

CHAPTER 5: DISCUSSION

Traditionally, brown bread is perceived to be of poorer quality than white bread. The two main contributors to bread quality are volume and crumb structure (Stauffer, 1998). Most consumers want a bread with a high loaf volume and a crumb structure which is uniform and fine (Van Vliet, 1999). These two quality parameters differ significantly between white and brown breads. Research has shown, that brown and wholemeal breads, containing non-endosperm components such as bran and/or germ, have significant lower loaf volumes when compared to white bread (Pomeranz et al, 1976; Pomeranz, 1977; Rogers & Hosenev, 1982; Moder et al, 1984; Barnes & Lowy, 1986; Bloksma & Bushuk, 1988; Galliard & Gallagher, 1988; Tait & Galliard, 1988; Lai et al, 1989a, 1989b, 1989c; Gan et al, 1989; Gan et al, 1992; and Nelles et al, 1998). In most countries, bran is not separated from the white flour, and brown or wholemeal breads are baked from flours of different extraction rates (Kent & Evers, 1994). Wholemeal flour in the U.K, for example, contains all the products of milled wheat, including bran and germ (100% extraction rate). Brown flours can be of extraction rates between 85% and 98%. In South Africa, brown bread is manufactured by adding bran components back to white flour, after which the dough is mechanically developed through high energy mixing. Despite these differences in processing, similar results were found in this study as were found before. The addition of bran to white flour, caused volumes of loaves baked from the resulting flour to be depressed, when compared to a white bread control (Tables 21 and 22; Fig. 20). A higher level of addition caused lower loaf volumes. This depression in loaf volume can not be explained solely in terms of the bran diluting the gluten proteins (Pomeranz, 1977).

In addition, added bran caused crumb structure to be coarse and open. This contrasted markedly with the uniform crumb of white bread, as was evident in the crumb structures during proofing (Fig. 34 to 37), as well as after baking (Fig. 30 to 33). Pomeranz (1977) and Gan et al (1989) found similar results. It therefore appears that bread quality, as measured by loaf volume and crumb structure, is seriously impaired by addition of bran. Ultimate bread quality depends on optimum properties in the dough matrix. Stauffer (1998) defined two characteristics of a "good" dough:

1. The ability to retain gas generated during fermentation in the form of numerous small gas cells;
2. A proper balance of viscous flow and elastic strength so that the loaf can expand adequately during proofing and the early stages of baking, yet retain its rounded form.

Gluten is the main component of dough that determines how well these requirements are met. Bran components appear to affect gluten functionality by changing its physicochemical characteristics. This hampers proper gluten formation, influences gas retention and eventually causes lower loaf volumes and poor crumb structures. The mechanism by which bran affects gluten functionality and influences bread quality can be explained by a subtle interplay of chemical and physical effects.

5.1 CHEMICAL EFFECTS OF BRAN ON BREAD QUALITY

Bran was subjected to a heat treatment in order to inactivate heat-sensitive components which could have had a detrimental effect on loaf volume. In previous studies (Galliard, 1986b; Nelles et al, 1998), this inactivation was brought about by a wet heat treatment. In this study, however, it was decided to use a dry heat treatment, the brans being autoclaved in air-tight containers. This particular treatment was chosen to ensure that the alteration in size and other physical properties of the bran particles would be minimised, as could not have been the case with a wet heat treatment. It was important not to change the physical properties of the brans, since it was attempted to separate chemical and physical effects. This part of the study concentrated on chemical effects, therefore all other parameters, including physical effects had to be kept constant. Results proved that apart from the colour, physical properties were not significantly altered (Tables 16 and 17). Moisture content was lowered only slightly, and particle size was not changed. Any changes in baking performance of the heat-treated brans could therefore only be attributed to chemical changes.

Dry heat treatment of different brans had at least two effects on chemical substances: It practically inactivated lipase activity in all bran types and brans from different sources (Tables 18 and 19) and significantly reduced TRS (Table 20), such as glutathione, possibly by oxidation. Nelles et al (1998) found similar effects on lipase and

lipoxygenase with a wet heat treatment, and on TRS with a wet oxidation method. Breads baked with heat-treated bran produced higher loaf volumes when compared to bread baked with the untreated bran (Tables 21 and 22; Fig. 22-29). This led to the conclusion that lipase and glutathione could at least in part be responsible for the reduction in loaf volume caused by bran addition. To develop a hypothesis concerning the mechanisms by which these substances cause loaf volume depression, it is necessary to look at what happens during dough formation.

The first basic step in breadmaking is combining water with wheat flour and imparting mechanical energy to the mixture to form an elastic dough. Wheat storage protein (gluten) possesses the unique ability to form a viscoelastic dough when wetted and kneaded. The strong, cohesive dough that is formed, is able to retain gas and a light, baked product is produced (Hoseney, 1994). Gluten is composed of two main groups of proteins: glutenin and gliadin. Glutenin consists of high molecular weight proteins in which individual polypeptide chains are cross-linked by the disulphide bonds of the amino acid cysteine. Gliadins are composed of lower molecular weight proteins in which the cysteine crosslinks are intra- rather than intermolecular, as shown in Figure 54 (Williams & Pullen, 1998). Although this is a very simplistic model for glutenin and gliadin structure, it serves to illustrate the point.

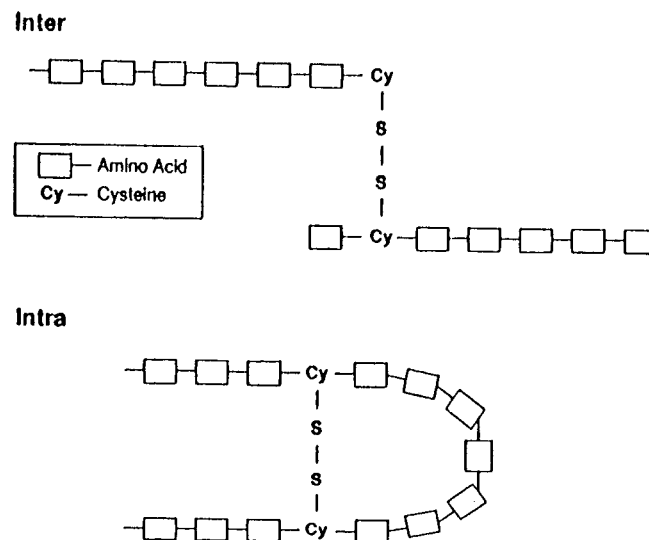


Fig. 54: Representation of cysteine crosslinks in gluten (Williams & Pullen, 1998)

Two groups of glutenin subunits have been identified: High molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS) (Stauffer, 1998). The molar ratio of LMW-GS to HMW-GS is 2:1 or higher; the amounts of the two kinds of subunits are roughly equal on a weight basis. One possible model explaining the elasticity of glutenin is shown in Figure 55.

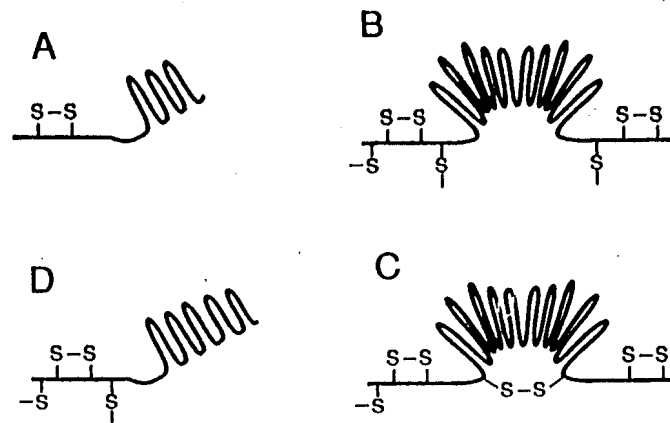


Fig. 55: Schematic depiction of gluten proteins. A) Gliadin, B) HMW-GS, showing possible action of molecular spring, C) HMW-GS, showing SS bond preventing extension of the spirals, D) LMW -GS (Stauffer, 1998)

The molecular architecture of glutenin subunits is unusual, as reviewed by Stauffer (1998). In HMS-GS, cysteine is concentrated in the regions near each end of the chain, with a long stretch of other amino acids between these two ends. LMW-GS have a similar concentration of cysteine residue, but at only one end of the chain (Fig. 55D). Thus, both ends of the HMW protein can enter into polymerisation reactions, while only one end of the LMW protein can react this way. The interior regions of both species, but the HMW-GS in particular, are postulated to form β -turn spirals, which in turn can fold into a helical sheet structure that can possibly be linked to a coil spring (Fig. 55B) and account for the elastic nature of glutenin. An intramolecular disulphide bond can retain this spring (Fig. 55C); this bond can be broken during mixing to develop the gluten structure. In fact, it seems clear that mixing breaks the HMW-GS into smaller units, which then reform to some extent. Graveland, Bosveld & Lichtendonk (1985) found that during short high-energy mixing the amount of LMW glutenin increased sharply, but decreased again when the dough was allowed to rest. The points of scission are

thought to be at the disulphide bonds, forming radicals ($SS \rightarrow 2S^*$). As disulphide bonds are broken, they reform between adjacent molecules that have been aligned along the lines of stress in the dough ($2S^* \rightarrow SS$) (Stauffer, 1998).

In addition to the crosslinked cysteine present in wheat protein, numerous low molecular weight SH compounds (reduced form) are present in flour which can cause SS/SH interchanges (Schofield & Chen, 1995). This exchange (Fig. 56) occurs at a rapid rate during mechanical dough development and subsequent resting, and is generally considered to influence dough rheological properties and breadmaking performance (Williams & Pullen, 1998). The radicals, can also react with these SH compounds in the following way: $S^* + SH \rightarrow SS + H^*$ (Stauffer, 1998).

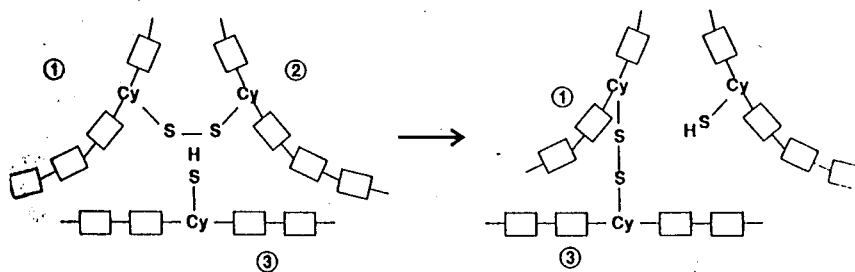


Fig. 56: Representation of SS/SH interchange (Williams & Pullen, 1998)

One of the substances present in flour, which could affect these redox reactions in glutenin, is glutathione (GSH) (Schofield and Chen, 1995). Substantial amounts of glutathione are present in the germ fraction of the wheat kernel (Bloksma & Bushuk, 1988). Even oxidised glutathione has been reported to affect dough in the same way as does the reduced form, although to a lesser extent (Ziegler, 1940). In the series of interchange reactions, rheologically ineffective mixed disulphides are formed at the expense of effective cross-links between protein chains. This could lead to a weak dough and poor baking quality. This is consistent with the work of Nelles et al (1998) whose results showed that a wet oxidation treatment of bran significantly reduced TRS, indicating oxidation of glutathione, and resulted in increased loaf volumes. Lai et al (1989a) also found that oxidising glutathione in whole wheat bread produced loaf volumes equal to that of a white control.

The presence (or absence) of oxygen in a dough system greatly influences SS/S_H interchanges and dough development. The CBP is the breadmaking system with the greatest need for oxidation (Williams & Pullen, 1998), since dough development occurs mainly during the mixing process and the rest stage thereafter. Oxidising agents, which are added to improve baking properties, are essential in the CBP. Even more so with the production of brown bread, where oxidants are added to help the gluten carry the bran (D.G. Carroll – Personal communication). The positive effects of, for example, ascorbic acid in mechanically developed doughs were described by Williams & Pullen (1998):

Oxidation of S_H groups to remove them from the system. This would benefit the dough structure by preventing them from preferentially reacting with the S_H group of the glutenin molecules exposed during the development period.

Causing a SS bond to be formed between two of the glutenin S_H groups exposed during the developing period. This would increase the elasticity of the dough structure.

Chemically, ascorbic acid is a reducing agent and can only function as an oxidising agent in a dough after it has been itself oxidised to dehydroascorbic acid, and to function efficiently requires the availability of atmospheric oxygen (Kent & Evers, 1994). The presence of oxygen in a developing dough, therefore, plays an important role in determining baking performance and ultimately loaf volume, because of its influence on ascorbic acid and gluten development.

It was found that at low levels of addition, Select and Digestive bran behaved the complete opposite to what was expected. At a 9% level of addition neither of the two brans depressed loaf volume. In fact, Digestive bran produced loaf volumes that were even somewhat larger than the white bread controls (Table 22; Fig. 20). This phenomenon could probably be explained by the large flaky shapes of the Digestive bran particles as could be observed by scanning electron microscopy (Fig. 19). It is possible that the flaky shapes of the bran particles were responsible for trapping or encapsulating air and oxygen and therefore providing extra gas cell nuclei in addition to

those formed during the mixing process. These nuclei would eventually expand because of CO₂ being produced by yeast cells during fermentation, leading to higher loaf volumes. The extra oxygen trapped by the Digestive bran could also have resulted in increasing the effective operation of the ascorbic acid and increasing gluten development by oxidation, which led to increased loaf volume.

It has been shown that wholemeal flour deteriorates rapidly on storage compared with white flour (Barnes & Lowy, 1986). Galliard (1986a) found that oxygen consumption values of aqueous suspensions of wheat wholemeal were substantially higher than those of white flours. The oxygen uptake was due primarily to the oxidation of unesterified, polyunsaturated fatty acids. In a subsequent study, Galliard (1986b) showed that the rate of oxygen uptake by aqueous suspensions of wholemeal flour increased linearly with increases in unesterified fatty acids. Warwick et al (1979) also found an increase in free fatty acids during storage of flour, with an accompanying loss in baking performance. Bell et al (1979) in addition showed that wheat fatty acids reduced loaf volumes. Galliard (1986a) provided evidence that the increase in unesterified fatty acids and oxygen consumption in wholemeal flour, as compared with white flour, is due to the presence of lipid metabolising enzymes present in the germ and bran fractions. The increased oxygen demand of wholemeal flour is due to higher levels of polyunsaturated free fatty acids (PUFFA) released by hydrolysis of triglycerides, catalysed by lipase. Lipase is a membrane-bound enzyme (O'Connor & Harwood, 1992) and 75-80% of its activity is located in the bran fraction (O'Connor et al, 1992). The polyunsaturated fatty acids are subsequently oxidised, catalysed by lipoxygenase that is concentrated in the germ fraction (Galliard 1986a). Proof of the lipase/lipoxygenase system being responsible for higher oxygen demands and accompanying poor baking quality of wholemeal flour was strengthened when Galliard (1986a) found that oxygen consumption of mixtures of bran and germ increased on storage more rapidly than that of bran or germ stored separately. Similar results were found by Barnes and Lowy (1986).

The principles of all of these findings were confirmed by this study. Brown bread was produced by addition of bran to white flour. This introduced a significant amount of lipase to the dough. Although the germ was removed during the milling process, it is possible that the flour, or even the bran could have been contaminated with germ



fractions, which in turn was responsible for the presence of lipoxygenase. Heat treatment of the bran practically inactivated lipase activity (and probably lipoxygenase as well) (Table 19). The breads baked with heat-treated brans, had higher loaf volumes than those baked with the untreated brans (Fig. 25 to 27) showing that the lipase/lipoxygenase system was partly responsible for the reduction in loaf volumes. This is consistent with work of Nelles et al (1998) who found that a wet heat treatment inactivated lipase and lipoxygenase and resulted in production of higher loaf volumes. Tait & Galliard (1988) also found that high lipase activity in wholemeal flour was associated with poor baking performance, while Lai et al (1989a) showed that lipoxygenase had a detrimental effect on loaf volume. The dry heat treatment used in this study also significantly reduced the level of oxidising activity (TRS) in the brans (Table 20), which produced higher loaf volumes, indicating that glutathione was probably another chemical constituent responsible for loaf volume depression brought about by bran addition. A summary of the proposed chemical reactions involved in loaf volume depression brought about by bran addition is shown in Figure 57.

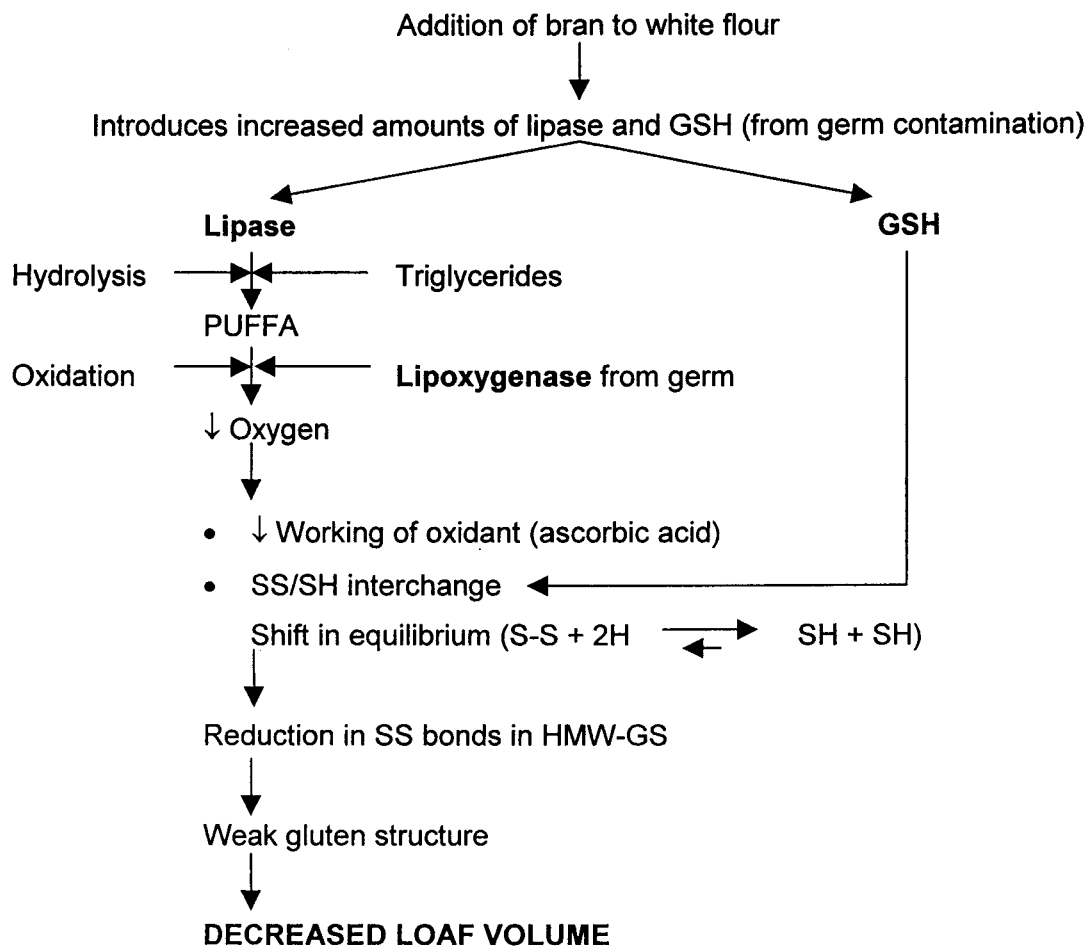


Fig. 57: Summary of proposed chemical effects of bran on loaf volume

Not all bran types depressed loaf volumes equally. Pollard produced smallest loaf volumes and Digestive bran the highest. During the milling process, Pollard is finely ground and contaminated by endosperm and germ (N. Dumas – Personal communication). Apart from having a smaller particle size (Fig. 17; Table 14), Pollard therefore also had a higher relative lipid content than the other brans (Table 12), with a consequently higher lipoxygenase concentration (Tait & Galliard, 1988). It is possible that the milling process was responsible for giving a higher *in vivo* lipase activity, because of more lipid substrate being made available, which caused more chemical deterioration in the resulting Pollard dough and lower loaf volumes. This explanation is consistent with work of Galliard (1986b) and Galliard & Gallagher (1988) who showed

that fine milling of bran and germ mixtures increased rate of deterioration (increased fatty acids and oxygen consumption) and baking performance. It seems that chemical composition of the bran play an important role in determining to which extent loaf volumes will be depressed by their addition. Digestive bran, for example, had the lowest fat content of all the brans (Table 12) and was the least contaminated with germ. Digestive bran, which constitutes the outer most layers of the bran, had the highest lipase activity before heat treatment (Table 19), which is in agreement with O'Connor et al (1992) who found that 75-80% of the lipase activity is located in the bran component of the kernel. However, because the Digestive bran had the lowest fat content and therefore lower lipoyxygenase concentrations than Pollard, it is possible that the *in vivo* lipase/lipoyxygenase system operated at a lower rate than in the Pollard, partly explaining the higher loaf volumes of breads with Digestive bran. These higher loaf volumes could however also be explained by the air/oxygen trapping mechanism described earlier. Pollard did not have the large, flaky shapes of the Digestive bran particles (Fig. 19) and therefore could not trap oxygen which could have improved baking performance, as could have been the case with Select and Digestive bran.

A difference in chemical composition could also be the reason why brans from different origins caused different loaf volume depressions, and why heat treatment affected loaf volume improvement differently (Table 21). Finney, Henry & Jeffers (1985) also reported that the effect of bran on loaf volume varies with the source of the bran. According to Oakenfull & Topping (1987), chemical composition of the bran could depend on the type of wheat milled, the mill stream from which the bran has been taken, as well as milling practices. Since all the wheat samples from different sources were milled on the same mill, and the bran was separated similarly, the only variable in this instance was the lipase activity of the different brans (Table 18), causing variations in loaf volume depression.

Heat treatment had the greatest effect on decreasing loaf volume depression in breads baked with Pollard and the least effect on breads baked with Digestive bran (Fig. 22 to 27). This indicated that the difference between loaf volumes of bread baked with heat-treated and untreated brans gets smaller as the bran particle size gets bigger, suggesting a greater chemical effect in brans with smaller particle size. These findings led to the investigation of the effect of bran size reduction and heat treatment on loaf

volume. When Pollard, Select and Digestive bran were milled, significant smaller loaf sizes were obtained (Tables 23 and 24). Overall, similar loaf sizes were obtained in breads produced with bran which was heat-treated and then milled, and breads produced with bran that had only be heat-treated. Therefore, it appears that thermal treatment of the bran counteracts its adverse chemical effect.

It can be concluded that the heat treatment probably inactivated all heat-sensitive components that might have had a chemical effect on baking performance. The improvement in loaf volume brought about by heat-treatment of the brans shows clearly that the heat-labile chemical substances in the brans were in part responsible for the depression in loaf volume. However, heat-treatment did not result in all the brans producing loaves of the same volume and height (Fig. 28). There were still some significant differences in loaf volumes between the different bran types after heat treatment, indicating that the depression in loaf volume brought about by bran is due to a physical effect of the bran as well as a chemical effect. This is in agreement with the work of Galliard & Gallagher (1988) who found that bran physically influenced dough properties and consequently caused a reduction in loaf volume.

5.2 PHYSICAL EFFECTS OF BRAN ON BREAD QUALITY

The first physical effect that bran has on dough properties, is increased water absorption. Pomeranz et al (1976), Pomeranz et al (1977) and Lai at al (1989b) showed that water absorption values increased in doughs with bran, as compared to a white dough, accompanied by increased mixing times (Pomeranz et al, 1976; Pomeranz et al, 1977; Cheetham, 1997). Optimum water absorption is important since it could determine dough handling, proofing and baking properties (loaf volume) and finished product characteristics (appearance and eating quality) (Stauffer, 1998). Nelles et al (1998), for example, found that hydrating bran before mixing and baking increased water absorption and loaf volumes significantly.

Four flour components absorb water: Protein, native starch, damaged starch and pentosans (Stauffer, 1998). The relative absorptions are given in Table 26.

Table 26: Influence of flour components on water absorption (Stauffer, 1998)

Component	Water per g component (g)	Amount per 100 g flour (g)	Absorption per 100 g flour
Protein	1.3	12	15.6
Intact starch	0.4	57	22.8
Damaged starch	2.0	8	16.0
Pentosans	7.0	2	14.0

Although pentosans are present at levels of only 2-3% in white flour, they can absorb seven times their own weight in water and therefore make a significant contribution to overall water absorption in the dough (Stauffer, 1998). About 65% of wheat flour pentosans are water-insoluble; these are almost exclusively xylans (Michniewicz, Biliaderis & Bushuk, 1990). The water-soluble pentosans are approximately half arabinoxylans and half arabinogalactans. The water-soluble pentosans have been shown to be important in producing optimum loaf volume (Hoseney, 1994). Bran contains 32-37% non-starch polysaccharides (NSPS) (Theander et al, 1993). Approximately 80% of the sugars present in NSPS are the pentoses xylose and arabinose (Stauffer, 1998). Addition of bran to white flour therefore substantially increases the water absorption of the resulting flour. According to Catteral (1998), the pentosan content in wholemeal flour can be up to 10% and can account for absorbing up to one-third of the water in the dough. This additional weight of the absorbed water could have been physically too great for the dough to handle, causing the observed lower loaf volumes and denser crumb structures obtained in breads with added bran. The effect of bran absorbing more water, is similar to the effect caused by flour containing high levels of damaged starch (Collins, 1971).

Lai et al (1989b), however, found that at normal absorption levels, the gluten in brown bread was not properly hydrated, because of the bran competing for and binding a relatively large amount of water. The use of inappropriate absorption levels therefore resulted in a reduction in loaf volume. Therefore, a 63% water absorption was used in this study in breads that were baked with bran, as compared to the 61% in the white bread controls. This higher water absorption resulted in a dough with optimum consistency as determined by dough handling (Ms. C. Hendriks, Wheat Board –

Personal communication), but could have been responsible for producing a heavy loaf with reduced loaf volume. Gan et al (1995) reviewed studies on the influence of pentosans on loaf volume and found them to be contradictory. Andersson, Hamalainen & Aman (1994) did predictive modelling studies of the baking properties of wheat, showing that inclusion of NSPS variables helped to explain some of the variation in dough rheological properties and contributed significantly to variation in flour water absorption, but did not explain variation in loaf volume. Therefore, it is possible that bran could also have been responsible for physically disrupting dough formation which caused ultimate depression in loaf volumes.

Research by Gan et al (1989, 1992) showed that the outer layers of the bran, especially the epicarp hairs, were responsible for loaf volume depression. The epicarp hairs appeared to play a prominent role in disrupting the gluten-protein matrix (Gan et al, 1989). It was shown that removing the epicarp hairs by pearling significantly improved the baking performance of the resulting flour, producing bread with 8.5% higher loaf volumes when compared to wholemeal bread. Incorporating the outermost bran fraction, containing the highest concentration of epicarp hairs into the breadmaking recipe, caused the most marked depression in loaf volume (Gan et al, 1992). Heat treatment of this fraction did not diminish its adverse effect on loaf volume, suggesting that heat sensitive components are not implicated. In addition, other work (Pomeranz et al, 1977; Moder et al, 1984; Lai et al, 1989b) showed that finely ground bran produced a higher loaf volume than coarse bran. It was therefore expected that the larger Digestive bran would cause the greatest depression in loaf volume, and Pollard the least. The opposite was found (Fig. 20, 21). Breads baked with Digestive bran had the highest loaf volumes and those baked with Pollard, the lowest. This is in agreement with the work of Özboy and Köksel (1997) who found that coarse bran showed unexpected strengthening effects on dough rheology and had a lessened deleterious effect on loaf volume. Furthermore, it is pertinent that none of the bran fractions used in this study were found to contain epicarp hairs (Fig. 19). In fact, the milling process used has been observed to separate the epicarp hairs in a different stream from the bran (N. Dumas – Personal communication). Thus the findings of this study cannot be compared directly with those of Gan et al (1989, 1992).

The possibility that larger bran particles (Digestive bran) had less of a detrimental chemical effect on loaf volume when compared to finely milled bran (Pollard), because of a difference in chemical composition and available substrate, has been described earlier. It has also been mentioned that the large, flaky shapes of the Digestive bran particles, could have been responsible for encapsulating air during the mixing process, providing oxygen for dough strengthening and nucleation sites for dough gas cell formation. It is here where the subtle interplay between chemical and physical effects become clear. Physically, because of its shape, Digestive and to a lesser extent Select bran trap air bubbles within the dough structure. Physically, these air bubbles provide gas cell nucleation sites, but chemically they also provide oxygen for effective gluten formation and functioning of the ascorbic acid.

The crumb structure of breads baked with all bran types was more open and irregular when compared to the uniform crumb structure of white bread, with Digestive bran having the most irregular structure (Fig. 30 to 33). These findings are consistent with work of Moder et al (1984) who showed that finely ground bran gave a finer and more uniform crumb grain structure, when compared to bread produced from a coarse bran. It was attempted to find the reason why coarse bran produced an open and unattractive crumb structure by looking at what happens during bubble formation in the proofing stage. CAT scanning (computer aided tomography scanning) of the proofing loaves provided a way to observe bubbles within the dough structure, without changing their sizes or shapes, which would not have been the case if the dough had to be sliced. This procedure preserved the essential nature of the dough and facilitated the detection of cells and computation of a number of bubble parameters that are directly interpretable and technologically relevant. It also provided a means to observe how bubble size and shape changed with proofing time. It was therefore possible to follow the progress of bubble formation and growth throughout the proofing period, which would eventually form the final product crumb structure. The influence of the different brans on bubble formation could also be monitored.

CAT scanning of proofing dough is quite a novel technique in evaluating baking quality, and therefore a few problems were encountered. One of them was logistical issues encountered at the hospital, which sometimes hindered scanning to be done at the precise times. Another was the time lapse that occurred during transport of the panned

loaves to the hospital. A more serious problem, however, came in the image analysis of the scans. The first scans were done before any proofing. The loaf, at this stage, only had a few bubbles and therefore had a very dense texture. To achieve the optimum image on the scan, gray level (GL) settings were adjusted so that a maximum contrast between dough and bubble could be observed. In theory, it was the plan to keep the same settings throughout for all the scans done at the four time intervals, in order to have the same GLs to work with during image analysis. This could, however, not be the case, since proofing changed the densities of the loaves. As proofing times increased, loaves became less dense and therefore the GL settings had to be altered in order to observe an image in which bubble still contrasted with dough (cell constituents). Furthermore, within the same loaf, different densities led to formation of a gray gradient (darker at the bottom of the loaf) (Fig. 34b, 35b and 36b) which complicated things even further. The end result was that GLs during binary imaging had to be chosen subjectively from a personal perspective. This might have led to some bubbles not being detected and therefore not being included in the bubble size and shape calculations, or that some cell constituents were mistakenly included in bubble calculations. For the same reason, the number of bubbles per unit area, which would have been of great interest, could not be determined accurately.

Sapirstein (1999) described the importance of generating a binary image by selecting a single appropriate GL threshold for image segmentation. Any pixels with GLs lower than threshold were deemed to be constituents of cells. The optimum GL threshold can be determined by the κ -means algorithm. Figure 58 shows the influence of choosing the correct GL threshold on imaging properties.

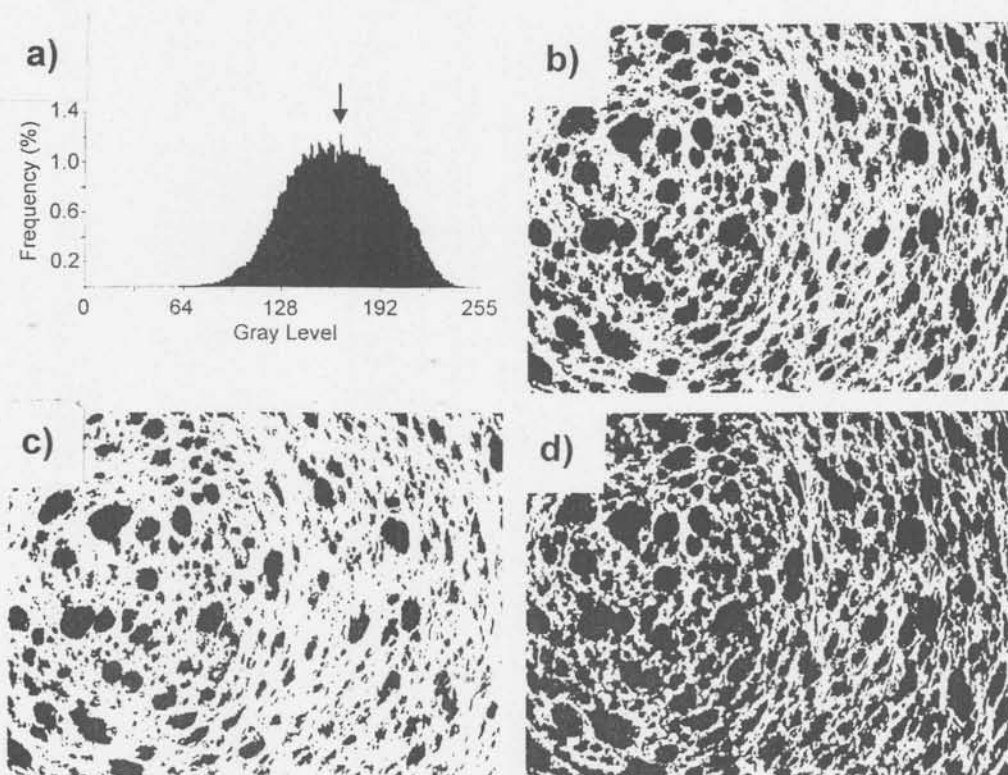


Fig. 58: a) Gray level histogram of bread crumb, arrow indicates optimum GL threshold = 172 determined by the κ -means algorithm; b) Binary image of bread crumb segmented at GL = 172; c) Binary image of bread crumb segmented at GL = 155; d) Binary image of bread crumb segmented at GL = 189 (Sapirstein, 1999)

Figure 58b corresponded to a segmented binary image based on the optimum GL level of 172. This binary image corresponded closely with that of the original. This is in contrast to the binary image results shown in Figures 58c and 58d where segmentation was based on GL thresholds selected at 10% below and above 172 respectively. The latter two binary images under- and over-estimated, respectively, the cellular composition of the bread slices. In the case of Figure 58c, the erosion of pixels which occurred in this image had a considerable effect on reducing cell sizes, altering shapes, along with increasing inter-cell distances. As cell wall thickness is a function of these distances, higher values than anticipated would be determined under these conditions. Similarly, Figure 58d considerably overestimated the cellular composition of the bread slice.

Whitworth & Alava (1999) also did CAT scanning on proofing loaves. They proofed the loaves inside the scanner which eliminated any problems that could have been encountered during transport. The authors imaged the internal structure of the proofing loaves and observed changes during proofing, but did not give any bubble measurement of the CAT scans by image analysis. It is suspected, however, that they would have experienced similar problems with GLs since gray gradients in their CAT scans were also observed. The size distribution of bubbles in their doughs was measured by image analysis of thin dough sections (frozen by immersion in liquid nitrogen) imaged with a microscope.

However, even with all the problems experienced during CAT scanning, valuable information was obtained from the CAT scans and image analysis thereof. The CAT scans showed an even and uniform crumb structure in white bread dough (Fig. 34). SEM and light microscopy of white and brown bread done by Pomeranz et al (1977) also revealed a major difference between the two crumb structures. The white bread had a fine crumb structure composed of thin sheets and filaments. Such a structure was essentially absent in brown bread. My work was novel in the sense that the structure of dough was examined, differing from other studies where the crumb structure of the baked bread was studied. It was shown that the structure of doughs made with Pollard closely resembled that of the white bread dough (Fig. 35). Bubbles in the breads made with Select and Digestive bran, however, were large, irregularly shaped and ununiformly distributed (Fig. 36, 37). It also seemed that more bubbles per unit area were present in the white bread than the breads with bran at any given time, with bread made with Digestive bran showing the least amount of bubbles. Image analysis of the CAT scans and consequent calculation of bubble parameters revealed a definite trend for bubble size (measured as bubble area, length and perimeter) to increase during the proofing period (Fig. 38-49). Furthermore, it seemed that bubbles became more elongated as proofing progressed (Fig. 50-53). Although this phenomenon was observed in all the breads, it was more pronounced in the breads made with Select and Digestive bran. The CAT scans also showed bran particles protruding through the bubbles, especially in the breads made with Digestive bran (Fig. 37d) and to a lesser extent in breads with Select bran (Fig. 36d). In order to explain how this phenomenon could physically affect

loaf volume, it is necessary to look at some physical aspects of bubble formation in foods.

Bread dough is a multiphase and multicomponent system composed of proteins, lipids, polysaccharides, water, gas and other minor nutrients and additives. It has a foam structure, with continuously growing gas bubbles dispersed in a semi-solid hydrated dough phase. As the volume fraction of the gas increases during fermentation, foam structure becomes increasingly fragile and unstable. To obtain a loaf of bread with a good loaf volume and a fine even crumb structure, it is crucial to promote the stability of the foam during the bread dough processing (Gan, van der Graaf, Leonard, Brooker, Parker & Schofield, 1999).

Cheftel, Cuq & Lorient (1985) gave an overview of food foams in general. Food foams are usually dispersions of gas bubbles in a continuous liquid or semi-solid phase that contains a soluble surfactant. In foams, a continuous phase of thin liquid layers, called lamellae, separates the gas bubbles. Mechanical energy is required for the creation of this interface. Maintaining the interface against coalescence of gas bubbles usually necessitates the presence of surface-active agents. These agents lower the interfacial tension and form a protective barrier between entrapped gas bubbles. Some proteins, e.g. gluten, are able to form a protective film by adsorbing at the gas/liquid interface. In this case the lamellae between two adjacent bubbles consist of two adsorbed protein films separated by a thin liquid layer. Because many foams have large interfacial areas, they are often unstable. According to Cheftel et al (1985), there are three main destabilising mechanisms.

- a) Drainage of lamella liquid due to gravity, pressure differences, and/or evaporation. The internal pressure P within bubbles is given by Laplace's capillary pressure equation:

$$P = P_{atm} + \frac{2\gamma}{R}$$

where P_{atm} is the atmospheric pressure (Pa), γ is the interfacial tension (N/m) and R is the bubble radius of curvature (m). Drainage is lessened when the bulk liquid

phase is viscous and the surface viscosity of the adsorbed protein film is great. The surface viscosity depends on the strength of protein-protein and protein-water interactions.

- b) Gas diffusion from small to larger bubbles. Such disproportionation results from solubility of the gas in the aqueous phase.
- c) Rupture of the liquid lamellae separating gas bubbles. Such ruptures result in an increase in bubble size through coalescence, and ultimately lead to a collapse of the foam.

The three most important attributes that serve to stabilise foams are low interfacial tension, high viscosity of the bulk liquid phase and strong, elastic films of adsorbed proteins.

During mixing of bread dough a small amount of air is entrapped, in the form of small spherical cells. A large number of these gas cells disappear during proofing and baking, because they are physically unstable or do not grow. According to Van Vliet (1999), of the three main destabilising mechanisms in food foams described by Cheftel et al (1985), two of them apply directly to bread, namely disproportionation, also known as Ostwald ripening, as well as coalescence. Van Vliet (1999) described Ostwald ripening as the growth of large gas bubbles at the expense of smaller ones due to the higher overpressure (Laplace pressure) of the gas in the small gas cells resulting in higher gas concentration in the vicinity of these cells. It causes diffusion of gas towards larger cells. Coalescence of gas cells is due to rupture of the dough film between them. Coalescence results in an irregular and coarse crumb structure.

According to Sapirstein (1999), the integrity of gas cells in weaker (unoxidised) dough compared to those in stronger (optimally oxidised) dough, cannot be maintained throughout the course of dough development, proofing and baking. As a consequence, small gas cells coalesce into larger ones. The greater the degree of gas cell coalescence during dough fermentation, the coarser will be the resulting bread crumb structure.

It seems that during the proofing of brown bread, the added bran physically ruptured gas cell walls of the foam structure leading to coalescence of the bubbles. This greater

degree of coalescence resulted in a coarser bread crumb structure, i.e. fewer and larger bubbles, compared to the smaller and greater quantity of bubbles observed in white bread. Coalescence caused bubbles with larger radii. Laplace's pressure equation states that pressure within the bubble is inversely proportional to its radius. The result is that the larger bubbles, formed by coalescence, had a lower internal pressure, possibly leading to the lower loaf volumes obtained in bread baked with bran. The large Digestive bran, with its jagged edges, had the worst effect, leading to large, irregular bubbles (Fig. 37). This is consistent with work of Moder et al (1984) who showed that finely ground bran had a uniform and fine crumb structure compared to the coarse structure of larger bran particles. Panelists in my study did not find the crumb structure of breads with Digestive bran to be objectionable, and indicated that it resembled the structure of an ideal brown bread (Table 25). This anomaly showed that a fine crumb structure in brown bread was not preferred. Breads with Digestive bran produced the highest loaf volumes when compared to Pollard and Select bran, in spite of the high degree of coalescence, which again supports the theory of oxygen being trapped and probably counteracting some of the negative effects of coalescence.

Baking of bread transforms the structure from a foam (containing discrete gas cells) into a sponge (containing a network of interconnected gas cells and a porous structure with a continuous gas phase), and sets the sponge structure (Cilliers & Sadr-kazemi, 1999). To produce a loaf of bread with a light and even crumb structure, the dough must be able to retain gases produced by yeast fermentation as discrete cells for a sufficiently long period (Bloksma, 1990). Gan et al (1995) proposed that soon after mixing, the dough consists of discrete gas cells lined with liquid films and embedded in a continuous starch-protein matrix. The matrix fails to enclose the gas cells completely at advanced stages of fermentation, leaving areas that contain only a thin liquid lamella. Baking increases the rate of expansion until the lamellar film is incapable of meeting the demand for new surface area generation, thus converting the foam structure of dough into an open sponge. The rupture of the liquid film is accompanied by a loss in gas retention. It is probable that the bran physically ruptured the liquid film surrounding the gas cells, causing reduced gas retention and lower loaf volumes. Pomeranz et al (1977) also found that loaf-volume-depressing effects of bran seemed to result from reduced gas retention.

The theory that higher gas retention causes higher loaf volumes, was supported further by Czuchajowska & Pomeranz (1993) who found that it was possible to compensate for a volume decrease as a result of poor gas retention by supplementing dough with high-quality gluten. Gas cell walls of gluten supplemented wholemeal bread appeared to be thicker, smoother and more continuous than those from unfortified flour (Gan et al, 1989).

A summary of the proposed physical effects of bran on loaf volume is shown in Figure 59.

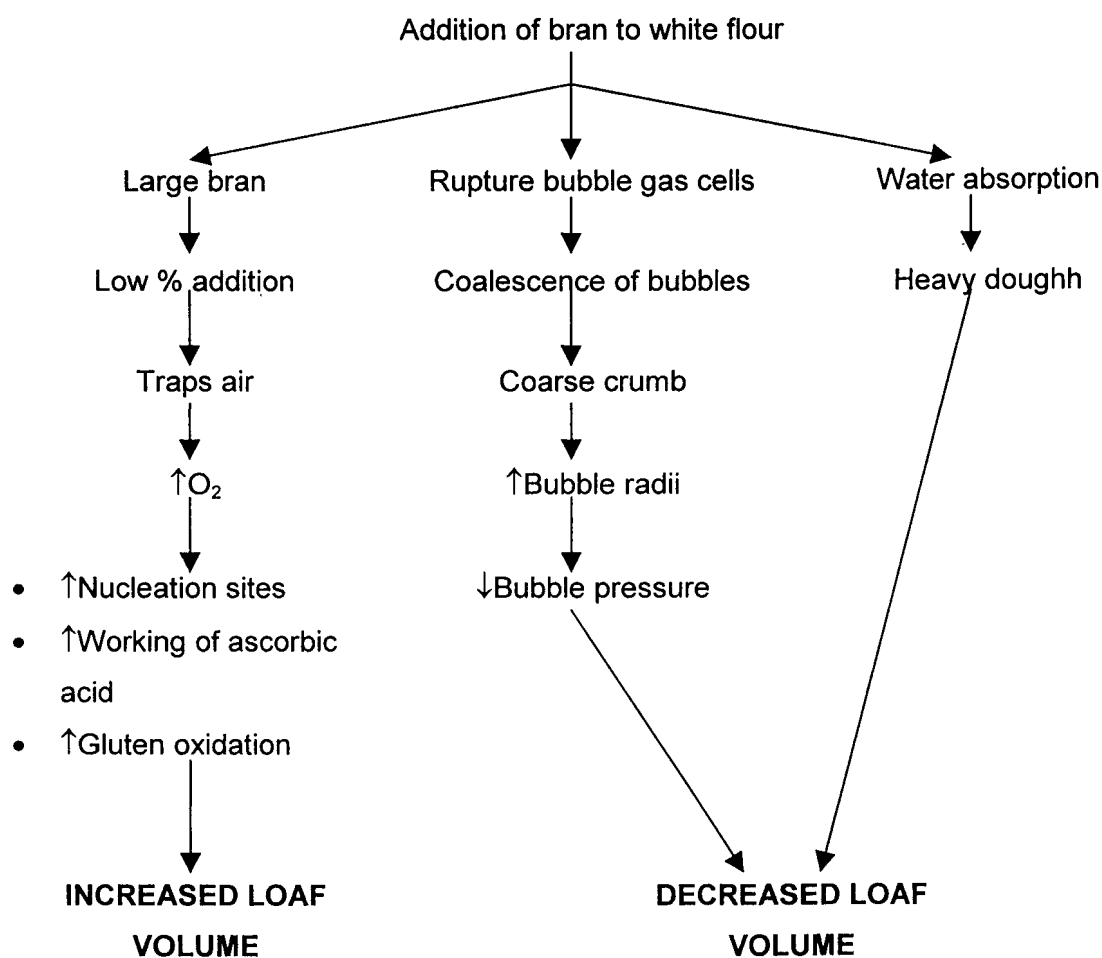


Fig. 59: Proposed physical effects of bran on loaf volume

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

Bread quality, as measured by loaf volume and crumb structure, is seriously impaired by addition of bran. Crumb structures of brown bread are coarse and open, and loaf volumes are depressed, more than would be expected from the diluting effect of the bran on the gluten proteins. Higher levels of bran addition cause lower loaf volumes. Bran components appear to affect gluten functionality by changing its physicochemical characteristics through a subtle interplay of chemical and physical effects.

In contrast to many other studies, this study showed that Pollard (smallest bran) depresses loaf volumes the most, and Digestive bran (largest bran) depresses loaf volumes the least. The chemical composition of brans, seem to play an important role in determining the extent of loaf volume depression. Pollard has a higher fat content suggesting more available lipid substrate than the other brans. Increased amounts of fat also indicate increased amounts of lipoxygenase. Lipase, present in the bran, catalyses hydrolysis of triglycerides in the fat, leading to the formation of predominantly polyunsaturated free fatty acids. Lipoxygenase catalyses the oxidation of these fatty acids, lowering the amount of available oxygen for gluten formation and functioning of ascorbic acid. This probably leads to the poor loaf volumes obtained in breads baked with Pollard compared to those baked with Digestive bran. Heat treatment of the brans practically inactivates lipase activity and probably lipoxygenase activity as well. Loaves baked with heat-treated Pollard, show significant increases in loaf volumes. A reduction in bran particle size in general decreases loaf volumes, but heat treatment of the smaller bran restore the original loaf volume, indicating that a chemical effect of the bran is at least in part responsible for depressing loaf volumes. Heat treatment of Select and Digestive bran also lessens loaf volume depressions, but to a lesser extent than in the Pollard. The improving effect of heat treatment on loaf volume, is the least in the Digestive bran, suggesting a smaller chemical effect in brans with larger particle sizes.

The difference in loaf volumes obtained with breads baked with brans, of the same nominal particle size range from different sources, also indicate that differences in chemical composition could account for differences in loaf volume depression brought about by bran addition.

In addition to the difference in chemical compositions of the different brans, the higher loaf volumes of breads with Digestive bran, compared to those with Pollard, could also possibly be explained by the large, flaky Digestive bran particles trapping air bubbles in the dough during the mixing stage. This is a perfect example of the interplay between the physical and chemical effects of bran on loaf volume. The trapped air bubbles probably fulfill two main functions which could lead to higher loaf volumes. They could:

1. Provide extra nucleation sites for gas cells which would later expand with the CO₂ produced by the yeast cells, causing increased loaf volumes;
2. Provide oxygen for the effective functioning of the oxidising agent ascorbic acid and for oxidation of the gluten to form disulphide links within the dough, causing a stronger dough and higher loaf volumes.

The theory that Digestive bran particles trap oxygen, could also explain why at low levels of addition, breads baked with Digestive bran have higher loaf volumes than white bread. However, for large bran particles to be able to increase loaf volume, they have to be free of epicarp hairs.

Another chemical constituent which is probably responsible for decreasing loaf volumes, is glutathione. Bran contains a considerable concentration of glutathione, coming from germ contamination. Addition of bran to white flour, possibly results in SS/SH interchange in the gluten, because of a shift in equilibrium towards the reduced form. This could lead to a weak gluten structure and decreased loaf volumes. Heat treatment significantly lowers TRS, of which glutathione is a part. The resultant increased loaf volumes in breads baked with heat-treated brans, indicate that glutathione also play a role in loaf volume depression.

Heat treatment does not result in all the brans producing loaves of the same volume and height, suggesting that the influence on loaf volume brought about by bran addition is due to a physical as well as a chemical effect. It is possible that bran physically disrupts the gluten-protein matrix by rupturing the gas cell walls of the foam structure, leading to coalescence of the bubbles. Coalescence causes larger and irregular bubbles, the effect becoming more pronounced during the later stages of proofing. Gas retention in these loaves is lowered, and internal pressure within the bubbles becomes less, possibly leading to lower loaf volumes in bread baked with bran. The bubbles in breads with

Digestive bran are the largest and the most irregular, indicating the severe effect of the large, flaky particles, causing coalescence. The negative effects of coalescence are, however, possibly counteracted to some extent by the trapped oxygen. A summary of the proposed interplay between chemical and physical effects is shown in Figure 60.

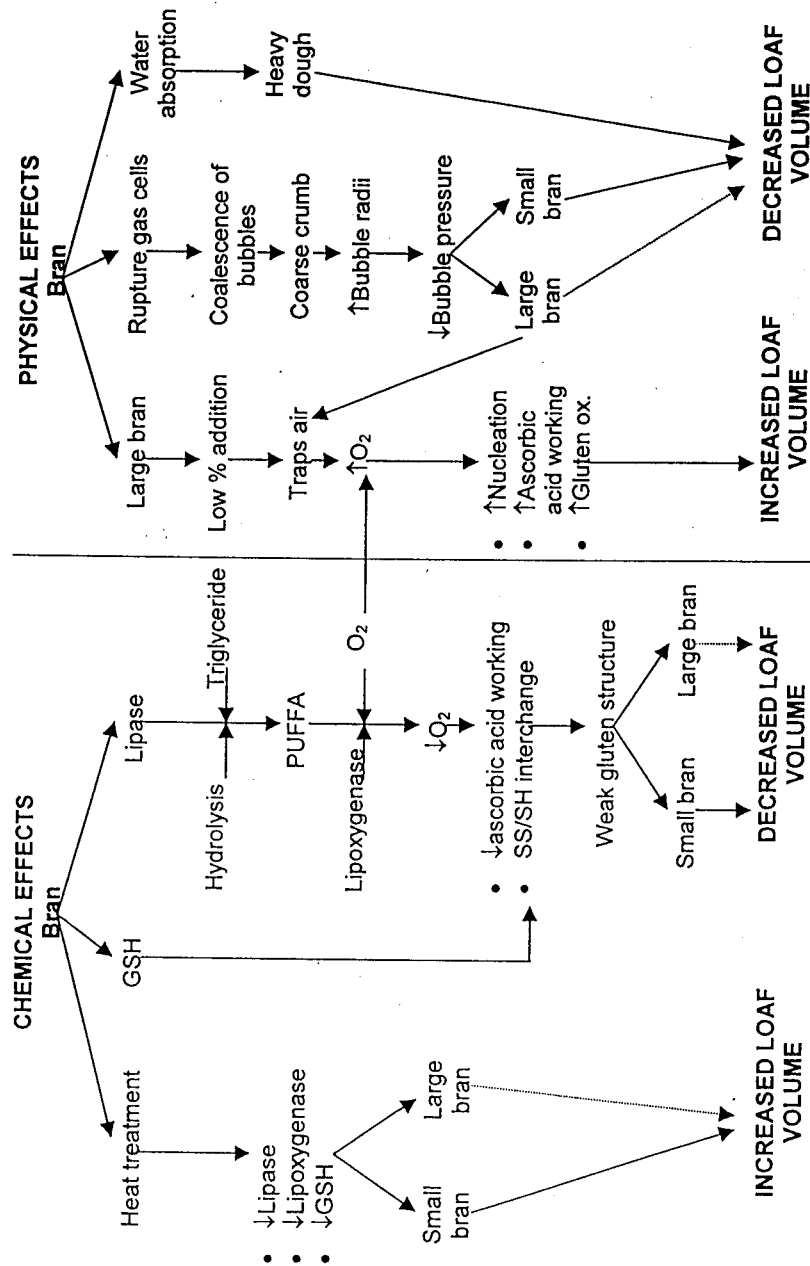


Fig. 60: Proposed interplay between chemical and physical effects of bran on loaf volume.

— Major effect; Minor effect

It is concluded that there is a subtle interplay between the chemical and physical effects of bran brown bread, which determines the extent of loaf volume depression. From a practical perspective, the addition of either large, epicarp hair free bran particles, or heat-treated smaller bran particles to white base flour, appear to have potential as methods of optimising brown bread loaf volume.

Further research is needed to test the suggested hypotheses. To show that the lipase/lipoxygenase system is in part responsible for loaf volume depression, free fatty acids could be added to the dough, and the resultant loaf volumes could be compared with controls which did not contain added free fatty acids. In addition, these loaf volumes could also be compared with breads in which heat-treated brans were used. If loaf volumes were lower in the breads with added fatty acids, and if loaf volumes increased with heat treatment, it could be concluded that the lipase/lipoxygenase system caused a depression in loaf volumes. To show that glutathione was also responsible for loaf volume depression, bran could be oxidised and loaf volumes of breads baked with unoxidised and oxidised brans could be compared. If oxidation caused a significant decrease in glutathione and higher loaf volumes were obtained with the oxidised bran, the adverse effect of glutathione on loaf volume would have been proven. To prove that the large Digestive bran encapsulated air and that the oxygen trapped with the air led to higher loaf volumes, bran could be treated with an inert gas such as nitrogen. If the breads which were baked with the inert gas treated bran had lower loaf volumes than the controls, it could be concluded that trapped oxygen was responsible for increased loaf volumes.