

## Safety of Asian soy sauces in a gluten-free diet

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### Introduction

One of the most frustrating aspects of the gluten-free diet is eating out. In a home situation most coeliacs are able to make informed choices based on food the label or by consulting gluten-free shopping lists; however, eating out in a restaurant can be a complicated matter. Patients then have to rely on information from the chef or attendants, who are not always proficient in questions relating to allergies.

from the  
fermentation  
process

The situation becomes even more complicated when food ingredients comprising hydrolyzed proteins - most often included for flavouring purposes - are used. One of these ingredients is the Asian soy sauce, which is available in treat varieties and differs depending to the ingredients used and geographical origin.

These sauces are produced either through microbiological fermentation or chemical hydrolysis. One of the disadvantages of chemical hydrolysis is the occurrence of toxic 3-MCPD (3-monochloropropane-1,3-diol) or 1,3-DCP (1,3-dichloropropane-2-ol). Asian soy sauces prepared through chemical hydrolyses are less complex than those prepared through fermentation. The dark color is often achieved by adding molasses or caramel.

Fermented soy sauces, as a rule, have a better quality, but are also more expensive. In these sauces ground steeped soybeans and wheat are the main ingredients. The fermentation process is rather protracted (1/2 yr) and leads to the proteins of the soy and wheat being hydrolyzed and deamidated. Wheat proteins contain a high level of glutamine which is deamidated to glutamic acid and thus acts as a flavour enhancer (the "umami" taste).

Coeliacs often avoid soy sauces if "wheat" is listed as an ingredient because they fear that intact gluten or smaller, but still toxic gluten peptides might be present. However, due to the extensive hydrolysis of the raw material this is not necessarily the case.

## **Materials and methods**

To elucidate the safety of soy sauces in terms of gluten-free diet we examined the following two aspects:

1. Via a number of coeliac mailing lists on the Internet as well as on the website of the Dutch Coeliac Society we asked patients how they dealt with the issue. We requested information about how they made their choice when shopping or eating out and whether they had ever developed symptoms after having eaten dishes prepared with soy sauces.
2. We sampled 22 soy sauces, either from supermarkets or Indonesian/Chinese shops (see Table 1). An aliquot of these sauces was sent to R-Biopharm and analyzed for gluten content, either through an R5 sandwich assay or R5 competitive ELISA assay. Extraction was done with ethanol or ethanol + gelatine. Some samples were spiked with gluten to check recovery. In both types of assays some samples were spiked with wheat flour (20 ppm gliadin) or with 20 ppm PWG gliadin.

The performance of several extraction solutions was investigated: 60% ethanol; 60% ethanol + 10% gelatine; ethanol + PVP + gelatine; cocktail solution; cocktail + gelatine and cocktail + skim milk powder for sandwich R5 ELISA, and in the case of the R5 competitive assay, 60% ethanol and 60% ethanol + 10% gelatine.

## **Results**

### **Results of questionnaire**

A general conclusion could not be drawn from the results of the questionnaire. As expected, most coeliacs stated that they avoided products if wheat was included in the ingredients list. Some reported having experienced symptoms after ingestion of soy sauces, but these symptoms were sometimes not typical and could not always be attributed per se to the soy sauce. Others, however, were at ease since they knew that the proteins in these sauces were almost completely degraded; consequently, they did not experience any symptoms. Given the results of our analyses, it is quite remarkable that symptoms occurred in some coeliacs after these products were eaten.

### **Analytical results**

All analyses done by means of the R5 sandwich assay proved negative. The results of the analysis through R-Biopharm R5 ELISA in competitive format are presented in Table 2.

TABLE 1. Properties of soy sauces (from the ingredient list)

No.	Name	Brand	Soy + or %	wheat	Salt	s = sugar m = molasse	acid	colour	preservative	flavouring
1	Dark soy sauce	Amoy	17%	+	+	s		+	s	
2	Ketjap manis	Improba	+	-	+	m	l/c		s/b	+
3	Soy sauce	Conimex	+	+	+				b	
4	ketjap asin (organic)	Conimex	24%	+	+	m		+	s	+
5	Ketjap manis	Conimex	26%	+	+	m		+	s	
6	Japanies soya sauce	Blue dragon	+	+	+				b	
7	Ketjap manis	Ah	+	+	+	m		+	s	
8	Ketjap manis	Improba	+	-	+	m	a	+	s	+
9	Soya sauce	Kikkoman	+	+	+					
10	Soya sauce	Blue dragon	+	+	40%	m	l	+	s	
11	Dark soya sauce	Panta-inorasingh	65%	+	+	m	c	+	b	
12	Ketjap manis	Rapindo	+	-	+	+			b	+
13	Dark soya sauce	Lee kum kee	72%	+	+	+		+	b	+
14	Light soya sauce	Chuew huad	35%	15%	17%	3.9%				
15	Dark soya sauce	Pearl river bridge	+	+	+	+				
16	Sweet soya sauce	Panta-inorasingh	35%	+	+	s/m				
17	Ketjap asin (organis)	Yakso	+ (tempeh)	-		m				+
18	Ketjap manis (organic)	Yakso	+ (tempeh)	-		m				
19	Light soya souce	Pearl river bridge	+	+	+					
20	Soya sauce	Mee chung	+	+	+	s			b	+
21	Ketjpa manis	Go-tan	22%	+	+	s	c	+		+
22	Mushroom soya sauce	Haday	+	+	+	s				+

**TABLE 2.** RIDASCREEN® Gliadin competitive assay (values in mg/kg)

Nr.		wheat present	extraction			
			peptide		gliadin	
			ethanol	ethanol + gelantine	ethanol	ethanol + gelantine
1	Dark soya souce, amoy	+	< 1250	< 1250	< 5	< 5
2	Ketjap manis, Improba	—	< 1250	< 1250	< 5	< 5
3	Sojasaus, conimex	+	< 1250	2340	< 5	12.9
4	Ketjap asin, conimex	+	< 1250	1388	< 5	9.1
5	Ketjap manis , conimex	+	1384	1519	< 5	< 5
6	Japanese soja saus, blue dragon	+	< 1250	< 1250	< 5	< 5
7	Ketjap manis, ah	+	1343	1290	5.9	< 5
8	Ketjap manis, improba	—	2541	1359	10.5	6
9	Sojasaus, kikkoman	+	1343	1421	7.1	5.8
10	Soy sauce, blue dragon	+	< 1250	< 1250	< 5	< 5
11	Dark soy sauce, pantainorasingh	+	1566	1463	8.1	9.3
12	Ketjap manis, rapindo	—	< 1250	< 1250	< 5	< 5
13	Dark soy sauce, lee kum kee	+	1738	1506	6.6	6
14	Light soy sauce, pearl river bridge	15%	< 1250	3364	5.6	5.7
15	Dark soy sauce, pearl river brdige	+	1513	1465	5.6	< 5
16	Sweet soy sauce, pantainorasingh	+	3141	2251	12.5	< 5
17	Ketjap asin (organic), yakso	—	< 1250	< 1250	< 5	< 5
18	Ketjap manis (organic), yakso	—	1800	1448	8.1	6.9
19	Light soy sauce, pearl river bridge	+	1845	1336	9.2	8.7
20	Soy sauce, mee chung	+	2147	< 1250	10.5	7.8
21	Ketjap manis, go-tan	+	1612	1578	6	5.2
22	Mushroom soya sauce, haday	+	3665	1430	9.6	5.3

gluten gluten  
 <10 <10 ppm  
 <10 <10  
 <10 <25.8  
 <10 <18.2  
 <10 <10  
 <10 <10  
 <11.8 <10  
 <21 <12  
 <14.2 <11.6  
 <10 <10  
 <16.2 <18.6  
 <10 <10  
 <13.2 <12  
 <11.2 <11.4  
 <11.2 <10  
 <25 <10  
 <10 <10  
 <16.2 <13.8  
 <18.4 <17.4  
 <21 <15.6  
 <12 <10.4  
 <19.2 <10.6

$$= \text{ppm} / 2 = \text{ppm} / 2$$

2 x gliadin = gluten

### Conclusions

#### (based on levels measured by the competitive assay with ethanol extraction)

Levels of QPFP peptide and gliadin were low in the 17 samples that contained wheat as an ingredient: the highest level found was 12.5 ppm gliadin, which is slightly higher than the gluten level of 20 ppm proposed as the Codex NFSDU standard. Given the amount of soy sauce used in Asian dishes this level might be irrelevant.

= 25 ppm

Of the five samples prepared without wheat two showed a level of gluten above the LOQ, and one slightly exceeded 20 ppm gluten. Gluten levels in these sauces were also low. There seems to be no correlation between gluten content and the declaration of wheat on the product label.

### Conclusions of spiking experiments

In a sandwich format the recovery was good. Overall recoveries with the respective solvents are: 88.6%; 92.6%; 95.3%; 105.9%; 95.2% and 91.4%

In the competitive format, recovery was also good, and gelatine was not needed for analyzing these types of samples. Overall recoveries were: 105.1% (60% ethanol) and 90.9% (60% ethanol with 10% gelatine).

### Discussion

In the EU, the following wheat-containing products are exempt from labelling: wheat starch-based glucose syrup; wheat starch-based dextrose; wheat-based maltodextrine; barley starch-based glucose syrup and cereal-based distilled spirits.

Wheat hydrolysis is not mentioned, thus the packaging of any food containing wheat hydrolysate has to list it as an ingredient on the label. However, this does not by definition mean that these foods are unsafe for coeliacs: the EU labelling directive is aimed not only at protecting persons with a wheat allergy, but also those with as coeliac disease, however, a safe level for these with wheat allergy has not yet been established.

The Codex draft standard as proposed at the Codex NFSDU meeting in November 2007, which is to be forwarded to the Codex Commission for final adoption at step 8, allows the presence of wheat hydrolysates in gluten-free products (para 2.1.1.b) provided the level of gluten is lower than 20 mg/kg. This raises questions about the suitability of analytical methods for all type of products. The proposed Codex Standard does not specify whether a particular method is especially suitable for hydrolyzed gluten proteins. Sandwich type assays are not suitable as they do not measure protein fragments which have only one epitope. By consequence, another type of analysis is required when hydrolysates or mixtures of hydrolysates and intact proteins are present. The RIDASCREEN® Gliadin competitive (R7011) assay is

designed to overcome these problems. However, the difficulty lies in calculating the results, because standard material is not yet available for this type of assay. Thus, it is difficult to evaluate the results within a regulatory framework, e.g., in comparison to the limit of 20 mg/kg, proposed by the Codex NFSDU committee as a statutory limit for gluten-free foods!

Provisionally in the R-5 competitive assay the results are expressed as  $\mu\text{g}$  peptide (QQFPF). In separate experiments it was found that an amount of peptide that was 250\* that of intact PWG gliadin produced the same response. This is also the case in our experiments. A ratio of 245\* (range 189-381) was found with ethanol extraction and a ratio of 258\* (range 152-590) was found with ethanol + gelatine extraction, both calculated using results higher than the detection level. Hydrolysates are usually present as a mixture with different degree of hydrolysis. Thus the stoichiometrical behaviour might be different and it is very difficult to validate a conversion factor in relation to toxicity.

### **Recommendations**

- We recommend that, as far as possible, coeliacs should choose Asian soy sauces which do not include wheat.
- If such information is not available, they should not be worried about the ingestion of gluten as investigations have shown that the levels of gluten are almost insignificant in relation to the safe dose as reported by Catassi et al. [1].
- Coeliacs should, however, still avoid dishes with very high levels of soy sauce.

### **Reference**

1. Catassi C, Fabiani E, Iacono G, D'Agate C, Francavilla R, Biagi F, Volta U, Accomando S, Picarelli A, De Vitis I, Pianelli G, Gesuita R, Carle F, Mandolesi A, Bearzi I, Fasano A. A prospective, double-blind, placebo-controlled trial to establish a safe gluten threshold for patients with celiac disease. *Am J Clin Nutr* 2007; **85**: 160-166.