Madras Agric. J., 101 (10-12): 401-405, December 2014
https://doi.org/10.29321/MAJ.10.001224

Standardisation of Glycerinisation Technique for Fragile Foliages

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Processing techniques in dry flower industry assume great significance in determining the final quality of dry flower products. Preserving plant materials by glycerinisation with glycols is experimented on fragile leaves. The treatment T_3 (Glycerine 30% followed Ethylene glycol 30%) resulted in enhancement of quality characteristics for *Myrtus* sp., *Thuja orientalis, Eucalyptus glaucescens* and *Asparagus virgatus* in terms of percentage of minimum moisture loss (34.22, 25.51, 26.81 and 9.15, respectively. The pigment concentration *viz.,* chlorophyll 'a', 'b' and total chlorophyll contents (0.877, 1.726, 1.685 and 1.406 mg g^{-1} respectively were also superior. The leaves scored better for quality parameters *viz.*, texture and shape retention. As an improved glycerinisation technique 30 % Glycerine followed by 30 % Ethylene glycol resulted in soft near to natural leaves with minimum moisture loss and with maximum colour and shape retention.

Keywords: Dry flower industry, Glycerinisation, Foliages, Quality

In the present era of eco-consciousness, use of plant-derived natural products like dry flowers has become the premier choice of the masses in their life styles for interior decorations. Dried and preserved ornamental products offer a wide range of unique qualities which include novelty, longevity, aesthetic properties, flexibility and year round availability (Ranjan and Mishra, 2002). Many types of foliage can be preserved by variety of processes that impart longevity to the product. Common methods of preservation include air drying, dehydration by desiccants (silica gel, borax, sand), freeze drying, coating with polymer dispersions and systemic or immersion glycol treatments. Leaves are best preserved with glycols. More and more foliages have shown to respond very well to this manner of preservation.

Preserving plant materials with glycols is called glycerinisation or glycol preservation. Foliages commonly preserved by glycol treatment using hydrophilic chemicals help to maintain the suppleness of dried plant materials by attracting water vapour from the surrounding atmosphere. Accordingly preserved materials are less brittle than dried materials. Thus it is less prone to shattering and mechanical damage and more lifelike in appearance. Leonard Karel (1973) stated that among the various methods of preserving foliages, glycerinisation affords perhaps greatest satisfaction. Glycerinization improves pliability, retains natural shape and makes the dried products last longer (Conder, 1979) . The merit of glycol preserving is replacing the volatile water in plant tissues with a non volatile liquid. If the internal moisture of plant parts are replaced with solvents like glycol to retain their original texture, shape and colour, the processed parts would look more natural than the air dried leaves (White *et al.,* 2007). However, a wrong choice of solvent can considerably influence the texture of the final product. Use of low volatile solvents like glycerine causes bleeding while high volatize solvents like ethylene alcohol and propylene alcohol causes complete shrinkage (Joyce, 1998). Hence, an appropriate combination of these solvents can be a reasonable choice. Therefore, there is a need to reconsider appropriate solvents and their concentrations by improved methods to address the challenge of reducing bleeding but retaining texture and shape of the foliages. With this objective the current study was taken to standardize the advanced glycerinisation technique for fragile foliages.

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Materials and Methods

Improved scientific glycerinisation study was carried out in the Tamil Nadu Agricultural University, Coimbatore during the year 2010 – 13 under completely randomized design. Matured leaves of four species *viz*., *Myrtus* sp*., Thuja orientalis, Eucalyptus glaucescen* and *Asparagus virgatus* were sourced from Yercaud and Niligiris. Mixtures of Glycerine, Ethylene and Propylene glycol were prepared using warm distilled water (20 $_0$ C). Treatment combinations were T_1 - Glycerine 30%

followed Glycerine 30%, T_2 - Glycerine 25% followed Ethylene glycol 25%, T_3 - Glycerine 30% followed Ethylene glycol 30%, T_4 - Glycerine 25% followed Propylene glycol 25%, T_5 - Glycerine 30% followed Propylene glycol 30%, T_6 - Ethylene glycol 25% followed by Glycerine 25%, T_7 - Ethylene glycol 30% followed by Glycerine 30% , T_8 - Propylene glycol 25% followed by Glycerine 25%, T_9 - Propylene glycol 30% followed by Glycerine 30%. To these mixtures, 0.1 per cent Sodium benzoate and 8 g of Apple green food dyes were added. The pH 4.0 was maintained 4.0 by adding citric acid. The systemic glycol preserving process adopted in this experiment is described below in different steps. Primary soaking - About 250 g each of mature fresh leaf stalks of the plant species were harvested and rinsed immediately in tap water. The leaf stalks were then given a slant cut of one inch with sharp knife without damaging the tissues, in order to ensure that air trapped inside is removed. The stalks were then placed quickly into the first step glycol preservative mixture as given in the treatment details in small mugs or jars. During the process of secondary soaking, the preserving durations were fixed as 2 days for *Asparagus virgatus*, 4 days for *Myrtus* sp., 4 days for *Eucalyptus glaucescens* and 8 days for *Thuja orientalis*. In the mid of preserving process (after completion of 50 % of the preserving duration), the foliages were removed and washed. The leaf stalks were again given a slant cut and immersed in the second step glycol preservative as mentioned in the treatment details. During both step at least 10 cm of the entire stem was totally immersed in the preservative solution with proper air circulation without overcrowding. The mugs were placed in dark room at ambient temperature (30±2 ^{0}C). Observations on percentage of moisture loss, pigment (chlorophyll) content and qualitative scores for texture and shape retention were measured. The percentage moisture loss was calculated by using the formula suggested by Ranganna (1977). The chlorophyll 'a', 'b' and the total chlorophyll contents in the preserved leaves were determined by using the method of Yoshida *et al.* (1971) and expressed in mgg^{-1} of preserved leaf sample. Sensory scoring was done for the quality characters texture and shape retention by a panel of 14 scientific persons including experts in dry flower technology on a 5 point Hedonic scale (Peryam , 1957). The data recorded were statistically analyzed for significance (Panse and Sukhatame, 1985).

Results and Discussion

Percentage of moisture content

All the parameters studied in the experiment were significantly differed by advanced glycerinisation technique. The glycerinisation of different foliages on percentage of moisture loss was presented in Table 1. Among different foliages employed in this study the minimum moisture loss

Table 1. Effect of glycerinisation on percentage of moisture loss of foliages

Treatment	Moisture loss (%)			
	Myrtus sp.	*Thuja orientalis*	*Eucalyptus glaucescens*	*Asparagus virgatus*
T_1	38.57_{bc}	29.06_{bc}	30.55_{bc}	11.57_{c}
T_2	36.06_{ab}	27.44_{ab}	28.50_{ab}	9.95_{ab}
T_3	34.22_{a}	25.51_{a}	26.81_{a}	9.15_{a}
T_4	42.67_{d}	30.89_{cd}	31.96_{c}	10.76_{bc}
T_5	40.25_{cd}	29.82_{cd}	30.15_{bc}	10.32_{b}
T_6	47.68_{ef}	35.82_{e}	41.59_{d}	13.97_{d}
T_7	46.83_{e}	31.55_{d}	41.45_{d}	13.54_{d}
T_8	53.49_{g}	38.19_{f}	48.46_{e}	17.02_{f}
T_9	50.23_{f}	37.26_{ef}	47.65_{e}	15.55_{e}
SEd	1.508	1.094	1.262	0.430
CD (p=0.05)	3.167	2.299	2.651	0.904

*Means followed by a common letter are not significantly different at the 5% level by LSD.

was recorded in T_3 (Glycerine 30 % followed Ethylene glycol 30 %) and T_2 (Glycerine 25% followed Ethylene glycol 25%) for *Myrtus* sp. (34.22 per cent and 36.06 per cent), *Thuja orientalis* (25.51 per cent and 27.44 per cent), *Eucalyptus glaucescens* (26.81 and 28.50 per cent) and *Asparagus virgatus* (9.15 per cent and 9.95 per cent). The sequence of solvent usage highly influences the rate of water replacement. Among the different treatments, minimum moisture loss was recorded in leaves of *Myrtus* sp.*, Thuja orientalis, Eucalyptus glaucescens* and *Asparagus virgatus* which were soaked in descending order of solvents (Glycerine > Ethylene glycol > Propylene glycol) with respect to their hydrophilic nature *viz.,* Glycerine 30 % followed by Ethylene glycol 30%. It is because, glycerine with presence of three –OH groups had strong affinity for water. Also being a low volatile liquid with high boiling point, it evaporates at a much slower rate than water at a given temperature (Raymond Chang, 1984). However, with the basic objective of reducing bleeding when the leaves were secondarily soaked in Ethylene glycol with two –OH groups, an optimal intermediate absorption isotherm is obtained (Mark Koch, 1995). Glycerinization of plant materials helps them to last longer apart from keeping them more pliable and retaining their natural shape. Further, it could be realized in the present study that, not only the choice of solvents, but also the sequence of solvents greatly influences the final quality of preserved botanicals. Improper choice of solvents and sequence of their usage can either cause bleeding or complete shrinkage of the plant materials. Hence, a combination of humectants with varied hygroscopic nature *viz.,* Glycerine, Ethylene glycol, Propylene glycol seemed to be a reasonable choice, as already observed by Joyce (1998).

Moisture loss in the leaves by preservation influences the shape of leaves. Intermediate absorption isotherms obtained by combining glycols in the descending order of high rate uptake humectants *viz.*, Glycerine > Ethylene Glycol enables softening of leaves, a capability resulting from its moisture retention capacity. This observation is in conformity with the view of White *et al.* (2007) who reported that treating foliages with glycols yields unique results that would enable plant parts to remain flexible and pliable indefinitely.

The treatment T_8 (Propylene glycol 25% followed by Glycerine 25%) recorded the maximum percentage of moisture loss for *Myrtus* sp. (53.49 per cent), *Asparagus virgatus* (17.02 per cent), *Eucalyptus glaucescens* (48.46 per cent) and *Thuja orientalis* (38.19). Maximum moisture loss was recorded in treatments where plant materials were primarily soaked in propylene glycol and ethylene glycol followed by secondary soaking in glycerine. It may be due to the reason that primary soaking in solvents with only two -OH groups which are high volatile in nature, the water being evaporated easily results in drying and shrinkage of leaves (Morrison and Boyd, 1959). Though further soaked in glycerine, initial drying must have resulted in the high rate of senescence and death of cells prevent or slow down the absorption of solvent in the second step (Ito *et al*., 2010). Uptake of humectants in the transpiration stream characteristically slows markedly over the first few days; presumably as solute accumulation reflect progressive closure of an increasing proportion of the stomatal population. Thereafter, the sustained and relatively low rates of uptake observed could be indicative of humectants uptake and driven by trans-cuticular water loss (Joyce, 1998). The difference in performance of the foliage can be attributed to the difference in genetic makeup of the plants. Among the four different leaves, minimum moisture loss in *Asparagus virgatus* and maximum moisture loss in *Myrtus* sp. recorded might be due to the narrow and broad leaf structure of the leaves respectively. This is in accordance with findings of Guerfel *et al.* (2008) who reported that the morphological and structural characteristics of leaves influence their response to processing methods.

Pigment concentration

Chlorophyll content exhibited significant differences for different treatments. The maximum chlorophyll 'a' content (Table 2.) was registered in T_3 (Glycerine 30% followed Ethylene glycol 30%) for all botanicals *viz.*, *Myrtus* sp. (0.206 mg g^{-1}), *Thuja orientalis* (0.758 mg g^{-1}), *Eucalyptus glauscens* (0.665 mg g^{-1}) and *Asparagus virgatus* (0.823 mg g^{-1}). This is followed by T_2 (Glycerine 25% followed Ethylene glycol 25%) by recording maximum chlorophyll 'a' content of 0.190 mg g^{-1} for *Myrtus* sp., 0.726 mg g^{-1} for *Thuja orientalis*, 0.623 mg g^{-1} for *Eucalyptus glauscencs* and 0.752 mg g^{-1} for *Asparagus virgatus*. The lowest chlorophyll 'a' content was recorded in T_8 (0.051 mg g^{-1}, 0.462 mg g^{-1}, 0.222 mg g^{-1} and 0.403 mg g^{-1}) for different foliages observed and T_9 is on par with T_8. Chlorophyll 'b' content of foliages on different treatments of glycerinisation exhibited significant differences (Table 3). Among the treatments, T_3 (Glycerine 30% followed Ethylene glycol 30%) registered the maximum chlorophyll 'b' content for different foliages, *Myrtus* sp. (0.797 mg g^{-1}), *Thuja orientalis* (1.066 mg g^{-1}), *Eucalyptus glaucescens* (1.058 mg g^{-1}) and *Asparagus virgatus* (0.603 mg g^{-1}) . T_2 (Glycerine 25% followed Ethylene glycol 25%) also registered higher chlorophyll 'b' content for all the foliages (0.658 mg g^{-1}, 0.943 mg g^{-1}, 0.948 mg g^{-1} and 0.543 mg g^{-1}) and is on par with T_3. The least chlorophyll 'b' content was registered in T_8 for *Myrtus* sp. (0.209 mg g^{-1}), *Thuja orientalis* (0.491 mg g^{-1}), *Eucalyptus glaucescens* (0.175 mg g^{-1}) and *Asparagus virgatus* (0.335 mg g^{-1}) respectively. When the mean effects of the different glycerinisation treatments were compared, foliages have significant effect on the total chlorophyll content (Fig.1). T_3 (Glycerine 30% followed Ethylene glycol 30%) recorded the maximum total chlorophyll content for *Myrtus* sp. (0.877 mg g^{-1}), *Thuja orientalis* (1.726 mg g^{-1}), *Eucalyptus glaucescens* (1.685 mg g^{-1}) and *Asparagus virgatus* (1.406 mg g^{-1}) while T_2 (Glycerine

Table 2. Effect of glycerinisation on chlorophyll 'a' content (mg g^{-1}) of foliages

Treatment	Chlorophyll 'a' content (mg g^{-1})			
	Myrtus sp.	*Thuja orientalis*	*Eucalyptus glaucescens*	*Asparagus virgatus*
T_1	0.097_{f}	0.555_{f}	0.357_{f}	0.490_{f}
T_2	0.190_{b}	0.726_{ab}	0.623_{b}	0.752_{b}
T_3	0.206_{a}	0.758_{a}	0.665_{a}	0.823_{a}
T_4	0.144_{d}	0.660_{cd}	0.557_{c}	0.623_{d}
T_5	0.164_{c}	0.682_{bc}	0.576_{c}	0.682_{c}
T_6	0.103_{f}	0.596_{ef}	0.443_{e}	0.509_{f}
T_7	0.123_{e}	0.613_{de}	0.487_{d}	0.557_{e}
T_8	0.051_{h}	0.462_{g}	0.222_{h}	0.403_{g}
T_9	0.076_{g}	0.504_{g}	0.271_{g}	0.423_{g}
SEd	0.005	0.022	0.018	0.022
CD (p=0.05)	0.011	0.047	0.038	0.046

*Means followed by a common letter are not significantly different at the 5% level by LSD.

Table 3. Effect of glycerinisation on chlorophyll 'b' content (mg g^{-1}) of foliages

Treatment	Chlorophyll 'b' content (mg g^{-1})			
	Myrtus sp.	*Thuja orientalis*	*Eucalyptus glaucescens*	*Asparagus virgatus*
T_1	0.320_{g}	0.563_{g}	0.456_{g}	0.387_{e}
T_2	0.658_{b}	0.943_{b}	0.948_{b}	0.543_{b}
T_3	0.797_{a}	1.066_{a}	1.058_{a}	0.603_{a}
T_4	0.503_{d}	0.790_{d}	0.732_{d}	0.494_{c}
T_5	0.590_{c}	0.854_{c}	0.889_{c}	0.521_{bc}
T_6	0.385_{f}	0.625_{f}	0.524_{f}	0.413_{e}
T_7	0.456_{e}	0.701_{e}	0.648_{e}	0.456_{d}
T_8	0.209_{i}	0.491_{h}	0.175_{i}	0.335_{f}
T_9	0.263_{h}	0.553_{g}	0.250_{h}	0.350_{f}
SEd	0.019	0.028	0.027	0.017
CD (p=0.05)	0.039	0.058	0.056	0.036

*Means followed by a common letter are not significantly different at the 5% level by LSD.

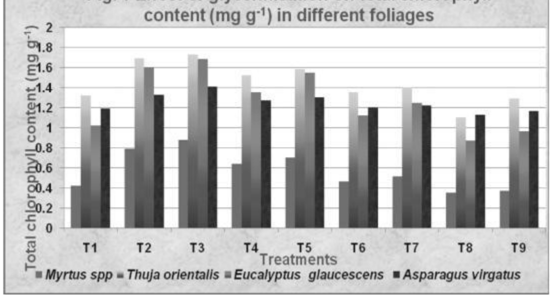

Fig. 1 Effect of glycerinisation on total chlorophyll content (mg g^{-1}) in different foliages

25% followed Ethylene glycol 25%) also registered higher total chlorophyll content for *Thuja orientalis* (1.693 mg g^{-1}), *Eucalyptus glaucescens* (1.594 mg g^{-1}) and *Asparagus virgatus* (1.325 mg g^{-1}) on par with T_3. The lowest total chlorophyll content was recorded in T_8 for Myrtus (0.348 mg g^{-1}), Thuja (1.104 mg g^{-1}), *Eucalyptus glaucescens* (0.873 mg g^{-1}) and Asparagus (1.126 mg g^{-1}). Chlorophyll is highly correlated with vitality and is an indirect measure of colour which is important for foliages (Pocklington *et al.,* 1974). The concentration of the pigments chlorophyll 'a', 'b' and total chlorophyll were high in preserved leaves (T_3).The chlorophyll being an oily pigment, minimum change in concentration might be due to minimum water loss, whereas, the low chlorophyll content in leaves treated with propylene glycol might be due to higher dehydration. Higher dehydration causes decomposition of chloroplast and disappearance of thylakoid membrane resulting in discolouration due to high leaching of pigment. Further, the pigments might have remained in the leaves but may be diffused due to senescence (Krishna Surendar *et al.,* 2013)

Sensory scoring

Various treatments of glycerinisation on foliages differed significantly for texture and shape retention. Glycerine 30% followed Ethylene glycol 30% (T_3) recorded (Table 4.) the highest score on texture for *Myrtus* sp. (4.67), *Thuja orientalis* (4.67), *Eucalyptus glaucescens* (4.33) and *Asparagus virgatus* (4.67). T_8 recorded the lowest sensory score of 1.00 for texture in Myrtus, Eucalyptus and Asparagus. T_3 registered the maximum score on shape retention for all the foliages tried *viz., Myrtus* sp. (5.00), *Thuja orientalis* (5.00), *Eucalyptus glaucescens* (4.67) and *Asparagus virgatus* (5.00) (Fig. 2).

Table 4. Sensory scoring on texture of foliages under different glycerinisation treatments

Treatment	Sensory scores			
	Myrtus sp.	*Thuja orientalis*	*Eucalyptus glaucescens*	*Asparagus virgatus*
T_1	3.00$_e$	2.33$_e$	3.00$_e$	2.67$_e$
T_2	4.33$_b$	4.33$_b$	4.00$_b$	4.33$_b$
T_3	4.67$_a$	4.67$_a$	4.33$_a$	4.67$_a$
T_4	3.67$_d$	3.67$_d$	3.33$_d$	3.33$_d$
T_5	4.00$_c$	4.00$_c$	3.67$_c$	4.00$_c$
T_6	2.33$_g$	2.00$_f$	2.33$_g$	2.00$_g$
T_7	2.67$_f$	2.33$_e$	2.67$_f$	2.33$_f$
T_8	1.00$_i$	1.33$_h$	1.00$_i$	1.00$_i$
T_9	1.33$_h$	1.67$_g$	1.33$_h$	1.33$_h$
SEd	0.120	0.118	0.112	0.117
CD (p=0.05)	0.252	0.248	0.236	0.246

*Means followed by a common letter are not significantly different at the 5% level by LSD.Scoring scale – 5 - I like extremely, 4 - I like moderately, 3 –I neither like nor dislike, 2 - I dislike moderately, 1- I dislike extremely

Qualitative criteria are centered around the visual look of the foliage, how it feels or how easy it is to work with as well as customer acceptance of the product. Visual scores were best for leaves preserved by primary soaking in Glycerine 30 % followed by secondary soaking in Ethylene glycol 30%. These leaves were associated with good texture and highest shape retention. Undoubtedly,

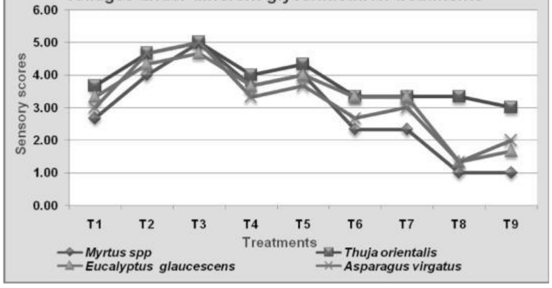

Fig. 2 Sensory scoring on shape retention of foliages under different glycerinisation treatments

the desirable quantitative criteria *viz*., less moisture loss and high pigment content might have naturally resulted in the high scores for the qualitative criteria.

Glycerinization of plant materials helps them to last longer apart from keeping them more pliable and retaining their natural shape. Further, it could be realized in the present study that, not only the choice of solvents, but also the sequence of solvents greatly influences the final quality of preserved botanicals. Improper choice of solvents and sequence of their usage can either cause bleeding or complete shrinkage of the plant materials. As an improved glycerinisation technique (T_3) 30 per cent Glycerine followed by 30 per cent Ethylene glycol resulted in soft near to natural leaves with minimum moisture loss with maximum colour and shape retention.

Acknowledgement

This study was funded by the National Agricultural Innovation Project - ICAR- NAIP – TNAU – "A value chain on flowers for domestic and export markets". The authors, therefore, acknowledge with thanks NAIP for the technical and financial support

References

Conder, S. 1979. In: *Commercial dried flowers***.** Merechurst Publication, London. pp. 60-75

Guerfel, M., Baccouri, O., Boujnah,D., Chaibi, W. and Zarrouk, M. 2008. Impacts of water stress on gas exchange, water relations, chlorophyll content and leaf structure in the two main Tunisian olive (*Olea europaea* L.) cultivars*. Scientia Hort.,* **119:** 257-263.

Ito, H., Hayashi,T., Hashimoto, M., Miyagawa, K., Nakamura, S., Mizuta, Y. and Yazawa, S. 2010. A Protocol for Preparing Preserved Flowers with Natural Color and Texture. *Hort Technology***, 20(2):** 445-448

Joyce, D. C.1998. Dried and preserved ornamental plant material: Not new, but oftern overlooked and underrated. Proc. Third Int. Symp. on New Floricultural Crops. Eds. J. A. Considine. J. Gibbs, ISHS, *Acta Hort.,* **454:**133-145

Krishna Surendar, K., Durga Devi, D., Ravi, I., Jeyakumar, P., Ramesh Kumar, S. and Velayudham, K. 2013. Impact of Water Deficit on Epicuticular Wax, Proline and Free Amino Acid Content and Yield of Banana Cultivars and Hybrids. *Plant Gene and Trait*, **4(6)**

Leonard Karel. 1973. Dried grasses, grains, gourds, pods, and cones. The Scarecrow Press Inc., Metuchen.

Mark Koch. 1995. Preserving Flowers & Foliage with Glycols & Dyes: A Manual for the Commercial Grower. Bennett, CO: Robert Koch Industries

Morrison, R. T. and Boyd, R. N. 1959. Organic chemistry. Allyn and Bacon, Inc., Boston.

Panse, V. G. and Sukhatame, P. V. 1985. Statistical methods for agricultural workers. ICAR, New Delhi.

Peryam, D. R. 1957. The 9- point hedonic scale. Introduction by Dr. Edgar chambers, I*V*. Peryam and Kroll research corporation .

Pocklington, T. E., Butler, J. D. and Hodges, T. K. 1974. Color evaluation of *Poa pratensis* cultivars. *J. Sports Turf Res. Inst.,* **66**: 134–140.

Ranganna, S. 1977. Manual of fruit and vegetable products. Tata Mc Graw –Hill Pub. Co. Ltd. New Delhi.

Ranjan, J. K. and Misra. S. 2002. Dried flowers: a way to enjoy their beauty for long period. *Indian Horticulture***, 47 (4):** 32-33.

Raymond Chang. 1984. Chemistry. Random House, New York.

White P., Tjia, B., Marion, R., Sheehan, R. and Brown, S. P. 2007 Drying and Preserving plant materials. Environment Horticulture Department, *Florida Cooperative Extension service*, Institute of Food and Agricultural Sciences, University of Florida. Document no. CIR495

Yoshida, T., Tada, K. and Arakawa, T. S. 1971. Vitamin B6 dependency of glutamic acid decarboxylase in the kidney from a patient with vitamin B6 dependent convulsion. *Tohoku J. Exp. Med.,* **104:** 195-198.

Received: August 21, 2013; Revised: October 24, 2014; Accepted: December 24, 2014