Solubility and Stability Characteristics of Proteins in Green Juice Prepared from Alfalfa, White Clover and Italian Ryegrass

Stanislaw GWIAZDA*, Akinori NOGUCHI**
and Kyoko SAIO**

* Agricultural University of Warsaw, 03-849
Warsaw, Poland

** National Food Research Institute, 2-1-2
Kannon-dai, Yatabe, Tsukuba, Ibaraki,
305 Japan

In Pro-Xan II process, green juice (GJ) extracted from leaves is heated first to 60°C, cooled and centrifuged. The supernatant i.e. clear brown juice (CBJ) is reheated to 80 to 85°C to prepare white leaf protein concentrate (LPC). This paper deals with solubility and stability of proteins in GJ and CBJ derived from alfalfa, white clover and Italian ryegrass. The pH solubility profiles from the three crops are almost similar in GJ but not in CBJ. The stability of protein in GJ and CBJ was low, depending upon the temperature and pH in storing and it is deeply related to the recovery of LPC. These results may suggest that LPC should be prepared with a keen attention to kinds of crop and to storing temperature and/or pH of GJ and CBJ.

The yield of leaf protein concentrate (LPC) is affected not only by the amount of pressed green juice (GJ) but also by the quality and quantity of extracted proteins, which depend upon macerating and pressing methods under consideration of plant species, varieties, stage of maturity, growing and harvest conditions¹⁾~³⁾. Moreover, the presence of oxidative and proteolytic enzymes in leave tissues may impair extractability of leaf protein and the yield of LPC. Phenoloxidase and peroxidase catalyse the reduction of *o*-diphenols to *o*-quinones which, in turn, react with proteins through polymerization to yield insoluble tannin-protein complexes. Endogenous proteolytic enzymes cause losses of extracted leaf proteins due to hydrolysis during storing⁴⁾~⁶⁾.

In Pro-Xan II process⁷⁾ to prepare white LPC, GJ is heated first to 60°C, cooled and

centrifuged. The supernatant, that is, clear brown juice (CBJ) is reheated to 80 to 85°C to precipitate white LPC.

This paper deals with solubility and stability of proteins in GJ and CBJ derived from alfalfa, white clover and Italian ryegrass.

Materials and Methods

Alfalfa (*Medicago sativa* L.), white clover (*Trifolium repens* L.) and Italian ryegrass (*Lolium multiflorum* L.) cultivated under normal agronomic conditions were used for the experiment. The plants were harvested at pre-bloom or early bloom stages. The juice was pressed with a Hans Vetter single screw press after adding 200 ppm of SO₂ with 5% Na₂S₂O₅ solution (the pH was adjusted to 6.1). Pressed GJ was filtered through a 100 mesh (0.149 mm) screen to remove residual fibrous materials and the filtrate was collected in ice bath. The GJ was heated in a heat exchanger so as to increase the temperature up to 59 to 60°C in 30 to 40 sec, held at the temperature for 30 min, and then centrifuged at 9 500 rpm (Sakuma CB-5-1 with continuous rotor). The obtained CBJ and initial GJ were analyzed for total nitrogenous compounds (TN) and proteinous compounds; nitrogenous compounds insoluble in 10% trichloroacetic acid (TCA) by the micro-Kjeldahl method.

Results and Discussion

The crops used in the experiment contained 21 to 25% of crude protein on dry basis as shown in Table 1. Using the screw press, 53, 47 and 58% of the total weight, respectively, for alfalfa, white clover and Italian ryegrass were transferred into each GJ. The concentration of TN of GJ from alfalfa, white clover and Italian ryegrass was 0.35, 0.31 and 0.19%, respectively, showing especially lower value in Italian ryegrass. With 10% TCA about 70% of TN was precipitated. These results agree with previous papers⁸⁾,⁹⁾.

As shown in Fig. 1-A, the solubility profiles on TN of GJ from three crops were almost similar. The minimum solubility occurred at around pH 3.0, where 40-45% of TN remained in the supernatant and the solubility decreased

Table 1 Composition of process fractions from alfalfa, white clover and Italian ryegrass

Fraction	Component (%)			
	pH	Nitrogen	Dry matter	TCA soluble N
Alfalfa				
leaves	—	0.62	18.1	—
press cake	—	0.82	28.7	—
green juice	5.80	0.35	7.5	30.7
clear brown juice	5.91	0.20	5.1	60.6
White clover				
leaves	—	0.53	13.4	—
press cake	—	0.72	21.3	—
green juice	6.06	0.31	5.9	28.7
clear brown juice	6.06	0.12	3.5	71.9
Italian ryegrass				
leaves	—	0.45	11.1	—
press cake	—	0.82	21.9	—
green juice	6.35	0.19	4.4	27.4
clear brown juice	6.40	0.08	3.3	75.0

* % of total juice nitrogen

Fig. 1 Nitrogen solubility of freshly extracted green and clear brown juices in the function of pH

●—●; Alfalfa, ○—○; White clover, ▲—▲; Italian ryegrass

slightly at pH 7 to 10 (5–10%) as observed before¹⁰⁾. These solubility profiles suggest that recovery of TN by acid precipitation method cannot be expected higher than 60% and that the extraction of fresh leaves with dilute ammonia solution, which is often used⁷⁾, cannot be effective to get high extractability.

On the other hand, the solubility profiles of TN of CBJ were rather different among crops as shown in Fig. 1-B. The solubility of CBJ from alfalfa showed rapid decrease in the pH range less than 6. It suggests that alfalfa is much easier to get white leaf protein concentrate as compared with white clover and Italian

Fig. 2 Protein retention in storing of freshly extracted green juice and clear brown juice

●—●; Alfalfa, ○—○; White clover, ▲—▲; Italian ryegrass

Fig. 3 Effect of pH on proteolysis of green juice proteins

●—●; Alfalfa, ○—○; White clover, ▲—▲; Italian ryegrass

TCA-soluble N was measured 6 hrs incubation at 25°C. That in freshly extracted green juice of alfalfa, white clover and Italian ryegrass was 30.7, 28.4 and 27.4% of total N, respectively

ryegrass.

The stability of protein in GJ and CBJ at 4 (suppose at cold room), 25 (room temperature) and 45°C (optimum temperature for clarification of heat treated juice at 60°C), were investigated. As shown in Fig. 2-A and B, as the temperature of keeping increased, the concentration of TN in juices decreased. The decrease shown in CBJ (Fig. 2-B) meant that heating at 60°C for 30 sec could not inactivate any proteolytic enzymes. For instance, when the CBJ of white clover was kept at 45°C for 4 hr., about 30% of protein was lost. Thus the recovery of white LPC significantly related to time and temperature of storing juice. The loss was enhanced at pH 5 to 6

as shown in Fig. 3, that is, natural pH of extracts (Table 1) and decreased at more acid or alkaline pH range. In practical preparation of LPC, it may be recommendable to adjust the pH of pressed juice to 3.5 or 8.5 immediately.

References

- 1) BYERS, M. and STURROCK, J. W.: *J. Sci. Food Agric.*, **16**, 431 (1965).
- 2) EDWARDS, R. H., de FREMERY, B. E. and KOHLER, G. O.: *Trans. Am. Soc. Agric. Eng.*, **20**, 423 (1977).
- 3) PIRIE, N. W.: Leaf Protein: The Agronomy, Preparation, Quality and Use, 1 BP Handbook **20**, Blackwell Scientific Publications, Oxford (1971).
- 4) SINGH, N.: *J. Sci. Food Agric.*, **13**, 325 (1962).
- 5) De FREMERY, D., BICKOFF, E. M. and KOHLER, G. O.: *J. Agric. Food Chem.*, **20**, 1155 (1972).
- 6) FINLEY, J. W., PALLAVICINI, C. and KOHLER, G. O.: *J. Sci. Food Agric.*, **31**, 156 (1980).
- 7) EDWARDS, R. H., MILLER, R. E., de FREMERY, D., KNUCHLES, B. E., BICKOFF, E. M. and KOHLER, G. O.: *J. Agric. Food Chem.*, **23**, 620 (1975).
- 8) KNUCHLES, B. E., BICKOFF, E. M. and KOHLER, G. O.: *J. Agric. Food Chem.*, **20**, 1055 (1972).
- 9) BRADY, C. J.: *Biochem. J.*, **78**, 631 (1961).
- 10) BETSCHAT, A. A. and KINSELLA, J. E.: *J. Agric. Food Chem.*, **22**, 116 (1974).

(Received Apr. 11, 1985)

アルファルファ、ホワイトクローバー、イタリアン
ライグラス葉搾汁中の蛋白質の溶解性と安定性

スタニスワフ ギワズダ*・野口明徳・斎尾恭子**

(* ワルシャワ農業大学, ** 食品総合研究所)

プロキサン第Ⅱ方式では、葉搾汁をまず60°Cまで加熱、冷却、遠心分離し、その上澄を80~85°Cまで加熱して、葉蛋白を沈澱、ホワイト濃縮葉蛋白を調製する。本報はこの調製法に関連し、アルファルファ、ホワイトク

ローバー、イタリアンライグラスから葉搾汁並びに60°C加熱後の上澄を調製し、その中の蛋白質の溶解性と安定性を調べた。三種の葉搾汁蛋白のpH溶解プロファイルは大きな差はないが、上澄液では若干認められる。葉搾汁また加熱後の上澄中の蛋白質は壊れ易く、貯蔵中のpH、温度に関連しながら減少し、最終濃縮葉蛋白の収率に影響する。これら結果より、濃縮葉蛋白の調製に当って、使用する草種および貯蔵中の温度やpHに細心の注意を払う必要があることが示唆される。