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Research Article

A comprehensive study on the production of potassium sorbate and sodium benzoate reference material in orange juice

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Abstract

Controlling the content of preservatives in soft drinks is of paramount important for assessing their quality during production and consumption, as well as for ensuring consumer health. To effectively enhance laboratory capacity in preservatives analysis, the requirement for reference materials (RMs) of non-alcoholic beverages containing common preservatives such as potassium sorbate and sodium benzoate is fundamentally essential. This research was conducted to provide a procedure for the production of reference materials for potassium sorbate and sodium benzoate in orange juice. The concentration of potassium sorbate and sodium benzoate in the reference materials complied with Circular No.24/2019/TT-BYT which regulates the management and use of food additives. The two RMs of natural and flavored orange juice were homogeneous and stable, however, their quality and stability were strongly affected by the storage temperature. At a particular condition of 5°C, synthetic orange juice witnessed a higher stability than natural at 532 days and 318 days, respectively. Furthermore, an accelerated aging study demonstrated that at -20°C, the integrity of potassium sorbate and sodium benzoate in natural orange juice was identically maintained for a substantial period of almost 3 years (expected shelf life 1,049 days).

Keywords: *Reference material, potassium sorbate, sodium benzoate, orange juice.*

1. INTRODUCTION

Food preservation plays an essential role in the production, processing, distribution and consumption of products. Food additions are widely developed in food production and processing, in order to ensure safety, extend shelf life, and maintain the desired characteristics of the products. However, scientific studies on the use of food additions in Vietnam [1, 2] indicated that there has been a considerable increase in the number of cases

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of unregulated and excess expenditure of preservatives, including potassium sorbate and sodium benzoate. Therefore, regulating the content of preservatives is a crucial requirement in both production and consumption in order to protect consumers health.

The tropical climate in Vietnam periodically facilitates an increasing demand for non-alcoholic beverages. Maintaining quality and extending expiration period of such products has been commonly achieved by using preservatives such as potassium sorbate and sodium benzoate. According to regulations in Vietnam, the threshold limits for potassium sorbate (calculated as sorbic acid) and sodium benzoate (calculated as benzoic acid) are 500 mg/kg and 250 mg/kg, respectively in flavored beverages while both the limits in fruit juice are set at 1,000 mg/kg [3].

Laboratory-available reference materials are crucial for all stages of measurement process, including method validation, calibration, and quality control. RMs are also employed in interlaboratory comparisons to validate methods and evaluate laboratory proficiency [4]. In Vietnam, there is currently no research on food-based preservative reference materials. Globally, in addition to the pure certified reference materials (CRMs) of potassium sorbate and sodium benzoate provided by reputable suppliers, a significant minority of RMs in food matrices for analyzing preservatives have been announced, e.g., the benzoate RM in Cola provided by the Food Analysis Performance Assessment Scheme (FAPAS) in the UK [5]. Consequently, research on the production of reference materials is fundamental to evaluate the conformity of preservative content in non-alcoholic beverage products.

Orange juice is famous in Vietnam and globally recognized for its thirst-quenching effect and appealing flavor. However, the difficulties in the production and preservation of orange juice are to eliminate the bitterness, prevent discoloration or separation [6], and to meet the quality requirements as prescribed, including microbiological criteria [7]. As a result, the main purpose of this study is to produce RMs of potassium sorbate and sodium benzoate in 02 matrices, namely flavored orange juice and natural orange juice, and also evaluate the characterization of the two RMs and their stability (accelerated aging and real-time) in according to ISO 17034:2016 [4]. The optimized process for RMs production will hence be carefully discussed and reported in this paper.

2. MATERIALS AND METHODS

2.1. Raw materials

The materials used in the study include natural orange juice (Vietnam) and orange flavored beverage (flavored orange) which was produced from white sugar (Vietnam), orange flavor (Singapore), and sunset yellow (China). The packaging material was 50 mL HDPE plastic bottle (China).

2.2. Chemicals, standards

The chemicals used in the study were provided by reputable companies and achieved the required purity such as: 99.7% of potassium sorbate standards from Sigma and 99.4% of sodium benzoate from Accustandard (USA); acetonitrile, methanol (gradient grade for liquid chromatography); purified water (from a Milli–Q Integral 3 purifier; Millipore, Billerica, MA), and others common laboratory chemicals.

2.3. Equipment

The equipment applied in RMs production includes: Automatic liquid sample mixing and capping system, Heat sealing and capping machine (LX 6000A) and other basic laboratory equipment. Analysis of potassium sorbate and sodium benzoate content was performed using high performance liquid chromatography (HPLC Water), PAD detector with wavelength range of 190 to 800 nm.

2.4. Analysis method

Potassium sorbate and sodium benzoate were analyzed at the National Institute for Food Control (NIFC) by HPLC refer to ISO 22855:2008 [8] and Chen *et al.* [9]. This analytical method has been accredited in accordance with ISO/IEC 17025:2017 [10]. After homogenization, the analytes are extracted from the sample matrix by purified water, following by 0.45 µm membrane filtration. Potassium sorbate and sodium benzoate were then quantified by HPLC with PDA detector at 223 nm (sodium benzoate) and 254 nm (potassium sorbate).

Microbiological tests (Total plate count, Coliform, *E. coli, Streptococci faecal, Pseudomonas aeruginosa, Staphylococcus aureus, Clostridium perfringens,* and Total yeasts and molds) were performed at NIFC according to the guidelines of current national standards [11-18]. These analytical methods have been accredited in accordance with ISO/IEC 17025:2017 [10].

2.5. Production of RMs

2.5.1. Investigation of production factors

On the basis of reference documents [19-21], pilot production was carried out to select raw materials and evaluate some vague factors which are appropriate for RM production. In this step, a small-scale batch (pilot batch) of natural orange juice was produced to evaluate the following factors:

- Preliminary assessment of homogeneity;

- Stability assessment at 3 storage temperatures: 5 ± 3°C, 25 ± 3°C and 45 ± 3°C (accelerated aging) in order to evaluate the impact of temperature on the stability of the preservative RM and optimize storage conditions;

- Experiment and selection of sterilization methods: (1) 120°C for 20 minutes and (2) 105°C for 10 minutes.

2.5.2. Production of RMs of preservatives in orange juice

Flavored orange juice contains sugar (8 ÷ 12% w/v), orange flavor (0.15% w/v) and sunset yellow colorant (50 ÷ 70 ppm) [9]. By consulting reference [20], the preparation of flavored orange is presented in Figure 1.

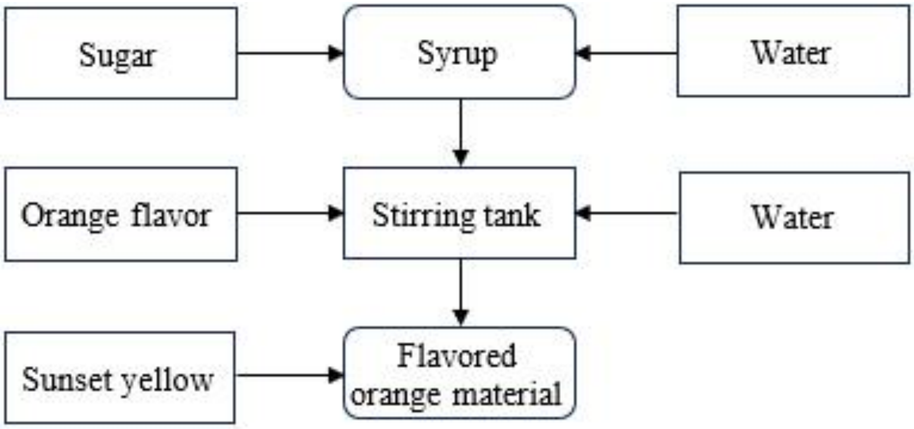

Figure 1. *Process of flavored orange material preparation*

The production process of orange juice RMs [21] (natural and flavored) is shown in Figure 2.

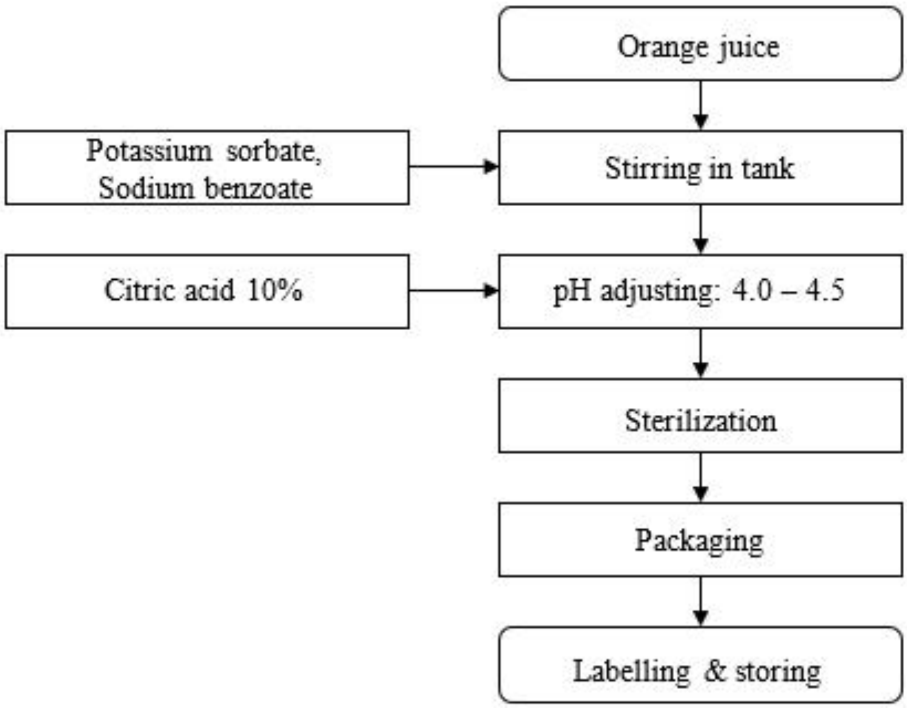

Figure 2. *Production process of orange juice RMs*

2.6. Evaluation of homogeneity

Ten items of each RM (natural orange juice and flavored orange juice) were analyzed for homogeneity assessment of preservative contents. Duplicate data is collected to evaluate the homogeneity of production batch following ISO Guide 35:2017 [22] and ISO 13528:2022 [23].

2.7. Organization of proficiency testing and evaluation of the characteristics of RMs

The orange juice RMs (natural and flavored) were utilized in the proficiency testing scheme (PT) organized by NIFC with 17 participants. The results of competent laboratories (accredited to meet the requirements of ISO/IEC 17025:2017 [10] for the analytical criteria or having participated in the PT organized by NIFC with satisfactory results of potassium sorbate and sodium benzoate in the soft drinks) whom value of (│z│ or │z'│) ≤ 1.0 were selected to calculate the certification value of the RMs.

The certification value of the RMs is calculated according to formula (1):

$$x_{CRM} = \frac{\sum x_i}{p} \quad (1)$$

Where: x_{CRM}: characteristic value of a certified reference material (CRM); x_i: value of the i-th laboratory and p: number of qualified laboratories selected.

The measurement uncertainty of the certification characteristic is calculated according to formula (2):

$$u_{CRM} = \sqrt{u_{char}^2 + u_{hom}^2 + u_{lst}^2} \quad (2)$$

Where: u_{CRM}: the standard uncertainty associated with property value of the CRM; u_{char}: the standard uncertainty associated with a value assigned in a characterization study; u_{hom}: the standard uncertainty associated with heterogeneity; u_{lts}: the standard uncertainty associated with long term stability.

2.8. Stability study

2.8.1. Assessment of long-term stability under low-temperature storage conditions

To evaluate the stability, the two RMs were tested for sodium benzoate and potassium sorbate content every 2nd week of the month from the production date within 4 months at the time points: 8, 37, 69, 99 and 128 days. The stability was studied in real time according to ISO Guide 35:2017 [22] and ISO 13528:2022 [23] by testing results of items stored at 5 ± 3°C.

2.8.2. Accelerated aging study

The RM items were studied under two storage conditions: room temperature (25 ± 3°C, humidity ≤ 70%) and storage in microclimate cabinet (45 ± 3°C, humidity: 75 ± 5%). The RMs were tested for sodium benzoate and potassium sorbate content at the same points as the real-time stability assessment.

The estimated shelf life of a RM is determined by formula (3):

$$F_1 = F_2 \times Q_{10}^{(\Delta T/10)} \quad (3)$$

Where: F_1: Shelf life at storage temperature; F_2: Shelf life at accelerated aging temperature; Q_{10}: Temperature coefficient; ΔT: Temperature difference between the two storage conditions.

3. RESULTS AND DISCUSSION

3.1. Investigation of production conditions

3.1.1. Evaluation of homogeneity

After the pilot production in natural orange juice, the samples were analyzed for homogeneity assessment of potassium sorbate and sodium benzoate, the results are presented in Table 1.

Table 1. *Homogeneity evaluation of potassium sorbate and sodium benzoate in pilot samples*

#	Content (mg/L)			
	Potassium sorbate		**Sodium benzoate**	
	1st time	**2nd time**	**1st time**	**2nd time**
1	97.1	98.7	101.0	98.8
2	98.5	95.9	96.6	98.8
3	96.5	96.3	97.6	98.8
4	97.1	97.1	96.5	98.9
5	97.6	96.5	101.0	98.8
	p = 0.7159		**p = 0.4981**	

The results of the one-way ANOVA analysis showed that p-value of potassium sorbate and sodium benzoate were greater than 0.05. Therefore, the pilot batch was homogeneity.

3.1.2. Estimate the impact of storage temperature on pilot samples and select the optimized storage temperature

To evaluate the impact of storage temperature on the stability of potassium sorbate and sodium benzoate content, pilot samples were studied at 03 different storage temperatures (5 ± 3°C, 25 ± 3°C and 45 ± 3°C) after 40 days and 76 days from the production date. The results are showed in Table 2.

The results of the two-way ANOVA analysis with 3-time repetitions (n = 3) showed that the storage temperature has an impact on potassium sorbate and sodium benzoate content.

Table 2. *Potassium sorbate and sodium benzoate in RMs at different storage temperatures*

Storage temperature	Content (mg/L)/ Storage time			
	Potassium sorbate		**Sodium benzoate**	
	40 days	**76 days**	**40 days**	**76 days**
5°C	96.05	91.20	98.55	96.30
	96.15	91.25	98.65	96.60
	96.50	92.15	97.80	97.15
25°C	93.70	92.35	96.95	94.65
	95.90	88.55	95.45	93.10
	93.95	87.55	96.15	94.55
45°C	90.55	70.05	94.40	87.35
	89.50	61.10	93.75	90.80
	91.30	56.65	94.30	88.15

Based on mentioned data, the linear equations of the analytes are obtained from multivariate linear regression analysis as follows:

Potassium sorbate: $y = 118.5 - 0.434\ t_1 - 0.348\ t_2$

Sodium benzoate: $y = 103.7 - 0.155\ t_1 - 0.087\ t_2$

Where: t_1: storage temperature; t_2: storage time.

The equations showed that the content of potassium sorbate and sodium benzoate reduce gradually when temperature and time of storage are increased. Therefore, the samples are stored at 5°C have the greatest stability of preservative content, comparing to those that are kept at higher temperature (25°C and 45°C). Thus, the temperature of 5 ± 3°C was selected for storing RMs of preservatives in orange juice matrices.

3.1.3. Evaluation and selection of sterilization

Pilot samples were sterilized by 02 methods: (1) 120°C for 20 minutes and (2) 105°C for 10 minutes. To evaluate the sterilization efficacy of these 02 methods, 08 microbiological tests such as: Total plate count, Coliform, *E. coli*, *Streptococci faecal*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Clostridium perfringens* and Total yeasts and molds were performed and assessed whether they were compliance with QCVN 6-2:2010/BYT [7] at 01 day, 40 days and 76 days from the production date. The results which were "Not detected" for all test samples indicates that both 02 sterilization methods were effective.

Method (2) was selected due to its efficiency in saving time and energy. Furthermore, high temperature sterilization might cause compositional changes in natural orange juice [20].

3.2. RM production

After considering the experimental results of the pilot samples, the RMs were produced following the processes presented in Section 2.5.2. With expected quantity of each RM sample is 120 bottles, the quantity of additional preservatives is presented in Table 3 and Table 4.

Table 3. *Quantity of additional preservatives in flavored orange juice sample*

#	Name of additional preservatives	Expected concentration (mg/L)	Total sample volume (120 bottles x 50 mL/bottle)	Quantity of additional preservatives (g)
1	Potassium sorbate	500	6.0 L	3.0
2	Sodium benzoate	250		1.5

Table 4. *Quantity of additional preservatives in natural orange juice sample*

#	Name of additional preservative	Expected concentration (mg/L)	Total sample volume (120 bottles x 50 mL/bottle)	Quantity of additional preservatives (g)
1	Potassium sorbate	1000	6.0 L	6.0
2	Sodium benzoate	1000		6.0

The final samples were then stored at temperature 5 ± 3°C.

3.3. Evaluation of homogeneity

Homogeneity is the prior and the most important criteria to ensure that all the items of finished products in a production batch have the same characteristics and assigned value. To evaluate the homogeneity, the content of potassium sorbate and sodium benzoate was analyzed. The testing results were presented in Table 5.

Table 5. *Concentration of preservatives in RM samples*

#	Concentration (mg/L)							
	Flavored orange juice				Natural orange juice			
	Potassium sorbate		Sodium benzoate		Potassium sorbate		Sodium benzoate	
	1st time	2nd time	1st time	2nd time	1st time	2nd time	1st time	2nd time
1	483	483	218	199	915	926	889	902
2	481	480	220	229	903	929	876	894
3	487	483	214	229	924	917	879	894
4	485	480	215	222	921	932	875	860
5	485	497	219	232	916	926	874	905
6	485	480	235	221	913	911	874	882
7	486	476	217	224	942	925	913	872
8	483	482	210	223	921	925	886	908
9	480	474	216	228	918	901	872	899
10	483	480	222	219	902	910	870	887
	p = 0.248		**p = 0.752**		**p = 0.287**		**p = 0.620**	

The results of one-factor ANOVA analysis showed that the p-value of potassium sorbate and sodium benzoate were greater than 0.05. The RM samples reach the required homogeneity.

The studied concentration of preservatives was significantly lower than expected concentration presented in section 3.2, revealed that beside the production error, sterilization process could be responsible for the content reduction of preservative in orange juice matrices.

3.4. Results of certification value

After passing the homogeneity assessment, RMs of natural orange juice and flavored orange juice were utilized in the PT organized by NIFC with 17 participants (who met the requirements as described in section 2.7). The results of this scheme were presented in Table 6.

According to ISO Guide 35:2017 [22], certified value of reference material can be determined by different methods. In which, using analysis data of proficient laboratory network is one of the most commonly used methods. In this study, the laboratories that were accredited in accordance with the requirements of ISO/IEC 17025:2017 [10] for the registered analytes or had satisfactory results of previous proficiency testing organized by the NIFC for potassium sorbate and sodium benzoate in beverage matrices were selected to participate in the scheme.

Table 6. *Content of potassium sorbate and sodium benzoate in RM natural orange juice and flavored orange juice*

#	Flavored orange juice				Natural orange juice			
	Potassium sorbate		Sodium benzoate		Potassium sorbate		Sodium benzoate	
	Result (mg/L)	z'-score	Result (mg/L)	z'-score	Result (mg/L)	z'-score	Result (mg/L)	z'-score
1	441	-0.53	228	0.57	885	-0.8	880	0.10
2	489	0.93	225	0.27	988	1.51	962	2.37
3	490	0.96	250	2.40	960	0.89	883	0.20
4	467	0.27	228	0.53	919	-0.03	939	1.75
5	420	-1.16	216	-0.49	863	-1.28	816	-1.66
6	480	0.67	582	30.57	954	0.75	883	0.20
7	447	-0.34	220	-0.19	832	-1.97	795	-2.24
8	453	-0.17	171	-4.33	895	-0.57	654	-6.13
9	449	-0.29	219	-0.21	903	-0.39	860	-0.45
10	468	0.28	218	-0.28	954	0.75	872	-0.12
11	431	-0.83	222	-0.02	944	0.54	891	0.42
12	482	0.71	222	0.00	905	-0.34	792	-2.33
13	423	-1.07	199	-1.92	865	-1.24	815	-1.68
14	500	1.26	235	1.13	926	0.12	856	-0.55
15	301	-4.76	188	-2.87	938	0.39	893	0.49
16	481	0.68	-	-	960	0.90	900	0.67
17	466	0.24	237	1.25	926	0.13	898	0.62

From the results of the laboratories with |z'-core| < 1, applying formula (1) in section 2.7, the certified value of potassium sorbate and sodium benzoate content of flavored orange juice RM were determined to be 468 and 222 mg/L, respectively, and those of natural orange juice RM were 928 and 882 mg/L, respectively.

3.5. Determination of estimated shelf life and uncertainty at storage condition (5°C)

RMs that were stored at 5°C were monitored for potassium sorbate and sodium benzoate content in 4 months at 04 sampling dates: 37 days, 69 days, 99 days, and 128 days from the production date. The data were used to establish the stability graph for the content of potassium sorbate and sodium benzoate of the RMs (for example, the potassium sorbate content in flavored orange juice RM is shown in Figure 3).

The estimated shelf life of the RM is determined when the linear line intersects the upper or lower acceptance range of the certified value (X_{CRM}). The uncertainty of the certified value is determined based on formula (2) in section 2.7. The results of the estimated shelf life, certified values , and uncertainties of the potassium sorbate and sodium benzoate RMs in natural orange juice and flavored ones were presented in Table 7.

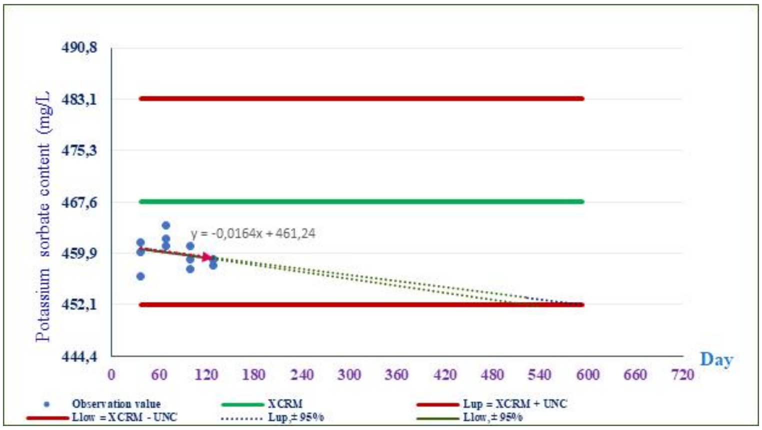

Figure 3. *Stability graph for potassium sorbate in flavored orange juice*

Long-term stability studies at storage conditions (5 ± 3°C) showed that the estimated shelf life of flavored orange juice RM was significantly longer than that of natural orange juice. This suggests that in addition to storage temperature, the stability of preservative content is also affected by the components of RMs, especially in natural orange juice RM.

Table 7. *Estimated shelf life, certified value and uncertainty of the RMs*

RM matrices	Estimated shelf life (day)	Certified value (mg/L)		Measurement uncertainty U_{CRM} (k=2)
Flavored orange juice	524	Potassium sorbate	468	24.6
		Sodium benzoate	222	15.6
Natural orange juice	318	Potassium sorbate	928	32.8
		Sodium benzoate	882	31.8

3.6. Accelerated aging study

In the accelerated aging study, the content of potassium sorbate and sodium benzoate were analyzed monthly under 02 storage conditions: room temperature (25 ± 3°C, humidity ≤ 70%) and in a climate chamber (45 ± 3°C, humidity: 75 ± 5%). The study is stopped when the RMs does not reach the stability for the preservative content.

According to formula (3) section 2.8, the Q_{10} coefficient of potassium sorbate and sodium benzoate in the RM flavored orange juice were determined to be 1.82 and 1.77, respectively, and in the RM natural orange juice, it is 1.61 and 1.58, respectively. The estimated shelf life of RM samples using the Q_{10} method is showed in Table 8.

Table 8. *Estimated shelf life of RM samples at different storage temperatures*

Preservative	Flavored orange juice		Natural orange juice	
	Estimated shelf life at 25°C (day)	**Estimated shelf life at 5°C (day)**	**Estimated shelf life at 5°C (day)**	**Estimated shelf life at -20°C (day)**
Potassium sorbate	159	526	320	1,049
Sodium benzoate	173	544	345	1,075
RM	**159**	**526**	**320**	**1,049**

The shelf life estimated from the accelerated aging study was similar to the long-term stability calculation (318 days for natural orange juice RM and 524 days for flavored orange juice RM). However, natural orange juice RM, if stored at a temperature of 5°C, has a shorter expected shelf life (318 days, equivalent to about 10 months) than the benzoic acid RM in Cola (stored at -20°C, shelf life 02 years) of FAPAS [5]. At a storage temperature of -20°C, the expected shelf life of the RM was greatly improved (about 1,049 days).

4. CONCLUSION

The study has established a standardized process for the production of RMs of potassium sorbate and sodium benzoate in matrices of natural orange juice and flavored orange juice. The estimated stability of these RMs is 318 days and 524 days, respectively, when stored at a temperature of 5 ± 3°C. The certified values and their uncertainties for potassium sorbate and sodium benzoate are 468 ± 24.6 mg/L and 222 ± 15.6 mg/L in the flavored orange juice RM, and 928 ± 32.8 mg/L and 882 ± 31.8 mg/L in the natural orange juice RM. To enhance stability, it is proposed that the natural orange juice RM be stored and monitored at -20°C, with an expected stability of approximately 1,049 days. Furthermore, due to the limitations of the study framework, future research should investigate the impact of the natural orange juice composition on the stability of the RMs.

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