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A review on different kinds of analytical methods are performed for the analysis of food preservatives in food

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Abstract

The onslaught of pathogens (disease-causing microbes) like bacteria and mold is responsible for the majority of cases of food deterioration. The various techniques for food preservation that have been developed are all intended to lessen or completely get rid of harmful ingredients. The term "food preservation" refers to methods for preserving food. Food preservative improves human health by eradicating or restricting the growth of bacteria and organisms responsible for food deterioration. There has been an increase in recent decades in the consumption of foods with high preservative content. By using several analytical techniques, including Uv-Visible, Calorimetry, HPLC, GC, LCMS, and Electrophoresis, the proposed methods were employed to identify various preservatives in a variety of food products.

Keywords: disease-causing microbes, LCMS, and Electrophoresis

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Introduction

Food additives are compounds that are added to food to preserve or enhance the food's safety, freshness, flavour, texture, or appearance. To guarantee processed food is safe and in good condition from factories or industrial kitchens, to transit to warehouses and stores, and finally to consumers, additives are required [1-2].

Any substance added to food is considered a food additive in the broadest sense. By definition, the phrase refers to "any substance whose intended use results in, or may reasonably be expected to result in—directly or indirectly—it's becoming a component of, or otherwise affects the properties of, any food." Any substance used in the preparation, treatment, packaging, handling, or storage of food is covered by this term. But the legal definition's main objective is to impose the need for premarket approval. Therefore, ingredients whose use is generally accepted as safe (where government approval is not required), ingredients whose use was authorised by the FDA or the U.S. Department of Agriculture prior to the enactment of the food additives provisions of law, colour additives, and pesticides where other legal premarket approval requirements apply are excluded from this definition [3].

The details of utilization of preservatives in some common products are mentioned in **Table 1**.

Table 1: Details regarding use of preservatives

Preservatives	Foods containing
Ascorbic acid (vitamin C)	Fruit products, acidic foods
Benzoic acid	Fruit products, acidic foods, margarine
BHA (butylatedhydroxyanisole)	Bakery products, cereals, fats and oils
BHT (butylatedhydroxytoluene)	Bakery products, cereals, fats and oils
Calcium lactate	Dairy products, olives, frozen desserts, jams, jellies
Calcium propionate	Breads and other baked goods
Calcium sorbate	Syrups, dairy products, cakes, mayonnaise, margarine
EDTA (ethylenediaminetetraacetic acid)	Dressings, margarine, canned vegetables
Methyl paraben	Beverages, dressings, relishes
Potassium propionate	Breads and other baked goods
Potassium sorbate	Dairy products, syrups, cakes, processed meats
Propionic acid	Breads and other baked goods
Propyl paraben	Beverages, cake, pastries, relishes
Propyl gallate	Cereals, snack foods, pastries
Sodium benzoate	Fruit products, margarine, acidic foods
Sodium nitrate and nitrite	Cured meats, fish, poultry
Sodium propionate	Breads and other baked goods
Sodium sorbate	Dairy products, mayonnaise, processed meats, fermented products
Sorbic acid	Dairy products, fruit products, syrups, sweets, beverages, fermented products
TBHQ (tert butyl hydroquinone)	Snack foods, fats, and oils
Tocopherols (vitamin E)	Oils and shortenings

The Scientific Committee on Food (SCF) is responsible for the safety evaluation of food additives in the European Union. The Commission of the European Union assigns E-numbers after the additive is cleared by the SCF. E numbers ("E" stands for "Europe") are codes for chemicals which can be used as food additives in the European Union and Switzerland, and are adopted by the food industry worldwide. The range of E-numbers assigned to the class "Preservatives" are 200 to 299. E-1105, lysozyme, is also included in the list of approved preservatives.

Table 2 Enlists preservatives approved by the European Union :8

E-Number	Name of Preservative
E 200	Sorbic acid
E 202	Potassium sorbate
E 203	Calcium sorbate
E 210	Benzoic acid
E 211	Sodium benzoate
E 212	Potassium benzoate
E 213	Calcium benzoate
E 214	Ethyl p-hydroxybenzoate
E 215	Sodium ethyl p-hydroxybenzoate
E 216	Propyl p-hydroxybenzoate
E 217	Sodium propyl p-hydroxybenzoate
E 218	Methyl p-hydroxybenzoate
E 219	Sodium methyl p-hydroxybenzoate
E 220	Sulphur dioxide
E 221	Sodium sulphite
E 222	Sodium hydrogen sulphite
E 223	Sodium metabisulphite
E 224	Potassium metabisulphite
E 226	Calcium sulphite
E 227	Calcium hydrogen sulphite
E 228	Potassium hydrogen sulphite
E 230	Biphenyl, diphenyl
E 231	Orthophenyl phenol
E 232	Sodium orthophenyl phenol
E 233	Thiabendazole
E 234	Nisin
E 235	Natamycin
E 239	Hexamethylenetetramine
E 242	Dimethyl dicarbonate
E 249	Potassium nitrite
E 250	Sodium nitrite
E 251	Sodium nitrate
E 252	Potassium nitrate
E 281	Sodium propionate
E 282	Calcium propionate
E 283	Potassium propionate
E 284	Boric acid
E 285	Sodium tetra borate (borax)
E 1105	Lysozyme

4. Harmful effects of Artificial Preservatives [9-12].

Although artificial preservatives are generally regarded as harmless, some have adverse and even fatal side effects.

Following intake, nitrates are transformed to nitrites, which can interact with haemoglobin to form methemoglobin, which can result in unconsciousness and death, especially in young children. Nitrosamines, which cause cancer, are created when proteins and nitrites react in the stomach. According to researchers, there is a strong correlation between nitrate levels in food and the number of people who die from Alzheimer's, Parkinson's, and type 2 diabetes. Monosodium glutamate-containing foods can cause headaches, sweating, skin redness, nausea, and weakness after intake (MSG) [13].

Sulfite containing food preservatives may cause severe allergic reactions and exacerbation of asthma. The toxic paraben chemicals are often used along with methyl chloroisothiazolinone and methylisothiazoline. These are reported to possibly cause neurological damage in rats and are potent irritants and allergens. The use of these toxic chemicals by pregnant women may adversely affect fetal brain development. Formaldehyde DMDM hydantoin, diazolidinyl urea and imidazolidinyl urea are all potent skin, eye and lung irritants. High levels of exposure to toxins like these can cause DNA damage to sperm14.

Table 3: Health hazards of some commonly used preservatives

Preservative	Hypersensitivity (H)	Asthma (A)	Cancer (C)
Potassium & Calcium Sorbates, Sorbic Acid	H	A	-
Benzoic Acid	H	A	-
Sodium Benzoate	H	A	C
Propyl paraben	-	A	-
Sulphur Dioxide	H	A	-
Sodium Metabisulphite	-	A	-
Potassium Bisulfite	H	A	-
Hexamethylenetetramine	-	-	C
Sodium Nitrite	H	A	C
Sodium or Potassium Nitrate	H	-	C
Calcium or Potassium or Sodium Propionates, Propionic Acid	H	A	-
Propyl Gallate	-	A	C
TertButylhydroquinone (TBHQ)	H	A	-
ButylatedHydroxyanisole (BHA)	H	A	C
ButylatedHydroxytoluene (BHT)	H	A	C

RISKS OF PRESERVATIVES

Despite the benefits attributed to food additives, for several years there have also been a number of concerns regarding the potential short- and long-term risks of consuming these substances. Critics of additives are concerned with both indirect and direct impacts of using additives. As for many of the benefits mentioned, there is not always adequate scientific proof of whether or not a particular additive is safe. Little or no data are available concerning the health risks or joint effects of the additive cocktail each of us consumes daily [15].

The indirect risks that have been described for additives are the converse of some of the benefits attributed to their use. While it is accepted that through additives a greater choice and variety of foods have been made available, there is no question that additives have also resulted in the increased availability of food products with a low density of nutrients. These so-called junk foods, which include many snack items, can in fact be used as substitutes in the diet for more nutritious foods. Recently the food industry has attempted to address this criticism by adding nutritional additives to snack items so that these foods are a source of selected vitamins and minerals. The long-term effectiveness of this is questionable.

Obviously, educational programs are needed to ensure that consumers select nutritious foods. Some scientists, however, feel that there is a place in the diet for foods that provide pleasure even if no direct nutritional benefit can be ascribed to their consumption [16]. Of greater concern than the indirect risks are the potential direct toxicological effects of additives. Short-term acute effects from additives are unlikely. Few additives are used at levels that will cause a direct toxicological impact, although there have been incidents where this has happened. Of particular concern are the hypersensitivity reactions to some additives that can have a direct and severe impact on sensitive individuals even when the chemicals are used at legally acceptable levels. The reactions to sulfites and other additives, are examples of such a problem. With proper labelling, however, sensitive individuals should be able to avoid potential allergens. Toxicological problems resulting from the long-term consumption of additives are not well documented. Cancer and reproductive problems are of primary concern, although there is no direct evidence linking additive consumption with their occurrence in humans. There are, however, animal studies that have indicated potential

problems with some additives. Although most of these additives have been banned, some continue to be used, the most notable being saccharin.

Balancing Risks and Benefits [20]

Due to the difficulties in precisely defining the risks and benefits of individual additives, a legal rather than a scientific decision is commonly made regarding the safety of a food additive. In such a decision, the potential risks must be weighed against the potential benefits. A common example of this balance is saccharin. Although there is no direct evidence that saccharin, in the low amounts consumed in foods, causes cancer in humans, risk evaluation in rats indicates a potential for cancer in humans. On the benefit side, saccharin is an excellent noncaloric sweetener that is useful for diabetics and those interested in reducing consumption of calories. Many consumers feel that the benefits of having saccharin available as a sweetening agent outweigh the risks. On the basis of available risk information, however, the FDA initially issued a ban on saccharin in the early 1970s. The U.S. Senate, recognizing the consumer demand for low-calorie foods, subsequently placed a moratorium on the ban, thus allowing saccharin's continued use. The moratorium Branen and Haggerty was essentially the first political recognition of the importance of balancing the potential risks of an additive against its perceived benefits and allowing the consumer the choice of consuming or not consuming the food. The moratorium has continued for several years and has undoubtedly had a significant impact on the continued and proposed use of additives.

Introduction to Instruments [21-24]

High Performance Liquid Chromatography

High Performance Liquid Chromatography (HPLC) is an analytical technique used for the separation of compounds soluble in a particular solvent. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres. That makes it much faster.

Principle

All chromatographic separations, including HPLC operate under the same basic principle; separation of a sample into its constituent parts because of the difference in the relative affinities of different molecules for the mobile phase and the stationary phase used in the separation.

Thin Layer Chromatography

Thin Layer Chromatography can be defined as a method of separation or identification of a mixture of components into individual components by using finely divided adsorbent solid / (liquid) spread over a plate and liquid as a mobile phase.

Principle

Thin-layer chromatography is performed on a sheet of glass, plastic, or aluminium foil, which is coated with a thin layer of adsorbent material, usually silica gel, aluminium oxide (alumina), or cellulose. This layer of adsorbent is known as the stationary phase. After the sample has been applied on the plate, a solvent or solvent mixture (known as the mobile phase) is drawn up the plate via capillary action. Because different analytes ascend the TLC plate at different rates, separation is achieved.It is thus based on the principle of adsorption chromatography or partition chromatography or combination of both, depending on adsorbent, its treatment and nature of solvents employed. The components with more affinity towards stationary phase travels slower. Components with less affinity towards stationary phase travels faster. Once separation occurs, the individual components are visualized as spots at a respective level of travel on the plate. Their nature or character is identified by means of suitable detection techniques.

Gas Chromatography

The mobile phase in gas chromatography is a gas, and the components are separated as vapours, which sets it apart from other types of chromatography. As a result, it is employed to distinguish between and find tiny molecular weight chemicals in the gas phase. In the injection port, the sample is either vaporised as a gas or a liquid. Helium is frequently used as the mobile phase in gas chromatography because of its low molecular weight and chemical inertness. The mobile phase pulls the analyte through the column as pressure is applied. Utilizing a column coated with a stationary phase, the separation is carried out.

Principle

The equilibrium for gas chromatography is partitioning, and the components of the sample will partition (i.e. distribute) between the two phases: the stationary phase and the mobile phase. Compounds that have a greater affinity for the stationary phase spend more time in the column and thus elute later and have a longer **retention time** than samples that have a higher affinity for the mobile phase. Affinity for the stationary phase is driven mainly by intermolecular interactions and the polarity of the stationary phase can be chosen to maximize interactions and thus the separation.

Capillary Electrophoresis

It is an analytical method used to separate ions according to their electrophoretic mobility using an applied voltage. There are various factors that can significantly affect electrophoretic mobility such as:

- Charge of the molecule
- Viscosity
- Radius of the atom

The rule of the thumb is that the greater the field strength the faster the mobility. There are different types of electrophoresis but the most predominant one is the

capillary electrophoresis because it yields faster results and provides a high-resolution separation.

Principle

Electrophoresis is a method in which the sample ion moves through the influence of applied voltage. It pertains to the migration of charged ions in the electric field. In a given solution, the electric current flows between the electrodes and carried by ions.

Looking at the principle, the charged molecules are placed in the electric field and migrate towards the pole of either positive or negative charged. A nucleic acid has a consistent negative charge imparted by the phosphate backbone and migrates towards the anode. Hence, the force will accelerate the movement of protein towards the cathode or anode depending on its charge. To sum up the process, the capillary is filled with a conductive fluid with designated pH value. The conductive fluid will serve as the buffer solution in which the sample will be separated. A sample is placed in the capillary through a pressure injection or through electro kinetic injection. A high voltage is placed over the capillary which will enable the sample to move through the capillary at varying speeds. The positive components travel to the negative electrode while the negative one's travel to the positive electrode. The principle of capillary electrophoresis as shown in the image where positively charged ions are called the anode and the negatively charged ions are called the cathode.

Nuclear Magnetic Spectroscopy

Nuclear magnetic resonance spectroscopy is one that studies the spin changes at the nuclear level. This spin change occurs when a radio frequency energy is absorbed by the nucleus in the presence of a magnetic field.

Principle

In an atom with an odd mass number, the proton (nucleus) spins on its own axis. When an external magnetic field is applied, the spin shifts to precessional orbit with a precessional frequency. But still, the nuclei are in the ground state with its spin aligned with the externally applied magnetic field.To this atom, if radio-frequency energy is applied such that the applied frequency is equal to precessional frequency, then the absorption of energy occurs leading to an NMR signal.

Since the energy is absorbed, the nucleus moves from the ground state to the excited state with its spin oriented in the opposite or anti-parallel direction.

1) Proton in ground state 2) Proton under the magnetic field and 3) Proton under radio-frequency + magnetic field.

If the application of radio frequency energy is stopped, then the nucleus returns to the ground state with parallel orientation spinning is precessional orbit. If even the magnetic field is removed, the nucleus will return to its normal spin on its own axis instead if precessional orbit.

Hence, application of magnetic field only makes the nucleus to spin in the precessional orbit while the application of radio frequency energy leads to NMR signal.

This indicates that both the application of the magnetic field as well as the radio frequency energy are needed to produce NMR signal.

MASS Spectroscopy

Mass Spectrometry (MS) is an analytical chemistry technique that helps identify the amount and type of chemicals present in a sample by measuring the mass-to-charge ratio and abundance of gas-phase ions. In this instrumental technique, the sample is converted to rapidly moving positive ions by electron bombardment and charged particles are separated according to their masses. A mass spectrum is a plot of relative abundance against the ratio of mass/charge (m/e). These spectra are used to determine the elemental or isotopic signature of a sample, the masses of particles and of molecules, and to elucidate the chemical structures of molecules and other chemical compounds.

Principle

In this technique, molecules are bombarded with a beam of energetic electrons.

1. The molecules are ionized and broken up into many fragments, some of which are positive ions. Each kind of ion has a particular ratio of mass to charge, i.e. m/e ratio (value).
2. For most ions, the charge is one, and thus, the m/e ratio is simply the molecular mass of the ion.
3. The ions pass through magnetic and electric fields to reach the detector where they are detected and signals are recorded to give mass spectra.

Atomic Absorption Spectroscopy

Atomic absorption spectroscopy (AAS) is another interesting type of spectroscopy.It is specifically designed for the analysis of the metals and metalloids substances. By definition, AAS is a quantitative analytical technique wherein the absorption of a specific wavelength of radiation by the neutral atoms in the ground state is measured. The more the number of the atoms in a given sample, the higher is the intensity of absorption and vice-versa.This is also called as metal analysis spectroscopy as it is mainly used for the analysis of metals.

Principle

The method relies on the principle of absorption spectroscopy. A liquid sample is allowed to convert into free atoms (desolvated and atomized). These free atoms absorb the light of a specific wavelength. The remaining unabsorbed light is detected and recorded. The intensity of absorption is directly proportional to the concentration of the sample. This was a descriptive study in which 50 articles from different journals were taken. Analytical information was

collected retrospectively from Analytical journals. In this study, it was found that, a number of studies were performed on food additives and on their limits for human use with different analytical techniques

Method	Matrix	Sample Preparation	Extraction	Detection
LC MS/MS	cow milk	Homogenized and centrifuged. The supernatant was filtered	0.1 M acetate buffer containing 1 M Nacl (pH 2.0)andMeoH (1:1) was used to extract nisin A and nisin Z from milk samples	The linearity of the analytical method had a high correlation coefficient (r≥0.9987). The limits of quantitation of nisin A and nisin Z were approximately 12.9and 10.9 μg/kg, respectively. The accuracy of the analytical method in milk ranged from 90.6 to103.4%fornisinAandfrom83.8 to104.4%for nisinZ.
GC MS/MS	Food products total of six producttypes, including "Pesto" sauce, tomato sauce containing basil, "Cola tasting" beveíages, Bolognasausage(polony),Vienna sausage(wuístel)andfíeshbasil.	dichloromethane as extraction solvent	Thedetectionlimits,determinedthinstandaídsolutions and in foods, weíe 10, 5 and 8 ng/ml foí estíagole, safíole and eugenol methyl etheí,íespectively	the calibíation cuíves showed a good lineaíity foí all the thíee compounds in theconcentíation íange 0.5–25 ppm, with coíielation coefficients íanging between 0.996 and 1.000. Ina numbeí of successive analyses, the estíagole peak aíea íepeatability (RSD) was 0.20 ng/ml
Ion-pair HPLC	Beverages,gelatine,syrups	Diluted with water and filtered	Nova-Pak c18 column with gradient elution(1.5ml/min) with methanol/phosphate buffer of pH 7 containing 5Mm tetra butylammonium bromide	520nm
Spectrophotometry	Commercially available dyes	Diluted with water and ultrasonicated	Computer programme that determines concentration of mixtures 4 compounds by comparing their spectra with standard spectra	MULTv3.0 Quimio program
Solid-phase spectrophotometry	Soft drinks,fruiliquors,ice creams	Filtered food samples were diluted to 100ml with the addition of 5ml 1m acetate buffer at pH 5 and 10ml ethanol	The mixture was agitated with 50mg Sephadex DEAE A-25gel.The solid phase was extracted and packed into 1mm cells for spectrophotometric determination.	487nm
Spectrophotometric	Soft drinks	Ion-pair formation with octadecyltrimethylammonium bromide at pH 5.6	Extraction of ion-pair into n-butanol	485nm

Solid-phase spectrophotometry	Soft drinks,sweetsanf fruit jellies	Samples dissolved in water and filtered	The colorants were fixed in Sephadex DEAE A-25gel at pH 2.0 and packed into 1mm cells for spectrophotometric determination	Between 400 and 800nm.Partial Least Squares (PLS) multivariate calibration used
RP-HPLC	Turkish yoghurt	methanol : water : acetic acid (12 : 8 : 1 v/v/v)	C8 column (150mm×4.6mm×5µm) at 1 mL/min flow rate	303 nm
RP-HPLC	carbonated and non-carbonated beverages, inaddition to cake, biscuit, yoghurt, sauce, ketchup, mayonnaise, croissant and jam products.	methanol : water(14 : 8 v/v)	C8 column (150mm×4.6mm×5µm) at 1 mL/min flow rate	530nm
HPLC	bakery productsbread, cake/rolls, burger/hotdogbuns and pitabreads	propionates to the non-ionized molecular form by adding glacialaceticacid, which is at the same time efficiently extracted into dichloromethane.	C8 column (150mm×4.6mm×5µm) at 1 mL/min flow rate	The levels of propionic acid plus propionates are 197-1273, 98-1846, 546-1932 and 479-1680 µg mL-1 , respectively
HPLC	cheese samples	50% () ACN-water containing 0.2 () glacial acetic acid.	C18 column of (150 mm × 4.6 mm) dimension and 5 µm of particle size	Sorbic acid levels in the cheese samples were found to be lower than the maximum acceptable limits (matured cheese: 1000 mg•kg−1 or L−1) of the Turkish Food Codex
UV	tomato products such as tomato juice, tomato concentrates and double concentrates, canned tomatoes, sun-dried tomatoes and ketchup	MeOH/isopropyl alcohol/THF (30:30:35) containing 250 ppm BHT and 0.05% TEA	carotenoid C30 reversed-phase column (250×4.6 id, 3 µm) from YMC corporation (Waters, Zellik, Belgium)	472 nm
liquid chromatographic (LC)	Gelatin-based and other water-dispersible beadlets, or tablets, capsules, and soft gels containing such product forms, were digested with protease.	alkaline sodium EDTA acetate bufferextracted from the resulting aqueous suspensions with dichloromethane and ethanol	Gelatin-based and other water-dispersible beadlets, or tablets, capsules, and soft gels containing such product forms, were digested with protease.	The within-day precision relative standard deviation (RSD) for the determination of total lycopene ranged from 0.9 to 5.7% over concentration ranges of 50–200 g/kg for raw materials and 0.3–24 g/kg for dietary supplements. The intermediate precision RSD (total RSD) ranged from 0.8 to 8.9%. Recoveries obtained for beadlet and tablet material for the different extraction variants ranged from 95.0 to 102.1% at levels of 0.02–20 g/kg for tablets and from 95.0 to 101.1% at levels of 1–200 g/kg for beadlet

				material.
RP-HPLC	FOOD products	extracted with hexanehe residues in evaporator was dissolved in a certain amount of water-C2H5OH (1:4, V/V) and filtered with 0.5 micron of filter membrane	Radial-PAK C12 column, methanol-water (92:8, V/V) mobile phase adjusted to pH = 3 with phosphoric acid and UV-280 nm detector	the coefficients of variation of PG, BHA and BHT were 0.61, 0.08 and 1.44 respectively, linear correlation coefficients were more than 0.999 and recoveries were 92%-98% (n = 6). The lowest detection limit was 0.5 mg/L

Conclusion

This review work has examined the various effects of food additives and preservatives on man. Additives have been used for many years to preserve, flavour, blend, thicken and colour foods, and have played an important and essential role in reducing serious nutritional deficiencies (Nutrition Supplement Additives). Additives help to assure the availability of whole some, appetizing and affordable foods that meet consumer's demands from season to season while also helping to preserve food from spoilage from microorganism. Food additives play a vital role in the food industries, but the various adverse effects associated with them remain a problem that need to be fought byus. Synthetic food additives react with the cellular component of the body leading to the various food disturbances (effects). If we must use food additives, because of their advantages, they should be the natural ones which have minimal effects andthose that are generally recognized as safe (GRAS) and in the case of those not generally recognized as safe (Non GRAS), the acceptable daily intakes (ADIs) should not be exceeded. To minimize the risk of developing health problems due to food additives and preservatives, one should avoid the foods containing these additives and preservatives. Before purchasing the canned food, its ingredients should be checked. Purchase only organic foods, which are free from artificial additives. Although it may seem difficult to change habits and find substitutes for foods that one enjoy, remind yourself that you will be adding to your diet some new whole some foods that you will come to enjoy even more. Look for foods that are not packaged and processed, but enjoy nature's own bounty of fresh fruits, vegetables, grains, beans, nuts and seeds. Find foods that resemble what they looked like when they were originally grown. If there are a number of alternative methods available for measuring a certain property of a food and additives, the choice of a particular method will depend on which of the above criteria is most important. For example, *accuracy* and use of an *official method* may be the most important criteria in a government laboratory which checks the validity of compositional or nutritional claims on food products and additives, whereas *speed* and the ability to make *non-destructive*measurements may be more important for routine quality control in a factory where a large number of samples have to be analyzed rapidly. So, this review gives a complete idea regarding the types of food additives used, analyzed and quantified.

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