THE UNIVERSITY OF MANITOBA

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

by

MARETTE KAREN DONALDSON

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE

DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF FOODS AND NUTRITION

WINNIPEG, MANITOBA

SEPTEMBER, 1978

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A dissertation submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

ACKNOWLEDGEMENTS

I would like to thank my advisor Mrs. M. Vaisey-Genser for her invaluable guidance throughout the completion of this manuscript. My appreciation is also extended to Dr. E. Hoehn and Dr. M. McDaniel for their helpful suggestions and continued interest in the project.

A most sincere thank-you for a job well done to my panelists;

Ms. Nora Chan

Ms. Janet Fabro

Ms. Kathy Harrison

Ms. Stacy Johnson

Ms. Marsha Leith and

Ms. Nancy Morgan.

My thanks are also extended to Dr. G. Paul and Ms. M. Leith for advice regarding statistical analyses.

I would also like to acknowledge Mr. and Mrs. T. Donaldson and Mr. and Mrs. R. Hay for their help and support during the last few months.

Two very special thank-yous are extended to Ms. Joyce Nelson for the typing of this manuscript and to my husband Dr. M.F. Erin, for his encouragement and understanding throughout this entire project.

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.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

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