Food Research International 60 (2014) 66–75

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Evaluation of methodologies to determine vegetable oil species present in oil mixtures: Proposition of an approach to meet the EU legislation demands for correct vegetable oils labelling

Maria Teresa Osorio, Simon A. Haughey, Christopher T. Elliott, Anastasios Koidis *

Institute for Global Food Security, School of Biological Sciences, Queen's University Belfast, BT9 5BN Belfast, Northern Ireland, UK

A R T I C L E I N F O

Article history:
Received 19 July 2013
Received in revised form 25 November 2013
Accepted 10 December 2013
Available online 18 December 2013

Keywords:
Authentication
Labelling
Vegetable oil
Blend
Palm oil
Legislation

A B S T R A C T

Refined vegetable oils are widely used in the food industry as ingredients or components in many processed food products in the form of oil blends. To date, the generic term 'vegetable oil' has been used in the labelling of food containing oil blends. With the introduction of new EU Regulation for Food Information (1169/2011) due to take effect in 2014, the oil species used must be clearly identified on the package and there is a need for development of fit for purpose methodology for industry and regulators alike to verify the oil species present in a product. The available methodologies that may be employed to authenticate the botanical origin of a vegetable oil admixture were reviewed and evaluated. The majority of the sources however, described techniques applied to crude vegetable oils such as olive oil due to the lack of refined vegetable oil focused studies. Nevertheless, DNA based typing methods and stable isotopes procedures were found not suitable for this particular purpose due to several issues. Only a small number of specific chromatographic and spectroscopic fingerprinting methods in either targeted or untargeted mode were found to be applicable in potentially providing a solution to this complex authenticity problem. Applied as a single method in isolation, these techniques would be able to give limited information on the oils identity as signals obtained for various oil types may well be overlapping. Therefore, more complex and combined approaches are likely to be needed to identify the oil species present in oil blends employing a stepwise approach in combination with advanced chemometrics. Options to provide such a methodology are outlined in the current study.

1. Introduction

The most commonly used vegetable oils include olive, sunflower, rapeseed, soybean and palm oil. Some of these oils are sold individually as commodities whereas others, such as palm oil, although not sold in retail, are essential ingredients in many processed food products such as pastries, butter, margarines and cookies. Refined oil blends are more often used by manufactures as the food industry is interested in the variations in physical parameters of foods and in creating different textures. The number of oils used in the various blends can range from two to four or even more including different oil species and oil derivatives. The formula of the oil blends depend on the processed food product to which it is applied to (bakery, confectionary, and dairy products, amongst others). In this respect, blend formulas for margarines are very different from blends to be employed in the production of ice cream. Oils can broadly be classified into two large groups, the hard and the soft oils. Soft oils are usually those that are liquid at room temperature such as sunflower, rapeseed, soybean, olive or corn oil, while hard oils or oil fractions are those that are solid at room temperature (e.g. palm oil).

With the introduction of new "Food Information" EU legislation (EU Regulation 1169/2011), due to take effect in 2014, a number of important changes in the labelling of foodstuffs will come into force. Regarding vegetable oil labelling as an ingredient in other foods, the Regulation states:

> *'Refined oils of vegetable origin may be grouped together in the list of ingredients under the designation 'vegetable oils' followed immediately by a list of indications of specific vegetable origin, and may be followed by the phrase 'in varying proportions'.' Point 8 Part A of Regulation 1169/2011*
>
> [(European Commission, 2011)]

This essentially means that in the case of blended vegetable oils used in food products, the type of vegetable oil must be clearly identified on the package in contrast to the previous situation where an oil blend could be labelled under the generic and often misleading term "vegetable oil". A pack of digestive biscuits, for example, if palm oil has used in a vegetable fat, should inform the consumer in the label as follows: "vegetable oil (palm oil)". These oils are industrially refined and come

* Corresponding author at: Institute for Global Food Security, Queen's University Belfast, 18-30 Malone Road, Belfast, BT9 5BN, Northern Ireland, UK. Tel.: +44 28 90975569; fax: +44 28 90976513.
E-mail addresses: t.koidis@qub.ac.uk, tassos.koidis@gmail.com (A. Koidis).

0963-9969/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodres.2013.12.013

from a number of different countries: palm oil usually originates from Indonesia and Malaysia, sunflower oil from Ukraine and Russia and rapeseed oil from Canada and USA (Food and Agriculture Organisation, 2009).

Palm oil became very popular due to the increased consumer awareness of trans-fatty acid formation during partial hydrogenation of other vegetable oils with higher unsaturated character such as sunflower oil. The industry responded by promoting palm oil, a physically saturated fat with desirable physical properties. Palm oil is now the world's most widely used oil, not only in food manufacturing but in other sectors (e.g. cosmetics, bioenergy) (United States Department of Agriculture, 2012). It is estimated that 7 in 10 products on UK supermarket shelves, from margarines and spreads to cooking oils and from chocolates and pastries to ice creams and biscuits, all contain palm oil (Bottriell & Judd, 2011). Almost three quarters of the world's palm oil supply ends up in the food industry and its traceability is complex (van Duijn, 2013). As the global demand for palm oil is increasing, producing countries suffer from intensive production and deforestation. It is believed that oil-palm agriculture is the greatest threat to biodiversity in these tropical areas (Wilcove & Koh, 2010) but huge economic interests (e.g. Indonesia exported $14.5 bn in palm oil related products in 2008) influence decisions. It is also the practices used to grow the crop that causes controversy (Wallace, 2012). These facts coupled by awareness of global warming, CO_2 emissions and evidence of poor working conditions in the palm producing sectors, initiated action in many countries. Nowadays, many leading food producers and retailers are members of the Roundtable on Sustainable Palm Oil (RSPO), an industry led organisation that, amongst others, certifies palm oil. Even if only a small portion of palm oil production is currently certified, sustainable palm oil comes in great demand from consumers, consumer organisations and experts who are increasingly becoming aware of the situation. Local food industries have played a significant role in this improving situation. For example, a number of companies operating in the UK have made commitments to source sustainable palm oil with many of them committed to 100% RSPO certified by 2015 and the progress is good (Bottriell & Judd, 2011).

As the enforcement date of the new EU Regulation is getting closer, regulators and industry must be tooled with the necessary analytical methodology to identify the nature of vegetable oils in a vegetable oil blend. An examination of the literature shows that most vegetable oil authenticity studies are related to crude high value vegetable oils such as olive oil and the detection of adulteration with lesser value oils (crude or refined). There are not many sources citing refined vegetable oils of interest: sunflower oil is one of the adulterants of olive oil, and some scientific data exist regarding this oil. Palm and corn oil have not been frequently investigated in authentication studies. Rapeseed oil was moderately studied in mixtures with olive oil or in its pure form. Acknowledging the complexity of the authenticity problem (many different types of vegetable oils, compositional and geographical variation and numerous oil mixture possibilities) it quickly became clear that there is no readily available method in the literature for this analytical problem and a new methodology has to be developed. Thus, there is a need to identify which analytical platforms can serve as a starting point in the development of the new approach. Development of such methodology however is not going to be an easy task. Several analytical techniques have been applied to oils and fats in order to detect adulteration or confirm authentication. These techniques include separation techniques (e.g. chromatography), which are based in the separation and detection of specific compounds that can act as individual markers, and non-separative/non-destructive techniques (e.g. infrared spectroscopy), which are based on physical properties and chemical footprint of the oil. Other promising techniques that have been investigated for this purpose include DNA-based techniques, direct mass spectrometry, and other emerging techniques such as dielectric spectroscopy.

In summary, the aim of this work is to systematically review and evaluate existing studies in the literature that could potentially aid in the development a new methodology in order to identify the nature of vegetable oils in a vegetable oil blend.

1.1. DNA-based identification methods

The main argument for using DNA typing methods is that the DNA sequence is exclusively connected with the species, and to a lesser extent with cultivars. Moreover, DNA techniques do not take into account the chemical composition of the samples (fatty acids, triacylglycerides) which might be altered by environmental factors, as well as by sample preparation protocol such as extraction (Zhang et al., 2012). However, DNA is more resilient to destruction by food processing (particularly cooking and sterilisation) than other marker substances (Woolfe & Primrose, 2004) which could be an important issue with oil samples as demonstrated below. DNA fingerprint typing methods have been used in oil authentication. There are three main molecular marker-based approaches: a) using Polymerase Chain Reaction (PCR), b) detecting single nucleotide polymorphisms (SNPs) that give rise to restriction fragment length polymorphism (RFLPs) which require short DNA amplicons for genotyping and c) detecting small sequence length polymorphisms (SSLPs). In general, molecular markers which require DNA amplicons of approximately 100 base pairs (bp) in length as templates are preferred for the identification of the botanical origin of highly processed food matrices such as edible oils.

DNA, although highly stable, is fragmented and is found only in trace quantities in oils. It therefore requires amplification which is not always successful and straight forward. The methods rely on the highly specific fragment amplification using polymerase chain reaction (PCR) and Real-time PCR in order to quantitatively and qualitatively analyse species-specific genes. Discussion in the literature is on-going over choosing the correct nuclear or plastid DNA target because amplicon size plays a significant role in authentication (Doveri & Lee, 2007; Wu et al., 2011). Recent advances show that DNA amplification from refined edible oils using PCR is sufficiently reliable to detect edible oil adulteration, such as genetically modified soybean DNA in refined oils or presence of refined oils in olive oil (Costa, Mafra, Amaral, & Oliveira, 2010). An interesting study on the detection of adulteration of higher value oils with palm oil was conducted by Zhang et al. (2009). Conventional and real-time PCR assays were used targeting the palm specific MT3-B sequence, amplifying a PCR product of 109 bp. The methodologies established were able to detect five haploid copies of palm DNA (10 pg) and were effectively applied to commercial edible vegetable oils, indicating the presence of unlabelled palm oil.

In contrast to nuclear, plastid/chloroplast DNA is more easily recoverable in oil than nuclear DNA and improves the DNA fragment extraction yield. Exploiting polymorphisms in chloroplast DNA to discriminate plant oil species on the basis of differential length amplicons was evaluated in two studies (Spaniolas, Bazakos, Awad, & Kalaitzis, 2008; Spaniolas, Bazakos, Spano, Zoghby, & Kalaitzis, 2010). Using the plastid region of the trnL (UAA) intron as polymorphic target, DNA templates from several vegetable oil species were mixed prior to PCR amplification, resulting in the detection of separate signals based on the combinatorial use of a PCR assay with a lab-on-a-chip capillary electrophoresis. In recent studies, PCR-CE-SSCP technique (PCR assay coupled to capillary electrophoresis and Single-Strand Conformation Polymorphism) with much lower limit of detection for DNA fragments compared to agarose gel electrophoresis was used (Spaniolas et al., 2010; Zhang et al., 2012). This technique was applied to identify several vegetable oils blended in olive oil, targeting the rbcL (ribulose 1,5-bisphosphate carboxylase large subunit) gene fraction of chloroplast genome. Applicability to commercial oils was demonstrated, but further improvements are required to increase sensitivity for vegetable oils for its effective use on olive oil authentication (Wu et al., 2011; Zhang et al., 2012).

Thus, both the PCR method of nuclei DNA and the exploitation of polymorphisms in chloroplast genes are promising approaches for identifying oil species in oil mixtures that warrants further work. Although