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Focused microwave-assisted Soxhlet extraction: an expeditive approach for the isolation of lipids from sausage products

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Abstract

A prototype of extractor based on the conventional Soxhlet principles but assisted in the cartridge zone by focused microwaves is proposed for accelerating the extraction of lipids from sausage products. The extraction process has been optimised using a multivariate design involving the main variables influencing the performance of the prototype (namely, irradiation power *P*, irradiation time *T* and number of cycles *C*). Under the optimum working conditions ($P=160$ W, $T=10$ s and $C=14$), the extraction of lipids from different Spanish sausage products is complete in 45 min. The extracts thus obtained have been compared with those provided by conventional Soxhlet extraction for 8 h by development of chromatographic analyses (namely, quantification of polar lipids by thin-layer chromatography, determination of free fatty acids by gas chromatography and quantification and distribution of total polar compounds by high-performance size exclusion chromatography), and not qualitative or quantitative differences have been found.

Keywords: Focused microwaves; Soxhlet extraction; Sausage products; Multivariate optimisation; Chromatographic analysis

1. Introduction

Interest in dietary fat is widespread, and the determination of fatty compounds is a basic requirement in testing food materials. Consumers demand reduction of both the total fat content and cholesterol in food in order to improve human health (Chao, Mulvaney, Bailey, & Fernando, 1991) thus forcing government agencies to the use of more precise methods for fat determination, which assure accuracy in labeling products. The determination of fat contents has so far been based on gravimetric measurements after leaching the sample with the suitable organic solvent. The most widely used procedure for fat removal from solid matrices is conventional Soxhlet extraction; nevertheless, new extraction methods are displacing conventional Soxhlet because of the drawbacks the later involves (García-Ayuso & Luque de Castro, 2001; Luque de Castro & García-Ayuso, 1998). The most significant of these drawbacks are the strong dependence of lipids extraction on the solvent used (Finney, Pomeranz, & Hoseney, 1976), the large volumes of organic solvents released to the atmosphere and the slowness of the procedure. Despite these drawbacks, Soxhlet extraction is used in a variety of official methods for determination of fats (ISO 659, 1988; Métodos Oficiales de Análisis, 1986).

The focused microwave assisted Soxhlet extractor used in this research is a patented device (PCT Application WO97/441009, 1998), which has been checked for the extraction of different compounds in solid samples such as pollutants (García-Ayuso, Luque-García, & Luque de Castro, 2000; Luque-García & Luque de Castro, 2001), and also in food analysis as an alternative to traditional methods for lipids extraction from different samples (García-Ayuso, Velasco, Dobarganes, & Luque de Castro, 1999a, 1999b; Luque-García, Velasco, Dobarganes, & Luque de Castro, 2002).

The key aspect of focused microwave-assisted Soxhlet extraction (FMASE) is that it maintains the advantages

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of conventional Soxhlet (namely: sample-fresh solvent contact during the whole extraction step, no filtration required after extraction and easy manipulation) and circumvents the shortcomings of conventional Soxhlet by accelerating the process and minimising environmental pollution due to the small amount of solvent released into the atmosphere.

This research was aimed at checking the performance of the microwave-assisted device for the extraction of lipids from sausage products with the dual objective of; (i) reducing the time required for the extraction of the total lipid contents with respect to conventional Soxhlet, and (ii) demonstrating the non influential effect of microwaves (under the working conditions used) on the degradation of lipids in these products as compared with conventional Soxhlet. An exhaustive study of the variables affecting the overall performance based on a multivariate design was carried out in order to determine both their influence and values maximising extraction efficiency. The efficacy of the extraction as well as the analytical results for fatty acid composition, polar compounds and the main groups of polar compounds in the extract were compared to those obtained by conventional Soxhlet extraction.

2. Experimental

2.1. Sampling

2.1.1. Types of sample

Three types of sausage products the (namely, Chorizo de pueblo, Chorizo empapelado and Salchichón, all of them of high quality) were used. Chorizo de pueblo and Chorizo empapelado have a similar composition and some differences in both spices contents and fabrication process. Salchichón has a different composition according to its quality regulation. All three products have in common the main raw material: meat and fat from pork.

2.1.2. Sample preparation

Each sausage product was cut into pieces which were ground independently in a mechanical mill, then placed in a plastic flask and stored at 4 °C until use. Chorizo de pueblo was used in the optimisation study, and the optimal values found were used in the extraction of fat from the other sausage products.

2.2. Determination of the moisture and volatile matter

Each of the three foodstuff under study was placed on a desiccated tared capsule that was transferred into an electrically heated oven with ±2 °C temperature control (Selecta, Bacelona, Spain) at 100 °C for 2 h. After this, the capsule was removed from the oven and cooled to room temperature in a desicsator. After weighing (Explorer analytical balance, Ohaus, USA), the procedure was repeated until the difference between two consecutive weightings was smaller than 2 mg. The free-humidity foodstuffs were used both to perform conventional Soxhlet extractions and to check that water removal is not necessary in the proposed method.

2.3. Conventional Soxhlet extraction

An amount of 5 g of the sample under study, mixed with 1.5 g of pre-washed sea-sand as a dispersant, was placed in a cellulose thimble (25×88 mm, Albet, Barcelona, Spain), which was fitted to a distillation flask containing 80 ml of *n*-hexane and 2–3 boiling glass regulators. After extraction for 8 h, the solvent was released in a rotary-evaporator (Rikakikai Co., Ltd., Japan) and the extract was dried under a nitrogen stream until the difference between two consecutive weightings was smaller than 2 mg.

2.4. Focused microwave-assisted Soxhlet extraction (FMASE)

The prototype patented by Luque de Castro and García-Ayuso was constructed from glassware similar to that of a conventional Soxhlet extractor but modified in order to facilitate accommodation of the sample cartridge compartment in the irradiation zone of a Microdigest 301 device of 200-W maximum power (Prolabo, France). The latter was also modified: an orifice at the bottom of the irradiation zone enabled connection of the cartridge zone to the distillation flask through a glass siphon. A Megal 500 thermometer (Prolabo, France) was used to monitor the extraction temperature. Two microprocessor programmers were used for time control of the microwave unit and the thermometer. The operation of the overall device is illustrated in Fig. 1. After the heat source was on for a time sufficient for distillation of enough extractant to cover the sample in the cartridge, the microwave source acted on the sample vessel for a time controlled by the programmer; then, the heated content of the vessel was allowed to remain for a preset time also controlled by the programmed for solid-extractant mass-transfer before siphoning. This constituted a cycle which was repeated as many times as required.

In the present method 100 ml of n-hexane and 2–3 boiling glass regulators were poured into the tared distillation flask. An amount of 5 g of the sample was mixed with 1.5 g of pre-washed sea-sand as a dispersion agent. The mixture was put into a cellulose extraction thimble (25×88 mm, Albet, Barcelona, Spain), which was covered with cotton wool and inserted into the quartz extraction vessel placed in the microwave-irradiation zone. The distillation flask was positioned on

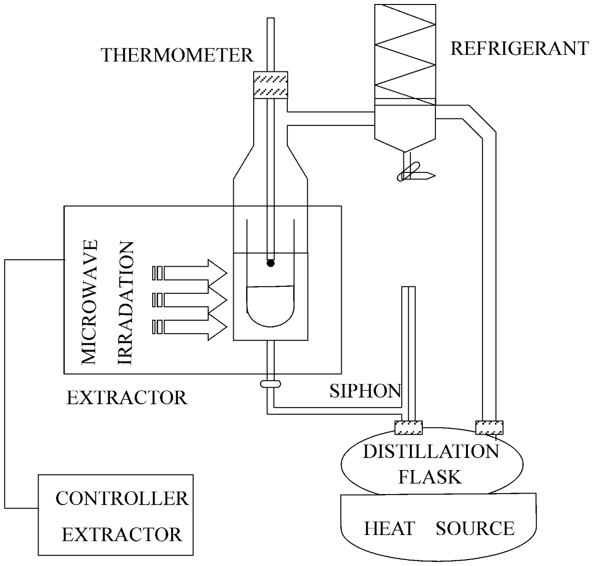

Fig. 1. Scheme of the prototype (for details, see Section 2).

the electrical isomantle (Prolabo, France) and connected to the sample vessel by the siphon and distillation tube. After the extraction step (eight cycles with 10 s of microwave irradiation at 160 W—80% of the maximum power—each cycle) the extractant condensated in the refrigerant was drained by actuating the valve at its bottom. The solvent (75–80 ml) was thus recovered. Removal of solvent traces from the extracted fat and gravimetric determination was performed as in the conventional Soxhlet procedure.

2.5. Chromatographic determination

2.5.1. Qualitative analysis

In order to know the neutral or polar character of the lipids, qualitative analysis of the extracts from the target samples was assessed by thin-layer chromatography. Solutions of lipids in diethylether (50 mg/ml) were prepared and 2 μl spots were applied onto the plate (20×20 cm^2) which was developed in a 70:30:1 (v/v/v) mixture of light petroleum–diethyl ether–acetic acid and revealed with iodine vapor.

2.5.2. Quantitation of polar lipids by thin-layer chromatography with flame ionization detection (TLC-FID)

Samples of the fat extracts (15 mg) were dissolved in n-hexane containing 5 mg/ml of squalane as internal standard and 1 μl was spotted on Chromarods S-III quartz rods with a coating of silica gel. The Chromarods were developed in 98:2:1 light petroleum ether–diethylether–acetic acid for 35 min and scanned in an Iatroscan MK-5 Analyser (Iatron Labs., Tokyo, Japan). The Iatroscan was operated under the following conditions: hydrogen flow rate 150 ml/min; air flow rate, 1500 ml/min; and scanning speed, 0.33 cm/s. Polar lipids remained at the origin of the rods and were quantified with respect to the standard. The samples were analysed in duplicate.

2.5.3. Determination of free fatty acids

Free fatty acids content, expressed as oleic acid percentage, was determined according to the IUPAC Standard Method (IUPAC, 1992).

2.5.4. Quantitation and distribution of total polar compounds

Evaluation of the extracts was approached by applying a methodology developed for quantitative analysis of different groups of alteration compounds in small samples (Márquez-Ruiz, Jorge, Martín-Polvillo, & Dobarganes, 1996). Briefly, 2 ml of a solution containing 50 mg of extracted oil and 1 mg monostearin, used as internal standard, was separated by solid-phase extraction (silica cartridge) into a first fraction (essentially unaltered triglycerides) eluted with 15 ml 90:10 hexane–diethyl ether and a second fraction, containing polar compounds and the internal standard, eluted with 15 ml of diethyl ether. Subsequently, fractions of polar compounds were analysed by HPSEC using a Rheodyne 7725y injector with a 10 μm sample loop, a Waters 510 HPLC pump (Waters Associates, Milford, MA, USA), two 100 and 500 Å Ultrastyragel columns 25 cm×0.77 cm inner diameter, packed with a porous, highly cross-linked styrenedivinylbenzene copolymer (<10 μm) connected in series, and a refractive index detector (Hewlett Packard, CA, USA). HPLC-grade tetrahydrofuran served as the mobile phase with a flow of 1 ml/min. The peaks resolved corresponded to triglyceride polymers, oxidised triglyceride monomers, diglycerides, monostearin, and a final peak constituted by free fatty acids and polar unsaponifiables.

2.5.5. Fatty acid composition

Fatty acids were analysed by GLC after derivatisation to fatty acid methyl esters (FAME) with KOH 2N in methanol, according to the IUPAC Standard Method (IUPAC, 1992). An HP 6890 chromatograph provided with an HP Innowax capillary column (polyethylene glycol, 30 m×0.25 mm i.d., film thickness 0.25 μm) (Hewlett Packard, USA), was used under the following temperature programme: 180 °C (4 min), 4 °C/min to 230 °C (15 min). The extracts were introduced on the column via a split injector (split ratio 1:40) at 250 °C and the flow rate of hydrogen, used as carrier gas, was 1 ml/min. Temperature of both the split injector and flame ionisation detector was 250 °C.

3. Results and discussion

First, and using as a model one of the target products—Chorizo de pueblo—a multivariate design was applied in order to both determine the influence of the experimental variables and maximise extraction efficiency. Then, the optimum working conditions were applied to the other samples in order to check the scope of application of the method. Finally, the results of the chromatographic analyses of the extracts were compared with those from extracts obtained by conventional Soxhlet.

3.1. Optimisation of the focused microwave-assisted Soxhlet extraction

The variables susceptible to be optimise in FMASE are always the irradiation power, the irradiation time and the number of cycles needed for the total extraction of the analytes.

For optimisation of the extraction conditions, a full factorial design at two levels, with an additional triplicate point located near the centre of the design, were used. The three factors included were: microwave irradiation power (*P*), irradiation time per cycle (*T*), and number of cycles (*C*). The levels of the factors and the responses obtained are shown in Table 1. The central value for the *P* factor is actually 110 W, however, 120 W was used instead since the microwave generator allows only setting percentages of the maximum power (200 W) in 10%-steps. The experiments were performed in a randomized order, and the main and second order effects between the variables were analysed with the Unscrambler 7.6 (Camo ASA, Oslo, Norway) software package.

From the results in Table 1 and the analysis of effects in Table 2, the following conclusions were drawn: (a) the differences between the points were small, which should be attributed to a high extraction efficiency at all the conditions studied, with several experiments giving yields close to the total fat content of the sample; (b) the main effect of *C* was significant and positive, but the main effects of *P* and *T* were not significant; (c) both the *PC* and *TC* second order interactions were significant, indicating weak antagonisms between these two factor pairs; (d) the *PT* second order interaction was not significant. Thus, the highest extraction yield should be obtained with a large number of cycles and, at the same time, with application of short irradiation periods at low or moderate microwave power.

Table 1
Factors levels and responses for a full factorial design plus a triplicate centre

Power (W)	Irradiation time/cycle (s)	Number of cycles	Fat (%)
60	30	4	40.65
60	30	8	48.19
60	90	4	43.92
60	90	8	44.86
160	30	4	45.15
160	30	8	46.49
160	90	4	44.26
160	90	8	44.00
120	60	6	42.28
120	60	6	42.02
120	60	6	42.63

Table 2
Analysis of effects for the 11 experiments in Table 1

Variable	Main effects and interactions (%)	*F*-ratio	*P*-value
Number of cycles (*C*)	2.39	3.66	0.31
TC	−2.05	2.69	0.35
PC	−1.85	2.19	0.38
Irradiation time/cycle (*T*)	−0.86	0.47	0.62
PT	−0.83	0.44	0.63
Power (*P*)	0.57	0.21	0.73

These conclusions were confirmed by examining the six faces of the cubic *CPT* experimental space. The corresponding six linear regression models of Table 3 (each one calculated at a different constant value of the variables) showed that the extraction yield increased always with *C* (which exhibited positive slopes), particularly at the low levels of the other two variables. Thus, when *P* or *T* were at their respective high levels ($P=160$, or $T=90$), the extraction yield slightly improved by increasing *C*; however, when *P* or *T* were at their low levels ($P=60$, or $T=30$), an increase of *C* lead to a much larger improvement of the extraction yield. Similarly, when *C* was at its low level ($C=4$), the extraction yield was significantly lower, but also improved by increasing any of the other two factors, *P* or *T*. These were logical results, since a low yield was found to be notably improved by increasing any of the three variables involved, but it was difficult to further increase percentages over 46–48% which probably approached the 100% extraction yield.

Surprisingly, the model obtained with $C=8$, indicated reductions of the extraction yield (negative slopes) when either of the other two variables, *P* or *T* increased. This was attributed to the formation of bubbles of the boiling solvent, which remained on the sample surface, thus obstructing its contact with solvent. The maximum extraction yield predicted for the models of Table 3 (i.e., 48%), corresponds to the following cases: $T=30$ with

Table 3
Linear regression models for the six *PTC* cube faces of Table 1

Cube face	Model	Min	Max
$P=60$	$38.08-0.0005\times T+1.06\times C$	42.3	46.5
$P=160$	$45.86-0.028\times T+0.14\times C$	43.9	46.1
$T=30$	$36.92+0.028\times P+1.11\times C$	42.2	48.0
$T=90$	$44.04-0.005\times P+0.09\times C$	44.0	44.6
$C=4$	$39.64+0.048\times P+0.020\times T$	41.7	45.3
$C=8$	$50.20-0.026\times P-0.049\times T$	43.8	48.0

$C=8$ and $P=160$, and $C=8$ with $T=30$ and $P=60$. Thus, the results indicated that the experimental region with $T<30$ while maintaining a high value of C should be also examined. For this purpose, another series of experiments, in which the number of cycles was maintained constant at $C=8$, and the response surface was investigated at $T<30$ and $P>160$, was performed. The experiments, which followed a full factorial design at two levels of P and T, with an additional duplicated point at the centre of the PT square, and the fat contents found, are shown in Table 4.

In agreement with the results given above for $C=8$ (last line of Table 3), the response decreased when either P or T increased. Thus, the optimum extraction yield was found for a large number of cycles (as $C=8$), a high power (as $P=160$ W) and a short irradiation time per cycle (as $T=10$ s), in order to prevent boiling of the solvent. The alternative, namely to increase the irradiation time per cycle and to reduce the power while maintaining a large number of cycles, has the drawback of increasing the total extraction time.

3.2. FMASE vs conventional Soxhlet

Concerning efficiency, the comparison of FMASE (under the optimum working conditions established in the previous paragraph) with conventional Soxhlet shows that the yields obtained by FMASE (average yield and standard deviation: 49.69 and 0.50%, respectively) were slightly higher than those provided by conventional Soxhlet using three portions of the same Chorizo de pueblo, subjected to extraction for 8 h each (average yield and standard deviation: 48.06 and 0.71%, respectively). Under the same working conditions other types of Spanish sausages (namely, Chorizo empapelado and Salchichón) were subjected to extraction, but a total of 14 cycles were necessary for quantitative removal of the lipid content, as shown in Table 5.

3.3. Qualitative aspects

One of the main aspects of the study here developed was to check if the proposed approach produced degradation of the lipids by the action of microwaves. With this aim, the extracts obtained by both FMASE and conventional Soxhlet were subjected to several chromatographic analyses and not differences due to the extraction system were found, as commented below.

Table 4
Factor levels and responses for a full factorial design plus a duplicate centre

Power (W)	Irradiation time/cycle (s)	Fat (%)
160	10	49.69
160	30	46.16
200	10	46.00
200	30	44.70
180	20	45.60
180	20	45.20

Table 5
Analysis of sausage products

Sample	Conventional Soxhlet	FMASE
Chorizo empapelado[a]	41.74	41.00
Salchichón[a]	32.60	35.70
Chorizo de pueblo[b]	48.50	49.69

All the results are the average of two experiments.

[a] $P=80\%$; $T=10$ s; $C=14$.

[b] $P=80\%$; $T=10$ s; $C=8$.

Thin-layer chromatography indicates that most lipids are neutral (namely, triacylglycerols, diacilglycerols and free fatty acids) and because polar lipids (phospholipids) stay at the beginning of the layer. Quantitation of polar lipids has been carried out by TLC-FID and the amounts are lower than 0.5% in both extracts (namely, from conventional Soxhlet and the proposed method), as shown in Fig. 2.

A small hydrolytic alteration produced in both extraction modes is shown by the presence of free fatty acids. Monitoring of the acidity is important because a high hydrolysis would indicate a higher sensitivity to oxidation during conservation of the product due to the fact that free fatty acids are oxidised quicker than triglycerids, meanwhile diglycerids have also prooxidant action (Mistry Min, 1988; Miyashita Takagi, 1986).

Finally, the determination of polar compounds is interesting because their content is related to the quality of the fat. The analysis of the polar fraction shows that most compounds are, in all cases, hydrolysis products.

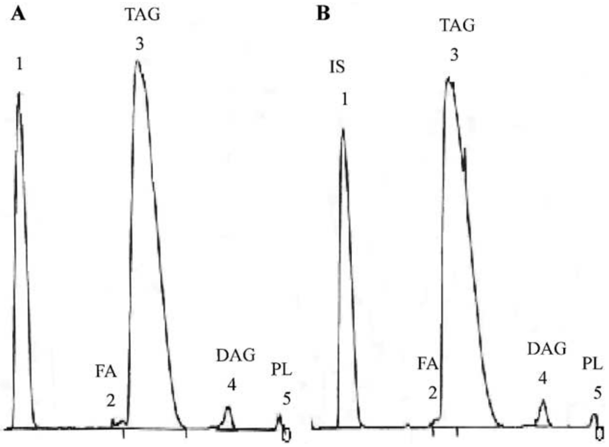

Fig. 2. Iatroscan TLC-FID chromatograms of Chorizo de pueblo extracted by (A) Soxhlet convencional and (B) FMASE. IS, internal standard; FA, fatty acids; TAG, triacylglycerols; DAG, diacylglycerols; PL, polar lipids; O, origin.

Table 6
Main polar compounds constituents (%)

Sample	FFA (% oleic acid)	PC (%)	TGD (%)	oxTGM (%)	DG (%)
1 Chorizo empapelado (Soxh)	2.5	8.2	0.5	1.9	1.8
2 Chorizo empapelado (Soxh + MW)	3.0	8.1	0.3	2.6	2.1
3 Salchichón (Soxh)	1.6	6.9	0.2	1.7	1.9
4 Salchichón (Soxh + MW)	1.2	6.7	0.2	2.2	1.8
5 Chorizo de pueblo (Soxh)	1.1	4.2	ND	0.9	1.3
6 Chorizo de pueblo (Soxh + MW)	1.1	3.8	ND	0.8	1.3

FFA, free fatty acids; PC, polar compounds; TGD, triacylglycerol dimers; ox-TGM, Triacylglycerol monomers; DG, diacylglycerols.

Combination of adsorption and size exclusion chromatography is mandatory in order to determine the quality of food lipids. First, total polar compounds should be low in high quality samples as they are mainly degradation compounds. As can be observed in the second column of Table 6, Chorizo de pueblo has the highest quality while Chorizo empapelado has the lowest. On the other hand, elimination of nonoxidised triglycerides by solid phase extraction allowed detection of the main groups of minor glyceridic compounds and there are not significant differences between the extract obtained by conventional Sohxlet and that obtained by FMASE, as illustrated in Fig. 3.

For quantitative purposes, not only total polar compounds are of interest but also the amounts of triacylglycerol dimers, oxidised triacylglycerol monomers and diacylglycerols, as they provide complementary information on polymerisation, oxidation and hydrolysis, the three main routes of lipid degradation. The last peak includes fatty acids and polar unsaponifiable compounds and it is excluded from calculations as fatty acids are better quantified by the standard IUPAC volumetric method.

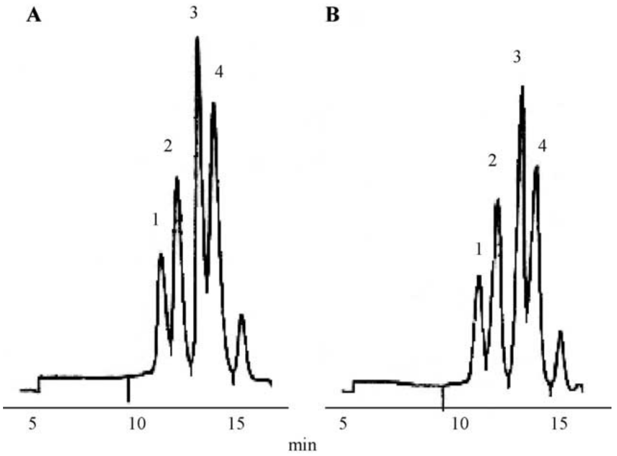

Fig. 3. Representative part of the HPSEC chromatograms of Chorizo de pueblo extracted by (A) Soxhlet convencional and (B) FMASE. Retention times: (1) 12.8 min, oxidised triacylglycerol monomers; (2) 13.3 min, diacylglycerols; (3) 14.0 min, internal standard (Monoestearin); (4) 14.5 min, fatty acids and polar unsaponifiable fraction.

4. Conclusions

The use of the prototype consisting of a conventional Soxhlet extractor assisted by focused microwaves on the extraction chamber for accelerating leaching of total fat from different types of sausage products has been checked. The main advantages provided by this device as compared to conventional Soxhlet for the target samples are as follows:

a. Substantial shortening of the extraction time (from 8 h to less than 1 h).
b. Saving of extractant (80–85% of the total volume was recovered by recycling)
c. Extraction efficiencies and precision comparable to or better than that provided by conventional Soxhlet extraction.
d. Use of the samples as received without the water adjustment usually required in conventional Soxhlet.
e. There is no difference in the fat composition obtained by FMASE and conventional Soxhlet.

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