ELSEVIER

Thermochimica Acta 357–358 (2000) 57–63

thermochimica acta

www.elsevier.com/locate/tca

Degradation of starchy food material by thermal analysis

Poonam Aggarwal*, David Dollimore

Department Of Chemistry, University Of Toledo, Toledo, OH 43606, USA

Received 15 September 1998; accepted 20 June 1999

Abstract

Raw starch has been used in recent years as a direct substrate for enzyme digestion in the production of fermentation products. The objective of this study was to examine the differences between the residual raw starch produced after partial hydrolysis with these enzymes and compare the enzyme treated and native material. In order to examine the porous starch granules produced due to partial hydrolysis, they were examined calorimetrically using DSC. A structural analysis was also made using SEM. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: DSC; Glucoamylase; Corn starch

1. Introduction

Enzymatic hydrolysis can help in studies of the physical and chemical structure of starch granules [1]. The structure of starch is such that it permits the degradation of the granules in certain areas when treated by hydrolytic enzymes and not in other areas. Previous research has investigated [2–4] the action pattern of various amylases on granular starches, with particular attention being given to the influence of the amylase source [5]. In this study quantitative analysis of the action of *Rhizopus* glucoamylase on corn starch was undertaken to determine the relative susceptibility and extent of the reaction. The partially degraded porous starch granules were then examined using SEM. This was done to study the morphology of the starch granules before and after treatment with glucoamylase by SEM, for different amounts of enzyme used.

Amylase is one of the earliest enzymes discovered and recognized as a bio-catalyst. The amylase family of enzymes have been well characterized through the study of various microorganisms. Two major classes of starch degrading enzymes have been identified in the microorganisms, alpha amylase (endo-1,4-α-D-glucan glucohydrolase, EC 3.2.1.1) which randomly cleaves the 1,4-α-D-glucosidic links between adjacent glucose units in linear amylose chains and glucoamylase (synonym amyloglucosidase and also referred as glucogenic enzyme, exo-1,4-α-D-glucan glucohydrolase EC 3.2.1.3) which hydrolyzes single glucose residues from the non reducing ends of amylose and amylopectin in a step wise manner. Unlike the alpha amylases, most glucoamylases can also hydrolyze the α-(1,6) linkages in the branch points of amylopectin, although at a slower rate than the α-(1,4) linkages. Both of these enzymes are produced by different microorganisms [6]. The amylases and related enzymes are probably amongst the most important enzymes industrially, especially in the food and pharmaceutical industry [7]. Amylases hydrolyze starch to produce glucose. Traditional acid hydrolysis of starch to glucose is now superseded by enzymatic processes. This is due to the fact that acid hydrolysis gives rise to undesirable by-products. The enzymatic

* Corresponding author. Tel.: +1-636-737-5986; fax: +1-636-737-6806.

0040-6031/00/$ – see front matter © 2000 Elsevier Science B.V. All rights reserved.
PII: S0040-6031(00)00368-3

hydrolysis of starch is one of the best established industrial processes for the conversion of starch to glucose [8]. There are different types of amylases and depending on the source from which they are extracted their mode of action differs. They are further classified as exo and endo acting amylases. The rate of hydrolysis of starch granules strongly depends upon the botanical source from which they are obtained. In this investigation, raw starch obtained from corn starch was subjected to hydrolysis by glucoamylase. The partially degraded product formed was then analyzed calorimetrically. After partial hydrolysis the residue was examined by SEM and a thermal analysis investigation was carried out to determine if there was any difference in behavior apparent after partial hydrolysis. These results were compared with values obtained for the intact starch and a comparison drawn. When starch is treated with glucoamylase, the native starch granules degrade, leaving behind porous starch granules. There have been a large number of studies conducted on the action of enzymes on starch, but a description of the partially degraded starch is limited and this was good material to obtain structural information about the starch. Enzymatic hydrolysis can help in studies of the physical and chemical structure of starch granules [9,10]. The structure of starch is such that degradation does not occur uniformly. SEM has been used earlier to examine the granules produced after amylosis. Gallant et al. [11] revealed a lamellae organization of the granules after enzymatic treatment. They also indicated that the nature of the degradation depended on the source and origin of the starch. This study was performed in order to obtain a better understanding of the effect of partial hydrolysis on starch due to the action of enzymes.

2. Materials and methods

In this investigation corn starch from Sigma (St. Louis, MO) was used. Glucoamylase (22,500 IU g^{-1}) was also from Sigma. (1 international unit (IU) of glucoamylase is that amount which releases 1 mg of glucose every 3 min).

2.1. DSC studies

The appropriate amount of starch was weighed and slurried with excess water at a ratio of 1:10. This mixture was stirred for 1 h prior to use. Of this slurry 10 mg was then weighed into an aluminum crucible and hermetically sealed using a sample encapsulation press. In the case of the partially hydrolyzed samples, starch was treated with glucoamylase overnight. The supernatant was decanted and the residual starch washed with distilled water and centrifuged. After decantation of the water, the partially hydrolyzed starch was dried at room temperature and stored in the desiccator. The gelatinization temperature was determined using DSC, from Dupont, model 990, calorimeter cell. The instrument was calibrated with pure indium. Each sample was analyzed against an empty reference pan, over the temperature range 20–100°C. A heating rate of 10°C min^{-1} was used for all the samples.

2.2. Raw starch digestion assay

In this 100 mg each of corn starch, were taken and suspended in 1.6 μl of 50 mM acetate buffer at pH 4.6. Three different concentrations of glucoamylase, i.e. 2, 20 and 200 IU ml^{-1}, (0.4 ml each) were added to this mixture to initiate the starch hydrolysis. The reaction was carried out at 37°C. Aliquots (0.3 ml) were withdrawn at various time intervals (from 0 to 258 h) after stirring, to obtain a homogenous mixture. The reaction was stopped by the addition of 55 μl of 0.2 M HCl to the aliquots. The supernatant solution and the unreacted granules were then separated by centrifuging the suspension for 1 min. The supernatant was then heated in a boiling water bath for 5 min to complete the inactivation of the enzyme. After cooling the solution, the pH was adjusted to neutral by the addition of 0.2 M NaOH containing 0.1 M tris-HCI. The granules were then washed by suspending them in 3 ml of distilled water and centrifuged. This washing was conducted three times. The washed granules were then filtered by vacuum filtration and dried at room temperature.

2.3. SEM micrographs

A JEOL JSM-6100 Scanning Electron Microscope was used for the electron microscopy data. Since the starch is a nonconductive material, the granules had to be lightly coated with a conductive material. Gold was used in this study. The starch sample was allowed to

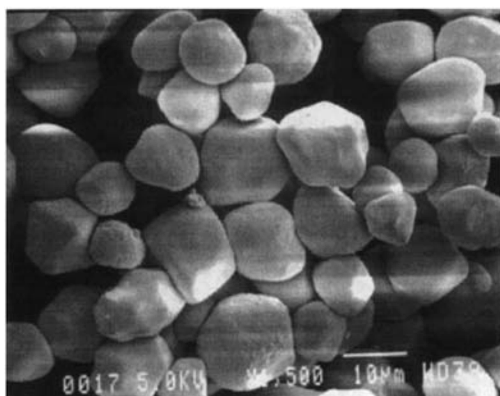

Fig. 1. SEM photograph of native corn starch granule.

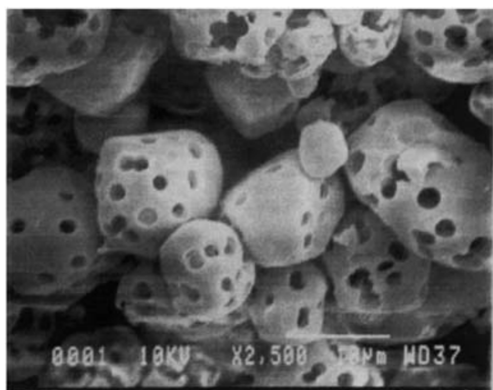

Fig. 3. SEM photograph of corn starch granules treated with 200 IU ml^{-1} glucoamylase for 32 h.

fall onto a double backed tape mounted on a graphite platform. The excess starch particles and clumps were removed, and the granules coated with a thin film of gold by vacuum evaporation. SEM photographs were taken using the native unmodified starch granules that were treated in exactly the same way except no enzyme was added and enzyme treated samples that contained 2, 20 and 200 IU ml^{-1} of glucoamylase and were incubated for 32–256 h of degradation.

3. Results and discussion

The action pattern of digestion that develops when certain starch granules are treated with amylases indicates that some areas of their surface are much more susceptible to attack than others. The SEM photographs of dry granules of commercial native corn starch (Fig. 1) can be grouped on the basis of morphology into three categories, generally spherical, angular dimpled and irregular [12]. Corn starch shows only a slight change in appearance on treatment with 2 IU of enzyme; on a 10-fold increase of enzyme concentration (20 IU), large ‘pin holes’ can be seen on the surface of the corn starch granules (Fig. 2). This picture clearly shows that the glucoamylase mainly worked by boring holes in the starch granules resulting in the formation of porous starch granules. On further increasing the enzyme concentration to 200 IU there are almost broken granules

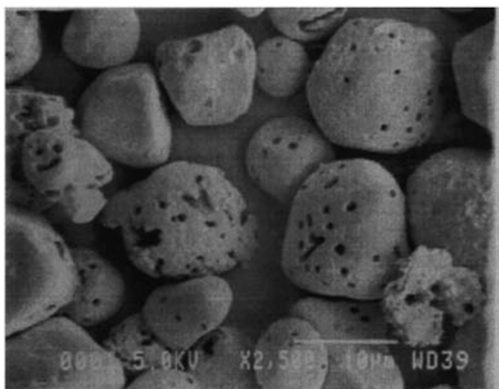

Fig. 2. SEM photograph of corn starch granules treated with 20 IU ml^{-1} glucoamylase for 32 h.

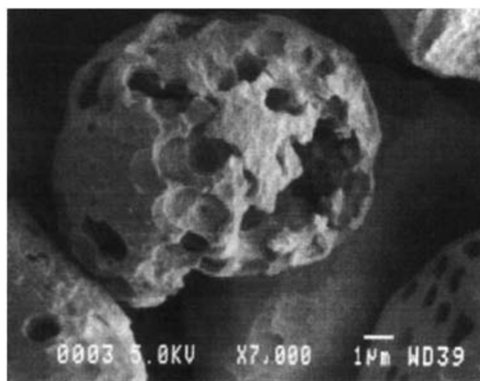

Fig. 4. SEM photograph of corn starch granules treated with 200 IU ml^{-1} glucoamylase for 32 h (closer view).

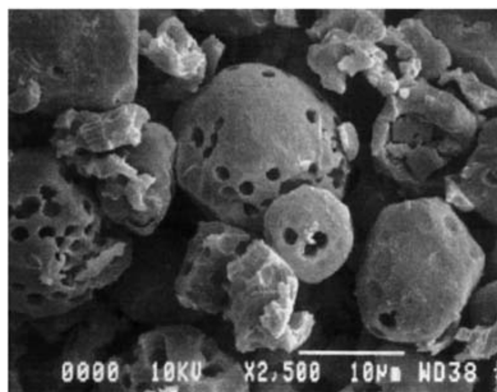

Fig. 5. SEM photograph of corn starch granules treated with 200 IU ml^{-1} glucoamylase for 48 h.

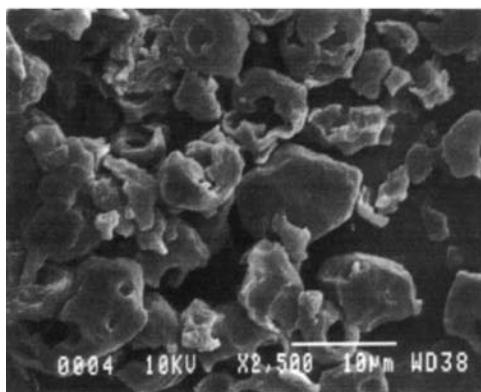

Fig. 7. SEM photograph of corn starch granules treated with 200 IU ml^{-1} glucoamylase for 128 h.

with the skeleton structure around it. (Figs. 3 and 4, closer view) The enzyme acts by first attacking the surface and forming pores on the surface [13]. The random canals are then enlarged until they begin to fuse, thus rupturing the starch granular structure [14]. When incubated for longer periods of time (48, 64, 128 and 256 h) the starch granules continue to degrade until there is complete collapse of the granule structure (Figs. 5–8). The morphological changes induced by amylosis have been largely explored [15]. Enzymes cause surface alterations and degrade the external part of the granule by exo-corrosion. When endo-corrosion occurs, the internal part of the granule is corroded through small pores by which enzymes penetrate the granule. In a given starch sample, small granules are more rapidly hydrolyzed than bigger ones, because of a larger available surface area as previously reported [16]. When the granules are small and round shaped, hydrolysis is more difficult than when they are polyhedric. The presence of truncatures lead to better susceptibility. Amylosis of isolated starch can occur by penetration of the enzyme into the granule, either by pitting or by fissures formed on the surface of starch. It appears that the starches which are readily or rapidly digested with enzyme are those whose surfaces are readily attacked, with the formation of canals (wheat, rice, corn and amylopectin). The rate of

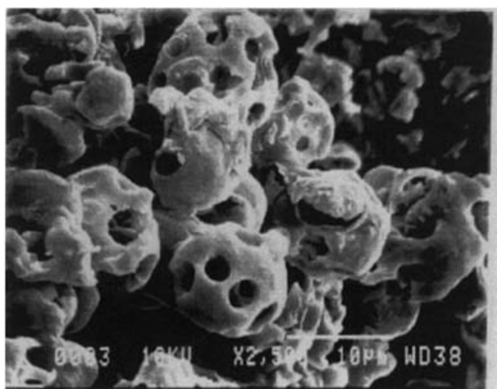

Fig. 6. SEM photograph of corn starch granules treated with 200 IU ml^{-1} glucoamylase for 64 h.

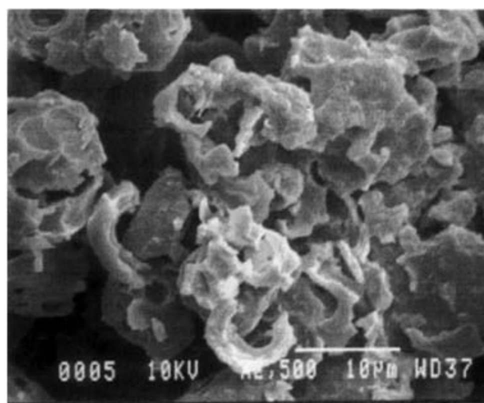

Fig. 8. SEM photograph of corn starch granules treated with 200 IU ml^{-1} glucoamylase for 256 h.

hydrolysis of non solution starch is dependent on the accessible surface area, SEM micrographs reveal that the surface of cereal starches is uneven, yielding a greater surface/volume ratio than that seen for smooth B-type starch [17]. Since the enzyme breaks the A-type starch granules into small pieces, this dramatically increases surface area and hence rate of hydrolysis.

When heated, an aqueous suspension of starch in excess water undergoes a co-operative endothermic transition known as gelatinization [18]. Its most prominent feature in DSC traces is an endothermic peak or peaks. The molecular events responsible for this transition are uncertain, but entail the melting of crystallites. The solute acts as a plasticizer in this process and influences to a high degree the thermodynamic parameters of this transition. At relatively high water levels, where the volume fraction of polysaccharide is less than 10%, a single endotherm is apparent. The precise position of the peak depends on the variety of starch being investigated. It has long been recognized that under real world heating conditions, gelatinization is a non-equilibrium transition [19–23]. The melting explanation of gelatinization suggests that crystalline (or helical) zones in different granules have different stability. It is suggested that water migrates from one location to another as the various granules gelatinize [24,25]. Thus, the DSC peaks correspond to the melting transitions of crystalline materials with different stability at different diluent levels. According to the model, if the water content of the suspension is sufficiently

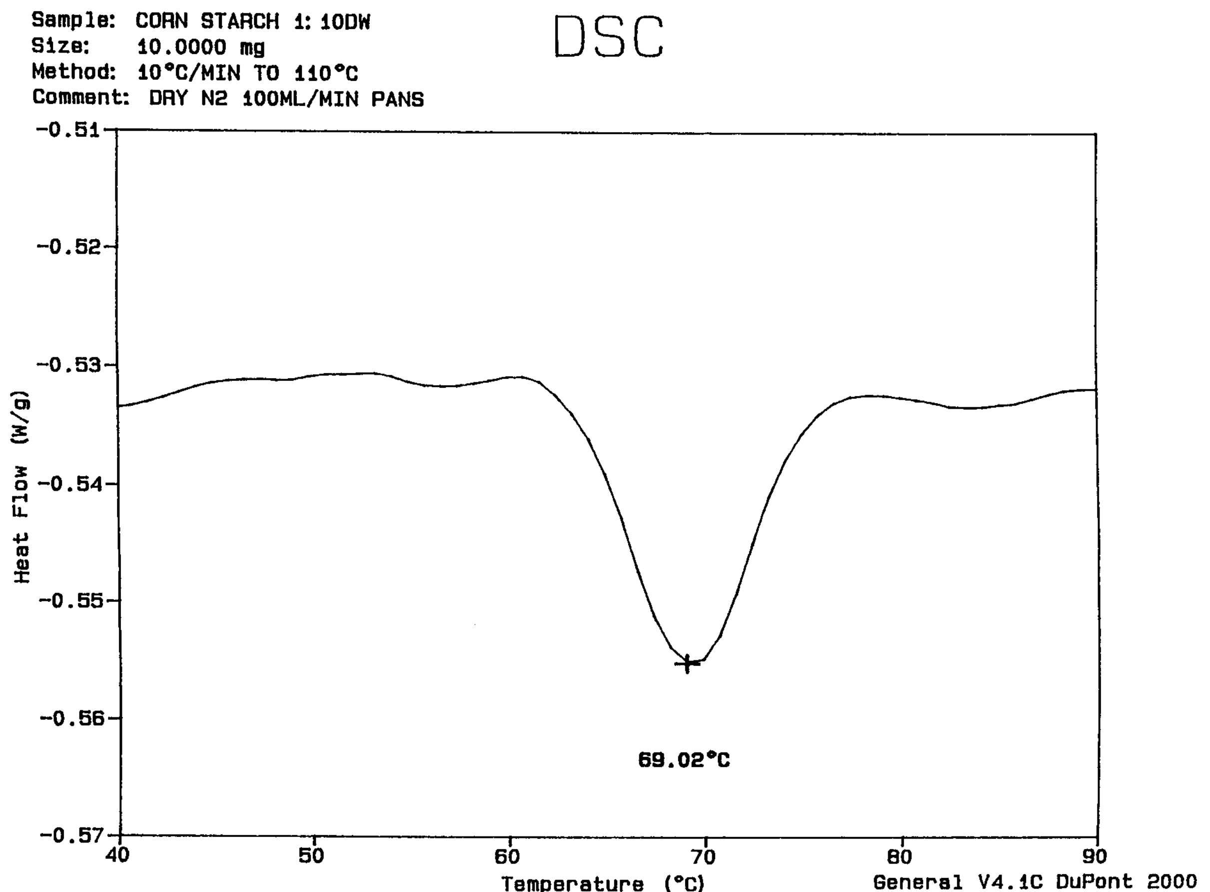

Fig. 9. DSC plot showing endothermic gelatinization for native corn starch granule in water.

high, i.e. above 65% each granule will absorb water without restriction and a single endothermic peak will be observed. If the water content is less than this level, there is competition by the granules for water. In this case the least stable granules melt first, absorb water and so deplete the remainder of the diluent. The latter particles melt at higher temperatures partly because they are more stable and partly because the effective volume fraction of diluent is reduced. In this study, excess water was used as the diluent and a comparison attempted between enzyme treated and native corn starch using the same level of water each time. The onset temperature, T_i, and the peak temperature T_p were determined by intercept of the baseline. As can be seen from the figure, (Fig. 9) untreated corn starch showed a characteristic melting curve, with T_p=69.02°C. A small shoulder prior to that was also seen. This is in agreement with the literature values. Partially hydrolyzed corn starch treated with glucoamylase showed an increase in the initial gelatinization temperature. Park et al. [26] reported that the swelling of larger starch granules occurred at a lower temperature than that required for the swelling of smaller starch granules, therefore, it is possible that for corn starch treated with glucoamylase, an increase in the relative number of smaller granules after hydrolysis would cause an increase in the initial gelation temperature. As can be seen from the table (Table 1), in each case studied, the partially hydrolyzed starch granules showed a higher peak temperature as compared to the undegraded starch granule. There was a decrease in melting enthalpy seen as well. This result is consistent with observations made on the SEM pictures and powder X-ray diffraction (XRD) results on native and enzyme treated starch granules.

Table 1
DSC results obtained for gelatinization of the native and porous corn starch granules due to glucoamylase action

1:10 Starch in DW	T_i (°C)	T_p (°C)
Corn starch (cs)	65.17	69.02
Cs after hydrolysis (32 h)	65.00	71.00
Cs after hydrolysis (48 h)	65.20	71.50
Cs after hydrolysis (64 h)	62.30	72.00
Cs after hydrolysis (128 h)	62.50	73.00

4. Conclusions

The partially degraded product obtained after hydrolysis by glucoamylase formed porous granules, as seen from the SEM micrographs. As the concentration of the enzyme was increased, the size of the pores formed increased, until they started fusing into each other and breaking the walls of the starch granule. Similar observations were made from the DSC data. As the pore size of the granules increased, the granules showed a higher gelation temperature than their native counter parts when examined using a DSC, thus indicating that enzyme treatment rendered the starch granules porous. The granules behaved like lower chain length polymers and more energy is required to break smaller size polymers as compared to bigger size polymers.

References

[1] V. Arasaratnam, K.J. Balasubramaniam, Microb. Biotechnol. 7 (1992) 37.
[2] T. Yamada, M. Hisamatsu, K. Teranishi, K. Katsuro, N. Hasegawa, M. Hayashi, Stärke 47 (1995) 358.
[3] Y. Tanaka, T. Ashikari, N. Nakamura, N. Kiuchi, Y. Shibano, T. Amachi, H. Yoshizumi, Agric. Biol. Chem. 50 (1986) 1737.
[4] T. Yamamoto, I. Miyahara, S. Yamamoto, K. Fujita, K. Mizokami, Denpun Kagaku 37 (1990) 129.
[5] D.M. Shetty, D.R. Lineback, P.A. Sieb, Cereal Chem. 51 (1974) 364.
[6] K. Kuip, in: S. Barenberg, G. Reed (Eds.), Enzymes in Food Processing, 2nd Edition, Academic Press, New York, 1975, p. 54.
[7] N.H. Aschengreen, Process Biochem. 10 (1975) 17.
[8] P.W. Lambert, J.L. Myers, in: B.S. Hartley, T. Atkinson, M.D. Lilly (Eds.) Industrial and Diagnostic Enzymes, The Royal Society, London, 1983, p. 25.
[9] A. Pandey, Städrke 47 (1995) 439.
[10] D.J. Manners, Carbohydr. Polym. 11 (1989) 87.
[11] D.J. Gallant, C. Mercier, A. Guilbot, Cereal Chem. 49 (1972) 354.
[12] D.M. Hall, J.G. Sayre, Text. Res. J. 40 (1970) 256.
[13] J.E. Fannon, R.J. Hauber, J.N. BeMiller, Cereal Chem. 64 (1992) 284.
[14] P.M. Baldwin, J. Adler, M.C. Davies, C.D. Melia, Stärke 46 (1994) 341.
[15] J.C. Valetudie, P. Colonna, B. Bouchet, D.J. Gallant, Stärke 45 (1993) 270.
[16] C.M.L. Franco, S.J. do Rio Preto, C.F. Ciacco, D. Tavares, Stärke 40 (1988) 432.
[17] G. Williamson, N.J. Beishaw, D.J. Self, T.R. Noel, S.G. Ring, P. Cairns, V.J. Morris, S.A. Clark, M.L. Parker, Carbohydr. Polym. 18 (1992) 179.

[18] N.I Davidora, S.P. Leontev, Y.V. Genin, A.Y. Sasov, Carbohydr. Polym. 27 (1995) 109.
[19] J. Leliévre, H. Liu, Thermochim. Acta 246 (1994) 309.
[20] J.W. Donovan, Biopolymers 18 (1979) 263.
[21] L. Slade, H. Levine, Carbohydr. Polym. 8 (1988) 183.
[22] J.J. Leliévre, Appl. Polym. Sci. 18 (1974) 293.
[23] I.D. Evans, D.R. Haisman, Stärke 34 (1982) 224.
[24] J. Leliévre, Stärke 37 (1985) 267.
[25] J. Leliévre, H. Liu, Carbohydr. Res. 219 (1991) 23.
[26] Y.K. Park, W.H. Bar, R.S. Papini, Rev. Bras. Tec. 2 (1971) 95.