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Post-harvest biotechnology of fruits with special reference to banana—Perspective and scope

K K Surendranathan*

Bioprocess Development and Biochemical Applications Section, Nuclear Agriculture and Biotechnology Division
Bhabha Atomic Research Centre, Mumbai 400 085, India

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Fruits, being highly nutritive, are important component of human diet but they possess very short post-harvest shelf life. As ripen, they become very soft and more prone to injuries, which makes them highly perishable. In India, over 30% of the annual produce is wasted due to spoilage. Hence, there is an urgent need to develop technologies to overcome post-harvest losses of fruits. Physiologists and biochemists attempted to extend the shelf life of fruits by different means though the results were not very satisfying. It was demonstrated recently that a judicious dose of γ-irradiation (0.1-0.5 kGy) could enhance the shelf life to fruits by about a week to a fortnight, which could help in minimising the spoilage during storage and transportation. However, stringent quality controls have to be strictly followed to get the best results. Studies revealed that γ-irradiation brings alterations/changes in metabolic pathways, which delay the production of essential precursors and energy required for ripening of fruits. Another strategy, to enhance the shelf life of fruits, could be adopted through regulation of endogenous ethylene production. Most recent studies have shown that it could be achieved by such genetically modified (GM) crops where gene expression of key enzymes responsible for ripening, like PG-ase, EFE and ACC-synthase, by means of antisense RNAs. However, adoption of this technology has so far been deterred due to apprehensions of safety issues associated with GM crops. An alternate method for prevention of spoilage of fruits as well as sustaining the interests in farmers could be the value addition of fruit commodities. This could be achieved by improving the conventional methods as well as development of non-conventional products of commercial interest. Nuclear Agriculture and Biotechnology Division, BARC has developed processes for the production of juice and powder from ripe banana, the largest produced and maximum wasted fruit, by creating an in-built mechanism to inactivate the pectin forming enzymes. With this process, over 60-80 % of the total moisture of the fruit is extracted out as juice. The commercially available variety, 'Harichal' (Mumbai kela) could give juice 550-640 ml /kg pulp. It is also demonstrated that a number of products of commercial significance, like banana nectar, carbonated juice and wine, from banana juice and biscuits, cakes, milkshakes, etc. from banana powder could be developed.

Keywords: banana juice, banana products, fruit ripening, mechanism of delay in ripening, post-harvest biotechnology

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Introduction

Fruits are considered to be one of the important constituents of human diet, which supply a number of vital nutrients essential to the body. Ripening makes the fruits edible and constitutes, physiologically and commercially, the most significant phase in their life. During the developmental phase, a fruit acquires proper shape, weight and volume, and attains maturity. Next is the ripening phase, during which the fruit acquires appropriate texture, colour, aroma and flavour. Senescence, the third and final phase, marks the beginning of the end of the life of a fruit where it undergoes degenerative changes. Physiologists and biochemists have attempted to define ripening in different ways[1,2]. However, one of the most accepted definitions describes the fruit ripening as a physiological process involving the induction /acceleration of a variety of metabolic processes, most or all enzymatically regulated and catalysed. The major degradation changes taking place during ripening are destruction of chloroplasts, breakdown of chlorophyll, catabolism of organic acids, inactivation of pectic compounds, break-down of starch, etc. Whereas, the major synthetic changes involve formation of carotenoids and other pigments, inter-conversion of sugars, formation of ATP, flavour volatiles, etc., and translation and transcription of several enzymes. Since ripening makes the fruit a

*Tel: 91-22-25593870; Fax: 91-22-25505151
E-mail: surikaimal@hotmail.com

Abbreviations: HMP, hexose monophosphate; TCA, tricarboxylic acid; Gy, gray; PG-ase, polygalacturonase; EFE, ethylene-forming enzyme; ACC, 1-amino cyclopropane 1-carboxylic acid commercial product, this phase has generated maximum interest into a grower, physiologist, biochemist, as well as a trader alike.

Fruit Production—Present Status

India is the second largest producer of fruits in the world[3]. In case of banana and mangoes, it is the largest producer. It is also the fourth largest producer of pineapple and sixth and ninth, respectively in case of citrus and apple production. The annual world production of fruits is around 370 million metric tons. Of which India contributes about 32 million metric tons, which accounts for 8% of the total fruit production of the world. However, it is shocking to note that not even 10% of the annual produce is commercially exploited. Over 30% of the total produce is lost annually due to post-harvest spoilage. To sustain and improve horticultural production, therefore, it is imperative to control these losses.

Post-harvest Biotechnology—A Necessity

Fruits become susceptible to damages on ripening for they soften drastically. A ripened fruit, depending on the variety, has a limited shelf life of a few days to one or two weeks. Besides, the soft and fleshy nature makes a ripened fruit more susceptible to injuries, increasing the losses further due to spoilage. Hence, it is necessary to develop appropriate technology and infrastructure for proper storage and transportation of the fruits.

One of the reasons for the low commercial activity in the fruit and vegetable sector is the lack of organised cultivation. As a result, the total domestic production of fruits and vegetables reaches only domestic market. Many a times, fruits like local varieties of mangoes, bananas, etc., do not attract commercial interests due to lack of proper post-harvest management. Some fruits, like jackfruit and cashew apple are under-utilised to the extent of 80% and spoiled as they fall around the tree causing environmental problems.

Thus, there is an urgent need to develop technologies to overcome post-harvest losses of fruits. One way of achieving this could be by developing feasible technology to extend the post-harvest shelf life. Another alternative is the value addition of the produce by developing innovative products of consumer interest. For either of these, it is important to have a fair assessment of the available technologies as well as an understanding of the physiology and biochemistry of fruit ripening.

Processes to Extend Shelf Life

Controlled Atmosphere

Some of the known conventional processes by which the shelf life of some fruits could be extended are available in the literature[1]. Storage of fruits under controlled atmosphere, such as low oxygen, removal of the endogenously produced ethylene, high CO_2 environment, etc., could achieve effective shelf life. However, fruits stored by these processes failed to ripen the normal way. Further, these fruits compromised on the aesthetic qualities of ripened fruits, such as development of appropriate aroma, flavour and texture. Hence, these processes could not survive as a commercially viable technique for extension of shelf life.

In the recent past, however, studies reported innovative approaches in this area, which could find useful applications in post-harvest preservation of fruits in fresh-like conditions. Controlled atmosphere storage, modified atmosphere storage, active and intelligent packaging, minimal processing and hurdle technology are some of them which may revolutionise the post-harvest preservation of perishable commodities, like fruits and vegetables. A number of reports during the recent past enumerate the challenging developments in this area. Hence, no detailed review is attempted here[4-12].

Radiation Preservation

Another process, which has emerged a few decades back, is radiation preservation. In this process, fruits are exposed to a judicious dose of γ-radiation (0.1-0.5 kGy), thereby making them undergo transient metabolic changes giving an extension of post-harvest shelf life. Radiation preservation is a cold process and does not leave any residual substance in the irradiated food. Perhaps, it is the most worked out process. Plenty of literature is available on the technology of radiation preservation of fruits[13-19]. Fruits belonging to the climacteric class, when exposed to γ-radiation, show delayed ripening process for a period of 5-12 days, thereby extending the post-harvest shelf life. This process could retain the tough texture of the fruit for longer periods and, thus, could be effective in reducing the spoilage losses during transportation and storage. However, limitations to this process are that fruits belonging to the non-climacteric class, such as lemon, oranges, grapes, lime, pineapple, blueberry, etc., do not get any extension of shelf life due to radiation-induced delay in the ripening. Citrus fruits as a class do not have physiologically or biochemically a clearly defined ripening phase. In them, ripening is a continuation of maturation and not a separate phase. However, this class of fruits also can be irradiated to obtain a shelf life extension by control of microbial infestation and the resultant spoilage[20].

Biochemical Mechanism

Though the technology of radiation preservation of fruits by γ-ray is well worked out, no detailed information is available on the mechanism, which brings in the delay in ripening. Hence, this review intends to consolidate the available information on the probable biochemical mechanism of delay in ripening of fruits. Studies reported on climacteric fruits by Surendranathan and Nair[21] proposed a number of transient metabolic alterations as some of the responsible factors for bringing in delayed ripening of γ-irradiated Cavendish banana. These metabolic alterations/changes delay the production of the essential precursors and energy (ATP) required for the ripening of fruits. One of the major and most visible changes taking place during ripening is the disappearance of starch with concomitant production of sugars. Most of the biochemical activities related to the ripening take place at the onset of climacteric and culminate within two to three days during which the fruits attain the climacteric peak. Just prior to the onset of climacteric, the endogenous ethylene level increases above the threshold level required for triggering a series of reactions, which initiates the ripening process[22]. In case of fruits exposed to an optimum dose of γ-ray, a shift in the endogenous ethylene peak as well as the onset of climacteric could be observed[23,24].

Detailed investigation of the starch metabolism and energy utilisation pathway showed transient alteration of the metabolic pathways in γ-irradiated fruits. Irradiated fruits during the pre-climacteric phase exhibited enhanced starch phosphorylase and isomerase activities[21]. Further, the accumulated fructose-6-phosphate is channelled through the HMP pathway by activated glucose-6-phosphate dehydrogenase and decreased phosphofructokinase activities. The sugar phosphates formed through HMP pathway are hydrolysed as free sugars[25,26].

Also, an investigation of the TCA cycle showed cleavage of isocitrate to glyoxylate and succinate through the glyoxylate shunt pathway due to the γ-ray induced activation of isocitrate lyase and malate synthase[25,27]. Thus, the activated glyoxylate shunt bypass the TCA cycle restricting the ATP production, which is vital for several cellular activities related to the ripening of fruit. The glyoxylate shunt also accumulates oxaloacetate, which is channelled through the activated gluconeogenic enzymes to form free fructose[26]. Thus, γ-irradiation of fruits cause an alteration of metabolic pathways effecting a partial arrest of catabolism of starch by the recycling of sugar phosphates to free sugars and preventing the ATP synthesis. However, these changes are reverted back as the fruit enters the climacteric phase. The delay in ripening observed was always found to be for the same period by which the pre-climacteric phase was extended or during which the metabolic pathways were altered. The major enzymes inhibited by exposure to γ-ray were hexokinase, phosphofructokinase, α-ketoglutaric acid dehydrogenase and succinate dehydrogenase[21]. In short, the control of starch utilisation by accumulation of free sugars during the pre-climacteric phase, resulting in the control of ATP production as well as other precursors required for ripening, could be one of the major reasons for the delay in ripening of climacteric fruits. A schematic account of the major alterations in the starch utilisation pathway in irradiated climacteric fruits is given in Fig. 1.

Precautions Required

In order to get the best possible result, however, the following precautions should be rigorously followed. Fruits of uniform maturity only should be selected as a batch for radiation processing. In a lot to be irradiated there should not be mix up between the topmost hand of a bunch and the middle or the lowermost bunch as they differ in maturity. The fruits should be irradiated during the pre-climacteric phase (preferably immediately after the harvest) after proper cleaning and sorting of the fruits. Fruits having even minor injuries should not be irradiated. As the time of endogenous ethylene production varies depending on the maturity of the fruit, the lots of identical maturity should only be kept together for storage after radiation processing. If all these precautions are followed the fruits will have an extension of shelf by about a fortnight during which period, the transportation to the destination can be carried out.

Radiation is also known to eliminate insect-pests from fruits[28,29]. Fruit flies are known to be one of the most important deterrents to the export of fruits, as strict quarantine rules for them are prevailing in several countries to which fruits are to be exported. Administration of γ-irradiation 0.15-0.5 kGy is known

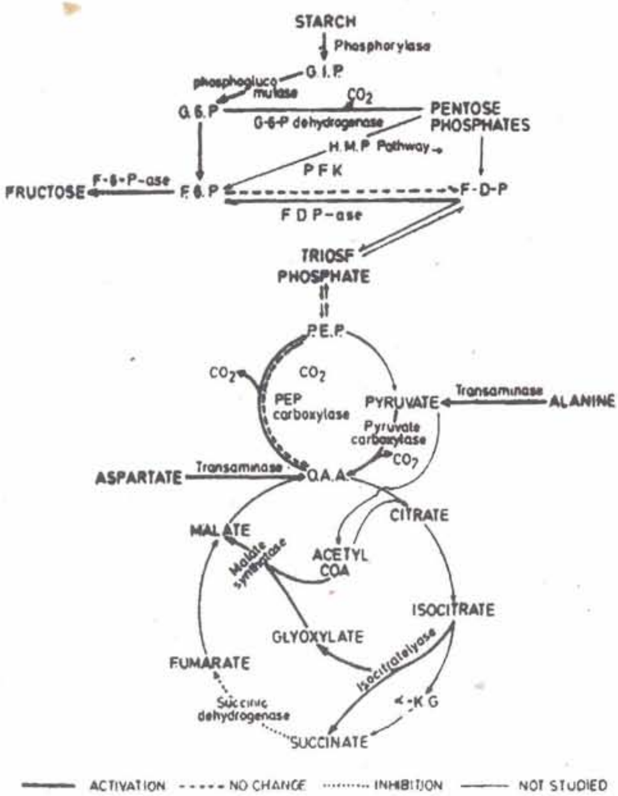

Fig. 1—Metabolic alterations in γ-irradiated pre-climacteric banana

to have a complete control on such pests. Thus, this treatment could be an excellent boost to the fruit export market if properly exploited. Currently, there is no commercial facility available for radiation processing of fruits in India. However, the recent installation of a low dose facility at Laslgoan, Nasik, Maharashtra for radiation processing of onion and potato (processing capacity of 10 tons/hr) could be made use of for fruit processing during off-season (as the dose requirement is closer to this). A sketch of the γ-ray processing unit is presented in Fig. 2.

Regulation of Endogenous Ethylene Production

One of the most recent methods by which an effective shelf life extension could be achieved is by genetically modifying the plant whereby the expression of the gene responsible for ripening could be regulated. This is achieved by effective turning off of the gene expression of a key enzyme of the ripening process by means of its antisense RNA. Antisense RNAs are long stretch of sequences that are complementary to the sense RNA. They are very specific to the target genes and cause reduction of sense RNA by interfering with transcription and translation process.

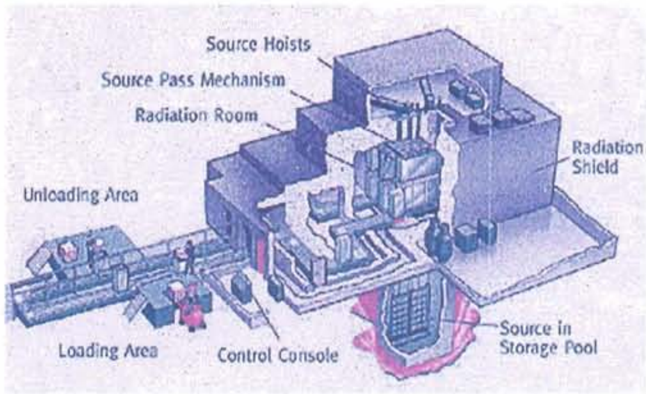

Fig. 2—Sketch of a radiation processing facility

A wealth of information is available in the literature on the development of antisense clones of key ripening enzymes in tomatoes[30-32]. Hence, only a brief mention of the highlights with respect to application is made here. Some of the clones effective in the control of ripening were of PG-ase, EFE and ACC-synthase.

PG-ase is one of the key enzymes involved in the ripening-associated softening of fruits[33,34]. The plants engineered to have the PG-ase antisense RNA did not express PG-ase activity, as a result fruits did not soften. However, the fruits could not obtain an effective delay in ripening. Hence, it could not be considered a completely successful technique but a strong textured tomato is very good for transportation and the loss due to spoilage could be minimised. The tomato clones having antisense RNA for EFE were capable of to control ethylene production only to some extent. Hence, these fruits also could not obtain a distinct delay in ripening.

In case of ACC-synthase clones, the ethylene production was reduced from 6-9 nl gm^{-1} hr^{-1} (control fruits) to < 0.1 nl gm^{-1} hr^{-1} even 96 days after inflorescence. Expression of PG-mRNA and its translation were also inhibited in these fruits even 180 days after inflorescence. The fruits started ripening only after exposure to externally administered ethylene. Besides tomatoes, this technology could successfully be translated to the other fruits, such as melons, squash, pineapple and papaya[35-38]. Thus, the development of a clone having antisense RNA of ACC-synthase could completely control ripening of tomatoes[31]. Though it is a path-breaking finding but apprehensions associated with the GM (genetically modified) crops deter it to gain public acceptance and it may have to wait a few more years to be taken up commercially. The major apprehension of GM crops stemmed from the observations that the modified genes could move to other organisms through the process of horizontal gene transfer, which could result in the formation of new biological entities. Even vertical gene flow transmissions could cause irreversible changes in closely related species which are sexually compatible[39,40]. Further, as there is no endogenous upsurge in ethylene production in non-climacteric fruits, this particular technology is restricted to climacteric class of fruits only. Thus, technologies to extend post-harvest shelf life of fruits have its own merits and limitations. Probably, it may take some more time to find of an appropriate technology, which could retain the freshness of fruits during storage and has consumer acceptability as well.

Value Addition—Process Development

Another alternative available to date, which can sustain interest amongst the fruit growers, is the conversion of their produce into value added commodities of consumer interest and commercial importance. A comparative evaluation of the post-harvest processing of agricultural produce at international level shows 23% value addition in China, 45% in Philippines, about 50% in European countries and over 55% in USA. While, India having a post harvest loss of over 30% is processing a mere 2-3% of its agricultural produce.

Thus, the need of the hour is to find sustainable technologies capable of value addition by developing new products of commercial importance. One of the important pre-requisites for attaining this goal will be the proper documentation and review of the existing, sustaining processes. Efforts to introduce improvements in the existing processing techniques and innovative approaches in bottling/packaging could improve the existing market and generate more demand for the produce.

Banana Products

Banana is the largest grown fruit crop of India. It is delicious and maximum consumed fruit and is available all through the year all over the country. However, for the short shelf life and soft and delicate texture, this fruit has maximum loss due to spoilage. If a proper technology is developed to design some products of consumer interest, not only the massive wastage of this fruit could be prevented but more demand for the fruit could also be generated. The conventional products of these fruits are wafers, puree and jam. Though some of these products are widely marketed in some parts of the country, it is not sufficient enough to create a sustainable demand for the produce. Another way could be the development of non-conventional products of novelty having good consumer interest.

Juice

Of the processed fruit products with respect to aroma, flavour and likeness, juice is the closest form to fresh fruits. Banana is known to have over 85% moisture when ripened. However, unlike other fruits, juice cannot be extracted from banana by any simple means, such as crushing, grinding or squeezing. If an appropriate technology is developed to extract juice from banana a highly marketable product having good consumer demand could be produced.

Nuclear Agriculture and Biotechnology Division, BARC has developed a novel and simple method by which, depending upon the variety of the banana used, over 60-80% of the total moisture of the fruit could be extracted out as juice[41,42]. A schematic sketch of the process is given in Fig. 3, which mainly involves creating an in-built mechanism to inactivate the pectin forming enzymes. No external additive is added to the fruit to increase extractability. An account of the material balance on processing of 20 kg pulp, and data on some physical and chemical characteristics of the juice are presented in Tables 1 and 2, respectively. The commercially available variety, 'Harichal' (Mumbai kela) could give juice 550 -640 ml /kg pulp (Fig. 4).

Ripe Banana Powder

On freeze drying/vacuum drying, the left-over pulp could produce a very fine powder having the aroma and flavour of banana. Ripe banana when dried and powdered generally gives a sticky lump only. In that context, the development of banana powder (Fig. 5), if developed as a commercial process, could also be a novel product[43].

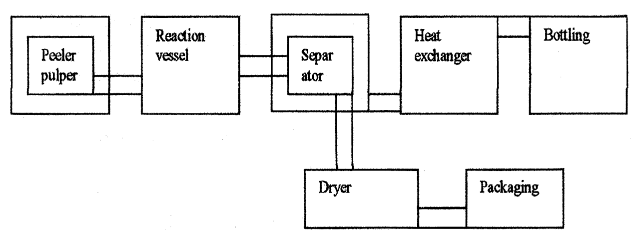

Fig. 3—Schematic diagram of the juice making process (The washed fruits are cut at both ends and peeled using an auto-peeler. The pulp obtained is blended thoroughly and transferred to the reaction vessel where it was treated and agitated for 6-8 hrs. The slurry formed in the reactor is transferred to a separator where the juice is separated. The separated juice is passed through an instant heat exchanger and bottled under aseptic condition. The left over pulp was dehydrated under vacuum and grinded to obtain a fine powder, which could be used as ripe banana powder.)

Table 1—Details of material balance when 20 kg pulp utilized for juice production

Fruit: 'Harichal/Mumbai kela' (*Musa cavendishii*)

Ripeness	Table ripe
Fruits required for twenty kg pulp	150-170 Nos
Amount of peel generated as waste	7-8.8 kg
Quantity of juice generated per 10 kg	10.00-13 l
Quantity of pulp generated per 10 kg	5-7 kg

The data presented are from several batches of fruits processed

Table 2—Analysis of banana juice

Sugar	20-27%
Solid content	15-22%
Specific gravity	1.1-1.16
*p*H	4.5-4.8

The data presented are from 10 batches of juice preparation

Fig. 4—Banana juice

Fig. 5—Ripe banana powder

Sub-products—Excellent wine comparable to those available in the market could be made from banana juice. The banana juice was inoculated with 2% yeast (*Saccharomyces cerevisiae* ATCC3177), obtained from Enzyme and Microbial Technology Section, Biotechnology Division, BARC, and kept at room temperature until the completion of the clarification to obtain very clear wine. The time required for completion of fermentation was less than 36 hrs and for clarification less than fifteen days. The wine did not contain any acidic principles or higher alcohols; *p*H was 4.3 and the alcohol content varied from 6-8%. Other products, which could be developed from banana juice, were banana nectar and carbonated banana drink (Table 3) The nectar prepared using 40% juice and carbonated drink with 30% juice were adjudged as 'excellent' and 'commercially viable drink' by a team of panellists.

Table 3—Ingredients of 'nectar' and 'soft drink' prepared from banana juice

Banana nectar		Banana soft drink (carbonated)	
Banana juice	40%	Banana juice	30%
Citric acid	0.6%	Citric acid	0.6%
Sugar	15%	Sugar	10%
		Carbon dioxide	1-2%

The final concentration for Banana nectar/soft drink was arrived at from the taste panel studies conducted amongst 20 expert panellists

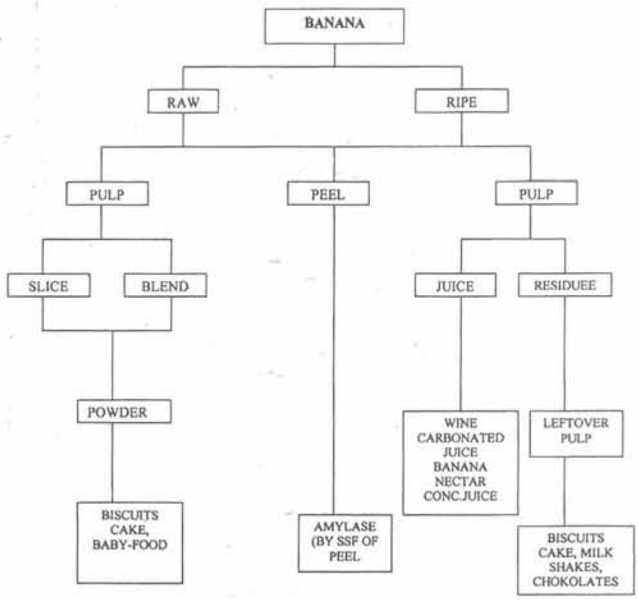

Fig. 6—Flow-chart on development of commercially important products from banana[41]

Banana powder, raw or ripe was found to be an excellent additive in confectioneries, biscuits or cakes[41]. Ripe banana powder could be used as an additive in chocolates and milk shakes, etc. On bulk production of these products, accumulation of large amount of banana peel could cause environmental problems. This peel could either be utilized for cattle feed or other suitable products, e.g. development of value added bioactive compounds, such as amylase, which is in great demand as an industrial enzyme[44]. Thus, by developing a number of value added products, banana could be developed as an industrial crop generating recurring and consistent demand for this crop. A flow-chart depicting commercially important products that could be developed from banana is presented in Fig. 6.

Studies in similar direction with respect to other fruits could also develop a large array of products and generate a sustaining demand for those produces. However, it is important to remember that developing products of commercially sustainable demand, proper understanding of physiology, biochemistry as well as the mechanism of ripening of fruits is most essential.

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