International Journal of
Food Science & Technology

www.ifst.org

1808 International Journal of Food Science and Technology 2013, 48, 1808–1814

Original article

# Physicochemical characteristics and shelf life estimation of maize/soybean extrudates added with bovine haemoglobin

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(Received 19 November 2012; Accepted in revised form 16 March 2013)

**Summary** Maize/soybean (88:12) extrudates fortified with bovine haemoglobin as a source of iron with the addition of iron absorption enhancers were developed. The aims were to evaluate physicochemical characteristics and to predict shelf life by sensory analysis. Extrudates were made using a Brabender 20 DN single-screw extruder. Specific mechanical energy consumption (SMEC), expansion ratio, specific volume, water solubility and water absorption were measured. Extrudates were stored at three temperatures (12.5, 30 and 45 °C) for 50 days. Colour, crispness and rancid flavour were evaluated by a trained panel. Both addition of haemoglobin and enhancers did not produce significant differences for SMEC, expansion and specific volume. Flavour was the attribute with the greatest degree of damage during storage, thus was used for predicting shelf life. The expanded product added with haemoglobin, and $Na_2EDTA$ could be kept for 15 months at 15 °C or 10 months at 22 °C without rancid flavour development.

**Keywords** Extrusion, haemoglobin, maize/soybean, rancid flavour, shelf life.

## Introduction

Iron deficiency affects global population. Risk groups are infants, children, adolescents, pregnant and reproductive age women. Main consequences of this deficiency include decrease in psychomotor and intellectual performance and increase in the incidence of premature and maternal or foetal mortality (Boccio & Bressan Monteiro, 2004; Akhtar *et al*., 2011).

The feasibility of food fortification implies a correct selection of a food vehicle which depends on food habits as well as population group considered at risk. Cereal flours and their products have been proposed as suitable vehicle for fortification with vitamins and minerals (Scrimshaw, 2005).

Iron compounds used for fortification should be carefully selected according to their bioavailability, the composition of the food matrix as well as technological processes used for food processing (Boccio & Bressan Monteiro, 2004). La Manna *et al*. (2008) showed that the addition of bovine haemoglobin (Hb) in some foods could be an alternative for iron (Fe) fortification. Hb is a by-product of slaughtering with low commercial value. However, it is a source of Fe with high good protein content. Moreover, its Fe bioavailability could be higher than others sources of this mineral (Vaghefi *et al*., 2002).

Fe absorption could be improved by using promotors. $Na_2EDTA$ can be used as Fe absorption enhancer, Fe: EDTA (1:1 molar ratio) being particularly effective in the presence of inhibitors. Studies with adults and children showed a significant increase in Fe absorption when EDTA was added to breakfast cereals and other cereal products (Davidsson *et al*., 2001; Visentín *et al*., 2009). However, Fe fortification could cause changes in sensory properties (Kiskini *et al*., 2007).

Soybean is widely used as a protein source because of its high protein content of good quality and functional properties. The limiting amino acid is methionine while the lysine content exceeds the requirements established by FAO, making possible its use for amino acid complementation of cereal proteins (Visentín *et al*., 2009). It has been shown that adding soy proteins to maize can be a strategy for improving the protein quality of maize and their products without changing its sensory characteristics (Pérez *et al*., 2008).

Extrusion cooking is considered to be an efficient manufacturing process. Extruded foods are formulated mainly of cereal, starch and vegetable proteins, which

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doi:10.1111/ijfs.12154

give structure, texture, mouth feel and others characteristics to specific finished products (Anton *et al.*, 2009).

Sensory quality is important to develop innovative products (Cayot, 2007). Therefore, it is important to estimate the shelf life of the fortified product to ensure food quality at the time of consumption (Singh, 1999).

The aims of this work were to obtain maize/soybean (88:12) extrudates added with bovine haemoglobin and iron absorption enhancers, to evaluate their physicochemical characteristics, sensory stability and to estimate their sensory shelf life.

## Materials and methods

### Raw materials

A commercial sample of 'Don Mario 2005′ soybean variety was selected to obtain the soybean grits according to Pérez *et al.* (2008). The beans were previously treated to inactivate lipoxygenase by immersing them in boiling water during 2 min and soon after cooling them with tap water. Treated beans were dried in an oven at 50 °C until they reached 9–10% of moisture. Using an air classifier and a roll mill, the grains were dehulled and ground (Vario Miag Germany). Final grits particle size was between 420 and 250 μm, less than 1% of particle size being below 250 μm.

Commercial maize grits provided by LITEX SH (Santa Fe, Argentina) and bovine haemoglobin provided by Yerubá SA, Esperanza (Santa Fe, Argentina), were used for extrusion experiments.

### Expanded samples

Maize/soybean (88:12) blend was selected as adequate to improve protein value without impairing sensory attributes of the expanded product (Fritz *et al.*, 2006). Addition of Hb was at 0.5% level. Fe absorption enhancers were added at the following ratio: $Na_2EDTA$ in Fe:EDTA (1:1) molar ratio and sodium citrate in Fe: citrate (1:50) molar ratios: equivalent to adding 13.5 mg $Na_2EDTA$ and 0.53 g of sodium citrate by 100 g of dry base mixture. The experimental formulated samples are shown in Table 1.

### Extrusion process

The extrusion process was carried out with a Brabender 20 DN (Duisbug-Germany) single-screw extruder, using a 4:1 compression ratio screw, a 3.5/20 mm (diameter/length) die, a 150 rpm screw speed and 15% feeding grits moisture. Extruder temperature was maintained at 160 °C (metering and die sections) by using the heat control device of the extruder. The grits mixture was conditioned to the extrusion moisture 1 h before each run. The feed rate of the extruder was at full capacity. The extruder feed section was maintained cool by circulating water through the jacketed device.

### Extrudate evaluation

Samples were obtained as soon as the stationary condition was reached, torque and mass output being simultaneously measured. For statistical analysis, two representative values of each extrusion response were taken, while each sample was obtaining. These values were used to determine the specific mechanical energy consumption (SMEC) using the following formula: SMEC ($J\ g^{-1}$) = k. T. N. $Qa^{-1}$, where $k$ is: $61.6 \times 10^{-3}$; T is torque in Brabender Units (BU); N is screw rpm and Qa ($g\ min^{-1}$) is the mass output, referred to feed moisture level. The value of k takes into account unit conversion and constants (González *et al.*, 2006).

All extruded samples were air-dried in an oven at 50 °C until a moisture content of 6% was reached, this

**Table 1** Experimental samples and codes. Water solubility (WS) and water absorption (WA) corresponding to experimental extruded samples

| Samples | Hb | Enhancers | Code | WS (%) | WA (g water/g solid) |
|---|---|---|---|---|---|
| Maize grit 88% + soybean 12% (Reference Sample) | – | – | M | 66.34[a] | 5.55[c] |
| Maize grit 88% + soybean 12% | – | $Na_2EDTA$: 0.135 g $kg^{-1}$ | M+E | 58.79[c] | 6.25[a] |
| Maize grit 88% + soybean 12% | – | Sodium citrate: 5.3 g $kg^{-1}$ | M+C | 63.21[b] | 6.10[ab] |
| Maize grit 87.5% + soybean 12% | 0.5% | – | MH | 59.16[c] | 6.44[a] |
| Maize grit 87.5% + soybean 12% | 0.5% | $Na_2EDTA$: 0.135 g $kg^{-1}$ | MH+E | 57.32[c] | 6.43[a] |
| Maize grit 87.5% + soybean 12% | 0.5% | Sodium citrate 5.3 g $kg^{-1}$ | MH+C | 64.65[a] | 5.70b[c] |

Different letters indicate significant differences ($P < 0.05$).
Hb: haemoglobin.

moisture level being considered adequate for texture evaluation. Each dried sample was divided in several portions and kept in plastic bags hermetically sealed until their evaluation. Diameters were measured with a Vernier caliper on ten pieces of sample, and radial expansion (E) was determined as the ratio E = D. $d^{-1}$, where D is the extrudate diameter (average of ten determinations) and d is the die diameter. Extrudate specific volume (SV) was obtained by calculating the volume/d.b. weight ratio ($cm^3$ $g^{-1}$), corresponding to an extrudate piece of about 10 cm long. This procedure was applied to ten pieces, and the average is reported.

Part of each sample was milled with a laboratory mill Ciclotec (UDY-Sweden), and solubility (WS) and water absorption (WA) were evaluated, in duplicates, according to González *et al.* (2002).

## Sensory evaluation of stability

To estimate degradation kinetics or shelf life during storage of samples, sensory attributes were taken as indicators, because they are the most sensible regarding quality.

### *Panel preparation*

The panel was integrated by 9 trained persons whose preparation taken 10 sessions. The methodology used was quality descriptive analysis (QDA) according to Murray *et al.* (2001). Nonstructured scales were used to assess 2 attributes: (crispness and colour) and 1 defect: (rancid flavour). Preliminary assays were done to establish the anchor extremes (1 and 9) of a 10 scale. In the case of colour, the extremes were 1 = pale yellow and 9 = dark brown. The value 1 corresponded to a cream snack and the 9 to a commercial snack oat based (brownish), which resembled the colour of sample containing haemoglobin. The anchors of the scale of crispness were 1 = almost nothing and 9 = great. It was established that a value of 5 was considered as desirable. The extremes for rancidity were 1 = almost nothing and 9 = very rancid. It was determined that the acceptable limit for rancid flavour was 2.

### *Sensory evaluation of selected expanded samples*

The extruded samples selected for the study of the stability were MS and samples with added haemoglobin (MH) and EDTA and haemoglobin (MH+E), the latter being the most interesting from the viewpoint of the nutritional improvement and M and MH were evaluated as controls.

New extruded samples were obtained for the estimation of kinetic parameters and shelf life. Samples coming from the extruder were dried at 50 °C until reaching a 6% moisture, packed in 80 g polypropylene–polypropylene–aluminium bags and stored in three chambers at 12.5 °C, 30 °C and 45 °C during 50 days. The evaluation was conducted in sensory evaluation room designed according ISO 8589 1988. Samples were presented with random codes, and the order of presentation was balanced (Sancho *et al.*, 2002).

During tasting, the panel scored, in each unstructured scale, the perceived intensity of colour, crispness and flavour. Then, the intensities of each attribute were measured in each scale, to assign a value for statistical analysis. Evaluation test for each sample was done in duplicates.

## Estimation of kinetic parameters

To estimate the values of kinetic parameters, $k$ (reaction rate) and $E_a$ (activation energy), the methodology proposed by Singh (1999) was used. The procedure is based on the concepts that reaction kinetics for an attribute of quality ($Q$), at a constant temperature can be expressed as:

$$dQ/dt = \pm kQ^n \quad (1)$$

where $\pm$ refers to the increase or the decrease in the attribute value with time $t$ (days), $k$ is the reaction rate constant ($day^{-1}$) and $n$ is the order of reaction.

The reaction rate constant $k$ can be calculated graphically using equations (2) and (3) for a zero-order reaction or first order, respectively:

$$Q_0 - Q = kt \quad (2)$$

$$In(Q_0/Q) = kt \quad (3)$$

where $Q_0$ is the initial value of the quality attribute.

An important consideration should be taken into account for the kinetic analysis. When the experimental results are plotted, the relationship quality attribute unit vs. storage time can be fitted for both zero- and first-order reaction.

There is an overlap between the two curves above 45% remnant quality, thereby demonstrating that for degrees of progress between 100% and 45%, either of the two models can be used to describe the change of quality attribute (Singh, 1999).

Temperature is a factor of great influence on the reaction rate constant and according to Arrhenius, the rate constant is expressed by the following equation (4):

$$k = k_0 exp(-Ea/RT) \quad (4)$$

where $k_0$ is a constant which represent the frequency and impact factor (independent of temperature), $E_a$ is the activation energy (kJ $mol^{-1}$), $R$ is the universal gas constant (8.314 J mol $K^{-1}$), and $T$ is absolute temperature (K). The activation energy can be obtained by plotting ln k vs. 1/T (K), where the slope of the line is the activation energy divided by the universal gas constant ($E_a/R$) (Singh, 1999).

*Shelf life*

To estimate the shelf life, a simple approach to determine the effect of temperature on the quality of food was used according to Singh (1999) and Santiago *et al.* (2004). For a given reaction order, $k$ can be calculated as:

$$k = \text{loss of quality at time} ts/ts \quad (5)$$

where $ts$ is the final time of life and the numerator is the loss of quality during $ts$. For a first-order reaction, the loss of quality is ln $Q_0/Q_e$ and for zero-order reaction is $Q_0$–$Q_e$. The term $Q_e$ is the attribute value reached at the same time as $ts$.

Taking logarithm on both sides of equation (5) and reformulating the Arrhenius equation (4) yields:

$$In\ ts = -In(\text{loss of quality at time} ts) + In\, k_0 - E_a/RT \quad (6)$$

According to equation (6), a semi-logarithmic graph of $ts$ vs. 1/T should give a straight line, and the activation energy is obtained from the slope: $+E_a/2.303$R. Where 2.303 is the conversion to decimal logarithm.

For small temperature ranges (less than ± 20 °C), $ts$ can be plotted directly against T without significant errors. Therefore, the equation of the straight line on semi-logarithmic graph can be written as follows:

$$t_{s\prime} = t_0 e^{-a\Delta T} \quad (7)$$

where $ts'$ is shelf life at temperature required, $t_0$ is the shelf time at a reference temperature ($T_0$), $a$ is the slope of the line, and $\Delta T$ is the temperature difference between the temperature at which $ts$ is calculated and the reference temperature (Singh, 1999).

### Statistical analysis of results

Statistical differences among samples were determined by ANOVA followed by LSD test (Least Significant Difference) to compare means at 95% confidence, using Stat graphics plus 5.1.

## Results and discussion

### Extrusion process and physical properties of extruded products

Addition of haemoglobin and iron absorption enhancers (EDTA and Citrate sodium) to maize/soybean (88:12) mixture did not significantly affect SMEC (615 ± 4 J g$^{-1}$), expansion (3.7 ± 0.04 cm cm$^{-1}$) or specific volume (9.7 ± 0.3 cm$^3$ g$^{-1}$). This indicates that the level of addition of Hb and those of Fe absorption enhancers used in this study would not be high enough to affect significantly extrusion responses.

Table 1 shows the values of WS and WA corresponding to extruded samples. It was observed that addition of Fe absorption enhancers and Hb decreased WS respect to M sample, except for MH+C sample. These results are difficult to explain because, as mentioned previously, there were not significant differences regarding SMEC, E and SV values. It is possible to argue that WS is much sensible to detect chemical changes produced by enhancers and Hb during extrusion than the measurements of SMEC, E or SV. Besides, addition of Citrate seems that overcome the negative effects produced by Hb, leading to a WS value similar to that of MS sample.

Regarding WA an inverse tendency was observed in comparison with that of WS. This is in agreement with what it is expected, because WS and WA are properties inversely related (González *et al.*, 2002).

### Sensory evaluation of extruded samples

*Evaluation of colour*

M sample stored at 12.5 °C showed a slight increase in colour during the 50 days of storage, showing significant differences between 20 and 40 days. However, M stored at 30 °C showed a reduction in the intensity of colour during storage, showing significant differences between 30 and 50 days. This sample stored at 45 °C showed a similar behaviour to that at 30 °C, but the colour loss was significantly different from 20 days storage.

The temperature had a significant effect on the colour, because the samples were statistically different at the end of storage period (Table 2). Therefore, it could be concluded that M sample stored at12.5 °C preserved its colour, while stored at 30 °C and 45 °C lost colour intensity with the time, the effect being greater at 45 °C. This discolouration is due to corn caratenoids oxidation.

MH and MH+E samples stored at 12.5 °C showed no change in colour during 50 days. In contrast, stored at 30 °C and 45 °C showed a gradual decrease in colour from 10 days storage. The loss of colour was higher in MH+E sample stored 40 days at 45 °C.The addition of Hb to the maize/soybean blend caused a colour change from yellow to brown, thus the colour of haemoglobin would mask the colour given by carotenoids.

Over time, these samples were also discoloured. This could be associated with haem iron oxidation. Furthermore, the addition of EDTA significantly affected the colour of the samples with haemoglobin.

*Evaluation of crispness*

M, MH and MH+E samples stored26 storage, and no significant difference between both temperatures was

**Table 2** Sensory evaluation of colour, crispness and rancid flavour of M, MH and MH+E extrudates stored at 12.5 °C, 30 °C and 45 °C during 50 days

| **Colour** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | M | | | MH | | | MH+E | | |
| Time | 12.5ºC | 30ºC | 45ºC | 12.5ºC | 30ºC | 45ºC | 12.5ºC | 30ºC | 45ºC |
| 0 | 5.0$^{efg}$ | 5.0$^{efg}$ | 5.0$^{efg}$ | 8.0$^{rstu}$ | 8.0$^{rstu}$ | 8.0$^{rstu}$ | 8.1$^{tu}$ | 8.1$^{tu}$ | 8.0$^{tu}$ |
| 5 | 5.0$^{fg}$ | 5.0$^{efg}$ | 5.0$^{efg}$ | 7.9$^{rstu}$ | 7.9$^{rstu}$ | 8.0$^{rstu}$ | 8.1$^{tu}$ | 7.9$^{rstu}$ | 8.0$^{rstu}$ |
| 10 | 5.0$^{fg}$ | 5.0$^{efg}$ | 4.6$^{de}$ | 7.8$^{qrst}$ | 7.7$^{pqrs}$ | 7.5$^{opq}$ | 7.7$^{pqr}$ | 7.7$^{pqrs}$ | 7.4$^{nop}$ |
| 20 | 5.0$^{fg}$ | 5.0$^{efg}$ | 4.1$^{c}$ | 7.9$^{rstu}$ | 7.4$^{nop}$ | 7.0$^{lm}$ | 7.6$^{pqr}$ | 7.4$^{nop}$ | 6.6$^{j}$ |
| 30 | 5.3$^{gh}$ | 4.9$^{efg}$ | 3.2$^{b}$ | 8.0$^{stu}$ | 7.3$^{mno}$ | 6.9$^{klm}$ | 7.8$^{qrst}$ | 7.0$^{lm}$ | 6.7$^{jkl}$ |
| 40 | 5.4$^{h}$ | 4.8$^{def}$ | 2.3$^{a}$ | 8.0$^{rstu}$ | 7.1$^{lmn}$ | 6.7$^{jkl}$ | 8.1$^{tu}$ | 6.8$^{jkl}$ | 6.1$^{i}$ |
| 50 | 5.5$^{h}$ | 4.5$^{cd}$ | 2.3$^{a}$ | 8.2$^{u}$ | 7.2$^{mno}$ | 6.6$^{jk}$ | 8.1$^{tu}$ | 6.7$^{jkl}$ | 6.1$^{i}$ |
| Crispness | | | | | | | | | |
| | M | | | MH | | | MH+E | | |
| Time | 12.5ºC | 30ºC | 45ºC | 12.5ºC | 30ºC | 45ºC | 12.5ºC | 30ºC | 45ºC |
| 0 | 7.0$^{lmnop}$ | 7.0$^{lmnop}$ | 7.0$^{lmnop}$ | 7.0$^{lmnop}$ | 7.0$^{lmnop}$ | 7.0$^{lmnop}$ | 7.0$^{lmnop}$ | 7.0$^{lmnop}$ | 7.0$^{lmnop}$ |
| 5 | 6.5$^{hijk}$ | 6.7$^{jklmn}$ | 7.4$^{pqrst}$ | 6.9$^{klmno}$ | 6.9$^{klmno}$ | 7.8$^{tuvw}$ | 6.9$^{klmno}$ | 7.2$^{nopqr}$ | 7.1$^{mnopq}$ |
| 10 | 6.3$^{fghij}$ | 6.6$^{ijkl}$ | 7.2$^{pqrst}$ | 6.9$^{klmno}$ | 6.7$^{jklm}$ | 7.8$^{tuv}$ | 6.4$^{ghij}$ | 6.6$^{hijkl}$ | 7.1$^{opqrs}$ |
| 20 | 5.9$^{def}$ | 6.0$^{efg}$ | 7.5$^{qrstu}$ | 6.0$^{efg}$ | 6.6$^{hijk}$ | 7.9$^{uvw}$ | 6.2$^{fghi}$ | 6.5$^{hijk}$ | 7.3$^{rstu}$ |
| 30 | 5.3$^{ab}$ | 5.5$^{bcd}$ | 7.6$^{stuv}$ | 6.0$^{efg}$ | 6.2$^{fgh}$ | 7.9$^{uvw}$ | 5.9$^{def}$ | 6.0$^{efg}$ | 7.6$^{stuv}$ |
| 40 | 5.1$^{a}$ | 5.3$^{ab}$ | 7.7$^{vw}$ | 5.8$^{cde}$ | 6.0$^{efg}$ | 7.9$^{vw}$ | 5.6$^{bcde}$ | 5.8$^{cde}$ | 7.7$^{uvw}$ |
| 50 | 5.2$^{ab}$ | 5.3$^{ab}$ | 8.0$^{w}$ | 5.2$^{ab}$ | 5.4$^{abc}$ | 8.1$^{w}$ | 5.3$^{ab}$ | 5.4$^{abc}$ | 8.0$^{w}$ |
| Rancid flavour | | | | | | | | | |
| | M | | | MH | | | MH+E | | |
| Time | 12.5ºC | 30ºC | 45ºC | 12.5ºC | 30ºC | 45ºC | 12.5ºC | 30ºC | 45ºC |
| 0 | 0.5$^{abc}$ | 0.5$^{abc}$ | 0.5$^{abc}$ | 0.5$^{abcde}$ | 0.5$^{abcde}$ | 0.5$^{abcde}$ | 0.5$^{abcd}$ | 0.5$^{abcd}$ | 0.5$^{abcd}$ |
| 5 | 0.5$^{abc}$ | 0.4$^{ab}$ | 0.5$^{abcd}$ | 0.8$^{bcde}$ | 2.0$^{h}$ | 4.0$^{i}$ | 0.5$^{abc}$ | 1.0$^{cd}$ | 2.0$^{h}$ |
| 10 | 0.6$^{abcde}$ | 0.5$^{a}$ | 0.8$^{bcde}$ | 1.0$^{de}$ | 4.0$^{i}$ | 5.8$^{kl}$ | 0.8$^{bcde}$ | 2.0$^{h}$ | 4.1$^{i}$ |
| 20 | 0.4$^{ab}$ | 0.5$^{ab}$ | 1.5$^{fg}$ | 1.0$^{cde}$ | 5.9$^{kl}$ | 6.5$^{mn}$ | 0.7$^{abcde}$ | 3.8$^{i}$ | 6.2$^{lm}$ |
| 30 | 0.3$^{a}$ | 0.6$^{abcde}$ | 2.0$^{gh}$ | 0.8$^{bcde}$ | 6.2$^{lm}$ | 7.2$^{op}$ | 0.8$^{bcde}$ | 4.6$^{j}$ | 7.5$^{pq}$ |
| 40 | 0.4$^{ab}$ | 0.6$^{abcde}$ | 4.0$^{i}$ | 0.8$^{bcde}$ | 6.9$^{no}$ | 7.9$^{qr}$ | 0.7$^{abcde}$ | 5.0$^{j}$ | 8.2$^{r}$ |
| 50 | 0.4$^{ab}$ | 0.6$^{abcde}$ | 6.5$^{mn}$ | 1.0$^{ef}$ | 7.0$^{o}$ | 8.4$^{r}$ | 0.7$^{abcde}$ | 5.5$^{k}$ | 8.0$^{qr}$ |

Different letters indicate significant differences ($P < 0.05$).

observed at 50 days. Moreover, the crispness at 50 days was within the acceptable limit (Table 2).

In contrast, samples stored at 45 °C increased crispiness at the end of storage period.

The loss or gain of crispiness could be explained by the passage of moisture (through the packaging film, which is not totally permeable to water vapour), from the samples to store environment and *viceversa* until the equilibrium is reached or the experiment is stopped. To explain that, we took into account that the average ambient air conditions during the 50 days storage time were 20 °C and 70% relative humidity (RH) and that the conditions inside the store chamber will change according to each store temperature. From the psychometric chart, it is found that the corresponding RH at 12.5 °C, 30 °C and 45 °C are 95%, 38% and 18%, respectively. On the other hand, water activities (aw) of maize at 6% moisture and at these three different temperatures would be around 0.05; 0.16 and 0.25, respectively (Herum, 1987). Thus, samples stored at 45 °C would loss moisture, while the opposite would happen with samples stored at 30 °C and 12.5 °C. According to González *et al.* (2004), crispness of expanded maize would be lost when product moisture is beyond 9%.

*Evaluation of rancid flavour*

M, MH and MH+E samples stored at 12.5 °C showed no development of rancid flavour during the 50 days of storage (Table 2) and maintained values equal or less than 1 from the beginning to the end.

MS sample storage at 30 °C had similar behaviour to that of 12.5 °C. This sample did not develop rancidity during storage. In contrast, M stored at 45 °C showed an increase in rancidity after 10 days. After 30 days, the development of rancidity exceeded the limit value given by the panel (2) and could be considered as not adequate.

MH and MH+E stored at 30 °C and 45 °C days showed an increase in rancidity reaching the limit value at 5 days, the intensity of rancid flavour at 45 °C being higher than 30 °C.

MH and MH+E samples stored at 45 °C had a similar evolution.

Both temperatures (30 °C and 45 °C) produced a rancid flavour development, which influenced sensory acceptance by the panellists.

## Shelf life prediction

Flavour was used to estimate the shelf life of the extruded samples, because it was the attribute that showed the highest changes related to acceptability during storage. Two extruded samples were selected: MH and MH+E because they were the most interesting regarding Fe fortification.

Table 3 shows *k* values corresponding to MH and MH+E extrudate samples at 12.5 °C, 30 °C and 45 °C storage temperatures, and estimation of sensory shelf life (SSL) for MH and MH+E extrudates at the same storage temperatures.

To estimate the kinetic constant (*k*), a plot with the amount of remaining quality attribute vs. storage time for each sample was made. The reaction order was obtained from the plot. As degree of deterioration is less than 55%, a zero-order reaction was considered. Besides, this order for reactions of lipid oxidation that leads to rancid flavours development is suggested (Singh, 1999). Shelf life (*ts*) was calculated from Equation (5) and considering a zero-order reaction and a value of $Qe = 80\%$, corresponding to a quality loss of 20%, because rancid flavour value equal or less than 2 was given by the sensory panel as accept limit value.

The MH+E extrudate would be stored at 12.5 °C during 18 months (540 days). But at 30 °C and 45 °C the decline of acceptability occurred at few days of the date of formulated. Similarly, MH at 12.5 °C could be stored during 13 months, but at other temperatures, the sample could deteriorate rapidly.

This estimation is in agreement with the results observed in the development of rancidity during storage (Table 2), when the rancidity average value exceeded the limit value 2, and these products would be no longer acceptable.

Using Equation (6), the activation energy was calculated resulting 26.9 kcal mol$^{-1}$ and 26.2 kcal mol$^{-1}$ for MH and MH+E extrudates. These values are within the expected values for lipid deterioration. Torres *et al.* (2001) indicated that activation energy for lipid oxidation could be between 10 and 25 kcal mol$^{-1}$, while for reactions of variations of colour, flavour and texture, the activation energy would be between 10 and 30 kcal mol$^{-1}$.

The activation energy allows the estimation of shelf life at others temperature. This estimation was made at 15 °C and 22 °C for both samples using 12.5 °C as reference temperature. These results are shown in Table 4. At 15 °C (refrigeration temperature), MH and MH+E could be stored during 338 days (11 months) and 459 days (15 months), respectively, whereas at 22 °C, 210 days (7 months) and 292 days (10 months) for MH and MH+E, respectively.

**Table 3** *k* Values corresponding to MH and MH+E extrudates samples at 12.5 °C, 30 °C and 45 °C storage temperatures, and estimation of sensory shelf life (SSL) for MH and MH+E extrudates at the same storage temperature

| Temperature (°C) | MH | | MH+E | |
|---|---|---|---|---|
| | *k* (day$^{-1}$) | SSL (day) | *k* (day$^{-1}$) | SSL (day) |
| 12.5 | 0.052 | 400 | 0.037 | 540 |
| 30 | 3.66 | 5 | 1.56 | 13 |
| 45 | 5.52 | 3 | 3.73 | 4 |

**Table 4** Prediction at 15 °C and 22 °C of Sensory shelf time for MH and MH+E extrudates using 12.5 °C as reference temperature.

| Temperature (°C) | MH (days) | MH+E (days) |
|---|---|---|
| 15 | 338 | 459 |
| 22 | 210 | 292 |

## Conclusion

Effects of the addition of haemoglobin and enhancers of iron absorption ($Na_2EDTA$) to maize/soybean blend did not significantly affect specific mechanical energy consumption, expansion or specific volume. On the other hand, water solubility and water absorption changed by the addition of Hb and Fe absorption enhancers. The extruded samples fortified with haemoglobin and haemoglobin plus EDTA could be stored for 15 and 10 months at 15 °C and 22 °C without deterioration, if packaging with a water-impermeable material is used.

## Acknowledgments

Financially supported by UNL-CAI+D 2009 and ANPCyT (PICT 1105). The authors thank the sensory evaluation panel from Instituto de Tecnología de Alimentos (ITA)–Facultad de Ingeniería Química–Universidad Nacional del Litoral and Adriana Bonaldo, Mario De Greef y Roberto Torres for participation in this research.

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