KINETICS OF HEAT-INDUCED COLOR CHANGE OF
A TUNA-VEGETABLE MIXTURE

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SUMMARY

Heat induced color change kinetics in a tuna-vegetable mixture was evaluated by measuring color parameter "L" (Hunter-Lab) and 5-hydroxi-methyl-furfural (5-HMF) accumulation. For this purpose small reusable stainless steel TDT cans were used and the kinetic studies performed in a temperature range characteristic of thermal processing of low acid canned foods (110-125°C). The color parameter L was better described by a pseudo zero order while a pseudo first order reaction was found for 5-HMF accumulation, indicating the progression of the Maillard reaction during heating. In both cases, temperature dependence of the rate constants followed the Arrhenius relationship, with Ea= 92.0 and 38.9kJ·mol^{-1} for lightness L and for 5-HMF accumulation, respectively. The color parameter L was more sensitive to temperature changes than 5-HMF accumulation, and therefore is suggested for evaluation of heat induced color changes in this product.

CINÉTICA DEL CAMBIO DE COLOR INDUCIDO POR EL CALOR EN UNA MEZCLA DE ATÚN CON VEGETALES

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RESUMEN

Se evaluó la cinética del cambio de color inducido por el calor en una mezcla de atún con vegetales mediante la medición del parámetro de color "L" (Hunter-Lab) y la acumulación de 5-hidroxi-metil-furfural (5-HMF). Los estudios cinéticos se llevaron a cabo en un rango de temperatura característico del procesamiento térmico de alimentos de baja acidez (110-125°C), empleando envases TDT reutilizables. El parámetro de color L tuvo un mejor ajuste a una cinética de pseudo orden cero, mientras que la acumulación de 5-HMF se ajustó a una cinética de pseudo orden uno, indicando el desarrollo de la reacción de Maillard durante el calentamiento. En ambos casos, la dependencia de la constante de la velocidad de reacción con la temperatura se ajustó a la ecuación de Arrhenius con Ea= 92,0 y 38,9kJ·mol^{-1} para la luminosidad L y para la acumulación de 5-HMF, respectivamente. El parámetro de color L fue más sensible a los cambios de temperatura que la acumulación de 5-HMF y por consiguiente se sugiere para la evaluación de los cambios de color inducidos por el calor en este producto.

Introduction

One of the recent worldwide developments in the canned tuna market has been the introduction of a wide range of tuna salads, tuna in sauces and tuna paste. Color is one of the most important quality attributes of tuna from the consumer point of view. Previous studies in traditional canned tuna (Banga, *et al*., 1993) have shown that product color does not appear to be largely affected by thermal processing over a wide temperature range.

In the case of vegetables, many natural pigments such as chlorophyll and carotenoids are easily degraded by heat; also, browning reactions such as the Maillard reaction and oxidation of ascorbic acid can take place (Barreiro *et al*., 1997). Haard (1992) pointed out that heat induced browning development is a complex process that can involve, besides the Maillard reaction between reducing sugars and fish proteins, other reactions such as protein-lipid interaction and degradation of fish haemoglobins and carotenoids. The design of thermal processes for mixed tuna-vegetable canned products should take into account the possible loss of quality by color reactions resulting from interactions between the basic ingredients as well as others commonly found in this type of product, such as sugar, salt, vinegar, oil and seasonings.

Banga *et al*. (1993) studied the kinetics of thermal induced surface color in canned tuna, using small cans (3.26cm radius and 3.0cm height) and heating temperatures from 110.6 to 139.2°C. These authors found that color changes during thermal processing were small and lightness (Hunter-Lab "L") was the color parameter that better modeled subjective color evaluation of this product, reporting a first order kinetics. Kong *et al*. (2007) determined the kinetics of salmon quality changes during thermal processing, including CIE L* value, using a small test cell (35mm diam by 6mm height) in a range of 100-131.1°C and processing time of 90-180min, finding a zero order kinetics for L*.

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CINÉTICA DA MUDANÇA DE COR INDUZIDO PELO CALOR EM UMA MISTURA DE ATUM COM VEGETAIS

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RESUMO

Foi avaliada a cinética da mudança de cor induzido pelo calor em uma mistura de atum com vegetais mediante a medição do parâmetro de cor "L" (Hunter-Lab) e a acumulação de 5-hidroxi-metil-furfural (5-HMF). Os estudos cinéticos foram levados a efeito em uma faixa de temperatura característica do processamento térmico de alimentos de baixa acidez (110-125°C), empregando envases TDT reutilizáveis. O parâmetro de cor L teve um melhor ajuste a uma cinética de pseudo ordem zero, enquanto que a acumulação de 5-HMF foi ajustada a uma

cinética de pseudo ordem um, indicando o desenvolvimento da reação de Maillard durante o aquecimento. Em ambos os casos, a dependência da constante de velocidade de reação com a temperatura foi ajustada à equação de Arrhenius com Ea= 92,0 y 38,9kJ·mol⁻¹ para a luminosidade L e para a acumulação de 5-HMF, respectivamente. O parâmetro de cor L foi mais sensível às mudanças de temperatura que a acumulação de 5-HMF e, por tanto, é sugerido para a avaliação das mudanças de cor induzidas pelo calor em este produto.

Banga *et al.* (1992) found that the lysine content of tuna meat was not significantly affected during thermal processing, which evidenced that the Maillard reaction did not contribute to thermal induced browning. This could be expected since the amount of reducing sugars in fishery products is negligible (Hurrel and Carpenter, 1977) and they are needed for the Maillard reaction to take place. On the other hand, the vegetable mixture usually found in specialty tuna products includes ingredients that provide a significant amount of reducing sugars. Consequently, a Maillard reaction between reducing sugars present in the vegetable mixture and tuna protein aminoacids is to be expected during thermal processing. Additionally, pH and a_w values of tuna-vegetable mixtures categorize the products as low-acid food requiring high temperature processing, which in turn enhances chemical reactions such as non-enzymatic browning.

The compound 5-hydroximethyl-furfural (5-HMF) is an indicator frequently used to study the development of the Maillard reaction in foods, including canned products and other thermally processed foods (Poretta, 1992). 5-HMF is an intermediate reaction product in the formation of brown pigments or melanoidins (Eskin, 1990).

Browning during thermal processing of canned tuna-vegetable mixture has been reported as a significant problem

by the industry. The knowledge of the kinetics of heat induced color changes in this product is important to optimize quality during commercial sterilization. No research work for this particular product was found in the literature, being the works of Banga *et al.* (1993) in tuna and Kong *et al.* (2007) in salmon the only research found for fishery products in the reviewed literature. Consequently, the aim of this study was to study color degradation kinetics using the parameters "L" (Hunter-Lab) and the accumulation of 5-HMF during heating of a tuna-vegetable mixture at temperatures representative of commercial thermal processing.

Materials and Methods

Sample description and preparation

The commercial mixture of tuna and vegetables used in this study was obtained from a local plant located near Cumaná, Venezuela. The mixture consisted on 70% (w/w) precooked yellowfin tuna (*Thunnus albacares*) and 30% (w/w) of a mixture of blanched frozen peas and carrots cut in cubes, seasoned with spices, sugar, and salt dissolved in water, vegetable oil and sugarcane vinegar (ratio filling liquid/tuna-vegetable mixture=0.12). This commercial mixture is usually canned and sterilized. However, in this study the raw product without thermal treatment was used.

About 2kg of sample (drained weight equal to 88g/100g) were ground and homogenized in a high speed blender (Ultra-turrax). The puree was strained using a kitchen colander. The prepared samples were kept frozen (-18°C) in glass jars, protected from light with aluminum foil, and properly closed until used for the experiments.

Physical and chemical characteristics of the tuna-vegetable mixture

The following analyses were carried out to characterize the tuna-vegetable mixture used in this study: Proximate analyses were done using the following procedures according to AOAC (1990). Moisture: vacuum oven method (<25mm Hg) at 70°C until constant weight (procedure 934.01); protein: total N_2 was determined by the Kjeldahl method for seafood (procedure 940.25); crude fat: by solvent extraction in a Soxhlet apparatus (procedures 954.02 and 963.15); ash: using a muffle furnace at 525°C for 12h (procedure 938.08); carbohydrates by difference. The mixture pH was determined by procedure 981.12 (AOAC, 1990) for acidified foods using a pH meter (Radiometer, PHM23). Water activity (a_w) was measured using psychrometric equipment (Decagon CX-2).

Lightness determination

After carefully mixing and thermally processing the

samples, lightness (color parameter L) was determined using a tristimulus colorimeter Gardner XL-23 (Gardner Instruments, Bethesda, MD, USA). The equipment was calibrated against a standard tile N° XL-23-137-C with the following color parameters: Y= 61.28; X= 59.65; Z= 43.12. A cylindrical glass cell (55mm diam) was used for the sample. Lightness measurements were done by triplicate.

Determination of 5-hydroxi-methyl-furfural

The formation of 5-HMF as an indication of the Maillard reaction was measured at each tested condition by duplicate according to the procedure described by Bravo *et al.* (2002). For each thermal treatment a small sample portion (2ml) was drawn and centrifuged at 16000g for 10min in a microcentrifuge (Eppendorf 5415). The aqueous supernatant was filtered through 0.45μm PVDF (polyvinyidene fluoride; Millipore). A volume of 50μl of each sample was injected in a HPLC apparatus (Waters LC module I), operating with a column Aminex HPX-87H, 300×7.8mm (BioRad) at 55°C; mobile phase of H_2SO_4 0.01N; flow rate of 0.8ml·min⁻¹; and a 283nm UV detector.

The 5-HMF concentration was calculated in each case from the area of the chromatographic peak (retention time: 23.7min), using a previously obtained calibration curve prepared using aqueous solutions

of 5-HMF (analytical grade, Sigma-Aldrich) of known concentrations.

Kinetic studies for browning during heating

To study browning developed in the samples taking place during heating, a modified thermal death time (TDT) method similar to that described by Stumbo (1973) was followed. For this purpose, stainless steal reusable TDT cans (74 and 69.4mm of external and internal diameter, and inner height of 17.1mm) were specially designed and built for this work. TDT cans were provided with screw caps and O-rings to assure hermetic closure and were filled with 20.0 ±0.5g of sample, avoiding the incorporation of air bubbles, in order to have enough quantity to allow the measurement of the color parameters in the colorimeter as indicated previously. The TDT cans were provided with a packing gland on top to fit a thermocouple. Two TDT cans were used for each time-temperature treatment tested. A thermostatic oil bath (2L, Technicon) provided with a digital temperature control (±0.5°C, Tecnologic, Italy) was used for the kinetic studies.

Kinetics of browning in the tuna and vegetable mixture was determined at four processing temperatures (110, 115, 120 and 125°C) and heating times from 5 to 60min. All time-temperature combinations were tested in duplicate. After the heat treatment was applied the TDT cans were removed and quickly cooled to -30°C in order to stop the heat treatment.

Once the samples were heated and cooled at the established time and temperature, measurement of lightness (L) and 5-HMF concentration were determined as indicated previously. The apparent order of reaction for these indicators was obtained by the adjustment of the experimental data to the integrated kinetic equations for orders 0 and 1, using regression analysis. In each case the best fit was selected using as criterion the linear correlation coefficient (R^2) and the rate constant (k) at each temperature determined from the slope of the straight line. The effect of temperature on the rate constant was calculated from the linear form of the Arrhenius equation

$$1n\ k= 1n\ ko - Ea/R\ T \qquad (1)$$

where k_o: pre-exponential factor (min⁻¹), E_a: activation energy (kJ·mol⁻¹), R: universal gas constant (kJ·mol⁻¹·K⁻¹), and T: absolute temperature (°K). The value E_a was calculated from the slope, and k_o from the intercept of the straight line given by Eq. 1, using a linear regression program. All the statistical analyses were performed by means of the Excel ver. 2003 package (Microsoft).

Results and Discussion

Physical and chemical characteristics

The results obtained for the proximate analysis of the tuna-vegetable mixture used in this work were (g/100g ±SD) moisture= 68.0 ±1.0, protein= 14.8 ±0.6, fat= 11.9 ±0.3, ash= 1.72 ±0.07, and carbohydrates =3.6. Other physical-chemical characteristics of the mixture
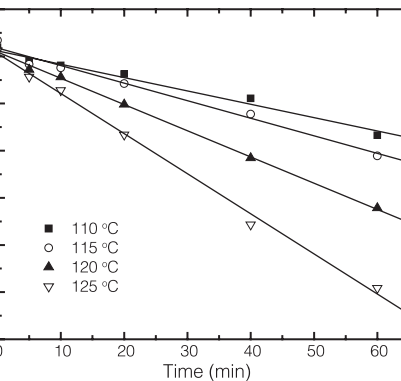

Figure 1. Kinetics of thermal induced browning in tuna-vegetable mixture measured by lightness evolution (L), in a temperature range of 110-125°C.

were (±SD): pH= 5.3 ±0.1, and $a_{w(23±0.6°C)}$= 0.983 ±0.002.

Kinetics of browning during heating

The change in color during thermal processing of foods is postulated to take place by various mechanisms, including the degradation of pigments, oxidation of ascorbic acid, and the Maillard reaction (Serkat and Luh, 1976; Okitani et al., 1983; Petropakis and Montgomery, 1984). These reactions can take place simultaneously but at a different reaction rate, depending on the color factor and the mechanism involved. Therefore, the color reaction measured shows an apparent order that accounts for the global result of heat-induced color change. Since there is probably more than one compound or color factor involved, the reaction does not necessarily take place through a single mechanism. Temperature induced browning during thermal treatment in a range of 110-125°C was assessed by measuring lightness variation and 5-HMF accumulation.

Color parameter L

Results obtained for lightness or color parameter L are presented in Figure 1. As observed in this figure, the L value diminished with time, indicating the progress of heat-induced browning in the mixture during heating. A slightly better adjustment for the L parameter was obtained for a pseudo-zero order kinetics, although a good adjustment for an apparent first order reaction was also obtained. Results obtained for both orders of reaction are presented in Table I. In all cases, highly significant linear regressions (p<0.001) were obtained, with high coefficients of determination. For the purpose of this research work a pseudo-zero order kinetics was selected.

An apparent zero order reaction for heat-induced changes of the color parameter L has been determined by various authors such as Kanterewicz and Chirife (1986) in cheese whey, using L* and other color parameters, Bates et al. (1998) in a starch-glucose-lysine system, Tosun (2004) in a Turkish food (zile pekmezi) at 55-75°C for nine days, Kumar et al. (2006) for the lightness value (L*) during deep-frying of Gulabjamun balls, and Kong et al. (2007) for L* values in salmon muscle during thermal processing at high temperature (100-131.1°C). On the other hand, apparent first order kinetics has been reported in the literature by other authors, like Banga et al. (1993) in canned tuna for L, Shin and Bhowmik (1995) in pea puree during heating and using Hunter Lab values, Kwok et al. (1999)

TABLE I

REGRESSION PARAMETERS FOR PSEUDO ZERO AND FIRST ORDER REACTIONS FOR LIGHTNESS COLOR PARAMETER IN A TUNA-VEGETABLE MIXTURE DURING HEATING

Parameter	Zero order				First order			
	Temperature (°C)				Temperature (°C)			
	110	115	120	125	110	115	120	125
k_o or $ln(k_o)$*	50.7	50.9	50.5	50.3	3.927	3.926	3.929	3.947
-k (min⁻¹)	0.145	0.187	0.279	0.426	0.003	0.004	0.007	0.012
R^2	0.983	0.983	0.997	0.997	0.972	0.985	0.997	0.997

* k_o for zero order kinetics and $ln(k_o)$ for first order kinetics.

for L* in soymilk in a temperature range from 80-140°C, and Demir *et al.* (2001) in hazelnuts during roasting (120-180°C) for color parameter L.

The value of the rate constant (-k) increases with a rise in temperature. The dependency of k on temperature for the pseudo-zero order reaction was determined using the linearized Arrhenius Eq. 1, as shown in Figure 2. A value of E_a= 92.0kJ·mol^{-1}, and lnk_o= 26.9min^{-1} were obtained. The linear regression analysis showed a significant (p<0.01) value with a determination coefficient R^2= 0.987. A similar value (87.99kJ·mol^{-1} was reported for L* in salmon by Kong *et al.* (2007) in a comparable temperature range (100-131.1°C). Banga *et al.* (1993) determined a z value of 44°C (E_a= 67kJ·mol^{-1}) for surface L values in canned tuna in a temperature range similar to that used in this work.

5-HMF accumulation

5-HMF accumulation was better described within the temperature range studied by a pseudo-first order reaction. The results obtained are presented in Table II and Figure 3. A similar reaction order has been obtained for 5-HMF accumulation in citrus juice concentrates stored for eight weeks in a range of 28-45°C (Koca *et al.*, 2003). Other authors, however, have generally reported a zero order reaction for 5-HMF accumulation during non-enzymatic browning (Cerrutti *et al.*, 1985; Tosun, 2004; Koca *et al.*, 2007). Since 5-HMF is an intermediate compound in non-enzymatic browning reactions, it is generated and at the same time consumed
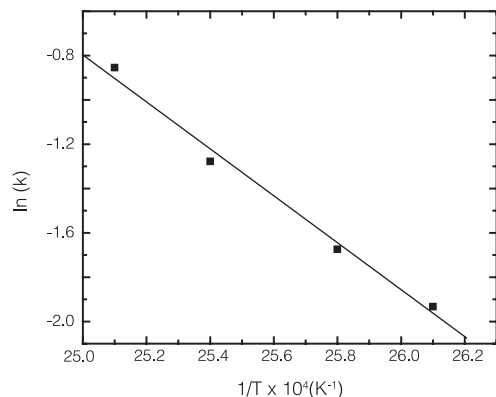

Figure 2. Arrhenius plot for color parameter L of a tuna-vegetable mixture.

TABLE II
PSEUDO FIRST ORDER REACTION RATE FOR 5-HMF ACCUMULATION IN A TUNA-VEGETABLE MIXTURE DURING HEATING

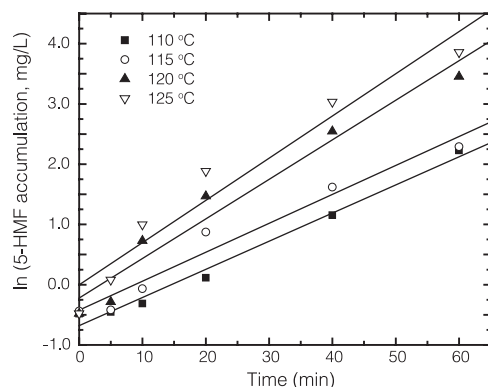| Temperature (°C)	-k (min^{-1})	R^2
110	0.047	0.987
115	0.048	0.966
120	0.066	0.956
125	0.071	0.944

Figure 3. Kinetics of 5-HMF accumulation during thermal treatment of a tuna-vegetable mixture, in a temperature range of 110-125°C.

to produce brown pigments. At any moment, the accumulated amount of 5-HMF represents a balance between its production and consumption in the global reaction. In this case, elevated sterilization temperatures as those used for low-acid foods were involved (110-125°C), something not usually found in this type of studies. Consequently, 5-HMF was pro-

Figure 4. Arrhenius plot for 5-HMF accumulation of a tuna-vegetable mixture.

duced at a faster rate in a relatively short period (up to 60min), showing an induction period of about 20min. A zero order reaction is the simplest model to describe the Maillard reaction without an induction period (Warmbier *et al.*, 1976; Labuza and Saltmarch, 1981; Baiser and Labuza, 1992; Narayan and Cross, 1992). Although a zero order reaction can be used to model brown pigment formation, provided that the amount of reactants are not limiting, the reaction is not strictly a zero order reaction because usually an induction period exists before brown pigment formation starts (Labuza and Saltmarch, 1981; Labuza, 1994; Bates *et al.*, 1998). In this case, when the induction period was considered, the best adjustment was obtained for a pseudo-first order reaction. In any case, browning is a very complex reaction that can be only modeled by a pseudo order of reaction.

The effect of temperature on 5-HMF accumulation could be taken into account by means of the Arrhenius equation (Figure 4), with values of E_a= 38.9kJ·mol^{-1}
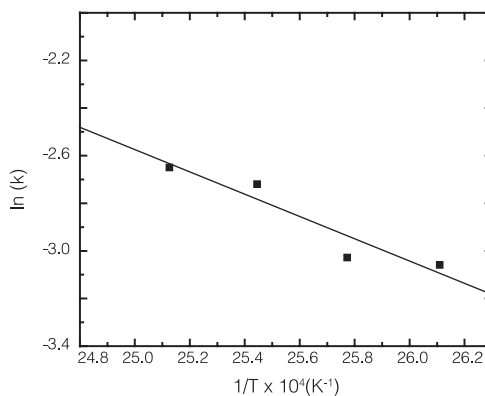
and lnk_o= 9.12min^{-1}. Linear regression analysis showed a determination coefficient R^2= 0.895 (p<0.05). No E_a values for fishery products could be found in the literature for this parameter.

The E_a values obtained for lightness or color parameter L (92.0kJ·mol^{-1}), and 5-HMF accumulation (38.9kJ·mol^{-1}) indicated that the latter is less sensible to changes in processing temperature. This could be expected since the chemical reaction involved in the formation of color compounds includes other temperature dependent reactions besides the formation of 5-HMF and, therefore, the decrease in the L value can be accounted by them in addition to the Maillard reaction. In this way, it can be stated that in this case the Maillard reaction took place, but it was not the only responsible for the change in color. Consequently, the L value is a more suitable parameter to follow color changes in a tuna-vegetable mixture during heating than 5-HMF accumulation.

Conclusions

The kinetics of color change in a tuna-vegetable mixture during heating was measured by the color parameter L and 5-HMF accumulation. In the first case the reaction was better described by a pseudo-zero order reaction although a pseudo-first order reaction could also adequately describe it. For 5-HMF accumulation, a pseudo-first order reaction was found, evidencing that the Maillard reaction was taking place. Ea values of 92.0 and 38.9kJ·mol^{-1} were obtained for lightness L (for a pseudo-zero order kinetics) and 5-HMF accumulation (for a pseudo-first order kinetics), respectively. The color parameter L was more sensitive to temperature changes than 5-HMF accumulation, and hence it is suggested for the evaluation of heat induced color changes in this product.

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