The Use of Polyclar AT (PVPP) in Brewing [1]

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ABSTRACT

A brief history of the use of Polyclar® AT (PVPP) in brewing is given. Its stabilizing effect, particularly concerning the interactions with beer polyphenols, is presented according to currently accepted views. Crucial points in the application of this processing agent in brewing, such as the stage at which it is added, the amount, and the contact time, are discussed. The methods for quality control testing of Polyclar AT and for determining the residual soluble polyvinylpyrrolidone (PVP) in beer were experimentally evaluated. The regulations governing the use of AT in brewing are also reviewed.

Key words: *Beer stability, Governmental regulations, Polyphenols, Polyvinylpyrrolidone (PVP)*

COLLOIDAL AND FLAVOR STABILITIES OF BEER

Colloidal (physical) instability of beer is caused by formation of hazes, basically through polyphenol-protein interactions. The interdependence of colloidal and flavor stabilities and the particular role of polyphenols in beer has been recognized (19). The processing conditions that give a built-in resistance to oxidation changes prolong the overall shelf life of beer. Demands for longer shelf life have been placed on brewers by today's trading conditions as part of a common trend in food and beverage industries. Bottled and canned beer may now be shipped to slow trade outlets or foreign markets. A six-month shelf life of beer thus becomes a requirement, and some brewers even strive for a year or more. Mass production and technological innovations have compounded the problem of beer instability. The trend toward lighter and paler beers, particularly in the United States, has rendered the colloidal and flavor stabilities the most important features of beer quality, in addition to the beer flavor itself.

The problem of beer instability may be approached at three different stages; before, during, or after the brewing process (19).

Measures Before Brewing

Measures before brewing include selecting barley malts with low tanninogen contents (20), alkali steeping of barley (24), and barley abrasion to remove husk polyphenols. Barley abrasion was originally intended to accelerate the absorption of water and gibberellic acid.

Measures During Brewing

During the brewing process, both additive and nonadditive measures are used. The nonadditive measures include a proper control of lautering and sparging (particularly the temperature, pH, time, and rejection of the last runnings containing high concentrations of condensed tannins) as well as controlled oxidation during mashing.

There are three general groups of additive measures (22). In the first, an effort is made to reduce the oxygen (air) level in beer by using oxygen scavengers or antioxidants, such as ascorbic or isoascorbic acids, sodium tetrathionate, and glucose oxidase. In the second, haze formation is accelerated by adding an active haze-forming agent, such as tannic acid (gallotannin, a hydrolyzable tannin). In the third, haze formation is delayed by reducing the concentrations of main haze-forming substances, namely proteins (fining or chillproofing agents such as proteolytic enzymes or silicates) and polyphenols (adsorbents such as Polyclar AT® or nylon 66). The use of proteolytic enzymes (papain and colupulin) has several disadvantages: the agent remains in beer; foam stability (which is essentially due to surface-active proteins) is reduced, requiring the addition of foam stabilizers; and some loss in beer body occurs. The use of bentonite, which is a nonspecific agent for protein removal, also leads to a reduction in foam stability and, because of sediment trapping, to high beer losses. Some silica gels are more specific for high molecular weight proteins and do not significantly affect the normal beer properties; however, rather high levels of use are required. Consequently, the removal of polyphenols and protein-polyphenol complexes with the aid of proper adsorbents is preferable. The first adsorbents used were the polyamide resins perlon, nylon 11, and nylon 66 (33), which form complexes with polyphenols in a manner similar to that of proteins. Disadvantages connected with their use were some loss in foam stability and isohumulone content, as well as the relatively high use levels required. Polyclar AT (PVPP) is superior to other polyamides, and its advantages in brewing are discussed later.

Measures After Brewing

After brewing, an effort is made to reduce the intrusion of air (oxygen), to which beer is particularly sensitive after the removal of yeast, which acts as a reducing agent. They include various mechanical gadgets, such as baffles in storage tanks, or procedures such as injecting water or beer at filling, high-pressure carbon dioxide, or nitrogen jetting.

PVP AND PVPP—BASIC DATA

The industrial production of polyvinylpyrrolidone (PVP) is based on work of W. Reppe (58). PVP is commercially available in various viscosity grades with corresponding molecular weights (eg, K-15, 10,000 daltons; K-30, 40,000 daltons; K-60, 160,000 daltons; and K-90, 360,000 daltons. K-values are derived from viscosity measurements and are calculated according to Finkentscher's (5) formula. The molecular weight of PVP is determined by various methods such as osmometry, ultracentrifugation, sedimentation constant determination, turbidity titrations, as well as viscosity, thermodiffusion, and light-scattering measurements.

PVP is a hygroscopic powder that darkens at elevated temperatures. It is freely soluble in water and in many organic solvents.

By contrast, the cross-linked PVP polymer is practically insoluble in water, ethanol–water mixtures, organic solvents, or strong alkalis and acids (3). It can be manufactured by polymerization of the monomer in the presence of an alkali metal or an alkaline earth oxide or hydroxide (29). In the brewing literature, cross-linked PVP usually appears under the names Polyclar AT (4) or PVPP (polyvinylpolypyrrolidone).

The use of the water-soluble PVP in brewing preceded the use of the insoluble Polyclar AT. Use is based on the property of PVP to form complexes with certain polyphenols. An early study (55) suggested dipole attractions and H-bond formation with vegetable tannins. PVP was shown to precipitate hydrolyzable tannins from solution at pH 3–4 at a ratio of 80–90%, based on the polymer. Condensed tannins, the group into which most beer polyphenols fall, were precipitated in the pH range of 2–7 to a degree of 90–100% (31). The complexing ability of PVP was attributed to the resonance of the $-C(=O)N$ group. The presence of imino hydrogen was deemed nonessential.

Gustavson (32) showed that the water-insoluble cross-linked PVP binds the vegetable tannins irreversibly through formation of H-bridges, whereby the phenol moiety donates hydrogen to the oxygen of the ketoimide group.

[1] Presented at the 49th Annual Meeting, Nashville, TN, April 1983.

Toxicological studies have shown that PVP is essentially an inert material with extremely low acute toxicity (5). A 3% aqueous solution of PVP of a low average molecular weight was fed to growing rats over a period of about 24 weeks. Weight increase was not retarded, nor was there evidence of PVP absorption in the gastrointestinal tracts of the animals (1).

Outside the beverage industries, PVP is also used as a blood plasma expander; in cosmetics; in pharmaceuticals (tablet binder, granulating agent, drug retardant, and stabilizer); in diagnostic laboratory procedures; and in human and veterinary medicine (6). In industry, they are used in adhesives and coatings, detergents and soaps, electrical components, fibers and textiles, lithography and photography, and paper and plastics (7).

USE OF PVP AND PVPP IN BREWING

An Overview

The use of both water-soluble PVP and the insoluble PVPP (Polyclar AT) as a processing agent in beverage clarification is based on ability to form insoluble complexes with the naturally occurring polyphenols (tannins) present in such beverages as beer, wine, whiskey, fruit juices, vinegar, and tea (9). Polyphenols are known to affect clarity, color, and flavor of these beverages. Chillproofing of an alcoholic beverage with PVP thus increases its shelf life.

Soluble PVP was initially used in brewing to form an insoluble complex, which was removed by filtration or centrifugation. It was subsequently replaced by insoluble PVP (PVPP), whose commercial food and beverage grades are available under the trademark Polyclar® (4). Because it is practically insoluble in beverages, it should be considered a processing agent and not an additive.

Polyclar AT (PVPP) offers decisive advantages over the other beer stabilizers, such as nylon 66 or silica gels. It is rather selective for polyphenols and is four to five times more adsorptive of these substances than nylon 66. It does not affect the foam stability or the isohumulone content of the beer at normal levels of use. It may be used alone or in combination with other stabilizers, such as proteolytic enzymes or silica gels. Like other polyamides, it acts through formation of H-bonds between its carbonyl group and the hydroxyl hydrogen of polyphenols. PVPP is more efficient than the other polyamides, such as perlon or nylon (33), because: it lacks an amide hydrogen and thus cannot form hydrogen bonds with itself; its molecular configuration makes it more open to attack by the polyphenol moiety; and it has a closer spacing of its amide groups than nylon, which allows for the formation of more H-bonds (Fig. 1).

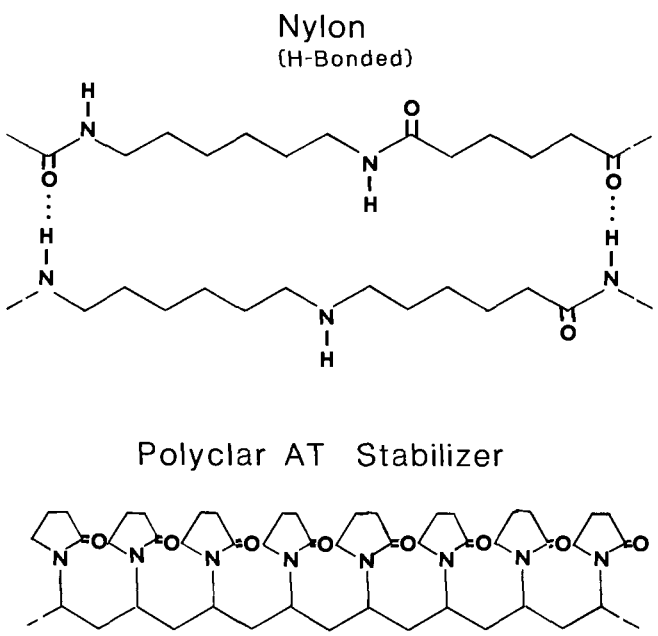

Fig. 1. Chemical structures of nylon 66 and Polyclar AT (PVPP) stabilizers.

In a typical U.S. brewery, PVPP is added uniformly and rapidly to filtered beer at a use level of 2–5 lb per 100 bbl (8–20 g/hl) for a contact time of 24 hr or less (9).

Laboratory trials showed that an AT–beer contact time of less than 24 hr may be sufficient to stabilize U.S. beers, while contact of as long as 28 days did not produce any harmful effects on beer. The optimal contact time may be very short and should be determined in plant trials for a particular type of beer. Before the AT treatment, the beer should be filtered, preferably using natural or slightly calcined, diatomaceous earth (kieselguhr). Otherwise, the desired colloidal stability is not achieved, or much higher use levels of AT are required. For U.S. and Canadian beers, a finely dispersed AT slurry (5–10%) allows uniform addition, employing a proportionating pump during transfer of beer following primary filtration. Another possibility is to add AT after cellaring, but before the final (polish) filtration. In this case, the beer is diverted to a surge tank to allow sufficient contact time. With European beers, a convenient stage to add AT is after the first filtration but before the polish filtration.

More recently, in-line addition with continuous body-feed of AT with recycling of the agent was introduced. The system uses very short contact times and results in good stability as well as significant cost savings. For easily stabilized beers, 2–3 lb of AT per 100 bbl was recommended; in combination with silica gel (3–4 lb per 100 bbl or 12–16 g/hl), only 1–2 lb of AT per 100 bbl (4–8 g/hl) were needed. When the two agents are combined, the resulting synergistic effect is caused by the removal of some high molecular weight protein that complete with AT for polyphenols. Consequently, when used in combination, it is preferable to add silica gel before AT.

A Brief History

McFarlane (39), in 1954, was the first to suggest the use of soluble PVP to stabilize and clarify "vegetable" beverages such as beer. Treatment with this agent precipitated a protein–tannin complex, thus improving physical stability, clarity, color, and flavor of the product. The optimal use levels of PVP varied with the type of beer. A beer was chillproofed with 1 lb PVP per 100 bbl (4 g/hl), with no adverse effects on its flavor or head retention (48). McFarlane et al (49) showed that a solution of PVP precipitated from beer chill-haze material containing tanninlike substances in addition to polypeptides and pentose carbohydrates. Later on, it was found (72) that PVP treatment of beer greatly reduced both its turbidity and its leucoanthocyanidin (anthocyanogen) content. Thus, the connection between the anthocyanogen content of wort and beer and the chill haze was established.

PVP precipitated tannins from hot wort, whereas proteins form a precipitate only on cooling (45). Adding PVP to hot wort in the kettle promotes a rapid and more complete separation of trub from the hot wort. The amount of "cold" trub is subsequently reduced. When increasing amounts of PVP were added to hot wort, the yield as well as the tannin content of the dry trub increased to a maximum and then decreased. On the other hand, the protein content steadily decreased to the point that PVP had almost completely replaced protein in the trub formation. At the optimal precipitation level, all of the PVP is removed with the trub. PVP could be added in cellar storage at a level of about 3 lb per 100 bbl (12 g/hl) of beer. This amount varied slightly with the yeast concentration.

McFarlane (40) also recommended treatment of hopped wort with PVP at a level of 1–5 lb per 100 bbl (4–20 g/hl), preferably during kettle boiling or when relatively hot before filtration. The precipitate, removed with the hot break, contained an increased content of chill-haze components, whereas the finished beer had a higher yield of hop-bitter substances.

In 1961, McFarlane and Bayne (43) introduced a water-insoluble polymer of PVP under the name of Agent AT-496, later to become known as Polyclar AT (4) or PVPP, in chillproofing of beer to replace the soluble PVP. Agent AT had all the advantages of water-soluble PVP, including a high selectivity for tannins, and none of the disadvantages associated with the presence of residual soluble PVP in finished beer. It adsorbed selectively the anthocyanogen-protein complexes in beer, and it had at least four times the activity of nylon 66 (33). Agent AT did not adsorb isohumulones even in amounts considerably in excess of those required for complete chillproofing. Beers treated with AT (2–5 lb per 100 bbl or 8–20 g/hl) showed a marked improvement in foam properties when compared with enzyme-treated beers; about 35% of the anthocyanogens was removed. A general improvement in taste, characterized by less astringent afterbitterness and increased isohumulone bitterness, was also observed. With the use of AT, the cold storage period could be shortened and the used agent itself could be restored to its original activity by washing with 1–2% caustic soda. AT-496 beer adsorbate was eluted with formamide or with *N*-methyl-2-pyrrolidone, and the anthocyanogens were chromatographed on a Sephadex G-25 column to yield leucocyanidin, leucodelphinidin and leucopelargonidin.

McFarlane (41) reported that treatment of filtered beer from brewery storage at the filter outlet with a 10% slurry of AT-496 in water removed about 25% of the anthocyanogen content with the precipitate. After the settling of the agent, the beer was filtered, carbonated, and bottled. It was shown that 1–4 lb of AT per 100 bbl effectively stabilized the beer, and its foam properties were superior to those of a papain-chillproofed beer. AT could be regenerated at a lesser cost than nylon 66. The eluate of an AT beer-adsorbate showed similar chemical composition to that of chill haze. The elution of anthocyanogens from the AT beer-adsorbate was done by heating with *N*-methyl-2-pyrrolidone and $FeSO_4$/HCl (2:1) solution (42).

Agent AT was found to be a more efficient and more selective adsorbent for beer anthocyanogens than nylon 66 (44). For determination of beer anthocyanogens, the commercial AT samples were purified by boiling in 10% hydrochloric acid for 10 min and washing free of acid because the impurities, such as the monomer, reduce the yield of the anthocyanidin pigment.

Stone (68) suggested that the molecular weight of PVP, used in treatment of fermented beverages, should be lower than that required for the formation of a precipitate with the tannins present.

McFarlane et al (46) used Polyclar AT chromatography to separate anthocyanogens from catechins. The latter were eluted from the column with 75% aqueous acetone. AT only partly removed catechins from beer. It is of interest that AT was reported to reduce wort color, which could be subsequently restored by adding catechins to wort (12).

McFarlane et al (47) chillproofed beer with mixtures of AT and papain, through which the amount of the enzyme could be reduced. This minimized the detrimental effect of proteolysis on the foam properties of beer.

Chapon and Chemardin (14) defined as tannoids the substances precipitable from beer with PVP under the test conditions. Tannoids are polyphenols that bind to PVP through hydrogen bridges to form insoluble complexes. Consequently, a method for determination of tannoids was based on adding a PVP solution to a tannoid-containing liquid such as beer.

Kringstad and Damm (37) treated beer separately with PVP and with a silica gel, subjecting the adsorbate subsequently to Sephadex G-25 chromatography. Elution of the silica gel beer-adsorbate with 60% formic acid yielded three fractions. The first fraction was haze-active, that is, it caused the development of chill haze from which it had been isolated. Elution of the PVP beer-adsorbate with 1.5*N* sodium hydroxide also yielded three fractions, one of which was particularly haze-active. The enzyme treatment significantly attacked the haze components adsorbed on silica gel, whereas the substances adsorbed on PVP were hardly affected. The former components were also especially rich in nitrogen. This proved that silica gel removed mostly the protein component of the beer haze (the molecular weight was estimated at about 30,000 daltons), while PVP attacked mostly the polyphenol component of the hazes (the molecular weight was estimated at 3,000–4,000 daltons corresponding to polymers consisting of more than 10 tanninogen units).

Steiner and Stocker (66) adsorbed the haze-forming substances from beer on Polyclar AT and eluted them under strict absence of oxygen successively with 50% aqueous dimethylformamide (fraction A), 0.2*N* methanolic potassium hydroxide (fraction B), and 0.2*N* aqueous potassium hydroxide (fraction C). Fraction A (33% of the eluable material) represented primarily inactive haze-forming material containing 32% of the protein and 95% of the anthocyanogens adsorbed on AT. Fraction B (37% of the eluable material) was very haze-active and consisted of a considerable amount of tannins as determined by vanillin and Folin's reagent tests, along with about 5% determinable anthocyanogens and 1.1% protein. Fraction C (30% of the eluable material), which showed a lower haze activity than fraction B, contained only 0.5% protein, traces of anthocyanogens, less vanillin binding, but more Folin's reagent-reactive tannins than fraction B. The composition of the fractions A–C was not altered by the isolation procedure since 97% of the original chill sensitivity of the treated beers was restored by adding back to them the corresponding amounts of the three fractions.

Singleton (64) discussed the adsorptivity of such polyamides as perlon, nylon, and Polyclar AT. He stated that these agents selectively adsorb tannins, leucoanthocyanidins, and other phenols by forming hydrogen bonds between the phenolic hydroxyl group and the amide bond of the agent. Their adsorptive capacity depends on the effective number of potential H-bonding sites per unit weight, while their selectivity is governed by the type, location, and geometry of the bonding site.

Jerumanis (36) reported that the adsorptivity of Polyclar AT for polyphenols decreased in the following order: anthocyanogens, catechins, flavonols, and phenolic acids.

Silbereisen and Kersting (63) evaluated several beer stabilizers, including AT powder, AT-496 paste, perlon, bentonite, silica gel, and proteolytic enzymes. Two procedures were used: a flow procedure with continuous feeding of the agent before the kieselguhr filtration, contact time with beer 1.5–3 min; and a long-contact procedure in which the agent was added during the transfer to another tank and the beer was filtered after a contact time of 11 days. The latter procedure resulted in a better beer stability with all agents except silica gel. For AT, the optimal dosage ran up to a massive 700 g/hl, and the optimal contact time was seven days.

In continuation of their earlier work (66), Steiner and Stocker (67) eluted two fractions from a Polyclar AT beer adsorbate (under exclusion of oxygen): a haze-inactive fraction (eluent 50% aqueous dimethylformamide) and a haze-active fraction (eluent 0.2*N* potassium hydroxide). The former fraction was related to the chill haze caused by aging of beer and was presumably formed through condensation and polymerization reactions of phenolic precursors; the latter fraction was unequivocally related to the chill stability of fresh beer.

The kinetics of tannic acid adsorption by Polyclar AT were found to be between the third and fourth orders in tannic acid, while the rate of tannin adsorption from a muscadine wine by AT was of about the fourth order in tannins (51). Because of a similarity between the two adsorptions, the fourth-order kinetics were taken as evidence for polymer formation before adsorption. Temperature has little effect on the rate of tannin adsorption, as evinced by a very small activation energy (0.7 kcal/mole), but a significant effect on the total amounts of tannins that can be adsorbed. The large ΔH (−12 kcal/mole) is caused by the great effect of temperature on the adsorptivity. A practical consequence of these findings is that the tannin removal (as from beer) can be quite efficient at refrigerator temperatures, as the adsorptive

capacity is gained by carrying the process at low temperatures.

Dadic (16) reported that an excess of Polyclar AT (1 g/L) removed 39.6% anthocyanogens and 52.1% catechins (or a total of 47.7% tanninogens) from beer. Using spectrophotometry, Dadic and Morrison (21) evaluated both AT and nylon 66 as adsorbents in the simultaneous analysis of anthocyanogens and catechins (tanninogens) in wort, beer, and brewing materials. AT yielded lower values because of its selectivity. For analytical purposes, where total tanninogens are determined, nylon 66 is thus recommended.

Dahlstrom and Sfat (25) treated eight commercial U.S. beers with Polyclar AT added in the last week of the ruh storage at a level of 1–6 lb per 100 bbl (4–24 g/hl). Following treatment, the beer was stored at 0°C for various periods of time (ranging from one day to two weeks), filtered, carbonated, bottled, and pasteurized at 60°C for 15 min. Generally, the effect varied from no improvement to significant improvements in colloidal and flavor stabilities, depending on the particular beer and the conditions employed. The results were considered encouraging.

Dadic (17) studied the adsorptivity and selectivity of Polyclar AT and nylon 66 for beer constituents, employing the following techniques: freeze-drying, gravimetry, thin-layer chromatography, ultraviolet and infrared spectrophotometry, as well as the analyses of anthocyanogens and catechins (tanninogens), protein, and bittering substances. The polyamide to beer ratios, resulting in removal of the largest proportions of tanninogens, were established for both adsorbents. The protein contents of the beers treated with AT did not vary significantly compared with those of the control beers. This confirmed the selectivity of the agent for the polyphenol component of the beer hazes. Bitterness was not largely affected even when excessive amounts of AT were used, whereas nylon 66 at similar levels removed almost all bitterness from beer (43).

Dadic and Van Gheluwe (23) treated samples of a Canadian ale with excessive amounts of both Polyclar AT and nylon 66. The treated beers were lyophilized and reconstituted with 5% aqueous ethanol. The colloidal stability was found to be directly proportional with the excessive tanninogen removal, but such beers lacked flavor and were paler than the beer control. The pH of the beer decreased proportionally with the tanninogen removal, while the protein content was reduced to a greater degree by excessive nylon than by excessive AT treatment. This confirmed the higher selectivity of AT vs nylon for the polyphenol constituents of beer.

Weigh et al (71) reported that filtered, matured beer, treated with 50 g AT/hl in plant-scale trials, had a shelf life of about six months and retained normal organoleptic properties. No changes in the volatile constituents were observed, but the total polyphenol contents were reduced. The analysis of individual phenolics (catechins, phenolic acids, flavanols), using gas chromatography of trimethylsilyl derivatives, showed that some compounds were adsorbed on AT and removed while the others remained in beer.

Sfat (60) found that removal of only 10 mg/L of anthocyanogens by PVPP treatment rendered the beer stable with good foam and taste properties. The stabilizing effect was better when the beer was prefiltered with diatomaceous earth. This was attributed to removal of nonturbid proteinaceous material that would compete with PVPP for the haze-controlling anthocyanogens. At the use levels of 3–4 lb of PVPP per 100 bbl (12–16 g/hl), the stabilizing effect increased with longer contact time and with the aged beers.

Dadic (18) employed Polyclar AT, along with two other polyamides (nylon 66 and Woelm 400) for the analysis of anthocyanogens and catechins (tanninogens) in beer, wine, and other substrates. AT was found to be four to five times stronger as a tanninogen adsorbent than the other two polyamides (43). Thus, at the analytical level of 1 g/60 ml beer, AT adsorbed 224.1 mg/L of beer tanninogens; nylon 66 at 6 g/60 ml of beer adsorbed 286.4 mg/L; and polyamide Woelm 400 at 5 g/60 ml of beer adsorbed 287.1 mg/L of tanninogens. The increased AT to beer ratios did not increase the amounts of tanninogens adsorbed from the beer, thus showing again a higher selectivity of AT for beer polyphenols in comparison with other polyamides (17,21,23). However, nylon 66 (or polyamide Woelm 400) should be used when total tanninogens are determined. The fact that much higher amounts of these agents are needed is of no consequence for analytical purposes.

Belleau and Dadic (13) identified phenolic and cinnamic acids present in beer with the aid of liquid chromatography on Polyclar AT, using an aqueous methanolic gradient elution.

Sfat (61) reported results on stabilization of nine U.S. beers with PVPP. Better colloidal stability was achieved when the treatment was done under the following conditions. The beer was prefiltered; contact time with PVPP lasted up to 6 hr; beer was agitated; PVPP was combined with either silica gel or with chillproofing enzymes; the use levels of PVPP were 2–3 lb per 100 bbl (8–12 g/hl) for easily stabilized beers, and 4–6 lb per 100 bbl (16–24 g/hl) for inherently unstable beers. PVPP treatment was not observed to have an adverse effect on beer quality.

Narziss and Bellmer (52) found that the use of PVPP at 10, 20, 40, and 80 g/hl of beer reduced the anthocyanogen and catechin contents and increased the polymerization index (PI = total polyphenols/anthocyanogens). Additionally, the residual polyphenols showed a stronger reducing power and a darker color. When a beer, forced at 50°C for five days, was treated with PVPP, an increased color because of polyphenols and a higher PI resulted.

Hodenberg and Sulke (34) suggested that PVPP should be added to beer after kieselguhr filtration and removed through a chamber filter with horizontal elements from which the agent could be regenerated. They listed a number of ways in which a beer could be stabilized with PVPP: the agent can be added to beer in the storage tank, or to the kieselguhr filter, or to the filtered beer in a buffered tank followed by a second filtration, or by using filter sheets impregnated with the agent. In the experiments, the automatic monitoring of the beer polyphenol content, or the cinchonine sulfate test, were used to control the stabilization process. The use of PVPP did not affect flavor, pH, foam stability, bitterness, or the coagulable nitrogen content of beer. The contents of anthocyanogens and total polyphenols decreased in PVPP-treated beers, resulting in a lighter color as compared with the beer controls. The authors concluded that beer stabilization with the aid of PVPP was less expensive than the treatments with silica gel or bentonite.

Sommer and Metscher (65) reported that Polyclar AT was generally superior to silica gel in stabilizing beer. Thus, 50 g AT per hectoliter gave a beer with better stability than did 150 g silica gel per hectoliter of beer. The optimum contact time for AT was set at 5–10 min. The presence of yeast did not appear to affect the results significantly. Head retention was normal, while the color of beer was significantly reduced. At 50 g AT per hectoliter of beer, the total polyphenols were reduced by 35–45% while, at 20 g AT per hectoliter this reduction amounted to 15–25%. Silica gel at 150 g/hl removed about 33% of the total polyphenol content of beer. AT and silica gel in combination were not effective in this case. The efficiency of AT also depended on beer turbidity.

Donhauser et al (26) monitored the effect of various beer stabilizers (PVPP, bentonite, hydrogel, Xerogel, and enzymes), using immunoelectrophoresis as well as more conventional analytical methods. PVPP was added to a pale beer at rates of 20 and 50 g/hl, while the other agents were employed generally at 70 and 140 g/hl. PVPP and enzymes gave the best stabilization effect as measured by a forcing test at 0/60/0°C. The use of PVPP also resulted in the best foam stability.

Van Gheluwe et al (69) studied the adsorptivity of Polyclar AT and nylon 66 for wort tanninogens at room temperature as well as at 80°C. Nylon 66 at 6 g/L adsorbed 100% tanninogens from wort at room temperature, but was somewhat less effective at 80°C. AT at 1 g/L adsorbed less tanninogens than nylon 66 at either temperature (the adsorptivity at 80°C was slightly lower than at room temperature). Thus, AT was again shown to be a more selective agent than nylon 66 (17,21,23,43).

Weigh (70) studied the effect of AT on beer flavor under rather

extreme conditions. Beers treated with 10 or 20 g AT per hectoliter and kept at 70°C for 1 hr, followed by a five-day storage at 50°C, only showed a stronger bitterness compared with the beer controls. Treatment with 100 g AT per hectoliter led to a slightly "impure" flavor, particularly when beer was stored at 20°C in the presence of air. Treatment with AT at 20°C yielded more stable beers with a slightly less "pure" flavor and a stronger bitterness in comparison with the treatment at 1°C.

Raible (57) noted that AT could be effectively combined with silica gel, particularly when silica gel is used before and AT after the filtration. This effect is similar to when the beer is prefiltered with diatomaceous earth (60), except that silica gel removes the proteinaceous material much more strongly than does kieselguhr.

Schaft et al (59) described an in-line beer-stabilization process with continuous body-feed use and employing 50 g PVPP per hectoliter with recycling of the agent. Shelf life of nine months to one year, and even longer, was reported. The system used at Lowenbrau brewery in Munich was described. It had been using an automated beer filter and a stabilizing station (350 hl/hr capacity) since early 1977. (The use of PVPP in brewing has been allowed in Germany since December 4, 1972.) A large PVPP dosing unit and a kieselguhr horizontal filter are inserted between the diatomaceous earth filter and the sheet filter. The agent is dosed into the prefiltered beer and collected on the horizontal filter. The kieselguhr horizontal filter was introduced to eliminate contact between water and beer, thus preventing oxygen from entering the stabilized beer. Additionally, no haze precursors will enter the stabilized beer from the filter bed during the filter discharge. Silica gel can be dosed with PVPP, either continuously or intermittently, if a greater reduction in protein content is required. This is particularly the case with beers of a high protein content. It was shown experimentally that PVPP and silica gel (Lucilite), used in combination, do not interfere with each other's action. This was very clearly illustrated by the analyses of total polyphenols (TP) and anthocyanogens (AV). These analyses are used to monitor the efficiency of PVPP treatment. For example: for 50 g PVPP per hectoliter of beer, TP 153 mg/L, AV 22 mg/L; for 50 g Lucilite per hectoliter of beer, TP 233 mg/L, AV 47 mg/L; for 50 g PVPP + 50 g Lucilite per hectoliter of beer, TP 151 mg/L, AV 23.4 mg/L; control (untreated beer), TP 240 mg/L, AV 48.8 mg/L. Lucilite, a silica gel, did not affect the polyphenol content of beer to any noticeable extent, whereas the combination of PVPP and Lucilite had the same effect on these substances as the PVPP alone. Obviously, Lucilite affected the protein component of the beer haze, whereas PVPP was selective for beer polyphenols. With this process it is basically unimportant which diatomaceous earth system is used upstream of the stabilizer, and the biological downstream treatment of the beer can be performed either by cold disinfection (sheet filter) or by pasteurization. After the treatment, the beer is purged from the filter containing the PVPP, which is then regenerated with a caustic soda solution. The regenerated agent is washed with fresh water, neutralized, and the water is removed (a fully automated control CIP system is used) before being returned to the dosing tank. A long contact time with beer is not required, as more than 80% of the PVPP adsorptive capacity is operative within as short a time as 2 min. However, this requires certain practical conditons, specifically, an intensive agitation; recycling in a laminar flow; a proper throughput rate (surface loading); and proper sedimentation of the agent. All these conditions were reported to exist in the described case, so the initial PVPP coating could be run through more than 1,000 regenerations with a consistently good beer stabilization.

Grosswiler and Meier (30) further studied the in-line use of PVPP with recycling and recommended it both for its stabilizing effect on beer as well as for the economy. PVPP at a rate of 30 g/hl yielded a beer stable for 22 days in a 40/0°C forcing test. Their equipment (all stainless steel) consisted of two separator centrifuges receiving the beer from the conditioning tanks, the stabilizing filter of a capacity of 150 hl of beer per week, a rest beer tank, and clear beer storage vessels. The treatment process starts by flushing the stabilizing filter, containing regenerated PVPP, with carbon dioxide. The filter is then filled with centrifuged beer for a treatment period of approximately 20 min, after which the beer is passed through the sheet filter and into the storage tanks. The PVPP is pumped into the holding tank with warm water (50°C) and is subsequently treated with hot, 1.5% aqueous sodium hydroxide solutions followed by a 2% aqueous sodium hydroxide solution. The process described (storage cellar to separator to PVPP stabilization to sheet filtration) could be varied in the following ways: (a) storage to sheet filtration; or (b) storage cellar to separator to PVPP stabilization to coarse perlite addition to sheet filtration. Variation "b" proved very suitable because perlite also removes some polyphenols, which results in a lesser load on filtration sheets than is the case in the original procedure or its variation "a".

The state of the art and the scientific basis for beer stabilization, using recycled PVPP, was reviewed by Hums (35). The use levels of 30–50 g of PVPP per hectoliter of beer with a contact time of about 5 min were recommended on the basis of polyphenol analyses. With the adopted system, the total polyphenols should be reduced by 40–50% and anthocyanogens by 60–70% for optimal beer stabilization. The use of PVPP in a brewery should be monitored by polyphenol analyses. Other beer quality variables to be checked are the colloidal stability (0/40°C and/or 0/60°C forcing tests), color (between 0.3 and 1.0 EBC units for light beers), and flavor. A combined treatment with 25–30 g of PVPP and about 50 g of hydrogel (a silica gel) per hectoliter of beer was also recommended.

Narziss and Gromus (53) reported the use of PVPP, alone or in combination with either silica gel or bentonite, to stabilize beer rich in polyphenols. All those agents attacked beer polyphenols and improved stability of the beverage. Silica gel removed predominantly high molecular weight polyphenols, whereas bentonite removed those of middle molecular weights. Thus, bentonite and silica gel may affect beer polyphenols in addition to beer proteinaceous material (59). PVPP also improved the heat stability of beer by removing the heat-sensitive polyphenols.

Narziss et al (54) reported that removal of polyphenols by PVPP was logarithmic: with high amounts of the agent, the removal rate drops and the adsorptivity curve flattens. This confirmed an earlier finding by Dadic (17). The use of PVPP at a level of 50 g/hl did not affect beer taste, but raising the level to 50–80 g/hl resulted in a slight decrease in fullness and overall flavor rating with the flavor stability remaining unchanged. In combined treatment, the following dosages were recommended (per hectoliter of beer): 20 g PVPP + 50 g hydrogel; 30 g PVPP + 50 g hydrogel; 20 g PVPP + 20 g Xerogel + 50 g hydrogel. The degree of stability improvement as a result of PVPP treatment was higher with beers rich in polyphenol than with beers poor in polyphenol. Anthocyanogen contents decreased by about 35% and total polyphenols by about 25% in PVPP-treated beers.

Meier (50) discussed the process-engineering aspects of beer stabilization with PVPP. The following problems in PVPP recycling should be considered: swelling, grain size distribution, precoating capacity, compressibility of PVPP cake, selection of a suitable filter element, and turbidity. The horizontal vs vertical (candle-type) filter was also discussed along with the cost of beer stabilization (amortization, regeneration, and PVPP losses).

REGULATIONS PERTAINING TO USE OF POLYCLAR AT (PVPP) IN BREWING

A maximum of 50 ppm soluble polyvinylpyrrolidone (PVP) in Polyclar AT (polyvinylpolypyrrolidone, PVPP) is allowed by the U.S. Food and Drug Administration (2,9). The finished cross-linked polymer, when refluxed 3 hr with water, 5% acetic acid, and 50% alcohol, gives no more than 50 ppm extractables with each solvent. It may be used under the U.S. Federal Food, Drug, and Cosmetics Act as a clarifying agent in beverages and vinegar,

followed by removal and filtration.

Regulations for the residual amount of soluble PVP in beer in the United States and in Canada differ. The F.D.A. allows a maximum of 10 ppm soluble residual PVP in beer (11), while the amount in Canada is restricted to only 2 ppm (10). In Germany, the use of PVPP in brewing has been allowed since December 4, 1972 (59), provided the following conditions are met: 1) PVPP is manufactured by polymerization of vinylpyrrolidone; 2) treating of 1.0 g of PVPP with 500 ml of a solution of 3% acetic acid, ethanol, and picoline (95:5:0.24) for 15 hr at room temperature does not yield more than 15 mg of solubles; 3) the soluble portion does not leave more than 5% ash residue; 4) a maximum of 50 g PVPP per hectoliter of beer is used.

ANALYTICAL METHODS FOR PVP IN POLYCLAR AT (PVPP) AND IN BEER—A CRITICAL EVALUATION

Food and Drug Administration regulations specify a maximum of 50 ppm soluble material in Polyclar AT (PVPP) stabilizer (2,9). The method for determination of soluble PVP in AT (9) consists of digesting the material with distilled water for 3 hr at 90–100°C, followed by cooling the suspension to room temperature and centrifugation at 2,500 rpm for 1 hr. After slurrying the supernatant liquid with filter aids, 71% perchloric acid is added, and the haze reading is done against a blank containing 50 ppm of PVP K-30. The haze reading of the sample should be below the haze reading of the blank.

McFarlane et al (45) discussed the existing methods for PVP determination, which included precipitation of PVP with trichloroacetic acid and measuring the nitrogen content of the precipitate (58), a reaction with Vital Red dye (15) and intensification of the iodine color by PVP (38). As they pointed out, the application of these methods to beer and wort analyses suffers from interference by protein and color, thus requiring a control sample (untreated with PVP), which may not be available. These methods also lack sensitivity, which the authors (45) proposed to enhance by separating PVP from proteins. Consequently, they suggested chromatography, the colorimetric method, or the nephelometric method.

Chromatography of the sample (such as beer) on a silica gel column, reacts the PVP with Vital Red dye, eluting the colored complex with *N*-methyl-2-pyrrolidone and determines the PVP concentration from a calibration curve (absorbance at 515 nm). This method is the most sensitive of the three and is applicable only to PVP K-90 type.

The colorimetric method, which is a modification of the Vital Red method of Chinard (15), suffers from protein and color interference.

The nephelometric method is based on measuring turbidity formed on tricholoroacetic acid addition when PVP is present, according to Reppe (58). It is simple and convenient but relatively insensitive with low molecular weight PVP such as K-30.

Shiraef (62) determined soluble PVP that remains in vinegar after treatment with Polyclar L (PVP K-30), or in beer after treatment with Polyclar H (PVP K-90). This method is based on precipitation of PVP with 18% aqueous perchloric acid. The clouding is proportional to PVP concentration and can be measured by spectrophotometry (absorbance at 500 nm read against a diluted beer blank), or by nephelometry. A calibration curve is prepared in water with amounts of PVP K-90 ranging from 0-20 mg/L. This method was later modified (8) to increase its sensitivity; thus the spectrophotometric readings were taken in a 5-cm cell (instead of the original 1-cm cell) against beer blank.

Postel (56) modified the chromatographic method of McFarlane et al (45). In this procedure, the beer is passed through a column containing coarse and fine silica gel layers. A solution of Vital Red dye is then applied: if soluble residual PVP is present, a red ring is formed on top of the column on the surface of the fine silica gel layer. For quantitative determination, the red zone is extruded from the column after washing with a borax solution, and the PVP-Vital Red complex is eluted with dimethyl formamide (*N*-methyl-2-pyrrolidone) (45). The absorbance is read at 530 nm (515 nm, in [45]). A calibration curve is made with aqueous solutions of PVP K-30 (PVP K-90 in [45]) ranging from 0 to 10 mg/L. The estimation limit was reported at 0.6 mg/L PVP and the detection limit even lower. Depending on the PVP concentration, the coefficient of variance varied between ±6 and ±15%. Beer constituents reportedly did not interfere with the analysis. In recovery experiments with regular light beers, however, an amount of approximately 0.8 mg/L of added PVP was reported bound to certain phenolics. Drawert et al (27) reported soluble residual PVP in some beers at levels below 0.5 mg/L, which is far below the allowed limit.

Frauenfelder (28) described another modification of the method of McFarlane et al (45) while still retaining the formation of the PVP-Vital Red complex of Chinard (15). This procedure was applied to the analysis of PVP in beer as well as in other beverages, foods, laundry products, and cosmetics. The soluble PVP from beer (and other samples) is adsorbed on a column containing a particular grade of silica gel and is then reacted with a solution of Vital Red dye. The colored complex is then eluted with *N*-methyl-2-pyrrolidone, and the absorption is read at 525 nm. Calibration standards are made with PVP K-90 (Polyclar H) for beer and with Polyclar L (PVP K-30) for other substrates.

We evaluated experimentally all the described methods. The method for determination of soluble PVP in AT (9) was found satisfactory. To determine soluble residual PVP in beer, we were looking for a method that is precise and accurate and also sensitive below the 2 ppm level to satisfy the Canadian regulations (10).

We found that the colorimetric and nephelometric methods of McFarlane et al (45) as well as the method of Shiraeff (62) and its modification (8) were too insensitive. The chromatographic method of McFarlane et al (45) and its modifications by Postel (56) and Frauenfelder (28) were much more sensitive but suffered from a lack of reproducibility. Thus, as in Postel's method (56), the borax washing of the colored complex is inadequate, and it is very difficult to determine how much of the red zone (due to the PVP-Vital Red complex) is to be removed, or how much eluent to use. Consequently, we developed a somewhat modified chromatographic procedure whose evaluation is in progress.

SUMMARY

Beer shelf life is affected primarily by polyphenol-protein interactions. Colloidal and flavor stabilities of beer are interdependent.

Beer can be stabilized by measures taken before, during, or after the brewing process, or by a combination thereof. Measures taken during the brewing process include the use of antioxidants as well as accelerating or delaying the haze formation. Haze formation may be delayed by reducing the concentration of protein (chillproofing with proteolytic enzymes or silicates) or polyphenols (polyamide adsorbents such as Polyclar AT or nylon 66).

The use of PVP and PVPP in brewing is based on the ability to bind polyphenols (tannins) through hydrogen-bond formation. The use of soluble PVP to stabilize and clarify beer was suggested by McFarlane in 1954. The use of insoluble Polyclar AT (PVPP) as a processing agent in brewing was introduced by McFarlane and Bayne in 1961. Suggested use levels of PVPP range between 1 and 5 lb per 100 bbl (4–20 g/hl) of beer with a contact time of 24 hr or less. The agent can be added before or after the secondary storage.

Recently a continuous in-line addition of Polyclar AT (PVPP) with recycling was introduced. In combined beer treatment, silica gel should be added before PVPP.

Analytical methods for determination of soluble PVP in AT (PVPP) as well as the residual soluble PVP in beer have been experimentally tested and evaluated.

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[Received April 27, 1983]