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IMPROVED QUALITY AND STORAGE STABILITY OF ENZYME PREPARED FRUIT SLICES AND SEGMENTS

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ABSTRACT

As reported in previous CFCS meetings peeling and sectioning of citrus is greatly improved by vacuum infusion of commonly available commercial enzyme solutions resulting in improved products of better appearance and flavor quality than those prepared by conventional methods. Commercial enzymes resulted in products with varying flavor, texture and storage stability. When treatments resulted in higher membrane strength in the sections, products dry-packed in shrink-film covered trays had improved texture and stability. In some products white crystals formed during storage. Analysis showed these were naringin and could be prevented by water spray or dips and rinses in dilute base solutions. The process provides more efficient approaches for preparation of prepared citrus segments and may be applicable to other types of fruit pieces as well.

RESUME

AMELIORATION DE LA QUALITE ET DE LA CONSERVATION DE MORCEAUX DE FRUITS PREPARES PAR TRAITEMENT ENZYMATIQUE

Comme il a été rapporté dans les précédentes sessions de la CFCS, le pelage et la découpe des citrons sont très améliorés par l'infusion sous vide avec des solutions enzymatiques. Ces dernières sont disponibles habituellement dans le commerce et conduisent à des produits améliorés, d'une meilleure apparence et d'une meilleure qualité aromatique que ceux préparés par des méthodes traditionnelles. Les enzymes du commerce conduisent à des produits à arôme, texture et stabilité à la conservation variables. Quand les traitements permettent un renforcement de la membrane des morceaux, les produits emballés par voie sèche avec films rétractiles

ont une meilleure texture et une plus grande stabilité. Dans certains échantillons, il apparaît des cristaux blancs au cours du stockage. A l'analyse c'est de la naringine et on peut empêcher leur formation par pulvérisation d'eau ou immersion et rinçage dans des solutions alcalines et diluées. Le procédé permet une meilleure fabrication pour la préparation de tranches d'agrumes et peut être appliqué aussi bien à d'autres types de fruits.

INTRODUCTION

Research at our laboratory has continued on methods to improve quality and storage stability of prepared, table ready cut fruit slices and segments using the «vacuum infusion» method we reported. In an earlier report, we showed how new lightly-processed fruit slices and pieces had a potential for new markets for the Caribbean and other tropical agricultural areas (Berry and Bruemmer, 1987). Last year, we described how this approach could be applied to small scale processes for «cottage industry» type applications (Berry, 1988). We have further studied details of the systems to improve the quality and storage stability of such products and this is a report of recent progress.

We have concentrated effort on the process as applied to citrus segments, since studies, inquiries and industry interest have indicated that these products have the best potential for immediate application. Briefly, the process consists of scoring the peel, submerging the fruit in an enzyme solution (commonly available, industrially-used pectinases), creating a vacuum around the solution, breaking the vacuum and allowing the surrounding solution to be drawn into the interior of the fruit through the scored peel. After a short time of incubation, the peel becomes very loose and is easily removed by hand, with very little effort. The fruit segments also separate very cleanly and easily, yielding a product of pleasing appearance and flavor/texture. However, research indicated considerable variation in ease of peeling and flavor/texture stability in storage, depending on conditions of preparation. Studies were carried out to improve quality and storage stability by optimizing conditions and preparations used for segment separation.

MATERIALS EN METHODS

VACUUM INFUSION OF FRUIT

Fresh washed grapefruit (*Citrus paradisi* Mac. F.) and oranges (*C. sinensis* L.) were warmed to about 30°C, briefly dipped in boiling water to sterilize the

surface, radially scored from stem to blossom end with a sharp knife by hand, just penetrating the flavedo and submerged in a bath of commercial pectinase in water. The container of fruit was then placed in a vacuum oven, evacuated to about 75 Torr, held for two minutes and the vacuum released slowly. This allowed the surrounding enzyme solution to penetrate into the interior of the fruit through the scored peel. The infused fruit were then stored at 30°C for 60 min and then peel removed and segments separated by hand. Fruit were evaluated and graded on a scale of 1-5 for ease and thoroughness of peel removal, and ease and efficiency of segment separation. A score of 1 was equal to untreated fruit and 5 indicated complete and thorough removal of peel or separation of segments with little effort and no remaining pieces of albedo, flavedo or rag.

Two general experimental studies were performed. In one, pectinase solutions were prepared at equal concentrations of total protein content. In the second, they were prepared in concentrations of each required to yield a peel separation score of 4.0. These were determined by testing each enzyme at several concentrations and using regression analysis to determine amounts needed to result in the desired peel rating score.

FLUID LOSS

Liquid lost during storage at 2°C was measured by weighing segments in plastic resealable bags, and weekly draining out any free liquid and reweighing. Loss was expressed as % of original weight. Percent retained was determined by subtracting % loss from 100.

EFFECTIVENESS INDEX

For directly comparing specific enzyme content of pectinases used vs. their effectiveness in removing peel and separating segments, and «Effectiveness Index» was developed. This consisted of adding the peeling score to the segmenting score, and multiplying this sum by the percent of fluid retention, thus : $(\text{peel score} + \text{segment score}) \times (100 - \text{fluid loss}) = \text{Effectiveness Index}$. A higher index indicates a more effective pectinase.

ENZYME ACTIVITY

Commercially available pectinases were obtained from Miles Laboratories, Elkhart IN, Novo Labs, Wilson CT and Rohm Tech, Malden MA. All samples were assayed for polygalacturonase (PG), cellulase and pectinesterase (PE), activities by the methods of Vas et al. (1967) as modified by Bruemmer et al. (1978). PG activity was measured and recorded both with 1.5 % polygalacturonic acid and with 0.45 % rapid-set lemon pectin as substrates.

Table 1 : Relative effectiveness of pectinases at equal concentrations of protein

Index (a)	Peeling	Segment Sep.	Fluid Retent.	PGA/P*	PGA/A*
7,7	4	4	95,7	214	1619
7	3,8	3,8	91,8	118	581
6,5	3,8	3,4	90,6	343	41
6	4	2,8	88,7	21	18
5,3	3	2,5	97,1	94	100

(a) peel rating + segment rating x fluid retention.

* Activity units of polygalacturonase assayed on pectin (P) or on polygalacturonic acid (A), at concentrations that resulted in peel ratings of 4.0.

Table 2 : Textural properties of stored segments

Effectiveness Index	Vesicle Comp. (lbs)	Membrane Shear (lbs)
5,3	164,9	151,3
6	98,7	108,8
6,5	121,9	106,7
7	107,1	90,9
9,7	96,9	73

Cellulase activity was measured using 2 % carboxymethylcellulose in water as substrate. A unit of activity was defined as the amount of enzyme necessary to bring about a 25 % reduction in initial viscosity of 6 ml of substrate in 10 min.

PE activity was determined with 15 ml of pectin solution as substrate adjusted to pH 4.0 prior to addition of the enzyme. Amount of 0.1 N NaOH solution neutralized at 50°C was plotted and the slope determined by the least squares method. PE units were calculated by the procedure of Rouse (1955).

TEXTURE MEASUREMENT

Texture was measured by determining the firmness of intact segments and the toughness of the carpellary membranes. For both determinations an Instron Model 1011 Universal Tester was used, set on compression mode (Instron Corp., Canton MA). Firmness was measured by crushing two segments to 3mm thickness in an Ottawa Texture Measurement System, and crushed segments were transferred to a Kramer Cell and the amount of force required to shear the membranes was determined (Kramer, 1960).

FLAVOR COMPARISONS

Flavor quality of segments which had been stored in sealed plastic bags of varying oxygen permeabilities was measured using direct rankings of three samples and comparing the sums (Kramer, 1960). Bags compared were : (1) Highly permeable, hand-pressure-closable polyethylene (2) composite heat-sealed intermediate oxygen permeability (3000 cc/sq. M/days) (3) composite heat-sealed very-low oxygen permeability (4-6 cc/sq. M/days). Segments in bags were stored at 2°C and sampled at 7 day intervals. All samples were tasted as juice by blending the segments, in order to avoid differences and variability due to the influence of individual segment texture, membranes etc. An experienced taste panel of 20 members was presented juices in red glasses, (under red lights to avoid influence of appearance), and requested to rank samples in order of preference.

RESULTS AND DISCUSSION

Considerable variability was observed in the effectiveness of different types of commercial pectinases for loosening and removal of peel, and separation of intact fruit segments. As shown in Table 1, three enzymes were fairly effective, at equal protein concentrations, for peel removal, and two of these were also very effective for segment separation. Four of the five reported

here resulted in retention of over 90 % of the fluid (juice) after several weeks storage. Table 1 lists the five pectinases studied in order of effectiveness indexes, which indicates overall usefulness, considering peeling, segment separation and fluid retention.

In order to develop a better measure of effectiveness, concentrations of each enzyme were adjusted to obtain approximately equal peel removal scores of 4.0, and the enzyme activities of these «equally effective» pectinase solutions were determined. Polygalacturonase, cellulase and pectinesterase activities were measured. However, no relationship was found between peeling/segmenting effectiveness and cellulase or pectinesterase activities. A relationship was observed with polygalacturonase (PGase) activity however and this varied depending on whether the PGase was measured on pectin or polygalacturonic acid (PGAcid) as a substrate. These values are shown in the last two columns in Table 1. Polygalacturonase activity on polygalacturonic acid appeared to be the most directly related to effectiveness of the enzymes for peeling and segmenting. The two preparations with highest effectiveness indexes were highest in this respect as well. As seen in the third preparation in Table 1, however, a relatively high PGase activity on pectin still marked a significantly effective pectinase for peel removal and segment separation. PGase activity on PGacid substrate may be the best measure of enzyme effectiveness, with PGase activity on pectin a secondary indicator. On the other hand, those pectinases that were low or lacking in both were not as effective for peeling and sectioning.

There is a near inverse relationship between effectiveness index and texture, which may indicate the enzymes which remove peel and separate segments best also results in the most «tender» or least «tough» membranes. The textural properties of the treated segments as determined by compression of vesicles and membrane shear are shown in Table 2. The enzymes with indexes of 6.0 and 6.5 were slightly out of order with the other values but this may be due to experimental error. The use of judgmental scores for ease of removal of peel and separation of segments is not very precise and points up the need for a better standardized system. This probably accounts for some of the observed variability.

Flavor stability of the segments dry-packed in plastic bags varied somewhat with the degree of oxygen permeability of the package. In highly oxygen-permeable bags, flavor deteriorated rapidly and after 3 weeks the samples were disliked by the flavor panel at the 99 % confidence level. In limited permeability bags all samples were still in the «like» category after two weeks at 2°C but after three weeks those in moderately permeable bags were preferred over those in almost impermeable bags. Thus, it appears a

restricted but not anaerobic environment may be the best for storage of these products.

Grapefruit segments stored as indicated, sometimes developed tiny white specks, mainly on the surfaces where peel had been removed. Microscopic examination of these specks, and their solubility and TLC behavior, melting point and IR spectrum identified them as naringin. Besides being a visual defect, naringin is intensely bitter and its presence on these segments is therefore very undesirable. However, we found washing with a gentle stream of warm water, or a brief dip in dilute NaOH solution followed by water misting, removed these naringin spots or reduced them to a tolerable level. They did not seem to be as prevalent with certain treated samples as with others, which may indicate a low level of naringinase in some of the pectinase samples, which helps reduce these specks.

In conclusion, the vacuum infusion of commercially available pectinases provides the basis for a new approach to preparation of citrus segments in a dry pack, plastic enclosed package. The product offers several advantages over conventionally prepared citrus segments, in avoiding exposures of the product to high temperatures or to hot lye solutions.

Flavor and texture of the products are better than those of conventional products and they can be prepared with a minimum of hand labor at reasonable cost. The resultant product is of high quality, nutritious and very highly increased in value, but needs to be transported and stored under refrigerated conditions. The product however, offers great convenience and citrus segments in as near natural form as possible. The process may be applicable to slices and cut pieces of other types of tropical fruits, as well.

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