Ripe Olives Storage Liquids Reuse During The Oxidation Process

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ABSTRACT

Storage of ripe olives in liquids with high acetic acid content under anaerobic conditions led to glucose accumulation, low ethanol, and high chemical oxygen demand (COD). In contrast, low acid concentrations and anaerobic conditions produced a high level of ethanol and slightly lower COD while aerobic conditions produced low glucose, ethanol and COD. Surface color of ripe olives improved when storage liquids were used in the second washing of the darkening step. Firmness also increased with increasing proportion of storage liquids reuse, although olives from highly acidic media were softer than those stored in low acid media.

Key Words: COD, oxidation process, reuse, ripe olives, storage liquids

INTRODUCTION

PROCESSING OF RIPE OLIVES INCLUDES A prior stage of preservation in liquid. In Spain the procedure normally used for this stage consists of putting the olives into 16000 L fiberglass fermentation vessels in 4–6 % (w/v) NaCl brine under aerobic conditions. Such brines are discarded when the oxidation step begins (Brenes et al., 1986; Garrido Fernández et al., 1997). In the USA a salt-free storage of olives combining acidulated water (lactic and acetic acids) and anaerobiosis is used (Vaughn et al., 1969). The American method was developed to alleviate the problems of brine disposal. The U.S. pickle industry has similar problems and is lowering the sodium chloride in its waste waters. Fleming et al. (1995) reported a fermentation method for cucumbers without sodium chloride, although sodium benzoate was needed to ensure microbial stability and calcium chloride to prevent loss of firmness (Fleming et al., 1996). In the salt-free storage method for ripe olives, sodium benzoate is also required (Vaughn, 1982) and calcium chloride is usually employed.

The limit of chlorides in freshwater bodies has been established at 230 ppm in the USA and 2000 ppm in Spain. However, in both countries organic contamination is strictly banned. For many years, the olive industry has discharged waste waters into city sewers or ponds. Seepage from the ponds has sometimes contaminated ground waters (Martin, 1992).

The predominant microorganisms during storing of ripe olives in brines or acidified media are yeasts (Brenes et al., 1986). The low growth of lactic acid bacteria in such liquids (Durán et al., 1994) has been related to antimicrobial polyphenols diffusing from olives into surrounding liquids (Fleming et al., 1973; Brenes et al., 1993a). Oleuropein is the main phenolic compound in olive fruits (Amiot et al., 1986), and during storage of ripe olives it is hydrolyzed under acidic conditions to yield hydroxytyrosol (3,4 dihydroxyphenyl ethanol) (Brenes et al.., 1993a). This compound and caffeic acid promote the dark color formed during the oxidation step of ripe olive processing (Brenes et al., 1992). Thus, if hydroxytyrosol is present in storage liquids it may contribute to a darker color of olives when reusing the liquids in the oxidation step.

Our objectives were to determine the feasibility of reusing storage liquids of ripe olives during the oxidation process and to evaluate the organic contamination from such method when using different kinds of storage liquids.

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MATERIALS & METHODS

Olives

Fruits were from Hojiblanca cultivar, obtained from local processors. Ripeness degree was such that 90 % of olives had a green-yellow surface color while the other 10 % had some pink color on the skin.

Storage procedures

Olives (30 kg) were placed in cylindrical PVC vessels and covered with 20L of different kinds of liquid (Table 1). Application of the anaerobic and aerobic conditions have been described (Brenes et al., 1986; Garrido Fernández et al., 1997). Experiments were run in duplicate at ambient temperature.

Darkening process

Oxidation tests were performed with olives stored for 180–210 days using four, 0.15-m dia, 0.3-m high, stainless-steel cylindrical containers with conical bases (García et al., 1991). The darkening process consisted of placing 1 kg of fruits in 1.5L of 0.5M NaOH solution (lye) for 4–5h, sufficient time for the lye to penetrate 1 mm of the olive flesh. It was controlled visually by cutting a fruit and treating with phenolphthalein. The olives were then covered with tap water, and air was bubbled through the mixture for 24h (García et al., 1991). After draining, olives were put in a new lye solution, which penetrated to the pit. Then, fruits were submerged in tap water with different percentages of storage liquids (0, 25, 50 and 75 %) through which air was bubbled for 24h. Olives were rinsed in aerated tap water for another 24h and CO_2 was introduced into the liquid until the pH in the olive flesh was 7.0–7.5. The liquid was then poured off and fruits were covered with a 0.08 % (w/v) ferrous gluconate solution (Fluka, Switzerland). The fruits/liquid mixture was aerated for 24h and olives were pitted and bottled (100g) in 250 mL glass containers with 125 mL of 0.4M NaCl and different amounts of ferrous gluconate [0, 0.02, 0.03, and 0.04 % (w/v)]. Finally, jars were sterilized at 121°C for 15 min in a computer-controlled Steriflow retort (Madinox, Barcelona, Spain).

Table 1–Characteristics of the storage process

	Initial conditions of storage liquids	
	NaCl (%, w/v)	Acetic acid (%, v/v)
Anaerobic	6	0.4
Anaerobic + acid + salt	6	1.5
Anaerobic + acid	0	1.5
Aerobic[a]	6	0.4

[a]The rate of air bubbling in the aerobic treatment was 0.3 L/ hr and liter of vessel capacity.

Chemical analyses

Sugars (glucose, fructose, sucrose and mannitol), alcohols (ethanol) and acids (lactic and acetic acid) were analyzed by HPLC, as described (Montaño et al., 1996). The pH of the flesh was measured in a slurry of 20g tissue using a Beckman Model 45 pH meter. Polyphenols in liquids were determined by HPLC. Samples (1 mL) were mixed with phosphoric acid to lower the pH to <3.0, centrifuged at 13000 × *g*, and injected into the chromatograph. These conditions have been described (García et al., 1996). Chemical Oxygen Demand (COD) was determined according to standard methods (APHA, 1985).

Product evaluation

Colorimetric measurements on olives were performed using a BYK-Gardner Model 9000 Color-view spectrophotometer. Interference by stray light was minimized by covering samples with a box which had a matte black interior. Color was expressed as percentage reflectance at 700 nm. Lower reflectance values indicate darker colors (Fernández and Garrido, 1971).

Firmness of fruits was measured using a Kramer shear compression cell coupled to an Instron Universal Machine, Model 1001. The cross head speed was 200 mm/min. Firmness was expressed as the mean of 10 replicate measurements, each of which was performed on 4 pitted olives (Brenes et al., 1994).

Informal sensory evaluation was carried out by a 16-member trained panel. Olives used for the tests had been stored in aerobic conditions and processed in the second washing of the darkening process with either 75 % (v/v) storage liquid or only tap water. A triangular comparison was carried out according to Kramer and Twigg (1970).

RESULTS & DISCUSSION

Chemical characteristics of storage liquids

Sodium chloride concentration in solutions with salt (6%) stabilized at 3.1–3.2% (w/v) after 2 mo, due to olive flesh absorption. Changes in pH depended on initial characteristics. With low acetic acid content (anaerobic and aerobic experiments, Table 1), pH was initially 3.1–3.2 and rose to 4.1–4.2 after 2 mo storage. In presence of high acetic acid content, corresponding values were lower (2.8 and 3.7–3.9, respectively). Salt (6%) and acetic acid (1.5%, w/v) yielded the lowest initial (2.5) and final (3.7–3.8) pH levels, due to the ionic strength of the NaCl. Changes in pH were negligible after the 2nd month. This initial pH increase in all storage conditions was produced by the absorption of the acid into the olive flesh and the diffusion of organic salts from the olive flesh into the surrounding liquid, which gave the solution a considerable buffer capacity (Brenes et al., 1986; Garrido et al., 1993).

No lactic acid was detected throughout storage in any experiment. This confirmed the pH stabilization observed after the flesh/liquid equilibrium was reached. Similar results were found when using high concentrations of salt or organic acid, when only yeasts were detected during storage (Brenes et al., 1986, Garrido et al., 1993). The added acetic acid in the storage liquids stabilized after the 2nd month, reaching 25–30 mM and 110-120 mM concentrations for low and high initial levels, respectively. Thus, there was no acetic acid increase during storage. Its formation has been reported only when the pH in the surrounding solutions was >4.5 (Garrido et al., 1993).

Changes of glucose in the storage solutions were compared (Fig. 1). High concentration of acetic acid led to glucose increase (higher in presence of salt) during the first 3–4 mo, reaching 80–100 mM. Then there was a reduction, which could be related to microbial growth (yeasts). This was normal, since no benzoic acid was used in these experiments, as compared with the salt-free olive storage in the USA (Vaughn, 1982). In low acid concentrations, glucose in liquids was considerably lower (especially in the aerobic medium), possibly due to abundant microbial growth, as has been reported in ripe olive aerobic storage experiments (Brenes et al., 1986).

Another sugar found in the storage liquids was mannitol, which increased during the first 3–4 mo to reach 40–50 mM. This level remained unchanged for the rest of the olive storage (data not shown). Apparently, mannitol was not well assimilated by the microorganisms (yeasts) usually found in these media (Garrido et al., 1993).

Changes in ethanol, a main end product in olive fermentation by yeasts (González Cancho et al., 1985) were compared (Fig. 2). In aerobic conditions, ethanol increased to 60–70 mM after 20–30 days and remained stable for the rest of the storage period. Aeration removes alcohol as well as the CO_2 produced by the microorganism (Garrido et al., 1993). In treatments with low initial acidity and anaerobic conditions, ethanol increased steadily throughout storage, reaching about 200 mM. Ethanol content in highly acidic media (anaerobic) was low during the first period (sugar accumulation), but increased as glucose decreased (Fig. 1).

Regarding phenolic compounds, hydroxytyrosol increased progressively to reach 10–12 mM (200 days after storing). Slight differences between treatments, particularly those of low and high acidity, were detected after 1 mo storage (data not shown). Hydroxytyrosol originates from acidic hydrolysis of oleuropein as suggested by Brenes et al. (1993) . In this case, there was no lactic acid formation during storage and hydrolysis of oleuropein could not be due to the enzymatic activity of lactic acid bacteria (Ciafardini et al., 1994).

Storage liquids are the main contributors to organic contamination in the ripe olive industries. COD changes with time were compared (Fig. 3) in the different storage liquids. Aerobic conditions had the lowest COD, which correlated with the lowest content in glucose and ethanol in such solutions (Fig. 1 and 2). The highest COD values were observed in strong acid solutions in presence or absence of salt. Thus, salt free storage was not an effective pollution abatement system since, while it diminished the NaCl content it considerably increased organic pollution. Aerobic storage, in presence of low acidity, may be considered as more convenient in this aspect. It yields storage liquids of considerably lower COD because high amounts of organic substances which contribute to such COD are metabolized during this preservation period.

Physicochemical characteristics of the darkening process

The contribution of the various liquids which originated in the darkening step to the total pollution was compared (Fig. 4). These data are from olives stored in anaerobic low acid liquids and processed using different proportions of storage solution in the second washing. In general, the higher COD contribution is due to the washing liquids. This was related to the longer period of time the olives were in contact with the reused waters. Total COD increased with the proportion of

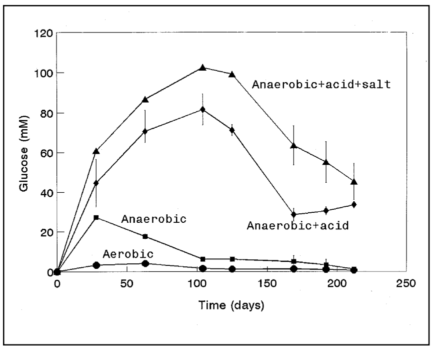

Fig. 1–Glucose changes in storage liquids of ripe olives.

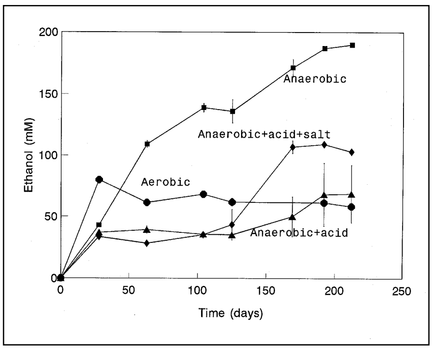

Fig. 2–Ethanol changes in storage liquids of ripe olives.

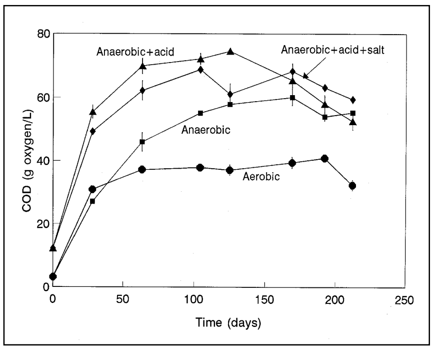

Fig. 3–COD changes in storage liquids of ripe olives.

storage liquid in the 2nd washing and the COD increment was notable not only in the second washing but also in the succeeding rinse liquids. Results with fruits from other storage treatments showed a similar trend (data not shown).

The COD generated in the darkening process was due, apart from the organics incorporated by the storage liquids reuse, to the diffusion of organic compounds from the olive flesh into surrounding liquids. The higher the COD in the storage liquid (and in the flesh) the higher the pollution produced in the darkening steps (Fig. 5). Aerobically stored fruits, independent of the percentage of storage liquids in the second washing, generated the least COD level, followed by the anaerobic system with low acidity, and fruits stored in highly acidic conditions. Total COD, independent of the storage system, increased with the proportion of reused storage liquid.

The aim of washing after the last lye treatment was to eliminate residual NaOH from the olive flesh. To achieve this, acidifying agents such as strong inorganic acids (HCl, H_2SO_4) or gaseous CO_2 are used (Brenes et al., 1993b). Reuse of storage liquids during the second washing can also contribute to neutralizing the olive flesh. The pH changed in olive flesh (Fig. 6) with progressively higher percentages of storage liquids in the second washing. Below 25% there was no effect on pH of the olive flesh by use of tap water. However, as the percentage of reused storage liquid increased, the pH in flesh diminished, particularly when using highly acidic solutions. Note that 1 unit of pH decrease may mean a considerable lessening of time, energy, and acidifying products during processing (Brenes et al., 1993b). There were also very noticeable differences in the surrounding liquids (Fig. 6), with only 25% of reused storage liquids. Differences between treatments were mainly on the basis of initial acid content. Highly acidic liquids led to lower pH. Thus, it is recommended that less than 50% of the storage liquid be reused in the second washing. Otherwise, washing liquids moved reach pH values <6.0, which can cause fruit discoloration and a sharp drop in the phenolic oxidation rate (García et al., 1992).

Surface color of olives (a main quality attribute) was, in general, improved by reuse of storage liquids. The 25% storage liquid reuse gave the best color (Fig. 7) in the case of highly acidified liquids. Higher percentages led to lower or negative effects on color (decreased color formation). Further improvements were not observed when reusing anaerobic low acid liquids in proportions >25%. In contrast, progressively increasing percentages of aerobic storage liquids (up to 75%) resulted in darker olive color (lower percentage reflectance).

The brown-black surface color developed during the darkening process of ripe olives is formed by oxidation and polymerization of orthodiphenols (Brenes et al., 1992). Garrido (1980) studied the addition of tannin (quebracho) to the solutions during washing, although results were not completely successful in improving color. Our results have confirmed the favorable effects that the presence of olive phenols (added by reusing the storage solution in the washing steps) has on enhancing surface color. Changes of olive orthodiphenols in the washing waters may be related to color development. The changes with time of hydroxytyrosol (main compound responsible for ripe olive color) in the second washing liquids were compared (Fig. 8) for different proportions of reused storage liquids. Fruits and liquids were from aerobic and highly acidified storage conditions. Concentrations of hydroxytyrosol increased with percentages of storage liquids added and the increases were similar in the two cases. However, changes during the subsequent washing period were rather different. Under aerobic conditions there was always a sharp drop

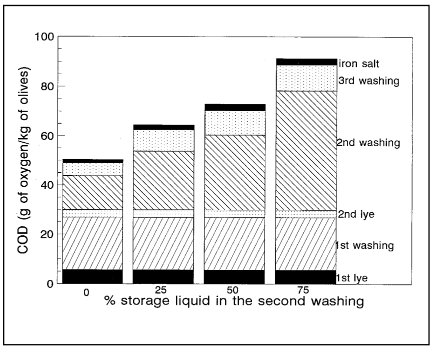

Fig. 4–COD produced in the different steps of the oxidation process of ripe olives. Fruits used had been stored in anaerobic conditions for 180 days and different percentages of storage liquids were used in the second washing of the oxidation process.

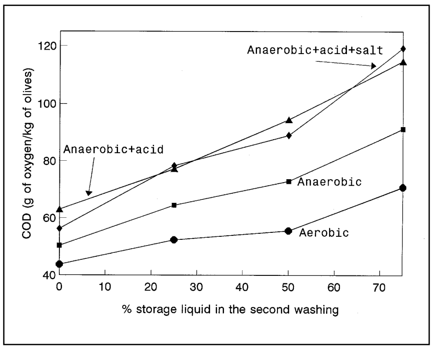

Fig. 5–Total COD produced in the oxidation process of ripe olives stored in the different liquids assayed. Fruits used had been stored for 180–210 days and different percentages of storage liquids were used in the second washing of the oxidation process.

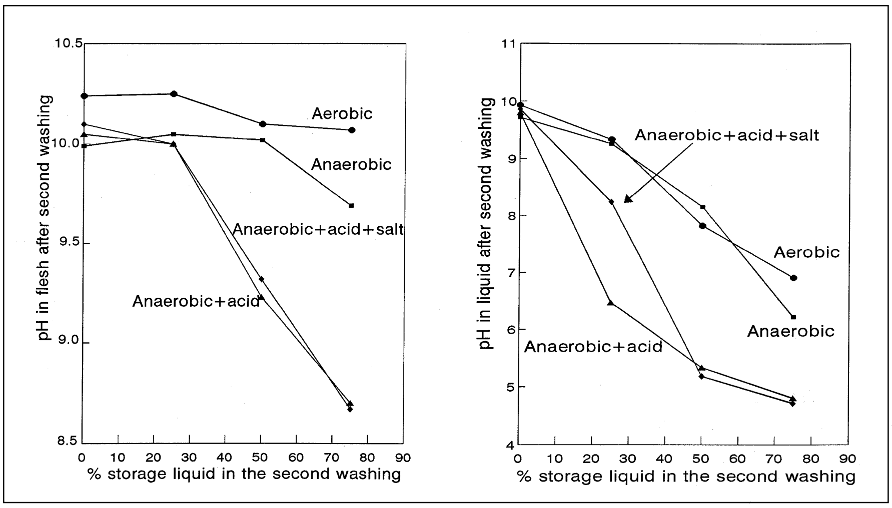

Fig. 6–pH changes in olive flesh and surrounding liquids during the second washing step. Fruits used had been stored for 180–210 days and different percentages of storage liquids were used in this step.

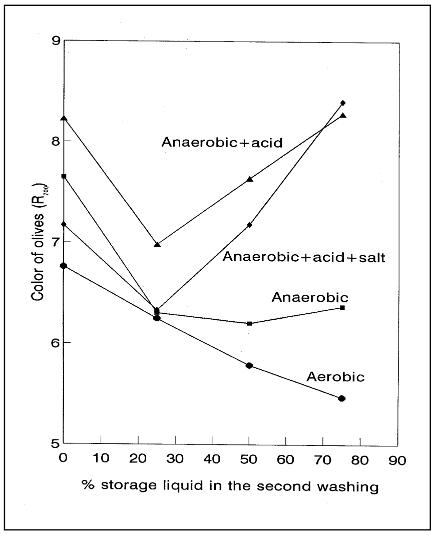

Fig. 7–Effect of reusing storage liquids in the second washing step of ripe olives processing on the color obtained after the darkening step and before packing. Fruits used had been stored for 180–210 days and different percentages of storage liquids were used in the second washing step of the oxidation process.

in this orthodiphenol with time. In highly acidified storage liquids, the decrease was considerably lower when 25% storage liquid was used, and there was no drop when the storage liquid was >50%. Thus, the low pH in the washing waters produced by the high proportion of acidified storage liquids (Fig. 6) prevented hydroxytyrosol oxidation, confirming the results of García et al. (1992), and consequently there was no favorable effect on color development.

Physicochemical characteristics of the packed product

The effect on the final color when reusing the storage liquid (aerobic treatment) during the second washing was compared (Fig. 9) using various iron concentrations in the packing solution. Surface color in the darkening process increased with reuse of storage liquids (Fig. 7) and this effect was maintained in the final product (after packing and sterilization). In general, color improved (lower percentage reflectance) with increasing concentration of iron, but curves showed a similar trend. We also observed that the reuse of storage liquids may reduce the concentration of iron needed to reach a visually good color.

Texture is also an important quality attribute for ripe olives. The firmness was compared (Fig. 10) on olives processed with different percentages of storage liquid reuse in the second washing of the darkening step. Firmness increased with percentage of reuse. This effect is not well known, although a similar result has been reported in the packing of green olives when the fermentation brines were reused (Brenes et al., 1989). Texture differences found due to storage conditions were remarkable. Olives stored in highly acidified media had markedly lower firmness than those in low acidity (anaerobic or aerobic). This confirmed the generally accepted principle that low pH produces a progressive softening in vegetable tissues (Brenes et al., 1994; Fleming et al., 1996). However, this negative effect of high acidity on ripe olive firmness could be minimized using calcium chloride, which decreases the softening rate (Brenes et al., 1994).

Sensory characteristics were not affected by reuse of the storage liquids during darkening (second washing). Informal tests showed no differences between treatments, by triangular tests (Kramer and Twigg, 1970). Reuse of storage liquids also had a marked effect on residual acetate content in the final product, although we used the final tap water washing and immersion in the iron salt solution. Increasing the percentage of storage liquids reused, increased residual acetate (Fig. 11), especially when working using highly acidified liquids. In those cases, acetate concentrations reached 10-20 mM, high enough to be considered an additive for product labeling purposes.

CONCLUSION

USE OF AEROBIC CONDITIONS DURING RIPE olive storage led to lower COD in storage liquids and waste waters produced in the darkening steps. In addition to more highly

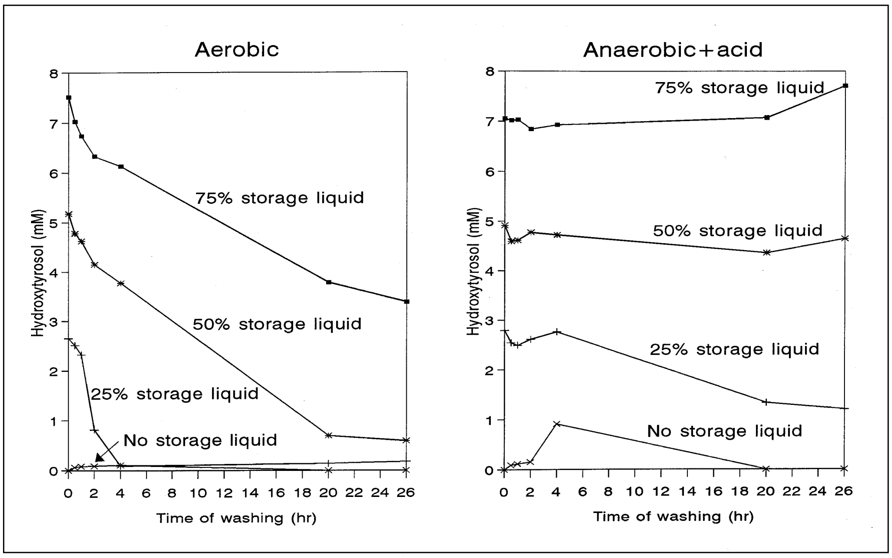

Fig. 8–Hydroxytyrosol changes in liquids during the second washing step. Fruits used had been stored for 200–210 days in aerobic and anaerobic+acid conditions. Different percentages of storage liquids were used in this step.

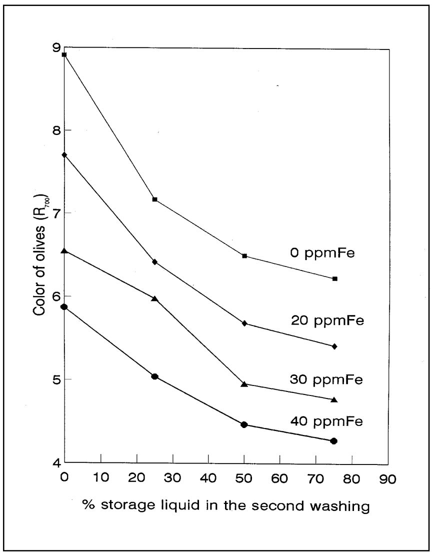

Fig. 9–Effect of reusing storage liquids in the second washing step of ripe olives processing on the color obtained after packing the fruits. Olives had been stored in aerobic conditions for 210 days and different amounts of iron (ferrous gluconate) were added to the cover solution during packing.

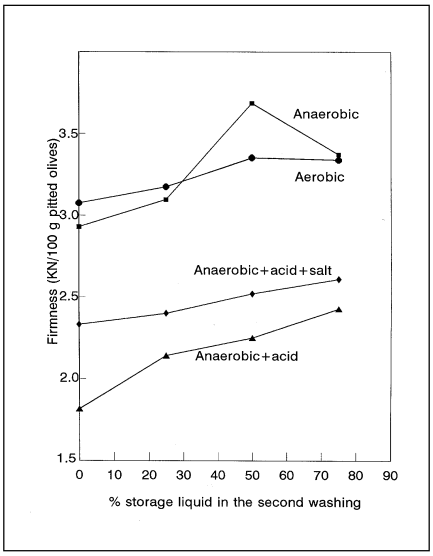

Fig.10–Effect of reusing storage liquids in the second washing step of ripe olives processing on the firmness of packed olives. Fruits had been stored for 180–210 days and different percentages of storage liquids were used in the second washing step of the oxidation process.

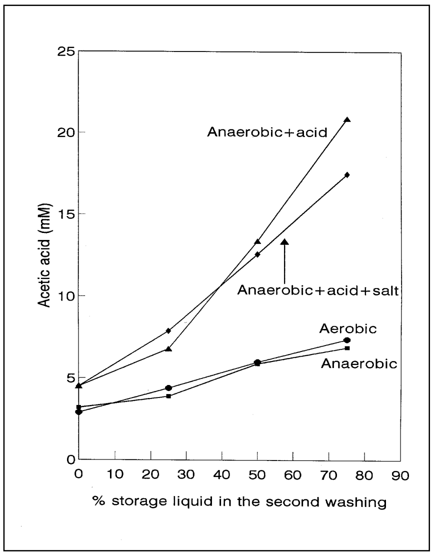

Fig.11–Effect of reusing storage liquids in the second washing step of ripe olives processing on the acetic acid condentration in the cover solution of packed olives. Fruits had been stored for 180–210 days and different percentages of storage liquids were used in the second washing step.

concentrated liquid wastes, highly acidified storage liquids resulted in softer olives. Reuse of storage liquids in the second washing of the ripe olive darkening process gave darker and firmer olives, especially under aerobic conditions.

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