REMOVAL OF PIGMENT GLANDS (GOSSYPOL) FROM COTTONSEED[1]

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ABSTRACT Cereal Chemistry 53(4): 549-560

Gossypol in pigment glands of cottonseed kernels is toxic to most monogastric animals and imparts undesirable color to oil and protein products produced from the seed. To produce a food-grade protein product from glanded cottonseed, a workable process for the removal of the pigment glands is prerequisite. The Liquid Cyclone Process (LCP) is the first economical and workable process capable of removing pigment glands from cottonseed to consistently produce high-protein, edible flour. Approximately 9000 lb of cottonseed flour containing 0.04% or less of free gossypol and more than 65% protein have been produced in the Southern Regional Research Center's pilot plant during the last 4 years. Evaluation of the flour in food applications was very successful. Flour produced by the LCP was approved as a food additive by the FDA as of July 13, 1972. Development of the LCP has led to construction of a plant to produce deglanded, high-protein cottonseed flour of edible grade at Plains Cooperative Oil Mill, Lubbock, Tex.

The cottonseed varieties predominantly grown throughout the world differ from other oilseeds in that they contain dark specks which are pigment glands. As can be seen in Fig. 1, these glands, 50 to 400 μ in diameter, are scattered throughout the kernel (1). These pigment glands, containing gossypol as the major pigment, are toxic to most monogastric animals, and very likely to man, and have precluded the use of cottonseed to any major extent as a source of protein to a food-hungry world. In addition, they have been a continuing problem for oil millers processing cottonseed into oil and meal. To make a quality cottonseed protein with high nutritional value and increased functionality available for human consumption, it is prerequisite that a means be developed for removing the pigment glands intact. This paper summarizes the results of research which culminated in the development of the Liquid Cyclone Process (LCP) for producing a deglanded, edible flour.

EARLY INVESTIGATIONS

Von Bretfeld in 1887 (2) and Hanausek in 1907 (3) were the earliest investigators of the structure of the pigment glands of cottonseed. They described the presence of a water-sensitive membrane which surrounded a greenish-black opaque secretion.

The major pigment contained in the glands was first isolated by Longmore in 1886 from soapstock obtained on refining cold-pressed oil (4). Marchlewski purified the same yellow pigment in 1899 and, because of its origin and his

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finding of a polyphenolic chemical nature, named it gossypol from gossyp (ium phen) ol (5).

Figure 2 shows the structural formulas for the tautomeric forms of gossypol.

In 1947 and 1948, reports by Boatner and others (6,7) were made on investigations of the nature of pigment glands and pigments of cottonseed. Figure 3 shows what Boatner and her group of researchers at SRRC observed in 1947, namely that the outer structure of the pigment gland was a rigid wall rather than a membrane, and that it was made up of five to eight irregularly shaped, thick, curved plates fitted together much the way a baseball cover is fitted together. They also found that the wall of platelets was composed of cellulose impregnated with pectin, hemicellulose, and uronic acid derivatives (6). Figure 4 shows the effect of water on pigment glands. The glands immediately rupture along the seams between plates and spew out their contents in the form of a colored, finely divided suspension of particles (7). As is evident in Fig. 5, nonpolar solvents such as hexane do not seemingly affect the glands or their contents (7).

Although gossypol is the major pigment of the glands (forming roughly 95% of

Fig. 1. Longitudinal section of a glanded cottonseed showing pigment glands as black specks.

the pigments by weight), in 1948 Boatner (7) reported the presence of at least 15 gossypol pigments or derivatives of gossypol in extracts of cottonseed or cottonseed oils and meals. Most of them have not been isolated or characterized as fully as gossypol. In the conventional processes for the production of oil and meal from cottonseed, the effect of conditioning prior to extraction is of prime importance. This conditioning influences the extent of rupture of the glands. The rupturing of glands allows the pigments to diffuse and react with extraglandular constituents during oil extraction and plays an important role in the final distribution of the gossypol pigments in the meals and oils.

The presence of gossypol pigments in meal presents two problems which are not easily separated. High levels of free gossypol cause unfavorable physiological effects in nonruminants such as swine, poultry, and rabbits (8). The reaction between gossypol and protein, forming "bound gossypol" during processing, reduces nutritive value by a reduction in the biological availability of lysine (8).

Although a great many studies have been published concerning the role of gossypol as it affects the nutritive value of cottonseed meal, most are studies with gossypol plus gossypol-like pigments. It is difficult to relate these studies to those conducted with purified gossypol.

Gossypol pigments are the chief cause of the dark color in commercially produced crude cottonseed oils. Although other nongossypol pigments and contaminants are known to be present in crude oil, these are easily removed in the normal refining and bleaching processes. It is the fixed colors derived from gossypol pigments subjected to heat over a period of time which are highly

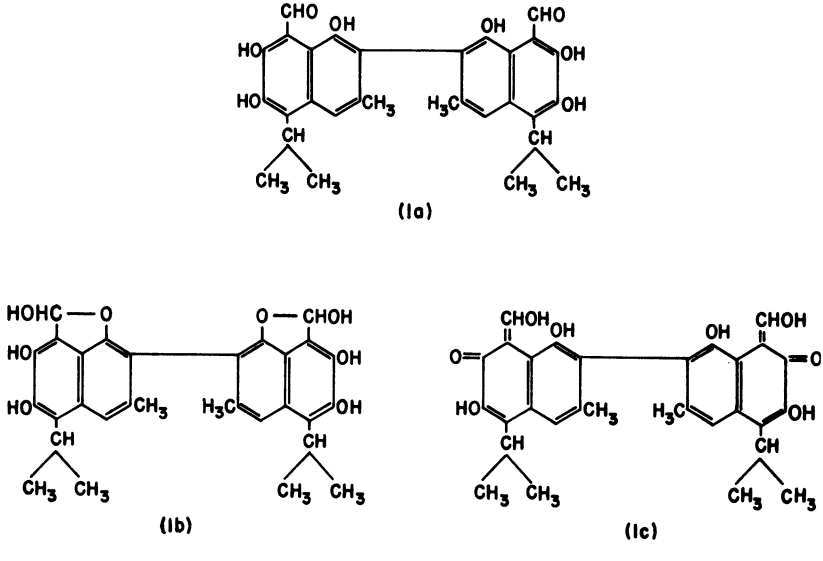

Fig. 2. Tautomeric forms indicating the polyphenolic nature of gossypol: 1a) hydroxyaldehyde tautomer, 1b) lactol tautomer, and 1c) cyclic carbonyl tautomer.

resistant to removal and can result in additional refining losses as well as reduction in market value (7).

To provide food-grade products from cottonseed, an excellent high-protein source, we must either breed the glands out of the seed, or remove them at the time of seed processing. Glandless cottonseed is available for planting, but until such time as the commercial cotton crops in the world are derived from glandless seed, processing methods will be needed to remove the pigment glands.

GLAND FLOTATION PROCESS

One such method, the Gland Flotation Process, was developed at the Southern Regional Research Center (SRRC) by Boatner's group in 1946 (9). During their research on pigment glands, they noted that the density of glands (1.35 g/cc) was lower than that of extraglandular kernel tissue (1.42 g/cc) or of hulls (1.45 + g/cc). They capitalized on these differences by violently disintegrating cottonseed flakes in a slurry of hexane and various other heavy solvents (like

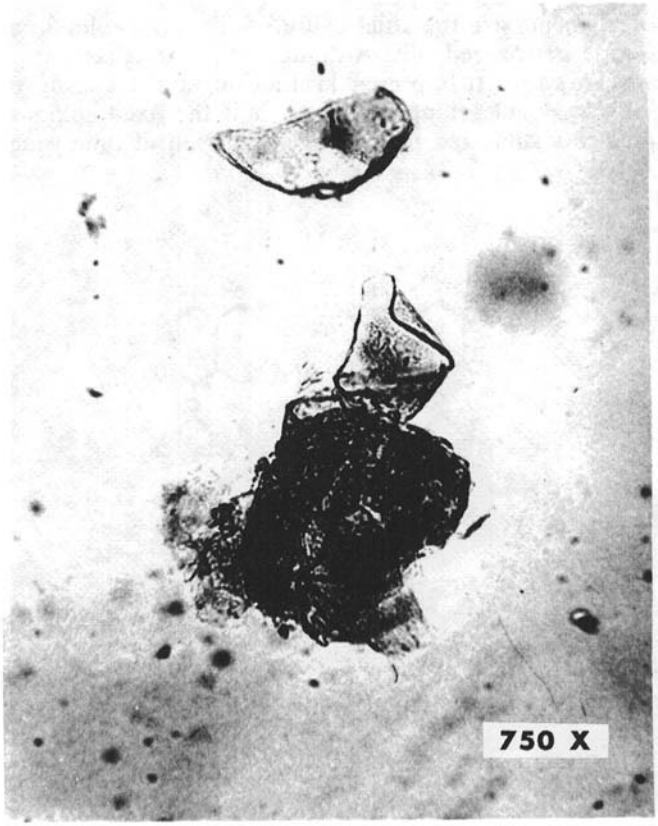

Fig. 3. An empty cottonseed pigment gland showing structural makeup of gland wall (platelets).

perchlorethylene, trichlorethylene, or carbon tetrachloride) mixed in a proportion so as to give a resulting specific gravity of 1.378 g/cc. Figure 6 depicts what occurred when the slurry was allowed to stand. The glands floated to the surface and were easily skimmed off, leaving meal that was essentially free of pigment glands. The process was extended to a small pilot-plant scale by the Engineering and Development Laboratory, whereupon a number of inherent disadvantages made it apparent that the process could not be commercialized (10). Comminution was one of the major problems: too little resulted in high gossypol contents in the meals, too much resulted in essentially the same density for meal and glands with no separation possible. The necessity of using heavy solvents which were high-boiling and toxic was another factor against commercialization. The observation of the settling characteristics of disintegrated components in the Gland Flotation Process did, however, lead to the idea of the Differential Settling Process.

DIFFERENTIAL SETTLING PROCESS

The Differential Settling Process depends primarily on the frictional resistance

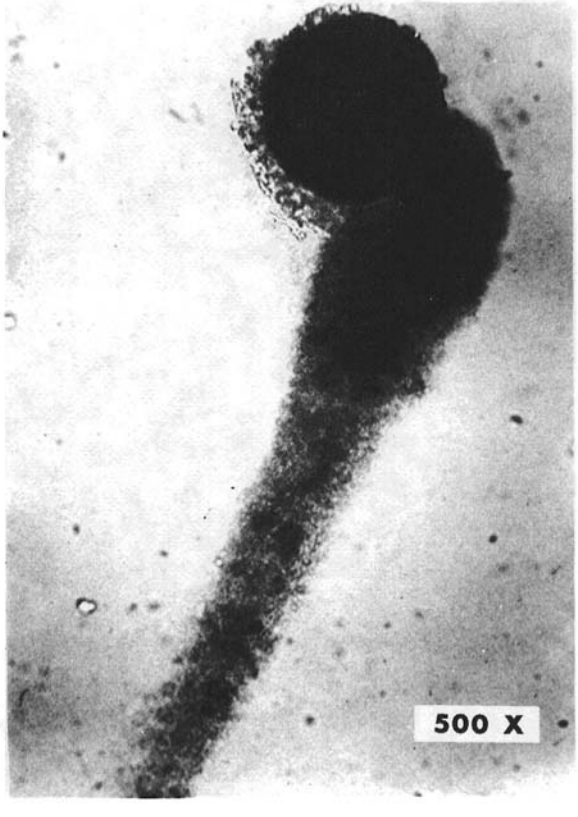

Fig. 4. Pigments streaming out of a ruptured pigment gland after contact with water.

between a solvent and a solid component in a slurry (11). Figure 7 demonstrates a typical laboratory differential settling of a slurry containing disintegrated cottonseed meats and hexane. The graduate on the left represents the slurry prior to settling, the middle graduate, after settling for a few minutes, and the graduate on the right, after settling for 1 hr. In the middle graduate, the hulls and coarse meal particles have settled rapidly to form a bottom layer. Above this is a whole pigment gland or top layer. Although the very fine meal particles (less than 40 μ) have a higher specific gravity than the glands (1.42 *vs.* 1.36 g/cc, respectively) they have not as yet settled out in the middle graduate. This is due to their fluffy, feathery nature which reflects itself in a relatively large surface area per unit weight and, thus, a very high resistance and a very slow settling rate. This laboratory development was scaled up to pilot-plant size, where tanks, a high-speed shearing mixer, centrifuges, and filters were used to produce a high-grade protein meal for industrial and feed use. The process, although not economically

Fig. 5. Hexane applied to milled kernels (glands unaffected).

attractive to industry, was the basis for the Liquid Cyclone Process which was to follow.

LIQUID CYCLONE PROCESS

The Liquid Cyclone Process (LCP), originally developed by Gastrock *et al.* (12) and refined to its present status by Vix *et al.* (13) and Gardner *et al.* (14), is the first economical and workable process capable of removing pigment glands (gossypol) from cottonseed to consistently produce a gland-free, high-protein, edible flour. Its successful development was dependent upon a scientific approach to the separation of cottonseed into its components. The Scanning Electron Microscope and other microscopic studies indicated that the morphology of cottonseed was such that the components could be dissected and easily separated. Hence the Liquid Cyclone Process.

Figure 8 shows a flowchart of SRRC's pilot-plant LCP (15). The LCP uses whole and cracked meats, essentially hull-free, obtained from prime-quality cottonseed uncontaminated with salmonella or aflatoxins. The kernels shown on the flowsheet contain less than 1% hulls. The kernels are first dried with air at 180° F to a moisture content of 1.5–2.0% on a pilot-plant scale, belt-type dryer.

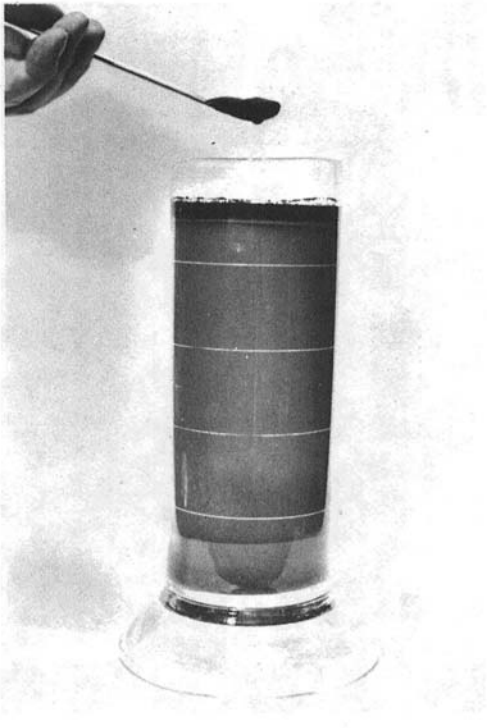

Fig. 6. Gravity separation by floating pigment glands, density 1.35 g/cc, in a solution of perchlorethylene-hexane, density 1.378 g/cc.

The purpose of drying is threefold: 1) to remove water which, as previously described, is an excellent solvent for rupturing the glands; 2) to toughen the pigment glands; and 3) to make the proteinaceous material more friable. The latter two factors are very important in comminution or milling, which is the next step and a critical one in the process. Without adequate kernel size reduction, the yield of flour will be low; with over-comminution, a significant number of pigment glands will be ruptured.

A sieveless, wide-chamber Alpine American Contraplex pin-mill was used in the comminution operation. Although the recommendation of using hull-free meats is stressed, the liquid cyclone can remove as much as a 3% hull content. Size reduction of these hulls not only requires additional power but, more importantly, causes both increased gland rupture and lower flour yields. In continuous operations, the milled meats go directly to a fluidizer where they are slurried with metered hexane. The discharged slurry with a solids level of 22% is then sent to an agitated liquid cyclone feed surge tank. The heart of the process—the liquid cyclone—is located in the center of the flowsheet. Although

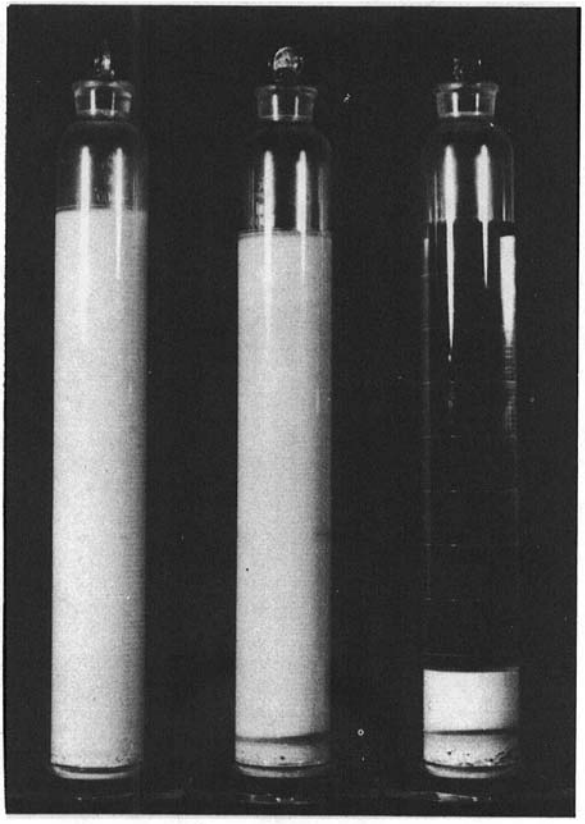

Fig. 7. Settling characteristics, at different time intervals, of a slurry consisting of comminuted cottonseed kernels in hexane.

it has no moving parts and is only 3 in. in diameter and 10 in. high, this liquid cyclone can produce a slurry fraction equivalent to approximately 12 tons of flour per day. Figure 9 depicts a sectional view of a liquid cyclone.

Basically, the liquid cyclone capitalizes upon the rates at which the different components of cottonseed settle out or classify in hexane (the Differential Settling Process) by using the action of centrifugal force which accentuates classification. The liquid cyclone classifies feed-slurry into a gland-free overflow slurry, containing 13 to 15% high-protein solids, and a gland-rich coarse meal underflow slurry, containing 43 to 45% solids. The classification is controlled by adjusting the rate of removal of the underflow slurry. This rate is usually the minimum obtainable without the appearance of pigment glands in the overflow or flour fraction. After adjustment of classification, the overs-unders fractions are directed to their respective recovery operations. High-protein, solvent-damp cottonseed cake is recovered from the overflow slurry on a rotary vacuum drum-type filter. During the filtration step, the lipids level in the solvent-free cake is reduced to approximately 0.60%. The filter cake is desolventized in a stainless-steel rotary twin shell "V"-type blender equipped for vacuum and solvent recovery operations. The cake is heated to 180°F, whereupon nitrogen gas is injected into the blender to strip solvent from the flour to a level below 50 ppm. During stripping, the flour temperature is allowed to rise to 200°F to obtain

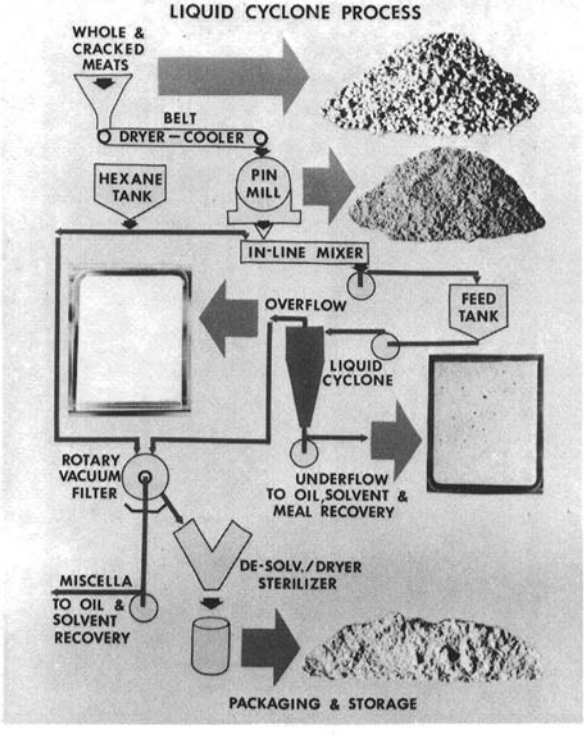

Fig. 8. Pilot-plant Liquid Cyclone Process flowsheet.

maximum bacteria kill. Because of the absence of moisture in the flour, this increase in temperature has little to no effect on protein quality or flour color. The flour is then cooled to 110°F and collected in drums with double polyethylene bag liners under aseptic conditions.

During the last 4 years, approximately 9000 lb of gland-free edible flour from glanded cottonseed have been produced in the SRRC pilot plant by using the LCP. Most of the production has been used in evaluations by the food industry and academic institutions. Composition of a cottonseed flour produced by this process is shown in Table I.

Moisture, lipids, free and total gossypol, nitrogen, fiber, and ash were analyzed by using official AOCS methods (16). Nitrogen solubility was measured by dispersion in 0.02*N* NaOH as suggested by Lyman *et al.* (17) and Martinez *et al.* (18). Available lysine content was determined by the method of Rao *et al.* (19). Residual hexane was determined by the rapid procedure of Fore and Dupuy (20). The flour is essentially gland-free, with a residual free gossypol content of 0.045% or lower. It was approved by the FDA as a food additive on July 13, 1972, and is bland in flavor, with an attractive, light creamy color. Its protein content ranges from 66 to 69% on a moisture-free basis, and therefore approaches that of a concentrate. The nutritional quality is excellent, with a protein efficiency ratio approaching that of sodium caseinate. Water absorption determinations indicate

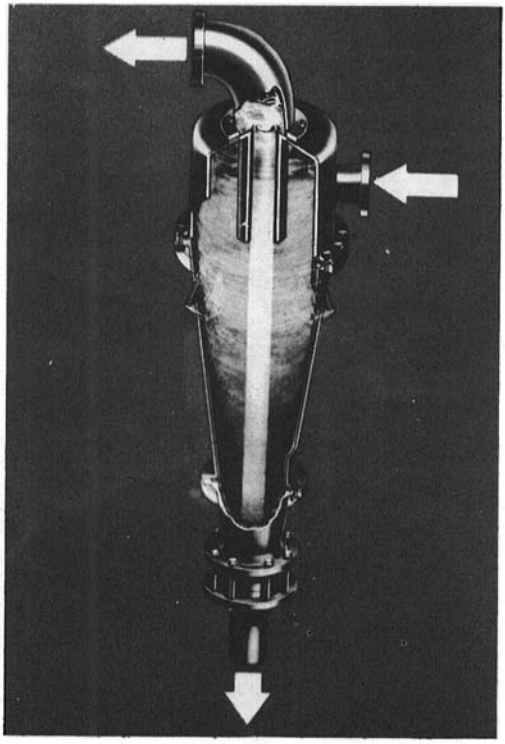

Fig. 9. Sectional view of a typical liquid cyclone.

that the flour absorbs 2.5 times its weight of water in comparison with soy concentrates and isolates, which absorb 5 to 6 times their weight. Oil absorption of the cottonseed flour is 1.5 times the weight of the flour, similar to that for soy products. Oil emulsification values were found to be generally higher than for soy, indicating a possible advantage for cottonseed flour in the formulation of weiners and sausage-type products (21). Table II shows some of the uses for LCP cottonseed flour. Although not shown, one use may be as a raw material in the production of protein isolates. Research at SRRC by Martinez's group has produced a cottonseed protein isolate, containing 95% or more protein, with almost complete solubility at pH 3.5. This product should have large market potential in the protein fortification of carbonated and citric acid based beverages (21, 22).

As a result of our research, Plains Cooperative Oil Mill, Lubbock, Tex., built a plant to produce deglanded, high-protein cottonseed flour. The plant, although experiencing problems, has produced a flour meeting specifications. This flour has been supplied (September 1975) to the Grain Processing Corporation, Muscatine, Iowa, for commercial evaluation.

Although it may take years to completely evaluate the impact of LCP flour on the food-processing industry, the removal of the pigment glands from cottonseed has opened the door to a new worldwide source of protein to feed mankind.

TABLE I
Chemical Analyses of a Typical LCP Cottonseed Flour

Components	%
Moisture	3.66
Lipids	0.62
Free gossypol	0.03
Total gossypol	0.12
Nitrogen	10.54
Protein (moisture-free basis), N × 6.25	68.40
N Solubility, 0.02*N* NaOH	99.49
Available lysine, g/16 g N	3.94
Fiber	2.4
Ash	7.54
Residual hexane, ppm	35

TABLE II
Uses for LCP Cottonseed Flour

Beef patties	Extruded flour (for meats and cereals)
Meatballs and gravy	Frozen desserts
Chili	Doughnuts
Beef broth	White bread
Fresh sausage	Devil's food cake
Frankfurters	Cookies
Imitation frankfurters	Crackers

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