Extraction Method by Water followed by Microwave Heating for Analyzing Sugars in Strawberry Fruits

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Summary

The sugar contents of strawberry fruits extracted by the conventional ethanol method were compared with those extracted with water followed by microwave heating. Sucrose, glucose, fructose and total sugar contents did not differ significantly between the two methods. The time for the water extraction method required approximately half that needed for the conventional ethanol method. Sugar metabolizing enzymes are inactivated by boiling in a microwave oven. In this method, frozen fruits should be chopped, quickly weighed at a low temperature, and the pieces immediately heated in a microwave oven. After strawberry fruits are heated in a microwave oven, they could be processed at a room temperature. Thus, the water extraction method followed by microwave heating is less time consuming but equally accurate for measuring the concentration and composition of soluble sugars in strawberry fruits.

Key Words: HPLC, microwave heating, strawberry, sugar analysis, water extraction.

Introduction

Sugars in fruit have been extracted from diced fruits using hot 80% ethanol, and the extracts analyzed by fractional assays, such as high performance liquid chromatography (HPLC) and gas chromatography (GC) (Yamaki et al., 1979; Reyes et al., 1982; Moriguchi et al., 1990; Ogiwara et al., 1998). However, these procedures are time consuming because after heating with ethanol, evaporation, fractionation, concentration, etc. are required.

Researchers have attempted to develop a faster, simpler extraction methods. Hamano et al. (1992) introduced an alternative method in which juice from freshly harvested strawberry fruits was extracted at a low temperature. The juice was then diluted with water and subjected to fractional determination by HPLC. Water extraction of sugars facilitates HPLC to perform more easily. Taira et al. (1986) and Zheng and Sugiura (1990) heated freshly harvested persimmon fruits in a microwave oven for a short period of time to inactivate the sugar metabolizing enzymes and extracted the sugars by using alcohol.

Based on these studies, we introduced a potentially quick, accurate method to measure the sugar content of fruits by heating strawberry fruits in a microwave oven and extracting the soluble constituents with water,

followed by HPLC analysis.

Materials and Methods

Experiment 1: Water extraction method followed by microwave heating for analyzing sugars.

Runners of the strawberry 'Nyoho' were planted on September 24, 1994 in the greenhouse; plants were covered by vinyl film on October 24, 1994. Fully ripe fruits were picked during January and February 1995, and immediately stored at -20°C for two months until analyzed. Ten to 15 frozen fruits were diced to 100 to 125 mm^3 at a low temperature. Those pieces were mixed and a randomized sample weighed; the samples were extracted for sugar under conditions in Fig. 1 and analyzed by HPLC. The samples were subjected to three treatments:

Treatment 1. Two treatments were tested to compare the sugar contents of strawberry fruits. One was the conventional ethanol extraction method (Fig. 1 Procedure A), and the other was as follows: 20g of chopped frozen fruits were added to 60ml of distilled water and the mixture heated in a microwave oven for 40 seconds up to a boil, and then the sugars were extracted with water (Fig. 1 Procedure B).

Treatment 2. To determine whether the sugar content of frozen fruits changes with time during cutting and weighing, chopped frozen fruits were put on a balance dish in a room at 18°C and left to stand at room temperature for 0, 60 or 180 minutes. The sugars were extracted and analyzed by procedure B. The change in the temperature of frozen fruit that was left for 200

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Fig. 1. The procedure of the ethanol extraction (A) and water extraction following microwave heating (B) for analysis of sugar contents in strawberry fruit. Condition of HPLC-analysis: Pump; LC-9A(Shimadzu), Detector; RID-6A(Shimadzu), Column; Shim-pack SCR-101N, Mobile phase; water, Flow rate; 0.5ml/min, Column temperature; 40 °C.

minutes was also measured. A temperature sensor (thermocouple thermometer) was inserted firmly 5mm under the skin of a fruit in a 25 g sample of freshly harvested fruit. The sample was put into a freezer at -20 °C for one day and then moved to the laboratory. The change in the temperature of fruit recorded at room temperature for 200 minutes with a thermogram (Yokogawa Seisakusho, mR-180).

Treatment 3. The change in sugar content after heating in a microwave oven was investigated by allowing the boiled samples to left at room temperature for 0 or 180 minutes. Sugars were extracted by the procedure B.

Experiment 2: Differences in sugar content of fruits of some strawberry varieties between the ethanol extraction method and the water extraction method followed by microwave heating.

Strawberry varieties 'Nyoho', 'Toyonoka', 'Himiko', 'Reiko', and 'Fresno' were planted on September 17, 1997 and covered by vinyl film on October 24, 1997. Fully ripe fruits were picked during January and February 1998. The average weights of 'Nyoho', 'Toyonoka', 'Himiko', 'Reiko', and 'Fresno' fruits were 21, 23, 29, 33, and 17g, respectively. A fruit of each variety was cut vertically in half and each half-fruit placed in a separate group. The samples were diced to 100 to 125mm³ and extracted by Procedure A; the others by Procedure B. Each sugar extract was analyzed by HPLC

as above.

Total sugar content, the sum of sucrose, glucose, and fructose, is expressed in milligrams of sugar per g of fresh weight.

Results and Discussion

Experiment 1: Water extraction method followed by microwave heating for analyzing sugars.

Treatment 1. Comparison of the ethanol extraction method with that of the water extraction method followed by microwave heating reveals that sucrose, glucose, fructose, and total sugar contents did not differ significantly between the two treatments (Table 1). When the fruits were heated in a microwave oven, the fruit tissue became soft and disintegrated and made the subsequent sugar extraction easy. Furthermore, water extraction method followed by microwave heating eliminates the need to perform procedures such as heating with ethanol, evaporation of the alcohol, and reducing the sample volume, etc. Thus, the extraction time for each sample was approximately one hour or about half of the time required for ethanol extraction.

Treatment 2. The individual sugar contents in the frozen fruit samples left to stand at room temperature for 0 or 60 minutes before heating did not differ significantly (Table 2). But in samples standing 180 minutes, fructose and glucose increased and sucrose decreased; the difference in total sugar content was not significant (Table 2). The temperature of the frozen fruit increased above 0 °C in 80 minutes and room temperature (18 °C) in 180 minutes so that invertase in the fruit hydrolyzed sucrose to fructose and glucose (Fig. 2). These findings indicate that frozen fruits should be cut, weighed and

immediately heated in the microwave oven.

Treatment 3. The sucrose, glucose, fructose, and total sugar contents of frozen fruits that had been heated in a microwave oven and left to stand at room temperature for 0 or 180 minutes did not differ significantly (Table 3). Zheng and Sugiura (1990) reported that sucrose in persimmon fruits are hydrolyzed relatively quickly, Matsui and Kitagawa (1988) found that invertase in persimmon fruits are not completely inactivated by hot

Fig. 2. Change in temperature of the frozen strawberry left to stand at room temperature (18 °C). The temperature of fruit was recorded by inserting a temperature sensor (thermocouple thermometer) 5 mm under the skin of a fruit, using a thermogram (Yokogawa Seisakusho, mR-180). The temperature sensor was immobilized using an adhesive before placing the fruit in the freezer (−20 °C).

Table 1. Sugar contents in strawberry fruit processed by the ethanol extraction method and by the water extraction method followed by microwave heating.

Treatment	Sucrose ^y	Glucose	Fructose	Total
Ethanol extraction method	22.33 ± 0.38 ^x	13.77 ± 0.19	14.38 ± 0.49	50.49 ± 0.95
Water extraction method followed by microwave heating ^z	23.13 ± 0.99	14.13 ± 0.21	14.67 ± 0.32	51.93 ± 1.50

^z Frozen fruits were chopped to pieces. Those pieces were weighed, added to distilled water, heated in a microwave oven for 40 seconds up to a boil, and then the sugars were extracted using water. The sugar contents were analyzed by HPLC.

^y The sugar contents are expressed as mg/g fresh weight.

^x Mean ± SD (n=3).

Table 2. Sugar contents in strawberry fruit that were left to stand at room temperature for different lengths of time before microwave heating.

Time (min.) ^z	Sucrose ^y	Glucose	Fructose	Total
0	23.13 ± 0.99 ^x	14.13 ± 0.21	14.67 ± 0.32	51.93 ± 1.50
60	22.47 ± 0.36	14.07 ± 1.31	15.10 ± 1.57	51.63 ± 4.06
180	11.80 ± 0.87	17.80 ± 0.61	19.73 ± 0.72	49.33 ± 0.92

^z Frozen fruits were chopped to pieces. Those pieces were put on a balance dish and left to stand at room temperature for 0, 60 or 180 minutes. The sugars were extracted and analyzed by procedure B in Fig. 1.

^y The sugar contents are expressed as mg/g fresh weight.

^x Mean ± SD (n=3).

Table 3. Sugar contents in strawberry fruit that had been heated in a microwave oven and left to stand for different lengths of time.

Time (min.) ^z	Sucrose ^y	Glucose	Fructose	Total
0	30.03 ± 0.98 ^x	15.05 ± 0.23	17.82 ± 0.37	62.90 ± 0.99
180	29.74 ± 0.28	15.62 ± 0.48	18.60 ± 0.50	63.96 ± 0.43

^z Frozen fruits were chopped to pieces. Those pieces were added to distilled water and heated in a microwave oven up to a boil. The boiled samples were allowed to left at room temperature for 0 or 180 minutes. Sugars were extracted by the procedure B in Fig. 1.

^y The sugar contents are expressed as mg/g fresh weight.

^x Mean ± SD (n=3).

Table 4. Differences in sugar contents of fruits of some strawberry varieties between ethanol extraction method and water extraction method followed by microwave heating.

Variety	Extraction method ^z	Sucrose ^y	Glucose	Fructose	Total
Nyoho	Ethanol	33.86 ± 5.59 ^x	16.72 ± 0.73	17.88 ± 1.27	68.47 ± 7.13
	Water	34.45 ± 4.88	16.82 ± 0.75	18.33 ± 1.16	69.60 ± 4.60
Toyonoka	Ethanol	28.02 ± 3.09	19.59 ± 0.80	21.91 ± 0.79	69.52 ± 3.79
	Water	26.94 ± 3.15	19.46 ± 0.80	21.59 ± 0.73	67.99 ± 3.20
Reiko	Ethanol	26.44 ± 2.52	12.03 ± 0.61	13.67 ± 0.64	52.13 ± 3.43
	Water	24.70 ± 1.83	12.27 ± 0.48	14.29 ± 0.83	51.26 ± 2.12
Himiko	Ethanol	26.97 ± 1.90	14.96 ± 0.46	17.25 ± 0.75	59.18 ± 2.15
	Water	26.16 ± 1.66	15.58 ± 0.47	18.27 ± 0.74	60.00 ± 2.31
Fresno	Ethanol	11.78 ± 1.11	19.59 ± 3.21	20.34 ± 3.24	51.72 ± 7.07
	Water	10.58 ± 0.64	18.45 ± 2.50	19.29 ± 2.85	48.32 ± 5.81

^z Ethanol; ethanol extraction, Water; water extraction followed by microwave heating.

^y The sugar contents are expressed as mg/g fresh weight.

^x Mean ± SD (n=5).

ethanol or heating in a microwave oven, contrary to our findings in this study. We found that after strawberry fruits are heated in a microwave oven, they could be processed at a room temperature.

Experiment 2: Differences in sugar content of the fruits of some strawberry varieties between the ethanol extraction method and the water extraction method followed by microwave heating.

The sugar contents in fruits of various strawberry varieties extracted by ethanol method and water method followed by microwave heating reveal that sucrose, glucose, fructose and total sugar contents of each varieties did not differ significantly between two treatments (Table 4). Furthermore, the sugar concentration within varieties were the same in the two methods except that total sugar of 'Nyoho' had the largest value by water extraction method, whereas 'Toyonoka' had the highest level in the ethanol extraction method. Standard deviations in sugar contents measured by water extraction method followed by microwave heating were generally smaller than those calculated for the ethanolic samples, indicating that the water extraction method is more stable and accurate.

Based on the above findings, we conclude that the water extraction method followed by microwave heating (Fig. 1 Procedure B) is a less time consuming but an accurate method to measure sugar content and composition of fruits. We believe that water extraction method followed by microwave heating is adaptable for determining the sugar contents in various varieties and strains of strawberries.

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電子レンジ処理と水を用いたイチゴ果実内の糖抽出法について

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摘 要

本実験は従来のアルコール抽出法と電子レンジ前処理水抽出法とによって測定したイチゴ果実内の糖含量を比較したものである。両方法によって抽出された全糖および組成別糖量には相違がなかった。電子レンジ前処理水抽出による抽出時間はアルコール抽出のそれに比べて半分程度であった。電子レンジ処理によって、糖代謝酵素は失活していた。よって、

冷凍果は低温下で細断、調整し、直ちに電子レンジ処理されるべきである。果実が電子レンジ処理されると、その後の抽出操作は室内で行うことができる。この電子レンジ前処理水抽出法は、従来のアルコール抽出法による糖含量の測定値と相違がなく、抽出までの時間が短かったことから、迅速かつ正確な抽出法であると思われる。