COMPOUNDS OF LOW MOLECULAR WEIGHT IN WASHED WHEAT GLUTEN*

By H. P. C. GALLUS† and A. C. JENNINGS†

Gallus and Jennings (1968) suggested that the rheological properties of a dough could be influenced by the nature and amount of the naturally occurring compounds of low molecular weight in the parent wheat flour.

There is a reasonably good correlation between the physical properties of gluten washed from a flour–water dough and the baking properties of the flour (Kent-Jones and Amos 1957, p. 143; Zeleny 1964). Thus the types of compounds of low molecular weight remaining in washed gluten were determined since it is likely that these adsorbed compounds would affect the rheological properties of gluten and dough.

Phospholipids, starch, proteins soluble in water and dilute salt solutions, enzymes, and non-starch carbohydrates have previously been detected in washed gluten (Kent-Jones and Amos 1957, p. 146; Pence, Nimmo, and Hepburn 1964).

Materials and Methods

All organic solvents were redistilled before use. The Sephadex G25 (fine) was supplied by Pharmacia, Uppsala, Sweden. The flour was prepared from the grain of *Triticum durum* cv. Dural.

The procedure described by Kent-Jones and Amos (1957, p. 601) was used to prepare the washed gluten from 200 g of flour. Immediately after preparation the gluten (61·0 g, containing *c.* 40 ml water) was macerated in 80 ml of phenol–acetic acid (1:1 w/v) and left at room temperature for 16 hr. The gluten dispersed to form a thick slurry which was transferred to Visking dialysis tubing (23/32 grade) and dialysed against three portions (1 litre each) of 10% (v/v) acetic acid for a total of 78 hr (Pusztai 1966).

The diffusates were bulked and evaporated to dryness in a rotary evaporator (Bagdasarian *et al.* 1964).

The residue was dissolved in 6 ml of phenol–acetic acid–water (1:1:1 w/v/v). The brown solution was eluted by the same solvent from a column of Sephadex G25 (bed volume 167 ml) and the effluent fractions assayed by the procedures of Gallus and Jennings (1968).

The various components were detected as follows:

(1) *Polypeptides.* By staining with nigrosine (Gallus and Jennings 1968).

(2) *Amino Acids and Peptides.* By their reaction with ninhydrin (Stepka 1957; Mabry and Todd 1963).

(3) *Lipids.* By staining with sudan black (Gurr 1960).

(4) *Polysaccharides and Lipids.* By their absorption of iodine from the vapour phase (Greenway, Kent, and Whitehouse 1953; Whitehouse, Bresler, and Staple 1958).

(5) *Carbohydrates.* By reaction with phenol and sulphuric acid (Gallus and Jennings 1968).

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† Department of Agricultural Biochemistry and Soil Science, Waite Agricultural Research Institute, University of Adelaide, Glen Osmond, S.A. 5064.

(6) *Reducing Sugars and Other Reducing Compounds.* By their reaction with silver nitrate (Trevelyan, Proctor, and Harrison 1950; Benson *et al.* 1952).

(7) *Individual Reducing Sugars,* neo*Inositol, and Glycerol.* By separation by paper chromatography (Jermyn and Isherwood 1949) then as for reducing sugars.

(8) *Inorganic Phosphorus.* By its reaction with ammonium molybdate (Harrap 1960).

(9) *Phenolic Compounds.* By paper chromatography with 0·25M formic acid and ethanol–0·25M formic acid (4:1 v/v), and reaction with ferric chloride–potassium ferricyanide reagent (Kirby, Knowles, and White 1953), diazotized *p*-nitroaniline (Swain 1953), and diazotized benzidine (Randerath 1963).

(10) *Aromatic and Heterocyclic Compounds.* By their absorption of, or fluorescence in, ultraviolet light, both before and after exposure to ammonia vapour and also after paper chromatography using the solvent systems as for phenolic compounds.

(11) *Pigments.* By visual observation.

Other preparations of washed gluten were extracted with ethyl acetate or n-butanol. The absorption spectra of the extracts were determined in a Unicam SP800 spectrophotometer.

Results

The volumes between which the various types of compounds were eluted are shown in Table 1. During elution the *neo*inositol was slightly retarded and the inorganic phosphorus to a greater extent, relative to the reducing sugars.

TABLE 1

RANGE OF ELUTION VOLUMES FOR VARIOUS COMPOUNDS FROM WHEAT GLUTEN

Compounds were detected by the indicated methods in the eluate obtained from a column of Sephadex G25 (bed volume 167 ml) on development with phenol–acetic acid–water (1:1:1 w/v/v)

Compounds	Method of Detection	Elution Volume (ml)	Compounds	Method of Detection	Elution Volume (ml)
Polypeptides	1	63–85	*neo*Inositol	7	160–194
Amino acids and peptides	2	63–85, 85–114, 114–137, 137–166	Glycerol	7	125–160
			Inorganic phosphate	8	177–211
Lipids	3	125–160	Phenolic compounds	9	125–160
Polysaccharides and/or lipids	4	125–160	Aromatic and heterocyclic compounds	10	63–125*; 125–166†
Carbohydrates	5	91–125, 125–171			
Reducing sugars	6	131–183	Pigments	11	63–85‡; 85–142§
Maltose	7	137–171			
Glucose	7	131–183			
Fructose	7	137–171			

* Faint fluorescence. † Bright light blue fluorescence. ‡ Salmon colour. § Orange colour.

The absorption spectra of the ethyl acetate and n-butanol extracts showed broad absorption peaks between about 265 and 285 nm and indicated that small amounts of free phenolic compounds were present.

Discussion

It is apparent that compounds of low molecular weight are not readily washed from gluten by water but are readily extracted during dialysis by the phenol–acetic acid–water solvent and 10% (v/v) acetic acid. The properties and use of this solvent

in cereal chemistry have been described elsewhere (Synge 1964; Gallus and Jennings 1968; Jennings 1968; Stanley, Jennings, and Nicholas 1968). The retention of several of these compounds in washed gluten is readily explained by strong interactions through either ionic, hydrogen, or hydrophobic bonds.

There is no ready explanation for the occurrence of reducing sugars, *neo*inositol, and glycerol in washed gluten. It is unlikely that they, or sucrose, would be strongly adsorbed to the macromolecular components of dough, and little, if any, enzymic activity occurs in the presence of the phenol–acetic acid–water solvent (Bagdasarian *et al.* 1964). Control tests showed that sucrose is rapidly hydrolysed in the eluting solvent used but maltose is stable.

Physical entrainment of the sugars, *neo*inositol, and glycerol within the pores of the gluten gel network would seem the most likely explanation although hydrogen bonding cannot be discounted completely. Disruption of the gel structure by the phenol–acetic acid–water solvent would release these neutral compounds.

Since there was no apparent specific retention of individual compounds in washed gluten, the amounts of the various types of compounds of low molecular weight present in dough or washed gluten were not determined.

The retention by physical adsorption or mechanical entrainment in washed gluten of apparently all compounds of low molecular weight occurring in flour or produced enzymically during the resting stage in the washing of gluten makes it impossible to predict which types of compounds are most likely to markedly affect the rheological properties. It is apparent that the effects of each type will have to be determined separately.

The retention of such a wide range of compounds of both high (Kent-Jones and Amos 1957, p. 146; Pence, Nimmo, and Hepburn 1964) and low molecular weight (Table 1) by adsorption and entrainment in washed gluten emphasizes the complex interactions which occur in dough and gluten.

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References

BAGDASARIAN, M., MATHESON, N. K., SYNGE, R. L. M., and YOUNGSON, M. A. (1964).—*Biochem. J.* **91**, 91.

BENSON, A. A., ET AL. (1952).—*J. biol. Chem.* **196**, 703.

GALLUS, H. P. C., and JENNINGS, A. C. (1968).—*Aust. J. biol. Sci.* **21**, 1077.

GREENWAY, R. M., KENT, P. W., and WHITEHOUSE, M. W. (1953).—*Research, Lond.* **6**, 6S.

GURR, E. (1960).—"Encyclopaedia of Microscopic Stains." (Ed. E. Gurr.) p. 376. (Leonard Hill: London.)

HARRAP, F. E. G. (1960).—*Analyst, Lond.* **85**, 452.

JENNINGS, A. C. (1968).—*Aust. J. biol. Sci.* **21**, 1053.

JERMYN, M. A., and ISHERWOOD, F. A. (1949).—*Biochem. J.* **44**, 402.

KENT-JONES, D. W., and AMOS, A. J. (1957).—In "Modern Cereal Chemistry". 5th Edn. (Eds. D. W. Kent-Jones and A. J. Amos.) (Northern Publ. Co.: Liverpool.)

KIRBY, K. S., KNOWLES, E. J., and WHITE, T. (1953).—*J. Soc. Leath. Trades Chem.* **37**, 283.

MABRY, C. C., and TODD, W. S. (1963).—*J. Lab. clin. Med.* **61**, 146.

PENCE, J. W., NIMMO, C. C., and HEPBURN, F. N. (1964).—In "Wheat Chemistry and Technology". (Ed. I. Hlynka.) p. 234. (American Association of Cereal Chemists: St. Paul, Minn.)
PUSZTAI, A. (1966).—*Biochem. J.* **101**, 265.
RANDERATH, K. (1963).—"Thin-layer Chromatography." p. 176. (Academic Press: New York.)
STANLEY, P. E., JENNINGS, A. C., and NICHOLAS, D. J. D. (1968).—*Phytochemistry* **7**, 1109.
STEPKA, W. (1957).—In "Methods in Enzymology". (Eds. S. P. Colowick and N. O. Kaplan.) Vol. 3. p. 504. (Academic Press: New York.)
SWAIN, T. (1953).—*Biochem. J.* **53**, 200.
SYNGE, R. L. M. (1964).—*Metabolism* **13**, 969.
TREVELYAN, W. E., PROCTOR, D. P., and HARRISON, J. S. (1950).—*Nature, Lond.* **166**, 444.
WHITEHOUSE, M. W., BRESLER, A. E., and STAPLE, E. (1958).—*J. Chromat.* **1**, 385.
ZELENY, L. (1964).—In "Wheat Chemistry and Technology". (Ed. I. Hlynka.) p. 33. (American Association of Cereal Chemists: St. Paul, Minn.)

CORRIGENDUM

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p. 532: *For* -Phe-X-Y *read* -Phe-X-Y
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