

Nonvolatile Flavor Components in Chinese Quince Fruits, *Chaenomeles sinensis koehne*

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모과의 비휘발성 Flavor 성분 에 관한 연구

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Abstract

This study was performed to identify detailed informations on the nonvolatile flavor of chinese quince fruits, *Chaenomeles sinensis koehne*. About 72% of the free amino acids were shown to be valine, asparagine, γ -aminobutyric acid, aspartic acid and serine. Arginine, tyrosine, methionine and tryptophan were not present. Glutamic acid and glutamine as a amino acid for peptides were the major components, whereas cysteic acid, methionine sulfone and tryptophan were not detected. The nucleotides attained were composed of cytosine, uridine-5'-monophosphate and cytidine-5'-monophosphate, and these were proved to be a very small quantity. Guanosine-5'-monophosphate, inosine-5'-monophosphate and adenosine-5'-monophosphate were not present. The major sugars were shown to be glucose, sorbose, sucrose and fructose. Fructose was the most abundant one among them. A total of 11 organic acids were identified by capillary gas chromatography and capillary gas chromatography-mass spectrometry. The major components identified were tartaric acid and α -ketoglutaric acid. The total content of vitamin C determined was 386.6mg%, and those of ascorbic, dehydroascorbic, and 2, 3-diketo-L-gulonic acid were 28.8mg%, 154.5mg% and 197.3mg%, respectively. Calcium and phosphorus were the major components, while heavy metals such as cadmium, copper and lead were determined to be a small amount. In the result of organoleptic test on the natural and synthetic extract of chinese quince fruits, the principal taste components consisted of free amino acids, sugars, organic acids, vitamin C and minerals. Five groups mentioned would have a favorable influence upon the taste of fresh chinese quince fruits. Key words: nonvolatile flavor components, chinese quince fruits

Introduction

The chinese quince trees, *Chaenomeles sinensis koehne*, which belong to a *Rosaceae* family, blossom out in May and produce the yellow elliptical fruits in November.

The fruits are unsuitable for eating raw

because of heavy astringent taste and stone cells. However it has been used as food materials for teas, syrups, gums, cakes and wines. The fruits have several pharmacological effects such as an accelerator for digestion, a sedative for vomiting and diarrhea, an antiberiberi, an antispasm for muscular system, an atineuralgia for the lower limbs, an antianemia, a cough remedy, and expectorant and so forth. Therefore dehydrated fruits have been largely used for medicinal purposes. Few reports on nonvolatile flavor compo-

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nents are observed although pharmacological researches on the useful components of chinese quince fruits have been much performed.

This study was undertaken to identify the nonvolatile flavor components and apply for the recombinational flavorings as a basal data.

Materials and Methods

Materials

Fully-ripened chinese quince fruits were purchased at Cheongdo market, Kyeongsangbuk-do, on November 19th, 1986. The tap water washed the dirt off from fresh fruits after purchasing, and put it into a polystyrene bag. These were stored at -20°C freezer.

Analysis of chemical composition

The moisture was determined by oven drying method at 105-110°C under an atmospheric pressure⁽¹⁾. Crude ash, crude fat, crude protein and crude fiber were measured by a conventional method, respectively^(2,3). Pure protein content was determined by Barnstein's method⁽⁴⁾. The total sugar and the reducing sugar were calculated by Somogyi-Nelson's method^(5,6).

Nonprotein-nitrogenous compounds

Five grams of fresh sample were repeatedly extracted by refluxing with 50ml each of 80% ethanol three times, and the combined extract was deproteinized by the addition of trichloroacetic acid and 5-sulfosalicylic acid. The deproteinized extract was used for the determination of extractive-N, free amino acid-N⁽⁷⁾, peptide-N⁽⁷⁾, ammonia-N⁽⁸⁾ and amide-N⁽⁸⁾.

Free amino acids

The above-deproteinized extract was demineralized through Amberlite cation and anion column chromatography. Free amino acids were determined by the method for physiological fluid

analysis using Hitachi 835 automatic amino acid analyzer.

Amino acids in peptides

The above-deproteinized extract was hydrolyzed by 6N hydrochloric acid at 110°C sand bath for 24 hours under a complete vacuum. The total amount of cysteine and cystine was determined by cysteic acid analysis after performic acid oxidation of the peptide⁽⁹⁾. Methionine was also determined as the sulfone after performic acid oxidation of the peptide. The content of tryptophan in peptides was measured with a spectrophotometric method proposed by Gaitonde *et al*⁽¹⁰⁾.

Nucleotides

The nucleotides extracted with 10% cooled perchloric acid proposed by Nakajima *et al*⁽¹¹⁾, were determined by HPLC equipped with Bondapak C-18 column.

Sugars

Ten grams of the fresh sample were homogenized in 5 volumes of 80% ethanol in a homogenizer, and the supernatant was freed from homogenate in centrifugal baskets at 3,000×g for 10 mins. The residue obtained after centrifugation was repeatedly extracted more twice as mentioned above. The combined supernatant was approximately adjusted to pH 7.0 with 0.1N diluted sodium hydroxide, and the enzyme in this supernatant was then inactivated by treating in water at 80°C, for 10 mins. The solvent was taken off on a flash evaporator, and the aqueous liquor was passed successively through columns of Amberlite IR-120 and Amberlite CG-4B. The effluent and washings were combined and concentrated to a constant volume under reduced pressure, and this was used for the analysis of sugars by HPLC equipped with Bio Rad HPX-87 column.

Organic acids

Titrateable acidity in 80% ethanolic extracts was expressed as milliequivalent of 0.1 N diluted sodium hydroxide required to neutralize to the end point of phenolphthalein. Total acidity was determined by a conventional method using a cation-exchange column chromatography. Volatile acidity was expressed by the difference between the nonvolatile and titrateable acidity after steam distillation at atmospheric pressure. Combined acidity was then obtained by subtracting titrateable acidity from total acidity⁽¹²⁾. Organic acids being adsorbed in the anion column during the pretreatment of the extracts for GC analysis were eluted with 1.5N ammonium carbonate⁽¹³⁾. The eluate was successively passed through the cation column in order to remove cations⁽¹⁴⁾. The effluent was evaporated to dryness under reduced pressure, and the residue was esterified with BF₃-methanol solution⁽¹⁵⁾. The resulting methyl esters were identified by GC and GC-MS techniques, and determined by external-standardization techniques⁽¹⁶⁾.

Vitamin C

The hydrazone derivative for measurement of vitamin C was analysed by a spectrophotometric method⁽¹⁷⁾.

Minerals

Cations in the sample prepared by dry and wet ashing method were measured with an atomic absorption spectrophotometer (Perkin-Elmer 2380) according to the conventional method. Phosphorus was determined by molybdenum blue colorimetry.

Natural extract

One hundred grams of fresh sample chosen was homogenized by using 2 volumes of deionized water. After centrifuging, the supernatant was treated with an equal portion of hexane in order to remove the turbid lipid fraction. The

aqueous layer being contaminated with trace amount of hexane during the separation was evaporated to an adequate volume under reduced pressure, and the total volume of solution was adjusted to 100ml with deionized water. This sample prepared was commonly referred to as the whole natural extract and also used for a sensory testing panel.

Complete synthetic extract

The complete synthetic extract was likewise formulated according to the analytical value obtained from the above experiments. The pH was exactly adjusted to 6.8 with concentrated hydrochloric acid in accord with that of the natural extract.

Taste panel assessment

The panel being made up of 7 members (7 women) was selected out of 100 applicants. The panel was trained to the standard level of proficiency in five-taste difference test and concentration difference test before all the other taste panel assessments. In the mode of the operation of a sensory test, a set of test solutions being composed of one odd and two duplicate samples was present at room temperature to each panelist, who was instructed to select the odd sample. In order to obtain a total of 21 responses, each assessment was repeated three times. The results were statistically treated by using the table of criterion for the triangle difference test.

Results and Discussion

Chemical composition

Results obtained are given in Table 1. As shown in Table 1, the moisture content showed the highest value of 71.03%. The other components were abundant in the order of total sugar, crude fiber, crude fat, crude protein, crude ash and pure protein. The crude fat content showed

Table 1. Chemical compositions in 100g of fresh chinese quince fruits

Compositions	Contents
Moisture	71.03%
Crude protein	1.30%
(Pure protein)	(0.74%)
Crude lipid	1.80%
Total sugar	15.02%
(Reducing sugar)	(4.94%)
Crude fiber	6.69%
Crude ash	1.04%
pH	6.8
Alkalinity	19.00
Acidity	-8.40

lower value than that of a seed plant in the vegetable kingdom. This would be able to support the opinion, whose many investigators have reported that non-oleaginous fruits have a great commercial value for their carbohydrate reserves. Alkalinity and acidity were 19.00 and 8.40, respectively. It is well known that alkalinity is dependent on the contents of alkaline metals such as Na, K, Ca, Mg and so forth in foods, while acidity is determined by the contents of S, P, Cl and so forth. Since alkalinity showed higher value than acidity, it would be regarded as alkaline foods. On the other hand, it would become an important source of alkaline metals to us.

Nonprotein-nitrogenous compounds

The analytical data obtained are listed in Table 2. Nitrogen content in the free amino acid showed the highest value of 37mg%. This value was equivalent to about 42.05% of the extractive nitrogen content. The contents of the remainder were decreased in the order of the peptide nitrogen, the ammonia nitrogen, and the amide nitrogen. It is well known that these nonprotein-nitrogenous compounds have been vitally concerned with the taste, the nonenzymic browning and flavor deterioration. However, these would

Table 2. Nonprotein-nitrogenous compound compositions of fresh chinese quince fruits (mg/100g of wet weight basis)

Compositions	Contents (mg%)
Extractive-N	88.0
Free amino acid-N	37.0
Peptide-N	21.0
Ammonia-N	19.0
Amide-N	11.0

have a significant effect on the taste.

Free amino acids

The compositions of free amino acid measured with automatic amino acid analyzer are given in Table 3. The major constituents indicating higher value than 10% were composed of asparagine, valine and γ -aminobutyric acid. Glutamine, glycine, methionine, tyrosine, β -alanine, tryptophan, lysine and arginine were present at an undetectable level. Glutamic acid being a delicious taste was found at an average level of 18.3%. Leucine concerned with a precursor of volatile flavor components, such as 3-methyl-1-butanol and so forth, was present approximately in the same as that of glutamic acid. However, free amino acids detected would play an important role in the taste of fresh chinese quince fruits from an independent or a complementary standpoint. On the other hand, not only bitter amino acids, such as proline, valine, isoleucine, leucine, phenylalanine and histidine but also a tasteless one, such as asparagine, would also play an important role as an enhancement in the natural extract.

Combined amino acids

The amino acid compositions in peptides measured with automatic amino acid analyzer are listed in Table 4. Peptides are poor in sulfur since cysteine, cystine and methionine are absent, and contains no tryptophan. On the other

Table 3. Free amino acid compositions in 100g of fresh chinese quince fruits

Amino Acids	Contents(mg%) ^{a)}	% to Total Amino Acids
Aspartic acid	36.4	9.5
Threonine	10.5	2.7
Serine	29.9	7.8
Asparagine	82.5	21.5
Glutamic acid	18.3	4.8
Glutamine	0	0
Proline	5.6	1.5
Glycine	0	0
Alanine	20.4	5.3
Valine	87.9	22.9
Cysteine	7.0	1.8
Methionine	0	0
Isoleucine	10.4	2.7
Leucine	15.2	4.0
Tyrosine	0	0
Phenylalanine	7.4	1.9
β -Alanine	0	0
γ -ABA ^{b)}	41.0	10.7
Tryptophan	0	0
Lysine	0	0
Histidine	10.8	2.8
Anserine ^{c)}	0	0
Carnosine ^{d)}	0	0
Arginine	0	0
Total	383.3	99.9

a) Mean value of triplicate determinations within 5% deviation.

b) γ -ABA is substituted for γ -aminobutyric acid.

c) Anserine means a common name for β -alanyl-L-methyl-L-histidine.

d) Carnosine means a common name for β -alanyl-L-histidine.

hand, peptides are rich in acidic and amide amino acids since glutamic acid, aspartic acid, glutamine and asparagine are abundant. As shown in Table 3, dipeptides such as anserine and carnosine are absent, and these may not be concerned with the taste. According to a result of Table 4, alpha-L-glutamyl dipeptide, such as glu-asp, glu-glu, glu-ser and glu-thr, known to be a source of delicious tasting could be in

Table 4. Amino acid compositions of peptides in 100g of fresh chinese quince fruits

Amino Acids	Contents(mg%) ^{a)}	% to Total Amino Acids
Cysteic acid	0	0
Methionine sulfone	0	0
Aspartic acid & Asparagine	21.8	11.0
Threonine	11.7	5.9
Serine	11.3	5.7
Glutamic acid & Glutamine	22.3	11.3
Proline	10.2	5.1
Glycine	10.9	5.5
Alanine	11.4	5.8
Valine	15.6	7.9
Isoleucine	11.3	5.7
Leucine	19.0	9.6
Tyrosine	5.2	2.6
Phenylalanine	14.9	7.5
Lysine	18.6	9.4
Histidine	5.7	2.9
Tryptophan	0	0
Arginine	8.3	4.2
Total	198.2	100.1

a) Mean value of triplicate determinations within 5% deviation

existence as shown in Table 4. The carboxyl-terminal end of delicious tasting α -L-glutamyl dipeptides is generally composed of hydrophilic amino acids. In addition, tasteless-tasting (e.g., glu-gly, glu-ala, glu-pro and glu-val) and bitter tasting α -L-glutamyl dipeptides (e.g., glu-Ile, glu-Leu, glu-tyr and glu-phe) may be also in existence. The carboxyl-terminal end of both tasteless-tasting and better-tasting α -L-glutamyl dipeptides, however, is composed of the hydrophobic amino acids. At any rate, α -L-glutamyl dipeptides of the three groups described would play an important role in the taste of the natural extract.

Nucleotides

Nucleotide components in the extracts are given

in Table 5. Cytosine, CMP, and UMP comprised the major part of the total nucleotides. These were found at almost equal levels in fresh chinese quince fruits. Guanosine-5'-phosphate, IMP, and AMP were present at an undetectable level. The total nucleotides of in far less quantity than those of both marine and animal food-stuffs. Such a tendency would have something in accord with many fruits and vegetable foods. Although the total nucleotides were present in very small amounts, they would enhance the flavor potentiating activity of fresh chinese quince fruits in the presence of other flavor potentiators.

Table 5. Nucleotide compositions in 100g of fresh chinese quince fruits^{a)}

Nucleotides	Contents (mg%)	Nucleotides	Contents (mg%)
Cytosine	3.5	GMP	0
CMP	1.5	IMP	0
UMP	2.0	AMP	0

a) Mean value of triplicate determinations within 5% deviation.

Sugars

Results of the sugar components are summarized in Table 6. Fructose was the most abundant in fresh chinese quince fruits. The contents of the remainder except fructose were decreased in the order of glucose, sorbose and sucrose. Such the composition of the free sugars seems to be in common with many fruits and vegetable foods. It is well known that the relative sweetness of sugar would be considerably dependent on the amounts of low molecular weight monosaccharides, the concentration of solids, the temperature at which they are used, and other substances such as free amino acids and so forth resulting from the extracting procedure which may be coexisted¹⁸⁻²⁰⁾. All of the free sugars analyzed in the present work exhibited a sweet taste, and these would directly or indirectly play important

Table 6. Sugar compositions in fresh chinese quince fruits (mg/100g of tissue)^{a)}

Sugars	Contents	Sugars	Contents
Sucrose	25.2	Sorbose	345.7
Glucose	630.1	Fructose	3609.2

a) Each value is the mean of the values for triplicate determinations within 5% deviation.

roles in flavor of fresh chinese quince fruits.

Organic acids

Individual acidities based on alkalimetry and concentrations or organic acids determined by calibration curves for authentic specimen are listed in Table 7. Total acidity, which represents the sum of all the acids (e. g., free or combined with cations) was 36.3 milliequivalent. Eleven components among the total of 24 peaks separat-

Table 7. Varieties of acidity and concentrations of organic acids in 100g of fresh chinese quince fruits

Compositions	Contents ^{a)}
Total	36.3 ^{b)}
Titrateable	33.5
Volatile	4.0
Nonvolatile	29.5
Combined	2.8
Lactic	1.0 ^{c)}
Oxalic	27.7
Fumaric	1.2
Succinic	25.2
Maleic	6.3
Malic	42.1
α -ketoglutaric	108.6
Palmitic	30.1
Stearic	27.3
Tartaric	122.9
Citric	6.2

a) Each value is the mean of the values for triplicate determinations within 5% deviation.

b) All the acidity expressed in 0.1N NaOH ml equivalent.

c) Concentrations of organic acids expressed in mg/100g of fruit.

ed on the gas chromatogram were identified by comparing with the retention times and mass spectra of authentic specimen. Tartaric acid was the most abundant organic acid, but lactic and fumaric acid were present only in traces. All of the organic acids present in fresh chinese quince fruits might play an important role in general metabolism, particularly in respiration, the synthesis of phenolic compounds, lipids, amino acids volatile aromas and so forth⁽²¹⁾. These would probably play an important role as the promoter of sweetness or the main source of acidity in fresh chinese quince fruits.

Vitamin C

The results of the vitamin c analogue analysis are summarized in Table 8. In the case of fresh chinese quince fruits, the inactive DKA (2, 3-diketo-L-gulonic acid), formed by hydrolysis of DHA (dehydroascorbic acid), showed 197.3mg% of fresh weight. The active ASA similar to the structure of D-glucose was the lowest content as 28.8mg% of fresh weight. As shown in Table 8, 2, 3-diketo-L-gulonic acid (DKA) was the major component with dehydroascorbic acid (DHA) being the next most abundant, while L-ascorbic acid (ASA) was not significant. Dehydroascorbic acid, being the main product of the autoxidation of ASA with oxygen in acid solutions and indicating biological activity equivalent to ASA in human being, roughly comprised 40.6% of the total vitamin C. It is well known that vitamin C

Table 8. Concentration of vitamin C in 100g of fresh chinese quince fruits

Vitamin C	Contents (mg%) ^{a)}
ASA	28.8
DHA	154.5
DKA	197.3

a) Each value is the mean of the values for triplicate determinations within 5% deviation.

ASA: L-ascorbic acid, DHA: dehydroascorbic acid, DKA: 2, 3-diketo-L-gulonic acid.

content of foods is highly variable, reflecting contributions of the food material, degree of ripeness, origin and storage conditions. The possibility that vitamin C exist overwhelmingly in the oxidized form might be caused by a deterioration in the quality of fresh chinese quince fruits during freezing preservation. On the other hand, ASA has a slight acidity, so that it would play a great role in flavor of fresh chinese quince fruits.

Minerals

The mineral content in the samples was given in Table 9. Calcium comprised the major part of the cations. Phosphorus was the next most abundant cation. Heavy metal contents such as cadmium, copper, lead and zinc which cause a great trouble in the food sanitation aspects were shown to be the minor cations. According to Shallenberger *et al*⁽²²⁾, it seems that the hydrate and the complex salt of a certain inorganic compound would have a sweet taste as a moderately diluted concentration. For such a back-

Table 9. Minerals in fresh chinese quince fruits (mg/100g of fresh weight)

Minerals	Contents (mg%) ^{a)}		
	1	2	3
Cadmium	0	0	0
Copper	0.1	0.1	0.1
Iron	2.6	2.6	2.6
Manganese	3.1	1.4	2.3
Magnesium	1.1	6.6	3.9
Lead	0.02	0.02	0.02
Zinc	0.7	0.4	0.6
Calcium	25.3	26.8	26.1
Vanadium	0	0	0
Phosphours ^{b)}	10.2	17.0	13.6

a) Each value is the mean of the values for triplicate determinations within 5% deviation.

b) Determined by molybdenum blue colorimetry.

1: Prepared by dry ashing method.

2: Prepared by wet ashing method.

3: The mean value between 1 and 2.

ground of useful information, the major or minor mineral constituents would be more or less effective to the taste of fresh chinese quince fruits.

Organoleptic test

The results of group omission test on the complete synthetic extract are given in Table 10. The test solution, which omitted amino acids from the complete synthetic extract, clearly showed a tendency to decrease sweetness and a delicious taste, but strongly increased sourness. In the case of nucleotides, no clear difference was observed between the test solution and the complete synthetic extract. Some panelists, however, pointed out a decline in the intensity of sweetness and a delicious taste. Such a tendency being not correlated to the extent to which nucleotides occur in fresh chinese quince fruits. Although sweetness, a delicious taste, complexity and the characteristic taste were markedly decreased, sourness and bitterness considerably strengthened when sugars were omitted. The test solution in the absence of organic acids appreciably lacked sourness, the characteristic taste and harmony. In the case of vitamin C, although a result of organoleptic test individually indicated a delicate nuance in expression, it seems to be similar to that of organic acids. In any event,

it would be evident that both groups play an important role as the main source of sourness in fresh chinese fruits. In the omission test of minerals, sweetness and a delicious taste suffered some deterioration, whereas bitterness slightly increased. The solution tested lacked to a certain extent in the harmony. Minerals would play an important role as a buffer substance in the taste of fresh chinese quince fruits. Thus, it was confirmed that the principal components responsible for the taste of fresh chinese quince fruits consisted of amino acids, sugars, organic acids, vitamin C and minerals. All panelists stated that the complete synthetic extract satisfactorily simulated the taste of the natural extract, but that the synthetic one had slightly less bitterness and mildness. Furthermore all panelists confirmed the conclusion that the aroma of the synthetic extract was also inferior to that of the natural one. It is natural that the synthetic extract had neither nonvolatile flavor components other than six groups analyzed above nor volatile flavor components. For these reasons, further work on the substances closely related with the taste and aroma, should be conducted in the future, as a continuation of this study.

Table 10. Group omission test on complete synthetic extract

Omitted group	No. of correct identifications (n=21)	Level of significance ^{a)}	Degree of dif. ^{b)}		
			2	1	0
Amino acids	21	***	7 ^{c)}	0	0
Nucleotides	5	(-)	0	0	7
Sugars	20	***	5	2	0
Organic acids	19	***	4	3	0
Vitamin C	14	**	0	4	3
Minerals	17	***	4	3	0

a) ***, P<0.001; **, P<0.01

b) 2, obvious; 1, slight; 0, indistinguishable

c) Number of panellists reporting each degree of difference

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요 약

본 연구는 모과의 비휘발성 성분에 관한 조성을 규명하기 위한 기초 연구 과제로써 행하여졌다. 주요 비휘발

성 성분은 유리 아미노산의 경우 valine, asparagin γ -aminobutyric acid, aspartic acid 와 serine 이 전체의 72%를 차지하고 arginine, tyrosine, methionine 과 tryptophan 은 거의 검출되지 않았다. Peptide 구성 아미노산으로는 주로 glutamic acid 와 glutamine 이었으며 cysteic acid, methione sulfone 과 tryptophan 은 검출되지 않았다. 핵산관련 물질의 분석 결과 cytosine, UMP, CMP 는 소량 존재하였으며 GMP, IMP, AMP 는 검출되지 않았다. 당 분석 결과 주 성분은 glucose, sorbose, sucrose, fructose 였으며 fructose 함량이 가장 높은 것으로 판명되었다. GC 및 GC-MS 방법에 의해 총 11성분의 유기산이 동정 되었으며 이들중 tartaric acid 및 α -ketoglutaric acid 가 주 성분이었다. 정량된 총 비타민 C 함량은 386.6 mg% 였으며 ascorbic acid, dehydroascorbic acid 와 2, 3-diketo-L-gulonic acid 는 각각 28.8mg%, 154.5 mg%, 197.3mg% 였다. 무기성분으로는 칼슘과 인이 주요 성분으로 나타났으며 카드뮴, 구리, 납은 소량 존재하였다. 모과의 천연 및 합성 extract 에 대한 관능검사의 결과 유리형, 아미노산, 당, 유기산, 비타민 C 및 무기질의 맛의 주성분으로 판명되었다. 따라서 모과의 향미 성분중 비휘발성 성분은 주로 상기 다섯 그룹에 의해서 나타남을 확인하였다.

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