Development of an assay for Soy Isoflavones in Women's Hair and validation of a French Dietary Habits Inquiry

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Abstract: Soy isoflavones have estrogenic and anti-thyroidal effects in animals and humans that can be beneficial or adverse, depending on consumers' physiological status. Hence, knowing the consumers' isoflavones exposure can help when looking for health issues related to estrogenic or thyroidal effects. Several extraction methods were tested on 60 volunteer's hair samples from a hairdresser and from the clinical trial 2017T2-29. The acidic digestion method was the most efficient regarding isoflavones. A dietary habit inquiry was developed based on the analysis of 12,707 food labels from France. A score for the consumer's exposure to isoflavones was built considering, among other parameters, soy-based diet and foodstuff containing soy as an ingredient, i.e. "hidden-soy". The correlation between this score and isoflavones measurements in hair reached 0.947; p<0.001. Therefore, providing that relevant data are considered to assess isoflavones exposure, hair which smoothen daily isoflavones intakes' variations, is a relevant tissue to assess human isoflavones exposure for subsequent health analysis.

Keywords: soy isoflavones; consumer exposure; hair samples; dietary inquiry; digestion method.

Abbreviations:

DAI: Daidzein aglucone form IFS: Isoflavone Food score GEN: Genistein aglucone form IHS: Isoflavone Hair score

Data presented:

Supplementary material S1: Dietary Inquiry Proposed to Volunteers

Supplementary material S2: Matrix used to Calculate IFS and IHS

Supplementary material S3: Evidencing a Questionable Answer to the Food Questionnaire

Supplementary material S4: Pearson Regression Correlation Between Individual Isoflavones

and HIS

Supplementary data S1: Dietary Inquiry

Sex of the volunteer Subject's age Casual treatment since Shampoo frequency Last colouring Casual product used Casual product used Last product used Last product used Last product used Casual product used Last product used Last product used Casual product used Last product used Casual product used Last product used Last product used Casual product used Last product used Casual product used Last product used Casual product used Casual product used Casual product used Last product used Casual product us	Subjet and study parameters Hair treatments questionnaire	Study acronyme Subject code Investigator ID		Promotor's name Inquiry date Investigator's name		
Hair length	Sex of the volunteer	s	<u></u>			
Food consumption profile I eat at home I eat in a canteen I eat in a restaurant Gooking pratices Frequency per were frough habits I eat in a canteen I eat in a restaurant Gooking pratices Frequency per were frough abits I eat in a canteen I eat in a restaurant Gooking pratices Frequency per were frough abits Frying Frying Vapor cooking Vapor cooking Lacto-vegetarian I go to the fast-food I eat in my office at least once a week Grill	Hair length		Last per	rm Casual p	product used	
Omnivorous 2 times a day 2 times a day Vapor cooking Vegetarian once a day once a day once a day Lacto-vegetarian I go to the fast-food I eat in my office at least once a week Grill	Food consumption profile		ome	I eat in a canteen	I eat in a restaurant	(number of times)
Lacto-vegetarian I go to the fast-food I eat in my office at least once a week Grill					֡֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓֓	Vapor cooking
Flexivegetarian I don't have 3 meals a day I have snacks all day I have food supplements	Vegan	once a	week		_ [Grill Wok/Oven
For how-long have you been on this diet? I am a small-eater I am a medium-eater I am a medium-eater I am a big-eater Which food supplements Which food supplements Use of the control of the contr	For how-long have you been on this	I am a If your c please te	small-eater ase is not considered Il us if you have a	_		

3 Supplementary data S1: Dietary Inquiry (continued)

Cons	umption of foodstuff based on "	plant-milk"												
1	Do you drink soy-milk?	Which season		Winter		Spring		Summer		Autumn		All year	Casual portion size	
	several a day	everydays	sometimes a week		once a week		< once a week		several a month		<pre> < once a month</pre>	never	1/2 1 2 3	spoon glass bowl plate
2	Do you eat soy-yogurts?	Which season		Winter		Spring		Summer		Autumn		All year	Casual portion size	
	several a day	everydays	sometimes a week		once a week		<pre>< once a week</pre>		several a month		<pre> < once a month</pre>	never	1/2 1 2 3	spoon glass bowl plate
3	Do you eat soy-cream?	Which season		Winter		Spring		Summer		Autumn		All year	Casual portion size	
	several a day	everydays	sometimes a week		once a week		<pre>< once a week</pre>		several a month		<pre> < once a month</pre>	never	1/2 1 2 3	spoon glass bowl plate
4	Do you eat soy-cheese or tofu?	Which season		Winter		Spring		Summer		Autumn		All year	Casual portion size	
	several a day	everydays	sometimes a week		once a week		< once a week		several a month		<pre>< once a month</pre>	never	1/2 1 2 3	spoon glass bowl plate
5	Do you drink "plant-milks" (coconut, haselnut, almond, ri	Which season		Winter		Spring		Summer		Autumn		All year	Casual portion size	
	several a day	everydays	sometimes a week		once a week		<pre>< once a week</pre>		several a month		<pre>< once a month</pre>	never	1/2 1 2 3	spoon glass bowl plate

6 Supplementary data S1: Dietary Inquiry (continued) Consumption of elaborated dishes

Con	sumption of elaborated dishes													
6	Do you eat soy-based product (Saussages, burgers, minced i			Winter		Spring		Summer		Autumn		All year	Casual portion size	
	several a day	everydays	sometimes a week		once a week		<pre>< once a week</pre>		several a month		< once a month	never	1/2 spo 1 gla: 2 bov 3 plat	ss vl
7	Do you eat meat-based elaborated dishes? (paté, saud	Which season		Winter		Spring		Summer		Autumn		All year	Casual portion size	
	several a day	everydays	sometimes a week		once a week		< once a week		several a month		< once a month	never	1/2 spo glas 2 bov 3 plat	ss vl
8	Do you eat fish-based elaborated dishes? (surimi, pa	Which season até)		Winter		Spring		Summer		Autumn		All year	Casual portion size	
	several a day	everydays	sometimes a week		once a week		<pre>< once a week</pre>		several a month		< once a month	never	1/2 spo 1 glas 2 boy 3 plat	ss vl
9	Do you eat vegetable-based elaborated dishes?	Which season		Winter		Spring		Summer		Autumn		All year	Casual portion size	
	several a day	everydays	sometimes a week		once a week		<pre>< once a week</pre>		several a month		< once a month	never	1/2 spo glas 2 bov 3 plat	ss vl
10	Do you eat pizzas?	Which season		Winter		Spring		Summer		Autumn		All year	Casual portion size	
	several a day	everydays	sometimes a week		once a week		<pre>once a week</pre>		several a month		< once a month	never	1/2 spo glas 2 bov 3 plat	ss vl

8

Supplementary data S1: Dietary Inquiry (continued) Do you eat fresh elaborated Which season Winter Spring Summer Autumn All year Casual portion size dishes? 1/2 spoon everydays several sometimes once a once a several < once a never 1 glass a day week week a month month 2 bowl a week 3 plate Casual portion size Do vou eat meat-based Winter Spring Autumn All year Which season Summer canned dishes? 1/2 spoon everydays several sometimes once a < once a several < once a never glass 2 a day a week week week a month month bowl 3 plate Do you eat nuggets Winter All year Spring Autumn Casual portion size Which season Summer frozen or not? 1/2 spoon several everydays sometimes once a < once a several < once a never 1 glass 2 a day a week week week a month month bowl 3 plate Do you eat Kebbabs or Which season Winter Spring Summer Autumn All year Casual portion size hamburgers? 1/2 spoon everydays once a several sometimes once a several < once a never glass a day a week week week a month month 2 bowl 3 plate Winter All year Do you eat meat-balls Spring Summer Autumn Casual portion size 15 Which season frozen or not? 1/2 spoon several everydays sometimes once a once a several once a never glass a day week week 2 a week a month month bowl 3 plate

12 Supplementary data S1: Dietary Inquiry (continued) 13 Winter Do you eat "escaloppe Spring Which season Summer Autumn All year Casual portion size cordon bleu" frozen or not? 1/2 spoon everydays sometimes several once a once a several < once a never 1 glass a day a week week week a month month 2 bowl 3 plate Winter Do you eat stuffed vegetables Which season Spring Summer Autumn All year Casual portion size frozen or not? 1/2 spoon everydays sometimes once a several < once a several < once a never glass 2 a day a week week week a month month bowl 3 plate Spring Winter All year Do you eat Which season Summer Autumn Casual portion size frozen ground beef? 1/2 spoon several everydays sometimes once a < once a several < once a never 1 glass 2 a day a week week week a month month bowl 3 plate Do you eat industrial Which season Winter Spring Summer Autumn All year Casual portion size biscuits? 1/2 spoon everydays sometimes once a several once a several < once a never glass a day a week week week a month month 2 bowl

141516

3

plate

17 Supplementary data S1: Dietary Inquiry (continued)

Legu	ime consumption raw or cooked	i											
20	Do you eat legumes like beans, lentils, peas	Which season		Winter		Spring		Summer		Autumn		All year	Casual portion size
	several a day	everydays	sometimes a week		once a week		< once a week		several a month		< once a month	never	1/2 spoon glass 2 bowl plate
21	Do you eat chick-peas?	Which season		Winter		Spring		Summer		Autumn		All year	Casual portion size
	several a day	everydays	sometimes a week		once a week		< once a week		several a month		< once a month	never	1/2 spoon glass 2 bowl 3 plate
22	Do you eat soybeans ?	Which season		Winter		Spring		Summer		Autumn		All year	Casual portion size
	several a day	everydays	sometimes a week		once a week		< once a week		several a month		< once a month	never	1/2 spoon glass 2 bowl glate
23	Do you eat mungo-sprouts?	Which season		Winter		Spring		Summer		Autumn		All year	Casual portion size
	several a day	everydays	sometimes a week		once a week		<pre>< once a week</pre>		several a month		< once a month	never	1/2 spoon glass 2 bowl plate
24	Do you eat alfalfa or clover sprouts?	Which season		Winter		Spring		Summer		Autumn		All year	Casual portion size
	several a day	everydays	sometimes a week		once a week		< once a week		several a month		<pre>< once a month</pre>	never	1/2 spoon glass 2 bowl 3 plate

19 Supplementary material S2: Calculation of IFS and HIS

Diet duration (months)

Canteen >3 times/week

Fast food >2times /w

Hair						Multiplying factor
		Short	Medium	Long	Δ	
	Length	0,5	0,2	0,1	A	
		<2 month	<4 month	>4 month	В	a = A + B + C
	Frequency of cutting	0,5	0,2	0,1	Б	a-A+b+C
		Shampoo	Colouring	Perm	C	
	Treatments	0,5	0,2	0,1	Ò	

Home > 3times/w

0,5

0,2

c = a + b

G=E/F

e

Vegan		Vegetarian	Lactovegetarian	Flexitarian	Omnivore	
vegan	1	0,8	0,8			a
Asian		Exotic	Gluten free	Lactose free	Other	b
	1	0,8	0,2	0		_
Wok/Oven		Vapor	Grill	Frying	Boiling	C
	1	0,8	0,5	0,2	0	
Ultratransformed		Industrial	Home-made			d
	1	0,5	0,2			u

Restaurant > 2times/w

0,8

Hair length

22 Supplementary material S2: Calculation of IFS and HIS (continue)

Soy-consumption

<i>J</i> 1										
	Every day		> once a week		< once a week		never		f	
Soy juice		2		1		0,5		0	1	
	Every day		> once a week		< once a week		never		σ	
Soy yogurt		0,8		0,3		0,1		0	g	
	Every day		> once a week		< once a week		never		h	
Soy cream and icecream		2		1		0,5		0	11	
	Every day		> once a week		< once a week		never		i	
Soy cheese and tofu		1		0,5		0,2		0	1	
	Every day		> once a week		< once a week		never		:	
Other plant juices		0		0		0		0	J	
	Every day		> once a week		< once a week		never		k	
Other soy products (miso, tempeh)		2		1		0,5		0	K	
	Every day		> once a week		< once a week		never		1	If
Meat based dishes		0,2		0,1		0		0	1	to
	Every day		> once a week		< once a week		never			I
Fish based dishes		0,2		0		0		0	m	to
	Every day		> once a week		< once a week		never			I
Vegetables based dishes		0,4		0,2		0		0	n	te
	Every day		> once a week		< once a week		never		_	I
Pizzas		0,1		0		0		0	0	te
	Every day		> once a week		< once a week		never			If
Elaborated dishes		0,3		0,1		0		0	р	to
	Every day		> once a week		< once a week		never			If
Canned recipes		0,2		0,1		0		0	q	to
·	Every day	,	> once a week	,	< once a week		never			I
Nuggets		0,2		0,1		0		0	r	1
00	Every day	,	> once a week	,	< once a week		never			I
Hamburgers / Kebabs		0,5		0,2		0,1		0	s	
	Every day	,-	> once a week	,	< once a week		never			I
							1		t	1

f Canteens, increased to the upper level If Canteens, increased to the upper level If Canteens, increased to the upper level f soy increased to the upper level f soy increased to the upper level f soy increased to the upper level

24 Supplementary material S2: Calculation of IFS and HIS (continue)

25

2627

	Every day		> once a week		< once a week		never		
Escalope cordon bleu		0,2		0,1		0	()	u
Stuffed vegetables	Every day		> once a week		< once a week		never		
(tomatoes, cabbages)		0,2		0,1		0	()	V
	Every day		> once a week		< once a week		never		-1-
Frozen minced steaks		0,5		0,2		0,1	()	W
	Every day		> once a week		< once a week		never		.,
Industrial biscuits		0,15		0,1		0	()	X
	Every day		> once a week		< once a week		never		
Other legumes		0,2		0,1		0	()	У
	Every day		> once a week		< once a week		never		_
Chick peas		0,2		0,1		0	()	Z
	Every day		> once a week		< once a week		never		
Soy seeds		3		2		1	()	aa
	Every day		> once a week		< once a week		never		l
soybean sprouts		0,5		0,2		0,1	(ba
	Every day	_	> once a week	_	< once a week		never		
Alfalfa or clover sprouts		3		2		1	(ca

If soy increased to the upper level
If Canteens, increased to the upper level
If Canteens, increased to the upper level
If Canteens, increased to the upper level

	Soy/Alfalfa/Clover	Others	None	da
Food supplements	1	0	0	ua

Isoflavone Food Score (IFS)

IFS=SUM(a:da)* c

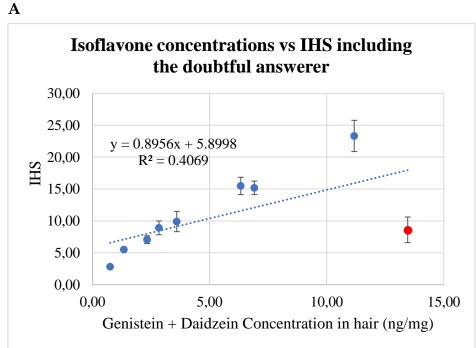
Isoflavone Hair Score (IHS)

IHS=IFS*G

3132

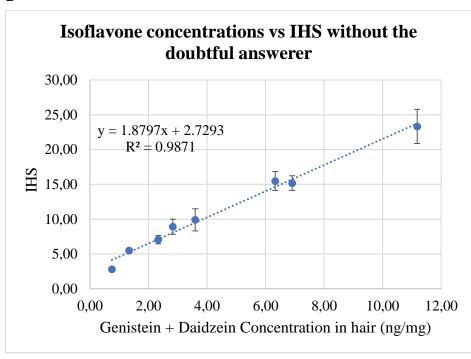
Supplementary material S3: Evidencing a Questionable Answer to the Food Questionnaire

30



N= 9; r = 0.638 p<0.1

В



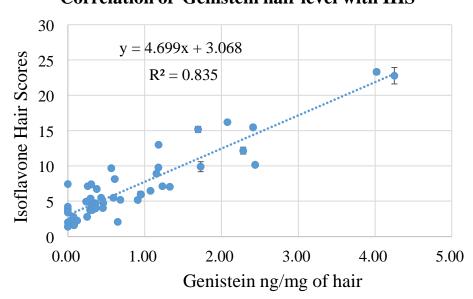
N = 8; r = 0.993 p<0.001

Supplementary material S4: Pearson Regression Correlation Between Individual Isoflavones and IHS

35

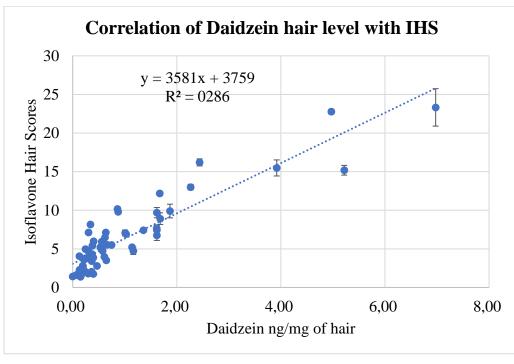
36 37 A

Correlation of Genistein hair level with IHS



$$N = 60$$
; $r_G = 0.914 p < 0.001$

В



$$N = 60$$
; $r_D = 0.91$; $p < 0.001$