A Protocol for Preparing Preserved Flowers with Natural Color and Texture

Hiroaki Ito^{1,5}, Takahiro Hayashi², Masaki Hashimoto³,
Katsuro Miyagawa³, Saori Nakamura³, Youichi Mizuta⁴,
and Susumu Yazawa⁴

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SUMMARY. A protocol for the preparation of preserved flowers retaining natural color and texture of 'Moondust Velvet Blue' carnations (*Dianthus caryophyllus*) was developed. This three-step process consists of soaking flowers in ethyl alcohol, then soaking them in polypropylene glycol, followed by a rinse with ethyl alcohol. Some kinds of flowers processed in this manner retained their natural color and texture for at least 6 months. The physicochemical properties of appropriate solvents used for retaining natural pigmentation and texture are discussed. This protocol is applicable to 13 kinds of flowers among 30 kinds of flowers tested and adds a new dimension to postharvest techniques for cut flowers.

Preserved flowers have a long ornamental period compared with fresh flowers, and can be more suited to flower arrangements, wedding bouquets, or store window decorations. In spite of their high processing costs, these flowers are in high demand. Preserved flowers were first developed in 1991, and are prepared from fresh flowers by replacing their internal moisture with polyethylene glycol (de Winter-Scaileur, 1991). These processed flowers can retain their fresh texture and flexibility for several years. However, preserved flowers do not retain their natural color. Flowers are artificially stained by soaking in polyethylene glycol with synthetic dyes. It is difficult to stain sepals, stems, and leaves separately from petals, thus multicolor flowers are usually stained monotone. If the internal moisture of fresh flowers can be replaced with solvents that allow petals to retain their original color, the processed flowers would look more natural than the currently available preserved flowers. There is a need to reconsider the soaking solvents to address the challenge of retaining petal color. We have studied the physicochemical properties of soaking solvents on petal tissues and have

screened solvents to determine which solvents allow the petals to retain their shape and color for a long period. This report introduces an established protocol for preparing preserved flowers retaining natural color and texture.

Materials and methods

PLANT MATERIALS. Flowers of 'Moondust Velvet Blue', a genetically engineered carnation variety with delphinidin-type anthocyanins (Fukui et al., 2003), were used to establish a protocol of three combined processes because its pigments have already been identified and it shows the highest pigment content among the three "Moondust" varieties. Cut 'Moondust Velvet Blue' flowers imported from Columbia were obtained from Suntory Flowers (Tokyo). The stem bases were inserted in a vase with 20 mL·L⁻¹ of a preservative containing sugars, inorganic ions, and antibacterial agents (Misaki; Otsuka Chemical, Osaka, Japan). Flowers after full bloom were used within 1 week.

Thirty kinds of flowers with different pigments such as flavonoids, carotenoids, chlorophylls, betalains, and anthocyanins were used for the applicability test of the established protocol.

Flowers were obtained from florists or experimental fields and were used immediately.

A PROTOCOL FOR FLOWER PROCESSING. Unless otherwise mentioned, ethyl alcohol was used to prevent petal shrinkage in the primary soaking process, followed by polypropylene glycol (diol type, average molecular weight: 400) to prevent petal discoloration in the secondary soaking process.

The protocol consisted of 1) soaking flowers in the primary solvent (ethyl alcohol) overnight; 2) soaking in the secondary solvent (100% polypropylene glycol) overnight or 2 d; 3) rinsing the flowers for 1 min with ethyl alcohol. The 'Moondust Velvet Blue' flowers with their stems trimmed to 10 cm were held upside down and the whole flower was soaked in 500 mL plastic containers containing 300 mL of primary or secondary soaking solvent. Thirty other kinds of flowers were soaked in the same way using 100 to 1000 mL of solvents, depending on the flower volume. The temperature of the solvents was 25 °C. The containers were covered with plastic wrap during steps 1 and 2. After the three treatment steps, the processed flowers were allowed to stand in a room at 25 °C and 45% ± 25% relative humidity (RH) for 1 d or more. Three replicate flowers were processed.

'Moondust Velvet Blue' carnation and the 30 other kinds of flowers were evaluated and classified into one of three groups: 1) petals that retained their color, 2) petals that retained their color only somewhat, and 3) petals that almost or completely lost their color.

Other preservation solvents such as 1-hexanol or methyl alcohol as the primary soaking solvents or polyethylene glycol (average molecular weight: 400); 2-methyl-2,4-pentanediol; or 1,3-butanediol as the secondary soaking solvents also were tested.

Results

FLOWER PROCESSING. A protocol for preparing preserved 'Moondust Velvet Blue' carnations so that they retain their natural color and texture

¹Koshien Junior College, Nishinomiya 663-8107, Japan

²Faculty of Agriculture, Kinki University, Nara 631-8505, Japan

³Suntorymidorie Limited, Osaka 618-8503, Japan

⁴Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan

⁵Corresponding author. E-mail: h-itou@koshien.ac.jp.

Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
29.5735	fl oz	mL	0.0338
7.8125	fl oz/gal	mL·L ⁻¹	0.1280
(°F - 32) ÷ 1.8	°F	°C	(1.8 × °C) + 32

Fig. 1. Outline in bold frames of a protocol to preserve natural flower color, with quality defects observed during each step if alternate solvents were used. The protocol consisted of soaking flowers in the primary solvent (ethyl alcohol) overnight, then soaking in the secondary solvent (100% polypropylene) glycol overnight or for 2 d, and then rinsing the flowers for 1 min with ethyl alcohol. Figure 2 references illustrate flower appearance at different point.

is detailed with bold frames in Fig. 1. Five serious quality defects were observed during and after the flower preservation process when solvents such as 1-hexanol or methyl alcohol were used instead of ethyl alcohol as the primary soaking solvent or polyethylene glycol; 2-methyl-2,4-pentanediol; or 1,3-butanediol was used instead of polypropylene glycol as the secondary soaking solvent (Figs. 1 and 2).

The quality defects observed were as follows: 1) Petal shrinkage occurred by soaking flowers in high-viscosity solvents (e.g., 1-hexanol) as the primary soaking solvent (Fig. 2C). The use of polypropylene glycol, polyethylene glycol, and glycerin also caused the same defects (data not shown). 2) Petal discoloration occurred by soaking flowers in high-hydrophilic solvents (e.g., methyl alcohol) as the primary soaking solvent or polyethylene glycol as the secondary soaking solvent (Fig. 2, D and E). The use of ethylene glycol, propylene glycol, and glycerin caused the same defects (data not shown). 3) Petal shrinkage occurred due to the volatilization of the primary soaking solvent when the flowers were kept in air before being transferred to the secondary soaking solvent (Fig. 2F). Flowers must be soaked in the secondary soaking solvent immediately after being soaked in ethyl alcohol as the primary soaking solvent (Fig. 2B). Severe petal shrinkage also occurred even when a low-volatile solvent (e.g., 2-methyl-2,4-pentanediol) was used as the secondary soaking solvent (Fig. 2J). The use of 2-(2-ethylhexyloxy)ethanol;

2,3-butanediol; and 4-hydroxy-2-butanone caused the same defects (data not shown). 4) The natural texture was not obtained just after soaking treatment with a nonvolatile sticky solvent (e.g., polypropylene glycol) because it remained on the petal surface (Fig. 2I). The flower needed to be rinsed with low-viscosity solvents (e.g., ethyl alcohol) to remove the secondary soaking solvent (Fig. 2G). The use of propylene glycol, glycerin, and 2-[2-(2-ethylhexyloxy)ethoxy]ethanol caused the same defects (data not shown). 5) Petals gradually lost their color after being processed using 1,3-butanediol as the secondary soaking solvent (Fig. 2K). The use of 1,3-propanediol; 1,4-butanediol; and 1,5-pentanediol caused the same defects (data not shown). Petal color was retained for at least 6 months by using polypropylene glycol as the secondary soaking solvent (Fig. 2L).

APPLICATION TO VARIOUS FLOWERS.

Similar to 'Moondust Velvet Blue' carnation, the flower shapes of most species tested were retained when the standard preserving protocol was used (Fig. 3). Flower species with thin petals that are particularly easy to deform were exceptions (Fig. 3, C2, D2, K2, L2, and S2). Compared with the color of unprocessed flowers (Fig. 3, A1-DD1), processed petals were grouped into those that retained their color well (Fig. 3, A2-L2), those that somewhat retained their color (Fig. 3, M1-O2), or those that nearly or completely lost their color (Fig. 3, P2-DD2). Some multicolor flowers

retained their color patterns (Fig. 3, B2, E2, F2, G2, and N2).

Discussion

Even under the best postharvest handling practices, the vase life of fresh flowers is relatively short and many of the flowers are lost during the marketing chain (Nowak and Rudnicki, 1990). Preserved flowers can retain their fresh texture and flexibility for several years but cannot retain their natural color. There is a need to reconsider the soaking solvents to address the challenge of retaining petal color. However, there are few reports on the effects of solvent treatment on petal tissues. It has been reported that processed flowers can be prepared by replacing internal moisture with 2-propyl alcohol or *t*-butyl alcohol (Romero-Sierra and Webb, 1982). However, these solvents volatilized from the petals, resulting in losing fresh texture. Replacement techniques have been developed for preparing micro- and macro-specimens from organs or whole bodies of animals, as well as processed flowers. Plastination (von Hagens, 1981) is an example technique of replacing the internal moisture with epoxy resin using an ascending series of acetone. However, this dehydration process results in the complete discoloration of tissues. A replacement technique using an ascending series of ethyl alcohol also causes pigments to leach out of petals during dehydration owing to the water in the solvent.

Fig. 2. Photographs of 'Moondust Velvet Blue' carnations at different processing stages. (A) Fresh flower. (B) The flower after soaking in ethyl alcohol. (C) Petal shrinkage soon after Step 1 when 1-hexanol was used as the primary soaking solvent. (D) Discoloration soon after Step 1 when methyl alcohol was used as the primary soaking solvent. (E) Discoloration soon after Step 2 when polyethylene glycol (average molecular weight: 400) was used as the secondary soaking solvent. (F) Petal shrinkage 1 d in air after Step 1 when ethyl alcohol was used as the primary soaking solvent. (G) Flower color immediately after soaking in polypropylene glycol as the secondary soaking solvent. (H) Flower color after Step 3 rinsing with ethyl alcohol. (I) Loss of natural texture after ≈ 1 month of storage in air after Step 2. (J) Petal shrinkage after ≈ 5 months storage of flowers that were prepared using 2-methyl-2,4-pentandiol as the secondary soaking solvent. (K) Discoloration after ≈ 2 months storage of flowers that was prepared using 1,3-butanediol as the secondary soaking solvent. (L) Processed flower prepared using ethyl alcohol as the primary soaking solvent, polypropylene glycol as the secondary soaking solvent, followed by rinsing with ethyl alcohol and being held in storage for ≈ 6 months in air.

Fig. 3. Photographs of fresh (1) and processed flowers (2) of various species. Processed flowers were prepared by soaking in ethyl alcohol overnight, followed by overnight or 2 d soaking in polypropylene glycol (diol type, average molecular weight: 400), before rinsing with ethyl alcohol. Photographs of processed flowers were taken 1 d after treatment except for A2 and B2, which were taken 15 and 13 months after processing, respectively. Plant species were as follows: (A) corn flower (*Centaurea cyanus*), (B) dwarf delphinium (*Delphinium grandiflorum*), (C) spiderwort (*Tradescantia obiensis*), (D) asiatic dayflower (*Commelina communis*), (E) pansy (*Viola $\times$ wittrockiana*), (F) lisianthus (*Eustoma grandiflorum*), (G) dutch iris (*Iris $\times$ hollandica*), (H) cockscomb (*Celosia cristata*), (I) bougainvillea (*Bougainvillea spectabilis*), (J) easter cactus (*Rhipsalidopsis gaertneri*), (K) portulaca (*Portulaca grandiflora*), (L) four o'clock (*Mirabilis jalapa*), (M) snapdragon (*Antirrhinum majus*), (N) cooktown orchid (*Dendrobium phalaenopsis*), (O) rose (*Rosa $\times$ hybrida*), (P) bigleaf hydrangea (*Hydrangea macrophylla*), (Q) daffodil (*Narcissus pseudonarcissus*), (R) carnation (*Dianthus caryophyllus*), (S) iceland poppy (*Papaver nudicaule*), (T) african violet (*Saintpaulia ionantha*), (U) salvia (*Salvia guaranitica*), (V) bigleaf hydrangea, (W) camellia (*Camellia japonica*), (X) geranium (*Pelargonium incrassatum*), (Y) tulip (*Tulipa gesneriana*), (Z) sunflower (*Helianthus annuus*), (AA) pansy (*V. $\times$ wittrockiana*), (BB) oil seed rape (*Brassica napus*), (CC) watermelon (*Citrullus lanatus*) and (DD), and sweet william (*Dianthus barbatus*).

The moisture replacement technique adopted here does not use water-containing solvents. In fact, pigments diffused but remained in the petals of dehydrated flowers. Some pigments such as flavonoids may get deposited on cell walls (Markham et al., 2000).

In this protocol, ethyl alcohol and polypropylene glycol were used as the primary and secondary soaking solvents, respectively. Primary soaking solvents with a low viscosity, such as ethyl alcohol, tended to prevent petal shrinkage (data unpublished). In addition, petal shrinkage induced by the secondary soaking solvents was greatly reduced if the petals were soaked preliminarily in such primary soaking solvents. Polypropylene glycol was selected as the most appropriate secondary soaking solvent because it retained the petal shape and color of 'Moondust Velvet Blue' carnations for a long time. Taking the cost, the safety, and waste disposal into account, the combination of ethyl alcohol and polypropylene glycol seemed to be a reasonable choice.

The flower color of 'Moondust Velvet Blue' carnation and 13 other flower species was retained well among 31 kinds of flowers tested (Figs. 2L and 3, A2–L2). Coexisting with polypropylene glycol, most flowers containing yellow flavonoids did not retain their petal color, except for yellow snapdragon (*Antirrhinum majus*). All flowers and leaves that included oil-soluble pigments, such as carotenoids and chlorophylls, did not retain their petal color. All flowers containing betalains retained their petal color vividly. Most flowers containing dephinidin-type anthocyanins retained their petal color, except for

bigleaf hydrangea (*Hydrangea macrophylla*) and salvia (*Salvia guaranitica*). Most flowers containing cyanidin-type anthocyanins did not retain their petal color, except for corn flower (*Centaurea cyanus*), which contains pigments that form metal complexes with calcium (Ca^{2+}), iron (Fe^{3+}), and magnesium (Mg^{2+}), and copigmentation with flavons (Kondo et al., 1998). Flowers containing peonidin-, petunidin-, and pelargonidin-type anthocyanins retained somewhat or did not retain their color.

Whether flower colors expressed by yellow flavonoids or anthocyanins were retained with polypropylene glycol depended on species. In addition to skeletons of flavonoids, their concentrations and formations may affect the stability of processed flower colors. Pigments were thought to exist in the solvent and to be deposited in cell walls in the processed petals. Hence, cell wall components, as well as solvents, may affect the formation and color expression of yellow flavonoids or anthocyanins.

The study showed that the processed 'Moondust Velvet Blue' carnation retained its petal shape and color over 6 months and the processing method can be applied to several kinds of flowers (Fig. 3). Some processed flowers, such as corn flower (Fig. 3A2; 15 months storage after processing) and dwarf delphinium (*Delphinium grandiflorum*) (Fig. 3B2; 13 months storage after processing), retained their flower color vividly for more than 1 year. The keeping quality of processed flowers seems to be influenced by many factors, such as the cultivation method, harvest maturity, and storage conditions after processing. Exclusion

of direct sunlight and high humidity are essential during storage because light, oxygen, and humidity cause the color to fade and the flowers to deform. Studies on cultivation method and storage conditions for processed flowers may lead to improvements in the keeping quality of processed flowers.

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