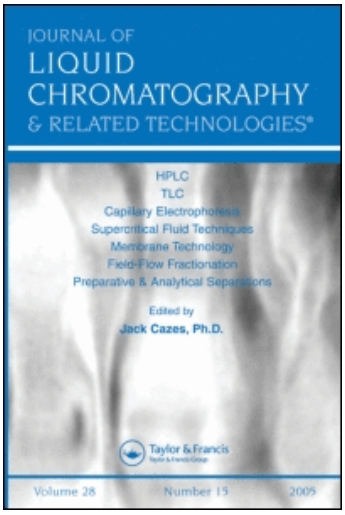

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Low Molecular Weight Peptides of Bread Dough and Bread. Dynamics During Fermentation and Baking

J. A. Prieto[a]; C. Collar[a]; C. Benedito De Barber[a]
[a] Laboraiorio de Cereales Institute de Agroquímica y Technologíc de Alimentos (CSIC) Jaime Roig 11, Valencia, Spain

To cite this Article Prieto, J. A. , Collar, C. and De Barber, C. Benedito(1992) 'Low Molecular Weight Peptides of Bread Dough and Bread. Dynamics During Fermentation and Baking', Journal of Liquid Chromatography & Related Technologies, 15: 2, 351 — 367
To link to this Article: DOI: 10.1080/10826079208017175
URL: http://dx.doi.org/10.1080/10826079208017175

JOURNAL OF LIQUID CHROMATOGRAPHY, 15(2), 351-367 (1992)

LOW MOLECULAR WEIGHT PEPTIDES OF BREAD DOUGH AND BREAD. DYNAMICS DURING FERMENTATION AND BAKING*

J. A. PRIETO, C. COLLAR,
AND C. BENEDITO DE BARBER
Laboratorio de Cereales
Instituto de Agroquímica y Technología de Alimentos (CSIC)
Jaime Roig, 11, 46010-Valencia, Spain

Summary

Dynamics in low molecular weight peptides (LMWP) during wheat bread dough fermentation and baking have been investigated by comparing the amino acid composition and the partial N-terminal amino acid sequence of the LMWP fraction of purified acetic acid extracts from unfermented, 2.5 hour-, and 24 hour-fermented straight doughs, and bread. Nutritional requirements on LMWP of yeast and lactic acid bacteria starting the fermentation are only evidenced when aspartic acid and asparagine levels are reduced. Specific endo and exoproteolytic activities releasing basic and hydrophobic nitrogen chains along breadmaking process, as well as level of hydrophylic residues in unfermented doughs affect browning and bread flavor.

* This paper is part of the doctoral thesis of J.A.Prieto
Offprint request to: C.Benedito de Barber

Introduction

The importance of low molecular weight peptides (LMWP) in the breadmaking process has been traditionally focused to their role as nitrogen source for microorganisms [1,2]. It is also well known their role in bread flavor as precursors in the formation of volatile compounds during fermentation [3] and baking [4,5]. Nevertheless, the direct contribution of LMWP to the flavor and taste of bread has not been considered so far.

It has been recognized however that LMWP can exhibit properties such as potential antioxidants, taste enhancers, sweeteners and bitter principles [6]. This latest aspect has received especial attention because the enzymatic hydrolysis of proteins from foodstuffs leads to the formation of bitter flavor [7,8,9]. Many bitter peptides from foods have also been detected and identified during fermentation and ripening processes [10,11].

The bitterness of LMWP appears to be closely related to the content and sequence of arginine, proline and hydrophobic amino acids [12,13]. By contrast, hydrophilic peptides, called "unami" peptides, especially with acidic character, exhibit bitter masking activity [14].

There are evidences that several wheat protein fractions could generate bitter peptides. Thus, the exhaustive hydrolysis of gliadin with proteases develops bitterness [15]. Grant and Wang [16] reported that the autohydrolysis of wheat flour released Phe-rich LMWP. It has also been observed the accumulation of hydrophobic amino acids, Pro-Pro, Leu-Pro-Phe, in partial sequences of corn zein [17] and of wheat gliadin [18]. Wheat proteins are also rich in hydrophilic amino acids [19] and therefore it is probably that "unami" peptides are present in the flour composition.

During bread dough fermentation, LMWP can be assimilated for growth [1], released from higher molecular weight peptides and hydrolyzed by the enzymatic activity of endoproteases from both flour [20] and lactic acid bacteria [21]. During baking, peptides can also participate in the Maillard browning reactions.

Umetsu and Ichishima [22] showed that a wheat carboxypeptidase, a serine type enzyme, is able to release selectively hydrophobic amino

acids from a casein bitter peptide fraction, resulting in a decrease of bitterness. Wheat amino peptidases show also a high affinity upon residues of Arg, Leu and Phe [23].

It is clear from literature references, that chemical and enzymatic reactions will affect the LMWP composition of wheat flour and therefore the contribution of these components to the sensory properties of bread.

In the present paper the LMWP fraction of bread dough and its changes during fermentation and baking have been studied. Data were used to obtain information on microbiological and enzymatic activities involved in the breadmaking process and to evaluate the role of LMWP in the taste and flavor of bread.

Material and methods

Reagents. Amino acids, dansyl amino acids and dansyl chloride (Dansyl-Cl) were purchased from Sigma (St.Louis,MO,U.S.A.). Dimethyl-aminoazobenzeneisothiocyanate (DABITC), phenylisothiocyanate (PITC), pyridine, triethylamine and trifluoroacetic acid (TFA), all sequencing grade were obtained from Fluka AG (Buchs,Switzerland). Acetonitrile (HPLC grade) and acetone (ultraviolet grade) were obtained from Panreac (Barcelona,Spain). All organic solvents used for sequencing were analytical grade from Merck (Darmstadt,F.R.G.). Buffers were prepared from chemicals of reagent grade or better, and ultrapure water delivered from a Milli Q system (Millipore,Bedford,MA,U.S.A.). Standard mixtures of dansyl- and dimethylamino-azobenzene thiohydantoin (DABTH-) amino acids were prepared as described previously [24,25].

Apparatus. The HPLC system (Waters Ass.,Milford,MA,U.S.A.) consisted of two 510 solvent delivery units, a 721 programmer and a U6K universal liquid chromatography injector coupled to a 490 variable wavelength ultraviolet detector. Data were simultaneously displayed and stored by a Waters 730 printerplotter automation system for subsequent integration. The column used was an ODS HS/3 (3µm,83x4.6mm,i.d.) from Perkin-Elmer (Norwalk,CT.U.S.A.), coupled to a precolumn (36x4.6mm,i.d.) packed with pellicular C_{18}.

Bread dough and bread preparation. Unfermented bread dough (UD) was prepared by a straight dough system from a commercial wheat bread flour as described previously [24]. UD was divided into 60 or 500g portions and fermented at 28°C and 80%rh for 2.5 ($FD_{2.5}$) and 24hr (FD_{24}). Bread was performed from 60g pieces of $FD_{2.5}$ baking at 220°C for 25min. Doughs were frozen, freeze-dried and then ground in a mortar to a fine powder before extraction.

LMWP extraction and initial purification. LMWP were extracted from doughs and bread freeze-dried samples (100g) with 0.01N acetic acid [26]. Extracts were successively purified of proteins and uncharged compounds by ultrafiltration on a 10.000 dalton cartridge and by strong cation exchange chromatography on a Dowex 50W-X2 [24].

Molecular exclusion chromatography. Doughs and bread extracts partially purified as described above, were freeze-dried, redissolved in 1N acetic acid and loaded into a column (2.5x80cm) of sephadex G-25 fine type, equilibrated with the same solvent. Samples were eluted with 1N acetic acid and collected in 5ml fractions at a temperature of 4°C and a flow rate of 1ml/min. Each fraction was analysed by the conventional ninhydrin method [27] before and after acid hydrolysis, and by the original cadmium-ninhydrin method [28]. Fractions containing LMWP (<1000 daltons) were pooled, evaporated to dryness and redissolved in 5 (UD), 2.2 ($FD_{2.5}$), 2.7 (FD_{24}) and 1.5ml (B) of 50mM $Na_2B_4O_7$ buffer (pH 11.0). Volume of buffer used in each sample was calculated according to its free amino acid content [24] (final concentration <4mg/ml), as was suggested by Rothenbühler et al.[29].

Ligand exchange chromatography. Aliquots of the samples purified by molecular exclusion chromatography were applied into a Cu^{2+}-sephadex G-25 column (1.5x11cm) prepared as reported [29]. LMWP and amino acids were eluted according to Fazakerley and Best [30]. The eluate was collected in 3ml fractions and analysed, after removing Cu^{2+} with a Chelex-100 resin, as it was mentioned before for the detection of nitrogenous material. Finally, the fractions containing LMWP were pooled, purified of salts by passing throught a Dowex 50W-X2 column, freeze-dried and redissolved in distilled water for amino acid composition and sequence analysis.

Amino acid composition. Amino acid analysis was carried out on peptide hydrolysates by RP-HPLC of their dansyl derivatives [31]. The dansylated extracts were redissolved in 1ml of 12mM HK_2PO_4, pH 7.0/acetonitrile (90:10,v/V) and filtered before the injection (20µl) into the chromatograph system. The separation, detection and quantification was performed as described [24].

Sequence analysis. LMWP (120-200nmol) of doughs and bread samples were coupled with DABITC/PITC and the released residues converted to DABTH-derivatives following the methodology of Chang [32]. Two sequencing cicles were made. DABTH-amino acids were redissolved in 50µl of aqueous ethanol (80%), and filtered. Volumes of 15 (UD), 6.6 ($FD_{2.5}$), 8.4 (FD_{24}) and 4.5µl (B) were injected in the HPLC system for analysis. Chromatography was performed as reported by Prieto et al.[25].

Results and Discussion

Chromatographic separation and quantification

Figure 1 shows RP-HPLC chromatograms of dansyl-amino acids released by hydrolysis of the LMWP fraction from UD,$FD_{2.5}$,FD_{24} and B. Separation was similar to that reported earlier for free amino acids analysis [24,33], although Dns-Arg which shows an especiall sensibility to any modification of the chromatographic conditions was eluted after Dns-Pro instead between the pair Dns-GABA/Dns-Pro. Nevertheless, this change did not affect the resolution of other Dns-amino acid.

Eighteen amino acids, including GABA, were detected in all samples (Fig.1). This amino acid formed from glutamic acid in flour by glutamate carboxylase activity [34] is coeluted along with LMWP in the ligand exchange chromatography [30], because its character of γ-amino. Results indicated also larger amounts of byproducts, especially Dns-OH and Dns-NH_2, than those found for free amino acid samples [24], because the higher molar ratio of Dns-Cl/Dns-AA. This finding is in agreement with our previous study of the derivatization conditions of Dns-amino acids [33]. Despite this, separations are still rather good to permit the resolution and quantification of all amino acids tested.

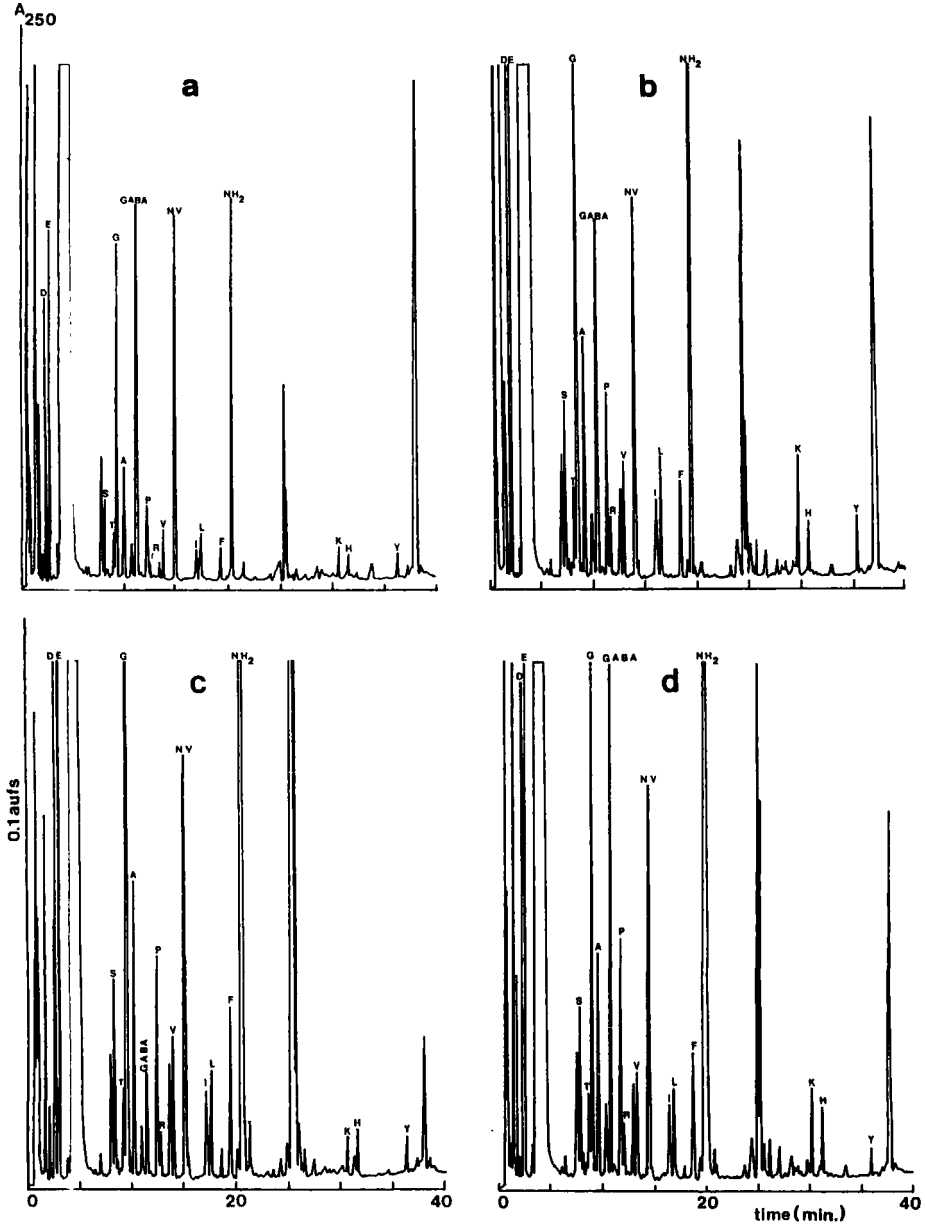

Figure 1.- RP-HPLC chromatograms of dansyl-amino acids released by hydrolysis of the LMWP fraction from UD (a), $FD_{2.5}$ (b), FD_{24} (c) and B (d). Chromatography as is described under Material and methods.

Reproducibility of the quantitative analysis of amino acids, was estimated by succesive injections of the derivatized extracts. Results (table I) revealed similar coefficients of variation to those for response factor in a standard mixture of dansyl-amino acids [24].

Amino acid composition of the LMWP fraction from bread dough. Biochemical changes during fermentation

Qualitative and quantitative amino acid composition of LMWP fraction isolated from UD,$FD_{2.5}$,FD_{24} and B is showed in the table I. Asx, Glx and Gly predominated in the amino acid composition of peptides of doughs and bread, accounting for 47% of the total amino acid content. Results stress the close relation between dough nitrogen compounds. Thus, Asp,Asn,Glu and Gln are the major free amino acids of UD [24].

During fermentation, the amino acid composition of LMWP fraction from bread dough showed important changes (table I). At the beginning of this period (0-2.5 hr) the majority of amino acids, except Asx (-20%) underwent a significant increase. Variations for Arg (+100%) and Lys (+78%) were remarkable, respect to those of other amino acids. Results can be explained by the activity of endoproteases over protein fractions of higher molecular weight. Peptide bonds next to Lys and Arg appear to have a higher grade of susceptibility. This is in agreement with earlier reported changes in the amino acid composition of the dialyzed LMWP fraction from hemoglobin after digestion with wheat flour proteases [16].

On the other hand, the shift in the Asx content and the non significant variation of Glx (table I), amino acid predominating in all wheat protein fractions, appear to reveal the activity of exoproteases since the beginning of fermentation. However, this behavior could be understood by the comsumptiom of Asx/Glx rich-small peptides by yeast. In this respect, it should be pointed out that Asp and Asn along with Trp showed the largest decreases from UD to $FD_{2.5}$ [24].

On the other hand, investigations on the transport and metabolism of LMWP by S.cerevisiae, indicated a low affinity by the uptake of acidic peptides [35]. A slight lag phase (5 to 7hr) when yeast are grown over peptides comparing to that on amino acids has

Table I. Amino acid composition of the LMWP fraction of unfermented bread (UD), 2.5-($FD_{2.5}$) and 24-hour (FD_{24}) fermented bread dough and wheat bread (B) samples.

Amino acid	Mean content[a], % CV							
	UD		$FD_{2.5}$		FD_{24}		B	
Asx[b]	2.61	0.38	2.08†	1.44	2.72†	0.36	1.33†	5.26
Glx[b]	3.37	5.04	3.50	2.29	4.90†	5.92	2.84†	2.46
Ser	0.93	1.07	0.97†	0.31	1.12†	0.89	0.59†	1.69
Thr	0.86	0.23	0.76†	1.32	0.76	1.32	0.44†	2.27
Gly	2.22	0.90	2.43†	1.23	3.18†	0.94	1.87†	0.53
Ala	1.04	0.96	0.99†	2.02	1.29†	0.78	0.62†	3.23
Pro	0.75	2.67	0.90†	1.11	1.11†	0.90	0.74†	1.35
Arg	0.41	2.44	0.82†	0.30	0.61†	0.27	0.46†	2.17
Val	0.63	3.17	0.67	1.49	0.88†	1.14	0.42†	2.38
Met		‡	0.16	6.25	0.22†	0.91	0.07†	2.85
Ile	0.52	7.69	0.61	9.84	0.64	1.56	0.42†	2.38
Leu	0.82	1.22	0.87	4.60	0.84	1.19	0.45†	4.44
Trp				n.d.				
Phe	0.71	1.41	0.84†	2.38	1.89†	1.06	0.81	0.25
Cys				n.d.				
Lys	0.28	10.7	0.50†	4.00	0.17†	0.48	0.22†	0.36
His	0.24	8.33	0.28†	3.57	0.22†	4.55	0.20†	5.00
Tyr	0.34	5.88	0.38	7.89	0.23†	4.35	0.10†	4.00

a. mg/100g sample, d.b.
b. Asx= Asp + Asn, Glx= Glu + Gln.
† Indicates a statistically significant change between mean values ($P<0.05\%$) for fermentation ($UB \rightarrow F_{2.5}$, $F_{2.5} \rightarrow F_{24}$) and baking ($F_{2.5} \rightarrow B$).
‡ Trace.
n.d. non determined

been also reported [36]. Besides, although LMW nitrogenous compounds do not show competence for the transport system, the regulatory control for them should be governed by the amino acid levels in the intracellular pool [35,37]. In this way, a rather high amount of free amino acids was found in bread dough [24].

Between 2.5 and 24 hours of fermentation, the amino acid analysis of the LMWP fraction (table I) revealed a further enhance in every

amino acid content, except for His (-21%),Arg (-26%), Tyr (-39%) and Lys (-66%). The change was especially important for Phe (+125%) indicating the existence of at least a second endoproteolytic activity in bread dough. This appears to differ from the earlier discussion about optime pH and especificity. The shift in the dough pH (5.5-4.5) along the fermentation process, probably promotes the activity of endoproteases with a higher specificity for peptidic bonds next to Phe.

The change in the Arg,Lys,His and Tyr content might be associated to the absorption of LMWP by lactic acid bacteria, more metabolically active at the end of fermentation. However, analysis of free amino acid composition revealed a high concentration of Lys and Arg in fermented bread dough (6 hours), which even increased at the last period of fermentation [24]. Besides, although lactic acid bacteria require Arg for growth, Lys is not essential [38]. A possible explanation could be if Lys and Arg belong to the LMWP which are metabolyzed as source of other essential amino acids. These could be Tyr and His [38], which amount as free amino acids is certainly low in 6-hour fermented dough ([24]. The uptake of these peptides will be accompanied by their hydrolysis and the release into the medium of the above amino acids which concentration in the intracellular pool is excessive [39], in this case Lys and Arg.

Changes could be atributed however to the activity of exoproteolytic enzymes. In fact, the increase from 0 to 2.5 hours in the content of Lys and Arg, and in smaller proportion of His and Tyr indicate that the number of peptide chains with these residues in terminal position should increase during this period. In this way, it is known that aminopeptidases [23] and carboxypeptidases [40,41] of wheat kernel have the ability to release most amino acids residues from the terminal of peptides and proteins. Therefore, the determination of the partial sequence of peptides and especially their changes along fermentation will permit us to obtain additional information to support and to clarify the above discussion.

Chemical changes during baking

During baking the amino acid content of the LMWP fraction isolated from $FD_{2.5}$ showed a strong decline (table I), indicating that peptides

participated actively in the development of bread flavor and taste supstances, through the Maillard browning reaction. The shift differed however among amino acids, varlyng from 18% for Pro to 74% for Tyr. These differences could be interpreted in terms of browning reactivity. However, LMWP can undergo important changes due to the enhancement of proteolytic activity as dough temperature increases. In this way, the absence of a significant variation for Phe is remarkable, because the result should be considered as a balance between peptide generation and transformation by the Maillard reaction and the activity of exoproteases.

Partial N-terminal amino sequence of the LMWP fraction of dough and bread. Changes during fermentation and baking

Figure 2 shows the separation by RP-HPLC of DABTH-derivatives released in the first cicle of sequenation of the LMWP fraction of dough and bread. Chromatograms revealed a good separation of DABTH-amino acids, similar to that reported earlier for a standard mixture [25]. All protein amino acids, except His, are present in variable proportion as first residue of the LMWP fraction isolated from dough and bread. N-glycyl,N-seryl,N-aspartyl and N-glutamyl peptides predominate however, in correspondance with the total amino acid composition described above.

Results indicated also a dynamic change during fermentation in the amino acid sequence of bread dough peptides. From 0 to 2.5 hours of fermentation there was a shift in the yield of the majority of DABTH-amino acids, altough those from non-polar amino acids, Tyr and especially Pro increased (Fig.2).

The decrease in the relative proportion of DABTH-Asp,-Glu,-Asn and -Gln is in good agreement with the variations observed during this period in the total content of Asx and Glx (table I). This result supports the activity of aminopeptidases at the begining of fermentation. It is clear also that the increase in the total amino acid content during this period results of the activity of endoproteolytic enzymes. The total balance of these activities is therefore a peptide fraction with a larger molecular size. The absence of variability of Arg

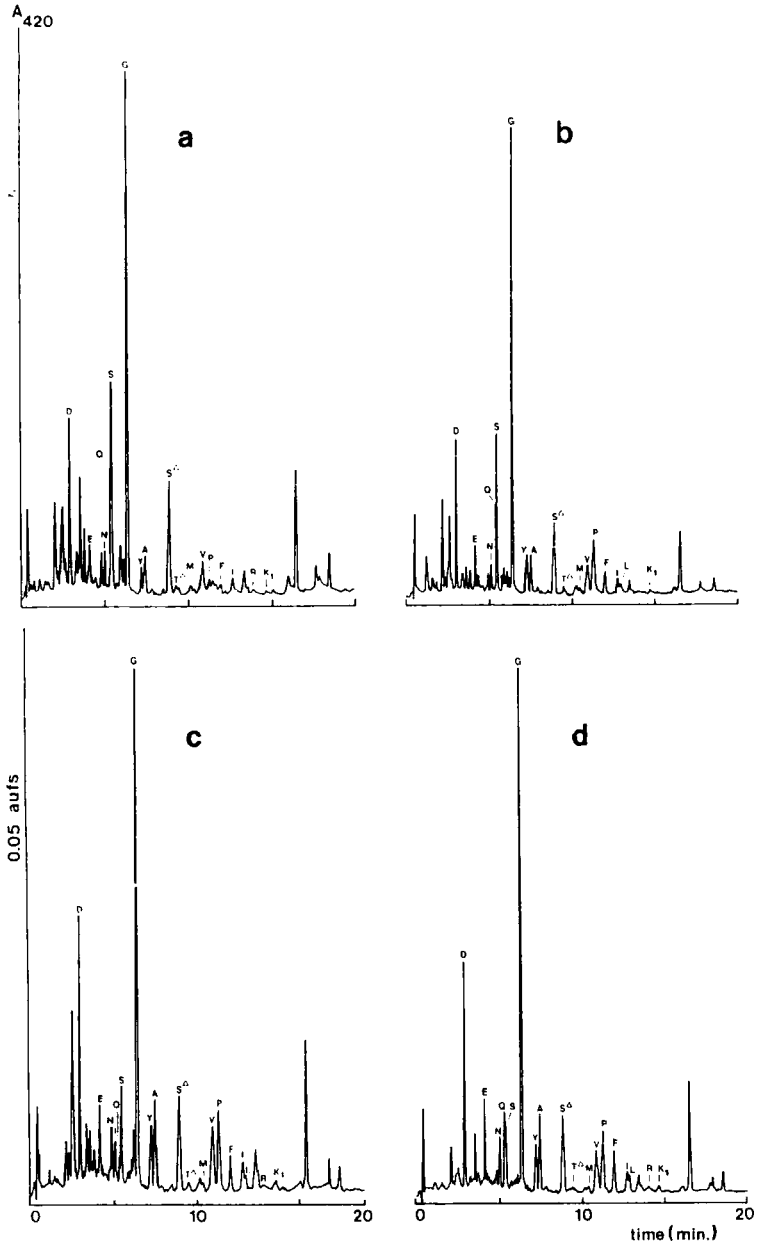

Figure 2.- Separation of DABTH-amino acids released in the first cicle of sequenation of LMWP from UD (a), $FD_{2,5}$ (b), FD_{24} (c) and B (d). Chromatography was performed as described under Material and methods.

and Lys as N-terminal residues appears to indicate also that this endoprotease activity shows a specificity for the carbonyl side. Similar results have been described for serine-type enzymes as trypsin.

At longer fermentation times ($F_{2.5}$→F_{24}), results revealed a higher DABTH-derivatives yield for the majority of amino acids, except for Gln. The variations have to be understood as discussed before as result of the activity of endoproteases which showed a higher specificity for peptide bonds next to Phe. This amino acid did not show however a remarkable enhance as N-terminal residue indicating that peptide bonds are cleaved by the carbonyl side of Phe, a chymotrypsin like activity.

On the other hand, the decrease in the DABTH-Gln content indicates that the aminopeptidase activity is active along the fermentation process. Finally, the increase in the DABTH-Tyr content supports that the changes in the total content of Tyr,Arg,Lys and His (table I) are due to the release of these residues by a carboxypeptidase. In fact, an enzyme with these characteristics has been isolated from wheat [40].

During baking (Fig.2b,d) the chromatograms indicated a similar behavior of DABTH-derivatives to that showed during longer fermentation period ($F_{2.5}$ to F_{24}). In fact, results clearly suggest an increase in the total number of peptide chains, which should be attributed to the activity of endoproteases. This result along with the shift in the total amount of amino acids (table I) indicates a peptide fraction with smaller chain length, definitely different to the changes observed from UD to $FD_{2.5}$. In this respect, it must be considered that other chemical reactions taking place during baking can affect the activity of enzymes. For instance, the increase of dough temperature and the denaturalization of proteins under the action of heat.

Second cicle of sequenation of the LMWP fraction of dough. Changes during fermentation and baking.

Analysis of the DABTH-residues released in the second cicle of sequenation of the LMWP fraction of UD,$FD_{2.5}$,FD_{24} and B (Fig.3) revealed a depletion in the yield of derivatives respect to that found for the N-terminal. Results indicate an important proportion of dipeptides in the

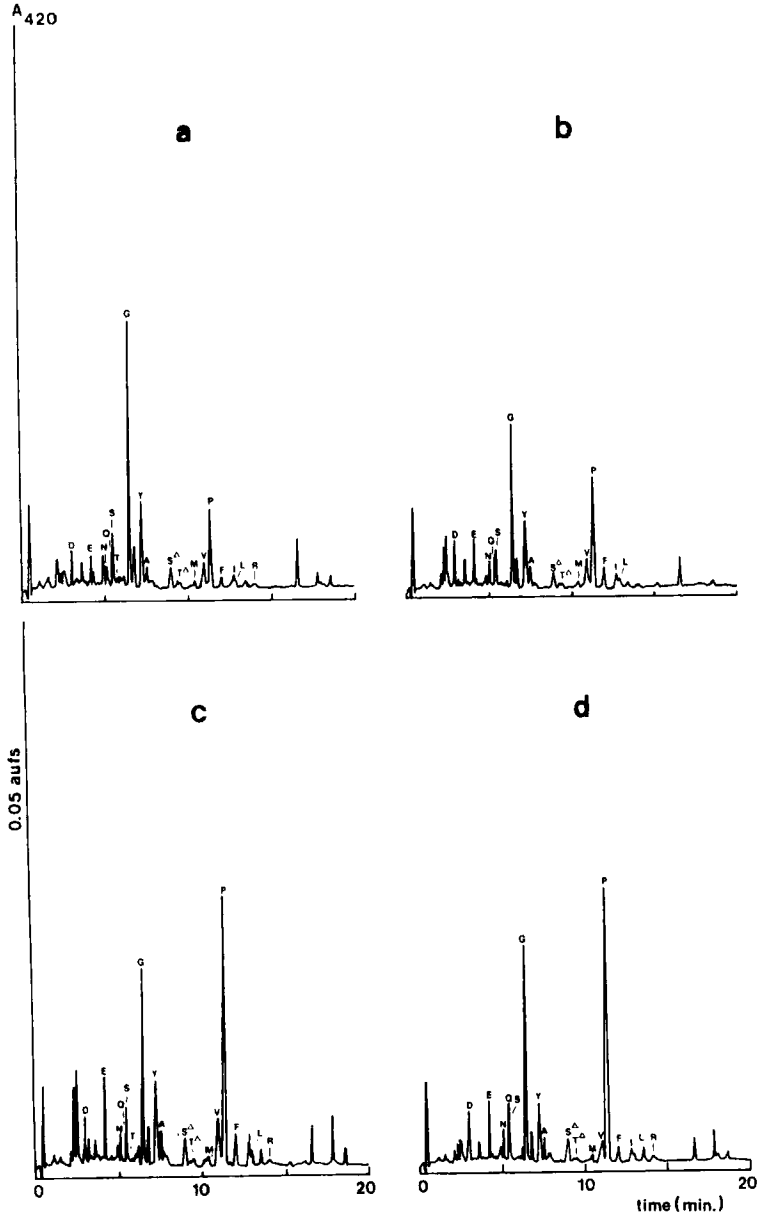

Figure 3.- Separation of DABTH-amino acids released in the second cicle of sequenation of LMWP from UD (a), $FD_{2,5}$ (b), FD_{24} (c) and B (d). Chromatography was performed as described under Material and methods.

fraction isolated from dough and bread . In fact, a sharp depletion in the derivation reaction yield has been reported when sequenation arrives to the C-terminus [42]. Conversely to this trend, chromatograms (Fig.3) revealed an increase in the content of DABTH-Tyr and especially of DABTH-Pro. This was remarkable for FD_{24} and B. Results observed for Tyr and Pro indicate that these amino acids are frecuently present as second residue from the N-terminus. This is in agreement with the frecuence of sequences found among peptides released by hydrolysis of wheat gliadins. Thus Gln-Pro accounts for 21% of total dipeptides [18].

The study of the changes, during fermentation and baking, of the DABTH-derivatives released in the second cicle of sequenation gives us more information on these sequences. Between 0 and 2.5 hours of fermentation (Fig.3), chromatograms showed small changes for the majority of derivatives, except for those of Gly and Pro. Pro underwent an important increase, indicating that it is frecuently present as third residue from the N-terminus. Otherwise, its yield would have been reduced due to that a part of Pro being located at second position has been already sequenced. These peptides should respond to the sequence X-X-Pro, because it is unlikely the accumulation of Pro (X-Pro-Pro); only 6% of Pro is distributed as Pro-Pro in the sequences of peptides released from wheat gliadin [18]. X represent mainly Gln, in the sequences of three amino acids obtained from gliadin and glutenin, accounting for 15 and 13% respectively [18]. However, as regard of the decrease in the DABTH-Gly yield, this amino acid is likely placed often as second residue from the N-terminus in X-Gly-Pro type sequences.

From $FD_{2.5}$ to FD_{24} results indicated a sharp enhance in the DABTH-derivatives content as could be expected from the increase earlier described in the number of peptide chains during this period. The larger variation was revealed again by DABTH-Pro, which was even higher than registered at the first cicle. Therefore the variation can not be explained by the simple activity of aminopeptidases, but should be attributed to the release by action of endoproteases of peptides accumulating Pro in this position. As it was discussed earlier, the activity of these enzymes showed a large specificity by Phe. In fact

the presence of Phe next to Pro in sequences from wheat gliadin is remarkable. For instance, Gln-Pro-Phe-Pro account for 7% of the sequences of four amino acids [18].

During baking the changes in the DABTH-amino acids composition were similar to those before described for the first cicle of sequenation (Fig.2,3). The results stress the importance that the endoproteolytic activity of bread dough can lead to the release of LMWP with hydrophobic sequences and therefore of potential bitter taste.

Conclusions

The role of the LMWP fraction of bread dough as a source of nitrogen for yeast and lactic acid bacteria appears to be insignificant. However, they could have some interest when the initial content of free amino acids is reduced and especially for those as Asp and Asn for which S.cerevisiae shows an especiall requirement.

The contribution of LMWP to the taste and flavor of bread will depend on, a) the content of peptides with an abundant composition of basic and hydrophobic amino acids, distributed in potentially bitter sequences released principally during fermentation and baking, b) the proportion of hydrophylic peptides in unfermented bread dough and c) the balance of endo and exoproteases activities, during fermentation and baking which originate mainly the changes in the peptide fraction.

Acknowledgement

This work was financed in part by the Comisión Interministerial de Ciencia y Tecnología (CICYT), Spain. We also appreciate the collaboration of Miss.Berta Barber by the German trasiation.

References

1. Payne JW (1976) Advances in Microbial Physiology 13:55-113
2. Faridi HA, Johnson JA (1978) Cereal Chem 55:7-15
3. Barber S, Benedito de Barber C, Martínez-Anaya MA, Martínez J, Alberola J (1985) Rev Agroquím Tecnol Aliment 25:223-232
4. Drapron R, Richard-Mollard D (1979) Buré J (ed) Le Pain, Centre Nat de la Rech Scient, pp 143-161
5. Maga JA (1974) Critical Reviews in Food Technology 5:55-142
6. Fujimaki M (1981) Rev Agroquím Tecnol Aliment 21:299-310

7. Arai S, Yamashita M, Kato H, Fujimaki M (1970) Agr Biol Chem 34:729-738
8. Schalinatus E, Behnke U (1975) Narhung 18:697-704
9. Sparrer D, Belitz H-D (1975) Z Lebensm Unters Forsch 157:197-204
10. Hamilton JS, Hill RD, Leeuwe HV (1974) Agric Biol Chem 38:375-379
11. Champion HM, Stanley DW (1982) Can Inst Food Sci Technol 15:283-288
12. Otagiri K, Nosho Y, Shinoda I, Fukui H, Okai H (1985) Agric Biol Chem 49:1019-1026
13. Ishibashi N, Ono I, Kato K, Shigenaga T, Shinoda I, Okai H, Fukui S (1988) Agric Biol Chem 52:91-94
14. Ohyama S, Ishibashi N, Tamura M, Nishizaki H, Okai H (1988) Agric Biol Chem 52:871-872
15. Petritschek A, Lynen F, Belitz H-D (1972) Lebensm-Wiss u Technol 5:77-81
16. Grant DR, Wang CC (1972) Cereal Chem 49:204-207
17. Wiesser H, Belitz H-D (1983) Z Lebensm Unters Forsch 177:205-
18. Wieser H, Seilmeier W, Belitz H-D (1984) Z Lebensm Unters Forsch 179:40-41
19. Wall JS (1979) Laidman DL, Wyn Jones RG (eds) Recent advances in the Biochemistry of Cereals, Academic Press Inc, New York, pp 275-311
20. Wang CC, Grant DR (1969) Cereal Chem 46:537-544
21. Kosmina NP (1977) Biochemie der Brotherstellung. VEB Fachbuverlag:Leipzig, G.D.R.
22. Jmetsu H, Ichishima E (1985) Hironori Shokuhin Kogyo Gakkaishi 32:281-287
23. Kruger JE, Preston KR (1978) Cereal Chem 55:360-372
24. Benedito de Barber C, Prieto JA, Collar C, Barber S (1989) Acta Aliment 18:53
25. Prieto JA, Collar C, Benedito de Barber C (1991) J Chromatogr Sci 29:205-209
26. Benedito de Barber C, Prieto JA, Collar C (1989) Cereal Chem 66:283-288
27. Spackman DH, Stein WH, Moore S (1958) Anal Chem 30:1190-1206
28. Doi E, Shibata D, Matoba T (1981) Anal Biochem 118:173-184
29. Rothenbühler E, Waibel R, Solms J (1979) Anal Biochem 97:367-375
30. Fazakerley S, Best DR (1965) Anal Biochem 12:290-295
31. Navarro JL, Aristoy M, Izquierdo L (1984) Rev Agroquím Technol Aliment 24:85-93
32. Chang J-Y (1983) Methods in Enzymol 91:455-466
33. Prieto JA, Collar C, Benedito de Barber C (1990) J Chromatogr Sci 28:572-577
34. Ponomareva AN, Kretovich VL, Kareva II, Yakubchik T (1964) Biochemistry (trans of Biokhimiya) 29:283-287
35. Nisbet TM, Payne JW (1979a) FEMS Microbiol Lett 6:193-196
36. Marder R, Becker JM, Naider F (1977) J Bacteriol 131:906-916
37. Nisbet TM, Payne JW (1979b) J Gen Microbiol 115:127-133
38. Spicher G, Schröeder R (1979) Z Lebensm Unters Forsch 168:397-401
39. Payne JW, Bell G (1979) J Bacteriol 137:447-455
40. Umetsu H, Abe M, Sugawara Y, Nakai T (1981) Food Chem 7:125-138
41. Umetsu H, Matsuoka H, Ichishima E (1983) J Agric Food Chem 31:50-53
42. Chang J-Y (1981) Biochem J 199:557-564

Appendix A
Key to Abbreviations

UD	unfermented bread dough
$FD_{2.5}$	2.5-hour fermented bread dough
FD_{24}	24-hour fermented bread dough
B	bread
Dns-Cl	dansyl chloride
Dns-AA	dansyl amino acid
Dns-OH	dansylic acid
Dns-NH_2	dansylamide
AA	amino acid
RP-HPLC	reversed-phase high-performance liquid chromatography
DABITC	dimethylaminoazobenzene isothiocyanate
PITC	phenyl isothiocyanate
DABTH	dimethylaminoazobenzene thiohydantoin
DABTC	dimethylaminoazobenzene thiocarbamoyl
PTC	phenylthiocarbamoyl
PTH	phenylthiohydantoin
LMWP	low molecular weight peptides
L-Lys-L-Asp	L-Lysil-L-Aspartic
ODS	octadecylsilane
AcN	acetonitrile
NaAc	sodium acetate
aufs	absorbance units full scale
CV	coefficient of variation
D	DABTH-Asp (-Aspartic acid)
E	DABTH-Glu (-Glutamic acid)
Q	DABTH-Asn (-Asparragine)
N	DABTH-Gln (-Glutamine)
S	DABTH-Ser (-Serine)
S^	dehydro-DABTH-Ser
T	DABTH-Thr (-Threonine)
T^	dehydro-DABTH-Thr
G	DABTH-Gly (-Glycine)
A	DABTH-Ala (-Alanine)
R	DABTH-Arg (-Arginine)
P	DABTH-Pro (-Proline)
V	DABTH-Val (-Valine)
M	DABTH-Met (-Metionine)
I	DABTH-Ile (-Isoleucine)
L	DABTH-Leu (-Leucine)
W	DABTH-Trp (-Tryptophan)
F	DABTH-Phe (-Phenylalanine)
C	DABTH-Cyst (-Cystine)
K_1	α-DABTH-ε-DABTC-Lys (-Lysine)
K_2	α-PTH-ε-DABTC-Lys
K_3	α-DABTH-ε-PTC-Lys
H	DABTH-His (-Histidine)
Y	DABTH-Tyr (-Tyrosine)