Yield and Nutritive Value of LPC Separated From Italian Ryegrass Juice Stored for One or Two Days

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Abstract Heat processing of plant juice is the most popular method to coagulate LPC but consumes energy. Alternative to the heat-coagulation method, fermentative coagulation of LPC has been proposed. But we have reported that recovery of protein in LPC was reduced by about 50% during 7 days' anaerobic fermentation of green juice. During the experiment, it was observed that the coagulation of LPC began within 1 day of the storage. So, in the present study, LPC was removed from Italian ryegrass juice stored for 1 and 2 days in sealed bottles. The juice acidified to pH 4 was also prepared and stored as well as the unacidified juice because most of the proteolysis during storage of plant juice can be attributed to the activity of plant enzyme which can be depressed by lowering the pH of the juice. Even when the juice was anaerobically kept only for 1 day, 20 and 27% of dry matter and crude protein, respectively, were lost from LPC. But when the juice was treated with HCl before the storage, the losses were reduced to about a half of those observed in the untreated juice. There was no obvious difference in the yield of LPC between the juices stored for 1 and 2 days. Diets containing 10% crude protein provided from one of the freeze-dried LPCs and methionine were prepared and restrictedly fed to rats weighing about 50 g for 10 days. Body weight gain, apparent digestibility of crude protein, nitrogen retention and the ratio of urinary nitrogen to digested nitrogen were determined by the rat trial. All the values were deteriorated by the storage. But the nutritive value of LPC separated from acidified juice stored for 1 day was not so low and significant reduction was only found in the nitrogen retention. While the digestibility of crude protein in LPC separated from the unacidified juice stored for 2 days was quite low compared with others. The lysine content of LPC reflected on the urinary nitrogen excretion of rats. The control LPCs were centrifugally separated from the acidified juice immediately after the acidification or after heating to 70°C with a gas burner. The heating process had no effects on both the yield and the nutritive value of LPC.

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Leaf protein concentrate (LPC) has ordinarily coagulated by thermal processing of plant juice making its cost too high to use as an animal feed. To reduce the cost, fermentative coagulation of LPC has been proposed by some workers¹⁻³⁾. But, in our previous studies^{4,5)}, it was found that the yield and the crude protein and the available lysine contents of LPC were reduced when green juice was subjected to anaerobic fermentation for 7 days without any other treatments. In the course of the studies,

it was observed that the coagulation of LPC began within 1 day. And in a preliminary study, adjustment of Italian ryegrass juice to pH 4 with HCl was found to be useful to coagulate LPC within 1 day without heating. From the facts, it was considered that 1 day's anaerobic preservation of green juice might be available to coagulate LPC, if the yield and the nutritive value of the LPC were comparable to those of thermally coagulated LPC. If some reductions were observed within 1 day, it could be attributed to plant enzyme actions which might be suppressed by acidifying the fresh juice to pH 4, because the optimum pH range for proteolytic plant enzyme is 5.0–6.0⁶⁾.

The present study has been made to prove the suppositions mentioned above. As the material crop, Italian ryegrass was used, because it ensured higher annual yield of LPC at Kagawa compared with lucerne⁷⁾ and ryegrass LPC had higher nutritive value than lucerne LPC in chicks^{8,9)}. As the controls, LPC separated from the acidified juice immediately after the acidification or after heating to 70°C were used. Separation of LPC from acidified juice is possible without heating if such a high speed centrifuge as 15,000 rpm is available. But in plant scale production, the separation of LPC from green juice is made using screw decanters of comparatively low speed and coagulation of LPC before the separation is essential.

Materials and Methods

Italian ryegrass (*Lolium multiflorum*) grown in the experimental plots of Kagawa University and harvested on April 9th and May 8th, 1985 at the second and the third regrowth, respectively, was used. The stage of maturity, the yield of the crop and the method of green juice extraction are obvious in a previous paper⁴⁾.

Two thirds of green juice was adjusted to pH 4 with HCl and then subdivided into four equal portions. LPC was centrifugally separated from green juice at 15,000 rpm immediately after the acidification (HCl-0), after heating directly with a gas burner to 70°C (HCl-Heat) and after keeping 1 (HCl-1) and 2 days (HCl-2) in sealed bottles attached with fermentation traps at room temperature (17–27°C).

Remaining one third of the juice was immediately poured into two bottles, anaerobically fermented for 1 (Fer-1) and 2 days (Fer-2) by sealing the bottles as mentioned above and then subjected to the centrifugal separation of LPC.

Every LPC was freeze-dried, made to fine powder with a ball mill, packed into bottles, sealed with stoppers and stored in a refrigerator of 5°C.

Rat trials were made as described in an earlier publication¹⁰⁾ using diets containing 10% crude protein provided from one of the LPCs and methionine. Methionine was added 0.4% of the diets.

Amino acid analysis of LPCs was made on the hydrolysates of intact and performic acid treated samples using Hitachi KLA-5 amino acid analyser¹¹⁾. The nitrogen content of LPCs, diets and excreta was determined by the Kjeldahl method. The significance of the differences of the data was analysed by the Tukey's multiple range test.

Results and Discussion

Effect of coagulation methods on the yield of Italian ryegrass LPC is shown in Table 1.

Heating of grass juice adjusted to pH 4 was ineffective in improving the LPC yield suggesting no necessity of the heating if such a high speed decanter as 15,000 rpm is available. But in practical process, it is difficult to use the high speed decanter and some additional treatments are required to coagulate LPC.

Whether the juice was adjusted to pH 4 or not, LPC was coagulated during the 1 day's storage with some reductions of dry matter (DM) and crude protein (CP) recoveries in it. The rate of the reduction was greater in Fer-1-LPC than in HCl-1-LPC. DM and CP recovered in HCl-1-LPC were 91 and 87%, respectively, of those recovered in LPCs separated from the juice on the day of extraction. They were 80 and 73% in Fer-1-LPC. These facts suggest that acidification of plant juice is effective in reducing the losses of LPC during the storage by more than 10%, but can not eliminate any losses of LPC. Further 1 day's storage had little effects on both

Table 1. Effect of coagulation methods on the yield of Italian ryegrass LPC¹

Coagulation method	Abbreviation	Dry matter distributed in LPC (%) ²	Crude protein content of LPC (%)	Crude protein distributed in LPC (%) ²
Adjusted to pH 4	HCl-0-LPC	100	55.4	100
and heated to 70°C	HCl-Heat-LPC	100	55.5	100
and left for 1 day	HCl-1-LPC	91	52.7	87
and left for 2 days	HCl-2-LPC	88	52.2	83
Fermented for 1 day	Fer-1-LPC	80	50.7	73
Fermented for 2 days	Fer-2-LPC	80	48.9	71

¹. Mean of LPCs prepared from 2nd and 3rd regrowth on April 9th and May 8th, 1985.

². Expressed as percent of the highest value.

Table 2. Effect of coagulation methods on the nutritive value of Italian ryegrass LPC in growing rats

Protein source ¹	Body weight gain (g/10 days)	Apparent digestibility of protein (%)	Nitrogen retention (%)	Urinary N/Digested N (%)
HCl-0-LPC	31.5 ^{b2}	81.4 ^d	64.2 ^d	21.7 ^{a b}
HCl-Heat-LPC	32.1 ^b	80.5 ^{c d}	63.4 ^d	21.3 ^a
HCl-1-LPC	30.9 ^{a b}	79.8 ^c	61.9 ^c	23.2 ^{b c}
HCl-2-LPC	29.8 ^{a b}	78.1 ^b	58.2 ^b	25.0 ^d
Fer-1-LPC	28.6 ^a	78.1 ^b	58.0 ^b	25.7 ^d
Fer-2-LPC	28.7 ^a	74.7 ^a	56.5 ^a	24.4 ^{c d}
Pooled standard error	0.61	0.21	0.32	0.40

¹. See Table 1. Each diet was supplemented with 0.4% of methionine. ². Means of 4 rats. Those with different superscript letters are significantly different at 5% level.

LPC Separated from Stored Grass Juice

Table 3. Effect of coagulation methods on the lysine and sulfur amino acid contents (g/16 gN) of Italian ryegrass LPC

	Coagulation method ¹					
	HCl-0	HCl-Heat	HCl-1	HCl-2	Fer-1	Fer-2
Lysine	6.71	6.68	6.32	6.28	6.19	6.26
Methionine	2.47	2.49	2.48	2.44	2.55	2.49
Cystine	0.90	0.89	0.94	0.96	0.94	0.96

¹. See Table 1.

DM and CP recoveries in LPC. But it was reported in a previous study⁴⁾ using 5 different cuts of Italian ryegrass that only 62-74 and 51-59% of DM and CP, respectively, of HCl-Heat-LPC were recovered in LPCs separated from the juices fermented for 7 days.

The result of the feeding trial is shown in Table 2. No significant differences were observed between the nutritive values of HCl-0- and HCl-Heat-LPCs suggesting that the nutritive quality of LPC was not affected by the direct heating of green juice with the gas burner.

During the storage of green juice, some reductions of the nutritive value of LPC have occurred but the rate of the reduction in apparent digestibility of crude protein (AD) and nitrogen retention (NR) was decreased by the acidification. By prolonging the storage period from 1 to 2 days, the nutritive value of LPC was further reduced. That is, NR of rats fed on HCl-2-LPC was similar to that of Fer-1-LPC, significantly higher than that of Fer-2-LPC and significantly lower than that of HCl-1-LPC. AD showed similar tendency to NR and was the lowest in Fer-2-LPC which corresponded to the value obtained in the LPC separated from ryegrass juice fermented for 7 days⁵⁾.

Reflecting these differences, the body weight gain of rats fed on HCl-0- and HCl-Heat-LPCs was significantly higher than that of rats fed on LPCs coagulated by fermentation. The value of rats fed on LPCs separated from stored acidified juice was between them.

Being able to suppose from the lysine content of LPCs shown in Table 3, the rate of urinary nitrogen excretion to digested nitrogen was greater in rats fed on LPCs prepared from stored juice than in those fed on LPCs prepared on the day of extraction. The reduction of lysine content of LPC during anaerobic fermentation of plant juice was already reported by the authors⁴⁾ and AMEENUDDIN *et al.*¹²⁾. But the rate of the reduction was far less in the present study because of the shorter period of storage. The acidification was not effective in preventing the reduction of the lysine content during the storage. Although there were no significant differences, in spite of being kept for the longer period with fermented juice, Fer-2-LPC contained more lysine than Fer-1-LPC and consequently the rats fed on the former showed less ratio of urinary nitrogen to digested nitrogen than fed on the latter. There might be some effects of microbial protein. Some workers^{2,13)} reported on the increase of sulfur amino acid contents of LPC during the fermentation of plant juice because of the

contamination of microbial protein, but it was not obvious in the present study.

From the above, it can be concluded that the best method among the procedures used in the present study for coagulating LPC without heating is to acidify the plant juice to pH 4 with HCl and separate LPC as early as possible when coagulation of it is observed. Even in that case, not only the yield of LPC, but also the nutritive value of it is reduced. Therefore, whether to choose the thermal coagulation or natural coagulation of LPC depends on the difference between the costs expended on heating (A) and loss by the declining yield (B). If (A)-(B) were a positive value, storage of the acidified juice should be chosen. Acidification of the juice is essential to depress the breakdown of LPC caused by plant enzyme. AJIBORA *et al.*⁹⁾ recommended the addition of culture of lactic acid bacteria to fasten the pH reduction and reduce the loss of LPC during the fermentation of plant juice. But it is obvious that the addition of HCl is more sure method to reduce the pH value of plant juice than the addition of inoculant. The cost of HCl is negligible. But the acidification of plant juice is not applicable to some species of lucerne because accumulation of medicagenic acid in LPC occurs by it¹⁴⁾.

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1 または 2 日間貯蔵したイタリアンライグラス緑汁液から 分離した LPC の収量と栄養価

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LPC は通常, 加熱凝固法により緑汁液から分離されるが, 加熱に多大の燃費を要する。緑汁液を嫌気状態に保つことによっても LPC は分離するが, 1 週間の貯蔵中に約 50% の蛋白質が LPC から失われることを前報で報告した。しかしその際, 貯蔵 1 日目ですでに LPC の凝固が観察されたので, 今回は, イタリアンライグラス緑汁液を 1 および 2 日間貯蔵したのち LPC を回収した。また, 貯蔵中の蛋白質分解の多くは植物酵素活性に帰するところから, それを抑制する目的で塩酸により pH 4 に調整した緑汁液も同様に貯蔵した。

緑汁液を 1 日嫌気条件下に保つ間に, 20 および 27% の乾物および蛋白質が LPC から失われた。しかし貯蔵前に塩酸処理することにより, 上記の損失は約 1/2 にとどまった。さらに 1 日貯蔵しても, LPC 収量に対する大

きな影響はなかった。

凍結乾燥 LPC とメチオニンを単一 N 源とする粗蛋白質含量 10% の飼料を, 体重約 50 g のラットに 10 日間制限給与した。体重, 蛋白消化率, 尿中 N 排泄量および N 保留のいずれの測定項目も, 緑汁液の貯蔵中に低下した。しかし塩酸処理 1 日貯蔵の場合の低下はわずかで, N 保留にのみ有意差が認められた。無処理 2 日間貯蔵の際の消化率の低下は, とくに著しかった。尿中 N 排泄量の増加と, LPC のリジン含量の低下はよく一致した。

なお, 対照として, 緑汁液を pH 4 に調整後ただちに直火で 70°C に加熱あるいは加熱することなく直接高速遠心により分離した 2 種類の LPC を用いたが, 両者の収量および栄養価に差は認められなかった。

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