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# CHANGES IN THE FREE AND TOTAL AMINO ACID COMPOSITION OF RIPENING CHESTNUT SEEDS

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**Key Word Index**—*Castanea sativa, Castanea crenata* × *Castanea sativa*, Fagaceae, chestnut, maturation, amino acid composition

**Abstract**—The distribution of free and protein amino acids during seed development is described for three chestnut varieties Asparagine accumulation was shown to correlate with protein biosynthesis Accumulation of free amino acids takes place before that of proteins in ripening seeds A temporary decrease was generally accompanied by protein biosynthesis and followed by final accumulation of both protein and free amino acids

## INTRODUCTION

The non-protein amino acids are well known [1–4] Although legume and cereal seeds have been extensively studied [5] because of their role in human nutrition, there remain many seeds such as chestnuts which have been largely ignored Amino acids and their by products asparagine and glutamine play an important part in the metabolism of ripening seeds since they are the precursors of storage proteins In the present study we report on the change in free and total amino acids in ripening chestnut seeds at three stages of maturity

## RESULTS AND DISCUSSION

### *Seed moisture content*

In order to eliminate the effect of variable moisture content in ripening seeds, all results are given in terms of dry weights Table 1 shows that for all three species studied the most mature specimens had the highest dry weights A previous study [6] has considered seed maturity as the stage of ripening at which maximum dry weight is obtained

### *Nitrogen content*

In ripening chestnut seeds (Table 2) the pool of free amino nitrogen represents a relatively small and constant portion of the total cotyledon nitrogen A slight reduction of this pool was observed in the three species studied at the second stage of maturity, followed by an increase at the final stage this implies amino acid incorporation into proteins It is well known that in maturing seeds such as the pea [7, 8], accummulation of free amino nitrogen takes place prior to accummulation of storage proteins, and it has been shown [9] that massive accummulation of free amino acids is generally associated with reduced growth and with the resultant decreased demand for nitrogenous compounds to build proteins

Table 1 Dry weight of seeds (g/100 g) of fresh chestnuts

| | Stage of maturation | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| Dorée de Lyon | 44 2 | 46 6 | 47 |
| Sauvage des Cars | 49 4 | 49 | 49 5 |
| Maraval 74 | 38 | 43 | 44 5 |

Table 2 Distribution of nitrogen in ripening chestnuts seeds expressed as mg N/g of chestnut dry wt (edible part)

| | Stage of maturation | Total N | Amino N |
|---|---|---|---|
| Dorée de Lyon | 1 | 11 9 | 0 6 |
| | 2 | 9 4 | 0 53 |
| | 3 | 11 2 | 0 7 |
| Sauvage des Cars | 1 | 8 5 | 0 8 |
| | 2 | 11 2 | 0 66 |
| | 3 | 9 9 | 0 76 |
| Maraval 74 | 1 | 7 3 | 0 44 |
| | 2 | 8 95 | 0 40 |
| | 3 | 11 8 | 1 02 |

### *Free amino acid content*

Among the more interesting variations in the level of free amino acids (Table 3) the following were notable Asp increased in the third stage of maturity The level of asparagine and γ-aminobutyrate varied Cystine became detectable during maturation Ornithine is low and it was often undetectable at maturity [10] Other amino acids showed no notable variations

### *Amino acid content of proteins*

Table 4 shows the change in amino acid content of proteins during maturation Some losses are known to occur during the hydrolysis procedure tryptophan is totally destroyed, asparagine and glutamine are respect-

Table 3 Free amino acids mg/g dry wt

| | | Asp | Thr | Ser | Asn | Glu | Gln | Gly | Ala | Val | Cys | Met | Ile | Leu | Tyr | Phe | GABA | Trp | $NH_4^+$ | Orn | Lys | His | Arg |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dorée de Lyon | 1 | 0 32 | 0 08 | 0 13 | 0 48 | 0 05 | 0 09 | 0 11 | 0 50 | 0 16 | 0 00 | 0 04 | 0 05 | 0 07 | 0 04 | 0 15 | 0 92 | 0 02 | 0 44 | 0 01 | 0 03 | 0 02 | 0 05 |
| | 2 | 0 16 | 0 07 | 0 13 | 0 54 | 0 14 | 0 06 | 0 11 | 0 45 | 0 11 | 0 01 | 0 03 | 0 03 | 0 07 | 0 03 | 0 06 | 0 80 | 0 05 | 0 38 | 0 02 | 0 03 | 0 01 | 0 03 |
| | 3 | 0 44 | 0 09 | 0 13 | 1 20 | 0 06 | 0 05 | 0 08 | 0 48 | 0 15 | 0 01 | 0 02 | 0 04 | 0 06 | 0 04 | 0 19 | 0 76 | 0 04 | 0 39 | 0 01 | 0 03 | 0 02 | 0 04 |
| Sauvage des Cars | 1 | 0 28 | 0 11 | 0 11 | 1 05 | 1 16 | 0 30 | 0 077 | 0 45 | 0 12 | 0 00 | 0 036 | 0 035 | 0 014 | 0 019 | 0 032 | 0 32 | 0 09 | 0 41 | 0 045 | 0 019 | 0 04 | 0 23 |
| | 2 | 0 21 | 0 11 | 0 15 | 0 88 | 0 58 | 0 10 | 0 07 | 0 58 | 0 12 | 0 02 | 0 03 | 0 04 | 0 01 | 0 03 | 0 02 | 0 53 | 0 07 | 0 29 | 0 02 | 0 02 | 0 05 | 0 13 |
| | 3 | 0 35 | 0 11 | 0 12 | 0 74 | 0 25 | 0 08 | 0 09 | 0 67 | 0 15 | 0 02 | 0 03 | 0 05 | 0 04 | 0 03 | 0 08 | 1 08 | 0 11 | 0 33 | 0 02 | 0 03 | 0 03 | 0 30 |
| Maraval 74 | 1 | 0 29 | 0 06 | 0 24 | 0 14 | 0 09 | 0 09 | 0 16 | 0 20 | 0 04 | 0 00 | 0 04 | 0 05 | 0 07 | 0 03 | 0 03 | 0 31 | 0 03 | 0 66 | 0 04 | 0 03 | 0 02 | 0 09 |
| | 2 | 0 23 | 0 04 | 0 14 | 0 61 | 0 06 | 0 04 | 0 08 | 0 17 | 0 03 | 0 01 | 0 01 | 0 03 | 0 04 | 0 02 | 0 02 | 0 25 | 0 02 | 0 57 | 0 03 | 0 02 | 0 01 | 0 08 |
| | 3 | 0 66 | 0 08 | 0 19 | 2 44 | 0 30 | 0 09 | 0 12 | 0 47 | 0 12 | 0 02 | 0 03 | 0 06 | 0 07 | 0 02 | 0 03 | 0 52 | 0 03 | 0 83 | 0 03 | 0 03 | 0 03 | 0 23 |

Table 4 Protein amino acids (mg/g dry wt)

| | | Asp | Thr | Ser | Glu | Gly | Ala | Val | Cys | Met | Ile | Leu | Tyr | Phe | $NH_4^+$ | Lys | His | Arg |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dorée de Lyon | 1 | 7 24 | 4 35 | 4 74 | 7 83 | 7 01 | 6 48 | 3 74 | 0 45 | 0 56 | 3 39 | 7 13 | 2 12 | 3 26 | 2 88 | 4 33 | 1 69 | 3 36 |
| | 2 | 5 85 | 3 72 | 3 94 | 6 17 | 6 04 | 5 11 | 2 97 | 0 30 | 0 79 | 2 07 | 4 84 | 1 81 | 2 31 | 3 12 | 3 05 | 1 16 | 2 63 |
| | 3 | 8 14 | 4 15 | 4 46 | 6 79 | 7 52 | 5 74 | 2 81 | 0 42 | 1 02 | 4 96 | 5 97 | 1 90 | 2 56 | 1 92 | 3 08 | 1 60 | 2 66 |
| Sauvage des Cars | 1 | 5 03 | 2 64 | 3 63 | 6 23 | 5 72 | 5 61 | 2 26 | 0 25 | 0 85 | 1 65 | 3 67 | 1 51 | 2 04 | 2 51 | 2 03 | 0 88 | 1 51 |
| | 2 | 5 67 | 2 60 | 3 65 | 5 57 | 6 38 | 4 46 | 3 24 | 0 20 | 1 29 | 4 05 | 7 90 | 1 89 | 3 65 | 5 93 | 4 35 | 2 83 | 2 16 |
| | 3 | 6 54 | 3 01 | 3 49 | 5 71 | 5 42 | 3 85 | 2 98 | 0 32 | 0 72 | 2 31 | 5 20 | 1 80 | 2 53 | 6 14 | 3 19 | 1 24 | 2 60 |
| Maraval 74 | 1 | 4 54 | 2 80 | 3 28 | 5 08 | 4 54 | 3 91 | 2 55 | 0 22 | 0 32 | 1 80 | 3 44 | 1 23 | 1 72 | 2 30 | 2 41 | 1 02 | 1 65 |
| | 2 | 5 72 | 3 34 | 3 66 | 6 85 | 5 16 | 4 71 | 3 15 | 0 33 | 0 13 | 2 45 | 4 71 | 1 62 | 2 17 | 3 00 | 2 92 | 1 06 | 2 39 |
| | 3 | 8 31 | 6 88 | 5 32 | 8 67 | 6 84 | 5 58 | 3 63 | 0 27 | 0 11 | 2 69 | 4 96 | 1 67 | 2 42 | 2 41 | 3 44 | 1 25 | 2 83 |

Table 5 Amounts of different nitrogen compounds during seed development expressed as mg/g dry wt

| | Stage of maturation | Protein | Free amino acids | Asn | Gln | Arg | | | GABA |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Free | Protein | Total | |
| Dorée de Lyon | 1 | 70 57 | 3 8 | 0 48 | 0 09 | 0 05 | 3 36 | 3 41 | 0 92 |
| | 2 | 55 42 | 3 32 | 0 54 | 0 06 | 0 03 | 2 63 | 2 66 | 0 80 |
| | 3 | 65 62 | 4 38 | 1 2 | 0 05 | 0 04 | 2 66 | 2 70 | 0 76 |
| Sauvage des Cars | 1 | 48 06 | 5 058 | 1 05 | 0 30 | 0 23 | 1 51 | 1 74 | 0 32 |
| | 2 | 65 86 | 4 143 | 0 88 | 0 10 | 0 13 | 2 16 | 2 29 | 0 53 |
| | 3 | 57 12 | 4 751 | 0 74 | 0 08 | 0 3 | 2 6 | 2 90 | 1 08 |
| Maraval 74 | 1 | 42 86 | 2 764 | 0 14 | 0 09 | 0 09 | 1 65 | 1 74 | 0 31 |
| | 2 | 53 40 | 2 544 | 0 61 | 0 04 | 0 08 | 2 39 | 2 47 | 0 25 |
| | 3 | 67 31 | 6 435 | 2 44 | 0 09 | 0 23 | 2 83 | 3 06 | 0 52 |

ively converted to aspartic acid and glutamic acid, and cysteine to cystine In the three species tested aspartic acid and isoleucine are higher in the third stage than in the first one Arginine increased in two species only: Sauvage des Cars and Maraval 74 The other amino acids showed no significant changes This slight variations in the amino acid composition of proteins came as a surprise because of previous work [10] showing a biosynthesis of basic proteins during maturation of *Castanea sativa* Even more interesting is a study of the content of four nitrogen compounds asparagine, glutamine [11–13], arginine (which are important in the synthesis of seed proteins) and γ-aminobutyric acid, which was considered because it represents an important portion of the free amino nitrogen (Table 5) Asparagine and arginine availability and protein biosynthesis seem to be closely related, i e in Maraval 74 protein accummulation and asparagine concentration increased from stage 1 to stage 3 Asparagine has already been shown to be a key element of seed nitrogen metabolism in lupin seeds [11] and in maturing cotyledons of soybean in culture [12] Developing seeds need to obtain the nitrogen required for storage protein synthesis and a large portion of this nitrogen probably comes from asparagine Less obvious is the role of glutamine in chestnuts seeds since its level is low Total arginine decreased in Dorée de Lyon and also proteins, whereas in the two other varieties they increased The level of γ-aminobutyrate varied A high level of this compound was found in the pool of free amino acids for the two varieties of *Castanea sativa* in the third stage of maturity (17 4% in Dorée de Lyon, 22 71% in Sauvage des Cars) This came as no surprise because of previously published work [14] The role of γ-aminobutyrate in maturing chestnut seed is not evident γ-aminobutyrate accumulation has been described as a response to anoxia [15] and as an index of cessation of growth and inhibition of protein synthesis [16] It was first detected in dormant cells of potato tubers [9] and then shown to be widely distributed in the plant kingdom [1] It could be taken as an indication of a dormant state in chestnut seed but the present work shows a high level of this compound in germinating chestnut cotyledons The role of γ-aminobutyrate in chestnut remain unexplained but its high level in *Castanea sativa* may be important for the development of this seed As stated recently by Rosenthal [4] "its overall physiological function in the plant is only poorly understood"

## EXPERIMENTAL

*Plant material* The three samples were collected in Limousin (France) in autumn 1982 *Castanea sativa* var Dorée de Lyon, *Castanea sativa* var Sauvage des Cars, Hybrid *Castanea crenata* × *Castanea sativa* Maraval 74 Three stages of maturity were defined as stage 1 seeds collected on tree (ten weeks after flowering), chestnut husk closed, chestnut pericarp white, stage 2 seeds collected on tree (11 weeks after flowering), chestnut husk open, chestnut pericarp brown, stage 3 complete maturity chestnut collected on the ground twelve weeks after flowering The different stages were preserved by freezing (−15°) before use

*Dry weight* Determination of the moisture content was performed with a Cenco moisture balance Moisture percentage allowed determination of dry wt 100–$H_2O$% = % dry wt (sample 5 g in duplicate analysis)

*Nitrogen content* The standard Kjeldahl method was used for the determination of total nitrogen in frozen samples In this study protein nitrogen is defined as the difference between the total nitrogen and amino (and amido) nitrogen

*Free amino acids (+ Asn + Gln) extraction and determination* Extraction was performed by crushing an aliquot of 5–10 chestnuts (10 g/30 ml) in HCl 10 mM, the three extracts were deproteinized with sulfosalicylic acid (3% w/v) and amino (and amido) acids determination was performed by reaction using ninhydrin [17], values in Tables 3 and 4 are the means of duplicate analysis Extracts were then concd by evaporation in Visking tubing (+4°) to about 5 mg/ml amino (and amido) acids were determined with a Technicon NC 2P system as described previously [14]

*Protein extraction and amino acid analysis* Protein estimation was calculated as follows protein = (total N × 6 25)-free amino acids Extraction was performed by crushing chestnuts (10 g/30 ml) in M NaCl (pH 7) 3 times successively Proteins were precipitated by sulfosalicylic acid, collected by centrifugation, then hydrolysed by heating in 6 M HCl for 24 hr at 100° Protein hydrolysates were analysed using the Technicon NC 2P system

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