TRYPTOPHAN, CYSTINE & CYSTEINE CONTENTS OF RAW AND GRANULATED POTATOES: QUANTITATIVE IMPORTANCE AND NUTRITIONAL VALUE.

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ABSTRACT

Attempts to assess the tryptophan levels in whole tuber by colorimetry using glyoxylic acid-tryptophan chromophore reaction gave inflated results. Hence the slow and tedious Spies W method had to be retained. However, cystine + cysteine determination by a simple colorimetric procedure using $NaBH_4$ and Ellman's reagent was successful for both raw and cooked potatoes.

INTRODUCTION

The two S-containing amino acids, methionine and half-cystine (cysteine + cystine), belong to the essential amino acids of the potato tuber, along with eight other acids (isoleucine, leucine, lysine, phenylalanine, tyrosine, threonine, tryptophan and valine). It has been reported that methionine and cystine are the limiting amino acids of potato protein (1). The most affected amino acids in free and bound forms during treatment of potatoes were methionine, glycine and lysine (losses of 86.0; 82.7 and 67.7%, respectively (2,3,8).

In any of the protein hydrolysis procedures (4) the original concentrations of cysteine and cystine cannot be estimated individually as both are determined as half-cystine, or cystein acid. Simpson et al. (5) introduced a method which allowed the discrimination of these two amino acids in a single hydrolysate. Averages of 52% methionine and 62% cysteine/cystine, and up to 90% tryptophan can be destroyed by conventional potato protein analysis (6,7).

Colorimetric determination of cystine and cysteine in proteins (4,10) involves several steps: protein unfolding with guanidine HCl (urea); reduction of disulfide bridges into sulfhydryl groups with Na-borohydride; followed by destruction of excess hydride and color development with Ellman's (11) reagent [5,5'-dithio-bis(2-nitrobenzoic acid)]. The success of disulfide groups determination by Ellman's reagent (10) depends greatly on removal of the excess of Na-borohydride with these precautions, the absorbance of blanks is usually low (0.03-0.05). The dianion readings are taken at 410 nm,

using a molar absorption coefficient of 13,600 M^{-1} cm^{-1} for calculation. The absorption spectrum of dianion 2-nitro-5-thiobenzoic acid (TNB) was also critically examined (12,13).

Spies (14,15) introduced two hydrolytic methods for the determination of tryptophan in plant proteins. As reviewed by Friedman and Finley (16) protein-bound tryptophan gives a greater color yield with p-dimethylaminobenzaldehyde than free tryptophan. Another simple method for determination of tryptophan in food was developed by Oste et al. (17). As found by many authors, carbohydrates resulting from acid hydrolysis of starch into smaller polysaccharides and glucose may be primarily responsible for the partial destruction of tryptophan (18-20). Protective agents such as p-toluenesulfonic acid (21), thioglycolic acid (22) and 3,3-indolypropionic acid and other reagents (23), or 4N methane-sulfonic acid containing 0.2% 3-[2-aminoethyl]indole (5) have been offered to attenuate tryptophan degradation during classical protein acid hydrolysis. The latter method has provided the best recovery of tryptophan from pure protein, however the loss of tryptophan is still appreciable in samples containing more than 20% carbohydrate.

The procedure, based on the reactivity of the indole-ring to develop a colored derivative with glyoxylic acid - Hopkins-Cole reaction (24) has been successfully applied as a rapid and simple method for the determination of tryptophan in cereal grains (25). As claimed by Concon (25), the results for cereal proteins agreed quite favorably with those of the Spies (14,15) method using pronase enzyme hydrolysates. When the Opienska-Blauth et al. (24) method is applied to unhydrolyzed cereals, as prescribed by these authors, serious browning reactions occur.

Cysteine/cystine will be assayed using Ellman's reagent after cystine is reduced by Na-borohydride, and tryptophan colorimetrically using glacial acetic acid + Fe^{+2} and concentrated sulfuric acid. If the above procedures are found to be easy, reproducible and rapid, they will be recommended as standard methods of quality control for the potato dehydration industry.

MATERIALS & METHODS

Potato tubers (Solanum tuberosum; cv. Russet Burbank) grown in Alberta were obtained from I & S Produce Ltd. (Edmonton) and were used throughout all experiments.

Medium-size tubers were peeled and sliced. After soaking in 500ppm $NaHSO_3$ solution, slices were freeze-dried (model FFD-42-WS). The addition of $NaHSO_3$ and EDTA was aimed at prevention of enzymatic browning induced by polyphenoloxidase. Tuber sap extracts were obtained by squeezing the cubes in a hydroulic press at 24,000 psi. Slices from the same batch were also subjected to two different

cooking methods: (1) precooking at 70°C for 20 min, cooling in tap water for 20 min, and boiling for 30 min; (2) steaming for 30 min, followed by cooling in an air-blast freezer.

The boiled and steamed samples were also freeze-dried. All samples were powdered in a Waring blender, and stored in brown bottles at -20°C. Granule preparation by the Freeze-Thaw (F-T) process developed by Ooraikul (26) was used for the preparation of dehydrated potato granules. Nitrogen content was determined by the standard micro-Kjeldahl method (9,27).

The freeze dried samples of raw and cooked tubers and from various steps of the F-T process were subjected to colorimetric cysteine determination using DTNB, 5,5'-dithio-bis(2-nitrobenzoic acid) and the absorption was measured at 412 nm (4).

Tryptophan content in protein extracts obtained without preliminary hydrolysis was determined by the procedure reported by Concon (25).

RESULTS AND DISCUSSION

Cystine + cysteine (or half-cystine) content was determined in tubers spectrophotometrically using Ellman's reaagent. Peptide chains were unfolded by urea and disulfide bridges were reduced by Na-borohydride. Though the analytical procedure used was based on that of Felker and Waines (4), but their recommended molar absorptivity of 12,000 M^{-1} cm^{-1} was not used. The accuracy of this and other molar absorptivities used in the past was questioned by Riddles et al. (12). Therefore, the molar absorptivity was determined in this study (Table I).

The values were quite consistent from 16.667-83.333 µmoles of cysteine, giving an average of 12,687.5, a value 5.7% higher than that used by Felker and Waines (4). The molar absorptivity did change at lower cysteine concentrations, varying from 11,151-11,769 below 16.667 µmoles. In this study all tuber samples were analyzed in the range of 16.6-83.3 µmoles half-cystine using a molar absorptivity value of 12,687.5.

In the reation mixture containing the Ellman reagent (0.833 mMoles), equimolar contents of cysteine were expected to react with reagent. However, this was only partly true. As shown in Figure 1, position A there was a linear relationship between cysteine and A_{412} up to 0.25 mMoles cysteine/L, after which there was a plateau of readings which clearly commenced at 0.8 mMoles cysteine/L. There was a linear relationship with cysteine concentration in the range of 0.417-83.33 µmoles/L, and this range was used as a calibration curve throughout the study (Fig. 1, position B).

Table I Molar absorptivity of cysteine as affected by cysteine concentration in the reaction mixture of the applied procedure.

Cysteine Concentration in Final Solution (C; µMole/L)	Molar Absorptivity[1] ε (M^{-1} cm^{-1})	
0.417	11151 ± 730	
2.083	11164 ± 805	
4.167	11645 ± 223	
8.333	11769 ± 148	
16.667	12762 ± 167	
25.000	12720 ± 163	
33.333	12606 ± 115	Average
41.662	12720 ± 134	12687.5
50.000	12802 ± 144	
58.333	12528 ± 439	SD
66.667	12472 ± 245	± 265.9
75.000	12721 ± 348	
83.333	12854 ± 495	

[1] ε was calculated from the equation:
$\varepsilon = A_{412} / C \cdot D$; with D=1 cm

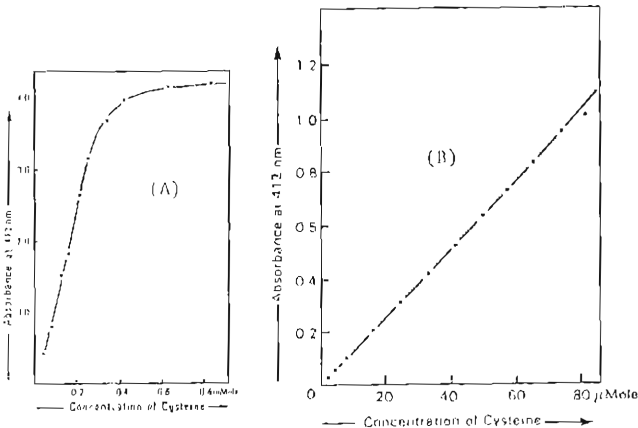

Fig. 1 Cysteine Ellman's reagent. (A), (B), Calibration curves.

As shown in Table II raw potato tuber total crude protein contained 1.22% half-cystine, agreeing with the content reported for tubers grown in Michigan by Kaldy and Markakis (9).

Steam-cooking of tuber and precooking followed by cooking in water resulted in a significant reduction of half-cystine content. As seen in Table II, steaming brought about 11% destruction of half-cystine, and precooking and boiling 24%.

Table II Total half-cystine content in potato tuber cv. Russet Burbank (Alberta) during its Freeze-Thaw processing into dehydrated granules.

Potato Sample	Total Half-cystine Content, (% of crude protein)
Whole Tuber	
Raw	1.22 ± 0.23
Steamed	1.08 ± 0.06
Precooked and Boiled	0.93 ± 0.23
Slicing	1.20 ± 0.08
Steam-Cooking	1.02 ± 0.05
Hot Mashing	0.92 ± 0.11
Freeze-Thawing	0.74 ± 0.01
Granulation	0.78 ± 0.05
End Product	0.78 ± 0.03
Discard Portion	0.74 ± 0.10

Destruction of half-cystine was also followed for various steps of a Freeze-Thaw granule process. As seen in Table II, the most significant reduction was found during steam-cooking, tuber hot mashing and freeze-thawing steps of the process. In subsequent dehydration steps no significant reduction was found, clearly emphasizing the need for involvement of water.

The calibration curve for tryptophan determination that was used in this study is shown in Fig.2,B.

The detection limit was close to 20 μg/ml and there was a linear relationship between absorbance and tryptophan concentration in a range of 80-200 μg tryptophan/ml at preselected reaction times of 70, 90, 110 or 130 min. The absorbance increased with increasing reaction time. The A_{545} increment for a 20 min time difference (for reaction times of 70-90 or 110-130 min) was 0.030 and was independent from tryptophan concentration in the 80-200 μg/ml range. The increment

readings between 90-110 min varied from 0.020-0.070 and were higher as tryptophan concentration increased.

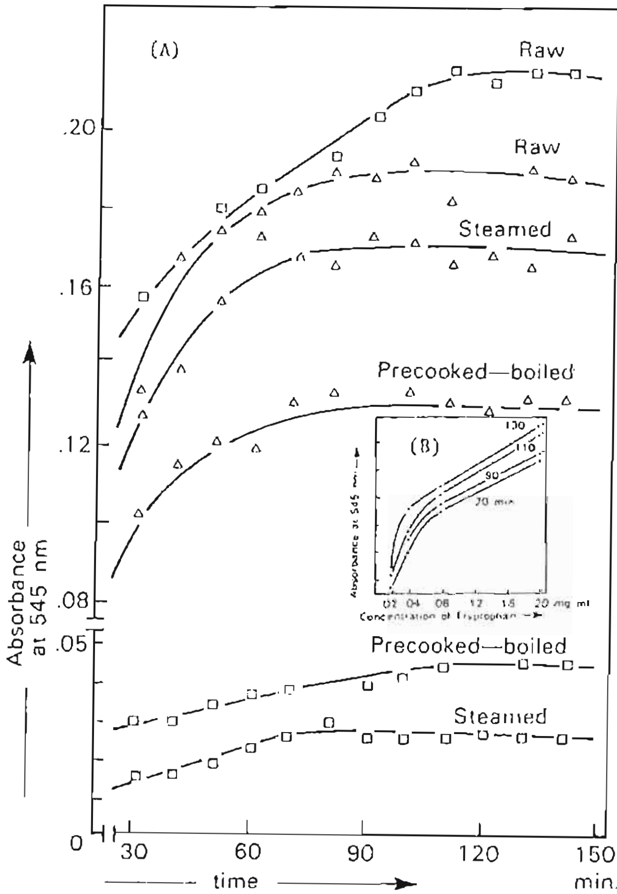

Fig. 2. (A), Color development of protein extracts by Concon's (1975) method. (B) Tryptophan

Tryptophan determination requires, as a first step, total-N (free and protein-bound amino acid-N) solubilization with a suitable solvent. Two solvents appear to be the most suitable for raw and cooked and freeze-dried and powdered potato samples. The first, dilute NaOH (0.075 N), solubilized up to 88% of the raw tuber total-N. However, it had a low efficiency in solubilizing total-N of steam-cooked potatoes (<33.5%) and of precooked and boiled samples (39.9%). The second solvent system, consisting of 70% ethanol and dilute NaOH in a ratio of 1:4 v/v, solubilized 91% of the available total-N of the raw tuber, while its efficiency was 58.2% for steam-cooked samples and 59.2% for precooked and boiled samples. When 0.5M NaCl was included into the solvent system (0.5 M NaCl:70% ethanol:0.075 N NaOH, 2:3:5 v/v), there was improved total-N solubilization (93.7% for raw, 80.1% for steam-cooked and 69.2% for precooked and boiled tubers).

When the potato tuber extracts were checked by iodine reaction for the presence of starch contaminants, the lowest amount of starch was detected with an ethanol:NaOH ratio of 1:4 v/v, while other ratios provided extracts with considerable starch content.

Concon (25) found that for cereals (wheat and rice) the maximum color development in tryptophan detection occurred after 20 min reaction time. In potato extracts the color development time was found to be much longer. As seen in Fig. 2,A, in extracts obtained with 70% ethanol the maximum color intensity was reached after 80 min for raw and boiled potatoes and after 70 min for steam-cooked samples. When dilute NaOH was used instead of ethanol, the time required was 110 min for raw and precooked and boiled samples, and 80 min for steam-cooked samples. Similar results were obtained when solvent mixtures were applied. Ethanol:dilute NaOH, 1:4 v/v, provided maximum color after 110 min for all samples, while extractant containing NaCl required times in excess of 120 min.

The Spies "W" results, when compared to those of colorimetric tryptophan determination (Tables III and IV), revealed that colorimetric data are inflated for cooked samples and low for raw tuber when readings were taken after 20 min reaction time. This pattern held regardless of protein extractant, except when NaCl was included, when the reverse trend was true (close to the correct tryptophan value with raw tuber and too low with cooked samples).

Tryptophan color reaction readings taken after 110 min, when the absorbance had leveled off, gave highly inflated tryptophan values, expressed as % of the available tuber protein. As seen in Table IV for raw tuber, this inflation was 26-31% for raw tubers and over 80% for cooked tubers.

Table III Tryptophan content of potato tubers cv. Russet Burbank grown in four locations in Alberta[1]

Sample Number	Location							
	Edmonton		Lacombe		Brooks		Vauxhall	
	mg/g	%	mg/g	%	mg/g	%	mg/g	%
1	1.27[1]	1.8[3]	1.10	1.2	1.51	1.8	1.54	1.7
2	1.16	1.2	1.26	1.6	1.19	1.5	1.34	1.5
3	1.46	1.9	1.35	1.6	1.19	1.6	1.46	1.8
4	1.18	2.0	1.36	1.8	0.98	1.3	1.08	1.5
Average:	1.27	1.7	1.27	1.6	1.22	1.6	1.36	1.6

[1]Analyses performed by Dr. M. Kaldy (Experimental Research Station, Agriculture Canada, Lethbridge)
[2]mg of tryptophan/g of potato dry matter
[3]Tryptophan percentage of the total protein

The source of error which contributed to inflated results was investigated. Reaction of other indoles in a glyoxylate mediated reaction was ruled out since no significant amounts of indoles other than tryptophan have ever been reported in potatoes. Unlike in the Spies and Chambers method (14,15), ethanol used in this study should not interfere. However, the possible interference of potato starch was assessed. Potato amylose was solubilized in 0.075 N NaOH (7mg/ml) and the tryptophan color developed (0.5 ml amylose solution + 0.5 ml tryptophan [180 μg/ml] + 3 ml reagent and 2 ml sulfuric acid). The A_{545} after 110 min was 0.134. When starch was omitted from the reaction mixture (blank), the reading was 0.122.

This difference accounted for an inflation in apparent tryptophan content of 0.04 μg/ml, i.e. only a 2.2% error. Therefore, starch contamination was ruled out as a major source of error. Finally, the water molarity of the reaction mixture was investigated as a possible source of error.

As outlined by Concon (25), the tryptophan determination reaction mixture consists of 1 ml aqueous sample solution, 2 ml of 25.8 N sulfuric acid and 3 ml of glacial acetic acid. In such a mixture the concentration of sulfuric acid was 8.6 N, and that of water 15.04 M. However, in this study the water content in this study the water

content in the solubilized samples, as well as in the final reaction mixture, was the variable component (as shown in Table V).

Table IV Tryptophan contents in raw and cooked tubers, determined by the colorimetric method of Concon (1975) using the suggested development time of 20 min

Tuber Sample	Extractant EtOH/NaOH ratio	Protein Extracted (%)	Tryptophan (% of protein)	Δ_{110}/Δ_{20} Q-value[1]
Raw	1:4	90.3 ± 0	1.096 ± 0.059	1.652
Raw	0:1	84.2 ± 5.9	1.142 ± 0.028	1.621
Steamed	2:1	51.7 ± 4.9	1.775 ± 0.152	---
Steamed	1:0	39.7 ± 1.40	2.270 ± 0.171	1.600
Precooked and Boiled	2:1	48.1 ± 1.03	1.699 ± 0.018	---
Precooked and Boiled	1:0	28.5 ± 0.91	2.562 ± 0.089	1.635
	Extractant NaCl/EtOH/NaOH Ratio			
Raw	2:3:5	93.69 ± 3.8	1.500 ± 0.16	2.033
Steamed	2:3:5	80.10 ± 2.6	1.352 ± 0.14	2.165
Precooked and Boiled	2:3:5	69.18 ± 4.8	0.972 ± 0.17	2.358

[1] Q-value = ratio of tryptophan color intensity reading at A_{545nm} after 110 min when color development levelled off and after 20 min, as used for cereals (Concon, 1975).

The water molarity varied from 8.55-15.04, a molarity change which closely followed the sigmoid curve for cereals, as established by Concon (25), i.e.

$$\text{Log } A = b \cdot S - C, \quad \text{with } b<0$$

where b is the slope of the curve (3.5), S is the sample reading and C is the blank. Hence, just changing the water molarity even within narrow limits would provide A_{545} readings inflated by 40% or more (last column of Table V). Taking into account such corrections, tryptophan contents in raw and cooked potatoes could be adjusted to nearer true values. However, the question would still remain, "Is the

partially extracted N of cooked tubers representative of the average tryptophan content of the tubers?"

Table V Water molarity in reaction mixture and calculated A_{545} in tryptophan color development for raw and cooked potato tubers

Protein Extractant	70% EtOH	0.075N NaOH	0.5M NaCl	Water in Extract (μl)	Molarity of Water Final Rxn. Mixture[1]	% of max. A_{545} Expected
Ethanol Dominant	1	0		300	8.55	>140
	2	1		533	10.71	>138
	3	1		475	10.18	140
	4	1		440	9.85	141
	5	1		417	9.64	142
Sodium Hydroxide Dominant	0	1		1000	15.04	50-90
	1	2		766	12.87	122
	1	3		825	13.42	115
	1	4		860	13.74	110
	1	5		883	13.95	110
Sodium Chloride Containing	1	5	3	922	14.31	100
	2	5	3	860	13.74	110

[1]Includes 624 μl water content from 2 ml of added 25.8 N sulfuric acid

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Accepted for publication: November 23, 1987.