Journal of Agricultural and Food Chemistry

NOVEMBER 1996
VOLUME 44, NUMBER 11

Protein in Varietally Derived Apple Juices†

Katharine M. Wall,‡ Valerie M. Tait,‡ Kenneth C. Eastwell,‡ Carol A. Reid,§ and Thomas H. J. Beveridge*,‡

Pacific Agri-Food Research Centre, Summerland, British Columbia, Canada V0H 1Z0, and Sun-Rype Products Ltd., 1165 Ethel Street, Kelowna, British Columbia, Canada V1Y 7N6

The effect of oxidation during apple juice manufacture on the levels of flavan-3-ol and protein was determined. Increased oxidation reduced levels of protein to 84−184 mg/L in oxidized juice from 133−424 mg/L in less oxidized product. Flavan-3-ol levels were also markedly reduced. Oxidation caused SDS-PAGE patterns to be increasingly smeared or streaked with increasing amounts of material trapped at the origin. Throughout these oxidative effects on juice protein and flavan-3-ol, there was persistence of a protein of approximately 32 kDa in the juices of McIntosh, Golden Delicious, and Jonagold apples. The possible relationship of this "32 kDa" protein to haze formation in clarified apple juice is discussed.

Keywords: *Apple juice; fruits; polyphenol oxidation; enzymes*

INTRODUCTION

There is little information in the literature regarding apple proteins and even less relating to proteins in apple juice. Apples contain about 0.2% protein measured as crude (Kjeldahl) protein nitrogen, but just 50−80% of this nitrogen is derived from protein (Hansen, 1970). These numbers suggest that the protein content of apple juice may be expected to range from 1000 to 1600 mg/L. These values are not consistent with experimentally reported protein levels in apple juice of 11−180 mg/L (Van Buren, 1992). The lower range of values perhaps reflects the oxidation reactions that occur during juice manufacture and the tendency for protein to tan to the pomace residue (Wall et al., 1996). The proteins extractable from fresh apple tissue or acetone powders include enzymes such as amylases, esterases, and phenolases, and other proteins (Clements, 1970). Proteins extracted under these conditions can be subjected to electrophoretic analysis (Clements, 1970), and a large number of individual proteins have been demonstrated (Lay-Yee et al., 1990). However, juice proteins vary significantly from these reports because juice does not extract all apple proteins quantitatively (Clements, 1970). Moreover, commercial apple juice is routinely made from apple mash that is actively oxidized in the presence of polyphenol oxidase; this reaction modifies both the protein and phenol content of the resulting juice (Johnson et al., 1969; Wall et al., 1996). Denaturing polyacrylamide electrophoresis revealed, in juice prepared from Granny Smith apples, protein with a broad range of subunit sizes ranging from <14 kDa to >200 kDa (Hsu et al., 1989). Additional proteins (~64−92 kDa) were detected in enzyme-clarified juice and were identified as proteins added during enzyme treatment. Evaluation of apple juice stability suggested that 21−31-kDa proteins were related to juice instability and haze formation (Hsu et al., 1989).

The purpose of this study was to examine variability in the electrophoretic patterns of proteins from juice of several apple cultivars, and to identify constraints to the routine electrophoresis of apple juice proteins.

MATERIALS AND METHODS

Apples were collected at commercial maturity from the Summerland Packing House or Summerland Research Centre

* Author to whom correspondence should be addressed.

† Pacific Agri-Food Research Centre Contribution 923.

‡ Pacific Agri-Food Research Centre.

§ Sun-Rype Products Ltd.

S0021-8561(96)00159-8 CCC: $12.00

Table 1. Protein and Flavan-3-ol in Apple Juice[a]

treatment	variety	protein (mg/L)	flavan-3-ol (mg/L)
fresh pressed, peeled apples	Granny Smith	372	1179
	Spartan	312	374
	McIntosh	187	84
	Golden Delicious	133	342
	Jonagold	194	521
	Red Delicious	424	399
fresh pressed peeled apples; aerated mash	Granny Smith	95	28
	Spartan	155	28
	McIntosh	84	18
	Golden Delicious	127	81
	Jonagold	143	264
	Red Delicious	184	68
fresh pressed unpeeled apples; aerated mash	Granny Smith	153	927
	Spartan	114	44
	McIntosh	109	147
	Golden Delicious	107	47
	Jonagold	115	89
	Red Delicious	171	79

[a] Values based on bovine γ-globulin standard for protein and catechin for flavan-3-ol.

orchards in the 1993 season. Apples were stored at 1 °C and utilized for juice production within 2−3 months of harvest.

Flavan-3-ol Analysis. Apples (~70 g) were blended with 150 mL of 100% methanol (HPLC grade) in a Waring blender at high speed for 5 min (Burda et al., 1990). The homogenate was vacuum filtered through Whatman No. 42 filter paper and diluted quantitatively to 250 mL with 100% methanol. Methanol extracts were stored at −40 °C for future analysis. Quantitative analysis of flavan-3-ol in the methanol extract was obtained by the vanillin-HCl (Price et al., 1978; Porter et al., 1986) colorimetric assays. Catechin was used as the standard, and results were reported in catechin equivalents. All samples were corrected for interfering anthocyanins in the vanillin-HCl assay with sample blanks (Lees et al., 1995).

Protein Analysis. Juice was prepared from apples (peeled or unpeeled) in a home-style centrifugal juicer and either frozen immediately or aerated for 20 min at room temperature prior to freezing. Samples were stored frozen at −18 °C until used for protein analysis. Frozen apple juice was thawed and immediately centrifuged (Eppendorf centrifuge, model 5415C) to pellet particulate. Juice protein was concentrated by centrifuging 1 mL of juice through a 5000 molecular weight cutoff dialysis membrane (Ultrafree-CL low binding cellulose membrane: Millipore); the retained suspension was dissolved in 0.1 N NaOH, and the relative amount of protein in the juice and protein isolates were determined according to the method of Bradford (1976; Bio-Rad) with bovine gamma globulin as standard. Qualitative protein assessment was achieved by sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE; 4% stacking gel and 17.5% resolving gel) (Laemmli, 1970), and the protein bands were visualized by silver staining (ICN).

RESULTS AND DISCUSSION

The increased oxidation produced by aeration of juice reduced the levels of both protein and flavan-3-ol in the resultant apple juice relative to nonaerated samples (Table 1). Protein levels in aerated juices ranged from 84−184 mg/L, results that are consistent with the previously reported levels of 11−180 mg/L (Van Buren, 1992). However, the results also indicated that if oxidation was less complete, then much higher protein levels in the juice were obtained, with values in this study ranging from 133 to 424 mg/L. Similarly, flavan-3-ol levels of juice were reduced markedly by aeration (Table 1) as would be expected (Johnson et al., 1969). Oxidation likely contributed to the lower protein levels in aerated juices through "tanning" of protein to the pomace (Lea, 1992).

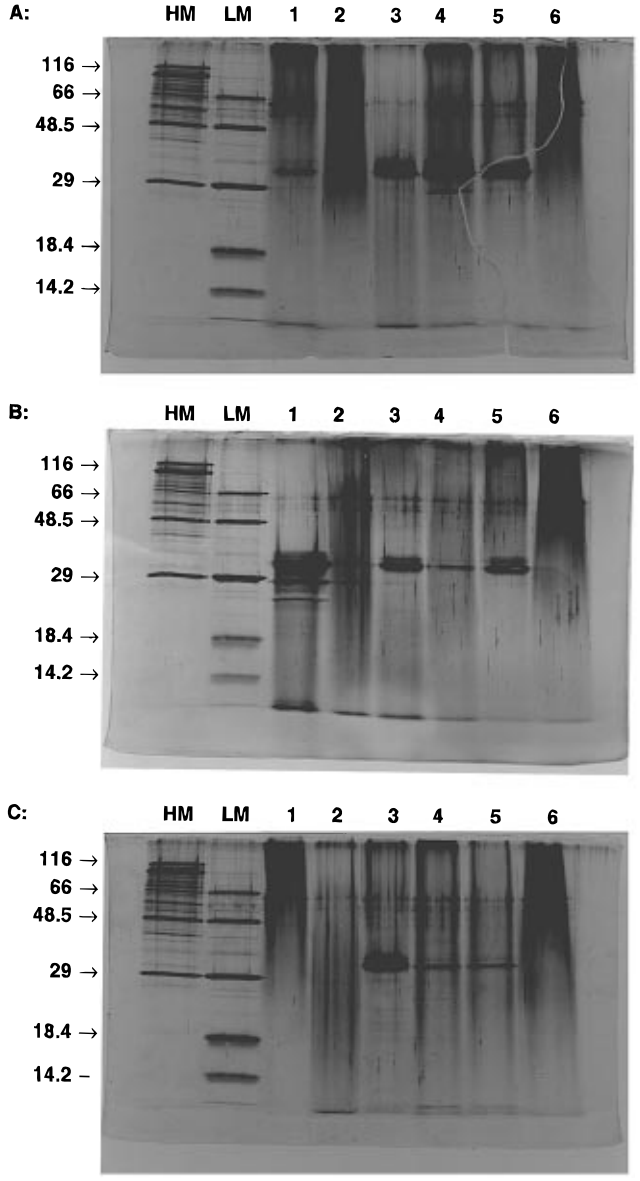

Figure 1. SDS-PAGE analysis of 3 μg of protein from apple juices: (A) juice from peeled apples; (B) juice from peeled apples, aerated for 20 min; and (C) juice from unpeeled apples, aerated for 20 min. Key: (HM) high molecular weight silver stain protein standards; (LM) low molecular weight silver stain protein standards; (1) Granny Smith juice; (2) Spartan juice; (3) McIntosh juice; (4) Golden Delicious juice; (5) Jonagold juice; Red Delicious juice.

Apple juice proteins differed in both quantity (Table 1) and quality depending upon variety (Figure 1), and proteins from each variety responded differently to oxidative conditions. Common to all electrophoretograms were the large amount of stain-reactive material that remained at the origin and the extensive smearing that occurred. Increased oxidation through aeration, especially in the presence of peel components, increased the extent of streaking. This correlation implicates enzymatic oxidation, which occurs in apple juices as a major contributing factor to band smearing. Peel contains more polyphenol oxidase than the flesh of apples (Burda et al., 1990), and the observations in the present study are consistent with this distribution. In spite of this smearing, fresh pressed juice of peeled apples of all varieties, except Red Delicious, gave bands in the region corresponding to 29−33 kDa (Figure 1A). Even in the absence of additional aeration, the Red Delicious pattern was dominated by smearing with no

discrete banding pattern. Aeration of juice from peeled Granny Smith apples provided a more complex pattern of proteins, in the region around 30 kDa, relative to the nonaerated samples (Figure 1B). The dramatic changes in the proteins of juice from Granny Smith apples were unique; changes in the electrophoretic protein pattern of juices from other varieties were minor in response to aeration. In the presence of peel, formation of high molecular weight complexes was enhanced (Figure 1C), and the 32-kDa band disappeared from Granny Smith and Spartan juice but persisted in McIntosh juice and, to a lesser extent, in Golden Delicious and Jonagold juice. The 32-kDa band was not detected in Red Delicious juice as would be expected considering previous results.

Throughout these complex reactions, there is an obvious persistence in the patterns of a protein of ~32 kDa in the juices of McIntosh, Golden Delicious, and Jonagold apples. Earlier studies have determined that proteins in the range 21−31 kDa were related to haze formation in Granny Smith apple juice stored 3 months (Hsu et al., 1989). The current study of Granny Smith apple juice proteins reveals the presence of a considerable quantity of a "32-kDa protein" in both the nonaerated and aerated juice of peeled apples, but little or none in aerated juice prepared from apple pulp and peel. This result probably relates to the increased levels of both phenol and polyphenol oxidase in peel compared with pulp and the more extensive browning reactions that ensue. These reactions "tan" protein to the pulp and, presumably the "32-kDa protein" is susceptible. Those apples in which abundant 32-kDa protein persists (Granny Smith, McIntosh, Golden Delicious, and Jonagold) may yield juices that have a greater propensity to result in haze formation than the juice from other varieties tested (Hsu et al., 1989).

In terms of the electrophoretic analysis of apple juice proteins, the oxidation reactions that occur normally during juice manufacture make this analysis problematic. However, reduced oxidation makes the patterns clearer, with diminished smearing, suggesting that the study of apple proteins in juice should begin with samples prepared without oxidation, from which clear patterns would be expected (Clements, 1970). We have provided a baseline of proteins extractable into apple juice from which the effects of oxidation can be assessed.

LITERATURE CITED

Bradford, M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248−254.

Burda, S.; Oleszek, W.; Lee, C. Y. Phenolic compounds and their changes in apples during maturation and cold storage. *J. Agric. Food Chem.* **1990**, *38*, 945−948.

Clements, R. L. Protein Patterns in Fruits. In *The Biochemistry of Fruits and their Products*; Hulme, A. C., Ed.; Academic: London, 1970; Vol. 1, pp 159−177.

Hansen, E. Proteins In *The Biochemistry of Fruits and their Products*; Hulme, A. C., Ed.; Academic: London, 1970; Vol. 1, pp 147−158.

Hsu, J. C.; Heatherbell, D. A.; Yorgey, B. M. Effects of fruit storage and processing on clarity, proteins, and stability of Granny Smith apple juice. *J. Food Sci.* **1989**, *54*, 660−662.

Johnson, G.; Donnelly, B. J.; Johnson, D. K. Procyanidins as related to apple juice processing and storage. *Food Technol.* **1969**, *23*, 1312−1316.

Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **1970**, *227*, 680−685.

Lay-Yee, M.; DellaPenna, D.; Ross, G. S. Changes in mRNA and protein during ripening in apple fruit (*Malus domestica* Borkh. cv Golden Delicious). *Plant Physiol.* **1990**, *94*, 850−853.

Lea, A. G. H. Flavour, colour and stability in fruit products: The effect of polyphenols. In *Plant Polyphenols*; Hemingway, R. W., Laks, P. E., Eds.; Plenum: New York, 1992; p 827.

Lees, G. L.; Wall, K. M.; Beveridge, T. H. J.; Suttill, N. H. Localization of condensed tannins in apple fruit peel, pulp and seeds. *Can. J. Bot.* **1995**, *73*, 1897−1904.

Porter, L. J.; Hrstich, L. N.; Chan, B. J. The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. *Phytochemistry* **1986**, *25*, 223−230.

Price, M. L.; Van Scoyoc, S.; Butler, L. G. A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. *J. Agric. Food Chem.* **1978**, *26*, 1214−1218.

Van Buren, J. P. Avoiding haze in clarified apple juice. In *Processed Apples Workshop*. Downing, D. L., Ed.; Special Report 65; New York State Agricultural Experiment Station, Ithaca, NY, 1992.

Wall, M. K.; Tait, V. M.; Eastwell, K. C.; Reid, C. A.; Beveridge, T. H. J. Haze development in aerobically or anaerobically produced clarified apple juices. *J. Food Sci.* **1996**, *61*, 1−5.

Received for review March 12, 1996. Accepted July 30, 1996.[⊗] This project was jointly funded by the Science Council of British Columbia, Sun-Rype Products Ltd., Kelowna, British Columbia, and Agriculture and Agri-Food Canada.

JF960159O

[⊗] Abstract published in *Advance ACS Abstracts*, October 1, 1996.