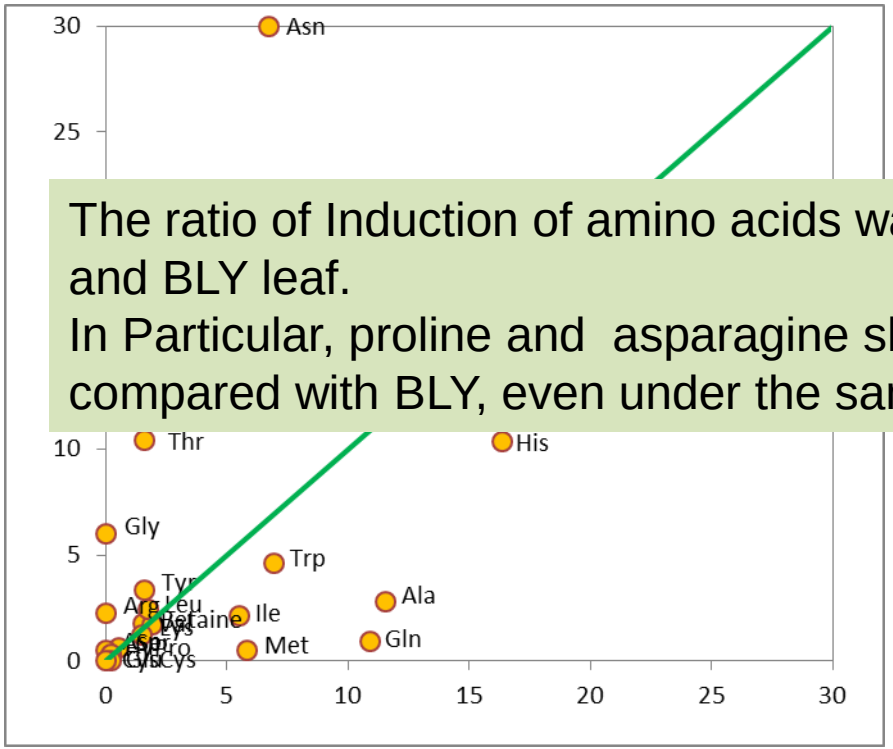
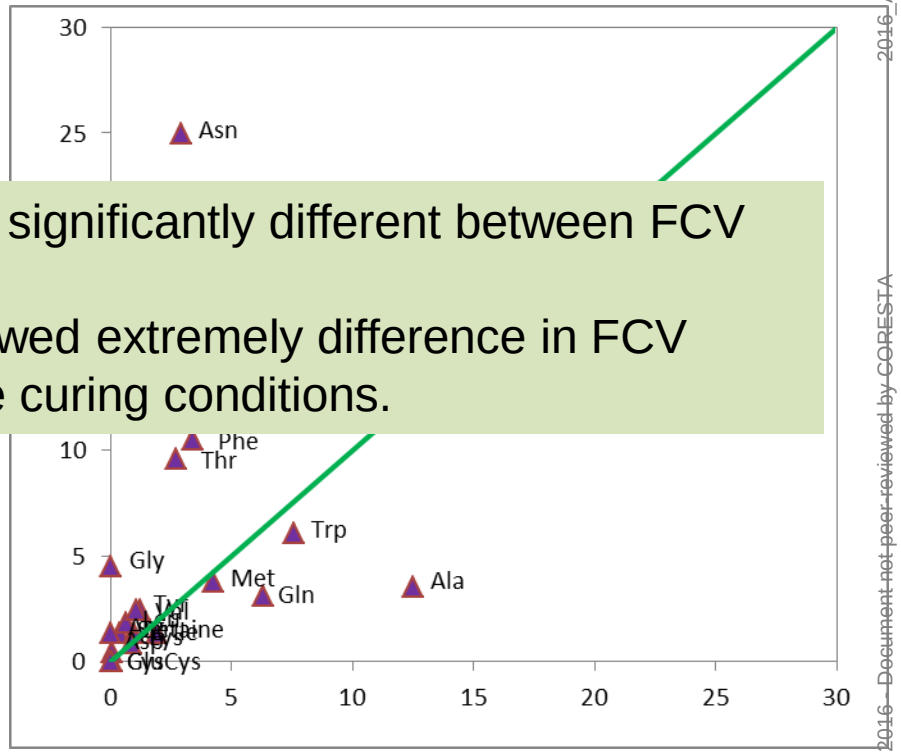
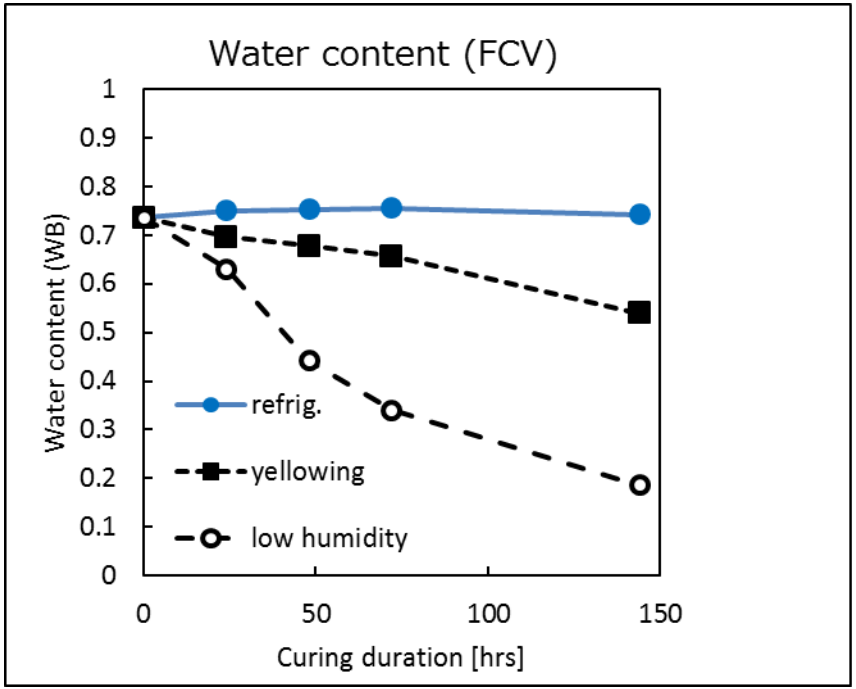


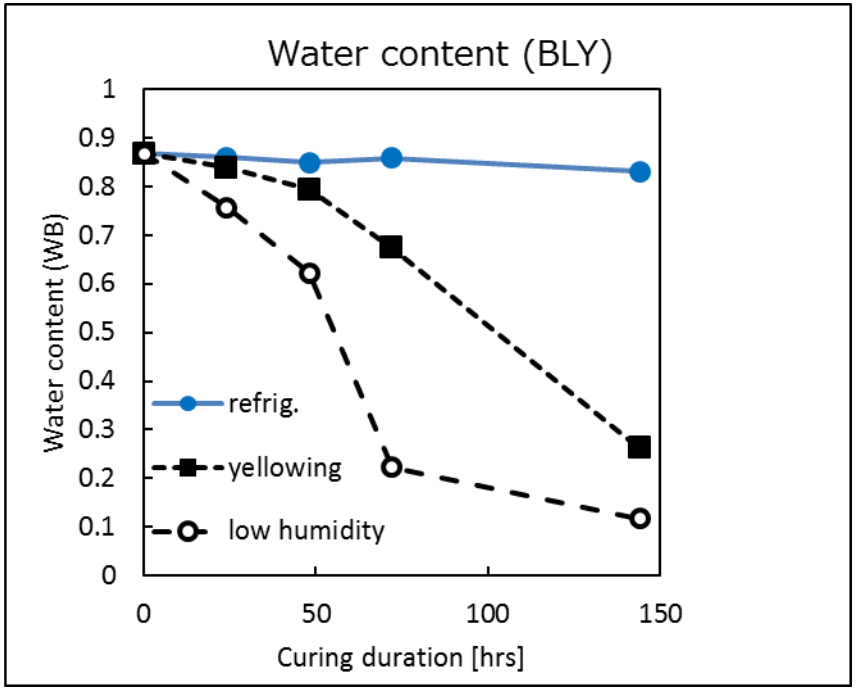


The ratio of Induction of amino acids was significantly different between FCV and BLY leaf.
In Particular, proline and asparagine showed extremely difference in FCV compared with BLY, even under the same curing conditions.

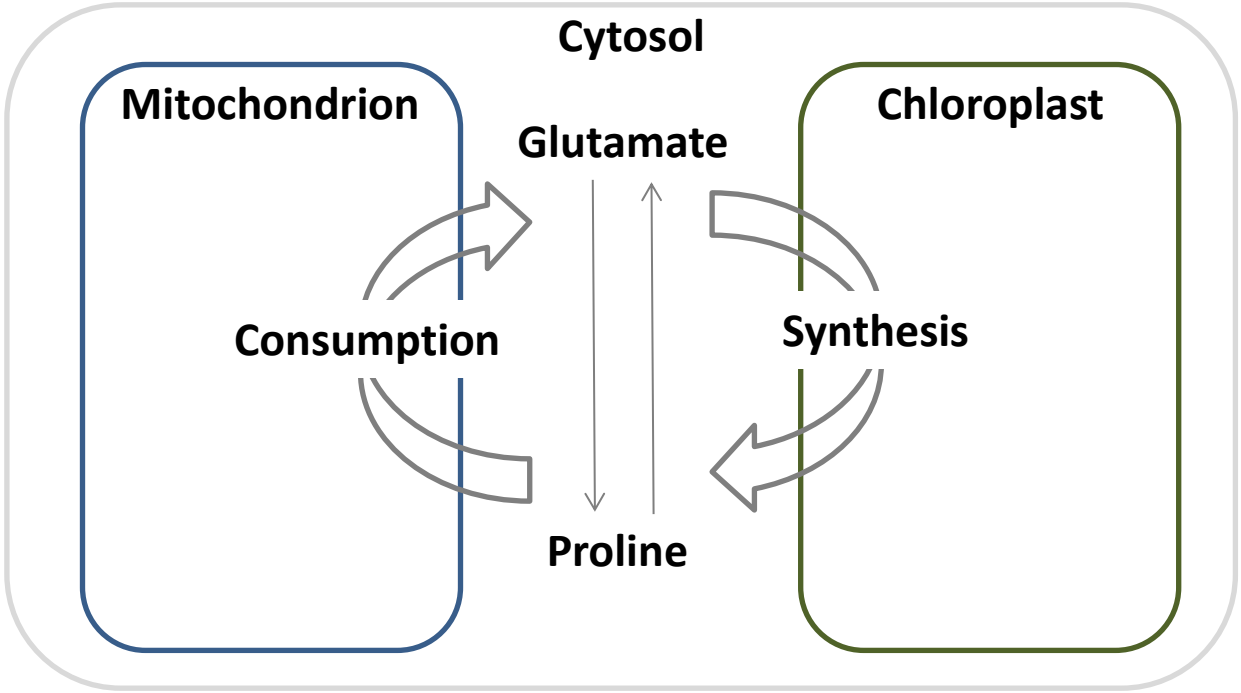
Discussion

Drought stress is a possible trigger for amino acid induction during curing. For a minimum of 24 hours, proline and asparagine were induced in FCV and BLY leaf at 38 ºC conditions (yellowing and low humidity).



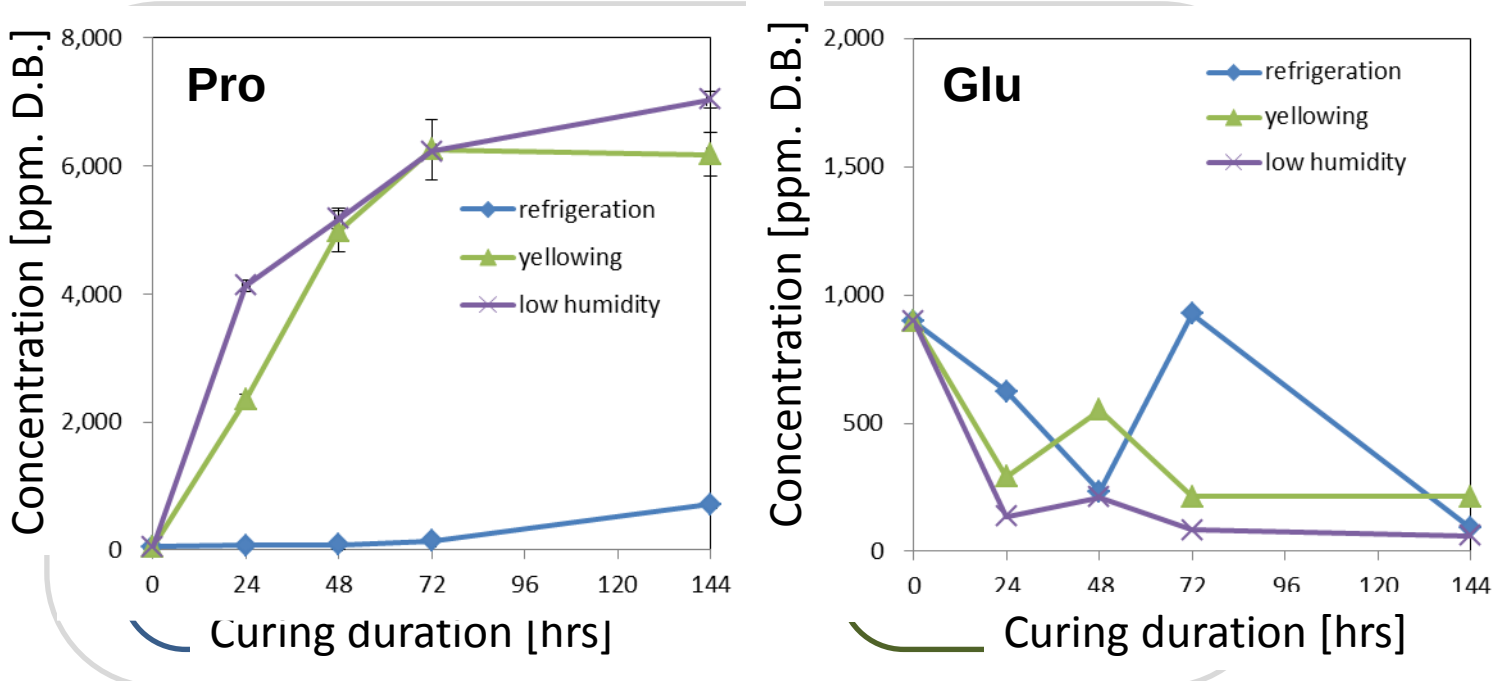


Discussion : Proline induction



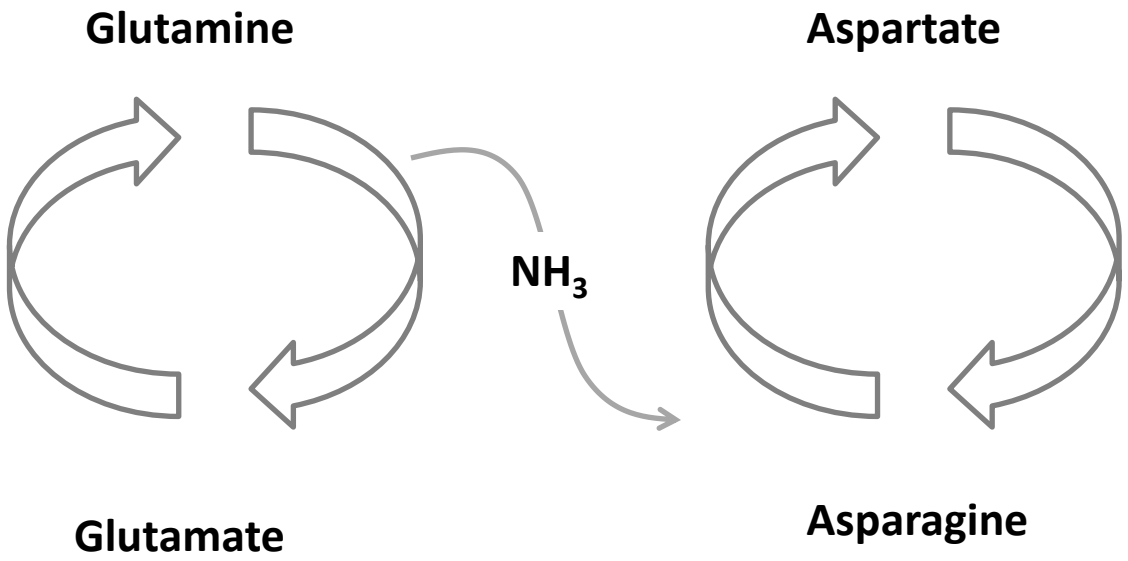
L. Szabados *et al.*, *Trends in Plant Science*, 2009, **15**, 89-97

Discussion : Proline induction



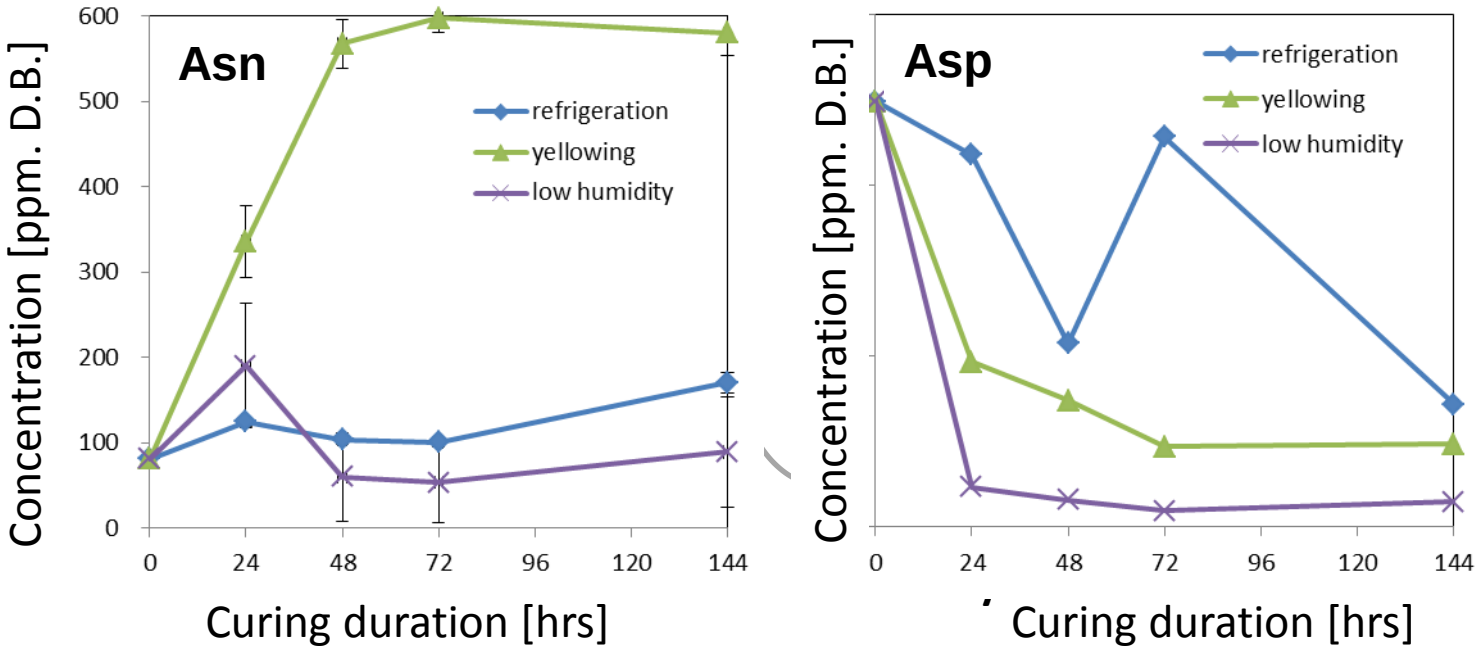
Glutamate decreased within 24 hours in heat-shock conditions in FCV leaf.
Initial proline induction can be interpreted as linked to glutamate consumption.
The late phase of the induction mechanism will be clarified in a future study.

Discussion : Asparagine induction



L. Gaufichon *et al.*, *Plant Science*, 2010, **179**, 141-153

Discussion : Asparagine induction



Aspartate dropped sharply within 24 hours in the low humidity condition in FCV leaf. BLY leaf contained 10 times more **Asn** than **Asp**. It is implied that there are underlying mechanisms in the metabolic pathway of **Asn** during curing in BLY leaf.

Discussion

The behavior of asparagine induction in FCV leaf in the yellowing condition showed a tendency similar to that in BLY leaf.

It is inferred that plant homeostasis (*e.g.* cell integrity and metabolism) is important to clarify when clarifying mechanisms of amino acid induction.

Curing conditions can contribute to improvements in the quality of tobacco leaf in terms of the amino acid induced during curing.

Summary

- The composition of amino acids in tobacco leaves showed a great difference between flue-cured and burley leaves.
- It is feasible that the central cause of this difference depended on leaf type.
- Similar tendencies in induction profiles were observed in the yellowing condition when comparing FCV and BLY leaf.
- Further study of amino acids would be of value to achieve a greater understanding of the role of the constituents in tobacco leaves.