CHEMICAL AND PHYSICAL PROPERTIES OF LIPIDS AND PROTEINS FROM Amaranthus cruentus SEED)【アマランタス・クルエンタス種子の脂質及びタンパク質の化学及び物理的特性に関する研究】

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論 文 内 容 要 旨

INTRODUCTION

Amaranth was an important food among Aztecs in Mexico and other cultures in Central America about 500 years ago (Ortíz de Montellano, 1978). Recently, amaranth seeds, which are still consumed and cultivated as a minor grain crop in Mexico and various other countries (Bestschart, 1985; Sánchez-Marroquín, 1983) have attracted much attention because of its exceptionally high lysine content, the limiting essential amino acid in corn-eating countries, and its uncommon high biological value as a vegetable protein. In Mexico, some efforts have been started for industrialized application of the amaranth crop (Soriano et al., 1987). However, research on amaranth seeds from both physico-chemical and nutritional stand points is limited. Investigations on *Amaranthus cruentus* seed oil are particularly scanty. For instance, it was reported that the major constituent fatty acids were oleic and linoleic acids, and that the fatty acid profile was similar to corn (Beadle et al., 1965; Sánchez-Marroquín, 1983; Weber, 1978). However, the knowledge about glycolipids and phospholipids, which are known to be very important for food processing such as baking, was very poor (Opute, 1979; Singhal and Kulkarni, 1988; Stoller and Weber, 1984; Teutonico and Knorr, 1985; Tudor and Bean, 1984; Tudor and Bean, 1985). With regard to the digestibility and nutritional value of amaranth seed crude oil, only one paper has been published (Garcia et al., 1987).

In Mexico mainly the traditionally consumed candies made of popped amaranth seed and meals from raw amaranth seed have empiricaly observed a very poor stability to oxidation and its shelf-life is short compared to other meals of conventional cereals. However, no basic information is available related to which cause this labile stability to oxidation.

Amaranth grains contain about 12~16% of crude protein which has a good balance of amino-acids and high lysine content compared with other cereals (Becker et al., 1981; NRC, 1984; Sánchez-Marroquín, 1983; Teutonico and Knorr, 1985). Leucine has been reported as the limiting amino-acid in amaranth protein grains (Becker et al., 1981).

Several reports have been published about the exceptional nutritional quality similar to milk-casein of amaranth grains protein (Singhal and Kulkarni, 1988). Nevertheless, detailed researches on isolation and characterization of amaranth grain proteins are scanty. In addition, the knowledge on amino-acid composition of each protein fraction of albumin, globulin, prolamin and glutelin from amaranth grains nowadays is limited (Abdi and Sahib, 1976; Bressani and García-Vela, 1990; Duarte, et al., 1986; Konishi et al., 1985). Up to the moment, only Konishi and co-workers have tried of elucidating the chemical structure of globulin isolated from *Amaranthus hypochondriacus*

(Konishi et al., 1985).

Therefore, this investigation was designed to obtain a more complete information on identification and quantification of whole lipid classes in *Amaranthus cruentus* (Mexican grain type) together with tocopherol content and oxidative stability of amaranth seed oil. On the other hand, this study was also undertaken to elucidate mainly the chemical rather that physical properties of both albumin and globulin from amaranth seed. This knowledge on lipids and proteins from amaranth would be required for a further application in the manufacturing of amaranth-based foodstuffs *e. g.* in Mexico or other developing countries where actually the search of new highly nutritive and cheaper protein sources have been intensified with regard of an enrichment of traditional diets of economically limited classes.

CHAPTER I. CHARACTERISTICS OF *Amaranthus cruentus* SEED OIL AND ITS OXIDATIVE STABILITY

The results of the present research (Table, 1~4) indicate that *Amaranthus cruentus* oil has a close similarity with the chemical composition of corn oil but both low tocopherol content and the occurrence of linolenic acid may contribute to its labile oxidative stability. Because of the very small size of amaranth seeds, approximately 1000-3000 grains per g, it is difficult to fractionate amaranth seeds into germ and bran, in which the lipid content and composition are entirely different. This work was performed to get a better knowledge of *Amaranthus cruentus* seed lipids, and its oxidative stability, which are important in order to consider the nutritional value and shelf-life of amaranth-based foodstuffs.

CHAPTER II. EXTRACTION AND SOLUBILITY OF PROTEINS FROM *Amaranthus cruentus* SEED

It was found a similar efficiency of protein extraction by using either NaCl or Na2SO4 at a concentration of 5% (Table 5). For this reason, sodium sulfate was used for isolating proteins from *A. cruentus* seed following the method of Padhye and Salunke (1977). Albumin was found as the main storage protein into amaranth seed used in this study. However, prolamin and glutelin fraction could not be entirely estimated because remained into protein residue(Table 6). It might be considered as an alternative method of complete extraction of proteins from amaranth seed, the extraction of saline proteins by means of Padhye and Salunke (1977) method and the method of Landry and Moureaux (1970) for isolation of prolamin and glutein fractions. In general terms, the solubility for albumin fraction

and saline soluble proteins fraction was very poor in either water or 1M NaCl. At a concentration of 0.4M NaCl globulin fraction showed to have a very good solubilization near to 100% at pH 9 (Fig. 1). The bad gel formation as a consequence of its poor solubility might complicate the direct application of amaranth protein isolate in amaranth-based beverages.

Chapter III. AMINO-ACID PROFILE OF *Amaranthus cruentus* SEED PROTEIN FRACTIONS

Whole protein from raw *Amaranthus cruentus* seed was limiting in leucine. Although threonine and valine were also detected in low amount when were compared with the FAO/WHO essential amino-acid reference profile through its respective chemical score. Amino-acid composition of albumin, globulin and glutelin showed to be very similar among them (Table 7). Albumin showed to content the highest value of lysine.

Prolamin had surprisedly a peculiar amino-acid profile. With the exception of its lysine content all other essential amino-acids were presents in superior amount than the FAO/WHO pattern. Contrarily to glutelin which had the lowest levels of essential amino-acids.

It would be possible enhanced by means of genetic engineering, the nutritional value of protein from amaranth increasing prolamin levels into the amaranth kernel.

After popping the seed the most limiting amino-acid observed was lysine (Table 8) followed by sulfur amino-acids and threonine in that decreasing order. The cysteic peak did not appear in the heat treated amaranth chromatogram but four unknown peaks were observed at the very end or run.

Neither loss nor racemization was observed in tryptophan content into amaranth popped seed. Methionine, phenylalanine, alanine, glutamic acid, and aspartic acid were partially racemized. This is important to consider because humans being can not utilize D-enantiomers.

CHAPTER IV. CHARACTERIZATION OF ALBUMIN AND GLOBULIN FROM *Amaranthus cruentus* SEED

Albumin from amaranth seed comprised the called albumin fraction-I, -II, and -III as were obtained by gel filtration. Each fraction revealed an entirely different polypeptide composition according to its electrophoretic analysis. Albumins showed a strong affinity to an anion exchange column (DE-52 Whatman). Complete elution of each albumin was achieved at 1M NaCl by using a 2M NaCl gradient elution system. Albumin is an oligomeric protein which consist of four main different polypeptides (Fig. 2, 3).

Globulin was separated in two fractions called globulin fraction-I and -II by gel filtration. Globulin fraction-I contains

three polypeptides of LMW and two VLMW polypeptides. On the other hand, globulin fraction-II contained only a VLMW polypeptides. The affinity of globulin fraction-I and -II on DE-52 cellulose column was weaker than that observed for albumin fractions (Fig. 4, 5).

Albumin fraction-I had a molecular weight similar to catalase and albumin fraction-II and -III both near to molecular weight of aldolase. Globulin fraction-I was similar to ferritin and globulin fraction-II to aldolase (Table 9).

From crude albumin fraction four LMW polypeptides were determined and three more polypeptides of VLMW. Crude globulin fraction was integrated for five LMW polypeptides and four polypeptides of VLMW (Table 10).

In the case of albumins had a more evident tendency to aggregate polypeptides than globulin according to their respective electrophoretic patterns with ME treatment or without it (Fig. 6).

The trypsin inhibitor activities from albumin or globulin fractions (Table 11) were stronger than corn or wheat but considerably less than raw soybean. On the other hand, crude albumin and globulin fractions were positive to PAS stain reaction (Fig. 7).

CONCLUSIONS

1. *Amaranthus cruentus* oil has a close similarity with the chemical composition of corn oil, but both low tocopherol content and the occurrence of linoleic acid may contribute to its labile oxidative stability.

2. The amount of polar lipids in amaranth seed oil was considerably low. The primary factor responsible for breadmaking quality should be proteins. However, the lack of polar lipids in amaranth seed may be an additional factor in the poor breadmaking quality of amaranth flour.

3. β-tocopherol was found as the main component into oil seed. This is unusual for vegetables oils. However, the tocopherol content was less than wheat germ, cottonseed, soybean, or whole corn kernel. That might explain, besides other factors, the poor oxidative stability empirically observed for processed foods based on amaranth in Mexico.

4. Some antioxidant activity was observed in oil extracted from 3 min steamed seed. Similar behavior was not detected in oil extracted from popped or boiled amaranth seed, probably because the antioxidative activity was lost by the severe thermical treatment.

5. It was found a similar efficiency of protein extraction by

using either NaCl or Na2SO4 at a concentration of 5%. For this reason, sodium sulfate was used for isolating proteins from *Amaranthus cruentus* seed.

6. In general terms the major storage proteins of legumes and other dicotyledonous plants are globulins and those of monocotyledonous plants are prolamins and glutelins. An interesting exception is oats, in which the major storage protein is a globulin. Similarly, amaranth is other exception for pseudo-cereal plants, because albumin is the main storage protein.

7. Saline soluble proteins from amaranth could be entirely quantified. Nevertheless, prolamin and glutelin could not be exactly estimated because remain into protein residue when the method of Padhye and Salunke is followed. It might be considered as an alternative method of complete extraction of proteins from amaranth seed, the extraction of saline soluble proteins by Padhye and Salunke method and the method of Landry and Moureaux for isolation of prolamin and glutelin fractions.

8. Solubility for albumin fraction and saline soluble proteins fraction was very poor in either water or 1M NaCl. Moreover, the bad gel formation as a consequence of its poor solubility might complicate the direct application of amaranth protein isolate in amaranth-based beverages.

9. Whole protein from raw *Amaranthus cruentus* seed was limiting in leucine followed by threonine and valine, when was compared with the FAO/WHO essential amino-acid reference profile through its respective chemical score.

10. Prolamin had surprisedly a peculiar amino-acid profile. With the exception of its lysine content all other essential amino-acids were presents in superior levels than the FAO/WHO pattern. It would be possible enhanced by means of genetic engineering, the nutritional value of protein from amaranth increasing prolamin levels into the amaranth kernel.

11. After popping the seed the most limiting amino-acid observed was lysine followed by sulfur amino-acids and threonine in that decreasing order.

12. The cysteic peak did not appear in the heat treated amaranth chromatogram but four unknown peaks were observed at the very end of run.

13. Neither loss nor racemization was observed in tryptophan

content into amaranth popped seed. Methionine, phenylalanine, alanine, glutamic acid, and aspartic acid were partially racemized. This is important to consider because humans being can not utilize D-enantiomers.

14. Albumin from amaranth seed comprised the called albumin fraction-I, -II, and -III as were obtained by gel filtration. Each fraction revealed an entirely different polypeptide composition according to its electrophoretic analysis. Albumin is an oligomeric protein which consist of four main different polypeptides.

15. Albumins showed a strong affinity to an anion exchange column. Complete elution of each albumin was achieved at 1M NaCl by using a 2M NaCl gradient elution system.

16. Globulin was separated in two fraction called globulin fraction-I and -II by gel filtration. Globulin fraction-I contains three polypeptides of LMW and two VLMW polypeptides. On the other hand, globulin fraction-II contained only a VLMW polypeptides.

17. Albumin fraction-I had a molecular weight similar to catalase and both albumin fraction-II and -III near to molecular weight to aldolase. Globulin fraction-I was similar to ferritin and globulin fraction II to aldolase.

18. In the case of albumins had a more evident tendency to aggregate polypeptides than globulin according to their respective electrophoretic patterns with ME treatment or without it.

19. The trypsin inhibitor activities from albumin or globulin fractions were stronger than corn or wheat but considerably less than raw soybean.

Table 1-Chemical analysis of amaranth seed and seed oil

Analysis	
Flour moisture (%)	10.1±0.1*
Ether extract (dry wt.%)	7.2±0.1
Acid value* (mgKOH/g)	6.2±0.1
Saponification value (mgKOH/g)	202.0±1.6
Unsaponifiable residue*	8.0±0.2
Iodine value	119.0±0.4

*Data are means±SD of triplicate determinations.
*Free fatty acids calculated as oleic acid.
*% in ether extract.

Table 2-Lipid composition of amaranth seed oil

Lipid class	% of total lipid	%
Neutral lipids	(92.4)	
Hydrocarbons	3.2	3.5±0.3*
Steryl esters	2.6	2.8±0.4
Triglycerides	86.6	93.7±0.6
Glycolipids	(2.6)	
ASG	0.5	19.2±0.1
MGDG	0.3	11.5±0.3
SG	0.7	27.0±0.0
CMH	0.3	11.5±0.4
DGDG	0.2	7.7±0.2
SQDG	0.2	7.7±0.1
TGDG	0.4	15.4±0.1
Phospholipids	(5.0)	
PG	0.3	6.0±0.4
DPG	0.4	8.0±0.8
PE	0.8	16.0±1.2
LPE	0.1	2.0±0.3
PI	0.5	10.0±0.4
PC	1.3	26.0±0.4
LPC	0.4	8.0±0.1
$UP_{1,2,3}$*	1.2	24.0

*Data are means±SD of triplicate determinations.
*UP means unidentified phospholipids.

Table 3-Fatty acid composition of each lipid class in amaranth seed oil

Lipid class	Fatty acid (wt %)							
	14:0*	16:0	16:1	18:0	18:1	18:2	18:3	20:0
Total lipids	0.1	19.0	0.7	3.0	25.0	43.0	2.0	7.2
Neutral lipids								
Steryl esters	---	15.0	8.3	4.3	23.5	42.5	---	6.4
Triglycerides	0.1	20.9	0.7	3.1	26.0	47.7	1.3	0.2
Glycolipids								
ASG	---	19.1	0.7	3.0	36.8	39.9	0.5	---
MGDG	---	14.9	1.3	0.9	23.3	59.2	0.5	---
SG	---	23.6	T	1.8	34.2	39.3	1.2	---
CMH	---	16.3	2.4	3.7	37.3	39.0	1.3	---
DGDG	---	16.1	1.1	0.7	38.0	42.1	2.0	---
SQDG	---	13.7	1.3	0.9	33.1	47.0	4.0	---
TGDG	---	35.2	1.2	3.3	26.2	34.1	T	---
Phospholipids								
DPG	---	23.2	0.9	0.8	35.8	39.3	T	---
PE	---	16.9	0.5	1.9	31.5	48.8	0.4	---
PI	---	16.7	0.4	0.6	34.1	46.8	1.4	---
PC	---	15.9	0.3	0.9	36.9	45.9	0.1	---
LPC	---	20.0	1.4	3.0	40.2	35.1	0.3	---

* The number indicates chain length and double bounds of fatty acid.
T Means trace amount (below 0.1%).

Table 4-Tocopherol composition of amaranth seed oil

Tocopherols	μg/g ether extract	%
ALFA	34.8±0.1*	14.7
BETA	101.4±0.3	43.0
GAMMA	14.7±0.0	6.2
DELTA	85.1±0.3	36.1
TOTAL	236.0	

*Data are means±SD of triplicate determinations.

Table 5-Efficiency of extraction of saline soluble amaranth proteins with different salt solutions

Salt solution	SSN[1]	PN[2]	NPN[3]	EPE[4]
	g/100g	g/100g	g/100g	%
5% NaCl	9.9±0.6	9.3±0.0	0.6	50.5
10% NaCl	10.5±0.0	8.0±0.7	2.5	43.5
5% Na_2SO_4	10.5±0.0	9.3±0.1	1.2	50.5
10% Na_2SO_4	10.5±0.0	8.6±0.6	1.9	46.7

[1] Saline soluble nitrogen = Protein nitrogen (albumin and globulin) + non-protein nitrogen.

[2] Protein nitrogen = Nitrogen precipitated with trichloroacetic acid.

[3] Non-protein nitrogen= TN - PN.

[4] Efficiency of protein extraction = 100 x PN/ protein content of amaranth defatted flour (18.4±0.5%).

Table 6

Protein yield of fractionation in two species of amaranth seed compared with different methods of protein fractionation

Protein fraction	A. cruentus		A. hypochondriacus		
	%	%	%	%	%
Fractionation method	A'	B''	C'	D''	E'''
Albumin	33.0	20.0	65.0	-	62.4
Globulin	17.5	19.1	17.0	-	17.5
Prolamin	6.9	2.8	11.0	1.0 - 1.6	9.2
Glutelin	1.7	46.3	7.0	22.4 - 29.4	10.9
NPN*	6.5	14.0	---	3:7[b]	---
Residue	34.4	13.4	---	9.0 - 13.0	---
N Recovery	94.6	--	---	90.0 - 105.0	---
Total N (g/100g defatted meal)	18.4	15.7	---	25.0 - 28.0	---
G/A ratio	0.5	0.9	0.3	2.1	0.3

(A) present study, (B) Bressani and Garcia-Vela (1990), (C) Duarte et al. (1986), (D) Konishi et al. (1985) and (E) Abdl and Sahib (1976). ' Method of Padhye and Salunke (1977). '' Method of Landry and Moureaux (1970). ''' Method of Mitchel (1948). * Non-Protein Nitrogen, [b] Ratio of NPN to Albumin-Globulin Nitrogen.

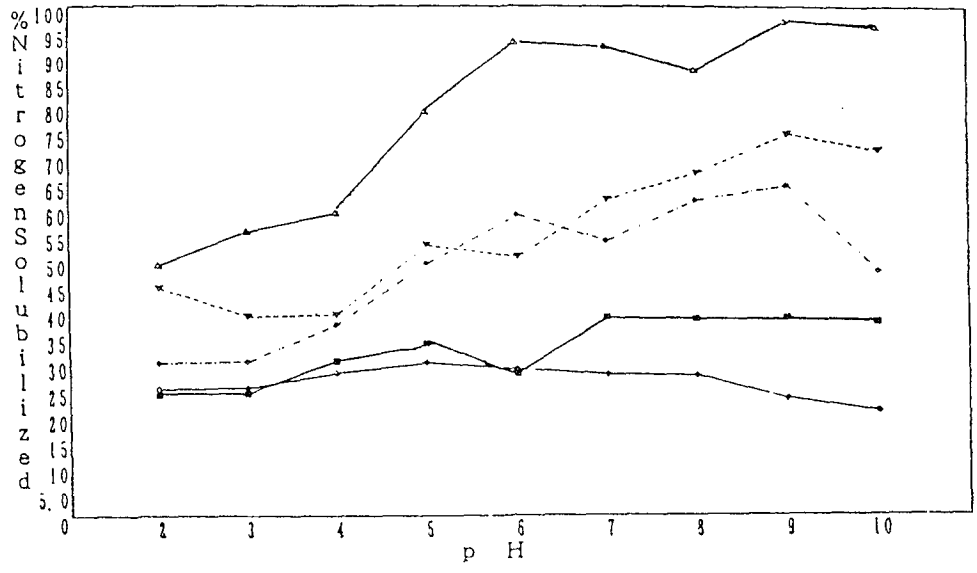

Fig.1

.-pH solubility profiles of saline soluble proteins from amaranth seed
Δ 1% (w/v) Crude Globulin in ionic strength of 0.4
▼ 1% (w/v) Crude water soluble protein extract in ionic strength of 0.4
♦ 1% (w/v) Crude water soluble protein extract in ionic strength of 1.0
■ 1% (w/v) Crude Albumin in ionic strength of 0.4
◇ 1% (w/v) Crude Albumin in water

Table 7

·Amino-acids composition of whole amaranth seed protein and its fractions

Amino-acid	Whole protein	Albumin	Globulin	Prolamin	Glutelin	FAO/WHO*
			g/100g protein			
Asp	8.8	7.2	7.3	9.0	8.8	-
Thr	3.7	4.3	3.2	5.8	3.8	4.0
Ser	6.7	5.6	5.8	8.5	6.1	-
Glu	17.2	20.1	20.0	10.2	20.9	-
Pro	4.5	4.3	5.9	4.8	1.8	-
Gly	8.4	13.1	9.6	16.0	12.1	-
Ala	4.0	6.1	5.0	7.5	7.2	-
Cys	3.5	3.7	3.5	3.0	1.0	-
Val	4.4	4.6	4.4	6.2	4.7	5.0
Met	2.2	2.1	3.4	1.4	3.1	3.5[b]
Ile	3.9	3.2	3.5	4.1	3.2	4.0
Leu	6.0	4.6	5.4	7.8	4.8	7.0
Tyr	3.8	2.5	2.4	4.3	3.5	6.0[c]
Phe	4.3	3.0	4.5	3.0	3.4	-
His	3.0	1.5	2.2	2.3	2.3	-
Lys	6.0	6.6	4.7	2.7	4.7	5.4
Arg	8.1	7.5	9.2	3.3	8.6	-
Trp	1.5	n.d.	n.d.	n.d.	n.d.	1.0

* Essential amino-acids reference pattern.
[b] Total sulfur amino-acids (methionine + cystine).
[c] Total aromatic amino-acids (phenylalanine + tyrosine).

Table 8

-Amino-acid composition of raw and popped amaranth by the direct contact method including their chemical score and net racemization[a].

Amino-acid	Amino-acid content		Chemical score[b]		Net racemization
	Raw	Popped	Raw	Popped	Popped
	g/16gN		%		%
Asp	8.8	9.0			23.9
Thr	3.7	3.8	92.5	95.0	0.0
Ser	6.7	6.5			0.0
Glu	17.2	19.3			3.1
Pro	4.5	4.6			
Gly	8.4	8.7			
Ala	4.0	4.4			0.7
Val	4.4	4.8	88.0	96.0	0.4
Met+Cys	5.7	2.9	162.9	82.9	6.4
Ile	3.9	4.3	98.0	108.0	
Leu	6.0	6.7	86.0	96.0	0.0
Phe+Tyr[c]	8.1	8.9	135.0	148.0	0.4
His	3.0	2.7			
Lys	6.0	3.0	111.0	56.0	0.0
Arg	8.1	8.0			
Trp	1.5	1.6	150.0	160.0	
Z_1		0.1[d]			
Z_2		0.3			
Z_3		0.3			
Z_4		0.1			

[a] Net racemization = [D/D+L x100) minus the racemization found in the raw seed due to the 6M HCl corresponding hydrolysates of digestion. D and L refer to the amino-acid enantiomers.

[b] Chemical score = $\frac{\text{amount of essential a.a. into the sample X 100}}{\text{amount of essential a.a. from FAO/WHO pattern}}$

[c] Racemization value refers only to phenylalanine.

[d] The four unknown peaks were calculated with the Leu color constant.

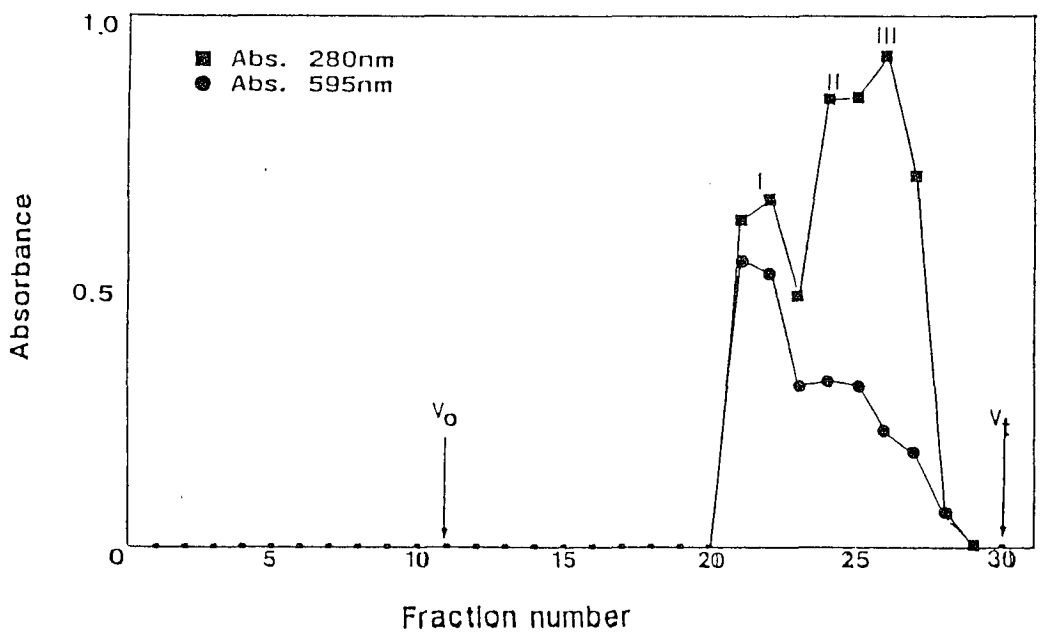

Fig 2.-Sepharose 6B gel chromatogram of albumin from A. cruentus. (I) fraction-I, (II) fraction-II, (III) fraction-III, (V_o) void volume, and (V_t) total volume.

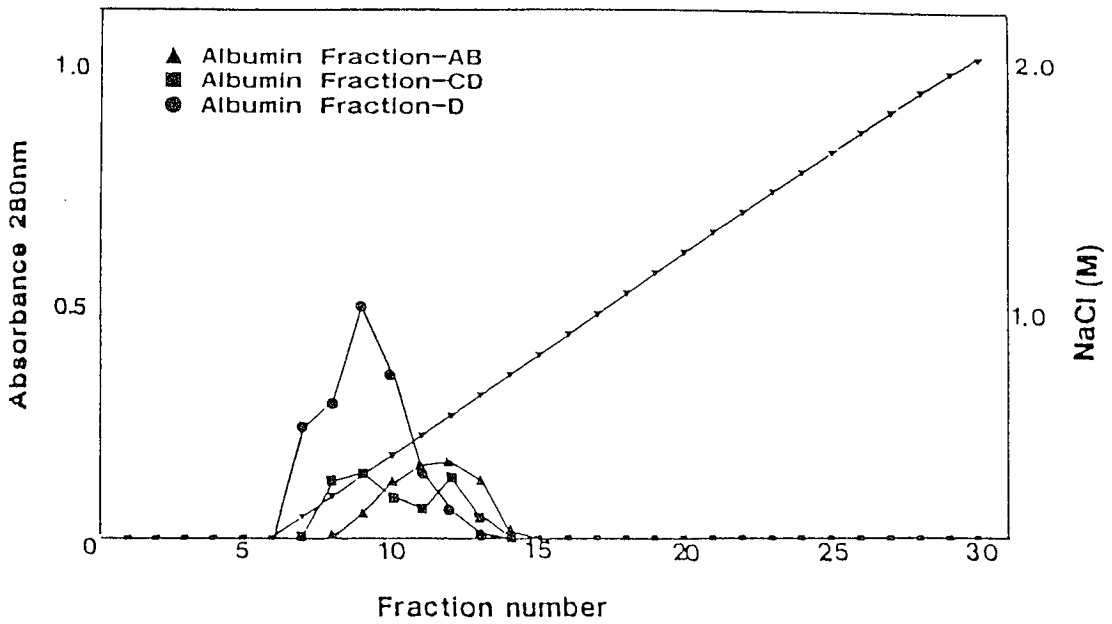

Fig.3 .-DE-52 cellulose column chromatogram elution profile of partially purified albumin from A. cruentus. Fraction-AB (fractions 21-23 in fig. 19); -CD (fractions 24-25) and D (fractions 26-28) were obtained by elution in Sepharose 6B column.

Table 9

Molecular weight of native albumin and globulin.

Protein fraction	K_{av}	Molecular weight
Albumins:		
Fraction-I	0.68	239 000
Fraction-II	0.75	190 000
Fraction-III	0.94	115 000
Globulins:		
Fraction-I	0.53	365 000
Fraction-II	0.78	181 000

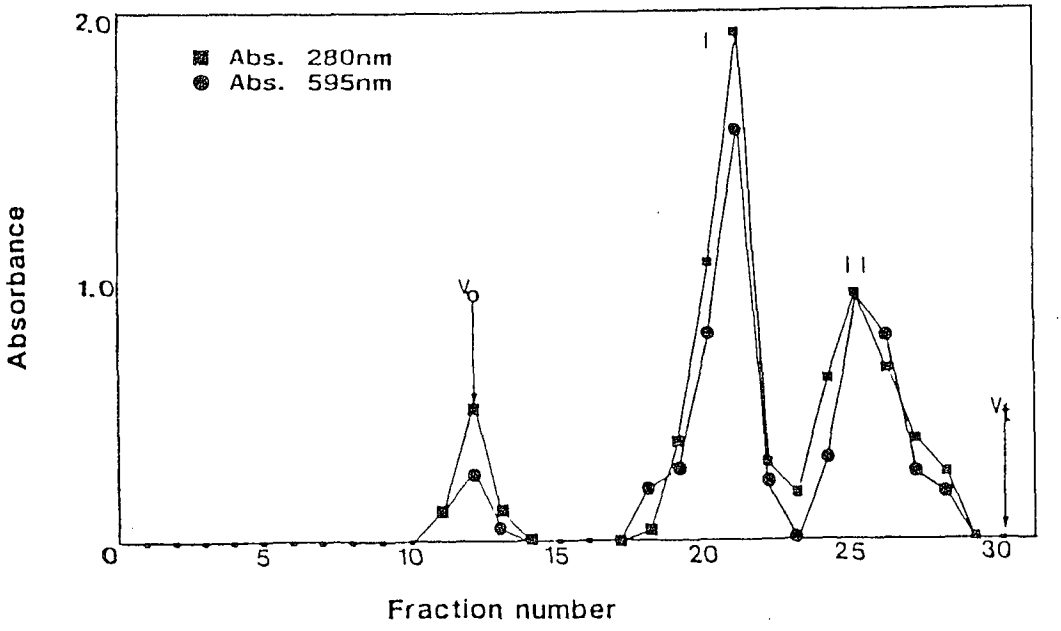

Fig.4 .-Sepharose 6B gel chromatogram of globulin from A. cruentus. (I) fraction-I, (II) fraction-II, (V_o) void volume, and (V_t) total volume.

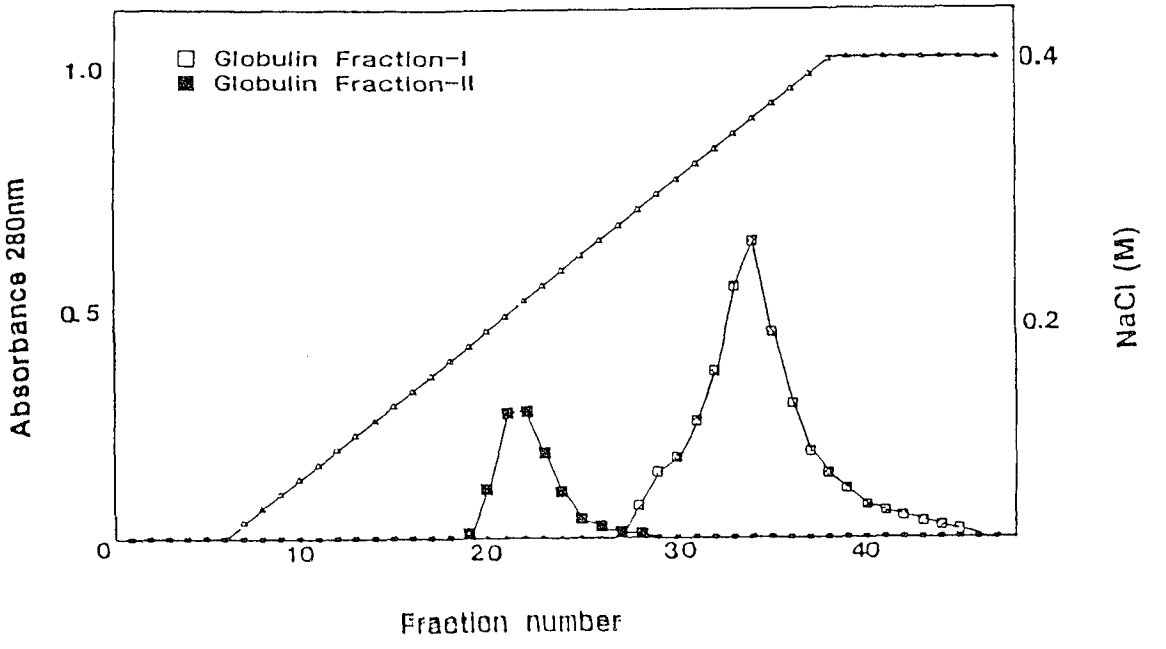

Fig.5 .-DE-52 cellulose chromatogram gradient elution profile of partially purified globulin from A. cruentus. Fraction-I and -II were obtained by elution in Sepharose 6B column..

Table 10

Molecular weight of detected polypeptides

Polypeptide	MW (daltons)	Polypeptide	MW (daltons)
Albumin:		Globulin:	
LMW		LMW	
α A	42 000	α G	59 000
β A	37 000	β G	56 000
γ A	35 000	γ G	50 000
δ A	31 000	δ G	42 000
VLMW		ε G	26 000
ε A	15 000	VLMW	
ζ A	2500-8400	ζ G	12 000
η A	1 800	η G	8 000
		θ G	5 600
		ι G	2 800

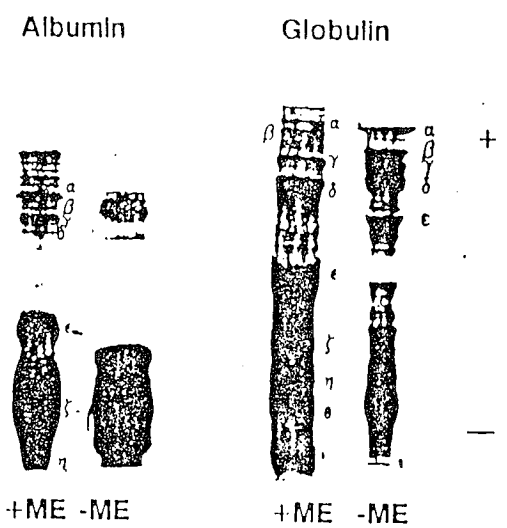

Fig. 6 Composed polypeptides of crude albumin and crude globulin. (+ME) mercaptoethanol treatment. (-ME) without mercaptoethanol treatment.

Table 11

Trypsin inhibitor activity of proteins from amaranth seed compared with other seeds

Seed	Protein fraction	TIU*/mg
A. cruentus	crude albumin	2.6±0.07
A. cruentus	crude globulin	1.2±0.09
Corn	crude enzymatic extract	0.4
Wheat	crude enzymatic extract	0.1
Soybean	crude enzymatic extract	90.0
A. hypochoncriacus	crude enzymatic extract	0.52
A. hypochondriacus	100-fold purified trypsin inhibitor	1200

* TIU means trypsin inhibitor unit. One TIU was arbitrarily defined as the amount of inhibitor which caused 10% inhibition of trypsin in 10 min under the described assay conditions.

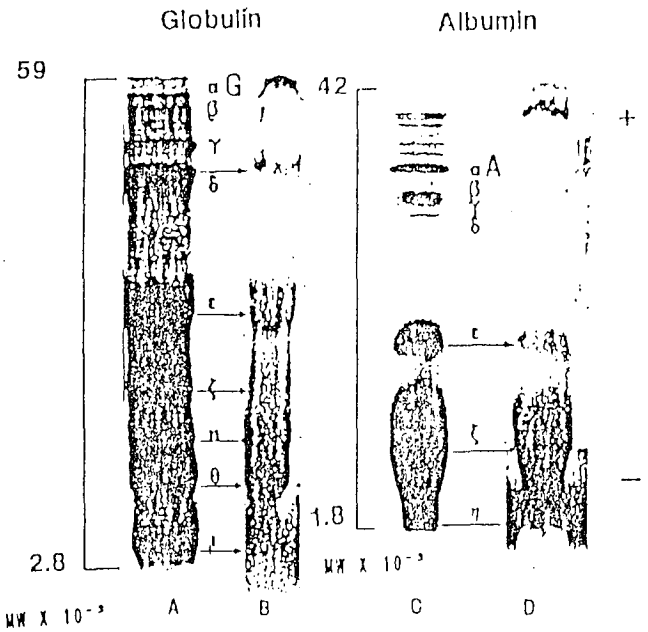

Fig.7 ..-Glycoprotein analysis. (A) globulin polypeptides, (B) globulin polypeptides positive to PAS stain reaction, (C) albumin polypeptides, (D) albumin polypeptides positive bands in SDS 20% polyacrylamide gel electrophoresis.

審査結果の要旨

アマランスはヒユ科の植物で，メキシコをはじめとするラテンアメリカ諸国では，重要な伝統的穀物であった。しかし，アマランスの食習慣が人身御供と深く結び付いていたことから，スペイン統括後は栽培が禁止され，ほとんど忘れられた存在となっていた。しかし，近年，アマランスのタンパク質にはリジン含量が高いことが示され，メキシコでの主食トウモロコシを補うには最適な作物であることがわかり，注目を集めている。しかし，現在の用途はポップして菓子に用いられているのみで，多様な食品への応用には，主要成分についての基礎的知見が必要である。そこで，本研究ではメキシコ産Amaramthus cruentus種子を用いて，ポップしたアマランスの酸化安定性が悪い原因の追及のために脂質成分を，また製粉後各種食品に加工した時の特性を知るためにタンパク質の組成および主要タンパク質の性質について検討した。

脂質については，総脂質の含量は乾物換算で７％と少なく，その90％はトリアシルグリセロールを主体とする中性脂質だった。糖脂質は10成分が同定され，主成分はスルホキノボシルジアシリグリセロールだった。リン脂質では，ホスファジルコリンが主成分で，10成分が存在した。

各脂質クラスを通して主要構成脂肪酸は18：２および18：１で，これは一般の穀物と共通していた。しかし，総トコフェロールは236μｇ/ｇと含量が低く，酸化安定性の劣る原因と思われたが，βが主成分という特異な組成を示した。

タンパク質の組成を調べると，アルブミンが主成分で，次いでグロブリン，プロラミンの順だった。一般に豆類など双子葉植物種子の主要貯蔵タンパク質はグロブリンとされており，本種子の組成は一般的でない。アルブミンは水への溶解性が悪く，これがアマランス分離タンパク質のゲル形性能が劣る原因となっていた。各タンパク質のアミノ酸組成は，プロラミンを除いて類似しており，第一次制限アミノ酸はロイシンだった。ポップすることにより，リジンは１/２に減少し，またラセミ化も進行することから，より温和な加熱条件による加工法の必要性が認められた。

アルブミンはゲル濾過法により分子量11万，19万，24万を与える三成分に分画されたが，各成分はSDS電気泳動分析により，相異なるポリペプチドから構成されていることが明らかになった。グロブリンは同様に２成分に分画された。また，アルブミン，グロブリンは共にトウモロコシよりも強いトリプシンインヒビター活性を示した。

以上の結果は，アマランス種子が他の穀物とかなり異なるタンパク質からなっていることを示し，今後実際の食品加工を行う上で，重要な知見を与えるもので，農学博士の学位を授与するに値すると判定した。