Research Note

Quantitative Determination of Conjugated Linoleic Acids in Hydrogenated Vegetable Oils Using Refractive Index

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Abstract Conjugated linoleic acid contents in hydrogenated vegetable oils were differentially determined using refractive indices when the iodine value could not be used. The refractive indices of soybean oil, cottonseed oil, and corn oil varied linearly with changes of linoleic acid contents of individual oils with determination coefficients of 0.91, 0.98, and 0.98, respectively. The refractive index can be used as a simple and fast method for control of the hydrogenation process of vegetable oils to obtain a desired conjugated linoleic acid content without fatty acid compositional analysis.

Keywords: refractive index, iodine value, conjugated linoleic acid, hydrogenation, vegetable oil

Introduction

Hydrogenation of vegetable oils is an important practice for modification of fats and oils. Hydrogenated vegetable oils are widely consumed worldwide in foods, such as margarines, shortenings, and confectionery fats. Hydrogenation eliminates some double bonds while many of the remaining double bonds are isomerized through *cis/trans* conversion and positional shifts of fatty acid double bonds (1,2).

It has been reported that conjugated linoleic acids (CLAs) have anti-cancer activity, diabetes curing activity, and anti-atherosclerosis activity (3-6). CLAs also contribute the enhancement of bone and muscle strength, and decrease of body adipose tissues (7-13).

A high level of CLAs can reportedly be formed during commercially selective hydrogenation of soybean oil and CLAs at levels of 98-230 mg/g of oil were reported in selectively hydrogenated soybean oils (14-17). It was reported that the substantial amounts of CLAs were produced during partial hydrogenation process of oils containing large amounts of polyunsaturated fatty acids, especially linoleic acid (13,14). The production rate and quantity of CLAs in hydrogenation process are greatly affected by the hydrogenation parameters, such as species of oils used, species and amount of catalyst used, pressure of hydrogen, agitation speed, and hydrogenation time (15-17). The fatty acid compositions of hydrogenated vegetable oils can be manipulated via control of hydrogenation conditions. There was

reportedly an increase in concentrations of stearic acid, oleic acid, elaidic acid, and *trans* CLAs, and a decrease in concentrations of linoleic acid and linolenic acid in fatty acid compositions at different stages of the soybean oil hydrogenation process (18,19). During the process of partial hydrogenation, a product is saturated to a certain iodine value (IV), and polyenoic fatty acids are also converted to monoenoic acids during selective hydrogenation (20). Analyses of fatty acid compositions and IV of oils have been used to control hydrogenation conditions (20). Analyses of fatty acid composition and IV, however, are time consuming and labor intensive.

The refractive index (RI) of fats and oils has long been used to determine IV during the hydrogenation process (2,21). Hydrogenation reduces the IV due to saturation of double bonds, and also reduces the RI of soybean oil (21). The RI changes with an IV change in a linear relationship to a certain extent, and the RI determination procedure is simpler and faster than titrimetric determination of IV. The RI has been used for practical control of hydrogenation conditions in place of IV determination. However, the IV reportedly does not vary linearly with the RI in several stages of the hydrogenation process (22).

Fatty acids reportedly have individual RI values and the longer a fatty acyl chain, the higher the RI. Higher RI values were detected when fatty acids had more double bonds, even with the same fatty acyl chain length (21,23). The objective of this study was to determine the different CLAs content in partially hydrogenated

vegetable oils using RI when the IV could not be used to distinguish materials with double bonds that were identical with isomers.

Materials and Methods

Materials Refined, bleached, and deodorized (RBD) soybean oil, cottonseed oil, and corn oil without additives were obtained from Wellga Oil and Fat Ltd. (Seoul, Korea). Peroxide value (PV) of three oils were less than 0.1 meq/kg of oil. Selective (SP-7) and nonselective nickel (N-222, N-325, N-545, N-655) catalysts were obtained from Engelhard (Jackson, MS, USA). All reagents and solvents were of analytical grade unless otherwise specified.

Hydrogenation conditions for preparation of partially hydrogenated soybean oils Selectively hydrogenated soybean, corn, and cottonseed oils were prepared following the method of Jung *et al.* (15). Hydrogenation was performed using a 1 L capacity hydrogenation reactor (HR1L; Next Instrument, Hwaseong, Korea) equipped with hydrogen pressure, temperature, and agitation rate controllers. Hydrogenation of corn, cottonseed, and soybean oils was carried out using a commercially available selective catalyst (SP-7) (Engelhard) at 230°C, a 0.024 MPa hydrogen pressure, and a 300 rpm agitation rate. Oil samples of 10 g were collected at predetermined intervals of 5–10 min during hydrogenation.

Preparation of fatty acid methyl esters Oil samples were methyl-esterified without pretreatment. Methyl esterification of hydrogenated soybean, corn, and cottonseed oils was carried out using 0.25 N sodium methoxide in methanol at 70°C for 20 min. Fatty acid methyl esters were extracted using 2,2,4-trimethylpentane containing an internal standard of heptadecanoic acid.

Gas chromatography (GC) Fatty acid compositions of vegetable oils were determined using a GC apparatus after methylesterification of fatty acids. Isolation of CLAs was carried out using GC. Fatty acid methyl ester (FAME) samples of 2–6 µL were injected into a GC equipped with a flame ionization detector (FID). A highly polar fused-silica capillary column (cyanopropyl siloxane phase, SP2380; 100 m × 0.25 mm, 0.25 µm thickness) (Supelco Inc., Bellefonte, PA, USA) was used with 100:1 split injection for oil samples. Helium was used as a carrier gas with a head pressure of 300 kPa. Temperatures of the injector and detector were 230 and 250°C, respectively. The initial oven temperature of 170°C was held for 1 min, then increased at 0.8°C/min to 200°C. Analytical results for fatty acid methyl esters of CLAs isomers were expressed as: weight of CLAs=peak area of CLAs/peak area of an internal standard normalized to the weight of the internal standard. CLAs contents of partially hydrogenated soybean oils were calculated as mg/g of oil using a previously reported formula (16).

Analytical methods IV and RI values of partially hydrogenated oils were determined following AOCS Official methods Cd 1c-85 and Tp 1a-64, respectively (24). The RI was determined at 60°C.

Statistical analysis All experimental values were expressed as average values of triplicate determinations, and standard deviation (SD) values were less than ±3%, unless otherwise specified ($n=3$). Respective SD values and error bars were not given when SD was less than ±3%. Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS, Chicago, IL, USA) to ascertain significant differences between treatments at $p<0.05$.

Results and Discussion

Fatty acid compositions of soybean, cottonseed, and corn oils are shown in Table 1. Major fatty acids present in three oils were palmitic, oleic, and linoleic acids. The CLAs content of soybean oil before hydrogenation was 0.86 mg/g of oil and was denoted as a trace amount below the 0.1% level. CLAs contents of cottonseed and corn oils were less than 0.1% and also denoted as trace. Fatty acid compositions of soybean, corn, and cottonseed oils were in agreement with previous results (25).

CLAs contents of soybean, cottonseed, and corn oils during the course of hydrogenation have been reported (16). The hydrogenation rate of soybean oil was highest, followed by cottonseed and corn oils in decreasing order. CLAs maxima during hydrogenation of different vegetable oils were almost the same, regardless of the oil type. CLAs maxima in corn, cottonseed, and soybean oils were 232.33±6.97, 237.24±7.11, and 233.33±7.01 mg of total CLAs/g of oil, respectively. The IV of corn, cottonseed, and soybean oils at maximum CLAs formation were 126.7±3.7, 119.0±3.4, and 132.1±3.9 meq/100 g, respectively. CLAs isomer compositions of different vegetable oils were similar to previous reports (16). During the hydrogenation process, CLAs contents of all oils initially increased, continuously

Table 1. Fatty acid compositions of vegetable oils before hydrogenation (% area)

Fatty acid	Soybean oil	Corn oil	Cottonseed oil
C14:0	ND ¹⁾	ND	0.8±0.1
C16:0	9.3±0.2	13.1±0.4	23.4±0.6
C16:1	ND	tr	0.6±0.0
C18:0	4.2±0.1	2.5±0.0	tr
C18:1	23.8±0.7	22.0±0.6	17.3±0.5
C18:2	54.5±1.5	61.2±1.7	57.6±1.6
CLA	tr ²⁾	tr	ND
C18:3	7.9±0.2	0.6±0.1	tr
C20:0	0.3±0.0	0.6±0.1	0.3±0.0
C22:0	tr	tr	tr

¹⁾Not detected.

²⁾Trace amount less than 0.1%.

Fig. 1. Relationship between refractive index and CLAs content of soybean oil (A), cottonseed oil (B), and corn oil (C) during the selective hydrogenation process.

reached maximum production, then decreased as hydrogenation proceeded.

The IV of all 3 oils; however, decreased continually, regardless of CLAs contents, as the hydrogenation time increased due to a decrease in numbers of double bonds in oils. A linear relationship between IV and CLAs contents was initially observed for hydrogenation; however, the linear relationship was not maintained after CLAs contents reached highest values (16).

The CLAs content could not be estimated using IV in all 3 oil types

after maximum CLAs production, probably because IV could not be used to distinguish CLAs from linoleic acid. In contrast, RI values changed linearly with CLAs contents in hydrogenated soybean oil even after maximum CLAs production (Fig. 1) because RI values of individual CLAs were different from linoleic acid.

The linear regression equation for CLAs content (%), Y and refractive index (X) of soybean oil was: $Y=5,235.89 X-7,640.68$ with a determination coefficient (r^2) of 0.91 (Fig. 1A). Linear regression equations for cottonseed and corn oils were: $Y=7,717.87X-11,252.64$, and $Y=7,695.79 X-11,231.31$, respectively, with r^2 of 0.98 for both oils (Fig. 1B and 1C). Determination coefficients of cottonseed and corn oils were higher than for soybean oil.

RI determination was a simple and fast analytical method for quantification of CLAs content with a determination coefficient higher than 0.91. CLAs contents in hydrogenated vegetable oils were conclusively determined within 2 min using simple and fast refractive index measurements. CLAs contents were not in a linear relationship with IV of oils during the entire process of hydrogenation. CLAs contents and RI values showed highly positive correlation coefficient values. The RI can be used industrially as an on-site continuous analytical method for monitoring and control of the hydrogenation process of vegetable oils for CLAs production. Time consuming IV and fatty acid composition analytical procedures can be eliminated for monitoring of hydrogenation conditions.

Disclosure The authors declare no conflict of interest.

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