Studies on the trans-fatty acids and the stability of the fats present in Indian bakery products

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RESUMEN

Estudios sobre ácidos grasos trans y estabilidad de las grasas presentes en productos horneados de India

La grasa es uno de los ingredientes más importantes de los productos horneados altamente susceptible al calor y la humedad. Se han realizado estudios para evaluar los cambios termoxidativos ocurridos en las grasas y también la presencia de ácidos grasos trans. Las grasas se extrajeron de productos como galletas, pan blanco, pan dulce, Dilkhush, pizza, pastel, rollo vegetal, gluco-galletas y barquillos. Se utilizaron en este estudio las grasas extraídas de los mencionados productos, así como nuestras control de shortening. Los valores encontrados de índice Totox fueron altos (4,0-30,9) en tanto que los porcentajes de ácidos grasos libres (%), índice de peróxido y anisídina fueron, 0,31-0,90, 1,2-11,0 y 11,6-8,9, respectivamente. Puesto que los productos se hornean entre 180-200 °C se determinaron los componentes polares, encontrándose entre 3,3-5,3% mostrando hidrólisis y cambios oxidativos moderados. El índice de yodo (51,0-73,3) y los valores del butiro-refractómetro (42,2-55,8) mostraron una insaturación moderada, todas las muestras fundieron por debajo de 37 °C. Los ácidos grasos trans variaron entre 35,5-46,2% también confirmado mediante FT-IR. Por lo tanto, los productos seleccionados se consideran seguros pero nutricionalmente inferiores a los productos preparados en mantecas líquidas.


SUMMARY

Studies on the trans-fatty acids and the stability of fats present in Indian bakery products

Fat is one of the most important ingredients in baked products which is highly susceptible to heat and moisture. Therefore, this study has been conducted to assess the thermoxidative changes occurring in the fat as well as the extent to which trans-fatty acids are present. Fats were extracted from products like rusk, white bread, sweet bun, dilkhush, pizza, plain cake, vegetable roll, gluco-biscuits and wafers. These fats and control shortening samples were taken for study. The totox value was found to be high (4.0-30.9) whereas the free fatty acid, peroxide and anisidine values were, 0.31-0.90%, 1.2-11.0 and 11.6-8.9 respectively. Since the products are baked at 180 to 200 °C polar components were determined to be between 3.3 to 5.3% showing a moderate hydrolysis and oxidative changes. Iodine value (51.0 to 73.3) and the Butyro-refractometer reading (42.2 to 55.8) showed moderate unsaturation and all the samples melted below 37 °C. Trans-fatty acid ranged from 35.5 to 46.2% as has also been confirmed by FT-IR. The products selected were considered safe but nutritionally inferior to products prepared with liquid shortenings.

KEY-WORDS: Baked products – Shortening – Thermoxidation – Trans-fats.

1. INTRODUCTION

The baking industry in India is the largest organized food industry, with an annual turnover of Rs. 800 crores. The industry mainly consists of bread, biscuits and cake manufacturing units. The production of bakery products has doubled over the last 10 years from 1.8 MT to about 3.5 MT. Bread and biscuits are the major products consisting of about 1.5 MT each. But a good amount of bakery products is made in small scale local bakeries in the towns where norms followed by major bakers are normally ignored.

Almost all bakery products require hydrogenated fats rather than liquid oils unlike the application of oil in a cooking medium, where only the oxidative stability is a concern. In baking, the specific physical properties of the fat are important because of the fact that fats are directly incorporated into the dough or cream. In order to enable processing such as layering and whipping to enhance aeration, physical properties such as the hardness, plasticity, spreadability and melting behavior of fats are crucial. Lard and tallow can also be used as bakery shortenings but they are not allowed in India. Hence, the Indian bakery industry uses hydrogenated fats like vanaspathi. As hydrogenated fats contain trans-fatty acids up to 45% (Patterson, 1983) which is proved to be detrimental to health (Alfonso Valenzuela et al., 1995; Kromhout et al., 1995) there was a necessity to study its presence in some of the bakery products commonly available in the Indian market. Efforts have also been made...
during the present study to evaluate the stability of fats at the baking temperature especially with respect to thermal oxidation and shelf life (Barrera-Arelliano et al., 1997).

2. MATERIALS AND METHODS

All the chemicals and solvents were of analytical reagent grade obtained from reputed chemical companies. Ethyl alcohol was refluxed with sodium hydroxide and subsequently in potassium permanganate before distillation to prepare aldehyde free alcohol. Iso-octane and para-anisidine were obtained from Qualigens. P-anisidine was recrystallized in hot water in the presence of sodium sulphite and activated carbon, before preparing the reagent for estimation. Wijs' solution was purchased from Nice chemicals, Kerala India.

The bakery products like rusk, bread, bun, dilkhush, milk bikkis, pizza, plain cake, vegetable roll and wafers were obtained from local renowned bakers. The samples like vanaspathi-1 and 2 were products of Ruchi vanaspathi and Dalda respectively. The bakery products were smashed before the extraction of fat.

The extraction of fat from the bakery products, the estimation of the fat percentage, free fatty acid percentage, peroxide value, anisidine value, iodine value, butyro-refractometer reading, slip melting point and volatiles and moisture percentages were determined following AOCS (2002-2003) methods. The totox value was calculated as follow:

\[ \text{Totox} = 2 \times \text{peroxide value} + \text{anisidine value}. \]

2.1. FT-IR Spectroscopy

FT-IR spectra were recorded on a Thermo- Electron Corporation Spectrometer (Model- NICOLET-5700 FT-IR, USA). Samples were applied on a potassium bromide cell and spectra were run between 800 and 4000 cm\(^{-1}\).

2.2. Determination of Polar Components

Polar components were determined according to the procedure given in the AOAC 2005 using column chromatography.

2.3. Preparation of fatty acid methyl esters

Fatty acid methyl esters were prepared in accordance with the AOAC 2005 procedure. A fatty acid analysis was carried out on a gas-liquid chromatograph, [FISCONS 8000 series Fiscos Instruments, Rodano (Milano), Italy]. The injection system and FID detector were maintained at 240 °C. The column temperature was 180 °C maintained within 0.1 °C (isothermal conditions). The GLC was equipped with a glass capillary column, length 55 meter with 0.25 mm internal diameter. The capillary wall was coated with butanediol polysuccinate (BP 21) from SGE, Australia with a coating thickness of 0.2 micron. The carrier gas was \( N_2 \) at a flow rate of 1 mL min\(^{-1}\) and hydrogen was fuel gas (gas used to burn the flame in the FID detector) at a rate of 40 mL min\(^{-1}\).

2.4. Statistical analysis

Data (3 replicates) were subjected to statistical analysis (Duncan, 1995) of variance (ANOVA) and Duncan’s multiple range test (DMRT) was applied to differentiate among the means of different samples at a probability of \( P \leq 0.05 \).

3. RESULTS AND DISCUSSION

The Indian bakery products chosen for the study were selected based on their popularity among the general public as they are economical and widely available in all bakeries including small bakeries and street vendors. These products like rusk, white bread, sweet bun, dilkhush (a local product made of pizza-like dough stuffed with grated raw coconut, fruits and plum pieces, nuts and baked as circles, very popular among the lower strata of people), pizza (pizza dough topped with tomato sauce, capsicum circles, cucumber and onion circles, shredded cheese and baked), plain cake (no topping), vegetable roll (a wheat flour roll filled with a mixture of cooked vegetables and spices ), biscuits and wafers were taken for the study. In all these products wheat flour dough is the base material where considerable amounts of vegetable shortening (hydrogenated fat) have been incorporated. They differ in the type and amount of toppings and stuffing materials which are mostly vegetables, spices and sauces. These toppings and stuffing materials do not contain any shortening, but sometimes salad oils. Hence the toppings and stuffings have been removed before extraction. It is only the dough portion, which contains shortening, which has been subjected to fat extraction. After drying, these extracted fats were taken for the experiments.

For the sake of comparison some of the branded hard fats like, shortening-1 and shortening-2 (vanaspathi, hydrogenated fats) were taken alongside with the fat samples isolated from the bakery products to carry out the estimations in order to ascertain the type of shortening used in these products by the bakers.

Since bakery products are prepared at a higher temperature (180-200 °C) and they carry good amounts of fat (10-15%) there is a possibility of thermal/oxidative changes taking place in the fat resulting in the formation of primary and secondary oxidation products (Baby Latha and Nasirullah, 2011). As these products are very popular among the population, it became necessary to ascertain their nutritional status which probably has not been attempted systematically until now.
3.1. Physico-chemical changes

Physico-chemical parameters which are important quality parameters for oils/fats have been investigated in the isolated fat samples from the corresponding products and compared with the control market shortening sample to ascertain the extent of damage done to the fat during preparation and storage (Table 1). Iodine value, which is a measurement of unsaturation, was found in the range between 51.9 and 73.7, indicating that perhaps two types of shortening namely palm stearin rich and another hydrogenated vegetable oil have been used for the preparation of these products. This has further been confirmed by the butyro-refractometer reading of the sample. The slip melting point of the extracted fat was found to be between 30.0 and 41.0 °C for the samples in comparison with 36.5 to 37.1 for the control shortening. This decrease may be attributed to the fact that while extracting fat from the samples other allied factors might have come along with the fat. Moreover low melting fat is a better option in a bakery product to facilitate its even spread.

3.2. Thermo-oxidative changes

The thermoxidative changes taking place in the fat (shortening) during the preparation and storage of these products are shown in Table 2. During the thermal and autoxidation, peroxides are the primary oxidation products which can be assessed by peroxide value whereas aldehyde, ketones and free fatty acids are the secondary oxidation products which can be estimated by anisidine value and free fatty acid %. The results indicate that the peroxide value ranged from 3.1 to 11.0 MeqO₂ Kg⁻¹, compared to the control sample with a peroxide value ranging from 2.5 to 2.8 MeqO₂ Kg⁻¹; while anisidine value and free fatty acid percentages ranged from 1.6 to 8.9 and 0.31 to 0.90 compared to the control sample which ranged from 5.8 to 6.1 and 0.25% respectively, indicating that oxidation was of a moderate nature.

Silica gel column was used to separate nonpolar components in the bakery fats isolated from the products. Non polar components comprised of triglycerides whereas polar components constituted diglycerides, monoglycerides, FFA and thermoxidative products containing peroxides, aldehydes, ketones etc. The polar components ranged from 3.3 to 5.3% in the fat isolated from the products and from 0.8 to 0.9% in the control shortening as expected (Table 3). The amount of polar components in the products is in the moderate range showing a smaller adverse effect of heat on the fat during baking.

### Table 1

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Sample</th>
<th>Fat %</th>
<th>Butyro refractometer reading</th>
<th>Slip melting Point °C</th>
<th>Iodine value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Rusk</td>
<td>5.8 ± 0.10a</td>
<td>53.5 ± 0.20a</td>
<td>31.5 ± 0.1a</td>
<td>73.5 ± 0.1d</td>
</tr>
<tr>
<td>2.</td>
<td>White Bread</td>
<td>6.1 ± 0.10b</td>
<td>57.1 ± 0.24a</td>
<td>31.0 ± 0.60a</td>
<td>73.7 ± 0.06d</td>
</tr>
<tr>
<td>3.</td>
<td>Sweet bun</td>
<td>7.1 ± 0.11c</td>
<td>50.3 ± 0.20d</td>
<td>30.0 ± 0.05a</td>
<td>58.8 ± 0.06b</td>
</tr>
<tr>
<td>4.</td>
<td>Dilkush</td>
<td>8.5 ± 0.15d</td>
<td>42.2 ± 0.20a</td>
<td>41.0 ± 0.10b</td>
<td>51.0 ± 0.06b</td>
</tr>
<tr>
<td>5.</td>
<td>Pizza</td>
<td>5.1 ± 0.10a</td>
<td>51.5 ± 0.20a</td>
<td>32.1 ± 0.11a</td>
<td>55.0 ± 0.06b</td>
</tr>
<tr>
<td>6.</td>
<td>Plain cake</td>
<td>9.7 ± 0.30a</td>
<td>48.5 ± 0.20a</td>
<td>34.0 ± 0.06a</td>
<td>61.3 ± 0.10b</td>
</tr>
<tr>
<td>7.</td>
<td>Vegetable roll</td>
<td>9.4 ± 0.11a</td>
<td>50.2 ± 0.20a</td>
<td>34.5 ± 0.06a</td>
<td>70.0 ± 0.06b</td>
</tr>
<tr>
<td>8.</td>
<td>Gluco-Biscuits</td>
<td>5.8 ± 0.11a</td>
<td>44.1 ± 0.06b</td>
<td>36.5 ± 0.10b</td>
<td>51.9 ± 0.06b</td>
</tr>
<tr>
<td>9.</td>
<td>Wafers</td>
<td>6.0 ± 0.11b</td>
<td>48.1 ± 0.22b</td>
<td>35.6 ± 0.06b</td>
<td>55.1 ± 0.10b</td>
</tr>
<tr>
<td>10.</td>
<td>Shortening-1</td>
<td>99.8 ± 0.15c</td>
<td>48.6 ± 0.06c</td>
<td>36.4 ± 0.32b</td>
<td>55.1 ± 0.10b</td>
</tr>
<tr>
<td>11.</td>
<td>Shortening-2</td>
<td>99.8 ± 0.20d</td>
<td>47.0 ± 0.06d</td>
<td>37.1 ± 0.10b</td>
<td>59.6 ± 0.40b</td>
</tr>
<tr>
<td>SEM</td>
<td>0.0125</td>
<td>0.0328</td>
<td>0.0052</td>
<td>0.0032</td>
<td></td>
</tr>
</tbody>
</table>

Estimations made in triplicate (n = 3), FFA-Free Fatty acid. Values in the same column with different superscripts are significantly (p ≤ 0.05) different. SEM-Standard Error Mean.
heat (baking temperature, 180-190 °C) on oleic (C18:1 cis) acid and linoleic (C18:2 cis cis) acids present in the shortening used for the product preparation. Oleic and linoleic acids ranged from 30.0 to 44.7 and 5.2 to 11.2% compared to the controlled shortening which ranged from 35.1 to 45.2 and 3.5 to 5.8% respectively. However elaidic acid (C18:1 trans) was found to be present in all cases (35.5 to 46.2%) indicating the presence of hydrogenated fats in all the samples, which is not desirable.

The fatty acid composition further showed that two sets of shortening samples were used for the preparation of these bakery products where one was liquid oil based and hydrogenated and the other palm stearin, liquid oil based and hydrogenated. In the first case, palmitic acid was present in lower amounts and ranged from 3.1 to 4.6% and in the latter case, the presence of palmitic acid was greater and ranged from 11.9 to 15.1%. This finding further confirmed the fact that the presence of palm stearin reduced the oleic acid content to a certain extent. This trend can also be noticed in the control market shortening samples. However, the trans-fatty acid contents remained similar (except in the sweet bun sample) in all cases.

4. CONCLUSIONS

It can be concluded that all the samples selected for the study, although very popular among the general public, do contain trans-fatty acids in appreciable amounts, which is nutritionally undesirable (Ascherio and Willet, 1997). Therefore, their consumption should be reduced or a trans-free shortening substitute could be made available.
The study on thermoxidative changes and physicochemical characteristics indicated that there was a minimal effect of heat and storage on the inherent fat in the product as has been confirmed by free fatty acid percentages, peroxide value, and anisidine value. The fatty acid composition of the fat sample isolated from the products showed that a reasonable amount of the oleic acid (a similar amount is present in palmolein) (Sikorski and Kolakowska, 2003) was present in all the samples along with the linoleic acid which is an essential fatty acid.

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