agriculture

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Analyzing the Environmental Impact of Chemically-Produced Protein Hydrolysate from Leather Waste vs. Enzymatically-Produced Protein Hydrolysate from Legume Grains

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Academic Editor: Brad Ridoutt
Received: 16 June 2017; Accepted: 24 July 2017; Published: 27 July 2017

Abstract: Protein hydrolysates are largely used as plant biostimulants for boosting crop growth, and improving crop tolerance to abiotic stresses and fruit quality. Protein hydrolysate-based biostimulants are mostly produced by chemical hydrolysis starting from animal wastes. However, an innovative process of enzymatic hydrolysis of legume-derived proteins has been recently introduced by few companies. The objective of this study was to evaluate the energy use and environmental impact of the production processes of enzymatically-produced protein hydrolysate starting from lupine seeds and protein hydrolysate obtained from chemical hydrolysis of leather wastes through the application of life cycle assessment (LCA). The LCA method was applied through the software GEMIS "Global Emission Model for Integrated Systems", elaborated at L'Oko-Institute in Germany, and the parameters taken into account were: CO_2 emissions in g per kg of protein hydrolysate; the consumption of fossil energy expressed in MJ per kg of protein hydrolysate; and water consumption reported in kg per kg of protein hydrolysate. In the case of legume-derived protein hydrolysate, the evaluation of the energy use and the environmental impact started from field production of lupine grains and ended with the industrial production of protein hydrolysate. In the case of animal-derived protein hydrolysate, the LCA method was applied only in the industrial production process, because the collagen is considered a waste product of the leather industry. The type of hydrolysis is the step that most affects the energy use and environmental impact on the entire industrial production process. The results obtained in terms of CO_2 emissions, fossil energy consumption and water use through the application of LCA showed that the production process of the animal-derived protein hydrolysate was characterized by a higher energy use (+26%) and environmental impact (+57% of CO_2 emissions) in comparison with the enzymatic production process of lupine-derived protein hydrolysate. In conclusion, the production of legume-derived protein hydrolysate by enzymatic hydrolysis is more environmentally friendly than the production of animal-derived protein hydrolysate through chemical hydrolysis.

Keywords: biostimulants; protein hydrolysates; hydrolysis; LCA; sustainability

1. Introduction

New agricultural strategies, such as the application of natural substances and/or beneficial microbials, have been evaluated as a means for reducing negative environmental impact and improving crop performance and sustainability under adverse ecological conditions [1,2]. As defined by the European Biostimulants Industry Council [3], plant biostimulants contain microorganism(s) and/or substance(s) whose function, when applied to plants or the rhizosphere, is to stimulate natural processes to enhance/benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, and crop quality [4–6]. Protein hydrolysate-based biostimulants contain free amino acid, oligo and polypeptides achieved through enzymatic and/or chemical hydrolysis of proteins, especially from vegetal or animal sources [7–13].

Currently, 90% of protein hydrolysate-based biostimulants in the market are obtained from chemical hydrolysis of cattle leather wastes; while only 5–10% is from enzymatic hydrolysis of plant biomass, especially *Leguminosae* crops. Traditionally, chemical hydrolysis is achieved with strong acids (e.g., chloride acid), and extreme temperatures (>130 °C), and generally yields products with low agronomic quality, since some important amino acids (e.g., tryptophan) and peptides are destroyed during the production process [13]. One way of reducing losses of amino acids and peptides during the process would be through digestion of proteins with enzymes [14]. An enzymatic hydrolysis system obtains high quality protein hydrolysates [15–17] using specific enzymes and low temperatures (<60 °C). The enzymatically-produced protein hydrolysates from plant residues contain not only free amino acids, but also soluble peptides that act as signal molecules regulating a broad spectrum of physiological processes [18–22].

A process-based approach such as life cycle assessment (LCA) provides a methodology for comparing the energy use, greenhouse gas emissions, and water use associated with protein hydrolysate production. The LCA approach applied for the comparison of production processes of hydrolysates from plant and animal origin would be a valuable tool for understanding the real convenience of the different industrial pathways. The results of the LCA will be a beneficial indication for industrial companies in order to evaluate the hydrolysis process efficiency, leading to the identification of more sustainable approaches. LCA method has been used successfully for evaluating the environmental impact of production processes in the field of food production [19,23–25]. However, no data are available on the environmental impact of production processes of protein hydrolysate-based biostimulants. Therefore, the aim of this paper was to apply an LCA approach for quantifying the energy use, greenhouse gas emissions, and water consumption associated with protein hydrolysate products starting from leather wastes or lupine seeds.

2. Materials and Methods

LCA Method

The work was carried out implementing the Life Cycle Assessment (LCA) approach to the two production chains of protein hydrolysate-based biostimulants as reported in Figure 1.

The process to biostimulant production includes the following phases: (1) dry milling and grinding of seeds, or cutting of leather waste; (2) water extraction, where the seed flour or shredded leather waste is dispersed in acidified water (pH 4.5, 50 °C) for 6 hours to extract the soluble compounds; (3) filtration and centrifugation, where the protein concentrate is separated from the other organic compounds in a centrifuge decanter; (4) enzymatic or acid hydrolysis at 60 °C or 130 °C, respectively; (4) centrifugation, where the soluble compounds like amino acids and peptides are separated from the insoluble residual compounds; and (5) product concentration, where the soluble compounds are concentrated ca. 6 times through water evaporation in a mechanical vapor recompression evaporator.

With the aim of relating each input datum to the corresponding impact in terms of GHGE and fossil energy requirement, the biostimulant production chains were implemented in Gemis 4.7 software (Öko Institut, Berlin, Germany). The sources of the main up-stream processes are reported in Table 1:

the majority of the processes are found in the Gemis database, with the only exception being protease enzyme, where emission factors taken from the scientific literature [20,21] were used.

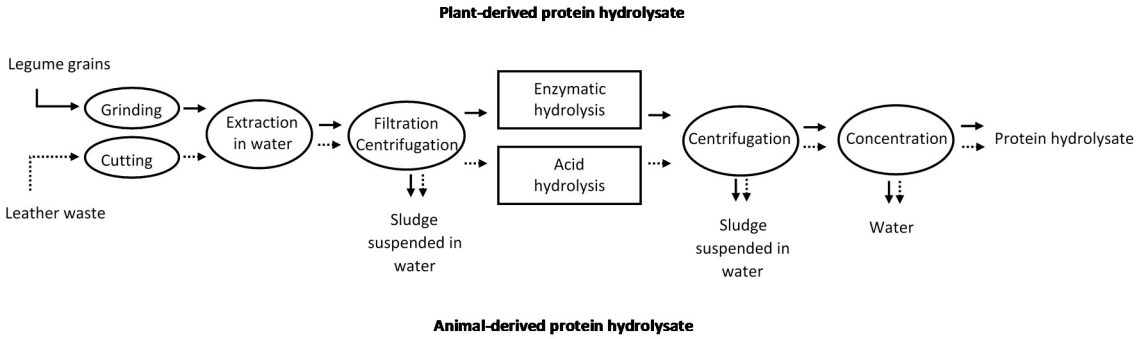

Figure 1. Production processes of protein hydrolysates: enzymatically-produced protein hydrolysate from legume grains vs. chemically-produced protein hydrolysate from leather waste.

Table 1. Up-stream processes used for the LCA implementation of the two production chains of biostimulants.

Up-Stream Production Chain	Source
N-fertilizer	Gemis 4.7—chem-inorgfertilizer-N-DE-2000
P_2O_5-fertilizer	Gemis 4.7—chem-inorgfertilizer-P-2000
K_2O-fertilizer	Gemis 4.7—chem-inorgfertilizer-K-2000
Pesticides	Gemis 4.7—chem-inorgpesticides-2000
Agricultural diesel	Gemis 4.7—dieselmotor-EU-agriculture-2010 (end-energy)
Grid electricity	Gemis 4.7—grid-el-IT-2010-local
Phosphoric acid (H_3PO_4)	Gemis 4.7—chem-inorgphosphoric acid-DE-2000
Hydrochloric acid (HCl)	Gemis 4.7—chem-inorgchlorine (membrane)-DE-2010ù
Protease enzyme	Nielsen et al., 2007; Nagaraju et al., 2013
Fresh water	Gemis 4.7—extra-drinking waterDE-2000
Heat (Natural gas boiler)	Gemis 4.7—gas-boiler-IT-2010
Waste-water treatment	Gemis 4.7—waste-water treatment-DE-2005

The developed approach for the environmental evaluation of the two production chains is based on the fundamentals of the ISO 14040 and 14044, aiming to promote a simplified comparative and attributional LCA. The LCA is a developed, standardized primary tool for environmental assessments, and LCA evaluates, from the environmental point of view, all the resources and inputs needed for the system studied, and all the outputs from the system, which include emissions to air, water and soil. In this way, first indications about the environmental pressures on the production chains were obtained. Considering the quality of the inventory data and their level of uncertainty, in this phase, the simplified LCA was limited to the calculation of three specific indicators, i.e., the GHGE with unit the CO_2 equivalent (CO_2eq), the Cumulated Energy Requirement (CER) and the Water Use (WU). In particular, the CO_2eq is a metric measure for comparing emissions from various GHGs on the basis of their global-warming potential (GWP) [22], the CER represents the fossil energy required for extracting, manufacturing and disposing raw and auxiliary materials all along each production chain; the WU is the sum of the fresh water used along all the production processes accounted in the LCA. The LCA method was applied for both production processes with reference to an output of 1 kg of protein hydrolysate-based biostimulant.

The LCA was modeled using the input data reported in Table 2: the first one uses lupine seeds as a source of proteins, the second one uses leather wastes. The data for energy, material inputs and outputs for both protein hydrolysate production systems were obtained from the literature [26] and interviews with experts, such as production managers of companies dealing with the production of protein hydrolysates.

The lupine production was assessed based on data collected in [23,26]: an inventory of the agricultural data used in the LCA is reported in Table 2.

Table 2. Inventory data for agricultural phase of lupine grain production [23].

Output	Value	Unit
Yield	2.7	t/ha
Inputs		
Seeds	0.040	kg/kg_{lupine}
Fertilizer-P_2O_5	45	kg/ha
Fertilizer-K_2O	80	kg/ha
Diesel	84	kg/ha
Electricity	15	kWh/ha

For the leather wastes, a specific analysis was carried out in order to calculate which impacts could be associated to these wastes. As reported in the COOP Environmental Product Declaration [21], beef meat production in Italy causes 23.8 kg CO_2 eq/kg of bone-free meat. This value is consistent with similar processes modeled in Gemis 4.7 for Southern Europe. Moreover, the amount of leather is equal to 7.9%, whilst the meat and other edible parts are about 92.1%. Then, the leather is tanned and the solid wastes of the process are about 43% of the raw material input. Therefore, considering the above mass allocation ratios in different animal tissues, 1 kg of leather gave a CO_2 emission of 0.958 kg CO_2eq, an energy consumption of 3864 MJ and water use of 1562 kg. However, the emissions associated with leather waste were set to zero in the LCA analysis because this waste does not have any market value.

Concerning the industrial phases for the production of protein hydrolysates, the inventory data are reported in Tables 3 and 4 were used.

Table 3. Key characteristics of the production process of protein hydrolysate-based biostimulant from lupine seeds (data refer to the production of 1 kg of protein hydrolysate).

Key System Inventory Characteristics	Unit	Value
Phase 1 (Dry Milling)		
Electricity	W·h	33
Phase 2 (Water Extraction)		
Chemical inputs (mineral acid, H_2SO_4 or H_3PO_4)	g	40
Water		0.7
Natural gas	W·h	320
Electricity	W·h	15
Phase 3 (Centrifugation)		
Electricity	W·h	35
Phase 4 (Enzymatic Hydrolysis at 60 °C for 6 h)		
Chemical inputs (protease)	G	11
Water	L	5
Natural gas	W·h	590
Electricity	W·h	55
Phase 5 (Centrifugation)		
Electricity	W·h	35
Phase 6 (Concentration)		
Electricity	W·h	300

Table 4. Key characteristics of the production process of protein hydrolysate-based biostimulant from leather waste (data refer to the production of 1 kg protein hydrolysate).

Key System Inventory Characteristics	Unit	Value
Phase 1 (Cutting)		
Electricity	W·h	16
Phase 2 (Water Extraction)		
Chemical inputs	G	115
Water		32
Natural gas (to keep the temperature at 90 °C for 1 h)	W·h	377
Electricity	W·h	15
Phase 3 (Centrifugation)		
Electricity	W·h	35
Phase 4 (Acid Hydrolysis at 130 °C for 4 h, and High Pressure 262 kPa)		
Chemical inputs	G	920
Natural gas	W·h	544.3
Electricity	W·h	70
Phase 5 (Centrifugation)		
Electricity	W·h	35
Phase 6 (Concentration)		
Electricity	W·h	300

3. Results and Discussions

The LCA results for the two-production process are reported in Tables 5 and 6.

Table 5. LCA results for protein hydrolysate production from lupine grains (data refer to the production of 1 kg protein hydrolysate).

Production phase	CO_2eq Emissions (g/kg)	Fossil Energy (MJ/kg)	Water (kg/kg)
Agricultural phase			
Seeds	6.011	13.974	0.071
Fertilizer-P_2O_5	18.984	0.278	0.132
Fertilizer-K_2O	32.275	0.516	1.640
Diesel	96.601	1.286	0.006
Electricity	2.419	0.033	0.005
Total (Agricultural phase)	**156.3**	**16.1**	**1.9**
Industrial phase			
Dry milling	14.182	0.194	0.026
Electricity	14.182	0.194	0.026
Water extraction	197.865	2.524	10.627
Phosphoric acid (H_3PO_4)	110.675	1.043	6.252
Water	0.254	0.004	0.638
Heat (Natural gas boiler)	80.490	1.389	3.725
Electricity	6.446	0.088	0.012
Centrifugation (protein separation)	15.041	0.205	0.028
Electricity	15.041	0.205	0.028
Enzymatic hydrolysis	215.852	3.561	11.666
Protease enzyme	42.000	0.650	0.200
Water	1.812	0.027	4.555
Heat (Natural gas boiler)	148.404	2.562	6.867
Electricity	23.636	0.323	0.044
Centrifugation (hydrolysate separation)	15.041	0.205	0.028
Electricity	15.041	0.205	0.028
Concentration (water removal)	128.924	1.760	0.241
Electricity	128.924	1.760	0.241
Total (Industrial phase)	**586.9**	**8.4**	**22.6**
Total (Agricultural phase + Industrial phase)	**743.2**	**24.5**	**24.5**

Table 6. LCA results for protein hydrolysate production from leather wastes (data refer to the production of 1 kg protein hydrolysate).

Production phase	CO_2eq Emissions (g/kg)	Fossil Energy (MJ/kg)	Water (kg/kg)
Industrial phase			
Cutting in pieces of 10–15 cm	7.564	0.103	0.014
Electricity	7.564	0.103	0.014
Water extraction	474.166	5.382	56.679
Phosphoric acid (H_3PO_4)	350.010	3.297	19.773
Water	12.755	0.187	32.066
Heat (Natural gas boiler)	104.311	1.801	4.827
Electricity	7.091	0.097	0.013
Centrifugation (protein separation)	16.545	0.226	0.031
Electricity	16.545	0.226	0.031
Acid hydrolysis	874.513	17.247	59.719
Hydrochloric acid (HCl)	690.828	14.196	52.688
Heat (Natural gas boiler)	150.595	2.600	6.968
Electricity	33.091	0.452	0.062
Centrifugation	16.545	0.226	0.031
Electricity	16.545	0.226	0.031
Concentration (hydrolysate separation)	141.817	1.936	0.265
Electricity	141.817	1.936	0.265
Waste-water treatment	1.097	0.020	0.028
Total (Industrial phase)	**1532.2**	**25.1**	**116.8**

The calculated indicators demonstrated a lower impact for the production process based on the enzymatic hydrolysis of lupine seeds than for that based on chemical hydrolysis of leather waste. This result is mainly due to the different processes of hydrolysis, which requires higher temperature, pressure and chemical inputs in a chemical hydrolysis process in comparison to an enzymatic hydrolysis process. In Figure 2, the CO_2 emission percentage for each production phase of enzymatically-produced protein hydrolysates from lupine grains is reported.

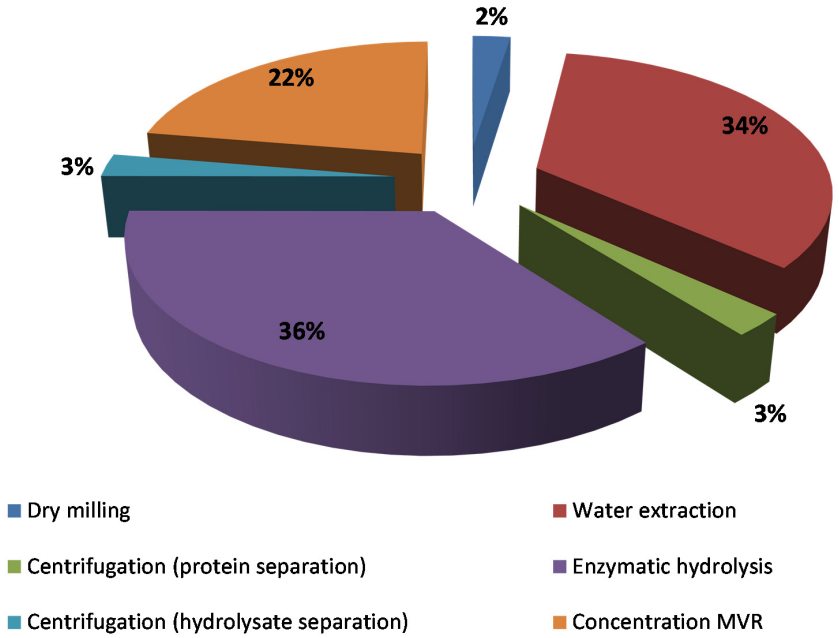

Figure 2. CO_2 emission percentages (on a total basis) for the industrial phases of enzymatically-produced protein hydrolysate from lupine grains.

In Figure 3, the CO_2 emission percentage for each production phase of chemically-produced protein hydrolysates from leather waste is reported.

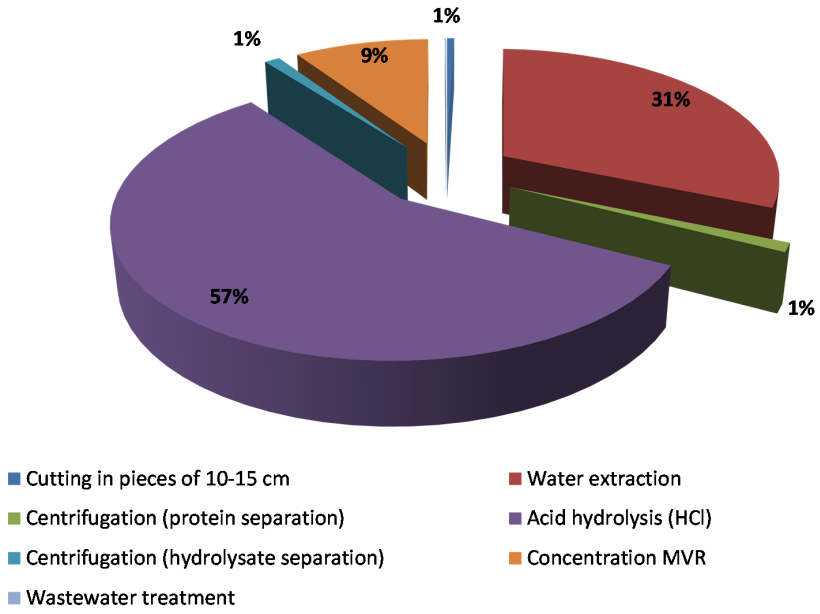

Figure 3. CO_2 emission percentages (on a total basis) for the industrial phases of chemically-produced protein hydrolysate from leather waste.

The results of this study showed that the production process based on enzymatic hydrolysis of lupine seeds had the lowest environmental impact. In particular, the greatest differences in CO_2 emissions between the production processes were observed in the hydrolysis phase (*n.* 4), with a saving of 57.03% using enzymatic hydrolysis (Figure 4). Concerning the energy consumption (Figure 5), enzymatic hydrolysis of lupine seeds required less energy in phase 4, while the opposite behavior was observed for phase 2 and 6, where the production of leather-derived protein hydrolysate by chemical hydrolysis reduced the energy needed by 8.5 and 13.1%, respectively.

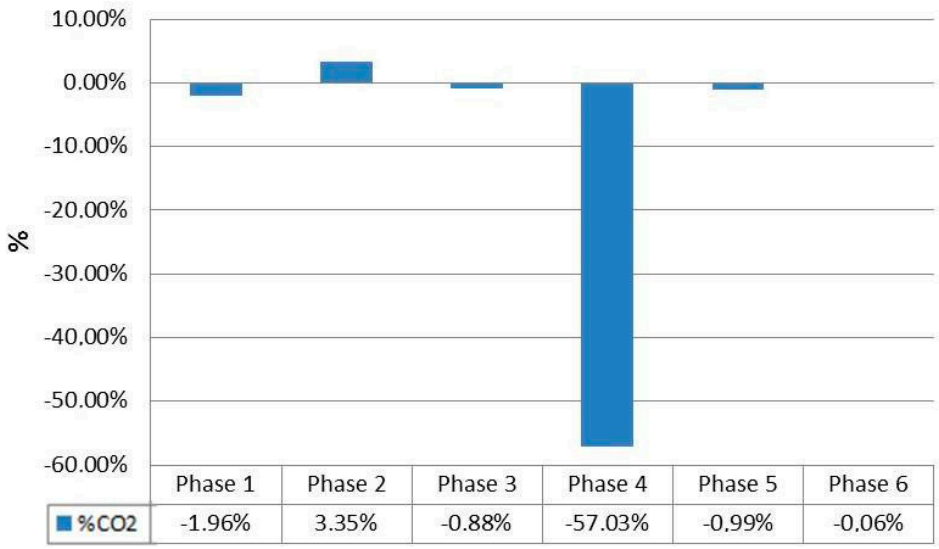

Figure 4. CO_2 emissions resulting from the difference between enzymatically-produced protein hydrolysate from lupine grains and chemically-produced protein hydrolysate from leather waste by industrial phase.

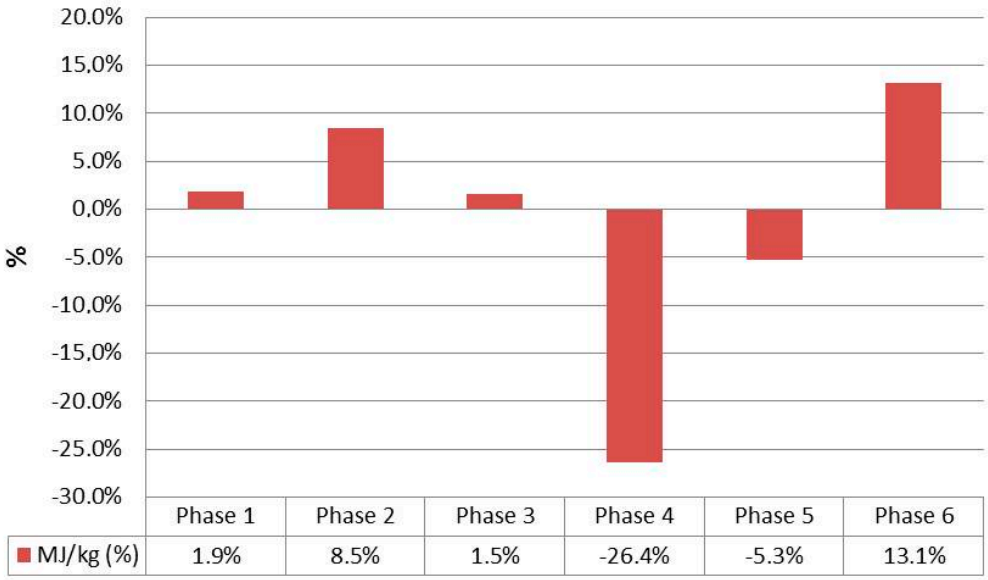

Figure 5. Energy consumption resulting from the difference between enzymatically-produced protein hydrolysate from lupine grains and chemically-produced protein hydrolysate from leather waste.

4. Conclusions

Overall, the results demonstrated that the production of legume-derived protein hydrolysate by enzymatic hydrolysis is more environmentally friendly than the production of protein hydrolysate through chemical hydrolysis of leather waste.

Author Contributions: The contribution to the programming and executing of this research must be equally divided by the authors.

Conflicts of Interest: The authors declare no conflict of interest.

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