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Application of Fixatives to Freeze Dried Rose Petals

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Abstract

The effect of freeze drying and fixatives in post-treating freeze drying on the morphological properties of the rose (*Rosa hybrida* L.) petal were investigated for the production of high quality of freeze dried rose. The morphology including form and color of the dried flowers of cut rose were depended on the drying methods. The drying time was extended due to their density and water content, and was shorter in the freeze drying than that in the natural and hot air drying. Freeze dried process for dried flowers took 2 days in a freeze dryer and did not cause shrinkage or toughening of rose petal being dried, preserving its natural shape and color. The diameter of freeze dried flowers showed little reduction compared to fresh flowers. In Hunter color values of petals of freeze dried flowers, L and a values were high and showed little variations in comparison to fresh petals. Freeze drying led to a noticeable increase in anthocyanin contents in petals, suggesting that anthocyanin contents play an important role in the acquisition of freezing tolerance. Exposure of flowers to freeze drying was accompanied by an increase in the carotenoid content. In the post-treating freeze drying, epoxy resin, a fixative, applied alone or in combination to petals of freeze dried flowers showed efficient coating for the protection from humidity and sunlight. Combined application of epoxy and acetone to freeze dried petals permitted maintenance of natural color and excellent tissue morphology, showing color stability and shiny texture in surface of petals. These findings suggest that application of fixatives to freeze dried rose petals improves the floral preservation and epoxy coating provides good quality in the freeze dried flower product.

Key Words : Coating, Epoxy resin, Fixatives, Freeze drying, Hunter color, Rose petal

1. Introduction

Freeze drying (Lyophilization) is a dehydration process where water vapor is removed by sublimation from materials, usually under conditions of low pressure and temperature[1,2]. The advantage of this process is mainly related to the high quality products with little or no change in chemical composition. Despite the excellent quality of final products, the cost of the freeze drying process is relatively expensive[3]. The major disadvantage of freeze drying is directly related to the long processing times caused by the high material resistance to heat transfer during the operation, the sample configuration, its own properties and the operating conditions[4]. Recently, freeze drying is applied in many industries such as phamaceutical and biotechnology, food industry, technological industry and floral industry as well as within laboratories, as a laboratory lyophilizer.

Freeze dried flower industry began in 1980s and provides one of today's most valuable business opportunities[5]. Extensive research continues on new products to produce perfectly preserved blooms for floral preservation[6]. The market potential of floral industry is literally staggering for floral freeze drying. A great advantage of being able to use freeze dried petals of floral varieties is that it increases the shelf life of prod-

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ucts and extends their season. Freeze drying is efficient natural means of preservation and it produces a result that favors extended storage for natural items, preserving the item's natural beauty[7]. Most botanical items such as flowers, fruits and vegetables can be preserved without change in shape, color or fragnance. The basic principle of floral freeze drying is the removal of water from frozen botanicals as water vapor. However, removing the water too fast may cause blistering, shrinkage and shriveling of freeze dried flowers.

Valuable knowledge for the freeze dry industry is gained from working directly with fresh product being prepared for freeze drying, pre-treating flowers for floral freeze drying and post-treating freeze dried flowers. In dried flower production, freeze drying can be used as late-stage of preservation procedure, because it can effectively remove solvents. Freeze dried flowers are fragile, shattered easily and have a tendency to change color. To solve these problems, post-treative chemicals are applied extensively to the freeze dried flowers. Chemicals with a full range of non-hazardous pre- and post-treatment are available for floral freeze drying. The application of fixatives are extensive and include coating, adhesive and composite materials[8]. In biology, a fixative is a solution to preserve or harden fresh tissue of cell specimens and they usually stabilize and firm tissues by denaturing or cross-linking constituent proteins. In drawing, a fixative is a liquid which is usually sprayed over a finished piece of artwork to better preserve it and prevent smudging. The post-treative chemicals used to meet the needs for color stabilization, durability and longevity in freeze dried flowers. Therefore, extensive research on both freeze drying and chemical pre- and post-treating freeze drying is needed for the floral freeze dry industry.

The objective of this study was to determine the effect of freeze drying and fixatives in post-treating freeze drying on the morphological properties of the rose petals with regard to flower preservation techniques.

2. Materials and Methods

2.1. Plant materials

Red rose (*Rosa hybrida* L. cv. Victor hugo and Sandra) and white rose (*Rosa hybrida* L. cv. Fabulous) obtained from a local farm were selected for dried flower preserving experiments. Cuttings were made from flowers of average size and they were dried before full blooming by different drying methods including natural, hot air and freeze drying. Cut rose flowers were hang upside down on wire and gently dried at root temperature for natural drying, or they were dried at 40℃ in a drying oven for hot air drying. Freeze drying of cut rose flowers was carried out in a vacuum freeze dryer (Samwon Co., Korea) and then allowed to dry for 48 h. For time-course analysis, the flowers were harvested at different time intervals.

2.2. Plant dry weights

The fresh weight of rose flower was determined immediately after harvest and the dry mass production was determined by drying samples at 80℃ for 72 h in a drying oven and reweighing.

2.3. Measurement of flower color

Each petal were analyzed for color variation with spectrocolorimeter (CR-200, Varian Co., Australia) and colorimeter (DM-200, Varian Co., Australia). The values of L (lightness), a (red to green) and b (yellow to blue) were measured.

2.4. Estimation of anthocyanins

Anthocyanins were extracted by submerging the flowers in acidified 70% methanol (CH_3OH) that contained 1% HCl at 4°C for 24 h. Anthocyanin content was determined by Francis[9] and estimated by measuring absorbance at 535 nm with UV/VIS spectrophotometer.

2.5. Estimation of carotenoids

Each preweighed petal was homogenized with a mortar and pestle in 80% (v/v) acetone and centrifuged at 10,000×g for 10 min at 0-4℃. The supernatant was brought up to a 1 ml volume, then the absorbance of the acetone extract was measured at 470 nm using a UV/VIS spectrophotometer. The carotenoid content was calculated according to the method of Arnon[10].

2.6. Application of fixatives

After freeze drying process, the flowers were handled with gentle hands and placed in room temperature to allow softening and each flower was then post-treated. A thin layer of fixative was coated to the surface of petal of each freeze dried flower for floral preservation. Epoxy resin, glycerine and toluene were selected as fixative coating and they were applied alone or in combination. Fixatives were sprayed with high pressure of butane or airbrushed over petals of freeze dried flowers.

3. Results and Discussion

3.1. Effect of freeze drying on morphological characteristics of cut flowers

Duration of drying of flowers depends on many factors including humidity, temperature, airflow and the type of flower. The time of drying and morphology of the flower of cut rose was affected by the drying methods. Under continuous drying of the flowers the time for dried flowers in natural drying and hot air drying was a minimum of 10 days and 3 days, respectively. On the contrary, the time for the dried flowers in freeze drying was approximately 2 days in freeze dryer. The visual symptoms of rose flower by freeze drying showed little reduction in flower size and complete coloration of the entire flowers, showing the subtlety of shading and natural look of a fresh petal. However, other methods such as natural and hot air drying resulted in rather shrivelled petal and had small size and darker color. The drying time was extended due to their density and water content.

Notable differences in dry weight between cultivars were not observed and the degree of reduction was upon the drying methods (Table 1). Dry weight was reduced in flowers exposed to natural and hot air drying, while those that had been exposed to freeze drying were less affected. The cut rose flowers exposed to natural drying had flower diameter less than half that of fresh flowers, whereas flowers of freeze drying had little change in flower diameter (Fig. 1) ; that is, the degree of shrinkage of the flowers was very low by freeze drying. The size of the rose petals was maintained in spite of removal of moisture from the petal as a freeze drying process, indicating that the extraction of moisture as water vapor may prevents shrinkage of the flowers[11]. The basic principle of floral freeze drying (lyophilization) is the removal of water from frozen botanicals as water vapor and collection of this water vapor as ice in a condenser. Therefore, shrinkage is eliminated or minimized and near perfect preservation will result. Freeze drying greatly increased the overall production costs and low temperature storage was important for retaining excellent functionality in freeze dried samples such as pharmaceutical products and some special food[12]. Freeze drying creates softer particles with a more homogenous chemical composition than traditional hot spray drying in advanced ceramics process.

In Hunter color values of dried petals, all samples exhibited some changes in L, a and b values according to the drying methods (Table 2). L and a values showed small variations compared to fresh petals,

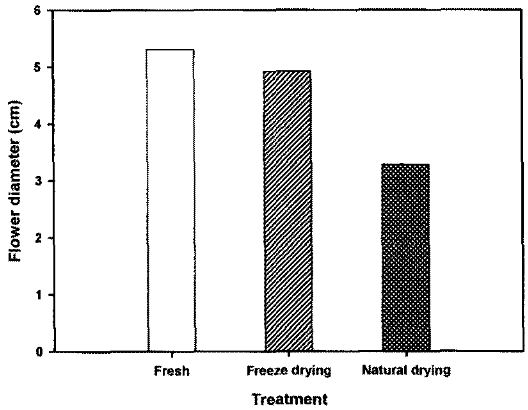

Fig. 1. The diameter of cut rose petals exposed to natural and freeze drying.

Table 1. Dry weight of cut rose petals exposed to various drying methods

Cultivar	Fresh weight(g)	Dry weight (g)		
		Natural drying	Hot air drying	Freeze drying
Rosa hybrida L. cv. Victor hugo	1.347±0.045	0.473±0.018	0.463±0.019	0.527±0.082
Rosa hybrida L. cv. Fabulous	1.125±0.062	0.390±0.052	0.489±0.083	0.600±0.048

Table 2. Petal color of cut rose exposed to various drying methods

Drying method	Hunter color value			
	L	a	b	△E
Fresh	38.0	+69.3	+30.8	-
Natural drying	17.5	+30.8	+3.5	51.45
Hot air drying	20.8	+35.7	+6.4	44.94
Freeze drying	36.5	+57.1	+14.5	20.41

whereas b values were considerably reduced in dried petals. The greatest degree of change was noted in the natural dried petals. On the contrary, both L, a and b values in the freeze dried petals remained relatively constant over time. Freeze dried petals had little change in L and a values with lower levels of b values compared to fresh petals. This changes were probably the result of a Millard reaction[13]. In the presence of heat and amino acids, sucrose is able to undergo a non-enzymatic browning reaction. The reaction rates were faster in the freeze dried samples because of the relatively high concentration of amino acids and sucrose due to the removal of the water. Another reaction that might have caused color changes is lipid oxidation. Highly reactive compounds could undergo oxidation over time, resulting in dramatic increases in the b values. The reaction can be slowed at low temperatures, thus the smaller degrees of change occurred in the samples stored in the freezer[14].

There were significant differences in anthocyanin content according to the drying methods and between cultivars (Fig. 2). Natural drying had minimum anthocyanin content compared to other drying methods. Notable differences in anthocyanin content were observed between fresh and freeze dried flowers. Anthocyanin content began to increase from the first day, reaching maximum level on the 3th day of exposure in freeze dried petals. Overall content of anthocyanin in red rose was much higher than that in white rose. The synthesis of anthocyanins is affected by environmental and growth conditions including photo-oxidation, low temperature, water and nutrient stress. Suboptimal temperatures experienced either as sudden, short-term cold spells induce anthocyanin synthesis[15,16]. The present observations showing a higher anthocyanin level in freeze dried petals suggest a protective role of the anthocyanin in the acquisition of freezing tolerance.

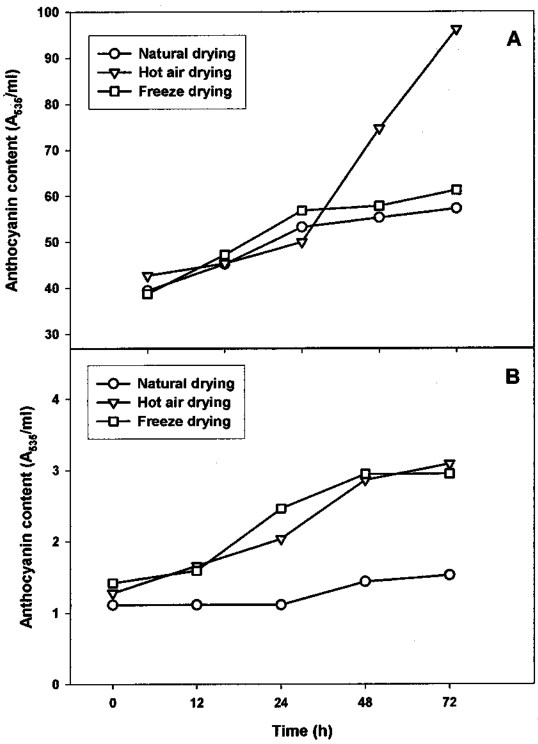

Fig. 2. Changes in anthocyanin content in cut rose petals exposed to various drying methods.
A, *Rosa hybrida* L. cv. Victor hugo ; B, *Rosa hybrida* L. cv. Fabulous.

Petals exposed to freeze drying had lower level of carotenoids than those from natural drying treated petals (Fig. 3). Carotenoid contents were almost 2-fold higher in petals of red rose than those of white rose. There is substantial evidence that carotenoids play an important role in protecting the photosynthetic system from photooxidative damage[17]. From the above results, it is suggested that freeze drying is the most perfect

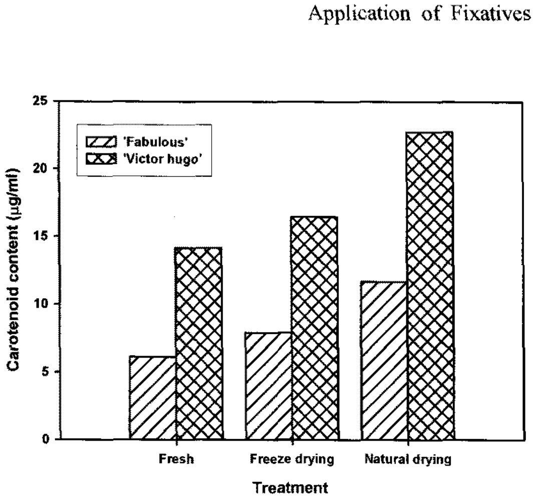

Fig. 3. Carotenoid content in cut rose petals exposed to natural and freeze-drying.

method of preservation with a laboratory freeze dryer, creating preserved flowers without change in shape or color.

3.2. Effect of fixatives on coating of freeze dried flowers

The freeze dried flowers were fragile, shattered easily and had a tendency to change color owing to removing of water too fast from petals. The requirements for long term storage of freeze dried materials is the prevention of moisture re-entering the product. These problems have all been addressed through chemical pre- and post-treatment. For the chemical post-treatment of the freeze dried petals, fixatives such as epoxy

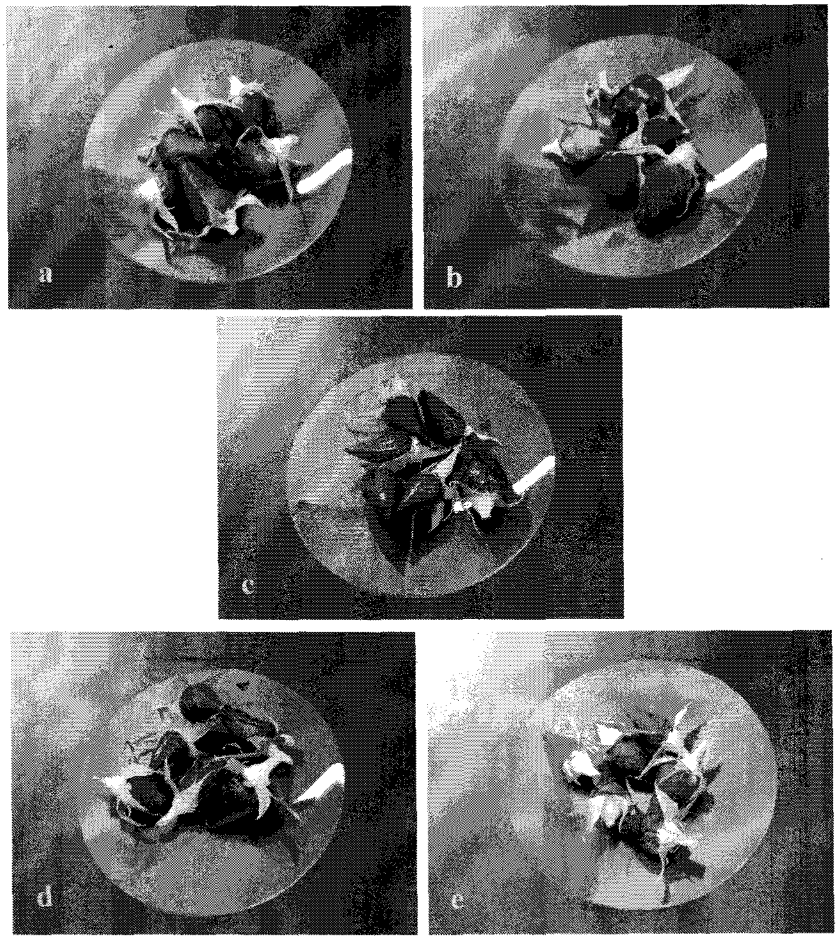

Fig. 4. Effect of fixatives on the morphological properties of freeze dried rose flowers. a, epoxy ; b, glycerine ; c, toluene ; d, epoxy + toluene ; e, epoxy + glycerine + toluene.

Fig. 5. Changes in shape and color of freeze dried rose flowers treated fixatives and solvent in combination. a, epoxy + acetone ; b, epoxy + glycerine ; c, epoxy + alcohol.

resin, glycerine and toluene were applied to the petal of each freeze dried flower (Fig. 4). The freeze dried petals coated with epoxy resin became more durable, elastic and color fast, creating a more distinct and longer lasting product as compared with other chemicals. Therefore, application of epoxy resin on freeze dried petal surfaces showed the most excellent coating and adhesive properties, suggesting protection from humidity and sunlight. When using epoxy resin and hardener such as acetone, alcohol and glycerine in combination, acetone was an effective solvent for dissolving epoxy (Fig. 5). Combined application of epoxy and acetone to freeze dried petals showed color stability and shiny texture in surface of petal, indicating the best quality for good freeze dried flower products.

Epoxy is a thermosetting epoxide polymer that polymerizes and crosslinks when mixed with a catalyzing agent or hardener. The chemistry of epoxies and the range of commercially available variations allows cure polymers to be produced with a very broad ranges of properties. In general, epoxies are known for their excellent adhesion, chemical and heat resistance, good-to-excellent mechanical properties and very good electrical insulating properties[18]. Epoxy coatings are widely used as primers to improve the adhesion of automotive and marine paints especially on metal surfaces where corrosion resistance is important. Since information on fixatives as floral coating to improve the flower preservation is very limited to date, it is highly recommended that further research is done in this area for good quality of freeze dried flower product.

4. Conclusions

The shorter drying time of the freeze drying process leaded to minimum damage to rose petals and retained their natural look compared to other drying methods. Freeze dried petals showed higher Hunter parameter L, a and b values than other dried ones. There was no significant difference between fresh flowers and freeze dried flowers in terms of L and a values of petal color. Freeze dried petals had maximum anthocyanin content during drying time compared to other drying methods. Using fixatives as coating to freeze dried petals, the most prominent effect was at epoxy resin, retaining natural color vibrancy and adding durability. Combination of epoxy resin and acetone to freeze dried petals showed more excellent tissue morphology permitting color stability and protection from humidity than epoxy alone. From the results, it can be concluded that the application of fixatives to freeze dried rose petals provides effective method for product of good quality in floral freeze dry industry.

Acknowledgements

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References

1) Mellor J. D., 1987, Fundamentals of Freeze Drying,

Academic Press, London, 16-337pp.
2) Franks F., 1989, Improved freeze-drying : An analysis of the basic scientific principles, Process Biochem., 24, 3-7.
3) Sadikoglu H., Liapis A. I., Crosser O. K., 1998, Optimal control of the primary and secondary drying stages of bulk solution freeze drying in trays, Drying Technol., 16, 399-341.
4) Sagara Y., Ichiba J., 1994, Measurement of transport properties for the dried layer of coffee solution undergoing freeze drying, Drying Technol., 12, 1081-1103.
5) Waterkeyn S., 1988, The Creative Art of Dried Flower, Kingdom, 282pp.
6) Park Y. J., Heo B. G., Kang Y. K., 2001, The present situation and prospect of dried flower and pressed flower processing, Kor. J. Hort. Sci. Technol., 19, 262-269.
7) Sho C. S., Park Y. J., Lee I. B., Heo B. G., 2001, Color and morphological characteristic of freeze-dried rose, J. Kor. Flower Res. Soc., 9, 87-90.
8) Yeo M. K., Cho E. J. 2004, Effects of TiO_2 photodegradation on leaching from epoxy resin chemical in water and biological toxicity, J. Environ. Toxicol., 19, 271-278.
9) Francis J. P., 1982, Analysis of anthocyanin, *In* Markakis P.(ed.), Anthocyanin as Food Colors, Academic Press, 181-208.
10) Arnon D., 1949, Copper enzymes in isolated chloroplast : Polyphenol oxidase in *Beta vulgaris*, Plant Physiol., 24, 1-15.
11) Sohn K. H., Kwon H. J., Kim E. Y., 2003, Effects of drying methods on shape and color of *Rosa hybrida*, Kor. J. Hort. Sci. Technol., 21, 136-140.
12) Krokida M. K., Maroulis Z. B., 1997, Effect of drying method on shrinkage and porosity, Drying Technol., 15, 2441-2458.
13) BeMiller J. N., Whistler R. L., 1996, Carbohydrates, *In* Fennema O. R.(ed.), Food Chemistry, 3th ed., Marcel Dekker, 157-223.
14) Reynolds J., Park J. W., Choi A. J., 2002, Physico-chemical properties of pacific whiting surimi as affected by various freezing and storage conditions, J. Food Science, 67, 2072-2078.
15) Leng P., Itamura H., Yamamura H., Deng X. M., 2000, Anthocyanin accumulation in apple and peach shoots during cold acclimation, Sci. Hort., 83, 43-50.
16) McKown R., Kuroki R., Warren G., 1996, Cold responses of *Arabidopsis* mutants impaired in freezing tolerance, Exp. Bot., 47, 1919-1925.
17) Demmig-Adams B., Adams W. W., 1996, The role of xanthophyll cycle carotenoids in the protection of photosynthesis, Trends in Plant Science, 1, 21-26.
18) Jolanki R., Kanerva L., Estlander T., 2000, Epoxy resins, *In* Kanerva L., Elsner P., Wahlberg J. R., Maibach H. I.(ed.), Handbook of Occupational Dermatology, Springer-Verlag, 570-590.