JARQ 49 (4), 313 - 318 (2015) http://www.jircas.affrc.go.jp

REVIEW

Recent Progress in Technologies for Enzymatic Peeling of Fruit

Masaki NOGUCHI[1,4]*, Yoshihiko OZAKI[2] and Jun-ichi AZUMA[3]

[1] Plant Physiology and Fruit Chemistry Division, NARO Institute of Fruit Tree Science, National Agriculture and Food Research Organization (NARO)
(Tsukuba, Ibaraki 305-8605, Japan)
[2] Faculty of Biology-oriented Science & Technology, Kinki University
(Kinokawa, Wakayama 649-6493, Japan)
[3] Graduate School of Engineering Osaka University
(Suita, Osaka 565-0871, Japan)

Abstract

Enzymatic peeling of fruit and vegetables is considered superior to conventional peeling methods in terms of minimizing product damage and indeed is expected to improve them. This technology was first proposed for the peeling of citrus fruit and numerous studies on its practical use have been conducted. However, its application to non-citrus fruit and vegetables remains limited. A novel process, including heat treatment prior to enzymatic treatment, is proposed to peel persimmon fruit. Heat treatment induces fine and even cracks on the fruit surface of certain persimmon cultivars, which act as channels via which enzyme solution can enter the peel tissue. Another function of the heat treatment is to inactivate polygalacturonase-inhibiting protein (PGIP), which is inherent in persimmon fruit. Inactivation of PGIP improves the efficiency of enzymatic peeling with exogenous polygalacturonase. Chemical pre-treatments with a food-grade emulsifier solution and a weak basic solution also enhance the permeability of enzyme solution into the peel tissue. These physical and chemical treatments are useful for applying enzymatic peeling to various persimmon cultivars. In future, enzymatic peeling technology is expected to be applied to various agricultural crops as the technology is further refined.

Discipline: Food
Additional key words: cuticle, emulsifier, persimmon, polygalacturonase, wax

Introduction

Enzymes are large molecules ─ mainly proteins ─ that catalyze specific chemical reactions. In contrast, most chemical catalysts catalyze a wide range of chemical reactions. Accordingly, in fruit processing, using enzymes has the advantages of preventing undesirable changes in material characteristics and the potential to retain the qualities of the raw materials.

Traditionally, application of enzymatic reactions in fruit processing was limited to liquid obtained from pressed, crushed or homogenized fruit. However, the development of enzyme infusion technology (Baker 1996, McArdle 1994) has expanded the applicable scope of enzymes to include solid fruit, to maintain their form. One example of the application of enzyme infusion technology is enzymatic peeling of citrus fruit rind (Adams 1991, Bruemmer 1981).

Enzymatic peeling of citrus fruit

Enzymatic peeling technology was first proposed for the peeling of citrus fruit prior to that of non-citrus fruit for three reasons (Pretel 2010).

The first reason pertains to fruit anatomy. Citrus fruit

This paper reports the results obtained in the joint project on “City Area Program 2009”, “Regional Innovation Cluster Program 2010” and “Regional Innovation Strategy Support Program 2011” sponsored by Ministry of Education, Culture, Sports, Science and Technology.
Present address:
[4] Collaboration and Public Relations Center, Headquarters, National Agriculture and Food Research Organization (NARO)
(Tsukuba, Ibaraki 305-8517, Japan).
*Corresponding author. e-mail: zhenji@affrc.go.jp
Received 28 April 2014; accepted 19 January 2015.

can be divided into the flavedo (the exocarp [epicarp] or zest, the peripheral and colored cell layers of the pericarp), albedo (the mesocarp or pith, the inner and white part of the pericarp), segment membranes and juice sacs (flesh or pulp), each of which differ in appearance and morphology. The citrus peel is generally easily separated from the flesh tissue, whereas the boundary between the peel and flesh tissue in non-citrus fruit is often difficult to distinguish. For this reason, citrus peel is easier to treat with enzymes than non-citrus fruit.

The second reason relates to fruit cell wall chemistry. Since the main component of citrus peel is cell wall pectic polysaccharides, the albedo and segment membranes can be degraded by treatment with hydrolytic enzymes that show polygalacturonase, cellulase and hemicellulase activities. Enzyme preparations possessing these activities are commercially available for industrial use, which makes the enzymatic treatment of citrus peel practically feasible.

The third reason is linked to fruit biochemistry. Polygalacturonase-inhibiting proteins (PGIPs) are present in the cell walls of vegetative and fruit tissues and inhibit polygalacturonase of fungal origin. PGIPs play roles in eliciting plant defense responses and avoiding the microbial decay of plant tissues (Lorenzo 2002, Matteo 2006), while those present in fruit tissues sometimes inhibit enzymatic processing such as enzymatic peeling of healthy persimmon fruit (Ozaki 2011). However, PGIPs may not affect the enzymatic peeling treatment of healthy citrus fruit since the expression of PGIP genes in citrus fruit is induced after wounding or inoculation of microorganisms (Barmore 1985, Gotoh 2002, Nalumpang 2002).

Enzymatic peeling of persimmon fruit

1. Importance of heat treatment prior to the enzyme reaction

The application of enzymatic peeling technology for non-citrus fruit has only been investigated for stone fruit. Toker (2003) studied the enzymatic conditions required to treat peach (*Prunus persica*), nectarine (*Prunus persica* var. *nucipersica*) and apricot (*Prunus armeniaca*) fruit successfully. Incubating these fruit in enzyme solution containing polygalacturonase, hemicellulase and cellulase activities at moderately high temperatures (around 45°C) facilitated their peeling. For vegetables, Suutarinen (2003) studied the enzymatic peeling of potato tubers (*Solanum tuberosum* cv. Asterix), carrot roots (*Daucus carota*), Swedish turnip roots (*Brassica napus*) and onion bulbs (*Allinum cepa*). In the procedures proposed for the above-mentioned stone fruit and vegetables, entry of the enzyme solution into the tissues was facilitated by vacuum infusion without any pretreatment of the plant materials.

For enzymatic peeling of persimmon (*Diospyros kaki*) fruit, Ozaki (2004) proposed employing heat treatment prior to the enzyme reaction (Fig. 1 A), which induces the formation of cracks in the cuticle peel. The cracks then act as channels through which the enzyme solution is introduced into the peel tissue. Moreover, the heat treatment effectively inactivates PGIP. Accordingly, the introduced polygalacturonase acts efficiently and degrades peel tissue within several hours in the outer region where PGIP was inactivated by heat treatment. In contrast, enzyme activity would be inhibited in the flesh, where PGIP remains active. These findings could be the main reason why enzymatic degradation of peel tissue occurs within a limited region and produces peeled fruit with a smooth surface.

2. Extension to other persimmon cultivars by perforation treatment

The above-mentioned enzymatic peeling method was applied to a limited number of persimmon cultivars, including ‘Hiratanenashi’ and ‘Tone-wase’. Further investigation revealed that the formation of cracks in the cuticle in response to heat treatment varied among persimmon cultivars. The cultivars were divided into three groups, based on whether the cracks occurred over the entire fruit surface, only in a limited area such as the fruit apex and immediate vicinity, or cracks barely developed at all (Ozaki 2011, Sakai 2012).

One reason for this phenomenon may be the mechanical strength of the cuticle layer on the outer surface of the peel. Tsubaki (2012) isolated cuticle membranes (CMs) from fruit of 27 persimmon cultivars and analyzed the relationships among the cultivars based on their chemical composition, density and mechanical properties. Wide cultivar-dependent divergence was observed in both chemical and mechanical properties, regardless of astringency. Correlation analysis of these parameters revealed that no single factor strongly affects the mechanical strength of the CMs. It was suggested that integrating both density and chemical constituents (polysaccharides, wax and cutin) of the CMs is linked to the expression of the mechanical strength of the cuticle.

Because the enzyme solution is introduced into the peel tissue through the cuticular cracks by simple diffusion, distribution of the enzyme solution is limited to the peel close to the cracks. For cultivars in which cuticular cracks rarely develop in response to heat treatment, a different strategy is required to improve distribution of the enzyme solution within the fruit. Sakai (2012) proposed perforating the peel with a needlepoint holder prior to the heat treatment (Fig. 1 B). The perforations were connected via cracks after heat treatment and the development of cracks was greatly enhanced. Subsequent enzymatic reaction treatment was also effective. The combination of perforation and heat treatments extended the applicable scope of enzymatic

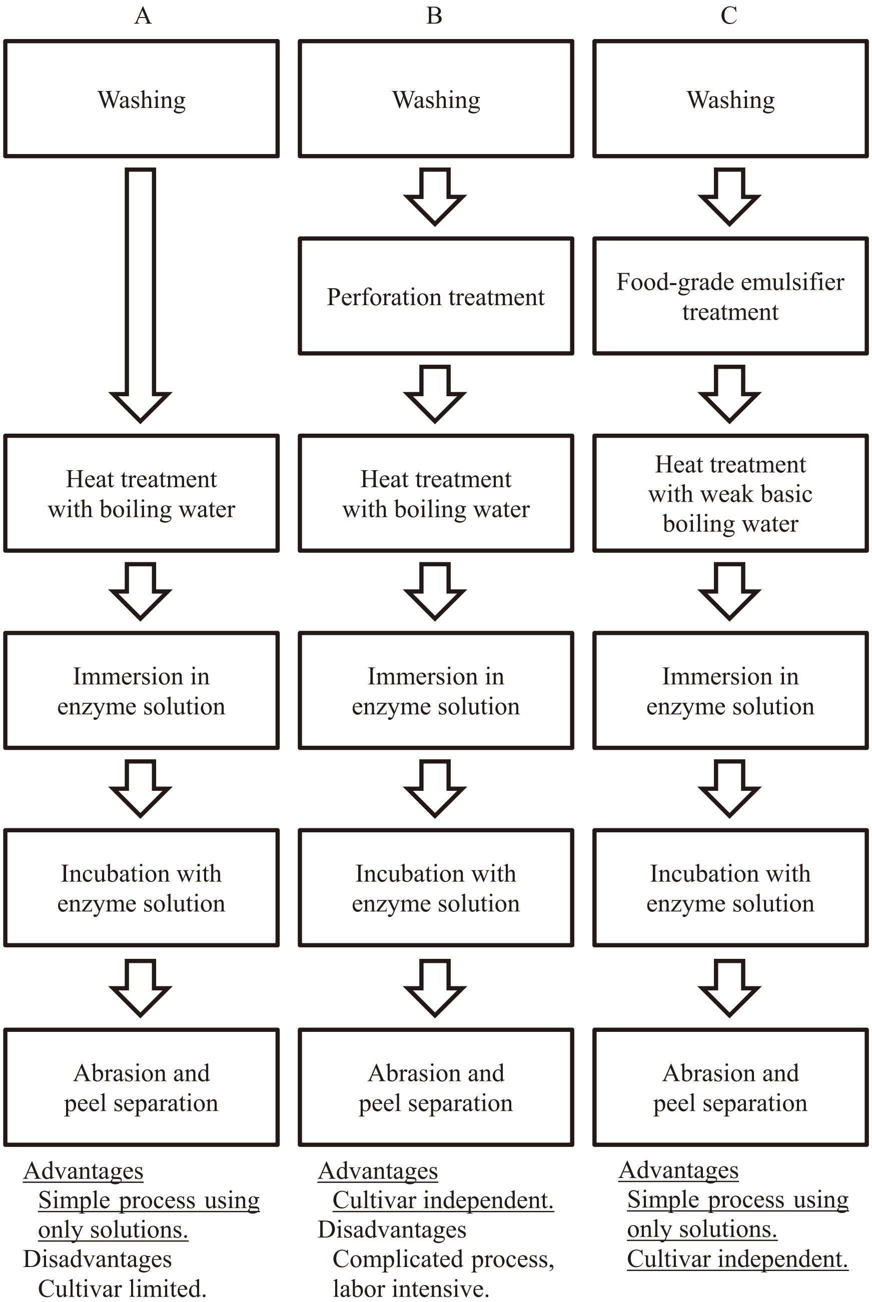

Fig. 1. Procedures to improve the enzymatic peeling of persimmon fruit

peeling technology to additional persimmon cultivars. For example, fruit of 'Fuyu', which is one of the main persimmon cultivars grown in Japan and in which cracks are rarely induced in the cuticle by heat treatment, can be peeled by combining perforation and heat treatment. However, the procedure is more labor-intensive, because the perforation treatment must be applied carefully to the whole fruit by hand. Mechanical perforation of the fruit surface has been proposed to improve the ease and efficiency of perforation treatment (Nakanishi 2008). However, it is difficult to adapt machinery to the various fruit shapes of different persimmon cultivars. Accordingly, simpler treatments rather than physical perforation treatment, such as immersing the fruit in fluid, are required.

3. Chemical treatment with weak basic and food-grade emulsifier solutions

The cuticle, which covers the outer surface of the fruit peel, mainly comprises wax and cutin. Wax is soluble in an organic solvent and comprises hydrophobic substances such as high-molecular-weight hydrocarbons and esters composed of high-molecular-weight fatty acids and alcohols. Cutin is a polymer of hydroxy fatty acids combined by ester bonds, which is degraded under basic conditions.

Noguchi (2013a) isolated fruit CMs from ‘Fuyu’ and ‘Hiratanenashi’ persimmon cultivars and analyzed their mechanical properties after physical or chemical treatment. Chemical treatment, which involved removal of wax with an organic solvent and further removal of cutin with a basic solution, reduced the breaking strength of isolated CMs more effectively than autoclave treatment (Table 1). This finding indicates that chemical treatment of the cuticle, as a pretreatment of the enzymatic peeling process, is effective in weakening the persimmon fruit skin and making cracking easier to induce with heat treatment.

The effect of a chemical treatment that removes or degrades the cuticle on the efficiency of enzymatic peeling has been tested with fresh fruit. The fresh fruit were treated with chloroform/methanol (1:1, v/v) to remove hydrophobic compounds and weaken the cuticle before heat treatment and showed enhanced surface cracking after the heat treatment and improved enzymatic peeling efficiency. Given that an effective process can be applied in industrial food processing, the combination of immersion in a food-grade emulsifier solution and heat treatment in a weak basic solution instead of organic solvents was proposed (Fig. 1 C) (Noguchi 2013b). Although treatment with some food-grade emulsifier solutions is not equivalent to removing wax with an organic solvent, it helped weaken the outer surface of the cuticle. Subsequent heat treatment in a weak basic solution may contribute to partial degradation of cutin. Using this novel treatment, the entire process for enzymatic peeling of persimmon fruit can be performed in solutions while eliminating the need for mechanical perforation (Fig. 2) (Noguchi 2013b).

Immersion in solutions is a simple and easy treatment, which is expected to improve the ease and efficiency of the peeling process for many agricultural crops. Additionally, by modifying and improving this novel process, enzymatic peeling technology can be extended to other agricultural crop species and cultivars.

4. Microbiological quality of enzymatically peeled persimmon fruit

The risk of microbial contamination of freshly harvested fruit should be controlled. Murakami (2012a) investigated changes in the microflora of persimmon fruit during the enzymatic peeling process and compared the microbial quality of enzymatically peeled fresh-cut slices with that of manually peeled fresh-cut slices using a knife.

Table 1. Effects of autoclave treatment and chemical treatments to remove wax and cutin on the mechanical property of the isolated cuticle membranes[1) 2)]

Treatment	Longitudinal breaking strength (MPa) [3)]				
	Cultivar				Simple main effects (Cultivar)
	Hiratanenashi		Fuyu		
Control	9.3	d [8)]	10.2	c	
Autoclave treatment [4)]	6.7	c	9.7	c	**
Chemical treatment to remove wax [5)]	3.4	b	4.2	b	*
Chemical treatment to remove wax and cutin [6)]	0.3	a	0.1	a	
Significance [7)]					
Treatment	***				
Cultivar	***				
Interaction (Treatment x Cultivar)	**				

1) This table was reworked from published data in Noguchi et al. (2013a).
2) Values are the mean of $n = 8$.
3) Measured as the mechanical strength of isolated cuticle membranes.
4) 120˚C, 20 min.
5) Treatment with chloroform/methanol (2:1, v/v) as the organic solvent.
6) Treatment with 1.0% (w/v) potassium hydroxide methanol solution after removal of wax.
7) Two-way factorial analysis of variance ($n = 8$).
8) Numbers followed by the same letter within a column do not differ significantly at $P < 0.05$ by the Tukey-Kramer test (post-hoc testing for simple main effects (Treatment)).
***, **, * Significant at $P < 0.001$, $P < 0.01$ and $P < 0.05$ respectively.

Fig. 2. Appearance of persimmon fruit peeled using the improved enzymatic peeling procedure
Upper images, unpeeled fruit; lower images, peeled fruit.
Cultivars: (a), 'Hiratanenashi'; (b), 'Ichidagaki'; (c), 'Kosyuhyakume'; (d), 'Saijyo'; (e), 'Atago'.

Although the peel of persimmon fruit had diverse microflora, heat treatment before the enzymatic peeling process reduced the microbial counts in both the peel and flesh of all fruit to levels below lower detection limits. When microbial contamination of enzyme- and knife-peeled persimmon slices was compared, the bacterial counts and diversity of the bacterial and fungal flora were lower in enzyme-peeled slices than in knife-peeled slices.

Another quality consideration, namely of physicochemical qualities such as texture, pH and surface color, of enzyme- and knife-peeled persimmon slices was evaluated (Murakami 2012a). It was revealed that the suitability of enzyme-peeling of persimmon fruit for fresh-cut slices was cultivar-dependent.

The microbiological quality and shelf life of enzyme-peeled fresh-cut persimmon slices were studied during storage in a high CO_2-controlled atmosphere and active modified atmosphere packaging (MAP) at 10°C (Murakami 2012b). The 20% CO_2 atmosphere was most effective for lowering the microbe counts of the enzyme-peeled slices in high CO_2 atmospheres (10, 15 and 20%). Texture, pH, surface color, sugar content and total ascorbic acid content of enzyme-peeled persimmon slices were unaffected by air or 20% CO_2 as the flushing gas. When enzyme-peeled fresh-cut persimmon slices were stored in an active MAP of 20% CO_2, the high CO_2 atmosphere reduced the frequency of bacterial growth and the shelf life was four days at 10°C.

Conclusion

In this paper, improvement in enzymatic peeling technology and its application to an extended variety of fruit species and cultivars was discussed. Peeling is an indispensable procedure when processing the majority of fruit and vegetables. However, there is still room to improve peeling technology. Currently, hand peeling is the most common method used, but is extremely labor-intensive and time-consuming. Enzymatic peeling technology is a promising candidate with which to replace hand peeling in future. Further extending the applicable scope of enzymatic peeling technology to cover additional species and cultivars of agricultural crops can be expected as a result of combining enzymatic, physical and chemical treatments.

References

Adams, B. et al. (1991) Process for enzyme peeling of fresh citrus fruit. US Patent No. 5000967.

Baker, R. A. et al. (1996) Current and potential applications of enzyme infusion in the food industry. *Trend. Food Sci. Technol.*, **7**, 279-284.

Barmore, C. R. & Nguyen T. K. (1985) Polygalacturonase inhibition in rind of Valencia orange infected with *Diplodia natalensis*. *Phytopathology*, **75**, 446-449.

Bruemmer, J. H. et al. (1981) Method preparing citrus fruit sections with fresh fruit flavor and appearance. US Patent No. 4284651

Gotoh, Y. et al. (2002) A cDNA Encoding Polygalacturonase-inhibiting protein induced in citrus leaves by polygalacturonase of *Alternaria citri*. *J. Gen. Plant Pathol.*, **68**, 57-61.

Lorenzo, G. D. & Ferrari, S. (2002) Polygalacturonase-inhibiting proteins in defense against phytopathogenic fungi. *Curr. Opin. Plant Biol.*, **5**, 295-299.

Matteo, A. D. et al. (2006) Polygalacturonase-inhibiting protein (PGIP) in plant defence: a structural view. *Phytochem.*, **67**, 528-533.

McArdle R. N. et al. (1994) Enzyme infusion: A developing technology. *Food tech.*, NOVEMBER, 85-89.

Murakami, Y. et al. (2012a) Microbiological and physicochemical quality of enzymatically peeled persimmon fruit for fresh-cut slices. *HortSci.*, **47**, 382-385.

Murakami, Y. et al. (2012b) Microbiological quality and shelf life of enzyme-peeled fresh-cut persimmon slices stored in high CO_2 atmospheres. *HortSci*., **47**, 1758-1763.

Nakanishi, H. et al. (2008) Machine for perforating persimmon fruit. JP Patent No. 4174440. 2008-10-29 published [In Japanese].

Nalumpang, S. et al. (2002) Functional characterization of citrus polygalacturonase-inhibiting protein. *J. Gen. Plant Pathol*., **68**, 118-127.

Noguchi, M. et al. (2013a) Pre-treatment with weak basic and detergent solutions improved enzymatic peeling process of persimmon fruit. *Nippon Shokuhin Kagaku Kogaku Kaishi* (J. *Jpn. Soc. Food Sci. Technol.*), **60**, 582-588 [In Japanese with English summary].

Noguchi, M. et al. (2013b) Method for peeling of persimmon fruit and peeled persimmons. JP Patent Publication No. 2013-243959. 2013-12-09 published [In Japanese].

Ozaki, Y. et al. (2004) Method for peeling of persimmon fruit, peeled persimmons and packaged peeled persimmons. JP Patent No. 3617042. 2005-02-02 published [In Japanese].

Ozaki, Y. (2011) *Food Enzyme Chemistry: its Cutting-edge Technology and Development in Food Proteomics.* CMC Publishing Co., Ltd., Tokyo, Japan, pp. 220-229 [In Japanese].

Pretel, M. T. et al. (2010) Enzymatic Peeling of Citrus Fruits. *Enzymes in Fruit and Vegetable Processing*: *Chemistry and Engineering Applications*, CRC Press, New York,
pp. 145-174.

Sakai, Y. et al. (2012) Method for peeling of persimmon fruit and peeled persimmons. JP Patent No. 4896651. 2012-03-14 published [In Japanese].

Suutarinen, M. et al. (2003) The potential of enzymatic peeling of vegetables. *J. Sci. Food Agric*., **83**, 1556-1564.

Toker, İ. et al. (2003) Enzymatic peeling of apricots, nectarines and peaches. *Lebensm. Wiss. Technol*., **36**, 215-221.

Tsubaki, S. et al. (2012) Mechanical properties of fruit-cuticular membranes isolated from 27 cultivars of *Diospyros kaki* Thunb. *Food chem*., **132**, 2135-2139.