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Lipids and Fatty Acid Composition of Barlely Grain

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보리의 지방질 성분에 관한 연구

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Abstract

Lipids isolated from three barley samples were identified and quantitated by column, thin layer and gas liquid chromatographic techniques. These lipids were shown to consist of 69.3-73.1% neutral lipids, 9.6-16.5% glycolipids, and 14.2-17.9% phospholipids. Among the neutral lipids, triglycerides were predominant (54.2 to 55.7%) with smaller amounts of 1,2-diglycerides, 1,3-diglycerides, free sterols, free fatty acids, steryl esters, and three unknown being present. Among the glycolipids, digalactosyl diglycerides (31.3 to 33.2%) and monogalactosyl diglycerides (26.2 to 29.6%) were the most abundant. Esterified steryl glycosides, steryl glycosides, cerebrosides, sulfolipids, and an unknown component were present as minor components. Of the phosopholipids, phosphatidyl cholines and serines, lysophosphatidyl cholines, and phosphatidyl ethanolamines were the major components, comprising over 80% of this class. The major fatty acids in the total and the three lipid classes were palmitic, oleic, linoleic and linolenic acids. However, the neutral lipids fraction contained more oleic acid than other lipid fractions, and the phospholipids fraction contained more palmitic acid than the other lipid fractions.

Introduction

The lipid content of barley represents a small fraction compared with carbohydrate and protein. However, the lipids are important nutritional components and impart major functional properties to barley products during processing and storage.

Most studies on barley lipids to date have been concerned with the fatty acid composition[1-3], the composition of lipid classes[4-5] and the distribution of lipids[3,6] in the grain. Changes in lipids during the malting and brewing process[3,7-11], and the lipid composition as a basis for increasing the lipid content of grain[12,13] have been studied. Lulai and Baker[14] reported the physiochemical characterization of barley lipoxygenase.

Very little is known about the nature of the lipids present in barley during processing and storage. This study was initiated to provide more detailed information of barley lipids as a preliminary study for effects of lipids in barley processing and barley products. In this paper,

lipid classes, fatty acid composition and lipid components of barley grains are reported.

Materials and Methods

Barley

Three dehulled barley samples were used in this study. These were obtained from the Kellogg Company (Battle Creek, MI, USA). *Morex* barley was grown in 1981 in Minnesota (Grain Pearling Company, Cannon Falls, MN, USA), Korean barley was grown in 1981 in South Korea (Nhong Shim Company, Seoul, Korea), while the blend of barley was a sample of dehulled U.S. grade No. 2 barley varieties used for the production of *"Nutri-Grain Barley"* at the Kellogg Company.

Lipid extraction and purification

Dehulled barley samples were ground in a Cyclone sample mill (UD Corp., Boulder, CO.), and lipid extraction carried out immediately to minimize oxidative and enzymatic activity. Crude lipids were extracted with a mixture of chloroform-methanol-water(1.0: 1.0: 0.9, v/v) as described by Bligh and Dyer[15].

Crude lipid extracts were freed from non-lipid contaminants by passing through a Sephadex G-25 column by the procedure of Wuthier[16]. The lipid extracts were evaporated to dryness under vacuum, weighed, redissolved in diethyl ether, and transferred to vials and stored at −15 °C until analysis.

Column chromatography

The Sephadex-purified lipids were subjected to column chromatography, using a silicic acid (Bio-Rad HA-325 mesh, Bio-Rad, Richmond, CA) column[17]. The lipid material (0.5-1.0 *g*) was applied in 2 *ml* of diethyl ether onto a column containing 10 *g* of silicic acid. Diethyl ether, acetone, and methanol were used sequentially to elute the neutral lipids (NL), glycolipids (GL), and phospholipids (PL), respectively. After evaporation of the solvent in a Büchi rotary evaporator at 35°C, the percent distribution of the three lipid classes was determined from the weights of each fraction, and transferred to vials and stored under nitrogen at −15°C until analysis.

Thin-layer chromatography and quantitative analysis of individual components

Lipid classes of the NL, GL and PL were separated by TLC on silica gel plates. For separation of NL, silica gel G plate (0.25 *mm*) and a solvent system containing petroleum ether-diethyl ether-acetic acid (90:10:1, v/v) was used. For separation of GL and PL, silica gel H plates (0.05 *mm*) and solvent systems, GL, chloroform-methanol-water (75:25:4, *v/v*), and PL, chloroform-methanol-water-28% aqueous amonia (65:35:4:0.2, *v/v*) were used. The spots were located and partially identified by spraying with 50% H_2SO_4 and subsequent charring at 110 °C. The lipids separated by TLC were identified by comparing R_f values with those of pure compounds (Supelco, Bellefonte, PA; and Applied Science Laboratories, State College, PA) and by using specific sprays, such as ninhydrin, Dragendorff's reagent, or molybdenum reagent for PL[18] and perchloric acid for GL[19].

The spots separated by TLC were quantitated by the following methods: the individual neutral lipids were quantitated by the procedure of Amenta[20]. The sugar content of each glycolipid band was determined by the orcinol procedure of Svennerholm[21]. The phosphorus content of each phospholipid band was measured by the method of Rouser *et al*[22].

Fatty acid analysis

The fatty acid compositions of the total lipid extract and of the three fractions were determined by converting to fatty acid methyl esters (FAME) as decribed by Morrison and Smith[23]. The FAME were analyzed using a Hewlett Packard 5840A gas chromatograph equipped with flame ionization detector. A glass column (2*m* x 2*mm* id) packed with 15% (w/w) DEGS on 80/100 mesh Chromosorb W (Supelco) was used for methyl ester separation. The column oven temperature was 190 °C, the injection temperature was maintained at 210 °C and the detector at 400 °C; the nitrogen carrier gas flow rate was 40 *ml* per min. The emerging peaks were identified by comparing retention times to those of a standard mixture of known fatty acid methyl esters (Supelco). Peak areas were integrated by a 5840A GC terminal Hewlett Packard integrator and the percentages of total fatty acids were determined.

The fatty acid content of the total lipid extract was determined by saponification, acidification, and extraction three times with petroleum ether[24]. The petroleum ether extracts were evaporated to dryness under vacuum and weighed.

Results and Discussion

Content of total lipid and lipid classes

The mean crude lipid content of the three barley samples used in this study varied from 232.5 to 370.3 *mg*/100 *g* of dry weight (Table 1). The lipid content of the two samples of *Morex* and the blend of barley were approximately similar, while that of the Korean barley was lower. These differences may be due to the varietal differences, degree of dehulling, environmental conditions and the length of the growth season. Purification with Sephadex G-25 reduced the crude lipid content of the three samples by from 18.2 to 29.7 *mg*/100 *g*. Bhatty and Rossnagel[3] reported that contents of crude and purified lipid in barley were different depending on the extraction and purification methods used.

Table 1. Content of total lipid classes in barley

	Morex	Korean barley	Blend of barley
Crude lipids, *mg*/100 *g*[a]	342.3±4.9	232.5±5.6	370.3±3.7
Purified lipids, *mg*/100 *g*[a]	312.6±2.6	214.3±3.8	342.3±4.5
Lipid classes, *mg*/100 *g*[a]			
Neutral lipids	220.4±5.2	148.5±3.6	250.2±4.1
Glycolipids	36.3±6.2	35.4±7.2	32.9±5.6
Phospholipids	55.9±7.1	30.4±8.4	59.2±7.6
Lipid classes, %			
Neutral lipids	70.5	69.3	73.1
Glycolipids	11.6	16.5	9.6
Phospholipids	17.9	14.2	17.3

[a] The values are means±SD of three replicate extractions on the dry weight basis.

The lipid class composition analysis as carried out by column chromatography indicated that the three samples contained almost similar amounts of NL (69.3-73.1%), but the samples contained different amounts of GL (9.6-16.5%) and PL (14.2-17.9%). The NL were completely free of phosphorus, whereas GL and PL contained same of it. The NL fractions were therefore not contaminated with polar lipids. However, the GL and PL fractions varied considerably among the three samples. Korean barley contained considerably more GL and less PL than did the other two samples. Barley lipids have been reported to be 65-78%, 7-13% GL, and 15-20% PL[12,13]. Weihrauch and Mathews[25] reported literature values for lipid classes in different cereals. However, these values may not be comparable because different extraction and separation procedures were employed and varieties within a cereal species may vary widely in lipid content and class.

Composition of neutral lipids

The neutral lipids from barley samples were resolved by TLC as shown in Fig. 1. The components were identified with the aid of pure standards such as cholesteryl palmitate, triolein, linoleic acid, cholesterol, 1,3-dipalmitin and 1,2-dipalmitin. The thin-layer chromatograms of the neutral lipid fractions in the three

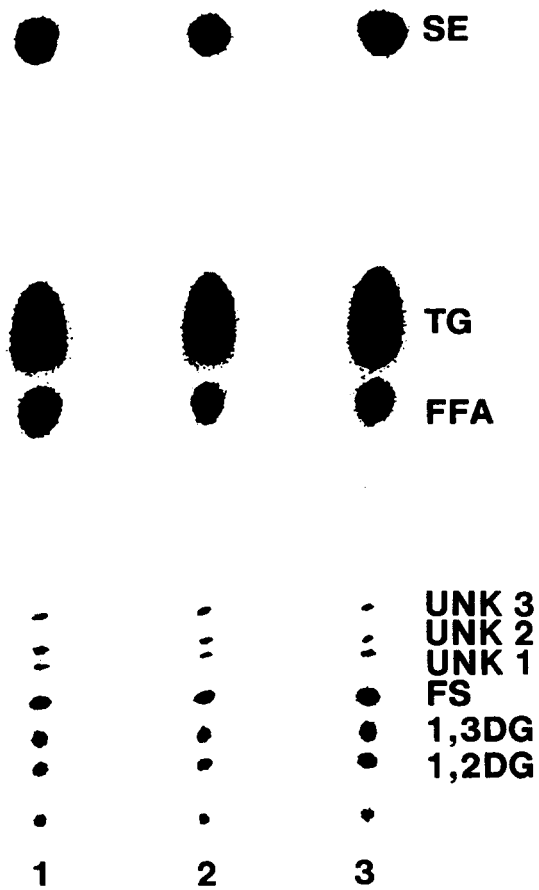

Fig. 1. Thin-layer chromatogram of neutral lipids in barley samples. 1,Morex; 2, Korean barley; 3, blend of barley. Adsorbent, silica gel G (0.25 *mm*); solvent system, petroleum etherdiethyl ether-acetic acid (90: 10: 1); visualization, charring by heating with 50% H_2SO_4. The spots were identified as follows: 1,2DG, 1,2-diglycerides; 1,3 DG, 1,3-diglycerides; FS, free sterols; Unk 1, unknown 1; Unk 2, unknown 2; Unk 3, unknown 3; FFA, free fatty acids; TG, triglycerides; SE steryl esters.

barley samples were qualitatively similar. Three unknown spots migrated ahead of the free sterols band. These qualitative results agree with the chromatogram of barley neutral lipid fractions reported by Price and Parsons[12] and Parsons and Price[13]. The neutral lipid fraction contained 54.2-56.3% TG, 9.6-10.3% SE, and 8.9-10.3% FFA. Smaller amounts of 1,2-DG, 1,3-DG, FS, and three unknowns were also present (Table 2). These quantitative results were similar to those reported by Price and Parsons[15]. The neutral lipid composition of the barley lipids was qualitatively similar to those studied on other low-fat cereals: wheat[26], millet[27], and rye[28], but quantitatively not similar to those of other cereal grains.

Composition of glycolipids

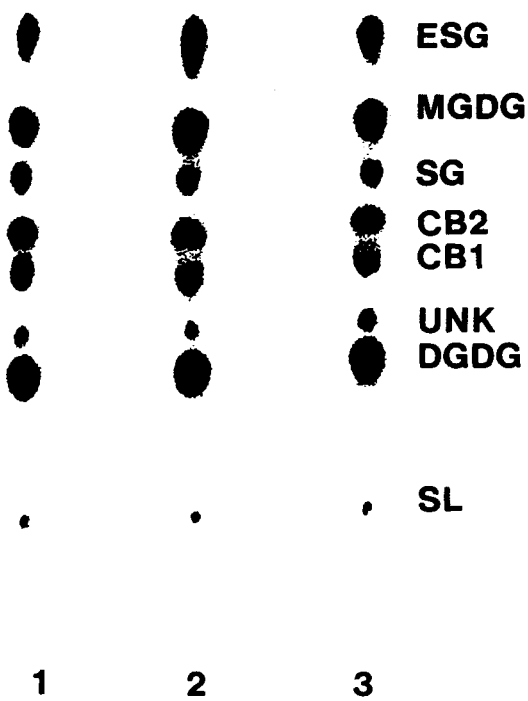

Fig. 2. Thin-layer chromatogram of glycolipids in barley samples. 1, Morex; 2, Korean barley; 3, blend of barley. Adsorbent, silica gel H (0.5*mm*); solvent system, chloroform-methanol-water (75: 25: 4); visualization, charring by heating with 50% H_2SO_4. The spots were identified as follows; SL, sulfolipids; DGDG, digalactosyl diglycerides; Unk, unknown; CB 1, cerebrosides 1; CB 2, cerebrosides 2; SG, steryl glycosides; MGDG, monogalactosyl diglycerides; ESG, esterified steryl glycosides.

The glycolipid fractions of the barley samples showed 8 distinct sugar-containing spots on spraying the TLC plate with 20% perchloric acid (Fig. 2). The components were identified by co-chromatography with glycolipid standards such as esterified steryl glycosides (ESG), monogalactosyl diglycerides (MGDG), steryl glycosides (SG), cerebrosides, digalactosyl diglycerides (EGDG) and sulfolipids (SL). There were no differences in chromatographic pattern among the three samples. The DGDG content was 31.3-33.2% of the total sugar of the glycolipids and it was the highest in this class. In grain, DGDG and MGDG rank first and second, respectively. This contrasts with photosynthetic tissue such as chloroplasts, where MGDG predominates[29]. Also, the glycolipid fraction contains ESG, SG, cerebrosides, SL and an unidentified lipid as minor components. The cerebroside standard (Supelco, 4-6008) when developed, showed to spots on the TLC plate. The standard cerebroside appears to be a mixture of ceramide hexosides or isomers of a cerebroside. These spots were tentatively identified as CB 1 and CB 2. The unknown spot reacted positively to a perchloric acid spray reagent; however positive identification was not achieved.

Composition of phospholipids

The phospholipids from the barley samples were resolved by TLC as shown in Fig. 3. The components were identified by co-chromatography with lysophosphatidyl cholines (LPC), phosphatidyl cholines (PC), phosphatidyl serines (PS), phsophatidyl inositols (PL), phosphatidyl ethanolamines (PE), phosphatidyl glycerols (PG) and diphosphatidyl glycerols (DPG). The specific phospholipids sprays of Dittmer and Lester[18] verified the identity of the phospholipids. The phospholipid fraction of the three samples gave the same chromatographic patterns. The spot representing both PC and PS was the largest in this lipid class. LPC and PE ranked second and third, respectively. Those compounds were the major phospholipids, comprising over 80% of this lipid class. Ukhun[30] indicated that those lipids containing a free-amino group participate in non-enzymatic browning and lipid oxidation during storage of processed cowpea products. Lesser amounts of PI, PG, DPG and an unknown phospholipid were present. The unknown spot reacted positively to a molybdenum reagent[18], but further identification was not carried out.

Fatty acid composition

The fatty acid composition of the total lipid extract was determined for the three barley samples (Table 3). Palmitic, oleic, linoleic and linolenic acids were the major fatty acids, while smaller quantities of stearic and myristic acids were also detected. Linoleic acid was the predominant unsaturated fatty acid and was present in amounts of 56.2-60.6% of the total fatty acids in the samples. Also, linolenic acid was found at levels at 4.4-4.8% in the three barley samples. The higher amounts of linoleic and linolenic acids may increase oxidative deterioration during barley processing and storage.

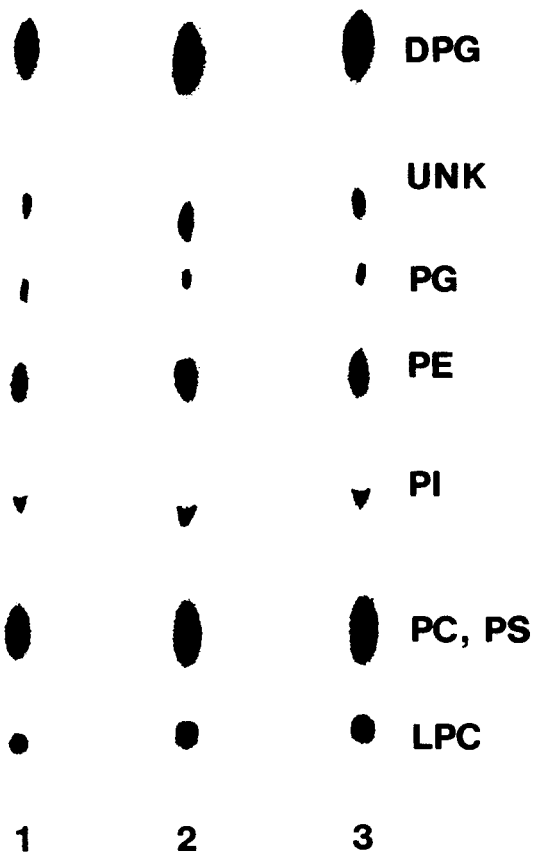

Fig. 3. Thin-layer chromatogram of phospholipids in barley samples. 1, Morex; 2, Korean barley; 3, blend of barley. Adsorbent, silica gel H (0.5 *mm*); solvent system, chloroform-methanol-water 28% aqueous ammonia (65: 35: 4.0: 0.2); visualization, charring by heating with 50% H_2SO_4. The spots were identified as follows: LPC, lysophosphatidyl cholines, PC, PS, phosphatidyl cholines and serines; PI, phosphatidyl inositols; PE, phosphatidyl ethanolamines; PG, phosphatidyl glycerols; Unk, unknown; DPG, diphosphatidyl glycerols.

Table 2. Composition of neutral and polar lipids in barley

Lipids classes[a]	*Morex*	Korean barley	Blend of barley
Neutral lipids[d]			
1,2-DG	7.1[b] (5.0)[c]	6.6 (4.6)	6.9 (5.0)
1,3-DG	6.2 (4.4)	5.8 (4.0)	5.4 (3.9)
FS	8.3 (5.9)	7.7 (5.3)	8.6 (6.4)
Unk 1	1.4 (1.0)	1.6 (1.1)	1.8 (1.3)
Unk 2	1.6 (1.1)	1.3 (0.9)	1.5 (1.1)
Unk 3	1.1 (0.8)	0.7 (0.5)	0.9 (0.7)
FFA	9.8 (6.9)	10.3 (7.2)	8.9 (6.5)
TG	54.2 (38.2)	56.3 (39.0)	55.7 (40.7)
SE	10.1 (7.1)	9.6 (6.7)	10.3 (7.5)
	99.8 (70.5)	99.9 (69.3)	100.0 (73.1)
Glycolipids[e]			
SL	1.8 (0.2)	1.3 (0.2)	1.6 (0.2)
DGDG	31.3 (3.6)	32.5 (5.3)	33.2 (3.2)
Unk	3.1 (0.4)	2.4 (0.4)	2.6 (0.2)
CB 1	9.4 (1.1)	8.3 (1.4)	7.8 (0.7)
CB 2	11.6 (1.3)	14.2 (2.3)	10.5 (1.0)
SG	3.8 (0.4)	3.4 (0.6)	4.4 (0.4)
MGDG	26.2 (3.0)	26.7 (4.4)	29.6 (2.9)
ESG	12.8 (1.6)	11.2 (1.9)	10.2 (1.0)
	100.0 (11.6)	100.0 (16.5)	99.9 (9.6)
Phospholipids[f]			
LPC	31.2 (5.6)	28.5 (4.0)	29.8 (5.2)
PC, PS	39.8 (7.2)	41.8 (5.9)	41.5 (7.2)
PI	4.1 (0.7)	5.3 (0.8)	4.6 (0.8)
PE	9.9 (1.8)	10.1 (1.4)	10.8 (1.9)
PG	3.3 (0.6)	4.1 (0.6)	3.1 (0.5)
Unk	5.1 (0.9)	3.2 (0.5)	3.7 (0.6)
DPG	6.3 (1.1)	7.0 (1.0)	6.2 (1.1)
	99.7 (17.9)	100.0 (14.2)	99.7 (17.3)

[a] Abbreviations are the same as in Fig. 1, 2 and 3.
[b] All values are the percent of each lipid class.
[c] All values in parentheses are the percent of total lipids.
[d] Individual components determined by duplicate Amenta method.
[e] Individual components determined by duplicate hexose analyses.
[f] Individual components determined by duplicate phosphorus analyses.

Table 3. Fatty acid composition of total lipids in barley

Fatty acid	*Morex*	Korean barley	Blend of barley
In purified lipids, (% of total fatty acids)			
Myristic	0.3	0.3	0.4
Palmitic	21.5	26.8	22.2
Stearic	1.6	1.2	1.7
Oleic	11.6	10.7	13.1
Linoleic	60.6	56.2	58.1
Linolenic	4.4	4.8	4.5
Fatty acid content			
In purified lipids, *g*/100*g*	69.8	63.9	71.5
In meal, *mg*/100*g*	2,373	1,469	2,646

The amount of fatty acids ranged from 63.9 to 71.52 *g*/100 *g* of purified lipids for the three barley samples. The fatty acid content also expressed as *mg*/100 *g* of meal generally reflects the difference in the total lipid contents of the three samples.

The fatty acid composition of NL, GL and PL fractions in presented in Table 4. Linoleic, palmitic, oleic and linolenic acids were the major fatty acids found in the three lipid fractions of the three samples. The NL fraction contained more oleic acid than did the GL and PL fractions. The GL fraction contained more linoleic acid than did the NL and PL fractions. The PL fraction generally contained more palmitic acid than did the NL and GL fractions, and the PL fraction contained generally less linolenic acid than the NL and GL fractions. These results almost agree with data reported by Price and Parsons[12] and Bhatty and Rossnagel[3].

요 약

3가지 보리로부터 추출한 지방질의 조성을 관, 얇은 막 및 기체-액체 크로마토그래피에 의하여 분석하였다. 총 지방질의 조성은 중성지질 69.3-73.1%, 당지질 9.6-16.5%, 인지질 14.2-17.9%였다. 중성지질 중에는 트리글리세리드의 함량이 가장 많았고 (중성지질의 54.2-55.7%), 그외에 소량의 1,2-및 1,3- 디글리세리드, 유리스테롤, 유리지방산, 스테릴에스테르와 미동정한 3가지 지방질을 함유하였다. 당지질중에는 디-갈락토오실 디-글리세리드와 모노 갈락토오실 디-글리세리드가 주성분이였고, 그외에 에스테르화 스테릴 글리세리드, 스테릴 글리세리드, 세레브로시드, 황지질 및 미동정한 한가지 지방질이 주성분으로 존재하였다. 인지질중에는 포스파티딜 콜린과세린, 라이소포스파티딜 콜린, 포스파티딜 에탄올 아민이 전인지질의 80%이상을 차지하는 주성분이었다. 총지방질 및 3가지 지방질 획분의 주요 지방산은 팔미트산, 올레산, 리놀레산 및 리놀렌산이었다. 그러나 중성지질 획분은 다른 지방질 획분보다 올레산의 함량이 많았고, 인지질 획분은 다른 지방질 획분보다 팔미트산의 함량이 많았다.

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Table 4. Fatty acid composition of lipid classes in barley[a,b]

Fatty acid	*Morex*			Korean barley			Blend of barely		
	NL	GL	PL	NL	GL	PL	NL	GL	PL
Myristic	0.3	0.2	0.3	0.4	0.2	0.5	0.3	0.3	0.5
Palmitic	21.6	17.9	27.1	27.7	21.5	26.6	21.3	18.8	26.6
Stearic	1.3	1.7	1.8	1.2	1.1	2.2	1.6	2.3	2.2
Oleic	12.9	6.6	8.3	11.5	7.1	9.0	14.8	6.3	9.0
Linoleic	60.3	69.3	60.5	54.5	65.3	58.9	57.3	67.7	58.9
Linolenic	3.7	4.3	2.0	4.7	4.7	2.9	4.6	4.6	2.9

a Expressed as area percentage of the total area from all methyl esters of each lipid fractions.
b NL, neutral lipids; GL, glycolipids; PL, phospholipids.

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