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THE STRUCTURE-FUNCTION RELATIONSHIP OF POLYMERIC SORBENTS FOR COLLOID STABILIZATION OF BEER

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Abstract

This review considers the research relating to the formation of non-biological haze in beer. The basic information regarding mutual interactions of haze-causing substances with precipitating, enzymatic, and adsorptive stabilizers of beer is discussed. The main haze-causing compounds in beer are polypeptides and polyphenols. To reduce the levels of these compounds in beer, adsorptive stabilizing agents are usually used during filtration. Electron microscopy and methods measuring the surface and dimensions of adsorbent particles and the volumes and diameters of their pores make it possible to assess morphological properties and suitability of the stabilizers for beer filtration. Determination of the sorptive effectiveness, the flow characteristics, and the wet volume secure a more comprehensive quality evaluation.

Information on morphological, sorptive, and filtering properties including structure as visualized by electron microscopy is presented on new types of polymeric sorbents developed in the author's laboratory to be active against beer polyphenols.

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Introduction

The beer consumer requires a clear beverage with fresh flavour and colour corresponding to the given beer type, and with sufficient and stable foam. The beer brilliance as well as other mentioned criteria deteriorate by the formation of microbial and/or non-biological haze of colloidal or physico-chemical nature generated during storage. Both haze types may be distinguished by light microscopy and better characterized by electron microscopy.

Securing the biological stability of beer has been technically resolved in practice. It is achieved by practising the principles of hygiene during the production process, by effective clarification of beer through filtration prior to filling of transport vessels and by pasteurization (Kahler and Voborský, 1981).

The physico-chemical or colloidal stability of beer is a substantially more complex phenomenon. This is mostly related to the formation of non-biological haze and encompasses additional criteria such as the stability of flavour, taste, colour, foam quality, and the problems of gushing (Dadič and Bellau, 1978; Dadič, 1974; Dalgliesh, 1977; Schur, 1979; Basařová, 1982). The colloidal haze is generated in bottled beer through interactions between molecules of organic substances, mainly polyphenols and polypeptides, but also polysaccharides and other soluble substances. The formation of this haze is enhanced in the presence of oxygen and minerals, by the effects of light, fluctuating temperature, motion, and the like.

A wide range of scientific findings has not yet led to an unambiguous theory which would explain the mechanism of the colloidal haze formation. It is, however, practically possible to produce beer in which the colloids have been stabilized for many months. Such technology is based on an effective reduction of the amount of haze-causing substances in the beverage using various stabilizing agents. The range, within which the stabilizers are efficient and provide colloidal stability, depends on the quality of the raw material, adherence to optimal processing conditions, and, if necessary, on the introduction of specially modified technological parameters and on the prevention of beer aeration in the final production stages (Basařová, 1964, 1966, 1968, 1977; McFarlane, 1968; Kirby *et al.*, 1977; Schur, 1979; Drew, 1981; Wackerbauer and Anger, 1984; Morton and Sfat, 1986; Pfenninger, 1987).

Haze-causing particles, colloids, and beer haze

Unfiltered beer contains various quantities of particles which cause haze. This haze consists of yeasts and bacteria, the sizes of which, as a rule, do not exceed 10 μm (Kahler and Voborský, 1981), colloidal particles from 1×10^{-3} μm to 1 μm (Wainwright, 1974), starch granules (between 1 and 100 μm in diameter as well as unique *omega granules* sized 0.5 to 2 μm) (Glenister, 1977), and a large quantity of dissolved colloids smaller than 10^{-1} μm (Wainwright, 1974).

Soluble organic compounds, particularly proteins, polyphenols, non-starch polysaccharides (β-glucans), and bitter hop substances in beer create complicated disperse colloidal salts or gel structures which may be distinguished by electron microscopy (Neumüller, 1973).

The minerals present in beer play an associative role with the colloids causing haze (Knorr, 1968). The ions of iron, copper, zinc (Gramshaw, 1969) and also of tin, calcium, magnesium, and oxalate (Schur, 1979; Gramshaw, 1970; Schildbach and Greif, 1977) are very reactive.

Colloidal hazes contain various concentrations of polypeptides, polyphenols, polysaccharides, and minerals (Wainwright, 1974), the amounts being dependent on the composition of the raw materials, processing conditions including the applied stabilizing treatment and the storage conditions of the beer. Chill and permanent hazes occur most frequently in beer. Their properties have been defined by Scriban (1959). The particles of the chill haze, which form in beer cooled below 0°C and dissolve when the temperature raises above 20°C, and the particles of the permanent haze, which dissolve when the temperature is raised to 40-70°C, may be distinguished by light microscopy and examined in greater detail by electron microscopy (Claesson and Sandegren, 1963, 1973). Buckee (1985) designed a system for the classification of haze particles in beer based predominantly on microscopical examination following specific staining procedures. In order to measure colloidal particles in the range from 0.01 μm to 2 μm, hydrodynamic chromatography has been recommended since the results are comparable in resolution with those obtained by electron microscopy (Leitzelement *et al.*, 1985; Lenoel *et al.*, 1986).

Particles of chill haze are of uniform round shapes, 0.1 to 1.0 μm in diameter. Their state of considerable hydration has been documented by electron microscopy (Claesson and Sandegren, 1969). These particles are larger in older beer haze than in fresh beer haze (Dadič, 1974). The difference may be associated with the condensation rate of polyphenols during storage. Different beers have different sensitivities to the formation of chill haze with respect to the quantity and the physico-chemical state of the haze-causing substances in beer (Chapon, 1968; Steiner and Stocker, 1969).

Permanent haze usually sets in after the formation of covalent bonds between the haze subunits. The particles are larger (1 to 10 μm in diameter) and less uniform than those in chill haze. Hazes, in which polysaccharides predominate, usually start developing before the chill haze is formed. The high-polysaccharide hazes result from the presence of unstable cleaved starch particles and soluble hemicellulose substances in the beer (Basařová, 1982).

The term *metal haze* is used to indicate hazes containing a high proportion of metals (Bengough and Harris, 1955). Hazes which contain higher concentrations of oxalate are called *oxalate hazes* (Gramshaw, 1970). *Silicic haze* is brought about by silicon oxide or silicates leached from the filtering or stabilizing materials into the beer. In exceptional cases, this kind of haze may result from a high content of silicates in the beer ingredients (Katayama and Horie, 1971). *Colloidal hazes* in beer may also be brought about by temperature effects during freezing and thawing. This latter type of haze which contains polysaccharides as the predominant and characteristic components, differs in microscopic images from the chill and permanent hazes.

The mechanism of colloidal haze formation

The basic mechanism in the formation of colloidal haze in beer (Bengough and Harris, 1955; Chapon and Chemardin, 1963; Chapon *et al.*, 1965; Gramshaw, 1968, 1969, 1970; Gardner and McGuinness, 1977; Dadič, 1974; Gracey and Barker, 1981) and also the importance of the connection between the colloidal and sensory beer stability (Dadič, 1974, 1980; Dalgliesh, 1977; Hardwick, 1978; Schur, 1979) are attributed to polyphenols, particularly their oxidation and association with polypeptides.

The preferential reaction of polyphenols with oxygen, compared to other beer components can be shown by measuring the incorporation of the ^{18}O-isotope from the headspace in beer bottles. After 8 months of storage, 65% of ^{18}O was found in the polyphenol fraction, 30% in free carbonyls, and 5% in beer isohumulones (Owades and Jakovac, 1966).

Polysaccharides are similarly capable of forming larger complexes *via* hydrogen bonds with polyphenols and polypeptides (Sommer and Metscher, 1978; Schur, 1979a). High-molecular mass glycoproteins, however, are regarded as having an inhibitive effect on the formation of haze (Lewis *et al.*, 1979).

The coacervation theory of de Jong (1923, 1927) can be applied to the formation of colloidal haze based on the dehydration effect of polyphenols or proteins (De Clerck, 1964). Chollot *et al.* (1962) ascribe the so-called increased tanning power of polyphenols to the nitrogenous substances and to the quantity of free hydroxyl groups in the polyphenol molecules. Similarly, the haze formation can be brought about by solid particles present in the beer (Chapon and Chemardin, 1963). In earlier as well as more recent papers, the prevailing view is that the oxidative polymerization of simple polyphenols into compounds of a higher polymerization degree capable of association with nitrogenous substances and the resulting precipitation of haze is of pivotal importance (Steiner, 1965; Woof and Pierce, 1967; Stocker, 1967; Jerumanis, 1969; Gramshaw, 1967, 1969, 1970; Kretschmer, 1980; Asano *et al.*, 1986). Polymerization induced by the acid medium in beer was mentioned by Gramshaw (1969, 1970) as one of the initiating factors in the haze formation but this hypothesis was scrutinized by Gomez and Lewis (1969).

There is no proof yet that polyphenols may polymerize during the storage of beer (Wackerbauer and Anger, 1984). Gupta and Haslam (1979) stated that polyphenol polymers originate as the result of intermediary metabolism only *in vivo* but not *in vitro*. In a hypothesis drawn by Gardner and McGuinness (1977), the initiation of haze formation is attributed to condensed polyphenols present in the beer ingredients which become activated by oxidative changes taking place during filtration and bottling. The polyphenols probably condense and react with nitrogenous macromolecules to form haze. Gracey and Barker (1981) assume that the haze is caused by macromolecules which have been brought into contact by calcium bridges developed between phosphates and protein carboxyls. Dimeric flavonoids probably also participate in these reactions. Delcour *et al.* (1982) attribute a significant role in the formation of colloid haze to aldehydes participating in the polymerization of polyphenols. The assumption is that haze formation increases with the increasing molecular mass of the polyphenols from dimers to polymers; monomers are very unlikely to participate. Chemical structure of the polyphenols also plays an important role (Derdelinckx and Jerumanis, 1987). For example, procyanidine B (catechin-catechin) increases haze formation more than procyanidine B4 (catechin-epicatechin) (Gardner and McGuinness, 1977; Delcour *et al.*, 1982). The number of hydroxyls in the molecule, their positions, and geometry are of additional importance (Singleton, 1967; Mulkay and Jerumanis, 1983).

The polypeptide association with polyphenols likely plays an important role in the haze formation. H-NMR spectroscopy studies (Outrup *et al.*, 1987) have shown that complexes consisting of small peptides (*e.g.*, [L-Pro-L-Pro-Gly]$_5$) and proanthocyanidins were soluble but that complexes consisting of high-molecular mass peptides (*e.g.*, [L-Pro-L-Pro-L-Pro]$_{26}$) and proanthocyanidins formed precipitates. The quantity of the haze formed also depends on the concentrations of the reacting substances, the length of the peptide chains, and the amino acid sequence in the peptides.

Beer stabilization

Stabilizing beer treatments using various preparations enjoy an ancient tradition. The treatments developed from using wood chips, saw dust filled with pitch, aluminum dust or foils, glues of vegetable and animal origins up to the recently used natural as well as synthetic stabilizers (Kamm, 1969).

In order to increase the colloidal stability of beer, the modern agents effectively decrease the contents of nitrogeneous and polyphenolic haze-causing substances in the beverage or prevent oxidative changes which would result from the aeration of beer in the final production phases. Stabilizers effective against polysaccharides and metal ions are not much used in practice.

Stabilizing preparations differ in their effectiveness which is defined as *precipitating, adsorptive, enzymatic,* or *antioxidizing* (De Clerck, 1964; Kamm, 1969). Depending on their ability to react with specific groups of haze-causing substances, the stabilizers may be divided into several groups: one group of *precipitants, adsorbents,* and *enzymes* decreases the amount of high-molecular mass peptides in beer, another group of adsorbents and enzymes is effective against beer polyphenols, and the third group consists of stabilizers which by their reducing ability limit the negative effect of oxygen dissolved in beer. An example of the latter group is ascorbic acid which acts as an indirect antioxidizing agent. Another example is glucose oxidase (EC 3.1.1.34) which exhibits direct reducing action. The last group of stabilizers includes preparations (*e.g.*, ethylene diamine tetraacetic acid - EDTA) which remove metal ions from beer in the form of insoluble complexes (Schur, 1979; Kahler and Voborský, 1981; Basařová, 1982).

In fact, the separation of haze-causing substances is facilitated by merely undercooling the beer prior to filtration but the extent of depletion is not sufficient to ensure long-term stability.

Amongst new procedures designed to improve colloidal stability of beer is the application of the static or dynamic effect of high pressure on beer. Pressure waves are capable of inducing precipitation of high-molecular mass polyphenols and polypeptides in beer prior to its filtration. Beneficial results have been achieved by applying pressure higher than 3 MPa (Basařová *et al.*, 1984). A lower effectiveness was achieved by lower pressures brought about by pulsation of the pressure waves or using the principle of cavitation effects in fluids (Chládek, 1988).

Precipitating stabilizers

Tannin belongs to precipitating preparations effective against nitrogenous substances and has been used in the brewing industry for the longest time. Tannin of plant origin (Fig. 1) has been used for this purpose because synthetically produced tannin is not suitable (Hummel, 1971). Commercial preparations contain gallic acid bound to glucose at a ratio 1:1. The free gallic acid content should not exceed 3-8% in the commercial stabilizer (Kamm, 1969; Van Dromme, 1979). The affinity of tannin to polypeptides is brought about by the large number of hydroxyl groups in the tannin molecules capable of forming hydrogen bridges with polypeptides. The resulting complexes are insoluble. Tannin primarily associates with nitrogenous substances having the isoelectric point in the range of pH 3.5 to 6.5 (Vancraenenbroeck *et al.*, 1977; Van Dromme, 1979). Tannin is effective by rapidly precipitating colloidal substances having their molecular masses between 10,000 and 30,000 daltons (Basařová, 1968). The overall specific effect of tannin on beer has been confirmed by gel chromatography. Following the treatment with tannin, all maxima on the Folin's reaction curve were lowered (Basařová, 1976). Even antioxidative effects have been attributed to tannin (Dadič and Belleau, 1978). Application of tannin has been tried during malt mashing (Karel, 1975), wort boiling (De Clerck, 1970), in cooled wort (Basařová, 1964, 1968), and in barrelled beer. The highest efficiency was noted at 5 to 10 g.hL^{-1} applied about 24 h before beer filtration (Van Dromme, 1979). The most recent papers by Chibata *et al.* (1986) and Delcour *et al.* (1988) confirm the permanent research interest in one of the oldest beer stabilizers.

Formaldehyde is a stabilizer with a precipitating effect on polyphenols (Macey *et al.*, 1964, 1966; Whatling *et al.*, 1968). It is primarily active against anthocyanogens (Pfenninger and Steiner, 1964; Schilfarth and Sommer, 1968) which are precipitated from beer due to the reaction with

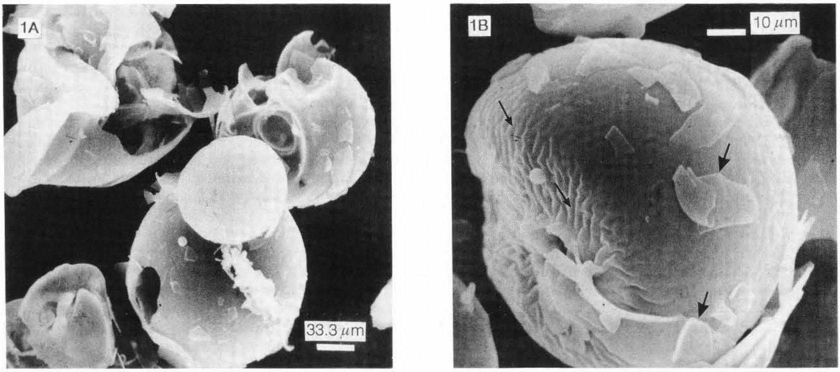


*Fig. 1. Natural tannin particles obtained by spray-drying are soluble in aqueous solutions. The ability of tannin to precipitate nitrogenous substances in beer depends on tannin purity (no synthetic substances are allowed) and on the number of free hydroxyl groups of gallic acid bound in the tannin molecules. Tannin particles are smooth (**A**) or finely wrinkled (**B**, small arrows); when milled or dissolved in aqueous solutions, the particles disintegrate into minute scales (large arrows).*

the condensation products of formaldehyde and amines (Macey, 1979). Formaldehyde is most frequently applied at mashing in powder or pill forms (paraformaldehyde). Although it is effective and inexpensive, its use is not permitted in the food industry of many countries (Thalacker, 1986).

Enzyme stabilizers

Pulverized and fluid proteolytic enzymatic preparations of plant, microbial, and animal origin have been used as beer stabilizers for a long time because of their ability to split high-molecular nitrogenous substances (Bamforth, 1985). The antioxidative effect of glucose oxidase (Hartmeier, 1979a) is rarely used in practice. Enzymatic degradation of polyphenolic haze substances also has not yet found any practical use, although experiments with tannase obtained from *Aspergillus flavus* have shown some potential (Masschelein and Batum, 1981).

Proteolytic enzymes are applied during or after completion of the fermentation process at 0.3 to 2.0 g.hL^{-1}. By analyzing the high-molecular mass polypeptide and amino acid contents in beer, the precipitating effect of the protease occurring in the course of the maturation process cannot be detected. This is because the values obtained are influenced by the removal of complex nitrogenous substances in the natural clarifying procedure and the amino acid spectrum is affected by amino acids produced by the yeasts or released by their autolysis (Basařová and Černá, 1973).

The active components of the majority of enzymatic beer stabilizers are papain (EC 3.4.22.2), chymopapain (EC 3.4.22.6), or lysozyme (EC 3.2.1.17) (Basařová and Černá, 1973). These stabilizers contain lactose or sorbitol as a filler (Hartmeier, 1979). Fycin (EC 3.4.22.3) and bromelin (EC 3.4.23.1) are proteases of plant origin and pepsin (EC 3.4.23.1) and trypsin (EC 3.4.21.4) are proteases of animal origin used in beer stabilizers. A proteolytic enzyme of microbial origin isolated from *Streptomyces fradiae* has also been tested (Posada *et al.*, 1979, 1981).

Proteases immobilized by anchoring on hydroxyacylmethacrylate gels (Basařová and Turková, 1977) have also been used to treat the beer. The advantages of this procedure consist of reducing the cost of the treatment and also of diminishing the contamination of the beer with degradation products of the enzyme preparations used. Another advantage is the possibility to adjust the number of the reactive groups, porosity and surface area of the gel. Glucose oxidase has also been anchored on the same carrier (Basařová, 1982). Beer stabilization by immobilized proteases was studied by Hansen (1981), Jin and Toda (1988), and Dellweg and Hartmeier (1983). A comparison of the effects of equal amounts of immobilized and free papain showed that free papain was more effective, probably because the residual activity of the dissolved enzyme delayed the association of polypeptide and polyphenol macromolecules in the beer during storage (Basařová, 1982).

The widespread application of immobilized enzymes in stabilizing procedures has been hindered by the need for special reactors and, primarily, by the unbalanced effectiveness in the course of beer stabilization caused by cumulative fouling of the active surface of the biocatalyst with beer haze particles.

New possibilities in enzymatic beer stabilization were discovered by studying the hereditary properties of brewery yeasts (Tubbs, 1984; Lawrence, 1986, Hammond, 1986; Lankashire, 1986; Franke, 1986). Possibilities of stabilizing beer without using special preparations may be studied by exploiting the potential of genetic engineering for enriching the genetic pool of yeast cells by making them produce active exocellular proteolytic or anti-oxidative enzymes (Franke, 1986; Young and Hosford, 1967; Sturley and Young, 1988; Bilinski *et al.*, 1988). This new trend is in full compliance with ecological intentions to reduce the use of auxiliary preparations in the production of foods and beverages.

Adsorptive beer stabilizers

The adsorbents of nitrogenous and polyphenolic substances are at present the most widely applied beer stabilizers. The following properties of the adsorbents are required (Hough and Lovell, 1979; Basařová *et al.*, 1979, 1984) for research work and for practical purposes:

1. Hygienically safe and acceptable.
2. Insoluble in beer, in alkaline and acid solutions and in currently used solvents.
3. Having a chemical structure that enables sorption of haze-causing substances.
4. Textural (morphological) properties of the sorbent which govern the effectiveness and sorption specificity (particle dimensions and surface area, and diameter and volume of the pores).
5. Minimal undesirable non-selective sorptive activity towards bitter hop substances, coloured beer compounds, foaming substances, and other extract components ensuring the so-called full taste of beer.
6. Technological properties warranting minimum beer losses at charge dosing and at dosing during filtration, not influencing the process smoothness (including the flow rate, wet volume, bulk weight, and filtration resistance).

Bentonites and siliceous gels belong to the group of adsorbents of nitrogenous substances. The bentonite (the group of montmorillonites) structure is made up by small crystals visible by electron microscopy (Kamm, 1969). The adsorption capacity is ensured by the large surface area but is limited by the degree of swelling in water solutions and by the exchange of calcium and sodium cations for the nitrogenous substances of the beer.

Basařová (1964), Nadzeyka *et al.* (1979), and Raible and Eichhorn (1964) attribute a lower stabilizing effectiveness to bentonites compared to siliceous gels. Narziss and Röttger (1973) state comparable results on the increased sorption of nitrogenous substances by bentonites over a wide range of molecular mass from 2,000 to 4,000 daltons and by siliceous gels over a range of 4,600 to 6,000 daltons. The differing views about the efficiency and specificity of protein sorption through adsorbents are obviously brought about by the diverse morphological properties (particle size and pore distribution) of the sorbents as well as the type of beer treated by the various authors in their work. Due to the considerable swelling capacity of bentonites, these stabilizers are not used in conventional filtration plants (Schur, 1979a, 1979b). The dosage is carried out by charges and beer losses amount to 1-3% (Schild and Weyh, 1963; Basařová, 1964).

Adsorbents of nitrogenous substances based on silicic acid have been widely used (Raible, 1961, 1979; Herlíková-Basařová, 1964). The main adsorbents used are xerogels (water content 4-5%), whereas hydrogels (water content 40-60%) are used to a lesser extent; silica sols are used the least (Wackerbauer and Anger, 1984b). For details on their preparation, see Diagram.

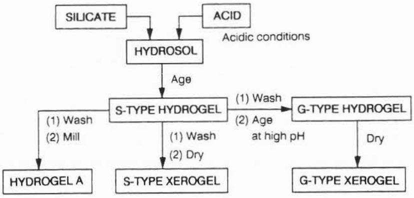


***Diagram**. Preparation of silica gels from silicic acid.*

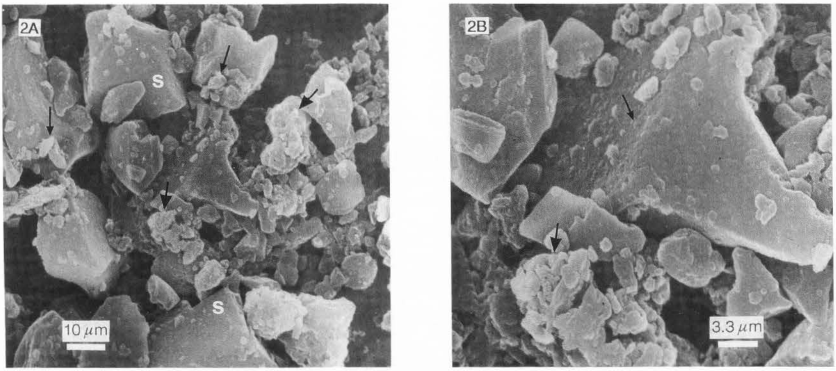
Table 1.

Filtering characteristics of polymeric sorbents and kieselguhr

Sample	Flow charact. $L.min^{-1}.m^{-2}$	Bulk weight $g.L^{-1}$	Wet volume $L.kg^{-1}$	Filtering resistance* $m^{-2}.10^{11}$
Kieselguhr Hyflo Super Cel	645	187	3.1	12
Filter Cel	23	131	3.9	160
Stabiquick (Silica gel)	16.7	340	2.3	620
Köstrosorb (Silica gel)	69	307	2.8	300
PVPP	264	200	7.3	18.5
Sorbamid	1720	290	4.2	2.3
Karap	1690	198	6.1	3.0
Polyamid 88	601	200	5.4	10.1

* Voborský, 1986.

The microstructure and technological properties of siliceous gels depend on the polymerizing and condensing processes taking place during their production. The concentration of the initial solutions is of the great importance as is the rate of adding acid, the reaction temperature, the pH (higher pH diminishes the surface area and increases the sorbent porosity), the procedure of washing and thermal processing, and grinding (Hough and Lovell, 1979; Kahler and Voborský, 1981). Sorbents with a wide range of properties can be made by changing the conditions listed above. For example, surface values of 200 up to 500 $m^2.g^{-1}$, pore volume of 0.4 up to 1.2 $cm^3.g^{-1}$, pore diameter of 3 up to 20 μm, and specific gravity of 300 up to 1,000 $kg.m^{-3}$ may be obtained (Hummel, 1971; Hough and Lovell, 1979). The



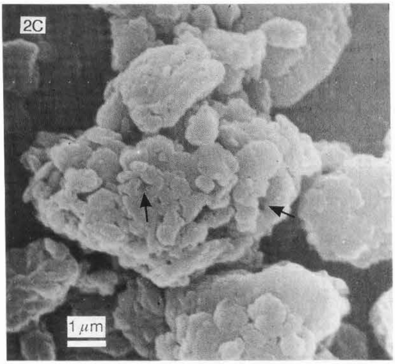


***Fig.** 2. Stabiquick (silicic xerogel) particles are of irregular shapes. **A**: Larger particles are smooth (S) or porous (large arrows). If the proportion of particles <5 µm (small arrows) is too high, the filtrability of beer stabilized using Stabiquick is reduced (Table 1). **B**: Detail of a smooth particle shows surface topography (small arrow); porosity of another particle (large arrow) is evident; **C**: Detail of pores (arrows) in a porous particle. The articulate surface of Stabiquick particles is formed by silicic acid aggregates containing hydroxyl radicals. The adsorption of beer polypeptides results from the formation of hydrogen bonds between the hydroxyl radicals of the adsorbent and the peptidic bonds of the polypeptides.*

sorption capacity of siliceous gels depends on the particle size and their surface and on the porosity and the number of free hydroxyl radicals in the silicic acid aggregates. In practice, a compromise is sought between the stabilizing effect or the particle size and securing the optimum filtration conditions. Small particles (<5 µm in diameter) are deleterious to the filtration properties of the sorbents. Filtration properties of the Stabiquick 360 siliceous xerogel are shown (Table 1) to be similar to the properties of very fine kieselguhr. Fig. 2 shows the structure of the particles of the xerogel. The structure of a similar xerogel (trade name Köstrosorb) is shown in Fig. 3. The sorption specificity first of all depends on the distribution of the pore volume (Schimpf and Runkel, 1962; Hough and Lovell, 1979; Kahler and Voborský, 1981). Nitrogenous compounds with a molecular mass over 10,000 daltons are mainly active in the formation of colloidal haze (Narziss and Röttger, 1973; Röttger, 1974; Bishop, 1975) which for adequate sorption means that the size of the pores should be 2.5 nm (Hough and Lovell, 1979). The sorption of peptides proceeds quickly and for a filtration time of 2-5 min, a dosage of up to 100 g.hL^{-1} of beer is sufficient.

According to Nadzeyka *et al.* (1979), siliceous gels adsorb about 38% of nitrogenous substances having molecular mass over 7,000 daltons, 45% of nitrogenous substances having molecular mass of 35,000-75,000 daltons, and 56% of nitrogenous substances having molecular mass of 13,500-35,000 daltons. The sorption of peptides with an isoelectric point in the range of pH 3-5 has been proved by isoelectric focussing. During the adsorption of nitrogenous compounds on siliceous gels also a certain proportion of polyphenols and polysacchartides (which are associated with polypetides) are also removed from beer (Ullmann, 1962; Weyh, 1979; Basařová, 1977; Narziss and Bellmer, 1975, 1976; Hough and Lovell, 1979; Narziss and Gromus, 1982). Stabilizing procedures have been proposed that combine the use of siliceous gels with ascorbic acid in order to prevent oxidative changes in beer caused by oxygen released from the space of the pores in the adsorbent (Raible, 1962; Herlíková-Basařová, 1964; Hug and Pfenninger, 1988). Other combinations consist of silica gel and proteolytic enzymes (Hummel, 1973), silica gel and polyvinylpolypyrrolidone (Jäger, 1980), and silica gel and bentonites (Raible, 1979).

The use of siliceous sols was tried out in the brewery industry in order to shorten beer maturation (Pfenninger

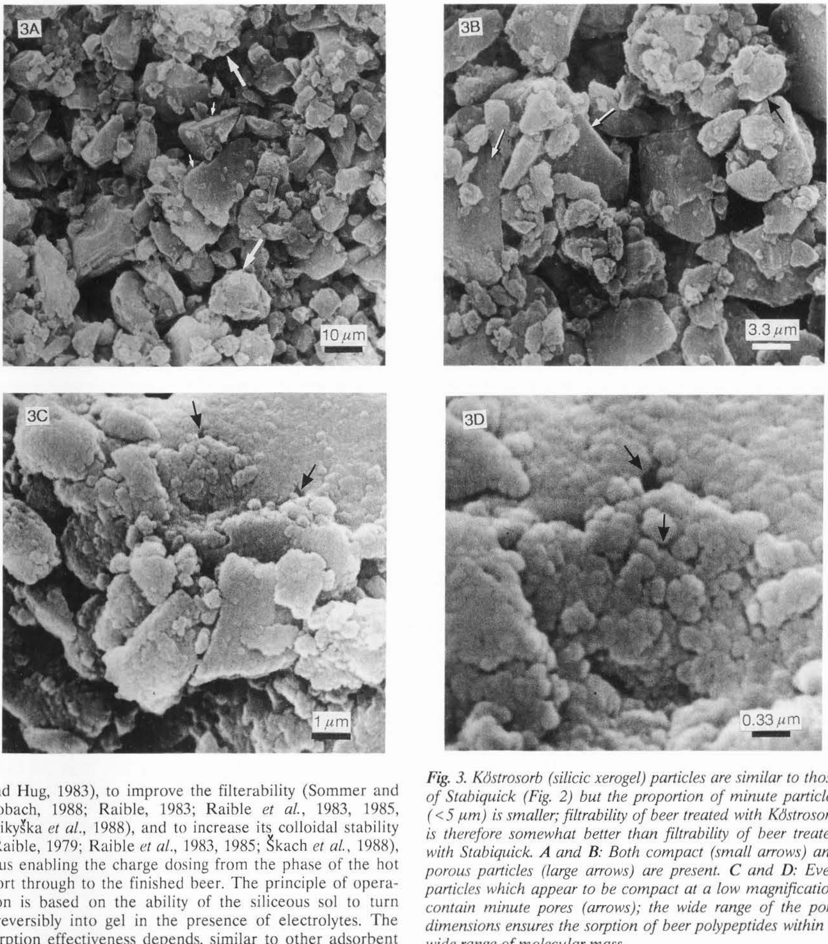


Fig. 3. Köstrosorb (silicic xerogel) particles are similar to those of Stabiquick (Fig. 2) but the proportion of minute particles (< 5 µm) is smaller; filtrability of beer treated with Köstrosorb is therefore somewhat better than filtrability of beer treated with Stabiquick. ***A*** *and* ***B****: Both compact (small arrows) and porous particles (large arrows) are present.* ***C*** *and* ***D****: Even particles which appear to be compact at a low magnification contain minute pores (arrows); the wide range of the pore dimensions ensures the sorption of beer polypeptides within a wide range of molecular mass.*

and Hug, 1983), to improve the filterability (Sommer and Bobach, 1988; Raible, 1983; Raible *et al.*, 1983, 1985, Mikyška *et al.*, 1988), and to increase its colloidal stability (Raible, 1979; Raible *et al.*, 1983, 1985; Škach *et al.*, 1988), thus enabling the charge dosing from the phase of the hot wort through to the finished beer. The principle of operation is based on the ability of the siliceous sol to turn irreversibly into gel in the presence of electrolytes. The sorption effectiveness depends, similar to other adsorbent types, on particle dimensions, porosity, differential pore distribution, and on the degree of aggregation and sol solubility. The cross-linking of the alkaline sol solution (70% are alkaline waters) starts as late as in the acidic regime in beer. Because of the transfer of alkaline water from the siliceous sol into beer and because of the concern that the sol might be incompletely precipitated and the beer loss at charge dosing could be increased, the usage of these sorbents in the brewery industry is still under discussion (Mayer, 1983).

Adsorbents of polyphenols

Due to the fact that research has improved the knowledge about the great reactivity of polyphenols in the formation of colloidal haze, breweries have geared their efforts up to a stabilizing procedure that reduces the concentration of these compounds in beer.

Polyamide derivatives structurally similar to peptides

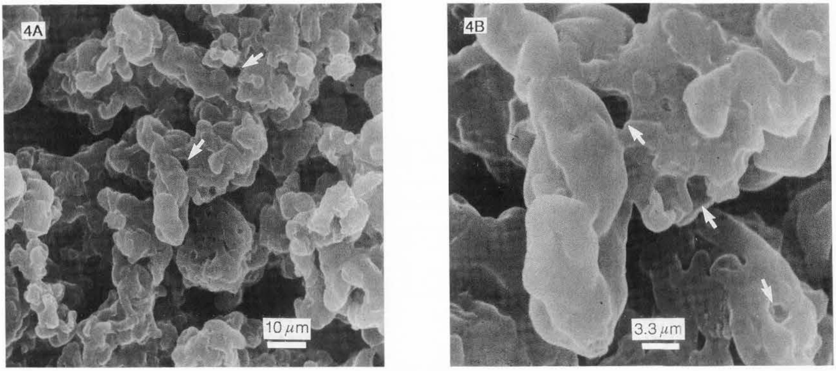


*Fig. 4. Particles of cross-linked polyvinylpolypyrrolidone (PVPP) have an articulate structure with a large active surface suitable for the sorption of beer polyphenols. The dimensions of the pores and their distribution do not ensure any strict specificity toward particular beer polyphenols. The sorption materializes via hydrogen bonds between the accessible peptide bonds of the stabilizer and active hydroxyl radicals of beer polyphenols. **A**: General view, **B**: Detail. Arrows point to pores of various dimensions. The volume of the pores is reduced after this stabilizer swells slightly in the beer treated.*

adsorb polyphenols effectively. The adsorption is performed primarily by the formation of hydrogen bridges between the hydroxyl groups of polyphenols and the polyamide peptide bonds (Hartl and Kleber, 1960; Singleton, 1967; Kamm, 1969).

Stabilizing compounds such as nylon (Harris and Ricketts, 1959, 1960; Schilfarth, 1960; Weymar, 1960; Kraus, 1961; Dadič and van Gheluwe, 1973) and perlon (Hartl, 1961) have been used with good stabilizing results but with very diverse findings concerning the side effects on beer quality (bitterness, foaming capacity, colour). These sorbents have not found wide application in practice.

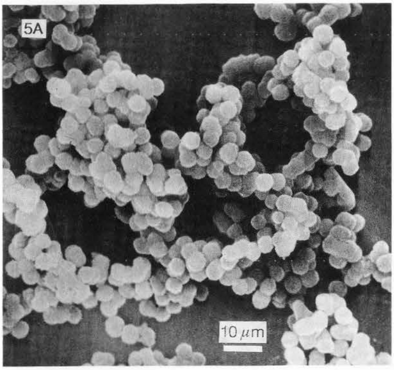
In the year 1955, McFarlane *et al.* provided information for the first time about the stabilizing effect of polyvinylpyrrolidone (PVP) which possesses a peptide bond in the molecule similar to polyamides. However, PVP solubility in beer induced the manufacturers to prepare insoluble cross-linked forms of polyvinylpolypyrrolidone (PVPP) under the trade name AT-496 (McFarlane, 1961) and later on Polyclar AT (McFarlane *et al.*, 1963).

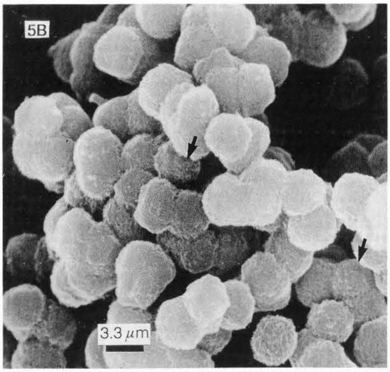
Many authors, *e.g.*, Drawert *et al.* (1973, 1974, 1975), Weyh *et al.* (1975), Weyh (1979), Narziss and Gromus (1982), Dadič and Lavalee (1983), Glynn and Wilkinson (1985), Meier (1987), and others have studied the impact of PVPP (Fig. 4) on beer quality and obtained differing results on beer stability. In addition to the markedly reduced polyphenol levels due to PVPP, the majority of authors also mentioned a slight decrease in nitrogenous substances. It has been unambiguously proved that the beer colour was slightly reduced (Drawert *et al.*, 1975; Narziss and Gromus, 1982, Škach, 1984; Meier, 1987). No substantial changes were recorded in any other analytical criteria.

PVPP adsorbs a wide range of polyphenol compounds very effectively. Some authors ascertained a lower effectiveness of PVPP towards condensed polyphenols (Gramshaw, 1967; Narziss and Bellmer, 1975, 1976; Basařová *et al.*, 1977; Sommer and Metscher, 1978). These data have influenced the methods for evaluating the PVPP effectiveness to a considerable degree (Narziss and Gromus, 1982). At a PVPP dosage of 50 $g.hL^{-1}$ of beer, Schafft (1978) stated that up to 60% of anthocyanogen and 40-50% of the total polyphenols remained as residues in beer. Wackerbauer and Anger (1984) stated the decrease of the anthocyanogen level in the range of 30-60%. Aeration exerts less of a negative influence on the colloidal state of beer when the beer is stabilized with PVPP in comparison with the stabilization aimed at nitrogenous substances (Hodenberg and Sulke, 1976; Drew, 1981).

The effectiveness of PVPP is, as with other adsorbent types, dependent on the degree of beer clarification prior to the stabilization procedure (Sfat, 1974; Drawert *et al.*, 1973, 1974, 1975; Sommer and Metscher, 1978). As an alternative to prefiltration by kieselguhr, separation by centrifugation is suggested (Donhauser *et al.*, 1988). PVPP dosage at wort boiling has also been used (Scholl *et al.*, 1988). There is a risk, however, that soluble monomers may be released from cross-linked PVPP during this procedure.

An advantage of PVPP is the ability for regeneration with a dilute alkali solution and the possibility of its repeated use (Schafft *et al.*, 1977). This fact inspired the development of special filtration and stabilizing equipment (Polster, 1986; Eppinger, 1987) with horizontal sieve filters (Schafft, 1978; Hodenberg and Sulke, 1979) and candle filters (Olbrich and Leipner, 1979; Schwinghammer, 1979; Grabmüller and Zítek, 1988) equipped with adaptable supporting partitions to allow for the dissimilar physical properties of PVPP in comparison with kieselguhr. For example, the swelling capacity of PVPP is 2.3 to 2.5 higher,





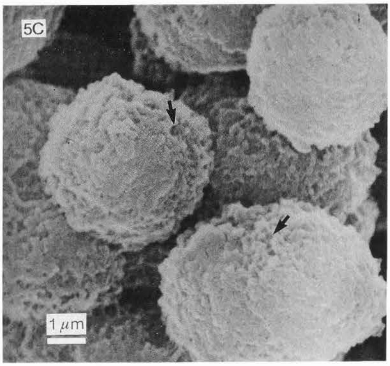


Fig. 5. The polyether sorbent Sorfix is in the form of very small spherical particles of uniform dimensions having a total surface of 590 $m^2.g^{-1}$. Sorfix contains no suitable groups which would participate in chemical sorption of nitrogenous or polyphenolic haze-causing substances from beer. The high porosity of the particles and a large surface are conducive to the formation of covalent bonds with active substances such as enzymes, microbial cells, etc. These materials are bound in large quantities. ***A:*** *Sorfix globules form rings.* ***B*** *and* ***C:*** *Detail of the porous (arrows) particle surface.*

the dimensions of the PVPP particles are up to 450 µm in diameter compared to 1-60 µm for kieselguhr, 30-40% of the PVPP particles are smaller than 60 µm in diameter but 8-10% are larger than 200 µm in diameter, and the specific (measurable) gravity of PVPP is 1.2 $g.cm^{-3}$, *i.e.*, about 4 times as high as that of kieselguhr (Meier and Domeisen, 1980). Filtration properties of PVPP are shown in Table 1 to be similar to the properties of fine kieselguhr. PVPP can be dosed together with siliceous hydrogels; these latter gels dissolve during the regeneration of the PVPP adsorbent. This combination provides better results for the stability of beer (Sfat, 1975; Beshed, 1985), but, generally, only at increased dosage (Sommer and Metscher, 1978). Filter plates designed to contain anchored PVPP can be used in current plate filters and can also be regenerated (Kipphan and Birbaum, 1964; Basařová and Faktor, 1969); Leipner, 1977; Kessler, 1988). The effectiveness, however, is unstable and decreases during the stabilizing filtration by fouling the active surface of the plate by up to 50%; each batch of treated beer has to be homogenized. New types of stabilizing plates are recommended for use in small to medium sized breweries (Kessler, 1988).

A new area in beer stabilization that offers economic, technological, and hygienic advantages, is the development of barley varieties with blocked anthocyanogen synthesis (von Wettstein *et al.*, 1977, 1980; von Wettstein, 1979; Ponton, 1988). The use of such varieties would lead, in practice, to the production of colloidally stable beers, the production of which would not need any special stabilizers and dosing equipment (Ahrenst-Larsen and Erdal, 1979; Sarx and Wischmann, 1985). Any wider application of the new barley varieties is impeded, for the time being, by the unbalanced distribution of the anthocyanogens in single grains. Whilst the corresponding malts and the beer produced from them possess a considerably reduced polyphenol content, these beers are prone to a darker (more noticeable) colour and to deviations in the composition of aromatic substances which is due to high amino acid levels in these specific barley varieties. This accounts for why in practice there is still a preference for tested stabilizers, primarily adsorbents of polyphenols and nitrogenous substances.

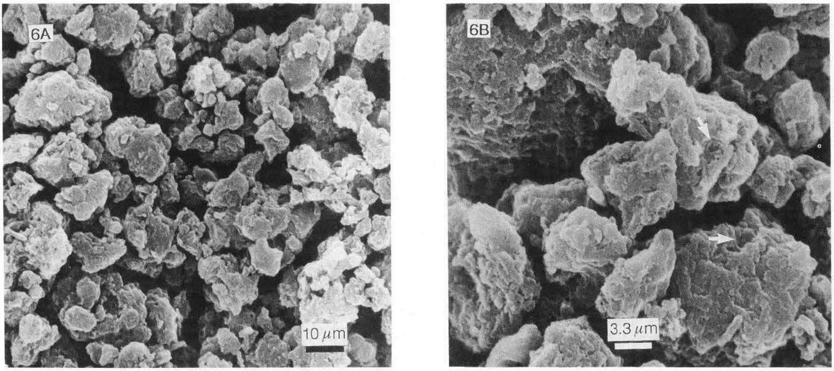
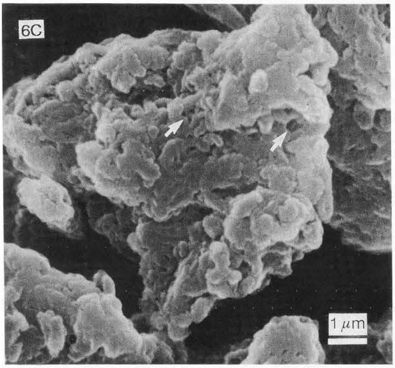


Fig. 6. Sorsilen consists of fine articulate particles (A and B) having a very large surface (50-100 $m^2.g^{-1}$). The high porosity (C, arrows) of Sorsilen facilitates the removal of compounds such as humic substances which react with the particle surface; however, there are not enough peptidic bonds to bind polyphenols from beer.



New adsorbent types for polyphenols

Within the framework of preparing and using various types of polymeric sorbents in various branches of the national economy including the brewing industry, a team specializing in basic research of these problems has been established at the Prague Institute of Chemical Technology. The following conclusions may be drawn from the results achieved so far: Polymers based on urea-formaldehyde resins and polymers of the acetylcellulose and polyphenoloxide types are totally ineffective in removing nitrogenous and polyphenolic substances that cause beer haze. Research of the morphological and sorptive properties has confirmed that these polymer types might be manufactured with a large surface and porosity but they lack active sites in the molecular structure that would be capable of mediating a competent physical bond with polyphenols or polypeptides. In a similar way, the polyetheric sorbent types which are marketed under the trade name Sorfix (Fig. 5) and used as carriers for immobilized enzymes and cells and their constituents (Jirků *et al.,* 1980; Jirků and Turková, 1987) do not exhibit any marked sorptive activity towards beer haze-causing substances. The surface area of Sorfix is 590 $m^2.g^{-1}$, particle diameter 0.2-1.2 mm, pore diameter 6.8 nm, and pore volume 0.467 $mL.g^{-1}$.

Based on the polyester polymers, the preparation Sorsilen has been developed (Fig. 6). The molecule of this basic component of polyethylene terephthalate consists of the repeating structural unit (OCH_2-CH_2-OOC-C_6H_4-CO). The molecular mass is about 20,000 daltons. The concentrations of the terminal hydroxyl and carboxyl groups are rather low (5-$15x10^{-5}$ equivalent of -COOH per gram of the polymer). That is why no effective chemical sorption can be assumed. Of the morphological properties, the most important is the large surface area (80-100 $m^2.g^{-1}$) and the considerable porosity (pore volume 2.7 $mL.g^{-1}$, interstitial volume 0.8 $mL.g^{-1}$, and particle diameter 0.1-0.5 mm). When dosing Sorsilen in beer, the sorptive activity against polyphenols is about 15-20% compared with an equal PVPP dosage (Basařová, 1977, Basařová *et al.,* 1977). The Sorsilen activity improves (reduces) the index value of polyphenol polymerization. The polymerization index is a problematic indicator of specificity but signals a different action of Sorsilen in comparison with PVPP and Nylon 66, which increase the index (Basařová *et al.,* 1977, 1979). Sorsilen effectively adsorbs humic substances and various types of aromatic compounds.

An unambiguous sorptive activity against beer polyphenols has been shown by the development of a series of chemically modified polyamides, the chemical structure of

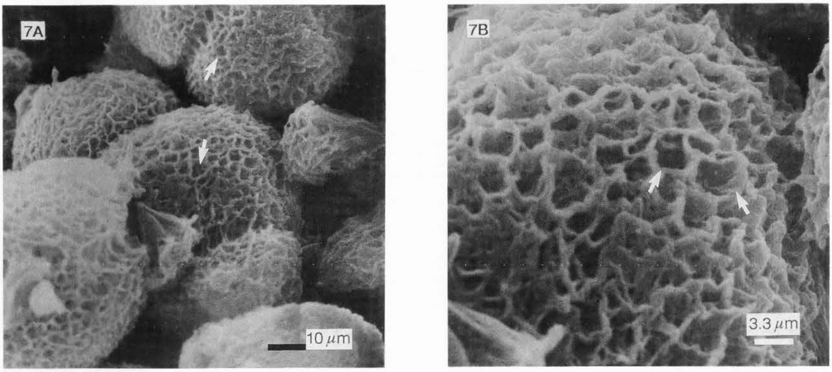


*Fig. 7. Sorbamid (stabilizer of polyamide nature) consists of regular spherical particles (**A**) having a large surface (5.8 $m^2.g^{-1}$, porosity ~45%). Regular shape and high mechanical stability ensure good filtration properties of this stabilizer (Table 1). The distribution of the pores (**B**, arrows) and their volume contribute to the specificity of Sorbamid as a sorbent for beer polyphenols.*

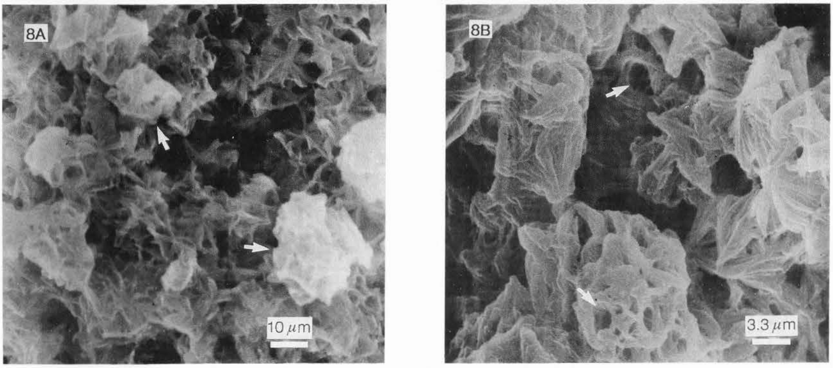
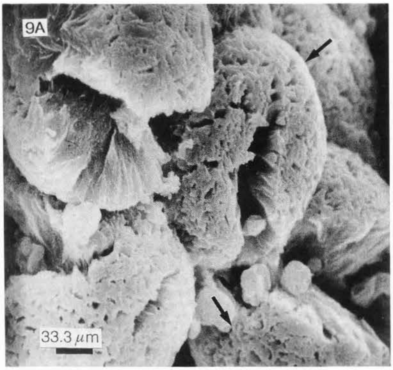
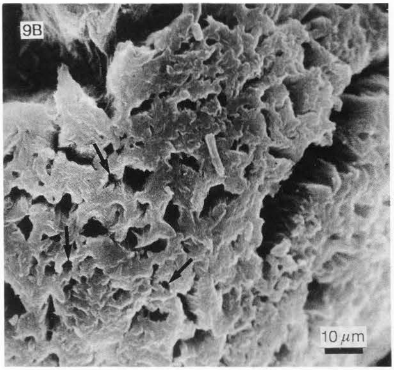


*Fig. 8. The incorporation of active charcoal (soot) (5% w/w) into the matrix of Sorbamid leads to the production of the adsorbent Karap. The change in chemical composition markedly affects the microstructure of the polymer: the particles are no more spherical but are finer and of irregular shape (**A**, arrows). The pores (**B**, arrows) are larger than in Sorbamid. Karap porosity (48%) and surface (7.8 $m^2.g^{-1}$) are higher than those of Sorbamid but sorption efficiency is lower because the introduction of charcoal reduces the mass proportion of the chemically active polyamide polymer.*

which offers the possibility of forming hydrogen bridges with polyphenols. Conditions of polymerization have been worked out in order to modify the morphological properties of these polymers over a wide range. All types of the prepared modified polyamides are capable of regeneration in similar conditions to PVPP with minimal losses and the frequency of their application is increasing. They also possess good filtration properties (Table 1). The sorbent prepared from specifically modified polycaprolactame through anion polymerization of 6-caprolactame is called Sorbamid (specific surface 5.8 $m^2.g^{-1}$, particle diameter <0.5 mm, pore diameter 440 nm, pore volume 0.545 $mL.g^{-1}$, porosity 45%). In beer stabilization, it has been found that Sorbamid has, on average, a 50% sorptive effectiveness against beer polyphenols compared with an equal PVPP dosage (Basařová *et al.,* 1983). Beer stabilized by Sorbamid also has a slightly decreased level of nitrogenous substances. It has not been exhaustively proved that the decrease in





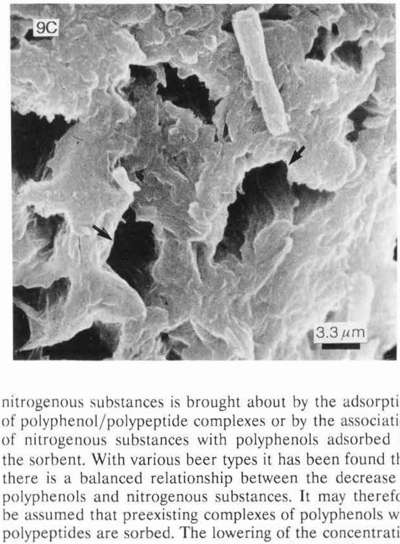


*Fig. 9. Chemically modified Polyamide 88 consists of spherical or ovoid particles (**A**). The surface (5.2 $m^2.g^{-1}$) is articulate. Higher specificity towards certain beer polyphenols as compared to PVPP depends on the high porosity and the suitable pore shapes (**B** and **C**: arrows); these properties limit the access and adsorption of beer polyphenols within a certain range of molecular masses.*

nitrogenous substances is brought about by the adsorption of polyphenol/polypeptide complexes or by the association of nitrogenous substances with polyphenols adsorbed on the sorbent. With various beer types it has been found that there is a balanced relationship between the decrease of polyphenols and nitrogenous substances. It may therefore be assumed that preexisting complexes of polyphenols with polypeptides are sorbed. The lowering of the concentration of nitrogenous substances is the same in stabilized beer which has not been aerated and in beer which has been artificially aerated. In the latter case it may be assumed that an increase of the so-called *tanning power* of sorbed polyphenols towards polypeptides is a result of the residues of nitrogenous substances of preexisting complexes (Škach, 1984). By the inclusion of 10% perlite (Perap) or active charcoal (Karap) into the matrix of the polymer Sorbamid already described, the surface of this polymer has been increased (Karap: specific surface area 7.8 $m^2.g^{-1}$, particle diameter 0.1-0.4 mm, pore diameter 212 nm, pore volume 0.822 $mL.g^{-1}$, porosity 48.6%), but the sorptive effectiveness towards polyphenols, as a consequence of the decrease of the number of bonding places per gram of a polymer, was reduced by about 5-10% (Kubánek *et al.,* 1984). Sorbamid is in the form of spherical particles (Fig. 7) generated most probably by the regular arrangement of macromolecules in the polymer. Polymers of an identical type, which contain built-in active charcoal (Karap), have an irregular particle shape (Fig. 8) with a very articulated structure. This arrangement is brought about by the presence of active soot interacting during polymerization and the formation of non-uniform particles.

An additional type of a modified stabilizer is the chemically modified polyamide sorbent prepared from activated anion polymerization of 6-caprolactame in the presence of polyacryloxide terminated by amino groups (Fig. 9). Compared with an equal PVPP dosage, this stabilizer contains about a 60-70% sorptive activity against beer polyphenols (Basařová and Škach, 1984; Škach, 1984). In contrast to PVPP, it adsorbs a wide range of polyphenolic compounds, and as has been proved by the HPLC method, the modified polyamide adsorbs a smaller number of polyphenolic compounds but has a greater affinity towards certain compounds. Both stabilizers are characterized by the same effectiveness against oxidized and oxidizable polyphenols, which participate in the colloidal changes of beer. The modified polyamide possesses a very articulated surface with a high number of large and small pores (specific surface area 5.2 $m^2.g^{-1}$, particle diameter 0.1-0.5 mm, pore diameter 380 nm, pore volume 0.40 $mL.g^{-1}$, porosity 47%).

This spatial articulation is obviously a limiting factor for the specific adsorption of certain compounds. The undesirable adsorption of other substances in beer was ascertained during the application of modified polyamide sorbents at current levels similar to those used with PVPP: a negligible sensory effect due to lowering of the isohumulone content and a moderate colour decrease. There was neither a statistically significant effect on foaming nor any additional analytical or organoleptic changes when stabilizing various beer types. The necessary contact time between stabilized beer and polyamide sorbents amounts maximally to 2 min (with PVPP, it is said to take 5-10 min). The real beer stability correlates not only with achieving a low polyphenol level but, as in the case of other sorbent types, depends on the physico-chemical state of treated beer and on conditions of its transport and storage. With Bohemian beers, at a dosage rate of the new polyamide of 40 g.hL^{-1}, there was, on average, a 3- to 5-month stability. If the levels of the nitrogenous substances are reduced in advance, this period extends to 9-13 months.

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Discussion with Reviewers

N.L. Davies: With many of the existing siliceous fining material or diatomaceous filters it is possible to create significant haze by mechanical breakdown of the fining or filter aid itself. How resistant are the newer aids described to handling during the fining process?

Author: Particles of the newer types of polyamide stabilizers are very resistant to mechanical effects. The present manufacturing practice, in which the new stabilizers have been used, has shown that the particles neither pass through the filter barrier nor damage the filtration layer. The new polyamide stabilizers have very good flow characteristics as shown in Table 1. Their values are considerably higher than the flow characteristics values of the siliceous xerogels or PVPP regularly used in practice. For these reasons, the risk that minute stabilizer particles will occur in draft beer is considerably smaller with the polyamide stabilizers than with very fine siliceous xerogels or PVPP.

N.L. Davies: How does the cost of the newer fining agents described compare to PVPP or siliceous compounds in terms or cost per charge and alteration in plant design?

Author: The manufacture of new polyamide stabilizers is being prepared. The production costs have not yet been established. As requested by the breweries, the stabilizers are made in 100-kg batches on semi-pilot plant scale. Even when considering a more laborious production and the doses which are higher by approximately 25% than the doses of PVPP, the costs of stabilization are about 15 to 20% lower in comparison with PVPP.

K.D. Zastrow: I don't agree with the author's viewpoint that wood chips *etc.* are serving, or were in the past considered to serve as *stabilizing* agents. These materials helped to clarify the beer, not to stabilize it.

Author: Good clarification of beer is the first condition which must be met in order to improve its colloidal state. When the beer is clarified using solid substances, nonstable colloidal compounds present in the beer become adsorbed on mechanically separated haze-forming substances. The use of wood chips in the clarification of beer was, therefore, included as the first attempt to improve the colloidal state of beer.

K.D. Zastrow: It should be mentioned in connection with the anthocyanogen-free barley that this concept for beer stabilization requires that also hops have to be free of anthocyanogens, something that could only be achieved by using hop extracts rather than the natural hop cones.

Author: The amount of polyphenols passing into the beer from hops can be reduced as you say. Single-component extracts which contain only the bitter hop substances have been used for this purpose instead of hops. The application of polyphenol-free malt and hops is still the subject of discussion. Many experts on brewing are concerned that the use of such ingredients will change the characteristic sensory attributes of certain beer types. In addition, nonoxidized polyphenols which originate from hops act as natural antioxidizing agents in beer. Thus, they have a positive effect on the colloidal stability of beer. Malt polyphenols enter the manufacturing process prior to hops polyphenols. During mashing, decanting of wort, and the subsequent manufacturing steps, these polyphenols polymerize and oxidize and are partly removed from the manufacturing process in the sludge.