# **Comparative Evaluation of Polyphosphates, Acids and Process Treatment Combinations for Tropical Preservation of Orange Juice**

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#### *A BSTRA CT*

*Fresh orange juice product formulations were prepared and subjected to preservative stability and organoleptic characterization tests, in order to assess the relative potential suitabilities of various agents as orange juice preservatives under the adverse prevalent tropical conditions of high humidity, high temperature and high environmental contamination situations. The preservative process treatments included the use of various chemical agents like food grade polyphosphates,*  $SO<sub>2</sub>$  *as a metabisulphite and organic acids* with or without prior pasteurization and storage under tropical ambient *temperature (30-35°C) or refrigerated storage at 12°C.* 

*Palatable and shelf-stable formulations were obtained by:* 

- *(a) the incorporation of 0"0025% (w/v) potassium metabisulphite without prior pasteurization or subsequent refrigerated storage;*
- *(b) pasteurization at 80°C for 1 min coupled with post-pasteurization addition of 0"05% (w/v) propionic acid under ambient storage*

*and* 

*(c) incorporation of 0"05% (w/v) propionic acid alone coupled with refrigerated storage at 12°C.* 

*Of the tested agents, propionic acid was the best acid for achieving increased preservative efficacy and organoleptic properties enhancement.*  Although satisfactory preservative efficacy was achieved by the combined use *of pasteurization, 0"05% (w/v) maleic acid incorporation and refrigerated* 

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*storage at 12°C, resultant formulations lacked satisfactory organoleptic characteristics.* 

*The polyphosphates were generally relatively ineffective at ambient storage conditions although their efficacies were enhanced under refrigeration storage at 12°C when counts were kept below 106 cfu/ml for 12 days. Fibrisol sodium tripolyphosphate-S-600 was foundmost effective of the tested polyphosphates and a combination of 0"025% (w/v) each of propionic acid and Fibrisol sodium tripolyphosphate-S-600 in conjunction with refrigeration could be*  substituted for 0<sup>.</sup>05% (w/v) propionic acid alone without undue compromise *of the preservative effectiveness. The chain lengths and relative stabilities of polyphosphates in the orange juice system and the relative residual molecular concentrations of the undissociated forms of the acids, as well as adaptive resistance capability of the natural contaminant cells to the acid agents, are factors considered to be at play in determining the demonstrated preservative efficacies.* 

### INTRODUCTION

Oranges *(Citrus sinensis)* are among the group of palatable citrus fruits consumed either as peeled whole fruit or in the form of extracted juice. The popular human consumption of orange juice is attributed to its favourable organoleptic characteristics which have been described by consumers as flavourful, aromatic, colourful, healthful, natural and refreshing (Cook, 1983). Orange juice constitutes a good nutritional source of ascorbic acid (vitamin C) whose deficiency causes scurvy (Duckworth, 1966). In addition, it could serve as a ready source of easily metabolizable sugars, principally sucrose and hexoses like fructose and glucose as well as pentoses, vitamins of the A and B types, especially  $\beta$ -carotene (pro-vitamin A) and folic acid, thiamine plus minerals like calcium, potassium and iron (Duckworth, 1966; McCready, 1977; ICMSF, 1980a; Cook, 1983).

The chemical characteristics of orange juice, including a low pH value of approximately 3, resulting from the organic acids contents, coupled with high sugar content, all combine with other antimicrobial factors to discourage, but not totally prevent, microbial spoilage (ICMSF, 1980a). Consequently, perishability in the fresh state, induced by microbial contaminants and inherent enzymes, constitutes a problem. Among the spoilage agents, yeasts predominate, being capable of turbidity development, flocculation, pellicles and clumping, while the pectinesterase-producing ones could destroy the natural pectin cloud (ICMSF, 1980a). Furthermore, yeast contaminants have been reported to be capable of degradation of component organic acids of the orange juice, thereby raising the pH value coupled with the formation of acetaldehyde which contributes a fermented flavour (ICMSF, 1980a). These derive from the innate physiological and

metabolic capabilities of yeasts for tolerating the ecological selective physiological conditions obtainable in orange juice (Marshal & Walkley, 1951a, b; Recca & Mrak, 1952; Mossel & Sholts, 1964; ICMSF, 1980a).

Published findings have also indicated that a good proportion of orange production may be lost through spoilage, especially in developing countries despite increasing demands (Duckworth, 1966). An alternative remedy is the post-harvest extraction and preservation of the juice for out-of-season consumption supply purposes, which constitutes an approach that is already being commercially exploited (ICMSF, 1980a). Nonetheless, investigations have continually been aimed at developing and improving methods for controlling deteriorative changes by using physical and/or chemical process treatments (ICMSF, 1980a). These have been focused in two major directions; namely, the production of frozen juice concentrates and the alternative shelf-stable juice products (Tillotson, 1984). Research efforts have also been directed toward processing and preserving juices for shelfstable products through formulation efforts and microbiological studies to determine the effectiveness of control measures against spoilage organisms while minimizing product damage and retaining consumer appeal (Tillotson, 1984).

Thus, in tropical regions with inadequate technological facilities, high tempecature and humidity conditions, as well as the prevalent high environmental contamination situations, research efforts should be directed towards the production of shelf-stable juice products. This is in view of the anticipated high energy costs that would be involved in the production and storage of frozen juice concentrates as alternatives. Thus, in the current study, investigations were directed towards assessing the extent of shelfstability derivable from formulations with chemical agents and process treatments aimed at preserving fresh orange juice as well as defining the associated influence of such on the organoleptic characteristics of resultant products.

Tested chemical agents were, however, limited to natural organic acid components of fruits, polyphosphates and some anti-spoilage agents permitted in foods, for comparative purposes. Particular interest in polyphosphate evaluation was motivated by their reported antimicrobial usage in other food systems, including bakery products and flesh food systems, for various purposes including mycotic inhibition (Ellinger, 1972).

## MATERIALS AND METHODS

#### **Orange juice preparation**

Freshly harvested firm, healthy and ripe oranges without any noticeable injury were obtained from a local farm. These were washed, peeled and sliced

using a pre-sterilized knife after which the component juice was extracted by manual squeezing into a sterile 3-1itre flask, taking realistic hygienic precautions to minimize extraneous contamination. After mixing, the whole extracted juice was immediately dispensed in 20-ml volumes into sterile plastic disposable bottles and stored frozen at  $-18^{\circ}$ C until required.

# **Chemical analysis of the orange juice**

The ascorbic acid content was determined by the 2,6-dichloroindophenol method described by the AOAC (1975) while the pH was measured on a Radiometer m-26 pH meter (Radiometer A/S, Copenhagen, Denmark). Total nitrogen was determined by the semi-micro Kjeldahl method (Pearson, 1976) and total soluble solutes was determined by the AOAC (1975) method while total ash was also determined as described by Pearson (1976).

# **Chemical agents preparation**

The chemical agents screened for their preservative efficacies included propionic, maleic and fumaric acids (Sigma Chemical Company, St. Louis, Missouri, USA) as well as citric and succinic acids (BDH Poole, Dorset). All the polyphosphates used were of food grade quality including Fibrisol-Sodium tripolyphosphate S-600, Curaphos-700-instant, Tetrasodiumpyrophosphate, Fibrisol N7 and Acid Sodium pyrophosphate. The potassium metabisulphite SLR grade, used in this study as  $SO_2$  source (Ough, 1983) was obtained from Fisons (Loughborough, Leicestershire). These were prepared as separate 10% stock solutions in deionized water prior to filter sterilization using  $0.45 \mu m$  membrane filters.

# **Orange juice preservative formulations**

Test samples equilibrated to room temperature were formulated in 10-ml volumes each by incorporating the preservative agents separately at 0.01% and  $0.05\%$  w/v concentration levels. This involved pipetting 0.1 ml and 0.5 ml of the filter-sterilized preservative stock solution into 9.9 ml or 9.5 ml of the juice, respectively, and mixing to ensure uniform distribution. When mixture combinations of two agents were to be evaluated,  $0.025\%$  w/v of each agent was added to give the total  $0.05\%$  w/v final concentration of the combination of the two agents. Potassium metabisulphite was subsequently tested alone at  $0.0025$  (w/v) concentration level after higher concentrations had proved too inhibitory to permit countable growth. All formulations were contained in sterile screw-capped glass bottles.

# **Pasteurization treatment**

Sample pasteurization was achieved by immersing the juice-containing bottles into a thermostatically maintained water bath at 80°C for 7 min with concomitant agitation. This treatment equated with an effective heat treatment at  $80^{\circ}$ C for 1 min at the sample thermal centre as monitored by an inserted thermometer.

### **Preservative efficacy evaluation procedure**

Immediately after formulations, samples were withdrawn and plated on appropriately dried tryptone soya agar (TSA) and malt extract agar (MEA) plates by a drop count method based on the Miles & Misra (1938) technique. Such plates were then incubated at 37°C until consistent counts were obtained. This was to determine the initial contamination level. Subsequently, the formulated test samples in duplicates were stored at both ambient temperature (30-35°C) and under refrigeration at 12°C.

At timed intervals during storage, the formulations were sampled and plated as above to determine the degree of growth of the contaminant population. The efficacy of preservative pasteurization, as described above, was also determined by sampling for cell count immediately after cooling to ambient temperature and at timed intervals in the course of storage. In addition, where the combination of pasteurization and chemical agents were under investigation, the latter agents were only added after carrying out the pasteurization treatment and rapid cooling to ambient temperature under running tap water. Routine sampling was also carried out in such cases as before.

### **Sensory evaluation**

A six-member taste panel was randomly constituted comprising workers, students and staff members. The panelists, randomly picked, were neither prescreened nor trained and consisted of three males and three females all aged between 20 and 40 years. Bulk fresh formulations of the orange juice were made, from which taste samples were dispensed in equal amounts into white plastic cups for taste assessment. Taste samples were randomly arranged and coded with three figure randomly chosen numbers. Panelists were independently required to score their overall acceptability of samples using a 9-point hedonic scale where 1 corresponds to 'dislike extremely' and 9 to 'like extremely', on the score sheets. The panel members were, in addition to overall acceptability scoring, asked to evaluate the organoleptic characteristics of samples in terms of appearance (colour), taste (flavour) and aroma (smell). Descriptive terms were supplied to aid in judging sample characteristics including whether sample colours were pale, normal or otherwise, whether samples smelled fresh, stale, pungent or otherwise and whether samples tasted bitter, sour, good or otherwise.

### RESULTS AND DISCUSSION

The chemical compositions of the fresh orange juice extracts, as analytically determined, are presented in Table 1. The values obtained fall well within

Chemical component	Value
Ascorbic acid (Vitamin C)	$35.2 \,\mathrm{mg}/100 \,\mathrm{ml}$
рH	$3-1$
Moisture content	88.6%
Ash content	$0.02$ g/g
Total nitrogen	$0.09$ mg/100 ml
Sugar content	$12.03$ g/100 ml

**TABLE |**  Analyzed Chemical Composition of the Orange Juice

reported ranges (Duckworth, 1966). Figure 1 shows the relative abilities of the different acids tested in suppressing the growth of the microbial population in the juice system at ambient storage temperatures while Fig. 2 shows the same effect under refrigeration storage. None of the tested agents shown was sufficiently active when used at the  $0.01\%$  w/v concentration level.

A close comparison of Figs 1 and 2 indicates that the microbial population increases rapidly under ambient storage faster than obtained under refrigeration, as would be expected. This therefore implies a faster growth rate, coupled with the depletion of consumable nutrients and accumulation of inhibitory metabolic products that perhaps lead to the subsequent fall in contaminant counts under ambient storage. The relatively slower rate of occurrence of these processes probably accounts for the lower earlier counts, but slightly higher subsequent counts, attained under refrigerated storage.

Propionic acid was consistently the most inhibitory under both storage conditions. Although, at ambient storage, the growth suppression was sufficiently significant, lowering the growth population by more than two log cycles, the inhibitory effect was even more dramatic under refrigeration



Fig. 1. Relative effects of different organic acids on the viable counts of contaminant microflora of orange juice at ambient temperature storage.  $\circ$  ---  $\circ$ , Counts on unadulterated orange juice;  $\triangledown$   $\cdots$ , Counts on orange juice +0.05% propionic acid;  $\Box$  Counts on orange juice + 0.05% maleic acid;  $\Box$   $\Box$  Counts on orange juice + 0.05% succinic acid;  $\blacktriangledown$   $\blacktriangledown$ , Counts on orange juice + 0.05% fumaric acid;  $x \rightarrow x$ , Counts on orange juice + 0.05% citric acid.

storage where growth was totally arrested and no further counts were obtained after 9 days of storage, suggesting lethal effects.

The obtained efficacy of propionic acid accords with its previously known activity against yeast cells (the suspected major spoilage organisms) leading to its incorporation in bakery products ingredient formulations for antimycotic activity (ICMSF, 1980b).

However, since the antimicrobial activity of acids generally are known to be pH-dependent coupled with their molecular concentrations in the undissociated form (ICMSF, 1980a), the inferior preservative effects of the other tested acids in this system, under identical pH and preservative concentration conditions compared to propionic acid, could be due to their probable greater magnitudes of ionic dissociation.

In addition, the undissociated molecular concentrations of the residual acids might also have been sufficiently low to permit adaptation by the contaminant cells. Thus, the poor preservative efficacies of the other acids obtained in this system might have involved one or both factors of low residual undissociated molecular concentration and adaptive resistance mechanisms by the natural wild contaminant cells. Although the verification



Fig. 2. Comparative effects of different organic acids on the viable counts of contaminant microflora of orange juice under refrigerated storage at  $12^{\circ}$ C.  $\circ$ — $\circ$ , Counts on unadulterated orange juice at ambient temperature storage;  $\triangledown \rightarrow \triangledown$ , Counts on unadulterated orange juice under refrigerated storage at 12°C;  $\Box$   $\Box$ , Counts on orange juice + 0.05% propionic acid at 12°C storage;  $\blacktriangledown$   $\blacktriangledown$ , Counts on orange juice + 0.05% succinic acid at 12°C storage;  $\times \rightarrow \infty$ , Counts on orange juice + 0.05% fumaric acid at 12°C storage;  $\bullet$   $\bullet$ , Counts on orange juice + 0.05% citric acid at 12°C storage.

of both speculations was beyond the scope of this current study, the possibility of the latter factor has also been previously highlighted (ICMSF, 1980a).

Furthermore, the latter suggestion of adaptive resistance mechanism is further strengthened by the very weak inhibition exhibited by citric acid, a major (orange) fruit component. It is to be expected that natural surviving contaminants within such a system must, however, have evolved genetic and/or physiological adaptations for coping with survival in such stressful environments, and thus reflect the levels of obtained resistance.

The results obtained for the polyphosphates were less promising, by contrast, with the growth population reaching  $10<sup>6</sup>$  cfu/ml for most within the first 48 h under ambient storage. Their growth inhibitory effects were, however, enhanced by refrigeration where growth populations remained below  $10^6$  cfu/ml for 2 weeks during storage at  $12^{\circ}$ C. Fibrisol-sodium tripolyphosphate-S-600 was the most effective of those screened. This is perhaps due to its longer chain length than most others that are pyrophosphates, excluding Curaphos-700-instant. It has earlier been suggested

by Ellinger (1972) and confirmed (Obafemi & Davies, 1985) that the antimicrobial activity of polyphosphates could be related to their component chain lengths within limits. The relative ineffectiveness of Curaphos-700-instant, although inexplicable, has, however, been previously similarly encountered (Obafemi & Davies, 1985). Although it contains longer component chain length mixtures than pyro- and triphosphates, it is suspected that its low activity may be due to probable relative instability of its chain length in food systems (Obafemi & Davies, 1985).

The ineffectiveness generally shown by the tested polyphosphates was, however, surprising in view of their previously known antimycotic activities (Ellinger, 1972).

Figure 3 shows the relative preservative efficacies of combining 0-025% each of propionic acid and Fibrisol sodium tripolyphosphate-S-600 compared to 0.05% of each, singly, at both ambient and refrigeration storage conditions. It is evident that, whereas a combination of both agents



Fig. 3. Comparative effects of combining 0'025% each of Fibrisol sodium tripolyphosphate-S-600 and propionic acid or their individual usage at 0.05% on viable counts in orange juice,  $\bigcirc \sim 0$ , Counts on unadulterated orange juice at ambient temperature;  $\triangledown \sim \triangledown$ , Counts on orange juice  $+0.025\%$  each of Fibrisol sodium tripolyphosphate-S-600 and propionic acid at ambient temperature ;  $\Box$   $\Box$ , Counts on orange juice + 0.025% each of Fibrisol sodium tripolyphosphate-S-600 and propionic acid under refrigerated temperature storage at 12°C;  $\nabla$ — $\nabla$ , Counts on orange juice + 0.05% Fibrisol sodium tripolyphosphate-S-600 at ambient temperature;  $\blacksquare$ . Counts on orange juice + 0.05% propionic acid at ambient temperature.

(the most effective in their groups) did not confer any enhanced preservative efficacy under ambient storage, there is a dramatic improvement under refrigeration. While it appears that propionic acid alone at 0.05% is better than combining  $0.025\%$  of both agents at ambient storage, a better preservation would be obtained by the combination coupled with refrigeration, under which the counts were consistently lower than  $10^3$  cfu/ml for the whole 30 days' storage test duration.

However, judging from a comparison of both Figs 2 and 3, it appears that the part-substitution of 0-025% propionic acid by the same concentration of Fibrisol sodium tripolyphosphate-S-600 does not enhance the preservative efficacy of the former. On the contrary, survival levels were actually slightly higher under ambient storage whereas counts obtained were constant under refrigeration, even after 9 days where the mixture was substituted for the propionic acid alone, suggesting a partial neutralization of the effects of propionic acid in the system.

The obtained results therefore suggest a mild reduction of the antimicrobial activity of propionic acid in the presence of Fibrisol sodiumtripolyphosphate-S-600. The observed phenomenon may be attributed to the alkaline nature of the polyphosphate which could perhaps have induced some measure of increased dissociation of the propionic acid, thereby reducing the molecular concentration of the residual undissociated propionic acid on which antimicrobial activity depends. Thus, for preservative purposes, the additional polyphosphate incorporation could be dispensed with, but where there is need for partial substitution of the propionic acid concentration without undue compromise of its preservative effectiveness, a mixture of the two might constitute a satisfactory alternative preservative combination.

As part of the bid to develop a suitable and workable preservative system (for the peculiar tropical conditions) for obtaining dependable and likeable shelf-stable product formulations, the next phase of the study also investigated the influence of combining physical and chemical process treatments. These had involved the use of pasteurization treatments either alone, or coupled with subsequent post-pasteurization incorporation of promising acids, with or without subsequent refrigeration storage. In addition, the relative efficacies of these compared to sulphur dioxide ( $SO<sub>2</sub>$ , a commonly used permitted preservative) as derived from a potassium metabisulphite source (Ough, 1983), are also included for comparative evaluation purposes. The results obtained for the preservative efficacies are shown in Figs 4 and 5 while the corresponding sensory evaluation results of the products derived from such process treatments, adjudged to be effective among those screened, are presented in Table 2.

It is evident from both Figs 4 and 5 that pre-pasteurization of samples



**TABLE 2**<br>Taste Panel Results

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Fig. 4. Viable counts of contaminant microflora of pasteurized orange juice samples and subsequent incorporation of succinic and maleic acid under storage conditions,  $\circ$ — $\circ$ , Orange juice + 0.05% succinic acid at ambient temperature (30–35°C) storage;  $\Box$ Orange juice + 0.05% succinic acid at 12°C storage;  $\triangledown \rightarrow \triangledown$ , Pasteurized orange juice + 0-05% succinic acid at ambient temperature storage;  $\blacktriangledown \longrightarrow \blacktriangledown$ , Pasteurized orange juice + 0.05% succinic acid at 12°C storage;  $\bullet \rightarrow \bullet$ , Pasteurized orange juice + 0.05% maleic acid at 12<sup>o</sup>C storage;  $\times \rightarrow \infty$ , Pasteurized orange juice + 0.05% maleic acid at ambient storage.

prior to additional incorporation of acids generally delayed the onset of countable growth in the respective samples. The shelf stability was also further enhanced by subsequent refrigeration storage.

Specifically, as can be seen in Fig. 4, the combined use of prepasteurization, incorporation of maleic acid  $(0.05\% \text{ w/v})$  and subsequent refrigerated storage, indicated good shelf stability with no countable growth within the first 3 weeks. Similar results were obtained for succinic acid except that the emergent microbial counts were much higher. Nonetheless, the chances for suitable industrial exploitation of the above two options are somewhat remote in view of their attendant unfavourable organoleptic properties (Table 2). In addition, succinic acid, being a natural component of fruits as well as having GRAS (generally recognized as safe; Gardner, 1972) status, could have been less objectionable compared to maleic acid which has a very restricted and conditional acceptable daily intake level (FAO/WHO, 1969).

Also in Fig. 5, the influence of propionic acid with or without previous pasteurization or cell survival is shown. It is evident that, where previous pasteurization is omitted for samples, incorporation of 0-05% propionic acid could only effectively inhibit the growth of contaminants if



Fig. 5. Comparative effects of pasteurization of samples, and/or subsequent incorporation of propionic acid or separate use of potassium metabisulphite on orange juice viable counts of contaminant microflora under storage conditions.  $\circ$ — $\circ$ , Counts on unadulterated orange juice at ambient temperature (30–35°C) storage;  $\triangle$  –– $\triangle$ , Counts on unadulterated orange juice under refrigerated storage at 12°C;  $\triangledown$ — $\triangledown$ , Counts on orange juice + 005% propionic acid at ambient temperature storage;  $\Box$   $\Box$ , Counts on orange juice + 0.05% propionic acid at  $12^{\circ}$ C storage;  $\bullet$  -  $\bullet$ , Counts on pasteurized orange juice at ambient temperature storage;  $\times$ — $\times$ , Counts on pasteurized orange juice at 12°C storage;  $\blacktriangledown$ — $\blacktriangledown$ , Counts on pasteurized orange juice  $+0.05%$  propionic acid at ambient temperature storage;  $\blacksquare$ II, Counts on orange juice + 0.0025% potassium metabisulphite at ambient storage.

accompanied by refrigerated storage at 12°C compared to its reduced efficacy at ambient temperature. On the other hand, effective growth suppression of contaminant cells was achieved either by pasteurization of samples followed by their refrigerated storage as before without propionic acid addition or, alternatively, post-pasteurization incorporation of 0.05% propionic acid followed by ambient storage. In both cases, no countable population was obtained for the first 3 weeks (Fig. 5) before a slow growth rate was obtained in which counts did not exceed 10<sup>4</sup> cfu/ml. However, under ambient storage, contaminant cells in propionate-free pasteurized samples rapidly increased after the first 16 days of non-detection.

Figure 5 also shows the microbiological profile of untreated unadulterated orange juice under both ambient (30-35°C) or refrigerated (12°C) storage, indicating rapid proliferation of contaminant cells. In contrast, formulated samples incorporating  $0.0025\%$  (w/v) potassium metabisulphate were found not to give countable colonies for the first 16 days, after which a Slow growth rate was observed in which counts still did not exceed  $10<sup>4</sup>$  cfu/ml after 32 days' storage (Fig. 5).

From the available findings of the current study, it could be inferred that some of the processing options evaluated clearly merit some further consideration for potential exploitation in the efforts aimed at producing shelf-stable and palatable orange juice. The continued use of  $SO<sub>2</sub>$  that is currently legally permitted is demonstrably supported by the current findings, in terms of the preservative efficacy and sensory evaluation results. Even so, the use of propionic acid (at the level tested singly or partly substituted with sodium tripolyphosphate at  $0.025\%$  (w/v)) also appears to be preliminarily justifiable on the basis of the demonstrated preservative efficacy and overall acceptability scores. Similarly, the combined use of previous sample pasteurization, equivalent to a heat-process treatment maintained at 80°C for 1 min at sample thermal centre with concomitant agitation, and coupled with the post-pasteurization incorporation of 0.05%  $(w/v)$  propionic acid, also offers potential promise. The latter, as well as the alternative use of  $0.0025\%$  (w/v) potassium metabisulphite, apart from giving organoleptically encouraging resultant products, also dispenses with the post-process refrigeration storage requirement and thus appears to be ideally advantageous for exploitation in some parts of the tropics. These methods therefore merit further consideration, particularly in view of the fact that propionic acid, together with its sodium, calcium and potassium salts, are currently not limited in terms of the acceptable daily intake level (FAO/WHO, 1969).

The case for the tested polyphosphate is also strengthened by its deductive exclusion from being technically regarded as a preservative by definition (Anon., 1972).

Finally, in view of the good solubility of the promising agents in orange juice, coupled with their demonstrated effectiveness against natural wild contaminants and the resultant organoleptic properties of the tested products, there appears to be some potential for exploitation in orange juice processing under tropical conditions.

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