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Studies on Pigment Destruction during Spaghetti Processing[1]

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ABSTRACT

The extent of pigment destruction during spaghetti processing under various conditions, i.e., with different substrates for lipoxidase, by mixing under an atmosphere of oxygen or with added lipoxidase, was studied. The greatest pigment loss occurred in doughs containing linoleic acid and mixed under oxygen. While inactivation of lipoxidase by steam-treatment of the semolina resulted in little or no pigment loss, increasing the enzyme activity by addition of purified wheat lipoxidase had no effect. Studies of pigment loss during various stages of processing showed that most of the pigment was lost during the drying cycle.

The reaction mechanism of pigment oxidation in spaghetti processing has not been elucidated as yet, but it is known to involve the lipoxidase-linoleate system (1). Friend (2) reported that the lipoxidase-linoleate system will oxidize beta-carotene, producing beta-carotene epoxide and three conjugated polyene ketones. Linoleic acid itself will undergo autoxidation in the presence of certain ions (3,4), and the oxidized acid in turn oxidizes carotenes. Dahle (5) reported that free polyunsaturated fatty acids play a significant role in pigment oxidation and that lipoxidase alone is an inadequate criterion by which to predict yellow pigment loss during processing of alimentary paste products.

According to Chichester and Nakayama (6), the oxidation of fatty acids such as linoleic acid is catalyzed by the presence of iron porphyrin compounds. Yet others have reported that lipoxidase requires no cofactors nor metal ions for activity (7). Recently Guss et al. (8) reported that wheat lipoxidase was relatively unreactive to trilinolein, methyl linoleate, and mono- and dilinolein.

This study was undertaken to investigate some of the factors associated with pigment oxidation in spaghetti processing. The effect of different substrates for lipoxidase added to semolina, the effect of mixing doughs under oxygen, and the effect of added purified wheat lipoxidase on pigment loss were studied. Secondly, the loss of pigment during various stages of processing by the micro macaroni method (9) was investigated.

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MATERIALS AND METHODS

Semolina milled from Stewart 63, a Canadian amber durum wheat variety, was used for most of the study. In a few experiments untreated commercially milled semolina was used.

For studying the extent of pigment loss during mixing with added substrates, doughs were mixed for 1.5 min. at 31% absorption in the micro macaroni mixer (9). Substrates used were linoleic acid, methyl linoleate, trilinolein, methyl linolenate, and trilinolenin; these were highly purified grade, 99.5% pure, from Nutritional Biochemical Corp. Ampoules of the substrates were divided into smaller volumes in small vials and sealed under nitrogen. To test for peroxide in linoleic acid, the thiobarbituric acid method as described and calibrated by Tsen and Hlynka (10) and the iodometric method for peroxide value described in "Standard Methods of the Oils and Fats Division of the I.U.P.A.C." (11) were followed. The level of peroxide in linoleic acid was found to be 21.0 μeq. of peroxide per g. of linoleic acid by the TBA method and 19.7 μeq. per g. by the iodometric method. To ensure that a homogeneous mixture of semolina and substrate was obtained, the substrate was added with a micropipet to about 15 ml. of petroleum ether. This solution was then added to 50 g. semolina and thoroughly mixed under nitrogen. The petroleum ether was then allowed to evaportate under a stream of nitrogen. After mixing, the dough was divided into three portions: The first portion was immediately frozen in liquid nitrogen, the second after 1 hr., and the third after 2 hr. Test pieces allowed reaction times were placed in a stoppered flask in a 30°C. water bath. These test pieces were then freeze-dried. The moisture and pigment contents of the ground freeze-dried samples were determined.

Pigment content was determined by measuring the absorption maximum of a water-saturated butanol extract at 445 mμ. Pigment content was expressed as p.p.m. lutein on 14% moisture basis using the extinction coefficient determined by Sims and LePage (12).

For studying the effect of oxygen on pigment loss, semolina samples were stored overnight in an atmosphere of oxygen, and then mixed in the micro mixer with oxygen passing through the mixing bowl at a rate of 1 liter per min. during mixing.

To inactivate lipoxidase, semolina samples were steam-treated in a manner described by Irvine et al. (13).

Purified wheat lipoxidase was obtained by ion-exchange column chromatography on DEAE (14). Lipoxidase activity was determined by the manometric method as described by Irvine and Anderson (15).

For studying the extent of pigment loss during various stages of processing, the micro macaroni method (9) was followed. Samples after various stages were frozen in liquid nitrogen and freeze-dried. Moisture and pigment contents were determined on the ground freeze-dried samples. Spaghetti was also processed from steam-treated semolina and the pigment of the dried product determined.

RESULTS

The effect of various substrates on pigment loss is presented in Table I. The pigment content of the semolina was the same as the control dough, since no loss in pigment could be detected after 1.5 min. of mixing. Pigment loss increases with

TABLE I. EFFECT OF VARIOUS SUBSTRATES, OXYGEN, AND ADDED LIPOXIDASE ON PIGMENT LOSS

Sample	moles	Pigment Content, Lutein Time After Mixing 0 hr. p.p.m.	1 hr. p.p.m.	2 hr. p.p.m.
Control		4.34 ± 0.07	4.12	4.06
Linoleic acid,	0.80×10^{-4}	3.88	3.68	3.60
Linoleic acid,	1.61×10^{-4}	3.82	3.62	3.50
Linoleic acid,	3.22×10^{-4}	3.70	3.52	3.42
Linoleic acid,	4.03×10^{-4}	3.62	3.46	3.25
Trilinolein,	0.92×10^{-4}	4.14	3.85	3.75
Methyl linoleate,	3.02×10^{-4}	4.30	4.09	3.82
Trilinolenin,	0.49×10^{-4}	4.32	4.10	4.00
Methyl linolenate,	3.4×10^{-4}	4.10	4.02	3.98
Dough mixed in O_2		4.28	4.06	3.95
Linoleic acid, mixed under O_2	3.22×10^{-4}	2.88	2.86	2.71
Steam-treated semolina mix under O_2		4.26	4.31	4.31
Steam-treated semolina with linoleic acid, mixed under O_2	3.22×10^{-4}	4.42[a]	4.21	4.21
Purified wheat lipoxidase		4.30	4.10	4.05
Linoleic acid, and purified wheat lipoxidase	3.22×10^{-4}	3.41	3.34	3.22

[a] A different sample of semolina was used. Pigment content = 4.42 p.p.m.

both increasing linoleic acid concentration and increasing reaction time. Adding 4.03×10^{-4} moles of linoleic acid, which is approximately a fourfold increase in the free linoleic acid concentration, in semolina, causes a 15% greater pigment loss than in the control dough after 2 hr. This level of peroxide present in this weight of linoleic acid was 1.95 μeq., which could contribute to some of the pigment loss.

With esters of linoleic and linolenic acids, the initial loss is much lower than with linoleic acid, but pigment loss increases with time. This time dependence might be attributed to lipase hydrolyzing the esters to release the free fatty acid.

Oxygen by itself has little effect on reactions leading to pigment oxidation. However, in the presence of free linoleic acid there is a significant pigment loss, with about one-third oxidized after mixing. Increasing the level of lipoxidase to twice the level of the control did not affect the extent of pigment destruction. However, in the presence of added linoleic acid, increasing the lipoxidase activity increases pigment loss. That lipoxidase is definitely involved in pigment destruction is shown by the results when steam-treated semolina was used. Steam-treatment effectively inactivates lipoxidase, and pigment loss 2 hr. after mixing is zero. Furthermore, even with added linoleic acid and mixing under oxygen, there is very little (4.5%) pigment loss.

These results indicate that the secondary reaction, that of pigment oxidation, is dependent, first, on lipoxidase, and second, on the levels of linoleic acid and oxygen. Lipids, particularly unsaturated fatty acids such as linoleic acid, do undergo autoxidation but the oxidation products do not appear to be involved in pigment destruction.

TABLE II. PIGMENT DESTRUCTION DURING VARIOUS STAGES OF PROCESSING

Stage	Pigment Content p.p.m.	Pigment Loss %
1	4.34	0
3	4.06	5.0
4	3.93	9.5
5 5 hr.	3.84	11.5
14 hr.	3.81	12.3
29 hr.	3.47	20.0

The micro macaroni method which was used to follow the extent of pigment loss during various stages of processing involves the following steps.

1. Dough is mixed for 1.5 min., the last 30 sec. under vacuum.
2. Dough is kneaded and subsequently rested under pressure (2,000 lb./sq.in.) for 10 min.
3. Dough is extruded at a pressure of about 600 lb./sq.in.
4. Spaghetti is allowed to dry at room temperature and humidity for 1 hr.
5. Spaghetti is then placed in humidity- and temperature-controlled cabinet for 29 hr.

Table II gives the percentage of pigment lost after various stages. Most of the pigment loss, about 75%, occurs after the sample is extruded (stage 3). The work input during extrusion accounts for only about 5% of the pigment loss. Tsen and Hlynka (10) showed that peroxides are formed during mixing and that the amount formed is dependent, among other things, on the time of mixing and the presence of oxygen. However, peroxides formed by autoxidation do not appear to be involved in pigment oxidation, since there is very little, if any, pigment loss in spaghetti processed from steam-treated semolina. Results indicate that reactions involving pigment oxidation involve primarily the lipoxidase-catalyzed system.

Results of this study support Dahle's statement (5) that free polyunsaturated fatty acids play a significant role in pigment oxidation. While lipoxidase is definitely involved in pigment destruction, its level of activity is not as important as the availability of substrate.

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