THE UNIVERSITY OF MANITOBA

SENSORY CHARACTERISTICS OF SOME UNPLEASANT TASTING AMINO ACIDS IN RELATION TO THE FREE AMINO ACIDS IN SELECTED PLANT PROTEINS

by

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ABSTRACT

Seven trained panelists assessed 3000 ppm aqueous solutions (pH 6.5) of ten amino acids for the presence of 13 flavor parameters and a measure of total flavor intensity. Five amino acids, arg-hcl, ileu, leu, phe and try, evaluated as possessing bitterness with accompanying astringency were further examined at four or more concentrations for bitterness, astringency, pleasantness and total intensity by six trained panelists using the method of magnitude estimation. Similarly binary mixtures of arg-hcl in combination with each of ileu, leu, phe and try were tasted at five concentrations for interaction effects in bitterness. The total bitterness intensity of each mix was formulated such that at every concentration each amino acid contributed approximately 50% of the total bitterness intensity, as determined from their individual bitterness power functions. The free amino acid content of 18 plant protein samples was determined and considered in terms of the sensory analyses of amino acids. Large differences in intensity existed between 3000 ppm solutions with try stimulating the most bitterness and ileu the least. Flavor profiles revealed that his, lys, met, pro and val induced complex sensations requiring several descriptors while arg-hcl, ileu, leu, phe and try were primarily bitter with accompanying astringency. The rate of growth (slope) of perceived bitterness was not significantly different among these five single amino acids and caffeine but in each case was greater than 1.0 indicating that bitterness increased as an accelerating function of concentration. Elevation differences indicated that try was most bitter (and not significantly different from caffeine), phe, arg-hcl, and leu were intermediate in bitterness and ileu was the least bitter. Astringency perception of ileu increased as an accelerating function of concentration. The rate of growth did not differ significantly from the alum reference but elevation differences revealed that alum was much more astringent than ileu. No other significant relationship between perception of astringency and amino acid concentration was established. Total intensity patterns revealed that ileu grew most rapidly in perceived total intensity followed by arg-hcl, try, phe and leu. The pleasantness of all amino acids declined as concentration increased. While the rate of decline was not significantly different among amino acids, the concentration at which unpleasantness became evident was lowest for try followed by phe, arg-hcl, leu and finally ileu. The rate of growth of bitterness intensity in binary mixtures either followed that of the component with the sharpest slope or was significantly greater in slope than either component. Suppression of bitterness was evident at low mix concentrations while additivity occurred at intermediate and possible synergism at the highest concentration. Free amino acid analyses revealed that the cereals durum, oats, rye, triticale and wheat, and the oilseeds, mustard, rapeseed, and sunflower, contained fewer amino acids in total in comparison to soy proteins and the legume proteins fababean, lupin and field pea. Quantities of bitter amino acids in the eighteen plant protein samples examined were insufficient to cause off-flavor to food products when considered on an individual basis.

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INTRODUCTION

At the present time North American food protein sources are in abundance, however, shortages may be a future food issue as it is presently in many other countries. On a world basis it has been projected from estimated supply and demand data that there will be a deficit of 30.2 million metric tons of animal protein sources by 1980 (Burrows, et al. 1972). It has been postulated that in the next few decades plant proteins will constitute up to two thirds of our food grade protein (Bird, 1974). At the present time cereal grains account for the major portion of consumed plant proteins. Oilseed meals and legumes offer potential new sources of fairly high and good quality protein.

Flavor is one of the most important determinants of the acceptance of plant proteins for human consumption. Generally cereals are considered to be neutral and bland in flavor in comparison to flours and concentrates of oilseeds and legumes. Much investigation has been conducted in regard to compounds responsible for vegetable protein off-flavor with the major emphasis being directed towards lipids and lipid degradation products. Little attention has been focused upon the role of free amino acids as possible contributors to plant protein off-flavor.

Several amino acids have been described as possessing unpleasant taste sensations (Solms et al.,1965 , Kirimura et al., 1969, Petritschek et al., 1972 and Schiffman and Dackis, 1975), including bitterness which is one flavor parameter frequently ascribed to plant proteins. Some amino acids have been reported to be compounds of high taste intensity (Solms et al., 1965) and have also been detected

in considerable quantities in the free form in some plant proteins (Bhatty and Finlayson, 1973). Whether or not unpleasant free amino acids are present in sufficient quantities to contribute to the off-flavor of plant proteins has not been examined in any detail.

The objectives of the present study were as follows:

1) To profile the flavor of amino acids reported in the literature to possess undesireable flavor properties.
2) To develop intensity patterns, using the method of magnitude estimation, relating perceived bitterness, astringency, pleasantness and total intensity to stimulus concentration for amino acids described in the profiles as bitter and astringent.
3) To examine interaction effects of binary amino acid mixtures.
4) To determine the free amino acid content of several plant proteins including samples of cereals, oilseeds and legumes.
5) To assess possible flavor implications of the free amino acids to plant proteins in light of the sensory information generated.

LITERATURE REVIEW

I Flavor Properties of Amino Acids

The flavor of individual amino acids has been well documented. Earlier studies produced conflicting results because differences in taste between L and D isomers had not been considered and pure L & D amino acids were not readily available. A summary of the most recent reports of the flavor properties of the naturally occurring L-amino acids is presented in Table 1. Reports in the literature are not entirely consistent. Taste properties of amino acids have been reported to vary with concentration (Solms, 1969) and pH differences have been demonstrated to alter threshold levels of compounds such as thiamine (Höhn et al.,1975). Thus methodology could account for some of the differences reported. Solms et al. (1965) used 3000 ppm aqueous solutions adjusted to pH 6.5, Petritschek et al. (1972) 3000 ppm aqueous solutions adjusted to pH 7.4 while Schiffman and Dackis (1975) examined undiluted amino acids presented in the powder form. Kirimura et al. (1969) report neither the exact concentrations of the solutions used nor any pH adjustment.

Generally it appears that some amino acids possess distinct flavor properties while others are slightly more complex. The sulfur containing amino acids, cysteine, glutamic acid and methionine, appear to be complex possessing sulfurous, meaty and glutamate-like taste properties. The sulfurous meaty sensations are reported to arise from decomposition products of amino acids rather than from original amino acid structure. Alanine, glycine and serine have been consistently reported to be sweet. Leucine, phenylalanine and tryptophane clearly possess bitterness while arginine, isoleucine, lysine, methionine, proline, tyrosine and valine

Table 1 Summary of the flavor properties of L amino acids as reported in the literature

L-Amino Acid	Flavor Properties			
	Solms et al. (1965)	Kirimura et al. (1969)	Petritschek et al. (1972)	Schiffman and Dackis (1975)
alanine	sweet	sweet	sweet	sweet
arginine	tasteless	bitter, slightly sweet	bitter	sharp, alkaline, bitter
asparagine	tasteless	sour, bitter	tastless	
cysteine	sulfurous		sulfurous	strong, nauseous, rotten eggs, sulfur, bitter
glutamic acid	glutamate	sour, glutmate sweet	glutamate	sweet, meaty, stale
glycine	sweet	sweet	sweet	sweet
histidine	tasteless	bitter	virtually tasteless slightly bitter	salty, sour, bitter, obnoxious, pungent
isoleucine	tasteless	bitter	bitter	weak, tasteless, flat, dry, alkaline
leucine	bitter	bitter	bitter	same as isoleucine
lysine	tasteless	bitter, sweet	virtually tasteless, slightly bitter	salty, bitter, sharp
methionine	sulfurous, meaty, slightly sweet	bitter, glutamate		repulsive, metallic, mineral, bitter, dry, smooth, nauseous

Table 1 cont'd

L-Amino Acid	Flavor Properties			
	Solms et al. (1965)	Kirimura et al. (1969)	Petritschek et al. (1972)	Schiffman and Dackis (1975)
phenylalanine	bitter	bitter	bitter	mineral, metallic, sharp stale, dry
proline	flat, slightly sweet	sweet, bitter	bitter	mineral, salty, sour, sweet
serine	tasteless	sweet, sour, glutamate	virtually tasteless, slightly sweet	sweet
threonine	tasteless	sweet, sour, bitter	tasteless	fatty, slightly sweet, mineral, stale
tryptophane	bitter	bitter	bitter	sharp, bitter, dry
tyrosine	bitter		bitter	dry, flat, stale
valine	tasteless	bitter	bitter	dry, flat, mineral, bitter, sour, sweet

have been implicated in stimulating bitterness as well as other flavor sensations. The unpleasant tasting L - amino acids arginine, lysine, histidine, phenylalanine and tryptophane have been reported to lose their unpleasantness and become weak when acetylated (Schiffman et. al., 1975).

Although limited, data regarding the taste intensity of some amino acids is available. Solms et al. (1965) reported that the bitterness of amino acids relative to caffeine was as follows: L-tryptophane one half, L-phenylalanine one quarter and L-tyrosine one twentieth. Thresholds of 30 and 50 ppm have been reported for aspartic and glutamic acids respectively and thresholds of 1900, 900 and 900 ppm reported for the bitter amino acids leucine, phenylalanine and tryptophane (Kirimura et. al., 1969). The taste intensity of the D isomers of the aromatic amino acids which are bitter in the L form is of interest. According to Solms et. al. (1965) D-tryptophane, D-phenylalanine and D-tyrosine are 35, 7 and 5.5 times as sweet as sucrose, respectively. Thus some amino acids appear to be compounds of high taste intensity.

In the above studies Solms et. al.(1965) and Petritschek et al. (1972) measured the intensity of amino acid solutions in comparison to a series of concentrations of standard compounds. Kirimura et al. (1969) measured intensity on a ten point scale while Schiffman and Dackis (1975) used semantic differential scales. None of the studies used the method of magnitude estimation which is presently considered the most appropriate method of measuring sensory intensity. The method permits the construction of a ratio scale between physical stimuli and psychophysical perception over a continuum which is represented by an equation; the power function (Moskowitz, 1975b). The equation permits an estimate of sensory intensity over the total concentration continuum of the physical

stimuli. This method was used in the present study to measure the taste intensity of amino acids.

II The Role of Free Amino Acids in the Flavor of Foodstuffs

The importance of free amino acids to taste was first recognized in 1908 when Ikeda discovered that monosodium L-glutamate was the essential component of the taste imparting ingredients of traditional Japanese food seasoners. (Kirimura et al., 1969). Free amino acids have since been demonstrated to be an integral part of numerous foodstuffs including sake, green tea, lobster and crab (Kirimura et al. 1969) as well as in potatoes (Buri et al., 1970) and cheese (Langler et al., 1967 and Dilanean, 1974).

Buri et al. (1970) demonstrated that a stepwise recombination of the three fractions: I nucleotides, II L-glutamic acid, and III other free amino acids previously determined to be present in the free form in potatoes, gave a distinct stepwise increase in potato flavor quality. A fully reconstituted potato flavor was not apparent due to the absense of the volatile fractions as well as other non-volatiles.

The best documentation of the role of free amino acids in the flavor of food deals with cheese. In an attempt to produce synthetic Swiss cheese Langler et al. (1967) evaluated mixtures of components known to occur in Swiss cheese. Sensory evaluation revealed that only upon the addition of free amino acids was a typical, full, sweet Swiss cheese flavor reported. The amino acids utilized included proline, glycine, serine, threonine, aspartic acid, cysteic, tryptophane and lysine. Proline at 3000 ppm was the dominating amino acid.

The flavor of different varieties of cheese appears to be

characterized by a typical profile and quantity of free amino acids (Dilanean, 1974). It was reported, for example, that the major free amino acids (31%) in Swiss cheese, glutamate and threonine, only constituted a small portion (3%) of the free amino acid content of Armyansky cheese. An analysis of Swiss cheeses of different qualities illustrated that those possessing total free amino acid contents of 2887 mg % were superior in quality to cheeses containing 4539 mg %. It was further demonstrated that alteration of typical amino acid patterns of Soviet cheese by utilization of different bacterial starters resulted in a reduction of the quality of the cheese.

Okhrimenko and Chebotaro (1975) reported the presence of peptides and free amino acids in blue veined cheese. According to these authors free amino acids possessing bitter flavors were present in quantities surpassing their threshold level. A direct relationship between total or individual amino acids and the degree of observed bitterness was established.

The role of free amino acids in the flavor of vegetable proteins has not received much attention. Fujimaki et al. (1970) reported the presence of free isoleucine, phenylalanine and valine in a peptic hydrolysate of soybean protein and suggested that these bitter amino acids might contribute to the bitterness of the hydrolysate.Honig et al. (1971) isloated tryptophane from soybean flakes. However, a quantitative determination revealed only 7 ppm and the authors concluded that this would not be sufficient to contribute to the bitterness of soybean products.

Bhatty and Finlayson (1973) determined the free amino acid content of 80% ethanol extracts of soy, rapeseed, and sunflower meals. Flavor

evaluation of amino acids was not the purpose of the study. However, quantities of some free amino acids which have previously been reported to possess bitterness were present in the meals in above reported threshold quantities (Kirimura et al., 1969). Free histidine was in above threshold quantities for rape meal and tryptophane was in above threshold quantities for soy and sunflower meals. Quantities of free arginine in fababean concentrate (Höhn, unpublished data) were present in amounts 6 1/2 times the reported threshold of arginine. Thus some amino acids which possess bitterness are present in plant proteins in above reported threshold quantities. Whether or not they are present in sufficient quantities to cause undesireable bitter flavors in these protein sources is not predictable from these data.

III Flavor Properties of Peptides

As of late much research has been directed towards the flavor of peptides, particularly those causing bitterness. Several attempts have been put forth to produce some classification system by which the taste of peptides may be predicted.

Kirimura et al. (1969) evaluated the taste properties of sixty dipeptides in 0.2% aqueous solutions. Results classified the peptides into three groups: sour, bitter and those having little or no taste. Sour peptides included those which contained a) 2 acidic amino acids b) an acidic and a neutral amino acid and c) an acidic and aromatic amino acid. Dipeptides in a) were more acidic than those in b) which were more acidic than those in c). Bitter peptides contained a) neutral amino acids with either large alkyl groups ($C \geq 3$) or a combination of large and small alkyl groups b) neutral and aromatic amino acids and c) neutral and basic amino acids. Peptides which had little taste

included a) two amino acids with small alkyl groups b) acidic and basic amino acids or c) two aromatic amino acids.

Twelve glutamyl oligopeptides examined for flavor properties were classified into three groups by Arai et al.,(1973). These included 1) brothy 2) flat and 3) bitter peptides. The glutamyl counterpart in these dipeptides was reported to be the more acidic for brothy peptides (aspartic acid, glutamic acid, serine and threonine), hydrophilic for flat peptides and hydrophobic for bitter peptides. However, Kirimura et al., (1969) had reported earlier that the dipeptides L-glutamyl-L-aspartate and L-glutamyl-L-glutamate possessed a sour taste which is in contradiction to the brothy flavor reported by these authors. This draws attention to one fault in Kirimura's classification, that being if a dipeptide possessed a taste other than bitterness it had to be sourness.

Schiffman and Engelhard (1976) examined forty-six dipeptides and observed no strict relationship between the flavor of a dipeptide and its constituent amino acids, however, they reported some trends. Most dipeptides were found to be predominantly bitter or weak. Weak peptides possessed constituent amino acids possessing hydroxyl groups or aliphatic side chains. All sweet peptides except one possessed a sweet tasting amino acid as their NH_2 terminal amino acid, however, this was found in bitter dipeptides as well. With one exception dipeptides with a sour component contained amino acids having acidic groups. No clear trends were observed for dipeptides with salty or bitter tastes.

A method for predicting the presence of bitterness of peptides on the basis of amino acid composition bas been set forth by Ney (1971). This method is based on a model proposed by Tanford (1962) for

calculating free energy changes (Δ F) between the native and unfolded forms of protein molecules in solution. The major contribution of the Δ F of proteins are the hydrophobic interactions of the non-polar side chains of the constituent amino acids which govern the stable globular form in aqueous solutions. Thus Δ F values for proteins and amino acids are a measure of the hydrophobicity of the non polar side chain (s) of constituent amino acid (s). Ney postulated that a value termed Q, representative of the average hydrophobicity of a peptide, may be calculated as the average Δ F values of contituent amino acids. Peptides possessing Q values less than 1300 were postulated to lack bitterness while those with values greater than 1400 would possess bitterness. Ney calculated Q values for twenty-one peptides ranging from di- to octapeptides and in all cases the tastes of the peptides complied with the theory.

It is well known that bitter peptides arise from hydrolyzed protein products. Guigoz and Solms (1976) systematically calculated Q values from identified bitter peptides isolated from casein, soy protein, zein, other food products and synthetic peptides as reported in the literature. From a compilation of 200 bitter peptides only 14 did not adhere to Ney's rule. That is, they tasted bitter, but possessed Q values less than 1300. These authors noted that several peptides with glycine residues (Δ F = 0 Cal/mole) did not adhere to the rule; however, if glycine residues were ignored in the calculation of Q only three of the peptides did not concur. Schiffman and Engelhard (1976) noted that several of their dipeptides departed from Ney's theory of bitterness, however, most of these were dipeptides

containing glycine.

Subsequently, this rule was extended to proteins and it was postulated that the occurrence of bitter peptides in hydrolyzed protein sources could be predicted from Q values of the protein (Ney, 1972). Q values of 1605, 1480, 1540, 1300 and 1280 were obtained for casein, zein, soy protein, muscle protein and collagen respectively. Thus, it was predicted that upon hydrolysis there was a greater probability of casein, soy and zein to form bitter peptides than for collagen and muscle protein. The authors stated that experimental data reported in the literature concurred with the predictions.

Few inconsistencies are apparent in regard to the taste of peptides. Ney's rule appears to be a useful tool in the prediction of the bitterness of peptides.

According to Kirimura et al. (1969) the taste intensities of dipeptides appeared to be greater than that of their constituent amino acids. Threshold values of L-Leucyl-L-Leucine and L-glutamyl-L-glutamate were approximately half those of L-leucine and L-glutamate respectively. Weiser and Belitz (1975) reported the thresholds for eight bitter peptides isolated from corn protein zein. It was observed that the threshold for bitter taste decreased for increasing numbers of hydrophobic side chains in a peptide. These same authors (1976) established taste threshold values for 80 peptide molecules and again the threshold was related to the hydrophobicity of the molecule. Thus the threshold may well depend upon relative hydrophobicity, the greater the hydrophobicity the lower the threshold.

Bitter peptides have received the most attention in the literature however peptides possess other flavor properties including sourness and

brothy tastes as previously enumerated. An extremely sweet dipeptide, L-aspartyl-L-phenylalanine methyl ester was discovered (Mazur, 1969) which may soon be adopted as a sweetening agent.

IV Taste Mixtures

The literature generally concludes that mixtures containing dissimilar taste components will result in a suppression of taste intensity while mixtures of similar stimuli are additive or synergistic in terms of intensity. Pangborn (1960) reported upon the effects of adding subthreshold, threshold and suprathreshold levels of secondary flavor components upon the intensities of primary flavor components present in aqueous solutions. All combinations of four chemicals representative of the basic taste sensations were examined. Generally, all compounds were found to reduce the intensity of the other, the most pronounced effect being the reduction of the sweetness of sucrose by citric acid and vice versa. Similarly Moskowitz (1972) reported that when suprathreshold quantities of sodium chloride, citric acid and quinine sulfate were each mixed with solutions of glucose and fructose the result was a reduction in the intensity of each taste in the mixture.

Mixtures of similar tastes are consistently reported to be additive or synergistic in regards to intensity. The most extensive research has been conducted with sweet tasting compounds. Evaluation of the intensity of binary mixes of dextrose in combination with each of fructrose, sucrose and calcium cyclamate as well as in mixtures of sucrose and fructrose (all present in suprathreshold concentrations) illustrated synergistic effects (Stone and Oliver, 1969). Additivity in sweetness was reported for combinations of dextrose with saccharin and the sweet

amino acids glycine and alanine. Certain binary mixtures expressed more synergism than others and in some combinations synergism was evident in mixtures containing the amino acids. Kamen (1959) also reported additivity between calcium cyclamate and sucrose and also observed synergistic effects at certain concentrations.

Combinations of acids have been shown to demonstrate additivity in sourness (Moskowtiz, 1974). Citric acid in combination with each of phytic, succinic and gluconolactone were additive in sourness while when in combination with hydrochloric acid synergism was apparent.

Two models have been proposed previously in the literature to account for the manner in which the taste system adds together mixtures of similar stimuli (Moskowitz, 1973). Type I additivity proposes that a summation of concentration occurs in the mixture and the taste system processes the mixture as a higher concentration of one of the components. Type II additivity proposes that there is a summation of perceived intensities of the compounds (simple additivity). After evaluations of the fit of both models to data of his own experiments on sweet tasting mixtures and sour mixtures along with data in the literature Moskowitz (1973, 1974) concluded that it generally appears that 'like tasting compounds add together according to a simple arithmetic manner (ie. simple additivity, type II) with synergism or supression simply phenomena that reveal a failure to account in an adequate way for the law of summation.'

Bartoshuk (1977) maintains that perceived intensity of a mixture of similar tasting compounds is not the simple sum of the intensity of the constituents but a reflectance of the psychophysical or power functions of its components. That is; the way a compound adds to itself

will determine the manner in which it adds to other substances. Thus mixtures of compounds with compressed power functions (slope $<$ 1) will also express compressed functions and thus illustrate supression Conversely, mixtures of compounds with expanded functions (slope $>$ 1) will in turn produce expanded functions and demonstrate synergism. Thus only mixtures of compounds whose individual functions are characterized by a slope of one will demonstrate simple additivity.

As predicted from power functions, acid and bitter substances when tasted by the dorsal flow method exhibited compressed functions and the mixtures illustrated supression; intensity was not equal to the additive sum of the components (Bartoshuk, 1977). Sugars tested by the dorsal flow procedure also demonstrated compressed functions but in this case simple additivity did account for the mix intensity. It was pointed out by the author that the slope of the compressed function was close to one which as previously stated would be the only instance in which simple additivity would account for mixture intensities. The same sugars examined by the sip and spit procedure exhibited expanded functions and in accordance synergism was observed in the mixes (mix intensity greater than the simple sum). In contradiction to Moskowitz's prediction, these results indicate that mixtures of compounds of similar qualities do not simply add their perceived intensities but show suppression, synergism or simple additivity depending upon their individual psychophysical functions. However, as stated by Bartoshuk, most of the power functions of the unmixed components examined by Moskowitz possessed slopes close to one in log-log coordinates. Thus in these instances simple addition would account for perceived intensity of the mixes and lead to his conclusions.

V Tasting Procedures and Taste Adaptation

As demonstrated by Bartoshuk (1977) the method of sample presentation in a tasting situation may greatly influence the slope or exponent of the power function. The sip and spit procedure resulted in a higher exponent than samples presented via a dorsal flow method. Similar results were reported by Meiselman (1971) who reviewed the literature examining the effects of tasting procedure upon the exponents of power functions. However, even when mode of presentation is controlled distorted results may be obtained if inappropriate interstimulus procedures are used resulting in the phenomena referred to as taste adaptation.

Adaptation is defined as a 'loss of sensitivity to a given stimulus as a result of continuous exposure to that stimuli or a similar one' (Anon, 1964). In a tasting situation adaptation may be partial or complete, the level depending upon interstimulus procedures utilized by the panelist. Common procedures, expectoration and mouthrinsing, have been demonstrated to be insufficient to clear the mouth of taste residuals (O'Mahony, 1972a). After exposure to a 1M NaCl solution for 15 seconds significant levels of residuals, determined by flame photometry, remained in the saliva of panelists for 7 - 17 minutes after rapid expectoration and for 6 - 10 minutes after a single mouth rinse. Five rinses were found to clear the mouth for most subjects, however, such stringent procedures are not adhered to in most taste panels.

Methods that allow panelists to judge for themselves when a stimuli has been cleared from the mouth (ad lib mouth rinsing) also do not appear to be reliable (O'Mahony, 1973). After expectoration of a

1M NaCl mouthrinse all judges reported the taste to have vanished from the mouth before the Na content of the saliva reached pre-experimental levels. Thus besides being inefficient in clearing stimulus residuals this method is uncontrolled.

O'Mahony (1972b) studied the effect of three interstimulus procedures, a 15 second and 2 minute rest and a mouthrinse, upon detection thresholds for sodium chloride. Ten ml samples of NaCl solutions were served in ten successive ascending series for each of the three test conditions. Detection thresholds increased in all three cases, however, the mouth rinsing procedure was the most effective and the 15 second rest the least effective in removing stimulus residuals.

Similarly both detection and recognition thresholds for sucrose, tartaric acid, quinine sulfate and sodium chloride have been reported to be higher upon presentation of successive ascending series when using either a mouthrinse or a no mouthrinse procedure between samples (O'Mahony and Dun, 1974). However, drifts in sensitivity and thresholds were significantly lower ($p < .05$) when using the mouthrinse for sucrose and sodium chloride solutions. The same trend occurred for tartaric acid and quinine sulfate but the differences were not significant.

Residuals may also affect intensity scaling judgements. Power functions generated from magnitude estimates of intensity of NaCl solutions were observed to possess greater exponents when obtained from procedures using a 15 second rest between samples and lower exponents when a mouthrinsing condition was used (O'Mahony 1973; cited by O'Mahony, 1974). These results supported the pre-experimental prediction that the no mouthrinse condition would result in a higher level of adaptation causing a decreased intensity perception of the

lower concentrations, consequently yielding a steeper slope and an elevated power function exponent. A similar effect had previously been reported for sodium chloride and sucrose power function exponents after adaptation to their respective stimuli (Meiselman, 1968). This trend is due to the non-linear nature of the logarithmic axis.

It thus appears that the lack of adequate rinsing procedures in a prolonged tasting situation could result in adapatation effects which in turn may increase threshold determinations, decrease intensity measures and produce elevated exponents in the generation of power functions. It has been reported that the after effects of bitter compounds are longer in duration than for sour and sweet components; they are the shortest for sweet stimulants (Krakauer and Dallenbach, 1937). Thus adaptation effects should be considered and appropriate inter-stimulus procedures not underestimated in panel tasting situations.

METHODOLOGY

On the basis of the information present in the literature, ten amino acids were selected for profile evaluation by a trained panel for the presence of thirteen flavor parameters. Of these amino acids, five: arginine hydrochloride, isoleucine, leucine, phenylalanine, and tryptophane were selected for further taste investigations. These five were evaluated at five or six concentrations by the method of magnitude estimation for the perceived intensity of bitterness, astringency, pleasantness, and total intensity. Subsequently, binary mixtures of arginine hydrochloride with each of the other four amino acids were tested at five concentrations for interaction effects in bitterness. The free amino acid content of eighteen plant protein samples was determined and quantitated to permit consideration of their flavor implications in light of the sensory information generated.

I Experimental Design

A Amino Acid Profiles

Seven trained panelists evaluated 3000 ppm solutions of ten different amino acids for two kinds of information. First the overall intensity was measured where a score of 100 was assumed to be equal to moderate intensity. Solutions were then examined for thirteen taste paramaters simply by establishing whether or not each was present (P) or not present (NP). Each amino acid profile was replicated twice by seven panelists for a total of fourteen judgements for each amino acid.

B Intensity Patterns

Single Amino Acids

Five amino acids, arginine hydrochloride (arg-hcl), isoleucine (ileu), leucine (leu), phenylalanine (phe) and tryptophane (try) were studied in detail. Six trained panelists evaluated the intensity of these amino acids for the taste parameters of bitterness, astringency, total intensity, and pleasantness in relation to identified references. The references used varied with the parameter tested. The concentration at which the amino acids were examined varied as illustrated in Table 2. Concentrations were selected in order to cover a range from below threshold to just below that concentration at which intensity starts to plateau. Due to solubility limitations in the cases of ileu, leu, and try this objective was compromised. While the aim was to have concentrations forming a geometric progression, in order to have at least four or five concentrations in the clearly perceptible range, departures from this were necessary because of panelist sensitivity. Each amino acid intensity pattern was replicated three times by six panelists for a total of eighteen judgements of each concentration in the intensity pattern with the exceptions stated in Table 2.

Amino Acid Mixtures

Six trained panelists evaluated binary mixtures of arg-hcl with each of ileu, leu , phe and try for bitterness and total intensity of five concentrations in relation to an 800 ppm caffeine reference. Mixtures were formulated so that the total bitterness intensity of each series of mixtures, assuming additivity between the two amino acids in

Table 2 Test concentrations used in the development of amino acid intensity patterns for bitterness, astringency, total intensity and pleasantness

Amino Acid	Taste Intensity Pattern	Concentration Used in Development of Intensity Patterns (ppm)							
ARG-HCL	Bitterness Astringency Total Intensity			1000	2000	4000	8000	12000*	16000
	Pleasantness	250	500	1000	2000	4000	8000		16000
ILEU	Bitterness Astringency Total Intensity			1000	2000	4000	8000	12000*	16000
	Pleasantness	250	500	1000	2000	4000	8000		16000
LEU	Bitterness Astringency Total Intensity			750	1500	5000	10000	12000*	15000
	Pleasantness	188	375	750	1500	5000	10000		15000
PHE	Bitterness Astringency Total Intensity			500	1000	2000	4000	8000	16000*
	Pleasantness	125	250	500	1000	2000	4000	8000	
TRY	Bitterness Astringency Total Intensity			500	1000	2000	3000	4000	
	Pleasantness	125	250	500	1000	2000	3000	4000	

* Only two replications were carried out for intensity judgements at these concentrations.

the mix, would be approximately equal to the bitterness intensity of arg-hcl at 2000, 4000, 8000, 12000 and 16000 ppm as determined from the arg-hcl power function, $S=kC^n$. Thus mixtures may be discussed in terms of arg-hcl equivalents. An arg-hcl equivalent may be defined as the amount of a compound necessary to elicit the same perceived bitterness intensity as one unit of arg-hcl. Each member of the pair of amino acids in the mix, at each concentration level, was expected to contribute approximately 50% of the bitterness intensity. The bitterness intensity functions of the single amino acids were utilized to determine the amount of each amino acid to be used in the mixture formulations. For example, to formulate a mixture of arg-hcl and ileu that would be approximately equi-bitter to arg-hcl at 2000 ppm one would proceed as follows:

1) The perceived bitterness of arg-hcl at 2000 ppm when the arg-hcl power function was $S = 6.35 \times 10^{-5} C^{1.102}$ would be $S = 6.35 \times 10^{-5} (2000)^{1.102} = 0.275$ bitterness units.

2) An additive mix with equal bitterness from each of the component amino acids would contain approximately

   .1375 bitterness units of arg-hcl

   and .1375 bitterness units of ileu

   = .275 total bitterness units

3) The amount of ileu necessary to elicit .1375 bitterness units where $S = 7.01 \times 10^{-7} C^{1.556}$ would be

$$C^{1.556} = \frac{.1375}{7.01 \times 10^{-7}} = 2520 \text{ ppm}$$

4) Similarly the amount of arg-hcl necessary to elicit .1375 bitterness units where $S = 6.35 \times 10^{-5} C^{1.102}$ would be

$$C^{1.102} = \frac{.1375}{6.35 \times 10^{-5}} = 1064 \text{ ppm}$$

Thus, the total mix would consist of 2520 ppm ileu, and 1064 ppm arg-hcl which represents 2000 ppm arg-hcl equivalents.

Table 3 shows the mixture formulations used in the development of the bitterness and total intensity patterns of the binary amino acid mixtures. Each intensity pattern was replicated three times by six panelists for a total of eighteen judgements of each concentration in the intensity pattern.

Caffeine and Alum

Panelists also evaluated caffeine for bitterness and pleasantness intensity in ratio to an 800 ppm caffeine reference and alum for astringency and pleasantness in ratio to an 800 ppm alum reference. The seven concentrations used in the development of the intensity patterns were the same for both parameters for both compounds (100, 200, 400, 800, 1600, 3200 and 6400 ppm). Each intensity pattern was replicated three times for a total of eighteen judgements for each concentration in the intensity patterns.

C Free Amino Acid Content of Plant Protein Samples

The free amino acid content of eighteen plant protein samples was determined from Ethanol (ETOH) extracts, and in some cases trichloroacetic acid (TCA) extracts, of the samples according to the methods of Bhatty and Finlayson (1973). Duplicate extracts were prepared and analyzed in all cases. Because of the tedious nature

Table 3 Amino acid mixture formulations

Amino Acids Combined AA1	AA2	Mix Concentration[1] in ARG-HCL Equivalents	Amount of Amino Acid in the Mix (ppm) ARG-HCL (50% Bitterness)	AA2 (50% Bitterness)	Total Bitterness Units (Assuming Additivity)
ARG-HCL	ILEU	1995	1050.40	2537.75	0.275
		4102	2149.84	4238.19	0.608
		8222	4304.76	6968.26	1.311
		12359	6452.49	9311.18	2.053
		16481	8576.44	11415.73	2.815
ARG-HCL	LEU	1989	1050.40	1015.81	0.274
		4064	2149.84	2214.50	0.603
		8128	4304.76	4714.39	1.293
		12161	6452.49	7323.42	2.018
		16180	8576.44	9981.53	2.760
ARG-HCL	PHE	1989	1050.40	598.19	0.274
		4064	2149.84	1275.36	0.603
		8147	4304.76	2657.09	1.297
		12217	6452.49	4075.96	2.027
		16255	8576.44	5506.43	2.776
ARG-HCL	TRY	1975	1050.40	665.81	0.272
		4045	2149.84	1084.30	0.599
		8109	4304.76	1739.78	1.290
		12162	6452.49	2291.91	2.016
		16181	8576.44	2781.97	2.762

[1] Mix concentrations in arg-hcl equivalents are not exactly equal among series of mixtures as the original calculations determining quantities of amino acid components were only carried to two decimal places in total bitterness units.

of the TCA extraction procedure it was only carried out for those plant proteins which showed an appreciable quantity of arginine in the ETOH extracts, and plant protein sources of interest. Thus, a TCA extract was prepared from rapeseed concentrate, soy flour, sunflower concentrate, fababean flour (-40°C), fababean concentrate (A/C, -40°C), fababean concentrate (C), lupin flour, pea flour and pea concentrate.

II Materials

A Source and Preparation Details

Table 4 lists the source and description of materials used in the preparation of samples for candidate screening. Chemicals were weighed, placed in volumetric flasks and brought to volume with glass distilled water. Solutions of citric acid, caffeine, sodium chloride and tannic acid were prepared a day ahead and left over night at room temperature until time of testing. Sucrose solutions were prepared just prior to testing.

Table 5 lists the sources and descriptions of amino acids for which profiles and intensity patterns were established. All amino acids were reagent grade. Arginine hydrochloride and lysine monohydrate were used in place of their free bases because it was easier to adjust to pH 6.5 using these compounds.

The preparation of amino acid solutions in glass distilled water was the same for both sections of the study. Amino acids were weighed into glass beakers and water was added to an amount 50 ml less than desired volume. Plastic coated magnets were placed in the beakers which were agitated on Corning magnetic stirrers until amino acids dissolved. The solutions were then removed and the pH adjusted to 6.5

Table 4 Description and source of materials used in candidate screening

Taste Sensation Stimulated	Chemical	Source
Sweetness	Sucrose	Manitoba Sugar Company Winnipeg, Manitoba
Sourness	Citric Acid	Matheson, Coleman and Bell Norwood (Cincinati), Ohio East Rutherford, New Jersey
Bitterness	Caffeine	J.T. Baker Chemical Company Phillipsburg, New Jersey
Astringency	Tannic Acid	Commercial Grade Source Unknown
Saltiness	Sodium Chloride	Windsor Salt, Canadian Salt Company Montreal, Quebec

Table 5 Description and sources of amino acids used in the sensory evaluation of flavor properties of amino acids

Amino Acid	Molecular Weight	Source
L-Arginine Hydrochloride (ARG-HCL)	210.7	Nutritional Biochemical Corporation (NBC) Cleveland, Ohio J.T. Baker Chemical Company Phillipsburg, New Jersey
L-Histidine (HIS)	155.2	Sigma Chemical Corporation (Sigma) St. Louis, Missouri
L-Isoleucine (ILEU)	131.2	Sigma; St. Louis, Missouri NBC; Cleveland, Ohio
L-Leucine (LEU)	131.2	Sigma, St. Louis, Missouri
L-Lysine Monohydrate (LYS)	182.7	NBC, Cleveland, Ohio
L-Methionine	149.2	NBC, Cleveland, Ohio
L-Phenylalanine (PHE)	165.2	NBC, Cleveland, Ohio Sigma, St. Louis, Missouri
L-Proline (PRO)	115.1	NBC, Cleveland, Ohio
L-Tryptophane (TRY)	204.2	NBC, Cleveland, Ohio Sigma, St. Louis, Missouri
L-Valine (VAL)	117.2	Sigma, St. Louis, Missouri

(range of 6.4 - 6.6) with either 1 N NaOH or 1 N HCl using a Beckman pH meter. The solutions were then transferred to volumetric flasks and brought to volume. All amino acid solutions were prepared a day ahead and left covered over night at room temperature until time of tasting.

A pH adjustment of 6.5 was decided upon as a practical step in the application of the sensory data to the possible flavor contributions of free amino acids to plant proteins. A survey of 4% slurries of several plant protein samples indicated that most were within a pH range of 6.0 - 6.75. Thus, a pH of 6.5 was selected for flavor investigations.

Table 6 lists the reference samples used to judge each sensory parameter along with its preparation details. The source and preparation of caffeine and alum listed in this table was the same as that used in the development of intensity patterns for these compounds.

Table 7 lists the eighteen plant protein samples selected for free amino acid determination and quantification. Information regarding processing techniques, source, age at time of determinations, storage conditions, and total nitrogen is also provided. Both extracting solvents, 80% ETOH (v/v) and 1% TCA (wt/v), used in free amino acid determinations of these samples were reagent grade chemicals prepared in glass distilled water.

Table 6 Source and preparation details of references for sensory parameters evaluated in amino acid taste profiling

Source Parameter	Reference Preparation[1]	Source
Astringency	5000 ppm solution powdered ammonium alum	Rexall Drug Co. Mississauga, Ontario
Alkalinity	50,000 ppm solution sodium bicarbonate	Cow Brand Baking Soda, Church and Dwight Ltd.
Putrid[2]	Undiluted trimethlamine hydrochloride stored in screw top vial	J.T. Baker Chemical Phillipsburg, New Jersey
Sulfurous[2]	50,000 ppm solution thio-acetamide, heated and placed in screw top vial	Fisher Scientific Chemical Manufacturing Division Fair Lawn, New Jersey
Metallic	Pineapple juice stored 10 days in open tin at refrigerator temperature, transferred to glass container until use	Del Monte Pineapple Juice, Canadian Canners, Hamilton
Rancidity[2]	38,000 ppm solution butyric acid stored in screw top vial	Matheson, Coleman, and Bell, Norwood (Cincinnati), Ohio East Rutherford, New Jersey
Mustiness	No reference apart from definition	
Staleness	Unsalted soda crackers	Busy Baker, Empress Foods Ltd. Vancouver, B.C.
Sweetness	20,000 ppm solution commercial sucrose	Manitoba Sugar Company Winnipeg, Manitoba
Saltiness	2000 ppm solution sodium chloride	Windsor Salt, Canadian Salt Co. Vancouver, B.C.

Table 6 cont'd

Source Parameter	Reference Preparation [1]	Source
Sourness	1000 ppm solution citric acid	Matheson, Coleman and Bell Norwood (Cincinnati) Ohio, East Rutherford New Jersey
Bitterness	800 ppm solution caffeine	J.T. Baker Chemical Company, Phillipsburg, New Jersey
Meatiness	1 beef bouillon cube in 250 ml boiling water	Oxo Foods Division Brooke Bond Foods Ltd. Belleville

[1] All solutions prepared in glass distilled water.

[2] Odor references.

Table 7 Description of plant protein sources analyzed for free amino acid content

Plant Protein	Source	Processing Technique	Storage Conditions and age	Total Nitrogen[1] (Dry Weight) %
a) Cereals				
Durum Flour (*Triticum durum*, Stewart)	Department of Plant Science Univeristy of Manitoba Winnipeg, Manitoba	Roller milled with bench equipment	5°C 5 months	2.45
Oat Flour (*Avena sativa*, L.)	General Foods	Experimental commercial sample	5°C	3.16
Rye Flour (*Secale cereale*)	Department of Plant Science University of Manitoba Winnipeg, Manitoba	Roller milled with bench equipment	5°C 5 months	1.87
Triticale Flour (Rosner)	Department of Plant Science University of Manitoba Winnipeg, Manitoba	Roller milled with bench equipment	5°C 5 months	2.26
Wheat Flour (*Triticum aestivum*)	Department of Plant Science University of Manitoba Winnipeg, Manitoba	Roller milled with bench equipment	5°C 5 months	2.48

Table 7 cont'd

Plant Protein	Source	Processing Technique	Storage Conditions and age	Total Nitrogen[1] (Dry Weight) %
b) Oilseeds				
Mustard Concentrate (*Sinapis, alba* L.)	Food Research Institute Ottawa, Ontario	Dehulled, heated, water washed, solvent extracted	5°C 1 year	9.74
Rapeseed Concentrate (*Brassica napus* L., Tower)	Food Research Institute Ottawa, Ontario	Dehulled, heated, water washed, solvent extracted	5°C 1 year	10.06
Soy Flour (*Glycine max.*)	Archer Daniels Midland Company Decatur, Illinois	Flaked, solvent extracted, ground, untoasted	5°C 2 1/2 years	8.61
Soybean Isolate (*Glycine max.*)	Unknown	Promine D		14.7
Sunflower Concentrate (*Helianthus annus* L.)	Crop Science Department University of Saskatchewan, Sakatoon, Saskatchewan	Diffusion extracted for four hours at pH 4.5 solvent extracted	5°C 3 years	11.11

Table 7 cont'd

Plant Protein	Source	Processing Technique	Storage Conditions and age	Total Nitrogen[1] (Dry Weight) %
c) Legumes				
Fababean Flour (*Vicia faba* L.(minor) Diana)	NRC Prairie Regional Labs Saskatoon, Saskatchewan	Dehulled, pin milled	-40°C 8 months	5.04
Fababean Concentrate (A/C) (*Vicia faba* L. (minor),Diana)	NRC Prairie Regional Labs Saskatoon, Saskatchewan	Dehulled, pin milled air classified	-40°C 8 months	10.80
Fababean Concentrate (A/C) (*Vicia faba* L. (minor), Diana)	NRC Prairie Regional Labs Saskatoon, Saskatchewan	Dehulled, pin milled air classfied	5°C 8 months	10.80
Fababean Concentrate (C) (*Vicia faba* L. (minor), Diana)	General Foods	Experimental Commercial sample	5°C 2 years	10.11
Fababean Isolate (*Vicia faba* L. (minor), Diana)	General Foods	Experimental Commercial sample	5°C 2 years	16.06

Table 7 cont'd

Plant Protein	Source	Processing Technique	Storage Conditions and age	Total Nitrogen[1] (Dry Weight) %
Lupin Flour (*Lupinus albus*)	Department of Plant Science, University of Manitoba Winnipeg, Manitoba	Roller milled with bench equipment	5°C 8 months	5.67
Field Pea Flour (*Pisum sativum* L. Trapper)	NRC Prairie Regional Labs Saskatoon, Saskatchewan	Dehulled, pin milled	5°C 8 months	3.91
Field Pea Concentrate (*Pisum sativum* L. Trapper)	NRC Prairie Regional Labs Saskatoon, Saskatchewan	Dehulled, pin milled air classified	5°C 8 months	9.22

[1] Using standard AOAC (1965) methods

III Sensory Evaluation

Seven females (ages 20 - 28) were selected from twelve male and female candidates to participate in the study. All selected panelists were students, graduate students, or staff of the Department of Foods and Nutrition.

As previously outlined, panelists evaluated ten amino acids for the presence of thirteen flavor parameters, constructed intensity patterns of five single amino acids for four flavor parameters and constructed flavor intensity patterns of four binary amino acid mixtures for two flavor parameters. Thus, panel screening, selection and training were conducted in light of these sensory tasks which require on behalf of the panelists the ability to recognize different flavor sensations and the ability to discriminate among intensities within sensory parameters.

All screening sessions and subsequent taste testing sessions were conducted in individual booths in a panel room in an air conditioned laboratory. Judgements in the screening sessions were made under red lighting to mask the color difference of tannic acid when examining candidates for taste recognition. Test session judgements were made under MacBeth Daylite fluorescent lighting. Training sessions were held at a large table in a foods laboratory. These were group sessions held with the purposes of agreeing upon definitions, clarifying the mechanics of tasting procedures and sharing cues in perception of flavor parameters.

A Panel Screening and Selection

When examining candidates for abilities of taste recognition, they were presented with six weak water solutions of chemicals representative of the four basic taste sensations and astringency and a water blank. Chemicals and concentrations used are shown in Table 8. Panelists were asked to identify the taste sensations present in randomized coded samples on the ballot provided (Figure 1).

In the second screening session candidates were instructed in the use of the method of magnitude estimation. They were then asked to measure the intensity of six randomly presented caffeine solutions (300, 600, 900, 1200, 1500 and 1800 ppm) in comparison to a 900 ppm caffeine reference. This test was completed twice by experienced tasters and four times by inexperienced tasters. The ballot used is illustrated in Figure 2. Ability to discriminate bitterness intensity was assessed from success in ranking as judged by Pages -"L" test (Page, 1963).

Performance on taste recognition and Pages L test are outlined in Table 9. Of the twelve candidates seven were selected as follows, N.M., S.J., J.F., N.C., K.H., M.L., and V.B. One panelist, V.B. was only available to complete the first section of the study.

B Panel Training

Five training sessions were held with periodic refresher sessions during the course of the experiment, ie. after a break in tasting sessions of greater than three weeks due to holidays or examination periods. The sensory analysis portion of this study was organized into two sections; amino acids profiling and intensity patterns of single amino acids

Table 8 Chemical solutions representative of the basic tastes and astringency used in candidate screening.

Taste Sensation	Chemical Solution [1] (WT/VOL)
Sweetness	10,000 ppm Sucrose
Sourness	600 ppm Citric Acid
Saltiness	1,000 ppm Sodium Chloride
Bitterness	300, 600 ppm Caffeine
Astringency	500 ppm Tannic Acid

[1] All solutions were prepared in glass distilled water.

Figure 1 Ballot for panel screening for perception of basic tastes and astringency.

BALLOT FOR SCREENING TESTS FOR TASTE

Instructions: In front of you are 7 cups containing weak water solutions of chemicals representing the basic taste sensations. One or more of these may be a blank or a repeat. Your task is to identify the dominant taste in each cup.

Please rinse your mouth with water before you taste each sample. Please taste the samples in the order indicated on this sheet. For each sample, record on the ballot below if the sample is tasteless or has a sweet, salty, sour, bitter taste or astringent mouth feel.

SAMPLE CODE NUMBER	TASTE DESCRIPTION
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____

Figure 2 Ballot used in panel screening for ability to discriminate differences in bitterness intensity of caffeine solutions

FLAVOUR EVALUATION

1. Taste the reference sample and assign it a score.
2. Taste each coded sample in the order presented and estimate the bitterness in relation to the reference.

   Assign a score to each sample.

SAMPLE	SCORE
R	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____

Table 9 Results of screening tests for basic taste sensations and intensity perception of a series of caffeine solutions

Candidate	Taste Sensation*							Pages L Score Maximum - 182.0 Minimum - 112.0 $L = \sum (\gamma_j \sum x_{ij})$
	Blank	Sweet	Sour	Saline	Astringent	Bitter 300 ppm	Bitter 600 ppm	
N.M.								179.5
S.J.								179.0
J.F.	X				X			179.0
N.C.					X			178.5
K.H.			X		X	X		176.0
R.L.				X	X	X		175.5[1]
M.L.						X		175.0
A.W.	X			X		X		173.5[1]
V.B.				X				171.5
D.J.			X			X	X	171.0[2]

Table 9 cont'd

Candidate	Taste Sensation*							Pages L Score Maximum - 182.0 Minimum - 112.0 $L = \hat{\Sigma}(y_j \hat{\Sigma} x_{ij})$
	Blank	Sweet	Sour	Saline	Astringent	Bitter 300 ppm	Bitter 600 ppm	
C.B.	X			X	X		X	169.0[3]
B.W.				X		X	X	163.5[2,3]

* X indicating an error in judgement

1 eliminated due to inconsistent availability

2 eliminated due to inability to recognize bitter sensation at either concentration level

3 eliminated due to low "L" score

and amino acid mixtures. Training for each section was held just prior to the actual testing because the tasks involved in each required slightly different skills from the panelists.

Training for Amino Acid Profiles

Three training sessions were held. The objectives were to acquaint judges with some of the sensations they might encounter while tasting amino acids, to establish or improve upon definitions appropriate to each sensation and to initiate the use of a time controlled tasting procedure.

In the first session panelists were introduced to definitions of flavor sensations with corresponding reference samples. Definitions originating from Amerine et al. (1965) were revised and more appropriate references selected during panel training sessions resulting in those shown in Figure 3 . No appropriate reference was identified for the sensation of mustiness nor was a definition formulated for the meaty taste sensation. Panelists, however, felt that they could recognize these sensations without any difficulty.

In the second and third training sessions panelists were introduced to amino acid solutions and a time controlled tasting procedure. Panelists were presented with a list of definitions, corresponding reference samples and two amino acid solutions. Panelists tasted the sample, held it in the mouth for 7 - 10 seconds and then expectorated. They then proceeded to identify the various sensory parameters in the test materials. The sample volume was not controlled in this sensory task. The time interval of 7 - 10 seconds had been chosen by the panelists.

Figure 3 Revised list of definitions for taste sensations and selected references

DEFINITIONS OF TASTE SENSATIONS

Astringency: Reference: Alum

Quality perceived through the complex of sensations caused by shrinking, drawing, or puckering of the skin surfaces of the oral cavity; dry feeling in the mouth.

Alkalinity: Reference: Baking Soda

A taste sensation usually attributed to a combination of sourness and bitterness (and possibly tactile) stimuli.

Putrid: Reference: Trimethylamine

Unpleasant flavor and odor usually associated with proteolytic spoilage.

Sulfurous: Reference: Hydrogen sulfide

Flavor similar to the odor of rotten eggs.

Metallic: Reference: Pineapple juice

Flavor defect suggesting iron or copper contamination.

Rancidity: Reference: Butryic acid

Having a rank odor or taste as that of old cheese or old oil.

Mustiness: Reference: None

Flavor similar to the odor of a damp, poorly ventilated cellar.

Staleness: Reference: Stale unsalted soda crackers

Not fresh; vapid or tasteless from age, such as stale beer, stale bread, or stale non-fat dry milk.

Figure 3 cont'd

Sweetness: Reference: Sucrose

A rapidly developing sapid sensation which is best tasted at the tip of the tongue.

Saltiness: Reference: Sodium chloride

A saline sensation best tasted at the tip and the sides of the tongue.

Sourness: Reference: Citric acid

A taste sensation usually caused by acids and is best tasted along the edges of the tongue.

Bitterness: Reference: Caffeine

A taste characterized by such compounds as caffeine, quinine, and certain alkaloids which is best tasted at the back of the tongue and may not be perceived until the solution is swallowed.

Meaty: Reference: Oxo

Training for Intensity Patterns

Two sessions were held before the tests measuring taste intensity patterns of amino acids and amino acid mixtures. Most panelists participating in the study were experienced tasters and familar with the method of magnitude estimation. The objectives of the training sessions were to reinforce the use of the method of magnitude estimation, to practice evaluating the intensity of a parameter in a mixture of taste sensations and to acquaint panelists with a controlled tasting procedure. Panelists were presented with increasingly difficult tasks and group performance was evaluated immediately after tasting in order to motivate panelists to perform well and to express any difficulties encountered.

In the first session panelists completed two matching standard tests in which randomized series of five concentrations of chemical solutions were matched to a known ascending series of samples of the same concentrations. The first test consisted of single stimulus samples (caffeine and water) and the second was a more complex mixture (caffeine in a citric acid stock solution).

In the second session panelists were asked to measure the intensity of a parameter in complex solutions of similar and conflicting taste sensations by the method of magnitude estimation. Panelists first evaluated the bitterness intensity of increasing amounts of caffeine in a sucrose stock solution and then increasing amounts of caffeine in a citric acid stock solution. When panelists completed these tasks with only two panelists erring by inverting the two lowest concentration levels, evaluation of amino acid solutions began.

The method of magnitude estimation requires that panelists score a sample for the intensity of a parameter in proportion to the intensity of a selected parameter in a reference sample (Moskowitz, 1975b). For example, if a panelist assigned a caffeine reference a value of 10 for bitterness intensity and then tasted a sample and found it to be one fifth as bitter as the caffeine reference he would assign the sample a score of 2 (10 x 1/5).

When magnitude estimation was used in the training sessions, a controlled tasting procedure was again introduced. This procedure was more stringent than the format used in the profile work due to the increased difficulty of the task of measuring intensity and the number of samples that would be evaluated in each session.

A modification of the taste procedure of O'Mahony and associates (1976) was adopted. Panelists placed 7 ml of sample in the mouth, held it there for 7 - 10 seconds and then expectorated. After the judgement was recorded, panelists rinsed their mouths with glass distilled water, ate a piece of cracker, rinsed again and waited 30 seconds before proceeding to the next sample. This procedure was adhered to for each sample and reference tasted. References were also 7 ml in volume. Judges aided in the development of this controlled procedure suggesting adequate sample volumes as well as appropriate interstimulus procedures.

C Sample Presentation and Tasting Procedures

Amino Acid Profiles

Amino acid profile taste sessions ran for a duration of two weeks. At each session panelists were presented with five amino acid solutions,

a taste or odor reference appropriate to each parameter being evaluated, a corresponding list of definitions, and a ballot. Figure 4 shows the ballot that was used for amino acid profiling. Order of amino acid presentation was randomized among panelists, however, the list of parameters and their corresponding references were always presented in a fixed order as illustrated on the ballot. The five amino acids served at each session were randomly assigned within replications as illustrated in Table 10.

Panelists were asked to taste the amino acid solution according to the procedure outlined on the ballot and to assess the total intensity where a score of 100 would indicate a moderate total intensity. Panelists were then to proceed down the list of descriptions indicating which, if any, were present in the samples. References for taste descriptions could be referred to at any time as long as panelists followed the controlled tasting procedure. The profile for each amino acid was completed before panelists proceeded to the next sample.

Intensity Patterns

Single Amino Acids

Each of the amino acids, arg-hcl, ileu, leu, phe and try were tested for bitterness, astringency, total intensity and pleasantness. All four parameters were tested at one time for each amino acid and panelists received only one amino acid per session. However, panelists received a separate set of solutions for each parameter to be judged and only one parameter was judged at a time. Within each series of amino acids, the different concentrations were randomly presented. The order in which parameters were judged was randomized between panelists at each session.

Figure 4 Ballot used for amino acid profiling

AMINO ACID PROFILING

In front of you are i) a set of references for different taste sensations.
ii) a corresponding list of definitions of taste sensations, and
iii) several amino acid solutions.

It is your task to profile the taste of each amino acid solution by stating whether each taste sensation stated below is present (P) or not present (NP)

Proceed as follows:

1. Taste the first amino acid solution holding it in your mouth for 7 - 10 seconds and then expectorate.
2. Assign the solution a score for total intensity where a score of 100 is moderate intensity.
3. Proceed down the list marking P or NP beside each taste sensation.

You may retaste the sample at any time but each time must control the length of exposure to 7 - 10 seconds. Definitions and references may also be used at any time. Do not limit yourself to the taste sensations stated if you perceive others. State them in the space provided. At the bottom please state the dominant sensation that you perceived. Complete the profile for one amino acid before you proceed to the next.

Taste Sensation	Reference						
Total Intensity	Moderate = 100						
Astringency	Alum						
Alkalinity	Baking soda						
Putrid	Trimethylamine						
Sulfurous	Hydrogen sulfide						
Metallic	Pineapple juice						
Rancidity	Butyric acid						
Mustiness							
Staleness	Stale crackers						
Sweetness	Sucrose						
Saltiness	Sodium chloride						
Sourness	Citric acid						
Bitterness	Caffeine						
Meaty	Oxo						
Others:							
Dominant taste:							

Table 10 Randomized ordering used for amino acid profiles

Replication	Session 1	Session 2
1	HIS	LYS
	PRO	ILEU
	LEU	PHE
	MET	VAL
	TRY	ARG-HCL
2	TRY	PHE
	HIS	ARG-HCL
	LEU	LYS
	ILEU	VAL
	PRO	MET

Within each of the three replications, the order in which the amino acids were examined was randomized as illustrated in Table 11. Amino acid intensity pattern tests ran for a duration of eight weeks. At each session panelists were presented with four series of solutions of one amino acid (a total of 22 - 25 samples), references appropriate to each parameter to be judged and a ballot (Figure 5). A list of definitions was not provided for taste sensations in this section of the study.

Table 12 lists the references used for each of the taste parameters that were judged. Caffeine was selected as a bitterness reference, alum as an astringency reference and internal references were selected for the evaluations of pleasantness and total intensity of each amino acid. An internal reference refers to an identified reference (R) which is the same as one of the coded samples. Internal references differed between amino acids but were the same for both evaluations of pleasantnes and total intensity for any one amino acid.

Amino Acid Mixtures

Each amino acid mixture was evaluated for the two parameters of bitterness and total intensity in reference to an 800 ppm caffeine reference. At each session panelists received six series of amino acid solutions consisting of two series of each of three amino acid mixtures. One series of solutions for each amino acid mixture was evaluated for bitterness intensity while the other was evaluated for total intensity. The samples within each series were randomly presented as was the order of parameter evaluation and amino acid series between judges. Panelists were not advised as to which series of amino acid mixtures were the same.

Table 11 Randomized ordering used in development of single amino acid intensity patterns

	Session				
Replication	1	2	3	4	5
1	ILEU	PHE	LEU	TRY	ARG-HCL
2	ARG-HCL	TRY	PHE	ILEU	LEU
3	ARG-HCL	PHE	LEU	TRY	ILEU

Figure 5 Ballot for assessment of taste intensity

Before you are a series of solutions each varying in intensity, and a reference sample (R). It is your task to magnitude estimate, against the reference, the intensity of each of the solutions. The procedure for testing is given below. Please follow the instructions as closely as possible.

INSTRUCTIONS:

1. Taste the reference, hold it in your mouth for 7 - 10 seconds, expectorate and assign it a value representative of its intensity.
2. Rinse, eat a piece of cracker, rinse again, wait 30 seconds, and proceed to the first (next) sample.
3. Place all the sample in your mouth holding it there for 7 - 10 seconds, expectorate, and assign the sample a value for intensity in relation to the reference.
4. Repeat steps 2 and 3.
5. Continue in this manner for each sample. You may retaste the reference at any time as long as you rinse as instructed.

SAMPLE	INTENSITY SCORE
R	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____

Table 12 References used for assessment of taste intensity patterns of single amino acids

Taste Intensity Pattern	Reference
Bitterness	800 ppm Caffeine
Astringency	800 ppm Alum
Total Intensity[1] and Pleasantness	4000 ppm ARG-HCL for ARG-HCL 8000 ppm ILEU for ILEU 5000 ppm LEU for LEU 2000 ppm TRY for TRY

[1] Total intensity and pleasantness references are only appropriate in assessing stated amino acid.

The amino acid mixtures served at each session were randomly selected as illustrated in Table 13 . Tests for mixtures ran for a three week period.

Caffeine and Alum

Caffeine and alum intensity patterns for bitterness and astringency, respectively, and pleasantness were established between the replications for the single amino acids. Caffeine intensity patterns were measured in reference to an 800 ppm caffeine reference and alum intensity patterns in reference to an 800 ppm alum reference.

At each session, panelists received four series of solutions, two of caffeine and two of alum. One series of each was evaluated for pleasantness and the other was evaluated for bitterness or astringency for caffeine and alum, respectively. Therefore, a total of twenty-eight samples was served at each session.

All intensity patterns were generated using the controlled tasting procedure enumerated under panel training. Additional 7 ml references and 7 ml aliquots of samples were available in the booths for retasting. Panelists could retaste either references or samples as desired so long as the interstimulus procedure was adhered to throughout. All panelists were equipped with stop watches to monitor the tasting procedure.

Table 13 Randomized ordering used for three replications of amino acid mixtures intensity patterns

Session			
1	2	3	4
LEU + ARG-HCL	PHE + ARG-HCL	ILEU + ARG-HCL	PHE + ARG-HCL
TRY + ARG-HCL	TRY + ARG-HCL	PHE + ARG-HCL	TRY + ARG-HCL
ILEU + ARG-HCL	ILEU+ ARG-HCL	LEU + ARG-HCL	LEU + ARG-HCL

IV Chemical Analysis of Free Amino Acids in Plant Protein Samples

The non-protein nitrogen of eighteen plant protein samples was extracted in duplicate, using 80% ETOH as the extracting solvent, according to the method of Bhatty and Finlayson (1973). These authors, however, reported that this solvent may give low yields of the basic amino acids, one in particular being arginine. A TCA (1%) extract was reported to result in a much better yield of the basic amino acids. Since sensory analysis revealed that arg-hcl possessed bitter taste properties it was of particular interest to determine accurately the arginine content of some of the plant proteins and thus TCA extracts were obtained from nine samples.

The ETOH and TCA extracts of the plant protein samples were used to determine their free amino acid content. An aliquot of each sample was used for amino acid analysis in a Beckman Model 117 Automatic Amino Acid Analyzer equipped with a Beckman 125 integrator. Only the basic amino acids present in the TCA extracts were determined and quantitated.

V Analysis of Data

A) Normalization of Magnitude Estimates

Two considerations must be recognized when analyzing sensory data obtained by the method of magnitude estimation. The first is that the variability in scores among panelists is large and the second is that magnitude estimates, which are ratios, are not normally but log-normally distributed (Moskowitz, 1975 b).

Panelist variability was reduced in two ways. Because panelists were free to assign any score to the reference, these scores were all brought to a common value of 10 and the intensity scores adjusted accordingly. Scores were further adjusted by calculating each panelist's geometric mean for each set of data and dividing it through each of the scores. This brought each panelist's scores into the same frame of reference reducing the effects of the different magnitudes of scale chosen by the panelists. The score of the reference past the point of being adjusted to 10 was not included in this second stage of normalization.

Because magnitude estimates are log-normally distributed, the appropriate measure of central tendency is the geometric mean rather than the arithmetic mean. To obtain this measure of central tendency for any set of magnitude estimates the ratios may be converted to logs and the arithmetic mean calculated which is analogous to determining the geometric mean of the antilogs. Data thus treated will be normally distributed and consequently any statistical analysis requiring a normal distribution may be applied (Moskowitz, 1975[b]). All sensory data obtained by the method of magnitude estimation was treated in this manner.

B) Handling of NP s in Magnitude Estimation

If a panelist did not perceive the parameter of interest in a sample, not present (NP) was recorded. This score is in essence a zero value. It is not possible to include zeros in the calculation of geometric means. As this is the desired measure of central tendency when using ratios, a suitable alternate for a zero value is required or the observation must be ignored.

Table 14 lists the parameters, by amino acid and amino acid mixtures, for which intensity patterns could be established. The concentrations listed are those tested which yielded data suitable for use in calculating intensity patterns. Concentrations in which greater than one third of the scores were NP were arbitrarily eliminated from the construction of the intensity patterns while concentrations in which at least two thirds of the scores estimated the parameter were retained. Thus upon occassion NP values did occur in concentrations retained for analysis.

When NP values occurred at the lowest concentration used in an intensity pattern, no score was entered in its place resulting in a different n for the concentration. However, when NP's occurred in the middle or higher concentration levels they were replaced with a calculated value if the panelist had scored at this particular concentration in the other two replications. The value substituted for NP was obtained by calculating a regression equation for the panelist on the basis of his other scores for the replication and generating a value from the equation of the line at which the NP occurred. An illustration of the method used to calculate NP is shown in Table 15 . Specific instances in which calculated NP values were used are shown in Appendix A.

This method of calculating NP was considered superior to a previously used practice of obtaining a number from each panelist which they felt was close to their zero value and substituting it for NP (Malcolmson, 1977). It does not appear that panelists can accurately estimate their NP value by this method. Examples of unadjusted scores for three panelists for the bitterness intensity of increasing concentrations of caffeine

Table 14 Concentrations of compounds used in the final determination of intensity patterns.

Stimulant	Flavor Parameter	Concentrations Used in Construction of Intensity Patterns (ppm)							
ARG-HCL	Bitterness				2000	4000	8000	12000	16000
	Astringency					4000	8000	12000	16000
	Total			1000	2000	4000	8000	12000	16000
	Pleasantness	250	500	1000	2000	4000	8000		16000
ILEU	Bitterness, Astringency					4000	8000	12000	16000
	Total			1000	2000	4000	8000	12000	16000
	Pleasantness	250	500	1000	2000	4000	8000		16000
LEU	Bitterness				1500	5000	10000	12000	15000
	Astringency						10000	12000	15000
	Total			750	1500	5000	10000	12000	15000
	Pleasantness	188	375	750	1500	5000	10000		15000

Table 14 cont'd

Stimulant	Flavor Parameter	Concentrations Used in Construction of Intensity Patterns (ppm)							
PHE[1]	Bitterness				1000	2000	4000	8000	16000
	Total			500	1000	2000	4000	8000	16000
	Pleasantness	125	250	500	1000	2000	4000	8000	
TRY	Bitterness				1000	2000	3000	4000	
	Astringency					2000	3000	4000	
	Total			500	1000	2000	3000	4000	
	Pleasantness	125	250	500	1000	2000	3000	4000	
Mixtures:[2]									
ARG-HCL + ILEU	Bitterness	2000	4000	8000	12000	16000			
	Total	2000	4000	8000	12000	16000			
ARG-HCL + LEU	Bitterness		4000	8000	12000	16000			
	Total	2000	4000	8000	12000	16000			

Table 14 cont'd

Stimulant	Flavor Parameter	Concentrations Used in Construction of Intensity Patterns (ppm)						
ARG-HCL + PHE	Bitterness		4000	8000	12000	16000		
	Total	2000	4000	8000	12000	16000		
ARG-HCL + TRY	Bitterness		4000	8000	12000	16000		
	Total	2000	4000	8000	12000	16000		
Caffeine	Bitterness	200	400	800	1600	3200	6400	
Alum	Astringency	100	2000	400	800	1600	3200	6400

1 Due to insufficient data the astringency intensity pattern was not constructed for PHE (Appendix B).

2 Concentrations stated for mixtures are expressed in ARG-HCL equivalents (approximate values).

Table 15 Illustration of the method used in calculating values for NP

	Bitterness Intensity Scores for Increasing Levels of Caffeine Concentration (ppm)				
Panelist	100	200	400	800	1600
A	1	3	"NP"	9	16
B	2	4	8	12	16
C	4	10	20	40	65

Panelist A's regression line considering the four assigned values:

$Y = 0.67 + .01 X$

To determine NP where $X = 400$

then $Y = 0.67 + .01 (400)$

$= 4.67$

are illustrated in Figures 6a - c. Linear regression analysis was applied to each of these sets of scores in order to obtain a line for perceived bitterness as a function of caffeine at concentrations at which panelists did perceive bitterness. NP values obtained from panelists are indicated on the figures. It is apparent from these illustrations that the NP replacement value stated by the panelist is very different from the value that would be obtained if the panelist's regression lines were extrapolated to the concentration at which the panelist used NP. In these three particular situations, the panelists all under estimated their NP value. Their replacement values would alter the data (inflate the scores) if they were included in the adjusting procedure used for magnitude estimates. It does not appear that substituting a panelist's stated NP is an accurate method of replacing NP scores.

C Statistical Analysis of Data

The scores for total intensity of the 3000 ppm amino acid solutions, obtained during amino acid profiling, were examined by an analysis of variance in order to determine if any significant differences existed. These were identified by testing multiple comparisons by the method of least significant difference (LSD). No statistical analysis was applied to the frequency data obtained for the sensory parameters evaluated in the flavor profiles.

Linear regression analysis was applied to taste intensity patterns of individual amino acids for bitterness, astringency and total intensity as well as bitterness and total intensity of amino acid mixtures. The caffeine intensity pattern for bitterness and the alum intensity pattern for astringency were also analyzed by linear regression. The linear

Figure 6a Percieved bitterness intensity as a function of caffeine concentration at levels at which no NPs occurred for a single panelist

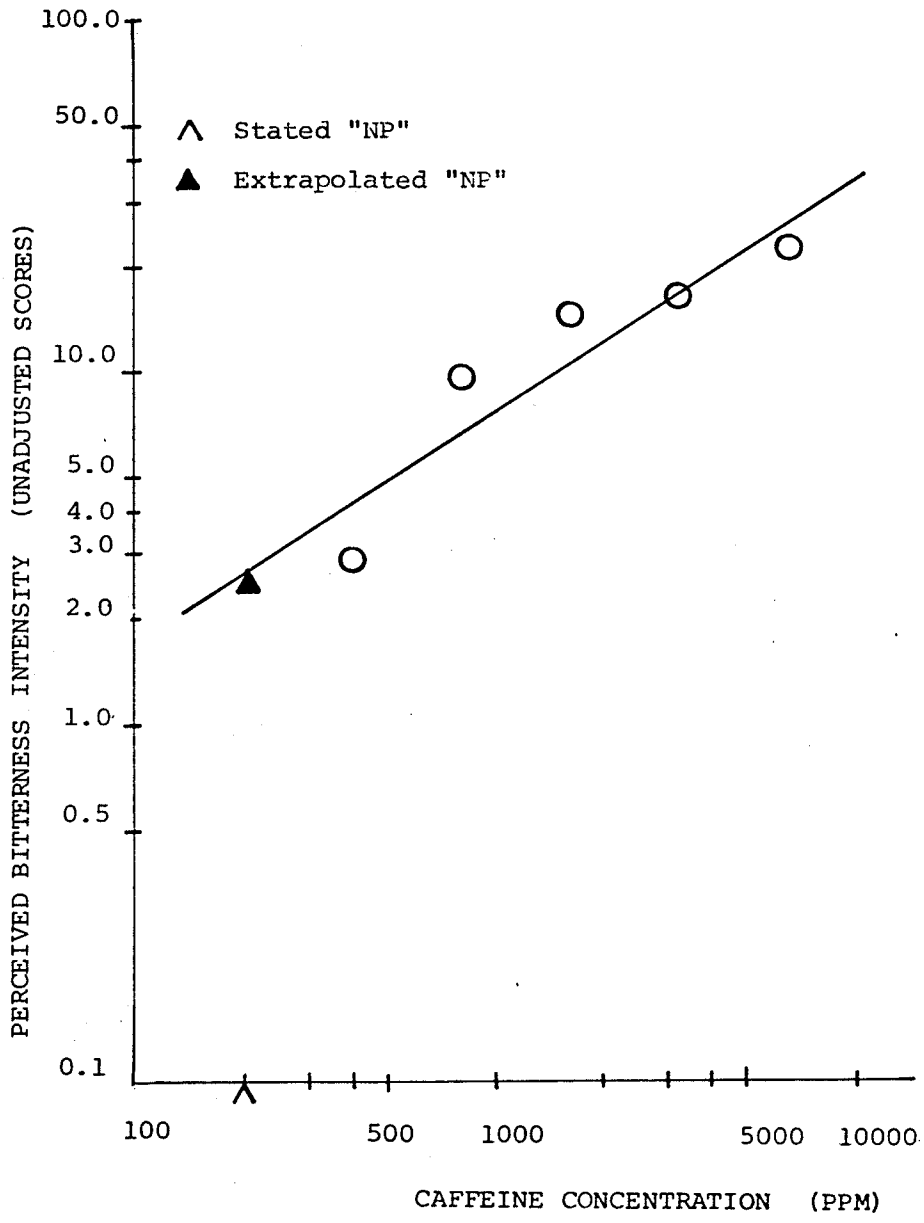


Figure 6b Perceived bitterness intensity as a function of caffeine concentration at levels at which no NPs occurred for a single panelist

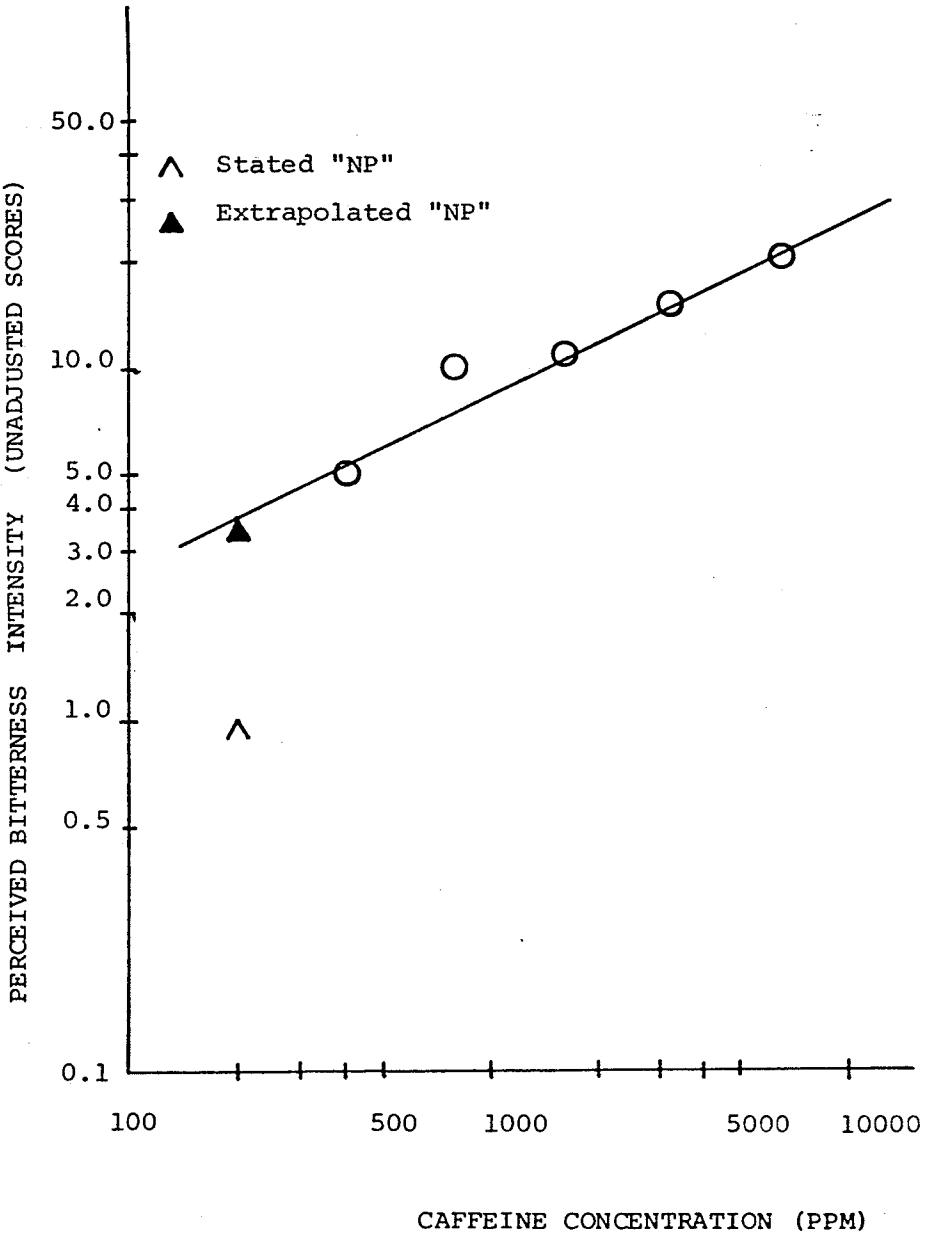
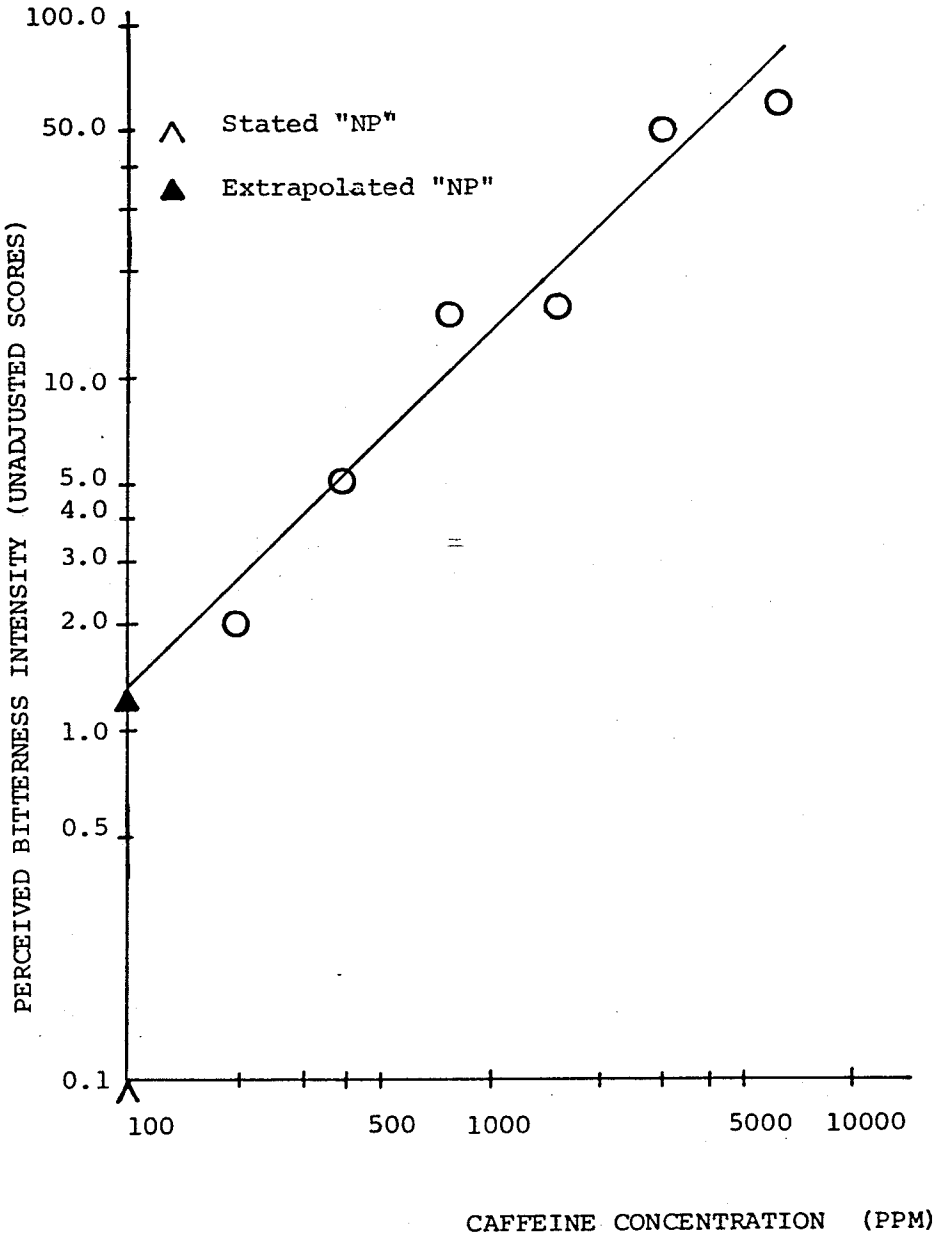


Figure 6c Perceived bitterness intensity as a function of caffeine concentration at levels at which no NPs occurred for a single panelist



regression equations ($Y = a + bx$) were used to generate power functions ($S = kC^n$) which relates sensory intensity (S) to physical concentration (C). The antilog of the intercept of the line is represented by k and the slope by the exponent n. The correlation coefficients (r) which measure the strength of the linear relationship between perceived intensity of a parameter and stimulus concentration were calculated and their significance tested. Only where the correlation coefficient was found to be significant ($p < .05$) was the power function calculated.

Analysis of covariance was used to test for differences among slopes and elevations in comparisons of interests. Where slopes were found to be homogeneous differences in elevation were determined. When slopes in comparisons of interest were found to be significantly different, a t-test was used to assess differences between treatment pairs ($p < .01$). When elevation differences occurred in comparisons of interest, where possible, 99% confidence intervals were constructed about the true difference of adjusted means of treatments.

The use of different reference samples among the amino acids for the evaluation of pleasantness and total intensity placed restrictions upon the analysis that could be applied to the data. Because the rate of growth of the intensity as a function of concentration (slope) is independent of the reference sample, slopes may be compared. Elevation judgements, however, are not independent of the reference samples and may not be compared. As previously stated the pleasantness reference and total intensity reference was the same for an amino acid but different among amino acids. Thus, elevation differences could not be compared among single amino acids for either total intensity or pleasantness intensity.

The comparisons of interest assessed by an anlysis of covariance were as follows:

a) the five single amino acids and caffeine for bitterness intensity

b) ileu and alum for astringency intensity

c) homogenicity of slopes of single amino acids for total intensity

d) homogenicity of slopes of each single amino acid for bitterness and total intensity

e) homogenicity of slopes of ileu for astringency and total intensity

f) homogenicity of slopes of ileu for astringency and bitterness

g) homogenicity of slopes of single amino acids, caffeine and alum for pleasantness discrimination lines

h) homogenicity of slopes of each single amino acid for pleasantness discrimination lines and bitterness

i) homogenicity of slopes of each single amino acid for pleasantness discrimination lines and total intensity

j) the four amino acid mixtures and arg-hcl for bitterness intensity

k) homogenicity of slopes of each amino acid mixture and their component amino acids for bitterness intensity

l) amino acid mixtures for total intensity

m) each amino acid mixture for bitterness and total intensity

Pleasantness data consistently showed 'neutral' reactions, ie. no differences among lower concentrations, and negative slopes at higher concentrations. Accordingly, two regression equations were fitted for each amino acid and their point of intersect was used to

determine an approximate threshold value for unpleasantness. One regression line (neutral) was generated for the points exhibiting the lowest and least significant r value (ie. no relationship between perceived pleasantness and amino acid concentration). The second regression equation (discrimination line) was generated from the points which exhibited the highest and most significant r value (ie. strong and significant relationship between pleasantness perception and amino acid concentration). These two regression equations were equated and solved for X (point of intersect of the two lines). This point of intersect was considered as an approximate estimate of unpleasantness threshold for each amino acid.

RESULTS AND DISCUSSION

I Amino Acid Profiles

Aqueous solutions of ten amino acids, arg-hcl, his, ileu, leu, lys, met, phe, pro, try and val were each evaluated at a concentration of 3000 ppm for a judgement of total flavor intensity and the presence of thirteen flavor parameters. The judgement of total flavor intensity was made in relation to a score of 100 which represented a moderate intensity. The flavor sensations were judged as either present or not present.

Significant differences ($p < .001$) were found to exist among the amino acids for total flavor intensity (Table 16). The treatment means of the amino acids for total intensity are listed in decreasing order in Table IV and significant differences are identified. Tryptophane was found to be significantly stronger in total intensity than all other amino acids. Phenylalanine, arg-hcl, met and lys were intermediate in intensity while ileu, his, val, pro and leu were quite mild.

The flavor profiles of each amino acid are illustrated graphically in Figure 7a-j. These profiles show the frequency with which a parameter was perceived in an amino acid out of a total of fourteen judgements (two replications of seven judgements each). The frequencies of the presence of all parameters in all amino acids are illustrated in Table 18 and the frequency with which each was stated to be the dominant sensation in Table 19. As illustrated some amino acids were quite complex while others appeared to be dominated by one flavor sensation.

The amino acids, his, lys, met, pro and val were not consistently reported among panelists to possess any one particular taste senstation. However, his was most frequently reported to possess bitterness and

Table 16 Analysis of variance of ten 3000 ppm amino acid solutions for total intensity

Source	DF	SS	MS	F
Amino Acids	9	180715.295	20079.477	17.267*
Panelists[1]	13	63194.361	4861.105	
Error	117	136058.934	1162.897	
Total	139	379968.590		

[1] Two replications of judgements by the same seven panelists.

* Significantly different ($p < .001$).

Table 17 Treatment means for total intensity of 3000 ppm amino acid solutions

Amino Acid (3000 ppm)	Treatment Mean For Total Intensity[1] (100 = Moderate Intensity)	
TRY	154	a
PHE	83	b
ARG-HCL	79	bc
MET	66	bcd
LYS	55	bcde
ILEU	44	cde
HIS	38	de
VAL	36	de
PRO	34	de
LEU	26	e

[1]Amino Acids with the same letter are not significantly different ($p < .01$)

LEGEND

ALK - ALKALINITY
AST - ASTRINGENCY
BIT - BITTERNESS
MEA - MEATINESS
MET - METALLIC
MUS - MUSTINESS
PUT - PUTRID
RAN - RANCIDITY
SAL - SALTINESS
SOU - SOURNESS
STA - STALENESS
SUL - SULFUROUS
SWE - SWEETNESS

Figure 7a Flavor Profile of Arginine-Hydrochloride

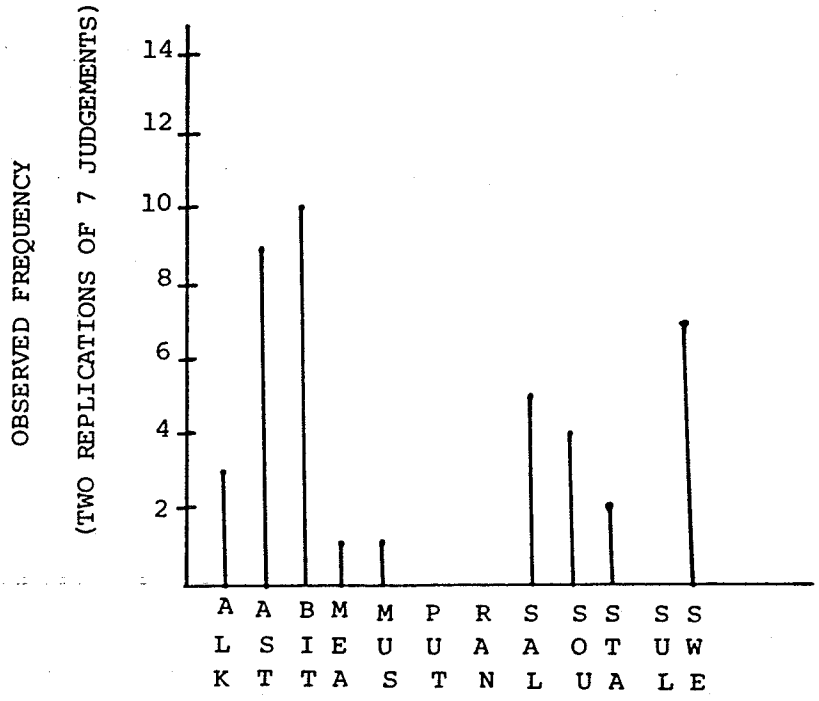


Figure 7b Flavor Profile of Histidine

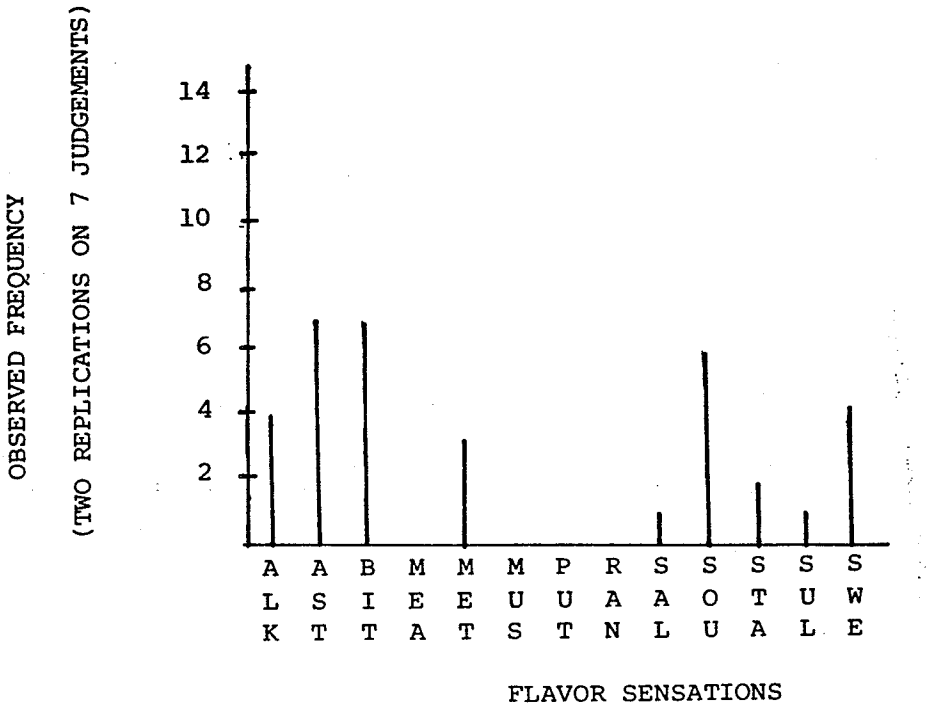


Figure 7c Flavor Profile of Isoleucine

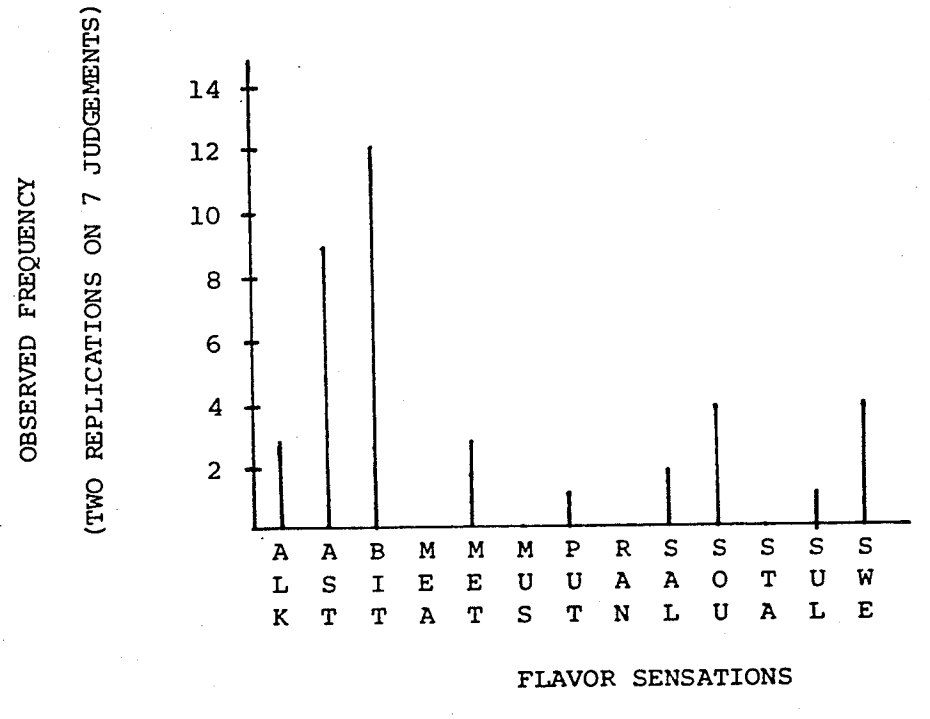


Figure 7d Flavor Profile of Leucine

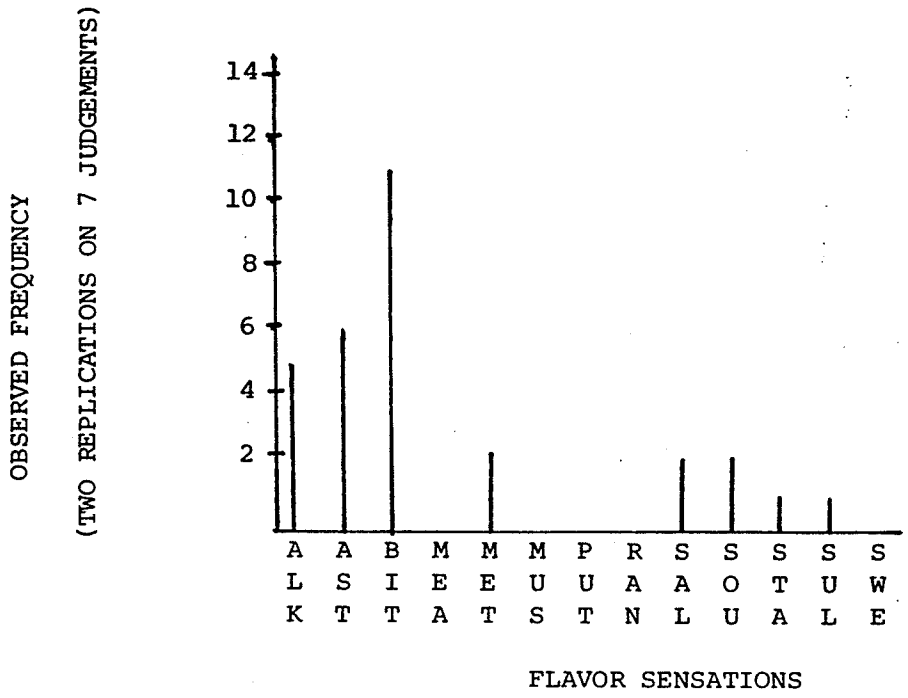


Figure 7e Flavor Profile of Lysine

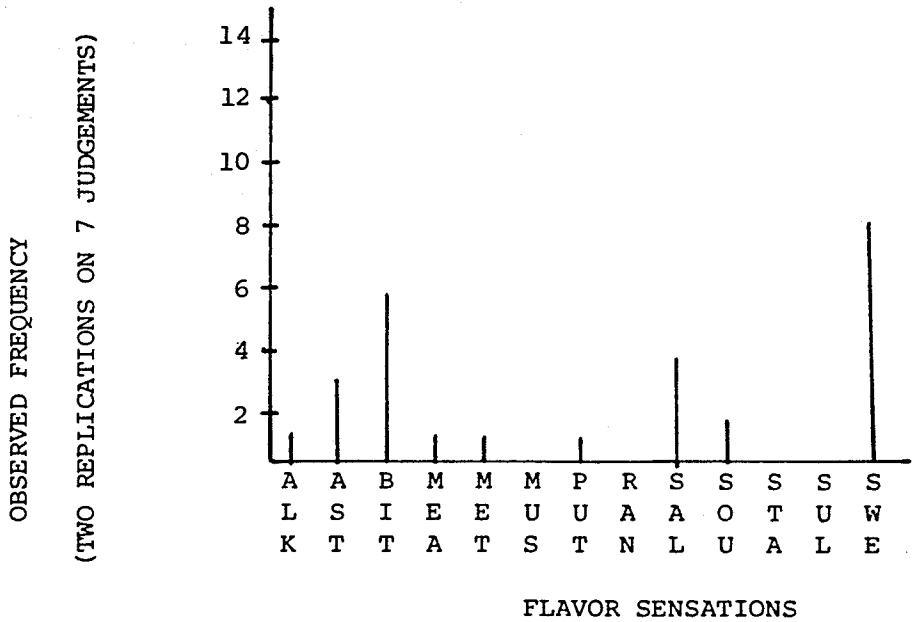


Figure 7f Flavor Profile of Methionine

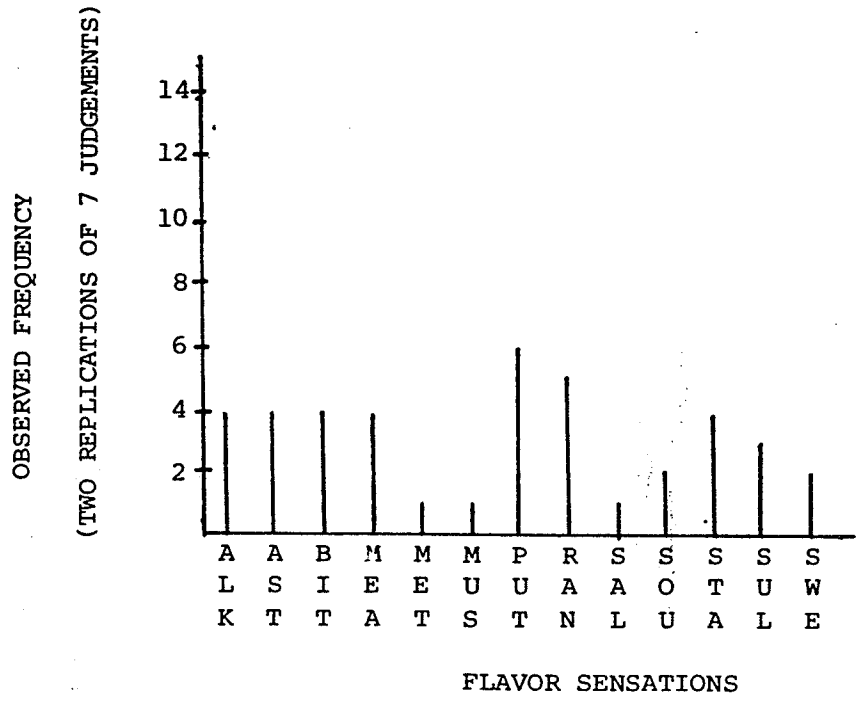


Figure 7g Flavor Profile of Phenylalanine

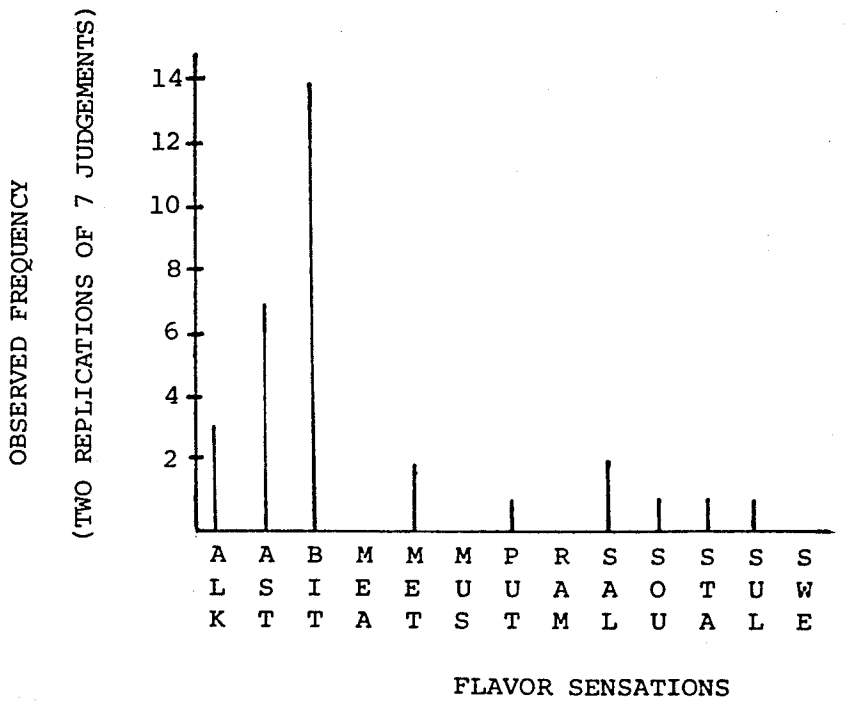


Figure 7h Flavor Profile of Proline

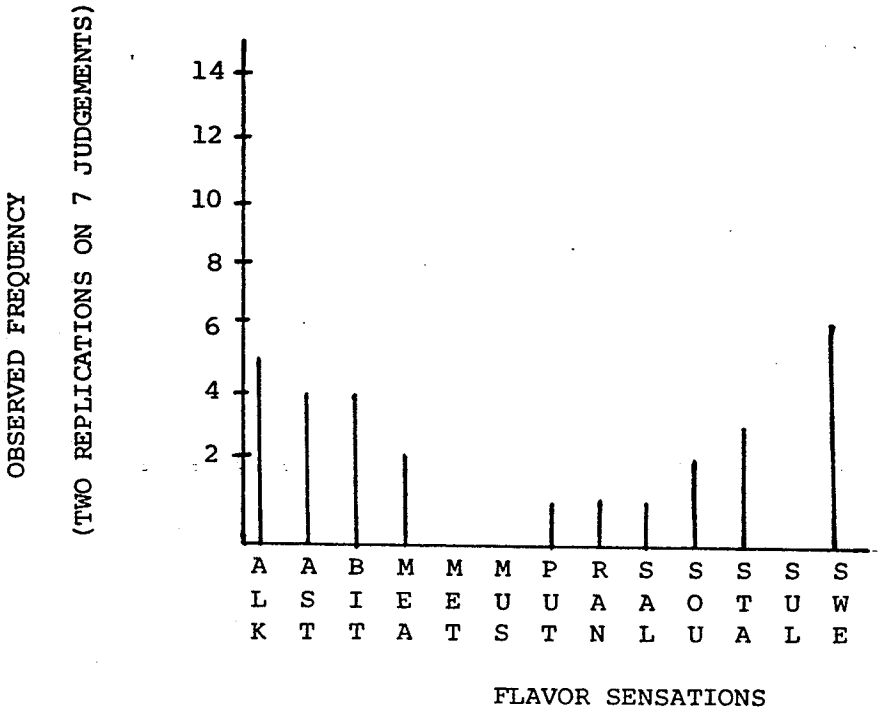


Figure 7i Flavor Profile of Tryptophane

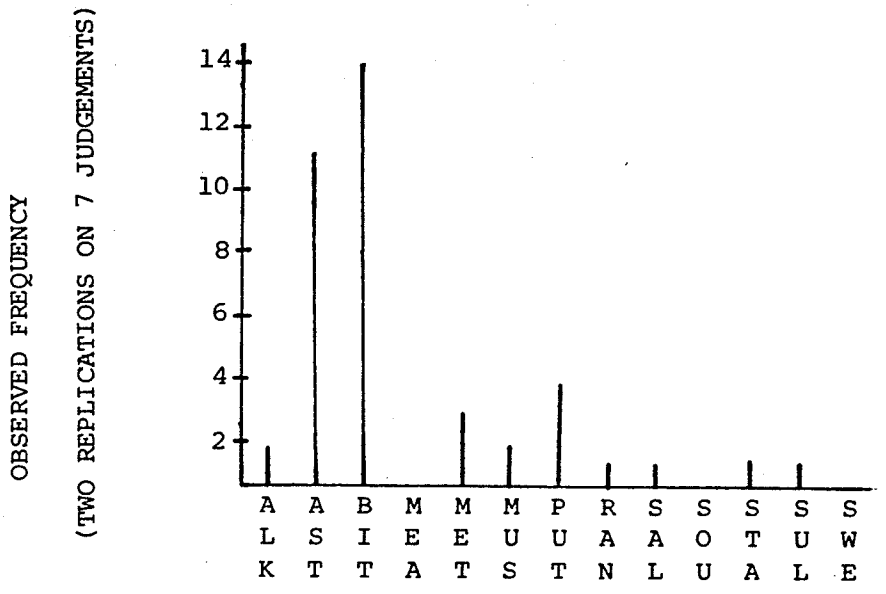


Figure 7j Flavor Profile of Valine

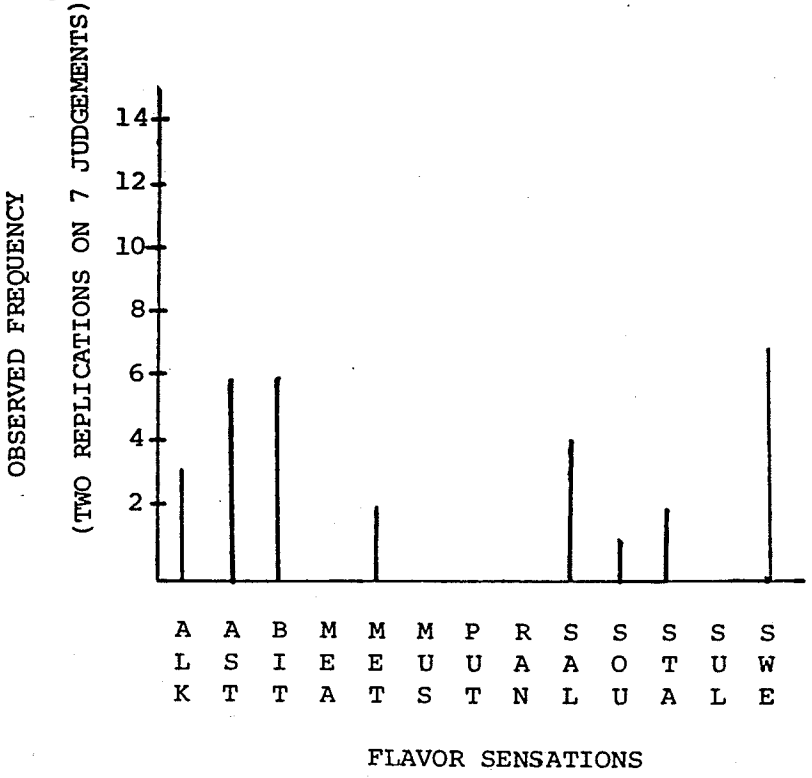


Table 18 Frequency of presence of all parameters in all amino acids[1]

	ALK	AST	BIT	MEA	MET	MUS	PUT	RAN	SAL	SOU	STA	SUL	SWE
ARG-HCL	3	9	10	1	1				5	4	2		7
HIS	4	7	7		3				1	6	2	1	4
ILEU	3	9	12		3		1		2	4		1	4
LEU	5	6	11		2				2	2	1	1	
LYS	1	3	6	1	1		1		4	2			8
MET	4	4	4	4	1	1	6	5	1	2	4	3	2
PHE	3	7	14	2			1		2	1	1	1	
PRO	5	4	4	2			1	1	1	2	3		6
TRY	2	11	14	3		2	4	1	1		1	1	
VAL	3	6	6		2				4	1	2		7

[1]Maximum of 14 judgements for each parameter of each amino acid.

Table 19 Frequency of parameters recorded as the dominant flavor sensation in all amino acids

Amino Acid	ALK	AST	BIT	MEA	MET	MUS	PUT	RAN	SAL	SOU	STA	SUL	SWE
ARG-HCL	2		6						2	2			5
HIS	1	2	4		1					2	1	1	1
ILEU	2	2	7		1		1			1			
LEU	1	2	4		1						1	1	
LYS	1		2				1		4				5
MET	1	1	1	2			4	2			1		1
PHE		1	11						1		1		
PRO	3	1	1	1			1		1	1			4
TRY		2	13		1		1						
VAL	1	2	4						1		1		4

astringency (7 judgements); lys, sweetness (8 judgements); met, a putrid sensation (6 judgements); proline, sweetness (6 judgements), and val, sweetness (7 judgements). The mildness of the total intensity of the samples; 38, 56, 66, 34 and 26 respectively (Table17) suggests that the samples that were tested were too weak for flavor characterization.

The amino acid met, appeared to possess unique flavor properties. Besides the putrid sensation it was reported with some frequency to possess a rancid sensation as well as alkalinity, astringency, bitterness, a meaty flavor and staleness (Table18). Thus, met appears to elicit a complex flavor sensation, the descriptors of which imply undesirable flavor properties.

Valine, also possesses an interesting flavor profile. Although sweetness was reported most frequently, bitterness and astringency were reported almost as often indicating complex and conflicting flavor properties (Table 18). Both sweetness and bitterness were reported with equal frequency as the dominant taste sensations (Table19).

Arginine hydrochloride, ileu, leu, phe and try were all found to possess bitterness as an important taste sensation as evidenced by the frequency of bitterness perception, 9, 12, 11, 14 and 14 judgements, respectively (Table 18). Bitterness was not only noted frequently, but in all cases was the taste sensation most often stated as being the dominant flavor present in these amino acid solutions (Table 19). In all cases, with varying frequencies, astringency appeared to be an accompanying flavor sensation, but was less important than bitterness.

Sweetness was reported quite often in arg-hcl (7 judgements) and in five of these was stated as being the dominant flavor parameter. Leucine was also found to possess alkalinity (5 judgements) but in only one case

was it stated as being the dominant taste sensation. It is of interest to note that try and phe, the strongest amino acids in terms of total intensity scores (Table 17), were those which appeared to be dominated by one taste sensation; bitterness.

The flavor properties of single amino acids as reported in the literature have been summarized previously in Table 1. The results of the amino acid profiles in the present study, as enumerated above, concur with those in the literature with a few exceptions which are outlined below. In most instances, concentration differences and differences between laboratories due to such factors as water and panelist sensitivity could account for conflicting results.

In the present study lys was found to be predominantly sweet with some bitterness components. Schiffman and Dackis (1975) reported only bitterness and saltiness in undiluted lys, while Solms, et al. (1965) and Petritschek et al. (1972) reported it was virtually tasteless. Valine was found to be sweet with accompanying bitterness and astringency. Petritschek et al. (1972) and Kirimura et al. (1969) reported only bitterness and Solms et al. (1965) reported tastelessness. Isoleucine was described in the present study as bitter possessing some astringency. In contrast, Schiffman and Dackis (1975) reported weak, tasteless, flat and dry sensations while Solms et al. (1965) again reported tastelessness. Leucine was also described as possessing bitterness in this study while Schiffman and Dackis (1975) reported it was indistinguisable from ileu.

Those amino acids in which nine or more bitterness judgements were recorded, arg-hcl, ileu, leu, phe and try, were selected for further taste investigations. These five amino acids were evaluated for both bitterness

and astringency as well as total intensity and pleasantness in order to establish growth patterns of each of these sensations as a function of concentration.

II Intensity Patterns

A Bitterness Intensity Patterns

The five amino acids, arg-hcl, ileu, leu, phe and try as well as caffeine were evaluated for bitterness intensity as a function of stimulus concentration in ratio to an 800 ppm caffeine reference. The correlation coefficients and power functions generated from linear regression analysis are shown in Table 20. The correlation coefficient (r) measures the strength of the linear relationship between bitterness perception and stimulus concentration. A significant positive linear relationship was observed in all cases; the relationship was highly significant ($p < .0025$) for all compounds except ileu ($p < .05$). This suggests that the measurement of bitterness intensity for ileu was more difficult for panelists to evaluate. This might be due to some interfering or conflicting taste sensation. Nonetheless, the relationship was significant at $p < .05$ thus ileu was included in subsequent analyses.

Figure 8 shows the linear relationship between bitterness perception and stimulus concentration of the amino acids and caffeine. Significant differences in slope (exponent of power function) were not apparent among the five amino acids and caffeine (Table 21). This homogenicity of slopes indicates that all treatments imparted similar changes in bitterness perception per unit change of concentration. Caffeine was included in these analyses to determine if differences existed between this well recognized bitter compound and amino acids characterized as possessing bitter taste properties.

Significant differences did occur among treatment means adjusted to the overall mean of the sample population of amino acids and caffeine. These adjusted means for each compound are listed in order

Table 20 Relationships between perceived bitterness intensity and concentration for single amino acids and caffeine

Treatment	Correlation Coefficient (r)	Power Function ($S = k\ C^{n}$)
ARG-HCL	.991**	$S=6.35 \times 10^{-5} C^{1.102}$
ILEU	.933*	$S=7.01 \times 10^{-7} C^{1.556}$
LEU	.983**	$S=1.28 \times 10^{-4} C^{1.009}$
PHE	.993**	$S=1.726 \times 10^{-4} C^{1.045}$
TRY	.996**	$S=3.459 \times 10^{-6} C^{1.627}$
CAFFEINE	.962**	$S=8.28 \times 10^{-4} C^{1.002}$

*, ** Significantly different $p < .05$, $< .0025$, respectively.

Figure 8 Perceived bitterness intensity of single amino acids and caffeine as a function of concentration

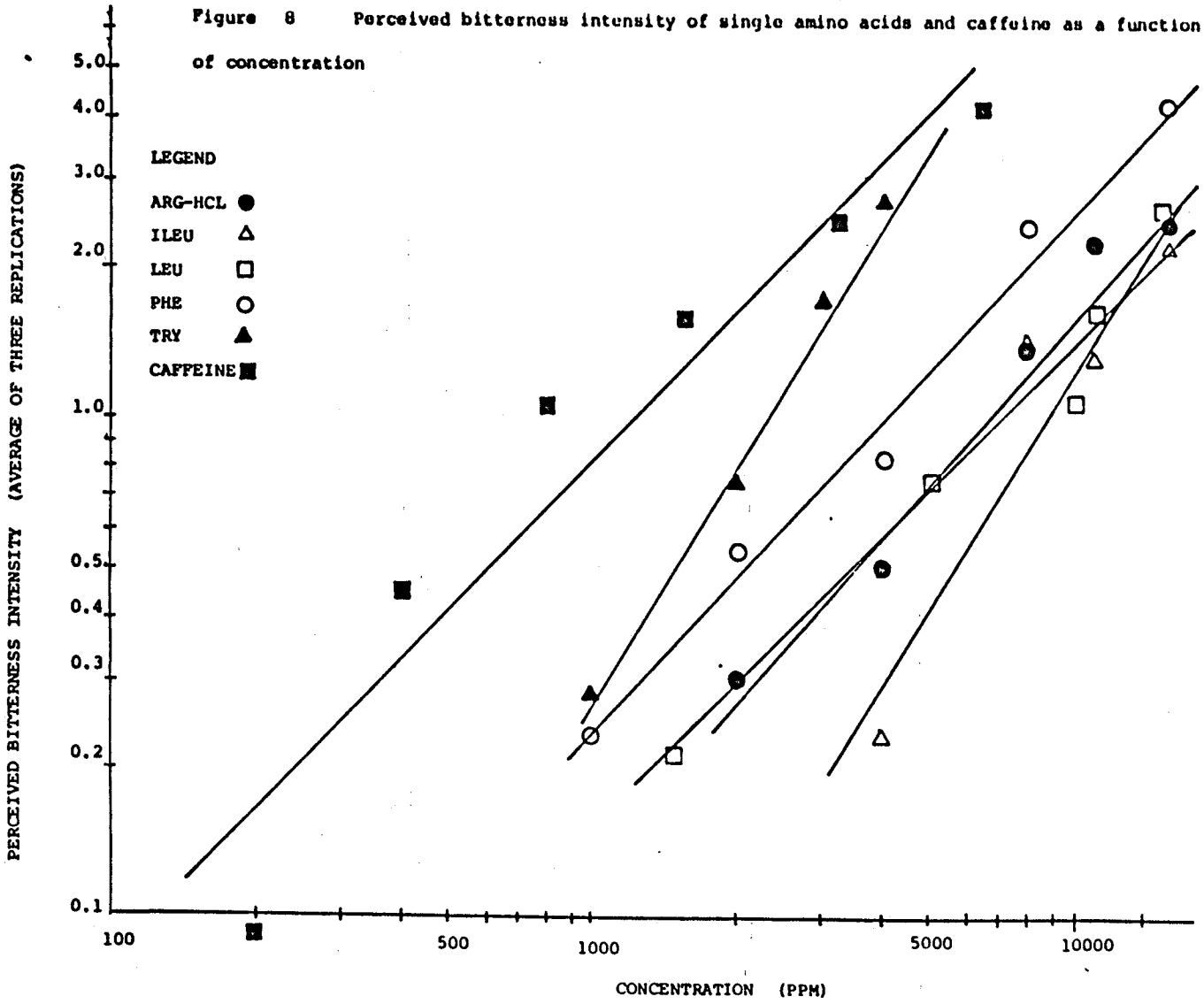


Table 21 Analysis of covariance for bitterness intensity of single amino acids and caffeine in relation to concentration [1]

SOURCE	DF	XX	XY	YY	SLOPE	SSR	SSE	DF	MS
ARG-HCL	4	.538	.593	.664	1.102	.654	.010	3	
ILEU	3	.204	.318	.569	1.556	.495	.074	2	
LEU	4	.655	.661	.691	1.009	.667	.023	3	
PHE	4	.906	.947	1.004	1.045	.990	.014	3	
TRY	3	.204	.333	.546	1.627	.541	.004	2	
CAFFEINE	5	1.586	1.589	1.722	1.002	1.592	.130	4	
POOLED							.255	17	.015
COMMON	23	4.094	4.442	5.195	1.085	4.818	.377	22	.0171
REGRESSION							.122	5	.024
ELEVATION							1.993	5	.399
TOTAL	28	7.104	4.486	5.203	0.632	2.833	2.370	27	

Differences among regression coefficients $F_{5,17} = \frac{.024}{.015} = 1.626$ NS

Differences among ajusted means $F_{5,22} = \frac{.399}{.0171} = 23.286$*

* Significantly different ($p < .001$).

1 This analysis of covariance and all others following were calculated to six decimal places and rounded to three after the total analysis was completed.

of decreasing bitterness intensity in Table 22. Caffeine and try were found to be significantly more bitter than all other treatments while ileu was found to be the least bitter. Phenylalanine was not significantly different from arg-hcl but was more bitter than leu. Arginine hycrochloride was not significantly different from leucine. Thus these amino acids possess different bitterness intensities, try being the strongest and not significantly different from caffeine, ileu being the least intense, and phe, arg-hcl and leu being somewhere in the middle intensity region of bitterness.

B Astringency Intensity Patterns

The five amino acids and alum were measured for astringency intensity as a function of concentration in ratio to an 800 ppm alum reference. Table 23 indicates the correlation coefficients and power functions obtained from linear regression analysis. Only alum and ileu were found to have a significant positive linear relationship between astringency perception and stimulus concentration (r with $p < .05$). This relationship is illustrated in Figure 9 . No significant relationship between astringency intensity and sample concentration was found for arg-hcl, leu, and try. Panelists' inconsistent perception of astringency in phe at all concentrations did not permit the calculation of an intensity pattern (Appendix B). Because of the lack of significant relationships between perception and stimulus concentration, only alum and ileu intensity patterns were analyzed further. The poor relationships obtained for astringency intensity of arg-hcl, leu, phe and try could be due to any one of several factors. Astringency does appear to be a distinguishable parameter as evidenced by the highly

Table 22 Adjusted treatment means for bitterness intensity of amino acids and caffeine

Treatment	Bitterness Intensity Adjusted Treatment Mean[1]
CAFFEINE	3.177 [a]
TRY	2.291 [a]
PHE	0.944 [b]
ARG-HCL	0.556 [bc]
LEU	0.522 [c]
ILEU	0.285 [d]

[1] Treatments with the same letter are not significantly different ($p < .01$)

Table 23 Relationships between perceived astringency intensity and concentration for single amino acids and alum

Treatment[1]	Correlation Coefficient (r)	Power Function[2] ($S = k\ C^n$)
ARG-HCL	.883 NS	
ILEU	.969*	$S = 1.658 \times 10^{-5}\ C^{1.207}$
LEU	.664 NS	
TRY	.414 NS	
ALUM	.996**	$S = 6.53 \times 10^{-4}\ C^{1.099}$

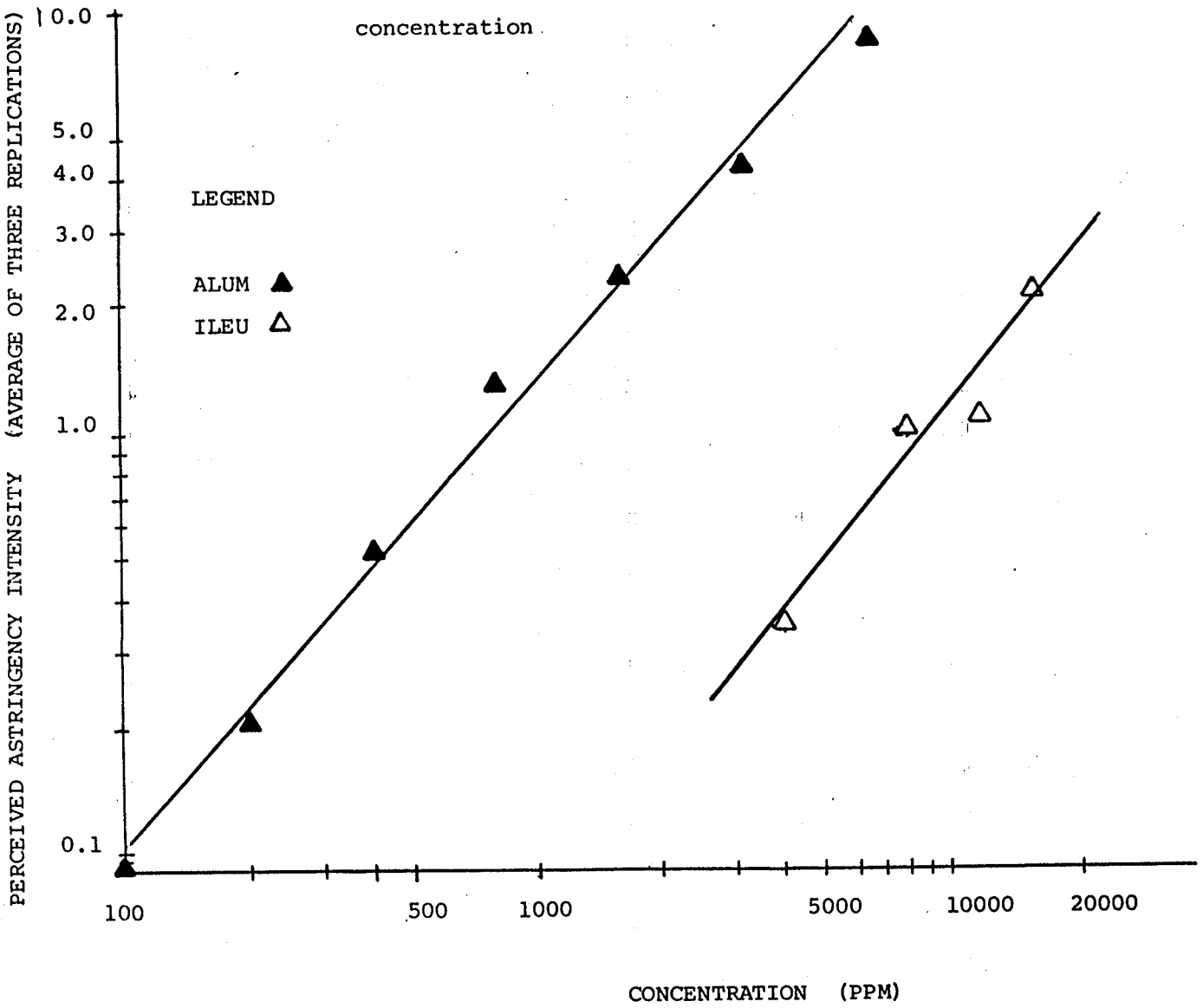
[1] Due to insufficient data, astringency pattern was not developed for PHE (see Appendix B).

[2] Because of the lack of a significant linear relationship between astringency perception and concentration power functions were not generated for arg-hcl, leu, and try.

*, ** Significant relationship $p < .025$, $< .001$, respectively

NS no significant relationship

Figure 9 Perceived astringency intensity of alum and isoleucine as a function of concentration



significant linear relationship between astringency perception and alum concentration. Astringency is defined as a quality perceived through the complex of sensations caused by shrinking, drawing or puckering of the skin surfaces of the oral cavity, a dry feeling in the mouth. Being such a complex sensation, astringency may be difficult to measure and could possibly be confused with bitterness. Another possible explanation for the inconsistent data could be that bitterness was the primary taste sensation and it dominated the astringency present in the samples. The fact that a significant positive linear relationship was established only for ileu which was found to be the least bitter amino acid seems to lend support to this idea. In turn the presence of astringency in ileu might account for the reduced precision of bitterness intensity judgements for this amino acid in comparison to the others. This is illustrated by the reduction in the strength of the linear relationship between concentration and bitterness perception of ileu ($p < .05$) in comparison to the other amino acids ($p < .0025$). The lack of consistent perception of astringency in the samples might also be due to the intensity of the alum astringency reference. In comparison to the 800 ppm alum reference sample the amount of astringency in the test samples might have been negligible as illustrated below.

Alum and ileu intensity patterns did not differ significantly in slope (Table 24). Both compounds induced similar changes (1.099 and 1.207, respectively) in perceived astringency per unit change in concentration. However, highly significant differences in elevation occurred, alum being extremely more astringent than ileu. The magnitude of the elevation differences as demonstrated by the values for the

Table 24 Analysis of covariance for astringency intensity of alum and isoleucine in relation to concentration

SOURCE	DF	XX	XY	YY	SLOPE	SSR	SSE	DF	MS
ALUM	6	2.537	2.789	3.091	1.099	3.066	.025	5	
ILEU	3	.204	.247	.317	1.207	.298	.019	2	
POOLED							.044	7	.006
COMMON	9	2.741	3.036	3.408	1.107	3.362	.046	8	.006
REGRESSION							.002	1	.002
ELEVATION							1.758	1	1.758
TOTAL	10	5.516	2.976	3.410	.540	1.606	1.804	9	

Differences among regression coefficients $F_{1,7} = \frac{.002}{.006} = .333$ NS

Differences among adjusted treatment means $F_{1,8} = \frac{1.758}{.006} = 293.0$*

NS No significant difference.

* Significant difference ($p < .001$).

adjusted treatment means for alum and ileu, 2.655 and 0.152 respectively, reinforces the possibility stated earlier that the 800 ppm alum reference was too intense for comparative purposes with the other amino acids.

C Total Flavor Intensity Patterns

The five amino acids were measured for total flavor intensity against internal reference samples. The correlation coefficients and power functions obtained from linear regression analysis are illustrated in Table 25 . In all cases the correlation coefficients showed a highly significant linear relationship existed between total intensity perception and stimulus concentration. The relationship for the five amino acids is illustrated in Figure 10.

The slopes of the total intensity patterns for amino acids were found to be significantly different (Table 26). Isoleucine had a significantly greater slope than either phe or leu but was not significantly different from arg-hcl or try. Arginine-hydrochloride had a significantly greater slope than leu but was not significantly different from try or phe. Tryptophane, phe, and leu were not significantly different from each other.

The rates of growth for bitterness intensity and total intensity were not always the same within every amino acid (Table 27). The slopes for bitterness intensity of leu, phe, and try were all found to be significantly steeper than the slopes for total intensity of each amino acid. This suggests that once bitterness begins to be perceived its intensity increases more rapidly per unit change in concentration as compared to the total intensity of these amino acids. Thus at higher concentrations it would appear that bitterness would

Table 25 Relationships between perceived total intensity and concentration for single amino acids

Treatment	Correlation Coefficient (r)	Power Function ($S = k\ C^{n}$)
ARG-HCL	.994**	$S=6.710 \times 10^{-4}\ C^{.869}$
ILEU	.963*	$S=1.420 \times 10^{-4}\ C^{1.062}$
LEU	.995**	$S=1.083 \times 10^{-2}\ C^{.535}$
PHE	.991**	$S=4.402 \times 10^{-3}\ C^{.700}$
TRY	.970**	$S=2.690 \times 10^{-3}\ C^{.800}$

*, ** Significant relationship $p < .005, < .001$ respectively

Figure 10 Perceived total intensity of single amino acids as a function of concentration

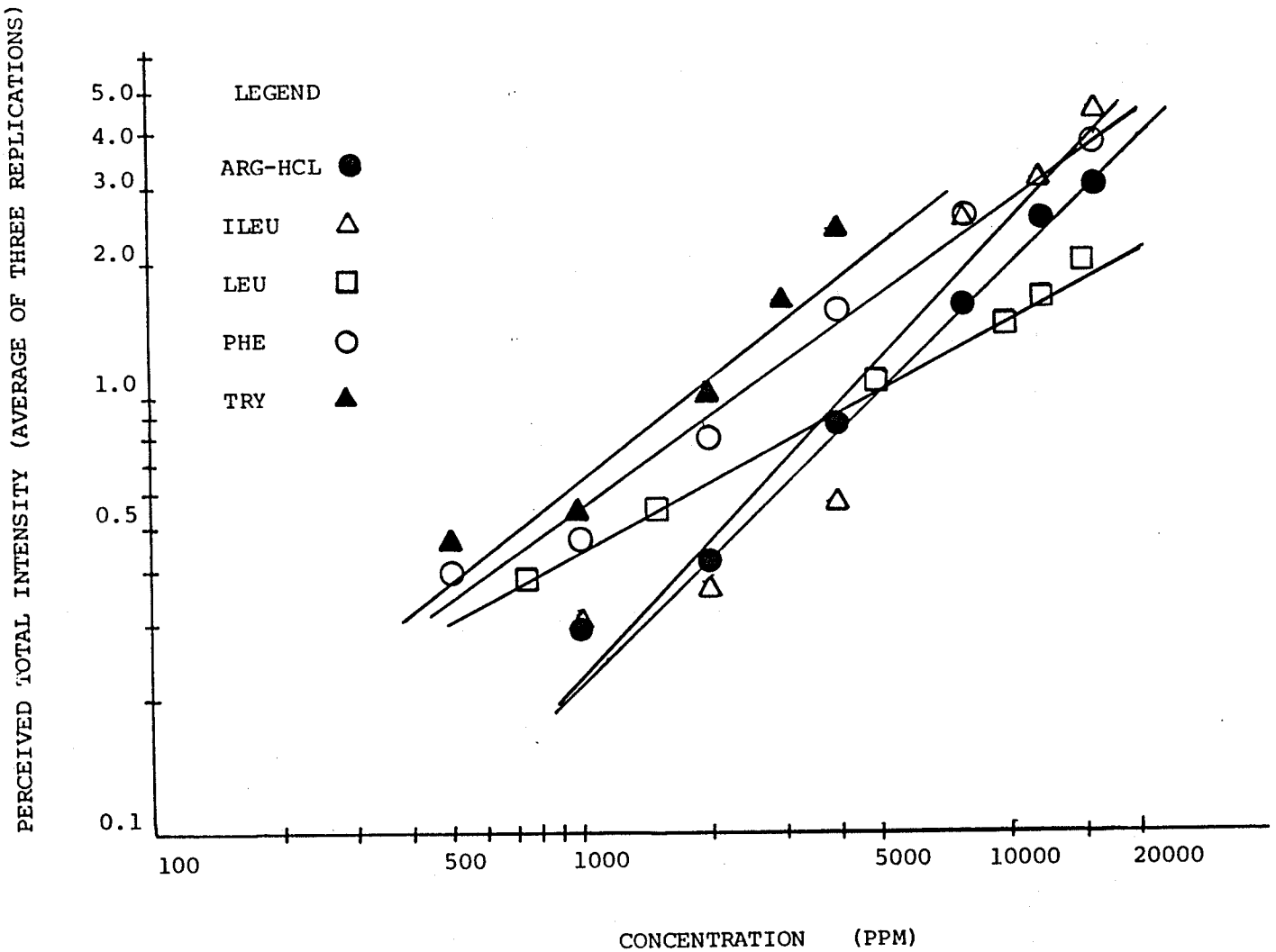


Table 26 Analysis of covariance for total intensity of single amino acids in relation to concentration

SOURCE	DF	XX	XY	YY	SLOPE	SSR	SSE	DF	MS
ARG-HCL	5	1.096	.952	.838	.869[ab]	.828	.010	4	
ILEU	5	1.096	1.164	1.333	1.062[a]	1.236	.097	4	
LEU	5	1.409	.754	.408	.535[c]	.404	.004	4	
PHE	5	1.586	1.109	.791	.700[bc]	.776	.015	4	
TRY	4	.538	.431	.366	.800[abc]	.345	.021	3	
POOLED							.148	19	.008
COMMON	24	5.724	4.410	3.736	.770	3.397	1.012	23	
REGRESSION							.864	4	.216
DIFFERENCE AMONG REGRESSION COEFFICIENTS[1]					$F_{4,19} = \frac{.216}{.008} = 27.00$*				

[1] Treatments with the same letter are not significantly different.

* Significantly different ($p < .001$)

Table 27 Analysis of covariance for bitterness and total intensity of each single amino acid in relationship to concentration

SOURCE	DF	XX	XY	YY	SLOPE	SSR	SSE	DF	MS
a) ARG-HCL									
Bitterness	4	.538	.593	.666	1.102[a]	.654	.010	3	
Total Intensity	5	1.096	.952	.838	.869[a]	.828	.010	4	
Pooled							.020	7	.003
Common	9	1.634	1.546	1.504	.946	1.462	.041	8	
Regression							.021	1	.021
Differences among regression coefficients F 1,7 = $\frac{.021}{.003}$ = 7.000 NS									
b) ILEU									
Bitterness	3	.204	.318	.569	1.556[a]	.495	.074	2	
Total Intensity	5	1.096	1.164	1.333	1.062[a]	1.236	.097	4	
Pooled							.171	6	.028
Common	8	1.300	1.481	1.902	1.139	1.688	.214	7	
Regression							.043	1	.043
Differences among regression coefficients[1] F 1,6 = $\frac{.043}{.028}$ = 1.536 NS									

Table 27 cont 'd

SOURCE	DF	XX	XY	YY	SLOPE	SSR	SSE	DF	MS
c) LEU									
Bitterness	4	.655	.661	.691	1.009[a]	.667	.023	3	
Total Intensity	5	1.409	.754	.408	.535[b]	.404	.004	4	
Pooled							.027	7	.004
Common	9	2.064	1.416	1.099	.686	.971	.128	8	
Regression							.100	1	.100
Differences among regression coefficients[1]					$F_{1,7} = \frac{.100}{.004} = 25.00^*$				
d) PHE									
Bitterness	4	.906	.947	1.004	1.045[a]	.990	.014	3	
Total Intensity	5	1.585	1.109	.791	.700[b]	.776	.015	4	
Pooled							.029	7	.004
Common	9	2.492	2.056	1.794	.825	1.697	.098	8	
Regression							.069	1	.069
Differences among regression coefficients[1]					$F_{1,7} = \frac{.069}{.004} = 17.25^*$				

Table 27 cont 'd

SOURCE	DF	XX	XY	YY	SLOPE	SSR	SSE	DF	MS
e) TRY									
Bitterness	3	.204	.333	.546	1.627[a]	.541	.004	2	
Total Intensity	4	.538	.431	.366	.800[b]	.345	.021	3	
Pooled							.025	5	.005
Common	7	.743	.763	.912	1.028	.785	.127	6	
Regression							.102	1	.102
Differences among coefficients[1]					$F_{1,5} = \frac{.102}{.005} = 20.40^*$				

1 Treatments with the same letter are not significantly different.

* Significantly different ($p < .01$).

account for greater and greater amounts of the total intensity. Significant differences in slope were not found to occur between these two parameters in arg-hcl and ileu indicating that for these two amino acids both bitterness and total intensity grow in a similar fashion.

The rate of growth of astringency intensity and total intensity was not significantly different for ileu (Table 28). Similarly, the rate of growth for bitterness intensity and astringency intensity of ileu did not differ significantly (Table 29). Thus for ileu, the intensity of all three parameters of bitterness, astringency and total intensity grew at an approximately constant rate.

D Pleasantness Intensity Patterns

The pleasantness intensity patterns for arg-hcl, ileu, leu, phe, and try are illustrated in Figures 11a - e. At lower concentrations neutral lines are evident showing no differences in perception as a function of concentration while at higher concentrations discrimination lines are apparent demonstrating negative slopes as a function of increasing concentration. Table 30 illustrates both linear regression equations (neutral and discrimination) for the pleasantness intensity patterns of each amino acid along with their correlation coefficients and calculated unpleasantness threshold values. Unpleasantness threshold values in this text refer to that point (concentration) at which pleasantness departs from neutrality to form a linear relationship with concentration. If the departure from neutrality is due to increased pleasantness a positive linear relationship will result.

Table 28 Analysis of covariance for astringency and total intensity of isoleucine in relationship to concentration

SOURCE	DF	XX	XY	YY	b	SSR	SSE	DF	MS
ILEU (Astringency)	3	.204	.247	.317	1.211[a]	.299	.018	2	
ILEU (Total)	5	1.096	1.164	1.333	1.062[a]	1.236	.097	4	
Pooled							.115	6	.019
Common	8	1.30	1.411	1.65	1.085	1.532	.118	7	
Regression							.003	1	.003
Differences among regression coefficients[1]					$F_{1,6} = \frac{.003}{.014} = .158$ NS				

[1] Treatments with the same letter are not significantly different.

NS No significant difference.

Table 29 Analysis of covariance for astringency and bitterness of isoleucine in relationship to concentration

SOURCE	DF	XX	XY	YY	b	SSR	SSE	DF	MS
ILEU (Astringency)	3	.204	.247	.317	1.211[a]	.299	.018	2	
ILEU (Bitterness)	3	.204	.318	.569	1.556[a]	.496	.073	2	
Pooled							.091	4	.023
Common	6	.408	.565	.886	1.385	.782	.104	5	
Regression							.013	1	.013
Differences among regression coefficients[1]					$F_{1,4} = \frac{.013}{.023} = .565$ NS				

[1] Treatments with the same letter are not significantly different.

NS No significant difference

Figure 11a Pleasantness intensity of arginine-hydrochloride as a function of increasing concentration

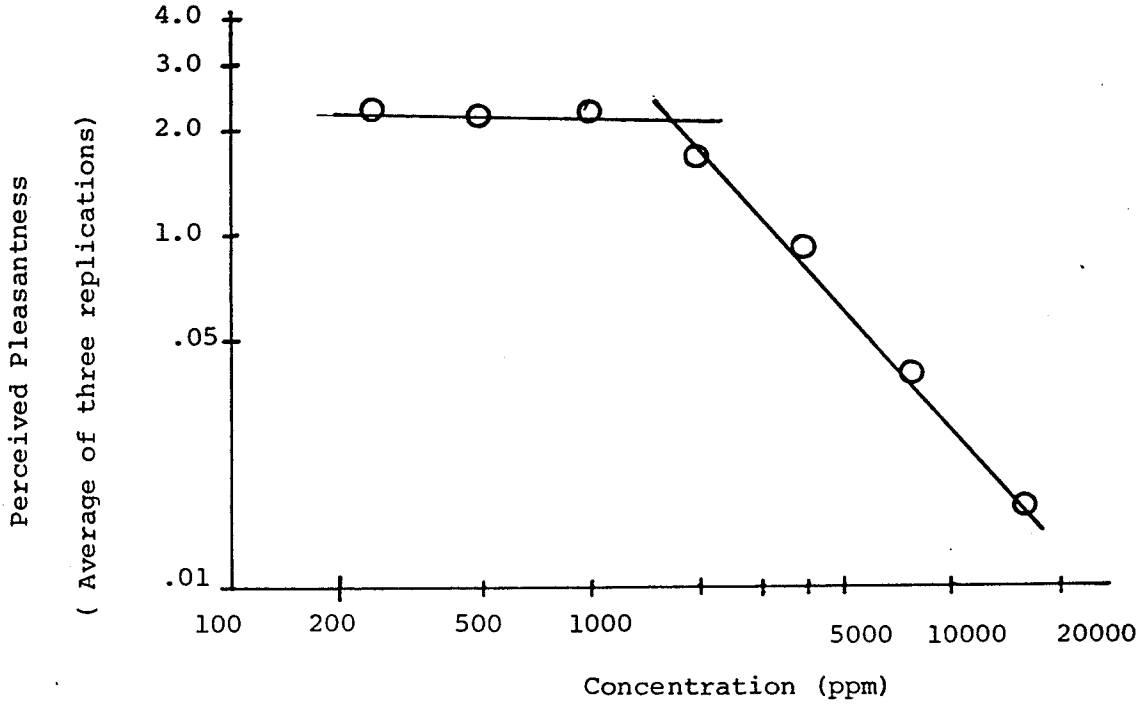


Figure 11b Pleasantness intensity of isoleucine as a function of increasing concentration

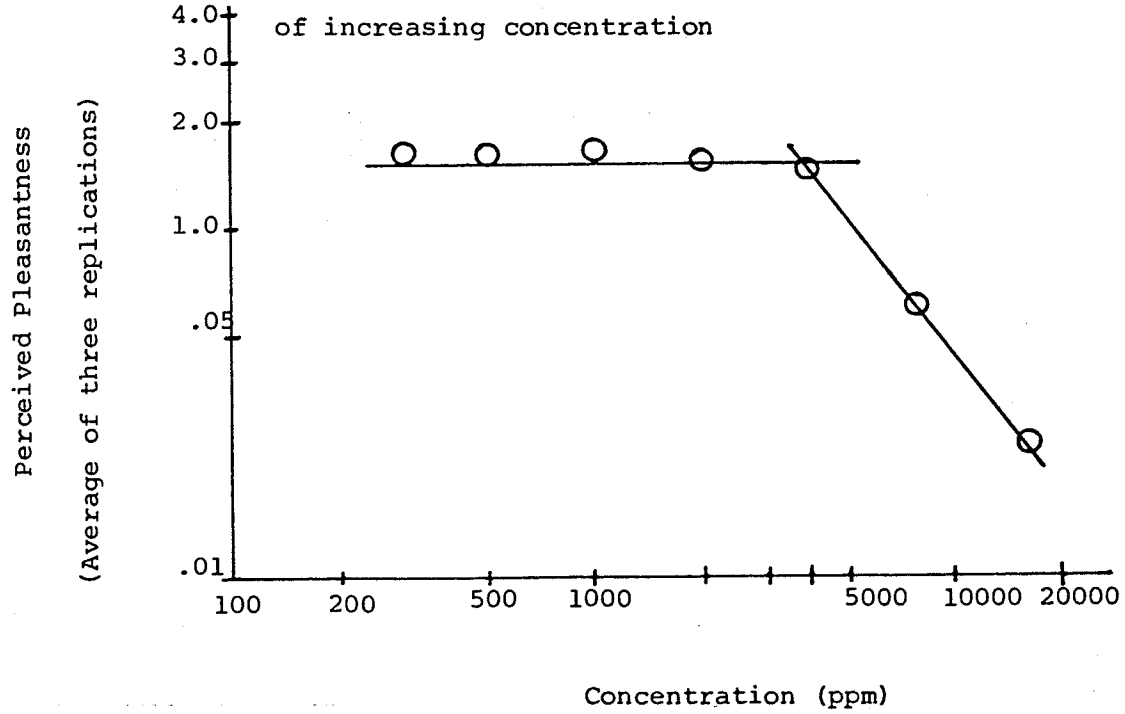


Figure 11c Pleasantness intensity of leucine as a function of concentration

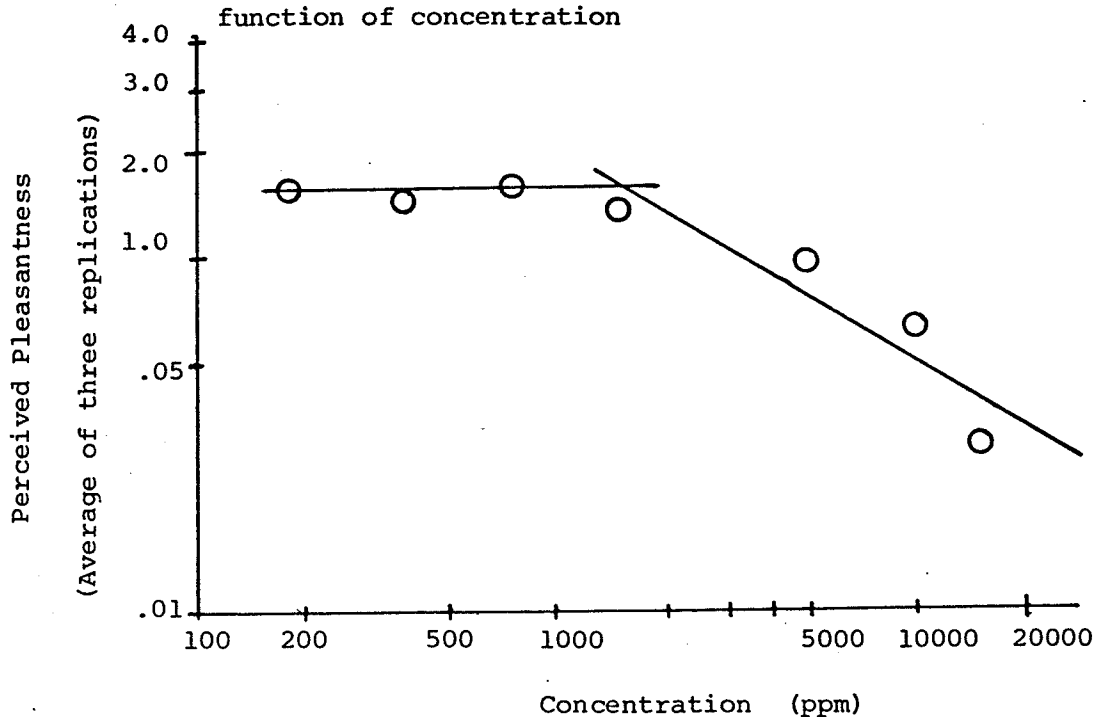


Figure 11d Pleasantness intensity of phenylalanine as a function of concentration

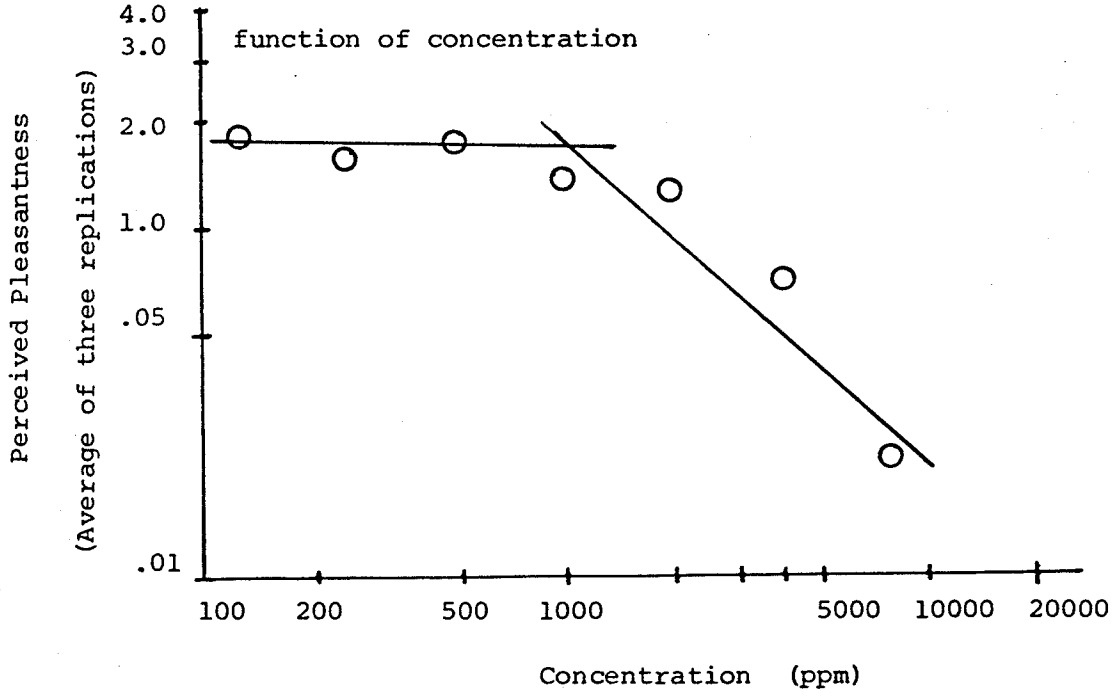


Figure 11e Pleasantness intensity of tryptophane as a function of concentration

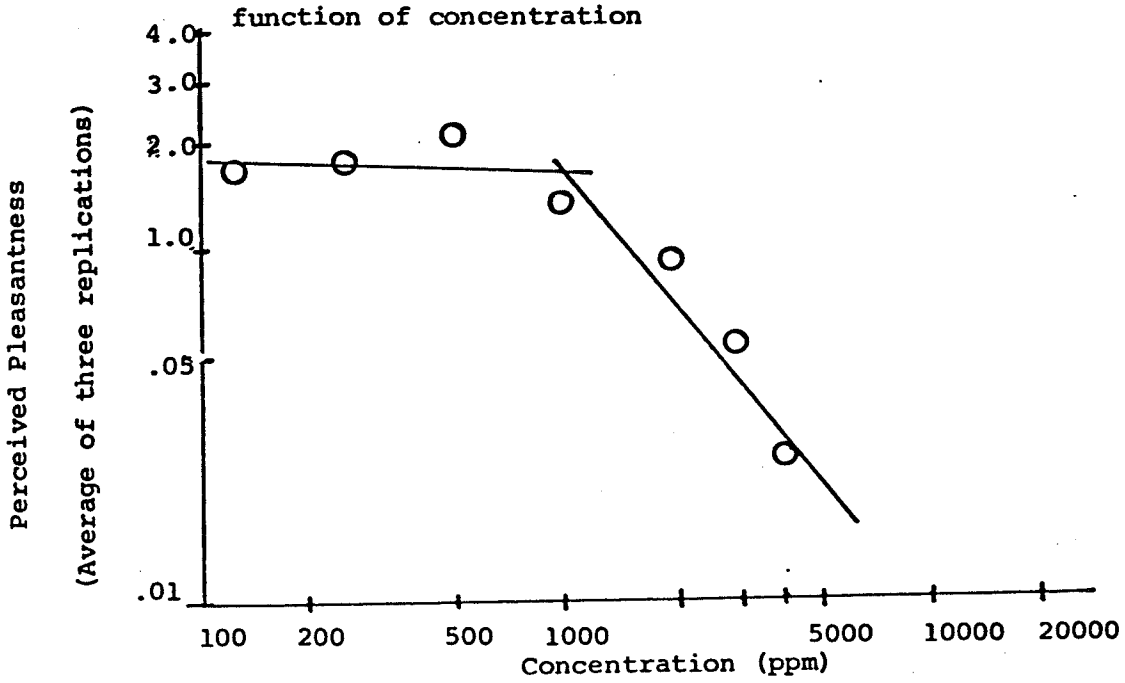


Figure 12 Pleasantness intensity of caffeine as a function of concentration

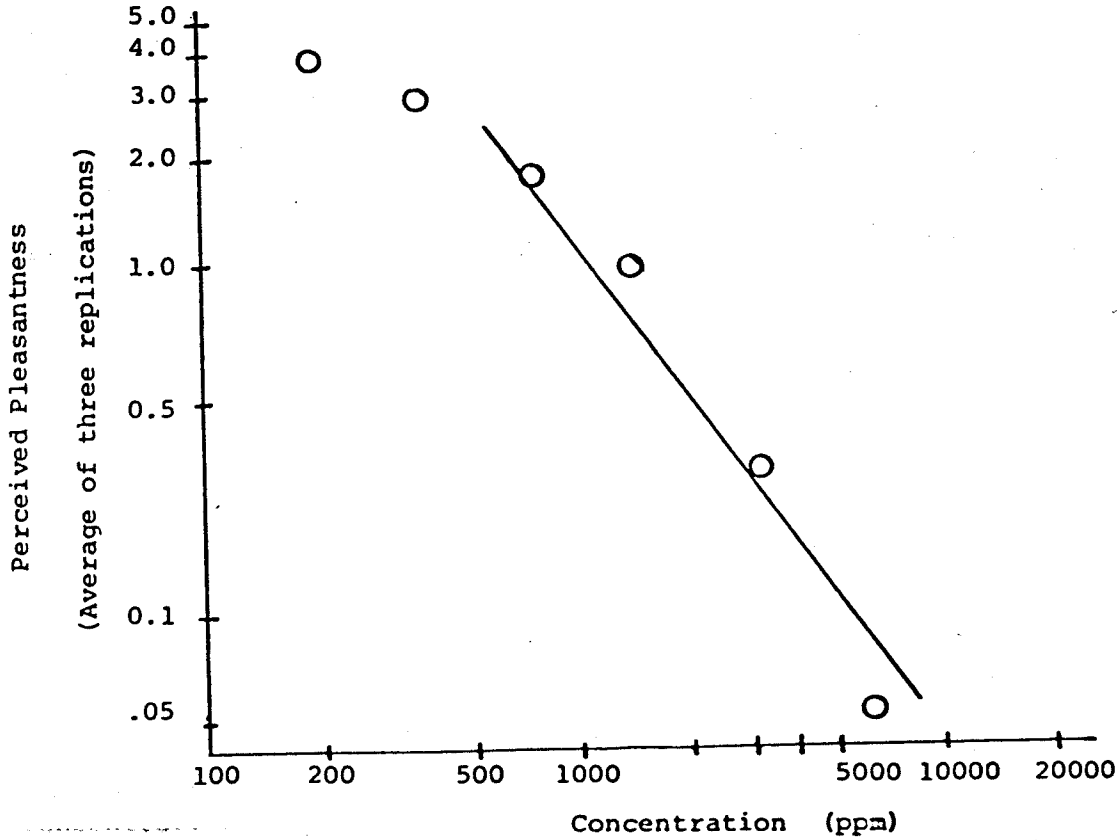


Table 30 Relationship between perceived pleasantness intensity and concentration; and calculated unpleasantness threshold values for single amino acids

AMINO ACID	NEUTRAL LINE REGRESSION EQUATION	r	DISCRIMINATION LINE REGRESSION EQUATION	r	CALCULATED UNPLEASANTNESS THRESHOLD[2]
ARG-HCL	Y = .386 - .015 X	.371	Y = 3.911 - 1.109 X	.996*	1662.185
ILEU[1]	Y = .215 - .009 X	.164	Y = 4.783 - 1.284 X	1.0**	3815.366
LEU	Y = .212 - .008 X	.131	Y = 2.131 - .604 X	.913*	1671.115
PHE	Y = .294 - .0266X	.257	Y = 2.912 - .891 X	.932*	1066.393
TRY[1]	Y = .372 - .054 X	.293	Y = 3.693 - 1.161 X	.945*	999.601

1 The two regression lines have a common point

2 Calculated as the point when the neutral and discrimination lines intersect

*, **Significant relationship $p < .05, < .005$, respectively

Conversely if it is unpleasant a negative linear relationship will occur. The latter was the case with all amino acids assessed in this experiment. It should be noted that both ileu and try share a common point for each of their regression equations (4000 ppm and 1000 ppm, respectively). These two points appeared to be in close proximity to the initial decline in pleasantness in each situation and thus were used as common points to both the neutral and discrimination lines. The calculated threshold values (Table 30) suggest that try has the lowest unpleasantness threshold closely followed by phe and then arg-hcl and leu and lastly ileu, possessing the highest unpleasantness threshold.

The pleasantness intensity patterns obtained for caffeine and alum did not permit the calculation of a threshold value. With both compounds the neutral perception line was not clearly evident indicating that the threshold values for both were close to initial concentration levels or lower. The discrimination lines, however, were evident and Figures 12 and 13 illustrate these relationships as a function of concentration. The regression equation for the discrimination line for each compound was calculated on that combination of points which produced the highest correlation coefficient. The regression lines and correlation coefficients are shown in Table 31.

Significant differences were not observed among the slopes of the perception lines for the amino acids, caffeine and alum (Table 32). The slope of the perception line indicates the relative change in pleasantness per unit change in concentration. All compounds possessed negative slopes indicating increasing unpleasantness in relation to increasing concentration levels. Elevation differences were not compared

Figure 13 Pleasantness intensity of alum as a function of concentration

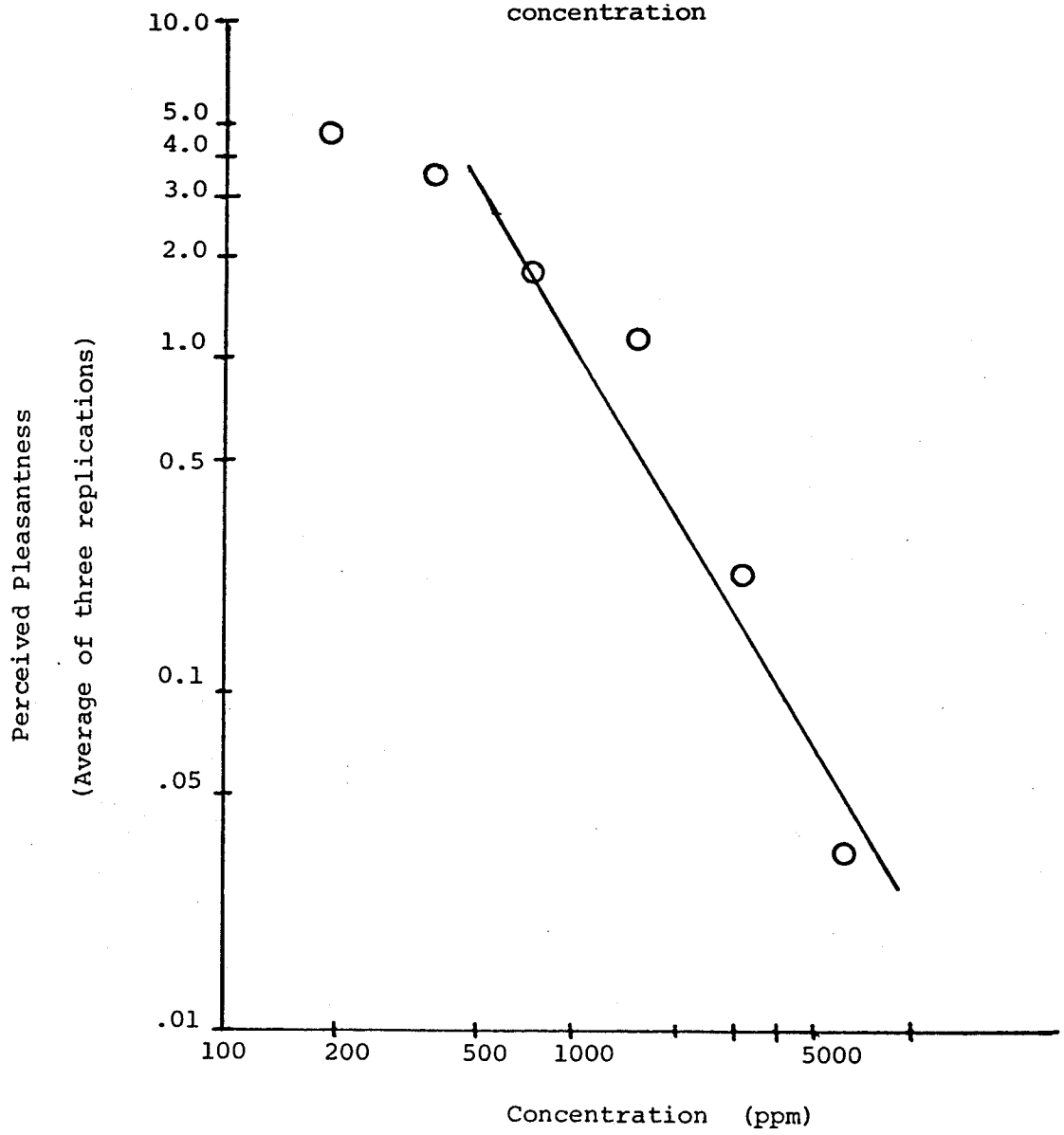


Table 31 Relationship between perceived pleasantness intensity and concentration for caffeine and alum

COMPOUND	PERCEPTION LINE REGRESSION EQUATION	r
ALUM	Y = 5.000 - 1.641 X	.926*
CAFFEINE	Y = 4.347 - 1.428 X	.974

* Significant relationship ($p < .01$)

Table 32 Analysis of covariance for pleasantness of single amino acids and caffeine and alum in relationship to concentration

SOURCE	DF	XX	XY	YY	SLOPE	SSR	SSE	DF	MS
ARG-HCL	3	.453	- .502	.561	- 1.109	.557	.00392	2	
ILEU	2	.181	- .233	.299	- 1.284	.299	.000	1	
LEU	3	.575	- .347	.251	- .604	.210	.042	2	
PHE	3	.453	- .404	.414	- .891	.360	.054	2	
TRY	3	.204	- .237	.309	-1.161	.276	.033	2	
ALUM	4	.906	-1.487	2.634	-1.641	2.439	.195	3	
CAFFEINE	4	.906	-1.294	1.946	-1.428	1.847	.099	3	
POOLED							.427	15	.0284
COMMON	22	3.678	-4.504	6.414	-1.225	5.516	.898	21	
REGRESSION							.417	6	.0785

Differences among regression coefficients $F_{6,15} = \frac{.0785}{.0284} = 2.786$ NS

NS No significant differences.

as each pleasantness intensity pattern was measured against a different reference compound.

No significant differences were identified between the rate of growth of bitterness intensity and the rate of decline in pleasantness in any of the amino acids arg-hcl, ileu, leu and phe (Table 33). This indicates that in these four amino acids the growth of bitterness intensity occurred at approximately the same rate as pleasantness decreased. This was not found to be the case for tryptophane. Tryptophane increased in bitterness at a significantly faster rate ($p < .05$) than it declined in pleasantness. The same comparison between pleasantness and total intensity indicated that increasing total intensity and decreasing pleasantness occurred at the same rate in the amino acids ileu, leu, phe and try (Table 34). Arginine-hydrochloride, however, was found to decrease in pleasantness at a significantly faster rate ($p < .05$) than it increased in total intensity.

For the amino acids ileu, leu, and phe the decrease in pleasantness occurred at the same rate as both the increase in bitterness and total intensity. In the case of try the decrease in pleasantness occurred at the same rate as the increase in total intensity rather than bitterness. Conversely, in arg-hcl the decrease in pleasantness occurred at a similar rate as the increase in bitterness rather than total intensity.

In order to obtain greater accuracy when establishing pleasantness intensity patterns it would be necessary to increase the number of samples and decrease concentration intervals between samples. This would provide more points for the generation of both neutral and discrimination lines thus yielding a stronger measure of the relationship

Table 33 Analysis of covariance for pleasantness and bitterness intensity of each single amino acid in relationship to concentration

SOURCE	DF	XX	XY	YY	SLOPE	SSR	SSE	DF	MS
a) ARG-HCL									
Pleassantness	3	.453	.502	.561	1.109[a]	.557	.004	2	
Bitterness	4	.538	.593	.664	1.102[a]	.654	.010	3	
Pooled							.014	5	.003
Common	7	.991	1.096	1.226	1.106	1.212	.014	6	
Regression							.000	1	.000
Differences among regression coefficients[1]					$F\ 1,5 = \frac{.000}{.003} = \infty$		NS		
b) ILEU									
Pleasantness	2	.181	.233	.299	1.284[a]	.299	.000	1	
Bitterness	3	.204	.318	.569	1.556[a]	.495	.074	2	
Pooled							.074	3	.025
Common	5	.386	.551	.868	1.428	.787	.081	4	
Regression							.007	1	.007
Differences among regression coefficients[1]					$F\ 1,3 = \frac{.007}{.025} = .289$		NS		

Table 33 cont'd

SOURCE	DF	XX	XY	YY	SLOPE	SSR	SSE	DF	MS
c) LEU									
Pleasantness	3	.575	.347	.251	.604[a]	.210	.042	2	
Bitterness	4	.655	.661	.691	1.009[a]	.667	.023	3	
Pooled							.065	5	.013
Common	7	1.231	1.009	.942	.820	.827	.115	6	
Regression							.050	1	.050
Differences among regression coefficients[1]					$F_{1,5} = \frac{.050}{.013} = 3.875$ NS				
d) PHE									
Pleasantness	3	.453	.404	.414	.891[a]	.360	.054	2	
Bitterness	4	.906	.947	1.004	1.045[a]	.990	.0138	3	
Pooled							.068	5	.014
Common	7	1.359	1.351	1.417	.994	1.342	.075	6	
Regression							.007	1	.007
Differences among regression coefficients[1]					$F_{1,5} = \frac{.007}{.014} = .529$ NS				

Table 33 cont'd

SOURCE	DF	XX	XY	YY	SLOPE	SSR	SSE	DF	MS
e) TRY									
Pleasantness	3	.204	.237	.309	1.161[a]	.276	.033	2	
Bitterness	3	.204	.333	.546	1.627[b]	.541	.004	2	
Pooled							.037	4	.009
Common	6	.409	.570	.854	1.394	.795	.059	5	
Regression							.097	1	.097
Differences among regression coefficients[1]						$F_{1,4} = \frac{.097}{.009} = 10.388^*$			

[1] Treatments with the same letter are not significantly different

NS No significant difference

* Significant difference ($p < .05$)

Table 34 Analysis of covariance for pleasantness and total intensity of each single amino acid in relationship to concentration

SOURCE	DF	XX	XY	YY	SLOPE	SSR	SSE	DF	MS
a) ARG-HCL									
Pleasantness	3	.453	.502	.561	1.109[a]	.557	.004	2	
Total Intensity	5	1.096	.952	.838	.869[b]	.828	.010	4	
Pooled							.014	6	.002
Common	8	1.549	1.455	1.399	.939	1.367	.033	7	
Regression							.018	1	.018
Differences among regression coefficients[1]					$F_{1,6} = \frac{.018}{.002} = 7.849^{*}$				
b) ILEU									
Pleasantness	2	.181	.233	.299	1.284[a]	.299	.000	1	
Total Intensity	5	1.096	1.164	1.333	1.062[a]	1.236	.097	4	
Pooled							.097	5	.019
Common	7	1.277	1.396	1.632	1.094	1.527	.105	6	
Regression							.008	1	.008
Differences among regression coefficients[1]					$F_{1,5} = \frac{.008}{.019} = .395$ NS				

Table 34 cont 'd

SOURCE	DF	XX	XY	YY	SLOPE	SSR	SSE	DF	MS
c) LEU									
Pleasantness	3	.575	.347	.251	.604[a]	.210	.042	2	
Total Intensity	5	1.409	.754	.408	.535[a]	.404	.004	4	
Pooled							.046	6	.001
Common	8	1.984	1.101	.659	.555	.611	.048	7	
Regression							.202	1	.002
Differences among regression coefficients[1]					$F_{1,6} = \frac{.002}{.046} = .041$ NS				
d) PHE									
Pleasantness	3	.453	.404	.414	.891[a]	.360	.054	2	
Total Intensity	5	1.586	1.109	.791	.700[a]	.776	.015	4	
Pooled							.069	6	.011
Common	8	2.038	1.513	1.204	.742	1.122	.082	7	
Regression							.013	1	.013
Differences among regression coefficients[1]					$F_{1,6} = \frac{.013}{.011} = 1.125$ NS				

Table 34 cont 'd

SOURCE	DF	XX	XY	YY	SLOPE	SSR	SSE	DF	MS
e) TRY									
Pleasantness	3	.204	.237	.309	1.161[a]	.276	.033	2	
Total Intensity	4	.538	.431	.366	.800[a]	.345	.021	3	
Pooled							.055	5	.011
Common	7	.743	.668	.675	.899	.601	.074	6	
Regression							.019	1	.019
Differences among regression coefficients [1]					$F_{1,5} = \frac{.019}{.011} = 1.764$ NS				

1 Treatments with the same letter are not significantly different

NS No significant differences

* Significant difference ($p < .05$)

or lack of relationship between perception and stimulus concentration. In order to gain a measure of variability about the threshold value, the intensity pattern would have to be replicated several times instead of the system used here.

This type of pleasantness intensity pattern could have widespread application in the food industry. As well as indicating the amount of compound that may be added without inducing any perceptual change in pleasantness it also indicates the rate of change of pleasantness per unit change of concentration once the compound is present in above unpleasantness threshold concentrations.

E Taste Intensity Patterns of Binary Mixtures of Amino Acids

The bitterness intensity of amino acid mixtures was measured against an 800 ppm caffeine reference. The correlation coefficients (r) and power functions generated from linear regression analyses are shown in Table 35 In all cases a highly significant positive linear relationship was found to exist between stimulus concentration and bitterness perception.

Significant differences ($p < .01$) among amino acid mixtures were found to be present in the rate of growth of perceived bitterness intensity as a function of concentration (Table 36). Ileu + arg-hcl and phe + arg-hcl had significantly sharper slopes than try + arg-hcl while the slope of leu + arg-hcl was intermediate. The rate of growth of perceived bitterness in binary amino acid mixtures appeared to vary in relation to the components.

All four mixtures were found to possess significantly ($p < .01$) sharper slopes than arg-hcl which was common to all mixtures (Table 36). Figure 14 illustrates the bitterness intensity patterns of arg-hcl and each mixture. It appears that the perception of bitterness intensity grew at a faster rate when arg-hcl was in combination with one of the other amino acids, than when it was the sole bitter ingredient.

When the slopes of the amino acid mixes and their other amino acid components were compared, differences in the growth of bitterness perception were not always observed (Tables 37 and 38). Table 38 lists the slopes of each amino acid mixture along with the slopes of each of their component amino acids. Significant differences in slopes between each mixture and its components are identified.

As illustrated, the slopes of the mixes of ileu + arg-hcl (b=1.842)

Table 35 Relationships between perceived bitterness and concentration among amino acid mixtures.

Amino Acid Mixture	Correlation Coefficient r	Power Function $S = kC^n$
ILEU + ARG-HCL	.999**	$7.924 \times 10^{-8} C^{1.842}$
LEU + ARG-HCL	.999**	$1.807 \times 10^{-7} C^{1.698}$
PHE + ARG-HCL	.994*	$6.949 \times 10^{-9} C^{2.058}$
TRY + ARG-HCL	.992*	$1.39 \times 10^{-6} C^{1.482}$

*, ** $p < .005 < .001$ respectively.

Table 36 Analysis of covariance for bitterness intensity of amino acid mixtures and arginine hydrochloride in relation to concentration.

SOURCE	DF	XX	XY	YY	SLOPE	SSR	SSE	DF	MS
ARG-HCL	4	.538	.593	.666	1.102[a]	.654	.012	3	
ILEU + ARG-HCL	4	.554	1.020	1.882	1.842[c]	1.879	.002	3	
LEU + ARG-HCL	3	.203	.345	.586	1.698[bc]	.585	.001	2	
PHE + ARG-HCL	3	.205	.421	.876	2.058[c]	.866	.010	2	
TRY + ARG-HCL	3	.205	.303	.456	1.482[b]	.449	.007	2	
Pooled							.032	12	.003
Common	17	1.704	2.683	4.467	1.574	4.222	.245	16	
Regression							.212	4	.053
Differences among regression coefficients[1]						$F_{4, 12} = \frac{.053}{.003} = 19.678$*			

1 Treatments with the same letter are not significantly different.

* Significantly different ($p < .001$)

Figure 14 Perceived bitterness intensity of amino acid mixtures and arginine hydrochloride as a function of concentration.

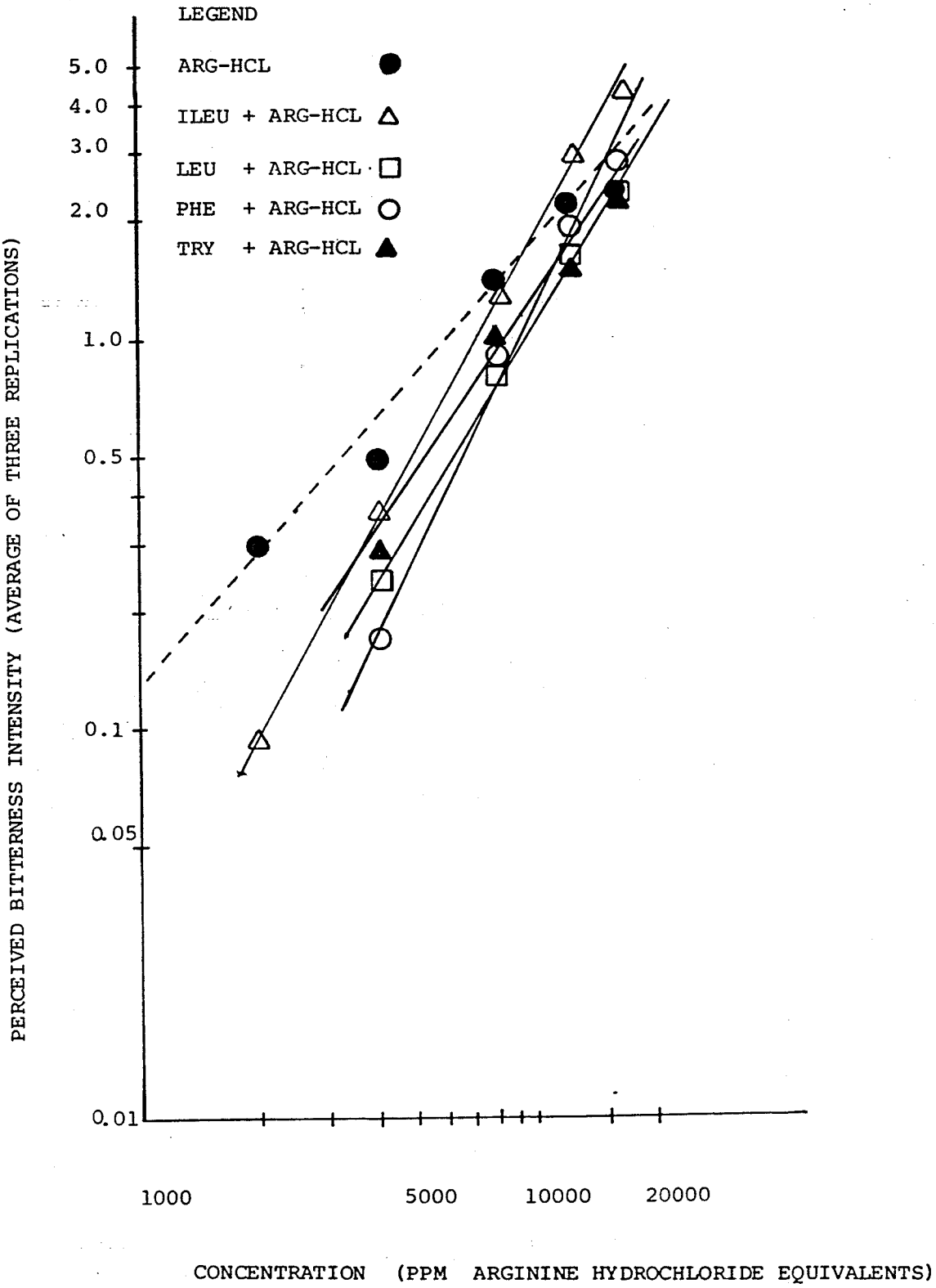


Table 37 Analysis of covariance for bitterness intensity of amino acid mixtures and their corresponding amino acids for differences in slope

SOURCE	DF	XX	XY	XY	SLOPE	SSR	SSE	DF	MS
ILEU	3	.204	.318	.569	1.556	.495	.074	2	
LEU	4	.655	.661	.691	1.009	.667	.023	3	
PHE	4	.906	.946	1.004	1.045	.990	.014	3	
TRY	3	.204	.333	.546	1.627	.541	.004	2	
ILEU + ARG-HCL	4	.554	1.020	1.882	1.842	1.879	.002	3	
LEU + ARG-HCL	3	.203	.345	.587	1.698	.585	.001	2	
PHE + ARG-HCL	3	.205	.421	.876	2.058	.866	.010	2	
TRY + ARG-HCL	3	.205	.303	.456	1.482	.449	.007	2	
Pooled							.135	19	.007
Common	27	3.136	4.349	6.610	1.387	6.030	.579	26	.022
Regression							.444	7	.063
Difference among regression coefficients[1]					$F_{7, 19} = \frac{.063}{.003} = 9.0$ *				

* Significantly different ($p < .0005$).

Table 38 Comparison of slopes of binary amino acid mixtures with each of their component amino acids [1]

Amino Acid Mixture	Bitterness Intensity · Slope of Mixture	Bitterness Intensity Slope of Components	
		Arginine-HCL	Other
ILEU + ARG-HCL	1.842[a]	1.102[x]	1.556[ax]
LEU + ARG-HCL	1.698[a]	1.102[x]	1.009[x]
PHE + ARG-HCL	2.058[a]	1.102[x]	1.045[x]
TRY + ARG-HCL	1.482[a]	1.102[x]	1.627[ax]

[1] Slopes with the same letter in a row are not significantly different from each other. Pairs of slopes were sequentially compared.

and try + arg-hcl (b=1.482) did not differ significantly from ileu (b=1.556) or try (b=1.627), respectively. In both cases the bitterness slope of the mixture followed that of the amino acid component with the greatest slope.

Leucine + arg-hcl and phe + arg-hcl did not follow the bitterness growth pattern of either amino acid component. As previously stated, both had significantly greater slopes than the arg-hcl component amino acid. Leucine + arg-hcl (b=1.698) also exhibited a sharper slope than leu (b=1.009) and similarly phe + arg-hcl (b=2.058) a sharper slope than phe (b=1.045). Thus the growth of bitterness intensity in these two mixes was significantly greater than that of either of the component amino acids when present as a single stimulus.

Arginine hydrochloride possessed a significantly flatter slope than any of the mixtures indicating a more gradual increase in bitterness perception as concentration increased (Figure 14). Although the slopes of the mixtures indicate a more rapid increase in bitterness perception, bitterness perception in arg-hcl appears to be evident at a lower concentration. This observation is supported by the fact that less than two thirds of the panelists perceived the bitter sensation in the three mixes, leu + arg-hcl, phe + arg-hcl and try + arg-hcl at the 2000 ppm (arg-hcl equivalents) concentration. Since mixtures were formulated to be approximately equi-bitter to arg-hcl, this indicates a suppressive effect (intensity of mix less than sum of the intensity of the components) between these amino acids at this concentration level. It should be noted that bitterness was detected in the ileu + arg-hcl mix indicating some additivity (intensity of the mix equal to sum of components) in bitterness between these two amino acids

at the mix concentration of 2000 ppm (arg-hcl equivalents). As evidenced in Figure 14 additivity in the mixes appears to come into play at slightly higher concentrations where there is little difference in perceived bitterness between mixes and arg-hcl. In fact a slight synergistic effect (intensity of mix is greater than sum of components) between the two mixes of ileu + arg-hcl and phe + arg-hcl appears to be evident for bitterness intensity. The lines for bitterness intensity of these two mixes at the higher concentrations cross over the bitterness slope for arg-hcl. Thus the general trend appears to be a lack of additivity in the mixes at the lower concentrations with it coming into play at the higher concentrations.

Amino acid mixtures were evaluated for total intensity against an 800 ppm caffeine reference. The correlation coefficients and power functions obtained from linear regression analysis are illustrated in Table 39. A highly significant positive linear relationship existed between perception of total intensity and concentration level.

Figure 15 illustrates the total intensity patterns for each amino acid mixture. No significant differences were apparent in either slope or elevation between the four mixtures (Table 40). Thus all four mixtures were found to be equi-intense.

Amino acid mixtures were found to be predominantly bitter in taste. No significant differences occurred in slope between bitterness intensity and total intensity for each amino acid mixture (Table 41). Elevation differences only occurred between bitterness intensity and total intensity of try + arg-hcl. This indicates that ileu + arg-hcl, leu + arg-hcl and phe + arg-hcl were generally found to be purely bitter with no other flavor attribute contributing to the total intensity. The mixture of try + arg-hcl

Table 39 Relationships between perceived total intensity and concentration among amino acid mixtures

Amino Acid Mixture	Correlation Coefficient r	Power Function $S = kC^n$
ILEU + ARG-HCL	.994**	$1.64 \times 10^{-6} C^{1.513}$
LEU + ARG-HCL	.970*	$3.53 \times 10^{-6} C^{1.424}$
PHE + ARG-HCL	.986**	$7.32 \times 10^{-7} C^{1.603}$
TRY + ARG-HCL	.992**	$2.18 \times 10^{-6} C^{1.476}$

*, ** $p < .005 < .001$, respectively.

Figure 15 Perceived total intensity of amino acid mixtures as a function of concentration

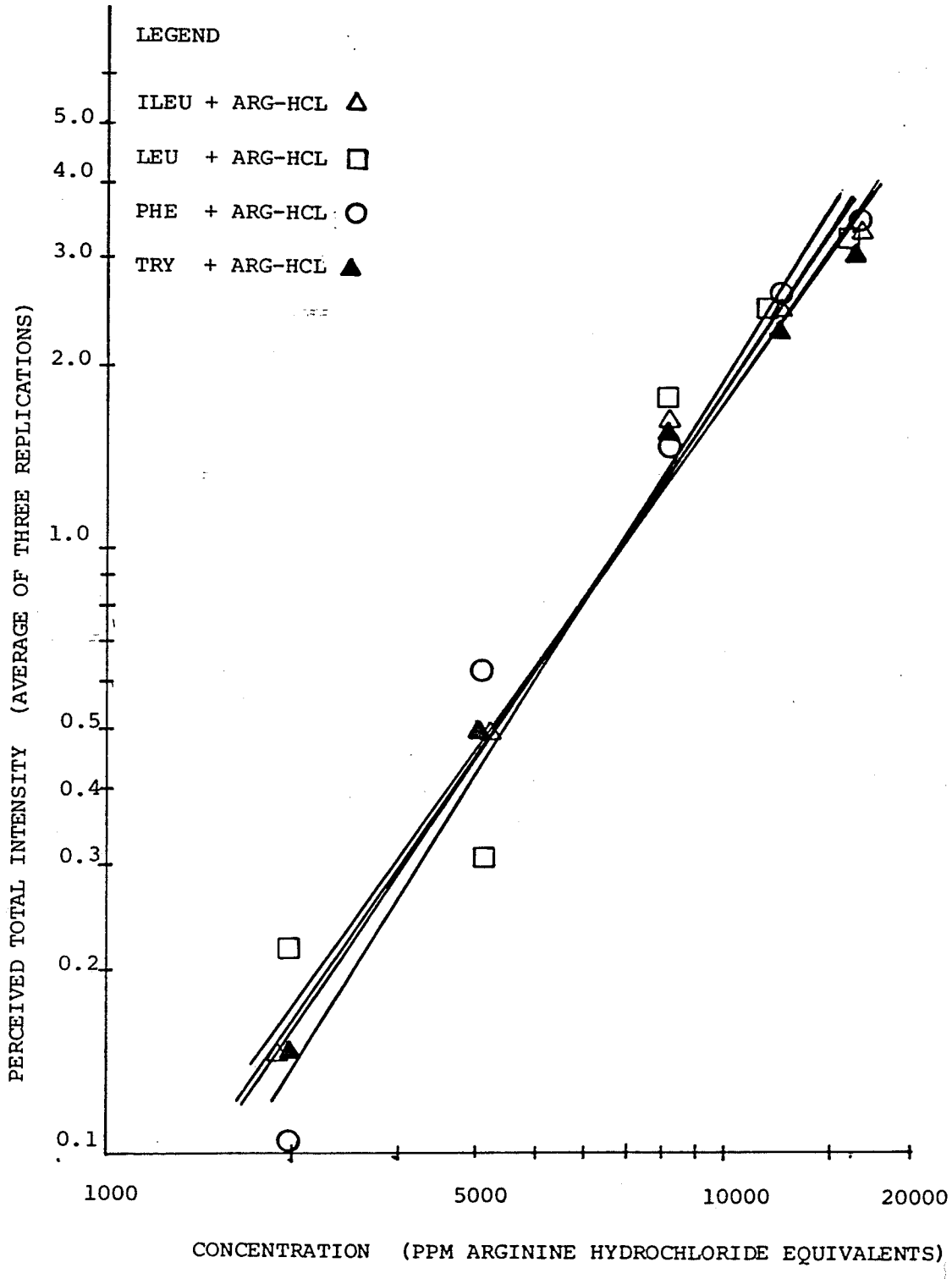


Table 40 Analysis of covariance for total intensity of amino acid mixtures in relationship to concentration

SOURCE	DF	XX	XY	YY	SLOPE	SSR	SSE	DF	MS
ILEU + ARG-HCL	4	.554	.837	1.282	1.513	1.267	.016	3	
LEU + ARG-HCL	4	.547	.779	1.178	1.424	1.109	.069	3	
PHE + ARG-HCL	4	.549	.881	1.453	1.603	1.412	.041	3	
TRY + ARG-HCL	4	.549	.811	1.214	1.476	1.198	.016	3	
Pooled							.143	12	.012
Common	16	2.199	3.308	5.128	1.504	4.976	.152	15	.010
Regression							.009	3	.003
Elevation							.000	3	.000
Total	19	2.199	3.308	5.129	1.504	4.976	.152	18	

Differences among regression coefficients $F_{3,12} = \frac{.003}{.012} = .260$ NS

Differences among adjusted treatment means $F_{3,15} = \frac{.000}{.01} = \infty$ NS

NS No significant differences

Table 41 Analysis of covariance for total intensity and bitterness of amino acid mixtures in relationship to concentration

SOURCE	DF	XX	XY	YY	SLOPE	SSR	SSE	DF	MS
ILEU + ARG-HCL (Total)	4	.554	.837	1.282	1.513	1.267	.016	3	
ILEU + ARG-HCL (Bitter)	4	.554	1.020	1.882	1.842	1.879	.002	3	
Pooled							.018	6	.003
Common	8	1.107	1.857	3.164	1.678	3.116	.048	7	.007
Regression							.030	1	.030
Elevation							.007	1	.007
Total	9	1.107	1.851	3.171	1.678	3.116	.055	8	.007

Differences among regression coefficients $F_{1,6} = \frac{.030}{.003} = 10.034$ NS

Differences among adjusted treatment means $F_{1,7} = \frac{.007}{.007} = .968$ NS

NS No significant differences

Table 41 cont 'd

SOURCE	DF	XX	XY	YY	SLOPE	SSR	SSE	DF	MS
LEU + ARG-HCL (Total)	4	.547	.779	1.178	1.424	1.109	.069	3	
LEU + ARG-HCL (Bitter)	3	.203	.345	.587	1.698	.585	.001	2	
Pooled							.070	5	.014
Common	7	.750	1.123	1.765	1.498	1.683	.082	6	.014
Regression							.011	1	.011
Elevation							.103	1	.174
Total	8	.788	1.116	1.766	1.417	1.581	.185	7	.026

Differences among regression coefficients $F\ 1, 5 = \frac{.011}{.014} = .784$ NS

Differences among adjusted treatment means $F\ 1, 6 = \frac{.103}{.013} = 7.592$ NS

NS No significant difference

Table 41 cont 'd

SOURCE	DF	XX	XY	YY	SLOPE	SSR	SSE	DF	MS
PHE + ARG-HCL (Total)	4	.549	.881	1.453	1.603	1.411	.041	3	
PHE + ARG-HCL (Bitter)	3	.205	.421	.876	2.058	.866	.010	2	
Pooled							.051	5	.010
Common	7	.754	1.302	2.329	1.726	2.247	.082	6	.014
Regression							.031	1	.031
Elevation							.121	1	.121
Total	8	.792	1.298	2.329	1.638	2.126	.203	7	.029

Differences among regression coefficients $F_{1,5} = \frac{.031}{.010} = 3.024$ NS

Differences among adjusted treatment means $F_{1,6} = \frac{.121}{.014} = 8.883$ NS

NS No significant differences

Table 41 cont'd

SOURCE	DF	XX	XY	YY	SLOPE	SSR	SSE	DF	MS
TRY + ARG-HCL (Total)	4	.549	.811	1.214	1.476	1.198	.016	3	
TRY + ARG-HCL (Bitter)	3	.205	.303	.456	1.482	.449	.007	2	
Pooled							.0233	5	.005
Common	7	.754	1.114	1.670	1.478	1.647	.023	6	.004
Regression							.000	1	.000
Elevation							.0650	1	.065
Total	8	.792	1.120	1.671	1.413	1.583	.088	7	.013

Differences among regression coefficients $F_{1,5} = \frac{.000}{.005} = \infty$ NS

Differences among adjusted treatment means $F_{1,6} = \frac{.065}{.004} = 16.719$ *

NS No significant differences

* Significantly different ($p < .01$).

was significantly less bitter than totally intense indicating the presence of some other taste parameter (s) contributing to total intensity.

III Free Amino Acid Content of Plant Protein Samples

The free amino acid content of ethanol extracts (80%) of the eighteen plant protein sources are listed in Table 42. Totals indicate that, in general, legumes possessed greater quantities of free amino acids than either cereals or oilseeds with no distinct differences being apparent between these latter two groups. Within the cereal sources, durum (457 ppm), triticale (662 ppm) and wheat (571 ppm) differed only slightly in total free amino acids while oats (1516 ppm) and rye (1913 ppm) contained approximately three times these amounts. In the oilseeds, mustard (160 ppm), sunflower (214 ppm) and soy isolate (330 ppm) possessed considerably fewer free amino acids than either rapeseed concentrate (1051 ppm) or soy flour (3271 ppm). Comparing the flour samples of legumes, fababean contained the fewest (2693 ppm), followed by pea (4310 ppm) and then lupin (6257 ppm). Processing effects were evident. In the fababean samples the isolate was found to contain the least amount of free amino acids, the flour was intermediate and the concentrate contained the greatest amounts. It should be noted that large differences in free amino acid content were not apparent between samples of fababean concentrate stored at the two temperatures of $-40^{o}C$ and $5^{o}C$.

The basic amino acids of rapeseed concentrate, soy flour, sunflower concentrate, fababean flour ($-40^{o}C$), fababean concentrates, lupin flour, and pea flour and concentrate were also determined and quantified from a TCA (1%) extract. A comparison of basic amino acids in both extracts and the total contents when basic amino acids were determined from TCA

Table 42 Free amino acid content of ethanol extracts of plant protein samples (average of duplicate determinations)

a) Cereals

Amino Acid	Free Amino Acid Content (ppm)				
	Durum Flour	Oat Flour	Rye Flour	Triticale Flour	Wheat Flour
Acidics & Neutrals					
Aspartic acid	30.61	94.50	185.67	55.24	75.87
Threonine	5.36	38.12	24.42	8.93	7.15
Serine	11.03	79.34	66.21	19.97	21.02
Asparagine	163.84	571.46	779.57	226.60	225.28
Proline	40.30	54.11	179.03	62.05	10.36
Glutamic Acid	49.29	206.72	187.59	38.25	51.50
Citrulline		4.38			
Glycine	7.13	29.28	30.78	9.38	8.26
Alanine	27.62	89.54	159.02	40.54	29.84
Valine	8.79	52.72	42.76	12.89	15.82
Cystathionine	9.99	12.21	21.09	16.65	13.32
Methionine	4.48	8.21	21.64	4.48	8.21
Isoleucine	6.56	26.24	20.99	6.56	10.49
Leucine	10.49	36.07	24.92	17.71	13.12
Tyrosine	11.78	35.33	18.12	11.78	9.06
Phenylalanine	14.87	32.21	20.65	16.52	10.74
β-alanine		3.12			
Total Acidics & Neutrals	402.73	1373.56	1782.46	550.55	510.04

a) Cereals

Amino Acid	Free Amino Acid Content (ppm)				
	Durum Flour	Oat Flour	Rye Flour	Triticale Flour	Wheat Flour
Basics:					
γ-aminobutryric acid	10.31	35.06	22.17	14.44	7.22
Ethanolamine		trace	trace	trace	trace
Ammonia	17.71	14.99	34.14	21.63	28.87
Lysine	3.65	30.70	12.43	4.39	4.39
Histidine	3.88	20.17	11.64	3.10	3.10
Tryptophane[2]					trace
Arginine	18.92	40.07	50.52	27.87	17.42
Total Basics	54.47	142.97	130.90	71.43	61.00
Total[1]	457.20	1516.53	1913.36	621.98	571.04

Table 42 Cont'd

b) Oilseeds

Amino Acid	Free Amino Acid Content (ppm)				
	Mustard Concentrate	Rape Concentrate	Soy Flour	Soy Isolate	Sunflower Concentrate
Acidics & Neutrals					
Aspartic Acid	5.99	113.14	383.33	21.96	
Threonine	2.38	trace	trace		7.74
Serine	4.20	9.46	36.78	2.10	4.20
Asparagine	64.74	197.53	698.31	89.84	11.89
Proline			70.80		
Glutamic Acid	30.16	587.05	484.79	75.56	19.86
Citrulline					
Glycine	trace	4.88	39.04	1.88	2.63
Alanine	1.78	9.80	196.89	8.46	9.35
Valine	2.34		50.37	4.69	19.33
Cystathionine			609.39	43.29	4.44
Methionine	trace	2.98	25.37		10.44
Isoleucine	1.31	2.62	42.63	1.97	15.09
Leucine	trace	1.31	46.57	5.25	42.63
Tyrosine	1.81	3.62	28.99	4.53	8.15
Phenylalanine	12.39	30.56	78.46	14.04	14.87
β -alanine			76.17		
Total Acids & Neutrals	127.10	1015.94	2867.89	313.67	170.62

b) Oilseeds

Amino Acid	Free Amino Acid Content (ppm)				
	Mustard Concentrate	Rape Concentrate	Soy Flour	Soy Isolate	Sunflower Concentrate
Basics:					
ν -aminobutyric acid	7.22		64.97	4.12	
Ethanolamine		2.44	42.76	1.83	
Ammonia	4.60	8.52	12.77	4.60	38.66
Lysine		3.65	11.70		1.46
Histidine		1.55	53.53	1.55	
Tryptophane [2]					
Arginine	20.90	19.16	217.75	4.36	3.48
Total Basics	37.72	35.32	403.48	16.46	43.60
Total [1]	164.82	1051.26	3271.37	330.13	214.22

Table 42 Cont'd

c) Legumes

Amino Acid	Free Amino Acid Content (ppm)				
	Faba Flour (-40°C)	Faba Concentrate (A/C,-40°C)	Faba Concentrate (A/C, 5°C)	Faba Concentrate (C)	Faba Isolate (C)
Acidics & Neutrals					
Aspartic Acid	202.33	85.84	89.84	175.69	7.99
Threonine	trace	trace	trace	trace	7.15
Serine	trace	10.51	33.63	35.73	7.36
Asparagine	690.38	1360.94	1321.05	1401.90	84.56
Proline	40.87	18.42	25.33	124.34	trace
Glutamic Acid	439.92	240.56	224.37	562.04	30.16
Citrulline	28.03	29.78	3.68	50.80	
Glycine	54.05	38.66	34.53	63.06	7.88
Alanine	99.34	84.64	78.84	555.92	26.28
Valine	19.33	53.89	42.14	110.71	9.37
Cystathionine	64.38	46.62	26.64	24.42	
Methionine	5.22	1.49	1.49		14.17
Isoleucine	15.09	22.30	22.30	43.29	8.53
Leucine	24.27	51.82	51.82	76.74	30.83
Tyrosine	25.37	178.40	52.54	94.22	15.40
Phenylalanine	84.25	trace	171.80	149.50	22.30
β-alanine	1.78			25.84	
Total Acidics & Neutrals	1730.23	2014.94	2171.00	3494.20	271.98

c) Legumes

Amino Acid	Free Amino Acid Content (ppm)				
	Faba Flour (-40°C)	Faba Concentrate (A/C,-40°C)	Faba Concentrate (A/C, 5°C)	Faba Concentrate (C)	Faba Isolate (C)
Basics:					
γ-aminobutyric acid	11.68	3.61	5.16	98.99	9.28
Ethanolamine	trace			26.26	12.28
Ammonia	14.63	19.50	19.58	25.97	5.62
Lysine	7.31	7.31	8.04	16.81	6.58
Histidine	13.96	12.41	7.76	24.83	3.88
Tryptophane [2]					
Arginine	850.97	1296.92	1121.85	1978.04	527.41
Total Basics	963.03	1339.75	1163.71	2170.90	565.05
Total [1]	2693.26	3354.69	3334.71	5665.10	837.03

c) Legumes

Amino Acid	Free Amino Acid Content (ppm)		
	Lupin Flour	Pea Flour	Pea Concentrate
Acidics & Neutrals			
Aspartic Acid	179.02	356.70	191.66
Threonine	63.73	trace	28.59
Serine	122.96	44.14	34.68
Asparagine	1404.54	2231.68	2176.84
Proline	155.43	49.51	33.63
Glutamic Acid	1371.99	969.59	423.73
Citrulline	trace	trace	46.42
Glycine	190.96	113.73	57.43
Alanine	177.73	77.51	50.34
Valine	80.25	41.59	37.49
Cystathionine	555.00	14.43	21.09
Methionine	5.97	5.22	11.19
Isoleucine	47.88	15.09	19.68
Leucine	60.34	20.33	43.94
Tyrosine	141.33	26.27	54.36
Phenylalanine	63.60	40.47	72.68
β-alanine	75.19	31.18	56.52
Total Acidics & Neutrals	4695.92	4037.44	3360.27

c) Legumes

Amino Acid	Free Amino Acid Content (ppm)		
	Lupin Flour	Pea Flour	Pea Concentrate
Basics:			
γ-aminobutyric Acid	81.46	14.95	10.31
Ethanolamine	19.24	1.70	trace
Ammonia	22.56	17.10	5.45
Lysine	39.47	12.43	21.93
Histidine	141.20	17.84	31.81
Tryptophane [2]	79.65	trace	38.30
Arginine	1177.59	208.46	637.57
Total Basics	1561.17	272.48	745.37
Total[1]	6257.09	4309.92	4105.64

1. Column totals may differ slightly from amino acid quantities stated. The amino acids O-phosphoserine, O-phosphoethanolamine, taurine, amino isobutyric acid, and ornithine occurred infrequently and in small quantities in some protein sources and were included in total values but are not stated in tables.
2. Tryptophane was not clearly resolved on the chromatograph and thus in most cases is not reported.

and ETOH extracts is shown in Table 43. As illustrated, the total free amino acid content increased in all cases when the basic amino acids were quantitated from the TCA extract. The increase in the total free amino acids ranged from 1.09 fold increase (pea flour) to a 10.75 fold increase (fababean concentrate, C). Generally the increase in total free amino acids appeared to be due to an increase in arginine content.

In light of the sensory information generated on the four amino acids, ileu, leu, phe and try and the free amino acid analyses of plant protein sources (Table 42 and 43), it does not appear that any of these four are present individually in sufficient quantities to elicit any undesirable flavor characteristics. Sensory analysis revealed that pleasantness dropped from a neutral response in ileu, leu, phe, and try at concentration levels of 3815, 1671, 1066 and 1000 ppm respectively. The levels of ileu, leu, phe and try present in the free form in plant proteins are several fold less than the estimated unpleasantness threshold values.

Arginine, however, was found to be present in some protein sources in amounts exceeding the unpleasantness threshold value of arg-hcl (1662 ppm). Ethanol extracts of plant protein sources revealed that only fababean concentrate (C) contained free arginine in a quantity (1978 ppm) above this threshold level. Trichloroacetic acid extracts, however, revealed that arg was present in above arg-hcl threshold quantities in fababean flour (3665 ppm), fababean concentrate A/C, -40^{o}C (9339 ppm), fababean concentrate C (8522 ppm), lupin flour (4219 ppm) and pea concentrate (2834 ppm). It is thus conceivable that the arginine content of these plant proteins could contribute to off-flavor characteristics of these sources.

Table 43 Free amino acid content of selected plant protein sources with a comparison of basic amino acid determinations from ethanol (80%) and trichloroacetic acid (1%) extracts

Amino Acid	Rape Concentrate		Soy Flour		Sunflower Concentrate		Faba Flour (-40°C)		Faba Concentrate (A/C,-40°C)	
	ETOH	TCA	ETOH	TCA	ETOH	TCA	ETOH	TCA	ETOH	TCA
Total Acidics & Neutrals	1015.94		3094.4		170.62		1730.23		2014.94	
Basics:										
γ-aminobutyric acid			64.97	44.34			11.68	7.22	3.61	6.19
ornithine				1.32				29.07		36.99
ethanolamine	2.44		42.76	17.71			trace			
ammonia	8.52	36.78	12.77	20.44	38.66	78.34	14.73	22.14	19.50	43.94
lysine	3.65	43.86	11.70	58.48	1.46	2.92	7.31	59.94	7.31	116.95
histidine	1.55	12.41	53.53	43.44			13.96	7.76	12.41	6.21
tryptophane[1]										
arginine	19.16	275.24	217.75	883.19	3.48	6.97	850.967	3665.17	1296.92	9338.86
Total Basics	35.32	368.29	403.48	1068.92	40.60	88.23	963.03	3791.30	1339.75	9549.14
Total[2]	1051.27	1384.23	3271.37	3936.81	214.22	248.84	2693.26	5521.53	3354.69	11564.08

Table 43 cont'd

Amino Acid	Faba Concentrate		Lupin Flour		Pea Flour		Pea Concentrate	
	ETOH	TCA	ETOH	TCA	ETOH	TCA	ETOH	TCA
Total Acidics & Neutrals	3494.2		4695.92		4037.44		3360.27	
Basics:								
γ-aminobutyric acid	98.99	74.25	81.46	193.87	14.95	12.37	10.31	12.37
ornithine		5.28		6.61		5.28		10.57
ethanolamine	26.26		19.24		1.70		trace	
ammonia	25.97	33.04	22.56	31.34	17.10	9.88	5.45	13.62
lysine	16.81	76.02	39.47	154.96	12.43	52.63	21.93	111.10
histidine	24.83	18.62	141.20	220.33	17.84	15.52	31.81	13.96
tryptophane [2]			79.65		trace		38.30	trace
arginine	1978.04	8521.86	1177.59	4219.12	208.46	566.15	637.57	2834.23
Total Basics	2170.90	8729.17	1561.17	4826.23	272.48	661.83	745.37	2995.85
Total [1]	5665.1	9001.06	6527.09	9422.15	4309.92	4699.27	4105.65	6356.12

1 The acidic amino acids were only determined and quantitated from the ethanol extracts; thus differences in totals for a sample are due to the differences of the extractions solvents on the basic amino acids.

2 Tryptophane was not clearly resolved on the chromatograph and thus in most cases is not reported.

GENERAL DISCUSSION

I Taste Properties of Single Amino Acids

Schiffman and Dackis (1975) summarized the taste properties of amino acids as follows: amino acids with aliphatic side chains including those containing OH groups tend to be pleasant or tasteless; amino acids containing an aromatic ring or sulfur tend to be unpleasant; the lighter weight amino acids taste sweet while the heavier ones tend to be bitter; amino acids with acidic groups tend to be sour and amino acids with basic groups have salty, bitter and sharp components in common. The profiles of the ten amino aicds in the present study generally adhere to the above guidelines with the exception that some aliphatic amino acids, ileu, leu and val were not found to be pleasant or tasteless. These amino acids were all found to possess bitterness which is considered to be an unpleasant taste sensation. Total intensity scores obtained from amino acid profiles, however, indicated that these three amino acids were relatively mild at the 3000 ppm concentration. Thus these three amino acids with aliphatic side chains were relatively mild but at high enough concentrations would elicit the undersirable taste sensation of bitterness. With this exception, the findings of the present study coincide with the above summary.

Of the five amino acids for which intensity patterns were established (arg-hcl, ileu, leu, phe and try) arg-hcl was found to be intermediate in bitterness intensity. No significant relationship was observed between astringency perception and stimulus concentration. Arginine hydrochloride was also intermediate in the rate of growth of total intensity (n = .869) which did not differ signficantly from the rate of growth of bitterness

intensity (n = 1.102). The unpleasantness threshold of 1662 ppm was the third lowest calculated for the five amino acids.

Of the five amino acids examined in detail, ileu was found to be the least bitter, to possess the sharpest slope for total intensity and to possess the highest unpleasantness threshold (3815 ppm). The higher the unpleasantness threshold the more pleasant the amino acid. Only for ileu was a significant positive linear relationship established between astringency perception and stimulus concentration. The three parameters of bitterness, astringency and total intensity were all found to grow at similar rates (n = 1.556, 1.207 and 1.062 respectively). Isoleucine appeared to be a fairly mild amino acid but once perceived, total intensity perception, consisting of bitterness and astringency, proceeded at a faster rate than for any of the other amino acids.

Leucine was found to be the second least bitter amino acid, of the five, to possess the most gradual increase in total intensity perception and the second highest unpleasantness threshold (1671 ppm). A significant relationship between astringency perception and increasing concentration was not observed. The slope of bitterness intensity (n = 1.009) was significantly greater than that of total intensity (n=.535) indicating that bitterness accounted for increasing proportions of total intensity perception as concentration increased. It thus appears that leu was fairly mild and once perceived the impact of total intensity proceeded in a decelerating rate as a function of stimulus concentration. The total intensity score obtained for ileu during amino acid profiling indicated it was twice as intense as leu while leu was found to be more bitter than ileu in the intensity patterns. The presence of astringency

in addition to bitterness in ileu could account for this difference.

Phenylalanine was the second most bitter amino acid and possessed the second lowest unpleasantness threshold (1066 ppm). A significant relationship between astringency perception and concentration was not observed. Although perception of unpleasantness in phe occurred at the second lowest concentration of the five amino acids examined, once perceived the total perceptual impact proceeded as a decelerating function of concentration (n = .700). Bitterness appeared to account for progressively increasing ratios of the total intensity as phe concentration increased as evidenced by a significantly greater slope for bitterness perception (n = 1.045). This might explain why astringency was identified in the amino acid profile and yet a siginifcant relationship was not observed between astringency perception and concentration. Perhaps as concentration increased bitterness dominated the presence of other taste properties.

Tryptophane was reported to be significantly more bitter than the other amino acids examined and was not found to be significantly different from caffeine in bitterness. Tryptophane possessed the lowest unpleasantness threshold (1000 ppm) of the five amino acids examined. This indicated that try induced an unpleasant response at a concentration lower than the other four amino acids. No significant relationship was established between astringency perception and try concentration. The total perceptual impact (total intensity) increased in a decelerating fashion (n = .80) while bitterness increased in an accelerating fashion (n = 1.627) indicating that bitterness accounted for increasing amounts of the total perceived intensity as concentration increased. As enumerated for phe, perhaps bitterness dominated

astringency as concentration increased thus not yielding a significant positive relationship between astringency perception and try concentration.

The amino acid profiles and intensity patterns established here along with the literature suggest that the flavor properties of amino acids vary with concentration. One of the clearest examples is the recognition of astringency in the 3000 ppm solutions of arg-hcl, leu, phe and try but the lack of any or consistent perception of astringency in the intensity patterns of these amino acids as concentration increased. Another example, appears in the case of lys. At a concentration of 3000 ppm Solms et al. (1965) reported lys tasteless, Petritschek et al. (1972) reported it virtually tasteless with slight bitterness and the present study reported primarily sweetness with bitter taste components. Kirimura et al. (1969) also reported sweet and bitter sensations. In the undiluted amino acid (Schiffman and Dackis, 1975) bitterness was reported with sharp and salty components. No sweetness was observed. It thus appears that at lower concentrations sweetness was apparent but at higher concentrations bitterness dominated.

The bitterness intensity of the amino acids examined in detail placed the amino acids in the following sequence in decreasing order of intensity; try, phe, arg-hcl, leu and ileu. Considering these five amino acids only, this sequence is in exact agreement with that established by Petritschek et al. (1972). Solms et al. (1965) examined the intensity of three of these amino acids and found try to be the most bitter, followed by phe and then leu. These results are again in accordance with those of the present study.

The slopes for bitterness intensity of try, ileu, arg-hcl, phe, leu and caffeine were 1.627, 1.556, 1.102, 1.045, 1.009 and 1.002, respectively. Meiselman (1971) reported the exponents for power functions of the bitter compounds quinine sulfate and quinine hydrochloride obtained by numerous different authors. These exponents ranged from 0.3 to 1.0 with a definite trend being evident between exponent size and tasting procedure. Exponents obtained by a sipping procedure were higher than those obtained by delivering the sample by a flow apparatus, generally being close to 1.0. Thus the exponents obtained for caffeine and the bitter amino acids arg-hcl, leu and phe are in fair agreement with the exponents obtained for quinine by the sipping procedures. Although no significant differences in slope were identified among amino acids and caffeine, ileu and try do appear to grow in perceived bitterness intensity at a faster rate ($n = 1.556$ and $n = 1.627$, respectively) than the other compounds tested and exponents reported in the literature.

The relationship between perceived bitterness intensity and the calculated unpleasantness threshold values is illustrated in Table 44. A negative linear relationship was observed between increasing bitterness intensity, as measured by adjusted treatment means for bitterness, and decreasing unpleasantness thresholds. That is, the higher the unpleasantness threshold (the amount of amino acid present at the point of perception of unpleasantness) the lower the bitterness intensity of the amino acid. This relationship may be expressed by the linear regression equation $Y = .314 - .00026X$. The correlation coefficient measuring the strength of the linear relationship between these two parameters ($r = .835$) was found to be significant ($p < .05$). This

Table 44 The relationship between perceived bitterness intensity and calculated unpleasantness threshold values

Amino Acid	Bitterness Intensity Values of Adjusted Treatment Means	Unpleasantness Threshold (ppm)
ILEU	0.258	3815
LEU	0.522	1671
ARG-HCL	0.556	1662
PHE	0.944	1066
TRY	2.291	1000

relationship is not surprising considering all five amino acids examined in depth had been established to possess bitter taste properties in the amino acid flavor profiles.

The pleasantness threshold as determined in the present study is thought to be analogous to the more commonly reported recognition threshold. The recognition threshold which is sometimes referred to as the identification threshold is defined as the minimum concentration at which a substance is correctly identified (Amerine et al., 1965). That is the concentration at which a taste property is correctly and or consistently ascribed to a compound. With the exception of leu, the calculated unpleasantness thresholds occurred at points below concentrations, but in close association, at which bitterness and astringency were perceived by at least two thirds of the panel. However, perception of total intensity of amino acid solutions increased with increasing concentration below levels at which either bitterness or astringency were perceived in any of the amino acids. Thus panelists perceived an increase in total intensity below the unpleasantness threshold indicating that the unpleasantness threshold is not analogous to the detection threshold. One of the clearest illustrations occurs with ileu. Total intensity was reported to increase with an increase in ileu concentration from 1000 ppm upwards while bitterness and astringency were not perceived until 4000 ppm. The unpleasantness threshold was identified at 3815 ppm indicating it is close to the test concentration at which perception of the two unpleasant sensations of bitterness and astringency occurred. It thus appears that the unpleasantness threshold may be analogous to a recognition threshold. Before this can be claimed with any confidence a more in depth examination comparing this method to more conventional

methods of measuring thresholds would have to be conducted.

The accuracy of the pleasantness thresholds calculated here is compromised by the small number of points determining each regression equation. As previously stated, a more accurate measure could be obtained by increasing the number of samples examined and decreasing concentration levels.

II Taste Properties of Binary Amino Acid Mixtures

In the present study it was observed that at the lowest test concentration (approximately 2000 ppm arg-hcl equivalents) binary amino acid mixtures did not illustrate the property of additivity. However, at slightly higher levels additivity was observed and possible synergistic effects were indicated at the highest concentrations. The taste mixture literature generally concludes that mixtures of components eliciting the same taste sensation (ie. sweetness) are additive or synergistic in regards to intensity. This has most frequently been demonstrated with sweet stimuli such as sucrose and cyclamate (Kamen, 1959); dextrose and fructose, and sucrose and fructose (Stone and Oliver, 1969); dextrose and fructose (Stone et al., 1969) and glucose in combination with fructrose, sodium cyclamate, sodium saccharin and saccharin (Moskowitz, 1973). Kamen (1959) observed additivity at low and high concentrations of sucrose and cyclamate mixtures while at intermediate concentrations synergism was observed. Oliver and Stone (1969) reported synergistic effects as high as 20 - 30% in sugar combinations but this was not

reported at all concentrations indicating that there are optimal mixture combinations for synergistic effects. These same authors also evaluated the sweetness intensity of dextrose with the sweet tasting amino acids DL alanine and glycine. Additivity was observed between the mixtures with synergistic effects only being evident at the highest dextrose level. This observation raises the question of whether bitter tasting amino acids will also express additivity or synergism when in the presence of different types of bitter compounds. In fact, an additive effect between try and ethyl α -D - galactopyranoside (a bitter compound extracted from soybean flakes) has been reported (Honig et al., 1971).

Additive and synergistic effects have also been observed between different sour stimuli (Moskowitz, 1974). Citric acid in binary mixes with each of gluconolactone, phytic acid and succinic acid demonstrated additivity while when in combination with hydrochloric acid a synergistic effect was observed.

Thus as demonstrated in the present study, compounds of the same taste sensation appear to be additive. Synergism has been reported with some frequency but as demonstrated by various sweetness studies optimal concentrations play a role in both the presence and degree of observed synergism. In the binary amino acid mixtures synergism only appeared to be evident at the highest concentrations.

A suppressive effect (intensity of mix less than the additive sum of individual components) at the lower mixture concentration was observed here and this has not been reported in the taste mixture literature reviewed. One possible explanation is that the amino acids constituting the mixtures were not always found to be purely bitter at

the 3000 ppm concentration. For example, arg-hcl was reported to be predominantly bitter but sweetness was also observed with some frequency and was thought to be the dominant sensation in 5 of 14 judgements at this concentration. Thus at the lower concentration conflicting taste sensations might account for a reduction in bitterness intensity as sweet compounds have been reported to reduce the intensity of bitter compounds (Moskowitz ,1972 and Pangborn, 1960). Such findings as in the present study and some reported in the literature identifying different interaction effects at different concentrations emphasize the importance of studying taste interactions at more than one concentration.

The method of magnitude estimation used in the present study is considered to be a superior method of measuring relative taste intensity in comparison to such methods as the GUST scale (Beebe-Center, 1949) and the matching method utilized by Solms et al. (1965). These two latter methods are similar. In both, a test sample is compared to a series of standards of different concentrations of a compound illustrating the basic taste sensation of interest. In essence, panelists are asked to match the intensity of the test sample to one of the standards. In magnitude estimation a number is directly assigned to reflect the sensory intensity of the parameter of interest in the sample in relation to a reference. This is considered to be less tedious than the process of tasting numerous samples and comparing intensities until a match is found.

When using the method of magnitude estimation, the intensity of the parameter of interest in a compound may be established at several concentrations permitting the calculation of a line reflecting sensory intensity

as a function of stimulus concentration. To obtain such a function by a matching procedure would require several times the number of judgements by the panelists. Consequently, the intensity of a compound established by such methods is usually determined at one concentration of the test sample as was done by Solms et al. (1965) for single amino acids. This single measure of intensity does not reflect the intensity of the test compound in comparison to the standard at all concentrations unless the growth curves of the parameter of interest in both the test and standard compound are parallel (ie. equal slopes). For example, if the bitterness intensity of leu at 5000 ppm was found to be 1/10 that of caffeine at the same concentration, this does not necessarily indicate that at 1000 or 10000 ppm leu will still be 1/10 as bitter as caffeine. The establishment of power functions for both compounds by the method of magnitude estimation would indicate the ratio of bitterness intensity of the two compounds across the total perceptual range. Thus, when obtaining intensity measurements the establishment of power functions by the method of magnitude estimation would yield the most accurate and complete information.

III Flavor Implications of the Free Amino Acid Content of Plant Protein Samples

As previously enumerated, of the five amino acids examined in detail only arg was present in the free form in above unpleasantness threshold quantities in any of the plant protein samples examined. Fababean concentrate contained more arg (9339 ppm) than any of the other plant proteins. The proportion of fababean concentrate which would contain quantities of free arg in excess of the arg-hcl unpleasantness threshold (1662 ppm) would be $\frac{1662 \times 100}{9339} = 17.8\%$. Thus a food product would have to contain at least 17.8% fababean concentrate for arg alone to be

implicated in its bitterness. Common substitution levels are lower than 17.8%. In fact this is a generous estimate because thresholds in food products are usually several fold higher than those in an aqueous solution.

Although intensity patterns were not established for asparagine, the quantity in the free form in the protein sources is worthy of notice. Solms et al. (1965) and Petritschek et al. (1972) found asparagine to be tasteless but Kirimura et al. (1969) characterized asparagine as tasting predominantly sour with underlying bitter components and as possessing a threshold value of 1000 ppm. This threshold value is not likely the unpleasantness threshold value as was determined in the present study. In regards to the plant protein sources examined, asparagine was present in above reported threshold quantities in the legume samples of all three fababean concentrates, lupin flour, pea flour and pea concentrate. Again, however, at common substitution levels it does not appear that this amino acid would influence flavor properties on an individual basis.

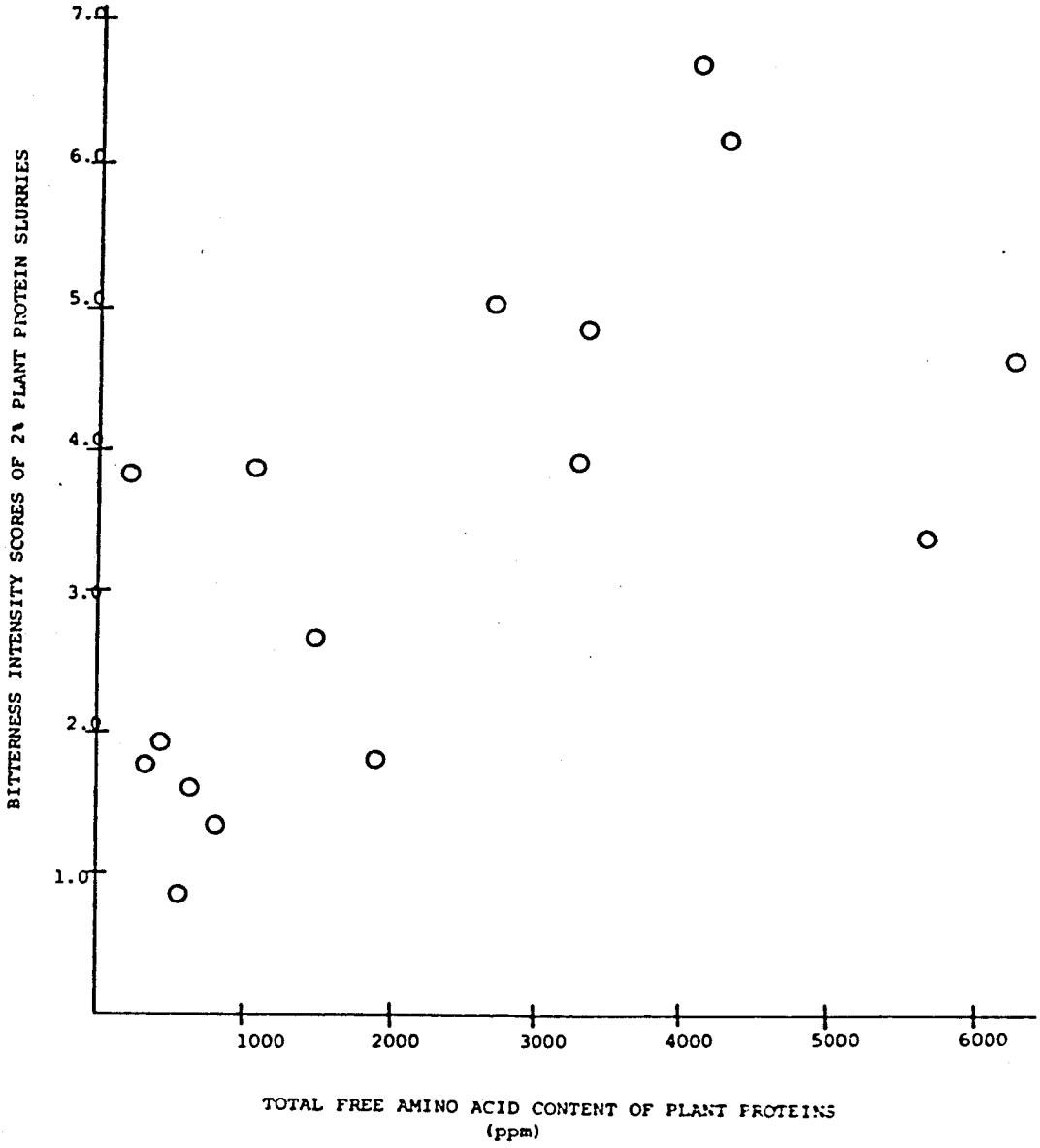
No single amino acid was present in the protein samples, in the free form, in sufficient quantities to elicit any undesirable flavor properties at common usage levels. Thus,additivity or synergistic effects must come into play if the free amino acid content of plant proteins plays a role in flavor. Additivity between binary mixtures of individual bitter amino acids has been observed in the present study. Höhn et al. (1975) reported observing additivity and potentiating effects in a mixed system composed of thiamine, thiamine diphosphate and several free amino acids.

Synergistic effects among α amino acids and other compounds have been reported in the literature. Tanaka et al. (1969,a) reported

ternary synergism between α amino acids, nucleotides and monosodium L-glutamate. L-glutamic acid was later reported as a suitable alternative for monsodium L-glutamate (Tanaka et al., 1969 b). Amino acid analysis revealed that considerable quantities of glutamic acid were present in the free form in several plant protein samples. In many cases glutamic acid was present in quantities several fold that of its reported threshold value of 50 ppm (Kirimura et al., 1969). It thus appears that the glutamic acid present in the samples could possibly produce synergistic effects among other amino acids and flavor compounds present in plant protein samples.

Figure 16 illustrates the relationship between the total free amino acid content of ETOH extracts of sixteen of the plant protein samples examined in the present study and bitterness intensity scores of 2% slurries of the same samples (Vaisey-Genser; unpublished data, 1978). Linear regression analysis between total free amino acid content and bitterness intensity scores yielded the following regression equation; $Y = 1.85 + .00074X$. The correlation coefficient (r), measuring the strength of the linear relationship between these two variables, of .798 was found to be significant ($p < .01$). Thus it may be concluded that in general, bitterness intensity scores increased as the total free amino acid content increased.

Figure 16 Bitterness intensity scores of 2% plant protein slurries as a function of total free amino acid content (ppm)



SUMMARY AND CONCLUSIONS

A trained sensory panel examined ten, 3000 ppm aqueous amino acid solutions of arg-hcl, his, ileu, leu, lys, met, phe, pro, try and val. These were evaluated for total flavor intensity and the presence of thirteen flavor parameters. Large differences in flavor intensity existed between amino acids. Tryptophane was scored the most intense (154) and leu the mildest (26) where a score of 100 was equal to moderate intensity. Flavor profiles divided these amino acids into two groups; those possessing complex flavor sensations including his, lys, met, pro and val and those which were primarily bitter with some astringency including arg-hcl, ileu, leu, phe and try.

Bitterness, astringency, total intensity and pleasantness intensity patterns were established for the five bitter amino acids using the method of magnitude estimation. All intensity pattern measurements were obtained using a controlled tasting procedure. The rate of growth of bitterness intensity was not significantly different among the five amino acids. However, in all cases the slope was greater than one indicating that bitterness increased as an accelerating function of concentration. Tryptophane was significantly more bitter and ileu significantly less bitter than the other amino acids. Phenylalanine, arg-hcl and leu were intermediate in bitterness intensity. A significant relationship between astringency perception and stimulus concentration was illustrated only for ileu. Due to inconsistent astringency perception a significant relationship was not apparent for the other four amino acids. Isoleucine possessed the sharpest slope for total intensity followed by arg-hcl, try, phe and then leu. Pleasantness intensity patterns indicated that all amino acids decreased in pleasantness as concentration increased but no significant differences existed in the rate of decline of pleasantness.

Unpleasantness threshold values obtained from pleasantness intensity patterns indicated that try was the least pleasant amino acid followed by phe, arg-hcl, leu and finally ileu.

Intensity patterns of binary amino acid mixtures of ileu, leu, phe and try each in combination with arg-hcl were established for the parameters of bitterness and total intensity. All mixtures were formulated such that each constituent amino acid contributed approximately one half of the total bitterness intensity of the mix as determined from their individual power functions.

The rate of growth of perceived bitterness in binary amino acid mixtures varied with the component amino acids. The mixes of ileu + arg-hcl and phe + arg-hcl possessed significantly greater slopes than try + arg-hcl while the slope of leu + arg-hcl was intermediate. The rate of growth of bitterness intensity in mixtures either followed that of the amino acid component with the sharper slope or was significantly greater in slope than either amino acid component (as determined from single amino acid intensity patterns). At low concentrations of the mixes additivity in bitterness was not apparent but appeared to come into play at intermediate concentrations with possible synergistic effects being evident at the highest concentrations. Total intensity patterns of the mixes were not significantly different in either slope or elevation.

Identification and quantification of the free amino acid content of plant protein samples revealed that the legumes examined generally possessed greater quantities of free amino acids in total than either cereals or oilseeds. Considering the sensory information obtained for the bitter amino acids only arg was present in plant proteins in sufficient quantities

on an individual basis to impart undesireable flavor properties. However, at common substitution levels it is not likely that this effect would be perceptible in a food product.

The present study is not conclusive as to whether or not free amino acids contribute significantly to the off-flavor of plant proteins. The investigations undertaken lay the groundwork for further examination of the role of amino acids in the flavor of these samples. Because additivity in bitterness among selected amino acids was illustrated there is a need for further investigations of amino acid mixtures.

Data obtained in the present study for astringency perception was not consistent. Being a complex sensation it appears that future studies examining this parameter should focus on it alone. Tryptophane was the most bitter amino acid identified from the sensory evaluations; thus quantification of this amino acid would appear to be worthwhile. More in depth investigations of amino acid mixtures might include evaluation of: flavor properties and intensities of model systems which profile the total bitter amino acid content and possible additive or synergistic effects between free amino acids and other bitter compounds, such as ethyl α-D-galactopyranoside, which have been identified in plant proteins.

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APPENDICES

Appendix A Total number of observations occurring at each concentration level used in developing bitterness and astringency intensity patterns of single amino acids

Amino Acid	Concentrations (ppm)	Total Number of Observations of Bitterness Intensity	Total Number of Observations of Astringency Intensity [1]
ARG-HCL	2000	12/18	--
	4000	16/18*	12/15
	8000	18/18	14/15
	12000	12/12	9/10
	16000	18/18	12/15
ILEU	4000	13/18	11/15
	8000	18/18	14/15
	12000	12/12	8/10
	16000	18/18	13/15
LEU	1500	12/18	--
	5000	15/18	--
	10000	18/18	11/15
	12000	12/12	9/10
	15000	18/18	12/15
PHE	1000	12/18	--
	2000	13/18	--
	4000	18/18	--
	8000	18/18	--
	16000	12/12	--
TRY	1000	15/18	--
	2000	18/18	12/15
	3000	18/18	13/15
	4000	18/18	10/15

* 1 calculated NP value

[1] One panelist's scores omitted due to confusion of taste sensation

Appendix B Mean scores and number of observations occurring at each concentration level in astringency intensity patterns of single amino acids

Amino Acid	Concentration ppm	Mean Astringency Score	Number of Observations
ARG-HCL	1000	1.193	7/15
	2000	0.667	8/15
	4000	2.140	12/15
	8000	2.207	14/15
	12000	2.900	9/10
	16000	3.267	12/15
ILEU	1000	1.153	9/15
	2000	0.680	8/15
	4000	0.910	11/15
	8000	2.567	14/15
	12000	2.230	8/10
	16000	3.700	13/15
LEU	750	0.560	4/15
	1500	0.753	11/15
	5000	0.793	8/15
	10000	2.040	11/15
	12000	1.533	9/10
	15000	1.907	12/15
PHE	500	0.573	4/15
	1000	1.027	9/15
	2000	1.001	9/15
	4000	1.473	12/15
	8000	1.573	12/15
	16000	1.260	5/10
TRY	500	1.147	8/15
	1000	0.867	8/15
	2000	2.533	12/15
	3000	2.247	13/15
	4000	2.507	10/15