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Psicose Contents in Various Food Products and its Origin

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To elucidate the history of human exposure of psicose, a determination method of psicose (D/L-psicose) in various food products has been developed: post column high-performance liquid chromatography (HPLC) using gel permeation column in ligand exchange mode coupled with pulsed amperometric detection (PAD). The calibration curve of D-psicose was linear in a concentration range of 5 to 150 μg/ml with a correlation coefficient of 0.9999. With ultrasonic extraction and C18 Sep-pack filtration methods, the samples for HPLC analysis could be prepared from various food products within 0.5–1.5 h without any interference materials. The psicose recovery ($n=5$) from coffee and corn-snack was found to be 96.6% with relative standard deviation (R.S.D.) of 1.9% and 96.7% with R.S.D. of 3.0%, respectively. The resultant psicose content varied from 0.5 mg/100 g (coffee) to 130.6 mg/100 g (Worcester sauce). In particular, confectionery products and seasoning sauces exhibited higher psicose content than other studied products. In high sugar food products, heat processing had a marked effect on the production of psicose. The psicose formation due to heating was suggested to be a non-enzymatic reaction.

Keywords: rare sugars, D-psicose, food products, high-performance liquid chromatography, pulse amperometric detection

Introduction

D-Psicose is a C-3 epimer of D-fructose, which is found in very small quantities in nature. Recently, Izumori and coworkers developed a new method for the mass production of D-Psicose (Takeshita *et al.,* 2000). Although D-Psicose may be useful as sweetening agent to reduce caloric intake (Matsuo *et al.,* 2002), its safety as a food additive has not yet been evaluated. Therefore, it is currently used mainly as a raw material for the production of some rare sugars (Sasahara *et al.,* 1998) or for research purposes. However, since sugars may be widely used in medical foods as a food additive, the safety of D-psicose should be established. The results of the determination of psicose content in food products should support the safety assessment of D-psicose products such as tagatose (Levin, 2002; Fifty-seventh report of the joint FAO/WHO Expert Committee on Food Additives, 2002).

It has already been reported that psicose is found in foodstuffs, for example, processed cane and beet molasses (Binkley, 1963), steam treated coffee (Luger & Steinhart, 1995) and wheat plant (Miller & Swain, 1960). However, little is known about the psicose content in food products and its daily intake. Various techniques have been used to determine psicose content, but they are laborious and very time consuming. Therefore, development of a routine, sensitive, rapid and accurate method for determination of psicose content in a variety of food products is required.

Recently, various highly sensitive analysis methods for sugars were reported (Chaplin, 1994; Nakamura, 1999; Yasuno, 1997). The high pH anion-exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD) is the most commonly used method for determination of sugars and it provides excellent sensitivity (Chaplin, 1994, Corradini *et al.,* 1997). Therefore, in the initial stage of this study, we discussed development of an optimum separation standard for D-psicose and D-fructose by HPAEC-PAD. During HPAEC-PAD analysis, D-psicose did not elute as a single peak, and thus the method was not sensitive enough to detect its amounts. Moreover, we recognized that D-fructose was partially converted to psicose during the chromatography. Andersen and Sørensen (2000) reported that due to the high pH, fructose partially decomposes during the separation process. Since D-Psicose is C-3 epimer of D-fructose, we employed a post column method, which combined size separation with ligand exchange column under mild alkaline conditions using 10^{-4} M NaOH solutions as eluent, and 0.3 M NaOH solution as post column reagent for PAD.

In this study, to elucidate the history of human exposure to psicose, we focused on determining the psicose content of various food products. In order to accomplish this, we have developed a sensitive and reliable HPLC-PAD method for determination of psicose, and reported the contents of psicose in several food products using this method.

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Materials and Methods

Chemicals D- and L-Psicose were supplied by Rare Sugar Research Center, Kagawa University (2393 Mikichou, Kita-gun, Kagawa). Unless otherwise specified, all other sugars and chemicals were obtained from Wako Pure Chemical Industries Co., Ltd. (Osaka, Japan). The HPLC-grade water used was from a Milli-Q water system (Millipore S.A., 67120 Molsheim, France).

Samples and Sample preparations Various food products and foodstuffs (confectionary products, dishes, fruits, seasonings and beverages) were purchased from local markets in Takamatsu area, Kagawa, Japan. The samples for analysis were prepared as follows: solid food products were cut into small pieces (<1 mm) and homogenized using a mini-chopping machine (Iwatani Int. Co., Tokyo, Japan). An appropriate amount of homogenates was precisely weighted in a beaker, to which 40–50 mL of HPLC grade water was added. Extraction was carried out using an ultrasonic treatment (Sharp UT-204, Sharp Co., Tokyo, Japan) at 20℃ for 10 min. The extract was centrifuged at 3500 xg for 5 min and filtered through four-layer cheesecloth. The resultant pellet was re-extracted twice with HPLC grade water and then the combined supernatants were diluted to 100 mL with water. For liquid foods, a specific amount of sample was diluted to 100 mL with HPLC grade water, and a chloroform treatment was used to remove the lipids from the samples. The extracts were filtered through a 0.2 μm syringe filter CD/X (Whatman Inc., New Jersey, U.S.A.). The extracts (*ca.* 10 mL) were then passed through a Sep-Pak C18 cartridge (Waters Co., Milford, Massachusetts, U.S.A.) as described in a manual (S9204b, p64; Japan Waters, Co., Tokyo, Japan) before their injection into the HPLC system.

Apparatus and measurements The HPAEC-PAD analysis was performed on a Metrohm MIC-8 system (817 Bioscan, 709 IC pump, 812 valve units; Metrohm Ltd, Herisau, Switzerland) with a Metrosep Carb 1 column (4.6 min i.d. x 250 mm; Metrohm). The analysis conditions of sugars are provided by the company at its website (http//www.metrohm.com/).

The post column HPLC system with PAD detector consisted of an L-6000 HPLC pump (Hitachi Co., Tokyo, Japan) for eluent and an 865-CO column oven (Jasco Co., Tokyo, Japan). A metal free-isocratic 709 IC pump (Metrohm) for post column reagent, an 812 Valve injection unit with a 20 μL loop, an 813 Compact Auto sampler and an 817 Bioscan PAD detector with a gold working electrode (all from Metrohm) were used. Chromatographic data acquisition and system control were performed using a Metrhom IC Net 2.3 (Metrohm). A GL-C611 column (10.7 mm i.d. x 300 mm; Hitachi) packed with polystyrene gel (Na^+ form) was used for gel permeation chromatography with ligand exchange mode (GPC-LEX) of sugars in this study. Elution was performed at a flow rate 1 mL/min at 60℃ using 10^{-4} M NaOH solution as eluent. The eluted samples from the column were mixed with 0.3 M NaOH solution at a flow rate 0.43 mL/min to form the post column reagent for PAD analysis. Approximately 50 μL of the mixture was passed through a coil at 32℃. The following pulse potentials and durations were used for detection of sugars: $E_1 = 0.05$ V ($t_1 = 0.40$ S), $E_2 = 0.75$ V ($t_2 = 0.20$ S), $E_3 = -0.15$ V ($t_3 = 0.40$ S) with the sample analysis time set to 0.10 S.

Samples (20 μL) were injected into the HPLC system, and the concentrations of various sugars such as sucrose, D-glucose, D-fructose, D-sorbitol and D-psicose were determined by comparing their retention times to the calibration curves of the standards. The standard sugars were dried in a desiccator containing diphosphorus pentaoxide until constant mass was achieved, which was estimated to be the net weight of each sugar. The standard curves were obtained by injection with six concentration levels and measuring the peak areas of their chromatograms.

Recovery and reproducibility D-Psicose (20.1 μg/mL) was added as a standard to both the corn snack and the coffee, which contained either high fat or high colored materials. Each sample was extracted, and the psicose content was determined according to the method described above. The reproducibility of the method was evaluated by the performing five successive runs of the extraction and determination of coffee and corn-snack.

Culinary treatments of high sugar foodstuffs The effect of culinary treatment on the formation of psicose in high-sugar foodstuff was examined as follows: The conversion of fructose to psicose during heating processing was conducted using a modification method of Binkley (1963): 3 ml of cane juice (Brix 16.4, pH 5.6) was heated at 98℃ in 5 ml sealed vessels. At precisely 0, 1, 2, 3 and 4 h, aliquots of the solution were collected and the psicose content was determined by the HPLC method. In addition, culinary treatment of a caramel sauce preparation was performed as follows: 12.5 g of sucrose (Mitsui Sugar Co., Ltd., Tokyo, Japan) or fructose (Danisco A/S, Langebrogade 1, DK-1001, Copenhagen, Denmark), and 3.75 mL of distilled water were weighed in aluminum cups. The uncovered cups were then put on a heated flat pan at 255–265℃ and the temperature of reaction solution was measured by a non-contact infrared thermometer SK-8700 II (Sato Keiryoki Mfg. Co. Ltd., Tokyo, Japan). The cups were collected a precisely 0, 1, 2, 3, 4, 5 and 6 min and 7.5 mL of distilled water was added to end the process. The caramel sauce in cup was further diluted to 100 mL before its psicose content was determined by the above given method. The color of the diluted caramel sauce was evaluated as YI (yellowness), which was measured by a color meter ND-300A (Nippon Denshoku Industries Co. Ltd., Tokyo, Japan).

Results and Discussion

Separation of psicose by HPLC with PAD In this study, we developed a sensitive HPLC technique as a reliable method for determination of psicose (D-, L-psicose) in various food products. During HPAEC-PAD analysis, D-psicose (0.4 μg) did not elute as a single peak, and thus the method was not sufficiently sensitive to its detect amounts. Moreover, we recognized that D-fructose (4.0 μg) was partially converted to psicose during the chromatography (Fig. 1-A).

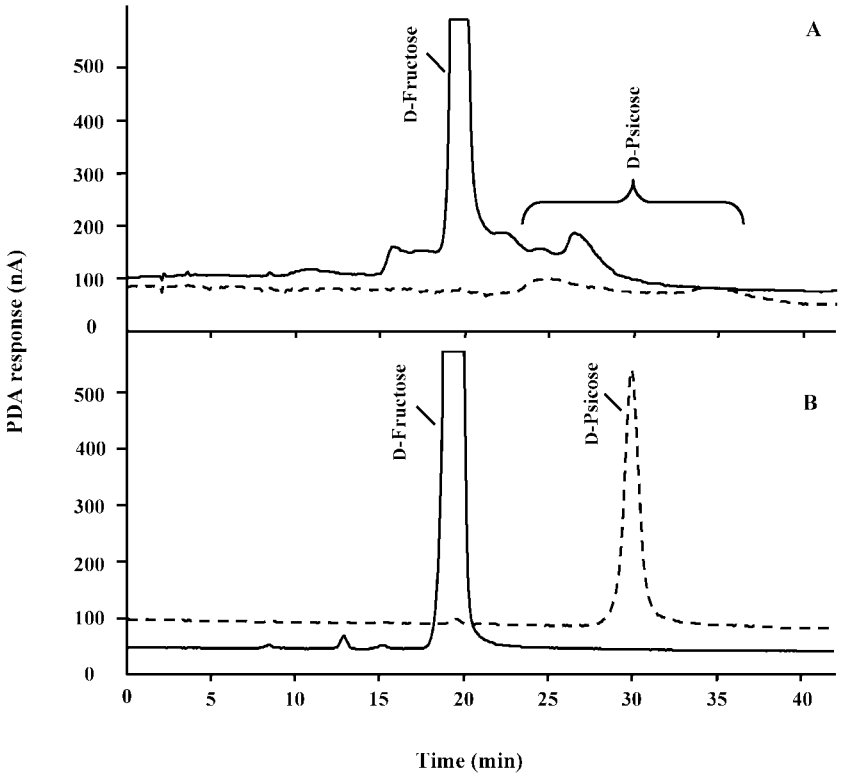

Fig. 1. Comparison of chromatograms of standard sugars obtained with two methods.
A, HPAEC-PAD; B, post column PAD; solid line, D-fructose; broken line, D-psicose Sample (20 μL) containing 0.4 μg of D-psicose or 4.0 μg of D-fructose was injected into the HPLC system.

Consequently, we employed a post column-PAD method with GPC-LEX column using 10^{-4} M NaOH solutions as eluent, under mild alkaline conditions. As expected, D-psicose (0.4 μg) and D-fructose (4.0 μg) eluted as a single peak with excellent sensitivity, and no other peaks were detected on the chromatogram (Fig. 1-B). Although Soga *et al.* (1992) have already reported carbohydrate analysis by hydrophilic interaction chromatography with PAD using post column pH adjustment, they did not discuss the separation of psicose on the chromatography. Moreover, the hydrophilic interaction chromatography used acetonitrile as eluent, which is unsuitable for routine analysis of sugars because of its high cost. In addition, the amino-derivative columns tend to lose their resolving power quicker than average due to stripping of amino-propyl ligand and Schiff base formation (Chaplin, 1994). Therefore, our method offers advantages in the determination of psicose in food products.

Figure 2 shows a chromatogram of various sugars: sucrose, D-glucose, D-fructose, D-sorbitol and D-psicose. While all sugars were effectively detected within 40 min, most were well separated, except for the coelution of L-psicose with D-psicose. Therefore, we could not determine the enantiomer mole fraction of D-psicose by this method. Recently, Shaw *et al.* (2003) developed a method to determine enantiomer mole fractions to determine sugars; however, the detection limit for the fructose was low at 0.1% (w/w) with enantiomeric excess of quantification at 0.5%. Therefore, this method was not applicable to the determination of psicose content in various food products.

Calibration curve and determination limit The calibration curves of D-psicose were linear in a concentration range of 5 to 150 μg/ml with a correlation coefficient (R^2) of 0.9999. Similar results were obtained with sucrose, D-glucose, D-fructose and D-sorbitol (Table 1). The relative standard deviation (R.S.D.) calculated from the lower limit concentrations of each sugar ($n=5$) was determined to be 2.2, 1.2, 1.5, 4.0 and 2.9% for sucrose, D-glucose, D-fructose, D-sorbitol and D-psicose, respectively. The R.S.D. and correlation coefficient values of our method were almost equal those of Soga *et al.* (1992). The detection limit for D-psicose was approximately 20 ng (signal-to-noise ratio of approximately 3).

Evaluation of the recovery and reproducibility of added D-psicose The extraction procedure using ultrasonic extraction and C18 Sep-pack filtration was very simple, and the samples for HPLC analysis could be prepared from various food products within 0.5–1.5 h. Moreover, the sample preparation method adequately isolated the HPLC analysis samples from other food components. Despite their high lipid content and color, the recovery and reproducibility of D-psicose from the corn-snack and coffee samples was excellent, as shown in Table 2 (coffee 96.6% and corn-snack 96.7%). The reproducibility of the results was also satisfactory with the R.S.D. of tested

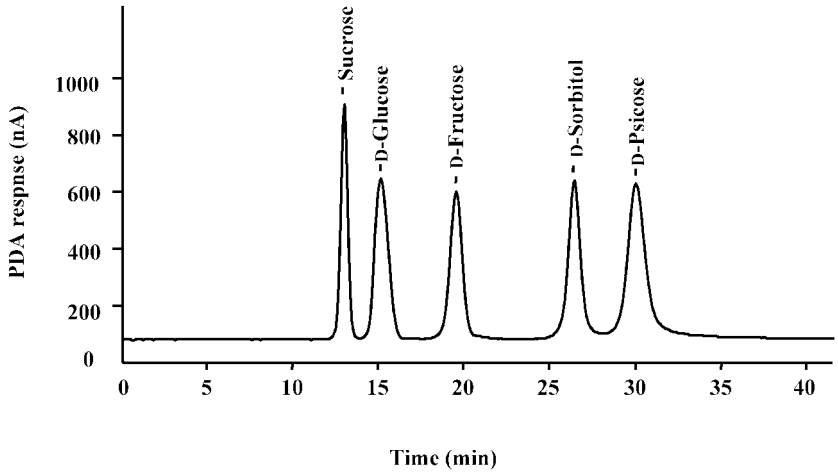

Fig. 2. Chromatographic separation of sugars on Hitachi GL-C611 column.
Sample (20 μL) containing 0.2 μg each of sucrose, D-glucose, D-fructose, D-sorbitol and 0.4 μg of D-psicose was injected into HPLC system.

Table 1. Determination of the concentration range of sugars and its reproducibility by the HPLC method.

	Determined lower limits (μg/ml)	Determined higher limits (μg/ml)	Correlation coefficient (R^2)	Relative standard deviation (R.S.D.)[1] (%)
Sucrose	1.0	50.1	0.9999	2.2
D-Glucose	2.5	75.0	0.9996	1.2
D-Fructose	1.0	75.0	0.9997	1.5
D-Sorbitol	2.5	100.6	0.9998	4.0
D-Psicose	5.0	150.1	0.9999	2.9

1) The R.S.D. values were calculated from the determined lower limits concentration of each sugar.

Table 2. Recovery of standard D-psicose from coffee and corn-snack samples.

	Coffee				Corn-snack			
Sample (n)	Blank[1] (μg/ml)	Added (μg/ml)	Found (μg/ml)	Recovery (%)	Blank[2] (μg/ml)	Added (μg/ml)	Found (μg/ml)	Recovery (%)
1	1.9	20.1	20.6	93.8	8.5	20.1	27.4	95.6
2	2.1	20.1	21.3	95.8	9.2	20.1	27.5	93.7
3	2.2	20.1	21.8	97.5	9.8	20.1	30.4	101.3
4	2.3	20.1	21.8	97.5	10.0	20.1	29.4	97.4
5	2.3	20.1	22.0	98.4	9.4	20.1	28.2	95.6
Average	2.1	20.1	21.5	96.6	9.4	20.1	28.6	96.7

1) Coffee was diluted to 1.25 folds with water, 2) Corn-snack was diluted to fifty folds with water.

coffee and corn-snack samples (n=5) at 1.9 and 3.0%, respectively.

Analysis of psicose content in various food products
Various food products from supermarkets were analyzed for their psicose content. Since the psicose contents of the food products varied widely, the samples were appropriately diluted to the analytical range of the HPLC, as shown in Table 1. The results of psicose content in various food products are summarized in Table 3, and a typical chromatogram is shown in Figure 3. There were no observed interference effects on chromatogram from the following food samples: confectionary products, seasonings, beverages and fruits. Since the C18 Sep-pack filtration avoided a gradual decline in the column resolution, no decrease in column performance was noted after as many as 400 injections. However, in the case of vegetable, there was some variation in retention times.

The psicose was widely distributed in various food products, and concentrations of the psicose in tested food products varied from 0.5 mg/100 g (coffee) to 130.6 mg/100

Table 3. D-Psicose contents in food products.

Confectionery Products	mg/100 g	Seasonings and Beverages	mg/100 g
Kasutera (sponge cake)	11.0	Caramel sauce	83.0
Yo-kan (Sweet jelly of beans)	11.2	Brown sugar	71.1
Corn-snack	47.0	Meat sauce	15.8
*Kawarasenbe*i (rice cracker)	27.3	Demiglace	16.3
Tinsukou (cookie)	26.7	Maple syrup	57.9
Brown sugar drop	76.5	Ketchup	39.8
Kurokarintou (fried dough cake)	95.6	Worcester sauce	130.6
Chocolate-chip cookie	6.4	Coke	38.3
Cereal	2.2	Coffee	0.5
		Fruit juice	21.5
		Tomato juice	2.4
Dishes	mg/100 g	Fruits	mg/100 g
Syouyu- mame (boiled beans)	5.7	Dried fig	29.6
Fish broiled with soy	39.1	Dried kiwi fruit	9.4
Simmered dishes of dried radish strips	8.1	Raisin	38.7
Fermented soybeans	7.8	Canned peaches	1.5
		Can of mandarin oranges	8.4
		Canned cherries	2.0

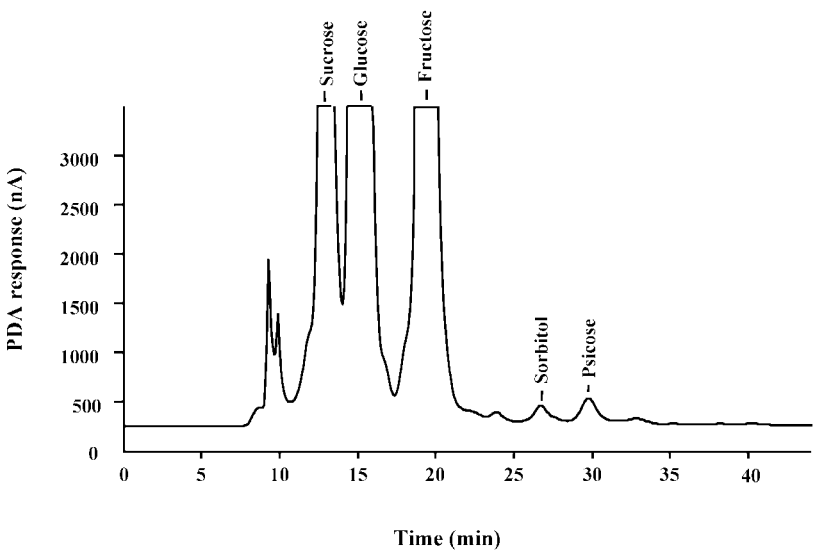

Fig. 3. Separation of sugars in a Worcester sauce extract.
Chromatographic condition and sample preparation procedure are described in the text.

g (Worcester sauce). Among high sugar foods, especially brown sugar and its related food products such as *kawarasenbei* (Rice cracker), *kurokarintou* (fried dough cake), brown sugar drop, which had been exposed to long time heating such during processing, Worcester sauce showed high psicose content (Table 3). These results suggest that the psicose contents in studied food products were closely related to the sugar concentration (sucrose and/or fructose), heating time and temperature during manufacturing process. Among high sugar foods, brown sugar and its related food products showed higher psicose content because they contained molasses. Binkly (1963) reported the D-psicose could be an artifact formed from D-fructose during molasses formation while heating of limed cane juice at 95–97℃ for 48 h. Worcester sauce contained highest psicose content among the studied food products. Fruits and sugar are used as common ingredients during long time simmering in manufacturing process of several foods. With long term heating, fructose in the fruits and sugar might be converted to psicose.

In contrast, Adachi (1958) reported the formation of D-tagatose, which is a C-4 epimer of D-fructose, from lactose in milk autoclaved at 120℃ for 10 h. The tagatose was unquestionably the de Bruyn and van Ekenstein reaction product of galactose. Moreover, Troyano *et al.* (1992) reported that the significant formation of tagatose takes place only under sterilization conditions. The rare sugars, psicose and tagatose, were formed in foods under heating conditions which were clearly different from the culinary conditions. Therefore, we studied the forma-

Table 4. Culinary treatment of high sugar foodstuffs.

Cane juice					Sucrose caramel							D-fructose caramel			
Time (h)	Sucrose	Fructose	Glucose	Psicose	Time (min)	Temp[3] (℃)	Sucrose	Fructose	Glucose	Psicose	YI[4]	Temp (℃)	Fructose	Psicose	YI
		(mg/100 g)[1]						(mg/100g)[2]					(mg/100g)		
0	14985.1	360.1	210.5	0	0	20	98923.1	12.3	12.9	0	1.01	20	101113.8	1.7	1.92
1	15181.3	307.6	159.2	0.2	1	105	98339.9	32.3	36.9	0	-0.20	106	98922.6	2.9	0.96
2	14753.3	304.8	157.0	0.6	2	113	95515.0	56.0	71.8	0	-0.28	118	99263.0	3.5	1.95
3	14404.9	297.6	158.2	1.1	3	145	97389.1	342.7	1442.5	0	-0.26	134	92592.3	3.4	1.28
4	14133.3	294.1	157.8	2.9	4	160	88164.1	1397.5	2136.2	0	-0.09	159	73064.3	11.4	3.86
					5	188	22049.2	10416.1	4160.3	3.4	11.0	178	32802.4	44.6	18.30
					6	200	17567.4	5625.2	29780.9	53.5	66.70	194	12784.4	40.7	72.03

1) Expressed as each sugar content/100g of cane juice, 2) Expressed as each sugar content /100g of dry matter of starting material, 3) Indicates of the temperature of the reaction solution, 4) Indicates of the color of the reaction solution.

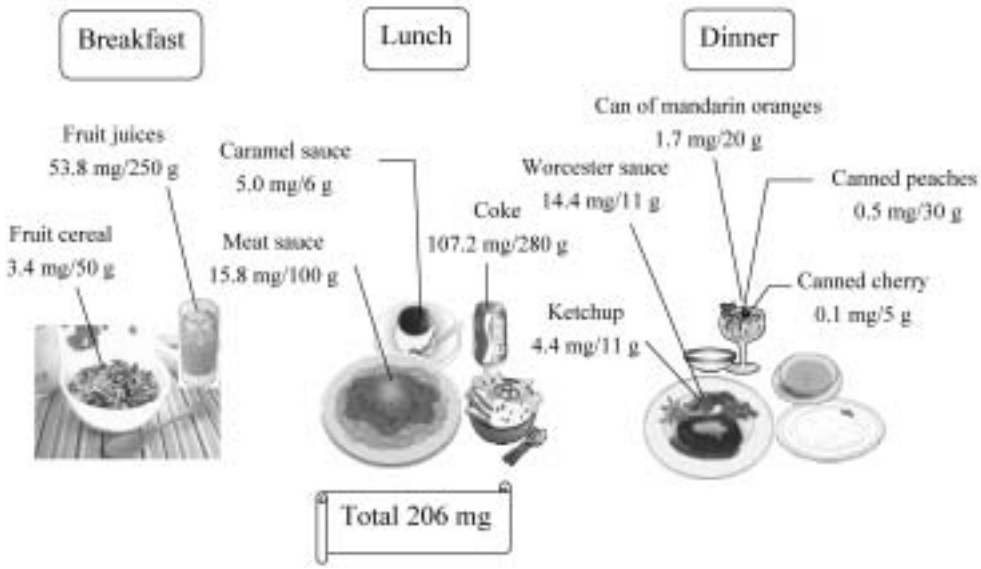

Fig. 4. Estimated daily intake of psicose from various food products.
The psicose content of each food was evaluated from the value of each food shown in Table 3.

tion of psicose under the ordinary culinary conditions.

Non-enzymatic production of psicose In high sugar foodstuff, heat processing had a marked effect on the production of psicose. For instance, the initial psicose content of cane juice was a trace (< 1.0 μg/mL), but it increased exponentially to 2.9 mg/100 g during the culinary treatment (4 h).

During caramelization of sucrose, the sucrose content of the solution was almost constant in early stage of the process (up to 160℃). However, at 188℃, the sucrose content decreased markedly and fructose and glucose contents increased. At 200℃, while the fructose content decreased rapidly, the psicose content increased remarkably in the reaction solution. The psicose contents of the solution also agreed with its YI values (Table 4). Thus, the produced psicose is suggested to be hydrolysis product of sucrose with heating. The non-enzymatic heating reaction results in formation of fructose, which is possibly converted to psicose on further heating.

When starting material of caramel sauce was replaced by fructose, psicose was produced in early stage of the process (at approximately 159℃). The psicose content was *ca.* 13 times higher than that of sucrose caramel at 5 min (178℃). Therefore, these results indicated that psicose was mainly produced from fructose and it was commonly produced in high sugar (sucrose and/or fructose) food products under to ordinary heating conditions. Additionally, the psicose content of fructose caramel solution at 178℃ was lower than that of the solution at 194℃, which was probably due to heat carbonization of the psicose (Table 4).

Interestingly, fructose, which is used as a starting material for caramel sauce, contained only a small amount of psicose (1.7 mg/100 g). However, psicose might be produced during storage or manufacturing process of fructose. Moreover, the psicose content of the caramel sauce was closely related to YI value of the caramel sauce. The result was in accordance with our data in Table 3, in which brown sugar and related food products showed higher psicose contents.

As shown in Table 3, people all over the world ingest small amounts of psicose from daily food products. Moreover, the psicose content in various food products was much higher than that of tagatose, which is generally

recognized as safe sweetener in foods (Levin, 2002). Using the data of Table 3, we estimated the daily intake of psicose. By assuming a daily diet consisting of fruit cereal, fruit juice, Bolognese spaghetti, creme caramel, coke, hamburger, fruit cocktail (Fig. 4), the psicose intake was estimated to be approximately 0.2 g. However, our estimation may be much lower than the actual intake because there are very few available studies on psicose contents in various food products.

Further investigations are in progress to study the enantiomer mole fraction to determine D-psicose, and to determine the psicose content in other food products. The kinetic studies of psicose production during heat-treatment of manufacturing processed foods are also under progress.

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References

Adachi, S. (1958). Formation of lactulose and tagatose from lactose in strongly heated milk. *Nature,* **181**, 840–841.

Andersen, R. and Sørensen, A. (2000). Separation and determination of alditols sugars by high-pH anion-exchange chromatography with pulsed amperometric detection. *J. Chromatgr.* A, **897**, 195–204.

Binkley, W.W. (1963). The fate of cane juice simple sugars during molasses formation IV. Probable conversion of D-fructose to D-psicose. *Int. Sugar J.,* **65**, 105–106.

Chaplin, M.F. (1994). Monosaccharides. In "Carbohydrate analysis," ed. by M. F. Chaplin and J. F. Kennedy. Oxford University Press, Oxford, pp. 1–41.

Corradini, C., Canali, G., Cogliandro, E. and Nicoletti, I. (1997). Separation of alditols of interest in food products by high-performance anion-exchange chromatography with pulsed amperometric detection. *J. Chromatogr.* A, **791**, 343–349.

Fifty-seventh report of joint FAO/WHO Expert Committee on Food Additives (2002). Evaluation of certain food additives and contaminates. WHO Technical Report Series, 909, I-x, pp. 29–32.

Levin, G.V. (2002). Tagatose, the new GRAS sweetener and health product. *J. Medicinal. Food,* **5**, 23–36.

Luger, A. and Steinhart, H. (1995). Carbohydrates in steam treated coffee. ASIC, 16°, Colloque, Kyoto, pp. 366–371.

Matsuo, T., Suzuki, H., Hashiguchi, M. and Izumori, K. (2002). D-psicose is a rare sugar that provides no energy to growing rats. *J. Nutr. Sci. Vitaminol.,* **48**, 77–80.

Miller, B.S. and Swan, T. (1960). Chromatographic analyses of the free amino-acids, organic acids and sugars in wheat plant extracts. *J. Sci. Food Agric.,* **11**, 344–348.

Nakamura, A., Hatanaka, C. and Nagamatsu, Y. (1999). Ultraviolet spectrometric determination of neutral monosaccharides by HPLC with ethanolamine. *Biosci. Biotechnol. Biochem.,* **63**, 178–180.

Sasahara, H., Mine, M. and Izumori, K. (1998). Production of D-talitol from D-psicose by *Candida famata* R28. *J. Ferment. Bioeng.,* **85**, 84–88.

Shaw, C.J., Huang, A. and Zhang, X. (2003). Quantitation and test of enantiomeric purity of the 1-ketohexoses by liquid chromatography with dual refractive index and laser-based chiroptical detection. *J. Chromatogr.* A, **987**, 439–443.

Soga, T., Inoue, Y. and Yamaguchi, K. (1992). Determination of carbohydrates by hydrophilic interaction chromatography with pulsed amperometric detection using postcolumn pH adjustment. J. Chromatgr., **625**, 151–155.

Takeshita, K., Suga, A., Takada, G. and Izumori, K. (2000). Mass production of D-psicose from D-fructose by continuous bioreactor system using immobilized D-tagatose 3-epimerase. *J. Biosci. Bioeng.,* **90**, 453–455.

Troyano, E., Martinez-Castro, L. and Olano, A. (1992). Kinetics of galactose and tagatose formation during heat-treatment of milk. *Food Chemistry,* **45**, 41–43.

Yasuno, S., Murata, T., Kokubo, K., Yamaguchi, T. and Kamei, M. (1997). Two-mode analysis by high-performance liquid chromatography of ρ-aminobenzoic ethyl ester-derivatized monosaccharides. *Biosci. Biotechnol. Biochem.,* **61**, 1944–1946.