POTENTIAL OF WPI EDIBLE OXYGEN BARRIER COATINGS FOR REDUCTION OF WALNUT RANCIDITY

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ABSTRACT

A methodology to evaluate rancidity in walnuts was established. This method was applied to compare the rancidity process in walnuts at two oxygen levels and two relative humidities. Significantly reduced rancidity at the lower oxygen level shows that there is considerable potential for the application of edible oxygen barrier coatings on walnuts stored at intermediate relative humidity.

Several approaches were taken to coat the walnut surface (highly hydrophobic) with a whey protein isolate (WPI) solution (water based). These included reducing coating solution surface tension, changing walnut surface characteristics and increasing coating solution viscosity. The last one was used as the basis for the coating procedure. By aging the WPI solution, it was possible to increase the viscosity enough to create a continuous layer around the nut. Furthermore, a special dipping/drying method was developed to obtain a uniform coating around the nut. Currently, different WPI-based formulations are being evaluated for ability to retard rancidity in walnuts.

OBJECTIVES

Rancidity is a major problem in walnuts during storage. Rancidity in walnuts is the development of an unacceptable off-flavor resulting from the oxidation of lipids. Oxygen concentration is one of the factors that affect lipid oxidation. Since WPI edible films have very low oxygen permeability, it was hypothesized that a WPI coating on walnuts could reduce their internal oxygen concentration and therefore delay the rancidity process. Towards testing this hypothesis, the following objectives have been achieved thus far in FY 94/95:

1.- Establishment of an accelerated methodology to evaluate rancidity in walnuts
2.- Assessment of the rancidity process in walnuts at low and high oxygen atmospheres
3.- Development of a methodology to coat walnuts with WPI solution

PROCEDURES

Peroxide value (PV) was determined by the method described by Chapman and Mackay (1949). Oil was extracted from the nuts by cold-pressing.

The method described by Frankel and Huang (1994) was used with slight modifications. Nut samples were ground in a regular coffee grinder. A 0.55 g sample and 1 mL deionized water were placed into 6 mL vials. Then, they were sealed with
silicone rubber Teflon caps and heated to 65 °C for 15 minutes. The headspace was analyzed using a Perkin-Elmer Sigma 3B gas chromatograph with an H-6 headspace sampler (Norwalk, CT) and a capillary DB-1701 column (30 m × 0.32 mm, 1 mm thickness; J&W, Folsom, CA) heated isothermically at 65 °C. Hexanal was identified by comparison with the retention time of reference components. Peaks areas were integrated electronically (C-R3A Chromatopac; Shimazu Co., Kyoto, Japan). Peak areas corresponding to hexanal was related to hexanal concentration by a standard curve with known added concentrations of hexanal.

Several 1.5 liter glass containers with airtight lids and saturated salt solutions in the bottoms were used to maintain a constant relative humidity (RH) around the walnuts. Potassium acetate (CH₃COOK) was used to provide a low relative humidity (21% at 37°C; ASTM, 1985) and sodium bromide (NaBr) was used to provide an intermediate relative humidity (53% at 37°C; Kitic et al., 1986). Preparation of the solutions was done according to standards (ASTM, 1985).

Oxygen concentration was measured using the method described by Saltveit and Strike (1989) with an oxygen analyzer model S-3A from Applied Electrochemistry Inc. (Sunnyvale, CA).

Surface tension was measured using a Fisher Surface Tensiometat (Fisher Scientific Co., Pittsburgh PA).

Viscosity was measured using a Rotovisco RV-20 (Haake Mess-Technik GmbH Co., Karlsruhe, Germany).

RESULTS AND DISCUSSION

Establishment of an accelerated methodology to evaluate rancidity on walnuts

This methodology consisted of a controlled accelerated rancidity test and two chemical analyses to quantify the rancidity level.

In order to establish a repeatable test of walnuts rancidity, temperate, RH, light and oxygen concentration were controlled during the experiment. Walnut were kept at 2°C until the moment needed for an experiment. Then, walnuts were placed in closed air-tight containers in which the relative humidity was controlled by means of saturated salt solution (Figure 1). These containers were placed in storage rooms with controlled temperatures (37°C or 29°C to accelerate the rancidity process). In order to eliminate the effect of light, containers were wrapped in aluminum foil. Oxygen concentration inside the containers were measured periodically using the method described above.

Two chemical methods were set up in order to quantify the rancidity level of walnuts. These methods were chosen based on the mechanism that Greeve et al. (1986) proposed to describe the walnut rancidity process: as a first step, polyunsaturated triacylglycerols react with lipases without the use of oxygen and to produce free fatty acids (FFA). As a second step, FFA react with lipoxygenase or heme or inorganic iron and oxygen. As a consequence, lipoperoxides and then rancid products such as hexanal are produced.
Since our purpose is to delay the rancidity process by reducing the amount of oxygen in contact with the walnut, the second step of the rancidity process was our target. Therefore, the production of FFA could not be used as a way to measure rancidity (as used currently by the industry). Measurement of hydroperoxides (PV) and volatiles (hexanal content in static headspace analysis) were considered directly related to the possible effect of a reduced oxygen atmosphere.

Assessment of rancidity at low and high oxygen content

The methodology described above was used to quantify and compare the rancidity process in walnuts stored in atmospheres with high oxygen content (21%) and low oxygen content (<2.5%). This comparison simulates the range of a possible coating performance. Oxygen content of 21% represents the uncoated nuts, and <2.5% oxygen represents an excellent oxygen barrier coating.

Two relative humidities were studied: 1) An intermediate relative humidity (=55%) commonly used to store walnuts (Anon, 1993), and 2) a low relative humidity (=20%) at which WPI edible films have optimum oxygen barrier characteristics (McHugh and Krochta, 1994).

Shelled Persian walnut halves (Chandler variety) were supplied by Diamond Walnut Growers, Inc. (Stockton, CA). This variety was chosen for its high polyunsaturated fatty acid composition. One glass container was assigned to each of the combinations of the variables that were studied: i) air (21% oxygen) and N2-flushed atmosphere (<2.5% oxygen). ii) 53% RH and 21% RH. A 350 g sample of walnuts were placed in each of the 4 containers (2 low oxygen containers and 2 high oxygen containers; Figure 1). Aluminum foil was used to cover all containers to provide dark conditions. All containers were placed into a controlled temperature room at 37°C for 12 weeks.

A 30 g sample was collected after 4 weeks. After this, samples were collected every 2 weeks to follow the evolution of the rancidity process. Moisture content, static headspace analysis and PV were performed in duplicate with all the samples. A low oxygen concentration was maintained in the low-oxygen containers by flushing with nitrogen after obtaining the samples. Samples of the air in the bottles before opening the lid and after the flushing were analyzed. The oxygen concentration was always below 2.5% during the experiment and was reduced to 0.1% after each flushing. Moisture equilibrium was reached before the first walnut sampling. Moisture contents for walnuts stored at 53% and 21% were 3.0% and 1.8%, respectively.

Hexanal content in a static headspace analysis and PV values were confirmed to be useful tests to quantify rancidity process in walnut (Figures 2 and 3). After 28 days of storage there was a clear difference between nuts stored at high or low oxygen concentration. The values for hexanal content and PV for high oxygen samples started increasing while the values for low oxygen content remained basically constant. This reflects an induction period of about 28 days for high oxygen samples.

The increase in hexanal content or PV was much more important in walnut stored at 53% RH than those stored at 21%. This indicates that walnuts are more
susceptibility to lipid oxidation at 53% RH than at 21% RH. As a consequence, there is greater opportunity for impact of an edible film at an intermediate relative humidity (53%) compared to at low relative humidity (21%) (Figures 2 and 3).

Development of a methodology to coat walnut with WPI solution

It was observed that after a walnut was dipped into a WPI solution (prepared by the procedure described by McHugh and Krochta, 1994), there was a process of dewetting that prevented the creation of a continuous layer around the nut. This showed that the surface tension of the walnut was lower than the surface tension of the solution. The low surface tension of the walnut surface reflected a hydrophobic nature that is a consequence of the high oil content of the nutmeat.

In order to coat the walnut (hydrophobic surface) with a WPI solution, the following approaches were evaluated:

1.- Reduced surface tension coating solution

Different food-grade surfactants were added into the protein solution to decrease the surface tension in order to ease the wetting process. Increasing amounts of each individual surfactant were added into the solution until the surface tension was not varying (i.e. until the concentration was above the critical micelle concentration; Figure 4). Span-20 was the surfactant that reduced the surface tension of the solution the most (from 56 to 32 dynes/cm). This low surface tension was not enough to avoid the dewetting process. The problem came specially from the hydrophobic nature of the pellicle of the walnut surface.

2.- Modified walnut surface characteristics

Increasing the roughness of the surface would help to maintain the solution on the surface. Walnuts were brushed with brass brushes. The results increased the roughness of the surface, but it was not enough to ease the coating process. Furthermore, this procedure would create injuries to the surface that could accelerate the lipid oxidation process.

3.- Increased viscosity coating solution

If the viscosity of a solution is high enough it will not move even when the surface tension of the solution is lower than the surface tension of the contact surface.

Hydroxypropylmethylcellulose was used as a thickener. Adding 0.25% to the solution increase the viscosity enough to create a continuous coating around the nut.

Later on, it was observed that the viscosity of WPI solutions increases with time. The larger the amount of the protein in the solution the quicker the increase. In order to quantify this observation, the viscosities of 11% WPI / 11% glycerol and 11% WPI/ 7.3% glycerol solutions were measured daily for 12 day using the method described above. Figure 5 shows the evolution of the rheological properties of the former solution. Since the viscosity decreases with shear rate, the solution can be
considered a shear-thinning fluid. At the same time, viscosity increased with time for any shear rate. Figure 6 showed the evolution of the viscosity for one particular shear rate (100 s⁻¹). Viscosity is higher and increased more quickly for a solution with more protein. Since the protein was denatured, it is possible that disulfide bonds are being formed slowly with time. These bonds increase the length of the chains of protein that are in the solution, and as a consequence the viscosity increases. After a certain time, the number of bonds is so high that the solution turns into a gel.

Since the viscosity increases with decreased temperature, 5°C was chosen as the solution temperature to perform the coating. It was found that allowing a 11% WPI/7.3% glycerol solution to increase in viscosity for 1 week was enough to maintain the coating on the nut surface, thus avoiding the dewetting process. It was necessary to wait 10 days for a 11% WPI/11% glycerol solution to be used as coating.

Once the solution had the required viscosity, walnuts were dipped into it and dried with hot air (30°C). In order to make the continuous layer as uniform as possible walnuts were dried and rotated at the same time thus the coating was distributed evenly during the drying process (Figure 7).

The thickness of the final coating could be increased not only by increasing the viscosity of the solution, but also by increasing the number of times the nuts were dipped into the solution.

CONCLUSIONS

An accelerated methodology to evaluate rancidity in walnuts was established. This methodology includes: 1) control of the main parameters that affect lipid oxidation, and 2) two chemical tests to quantify rancidity in walnuts. It is important to point out that hexanal content in static head space analysis was found to be easy, quick and reliable to monitor the rancidity process.

Results showing significantly lower rancidity in low-oxygen atmosphere point to the potential advantage of an edible coating as oxygen barrier on nuts. This is especially true for intermediate RH at which walnuts are commonly stored. There is also a potential for lower RH, although the range of oxygen-barrier-coating action is smaller.

A procedure was developed to coat walnut with a WPI solution. This includes a waiting period for the WPI solution to increase in viscosity. Dipping was used to coat the walnuts with the solution, and drying with hot air while the nuts were rotating improved the uniformity of the coating on the walnut.

At this moment different coating formulations are being tested for ability to reduce walnut rancidity. Preliminary Probably results may be available for the Annual Walnut Research Conference.
REFERENCES


Figure 1: Containers for high and low oxygen contents
Figure 2: Hexanal content in static headspace analysis for Persian walnuts stored at 37°C.
Figure 3: PV for Persian walnuts stored at 37°C
Figure 4: Apparent surface tension of a 10% WPI/6.67% glycerol solution with different surfactants above their critical micelle concentration at room temperature.
Figure 5: Variation of the rheological properties measured at 5°C of a refrigerated 11%WPI / 11% glycerol solution with time
Figure 6: Effect of time on the viscosity of WPI solutions maintained refrigerated (viscosity measured at 5°C and shear rate of 100 s⁻¹).
Figure 7: Procedure used to dip and dry the coatings on the walnut surface.