4th ICPPAM
Rostock, G.D.R., September 4-8, 1989
4th International Conference
Physical Properties of Agricultural
Materials and their Influence
on Technological - Processes

IMPACT BRUISES IN POMACEAE FRUITS: EVALUATION METHODS AND STRUCTURAL FEATURES

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SUMMARY

Using a laboratory impact tester, impacts were applied to fruits of different varieties of apples and pears. The response to impact was analized, and many parameters were recorded, to be correlated to bruise susceptibility and to ripeness changes. Different methods for the detection and evaluation of the bruised area and its features were studied, using direct observation and various reactives. Different types of bruises were established.

Bruising, impact, fruits, apples, pears, detection.

1.INTRODUCTION

In recent years, a great effort is being carried out to investigate into the relations between the parameters which characterise mechanical impact and the appearence and developement of bruises in different fruits. These studies are necessary, considering the objective of controlling the important problem of fresh produce quality losses ; another objective of these same studies is to obtain data on impact resistance of these fruits,in relation to the development of mechanical harvesting in the future . Both problems are especially critical for apples and pears.

The most recent publications on impact in fruits (1,2,3,4,5) , try to get indepth knowledge on the relations "impact vs. bruise development", once there exists the possibility of using precise instruments to measure the short-time impact history with accuracy. Some of the research results obtained formerly by different investigators with various types of impacters are not very consistant, and often contradictory; there is an evidence that impact tests need to be very broad, using many fruits and varying the impact conditions, as well as the conditions of the fruits themselves, in a very wide range. Garcia et al.(4) show that the physical phenomenon of an impact on a pear or an apple is considerably different for different ripeness stages of the fruit, varying from a mainly elastic response, with over 60% of rebound energy from the total input energy, to a mainly plastic response (less than 10% of rebound energy). The resulting bruises are supposed to be very different for these different types of impact response.

The other fundamental aspect of these studies to be solved is to develop the appropriate techniques for observation of the bruises in the fruit tissues with sufficient accuracy, in parallell to the accuracy obtained with the impact testing device. There is the need to evaluate on one side the magnitude of the bruise, to be related to the mechanical parameters of the impact, and on the other side the structural characteristics of the same bruise , which define the specific impact response for different fruit varieties and fruit conditions.

The direct visual aspect of a bruise is easilly detected from the softening and brown color of the affected tissue. Softening is due to degradation of the cellular walls and of the middle lamellae by different enzimes. Browning is known to be due to oxidation of poliphenols, in presence of the enzimes poliphenoloxidases (PPO). Oxigen exists primarily in the intercellular spaces. It is not completely cleared yet what is the actual site where the oxidative reaction takes place. We know that poliphenols are located primarily in the vacuole and the enzime PPO in the cloroplasts in the citoplasm, therefore both inside the cell wall. As stated by Shohei (6) and Wilner (7), cited by Rodriguez (8), browning reaction can take place outside as well as inside the cell. Therefore, for the development of a bruise, cell-wall rupture is not indispensable. On the other hand, other authors (9) have stated that the browning reaction develops in the intercellular spaces, after cell rupture occurs due to mechanical action. It is therefore suitable to study the structure of the bruised tissue in the different types of observed bruises.

During the last years, researchers (10) have used various fast and simple colorimetric methods, to evaluate bruise severity and to estimate browning potential.

2.MATERIALS AND METHODS

The impact testing device used in the tests has been described previously (1,2,4,5,11). The impacting device consists basically of a steel rod of 50.6 g mass , with a spherical tip (19 mm diameter), with adjustable height of fall (2 to 20 cm were used), and connected to a computer. In two different sets of tests, five varieties of fruits were tested; three pear - Limonera (Williams' type), Blanquilla and Decana, and four apple (Golden Delicious and Starking) varieties. After harvesting, the fruits were maintained in cold storage before testing, and other samples were ripened in a 20°C room for 48 and 96 hours, so as to get a wide range of ripening stages in each fruit variety. The smallest number of fruits impacted for each combination of treatments was 10 in all tests, and a total of over 900 impacts were tested in each variety. On a second set of tests, Blanquilla pears were ripened and tested until overripe (senescent). The mechanical parameters of the impact were calculated from the one measured (deceleration) and all of them recorded, using them later for complete statistical analysis.

Different techniques were used to evaluate the bruises, which are summarized as follows:

- Direct visual appreciation and measurement: with a scale, depth and width of the centered cross-section of the bruise were measured; these two parameters were treated independently in the analysis.
- Stereoscopic microscope (NIKON, SMZ-2T,1-63 XX) with conventional (trough and reflected light) and glass-fiber cold light illuminators. It was used to observe the bruised area, in the fruit and in manual cuts (1-3mm thick), fresh or stained with histology dyes like Saphranine.
- Fast colorimetric techniques: some of the reactives used for browning evaluation applied as follows:
- Cathecol: prepared in a 0.1 M sol on, with citric acid buffer (pH 6.2) (10). When a drop is placed on the su.ace of the fruit flesh, bruised tissue is stained very dark brown. This technique was applied in two different ways:a) whole fruits were dipped into the solution to reveal skin ruptures; b) whole-fruit slices were dipped into the solution to reveal bruised areas near the surface.
- Nitrose test: a solution of sodium nitrate, urea, acetic acid and sodium hidroxide; it has been used (10) to evaluate phenolic concentration in peaches.
- Epinephrine: prepared in a 0.01 M solution, with phosphate buffer 0.02 M (pH 7) (12). It gives a reddish coloration of bruised tissue.
- Saphranine: It is a very common Histology dye. It dies specifically cell walls and nuclei.

3.RESULTS AND DISCUSSION

1. Impact parameters.

Results of the relations between the mechanical impact parameters and bruise depth and width for each variety tested have been previously published (4,5,11). Some parameters ("first group") appear to be closely related to impact energy: maximum deformation, permanent deformation, maximum impulse, and depth and width of bruise. Some parameters, though,("third group") appear independent from impact energy, and closely dependent on ripeness level of the fruits: total impact duration, time to maximum force, slope force/deformation, apparent dynamic modulus of elasticity. The so-called " second group" of parameters are related to both independent parameters i.e.impact energy and ripeness level, and those are: maximum impact force, force/time slope, rebound velocity, percentage of elastic (rebound) energy.

The results of the second set of tests, on Blanquilla pear, showed that the senescent fruits behaved differently than the non-senescent fruits. Linear multiple regression models applied to these data showed that depth and width of the observed bruises could be explained for all data in each group in a 50% (only) by linear combinations of following parameters: maximum impact force, total impact duration and ripeness level parameters like firmness, acidity and sugar content (8,13).

All these results at the present stage of analysis lead us to the conclusion that in the apple and pear varieties tested, bruise size, as measured independently by the depth and the diameter (width) of its centered cross-section, depends mainly on the impact energy applied; the size of the bruise relates also to the ripeness stage of the fruits when ripeness levels include a wide range of variation.

2. Bruise features.

Appart from these results, based on direct measurement of bruise size, closer observation of the bruises showed that different varieties show different bruise patterns, and these, in the absence of important variations in other relevant parameters (like it might be the radius of curvature of the fruits) seem to be related to the structural differences between these fruits, which are remarcably different. Table 1 includes the differences in the structures of the fruit tissue gathered for both types of fruits.

Figure 1 shows models of average bruises observed in th varieties studied. The differences between them affect:

a) the relation depth/width, significantly higher in pears than in apples. For 12 cm drops (0,06 J), the average depth is similar for both apple varieties and Decana and Blanquilla pear, 2.5 - 3.5 mm, with no significant change for an 11-week period of cold storage. Bruise width, on the other hand, is significantly wider (7-8 mm) for both varieties of apples (pears: 3-5 mm)

b) the size, shape and number of fractures observed inside the bruised tissue. The close microscope direct observation of the bruises makes it possible to identify the presence of discontinuities, eventually fractures, in what may be the maximum stress and/or strain location in the bruised area when impacted. They appear always some mm below the skin, and centered in the bruise itself. In the case of the apple varieties, somewhat curved, horizontal fissures appear (14), not completelly separated, but rather showing cell walls like bridges between both sides; the cells are crushed together, with no evidence of having slided so that they may not be considered properly fractures. For low-energy impacts, although a bruise appears (of smaller size) no discontinuity can be observed. It is remarcable that after some time, the totality of the stressed tissue becomes brownish, and not only the ruptured spot or surface. Also, the specific reactives for poliphenoloxidase activity become active in the total "affected" area, delimitating very clearly the tissue that has been damaged. From electron-microscope complementary studies published elsewhere (8) we know that after a few hours most of the cells of the bruised area are soft, metabolized by the processes that initiate as a consequence of subjecting the tissue to stresses: disgregated middle lammellae and cell walls; precipitated citoplasm. When using the reactives, it is remarkable how the bruised area dyes immediately, showing a very distinct frontier between affected and non-affected surrounding tissue; also, this area does not increase with time, even after months of cold storage. From this, it appears to be clear that a distinct action has been applied to all the cells on the tissue affected by mechanical impact, so that it develops the bruising process, and not to the surrounding tissue.

Another conclusion of all these observations is that bruising may develop with or without cell rupture; we are able to affirm that cell compression can cause the iniciation of metabolic bruising processes inside the cell-wall; at the same time,it is fairly evident that broken cells accelerate the bruising metabolic processes in the area close to them.

The larger intercellular spaces of the apple tissue, which are the cause of its lower density (see Table 1) give rise to the formation of subsecuent transversal failure surfaces (Fig.3), which are able to absorb much of the strain during impacter penetration, dissipating the corresponding stresses, and therefore preventing the formation of deep bruises; these rather evolve into wide bruises.

In the case of Decana pear, with a much denser type of parenchima, real conical and vertical fractures are observed (Figs 1 and 2), starting at a point of maximum concentration of stresses, suggesting stress failure as described for some fragile materials.

Figure 1-e shows a closer modelization of the bruises of Blanquilla pear, including senescent fruits. The bruises show some interesting features:

a) The shape tends to become wider and flatter as softening increases from preclimacteric to climacteric and to postclimacteric (senescent) fruits.

b) For the harder fruits, a conical shape including discontinuity/fracture appears , but not in riper fruits.

c) Differences appear in the browning of different areas, being the intermediate (numbered '2') the most affected, when observed right after impact. The bruise develops later further to the whole of the affected tissue, as stated before.

3. Fast colorimetric techniques.Cathecol

This reactive resulted the most appropriate for the different applications studied. For delimiting with high contrast the bruised area and therefore, measuring it with better accuracy, it is enough to place a drop of the prepared solution , wait 6 minutes and then measure with a scale, and observe the shape. Fractures and fissures can be easily observed by cutting a thin slice of the section of the bruise being studied; the reactive flows trough the fissure and shows it very clearly in the cut slices.

It can be used on whole fruits, bruised or not, by dipping fruit slices during 1-2 minutes ; wait 6 minutes; cut a thin slice from the (stained) surface and measure the bruises, if present (Fig 4). This last procedure may be a way to automatically detect bruised fruits in large samples. This extreme is being studied right now.

Epinephrine gave good results in revealing bruises in pears and can be used on cut tissues like cathecol. It is not a good reactive for apples.

Saphranine is a very good reactive for the microscopic observation of directly cut tissue slices; it reveals very clearly the areas where the cell-walls are compressed together around the fractures.

Acknowledgements

This Project was financed by the U.S.A.-Spain Joint Committee for Scientific and Tecnological Research.

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Table 1. Structural differences between apples and pears.

APPLE	PEAR
Epidermis	
Squared cells	Flat cells
Many pores	No pores
Suberised cells	
Thinn walled hairs	Thick walled hairs
Hipodermis	
Isodiametric cells of variable size	Poligonal cells with thick walls
Large intercellular spaces	Few small intercellular spaces
Parenchima	
Enlarged irregular cells	Polyedric cells of smaller size
More and larger intercellular spaces	Esclereids:thick walled cells in groups
Many fibers	Calcium oxalate (hard) cristals
Lower (apparent) density	

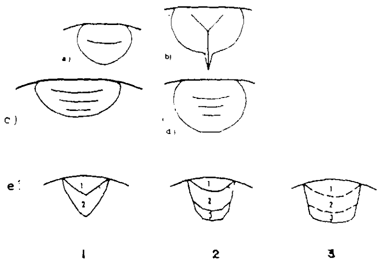

Fig. 1. Types of bruises and fractures or fissures observed in the varieties of - apples and pears tested. (12 cm drops). a) Limonera pear; b) Decana pear; c) Golden and Starking apples; d) Blanquilla pear; - e) Blanquilla pear: 1: hard fruits 2) ripe fruits, 3) **soft** fruits.

Fig. 2. Microphotograph ([illegible] XX) of fissure in Decana pear. 6 cm impact.

Fig. 3. Microphotograph (16 XX) of fissure in Starking apple. 8 cm. impact.

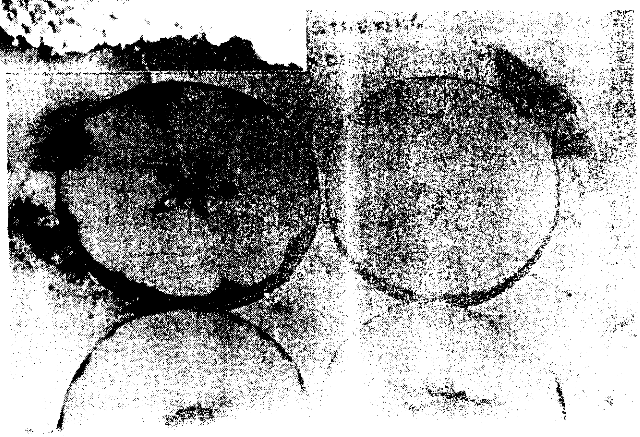

Fig. 4. Cathecol-dyed bruises of different sizes in - Starking apples.